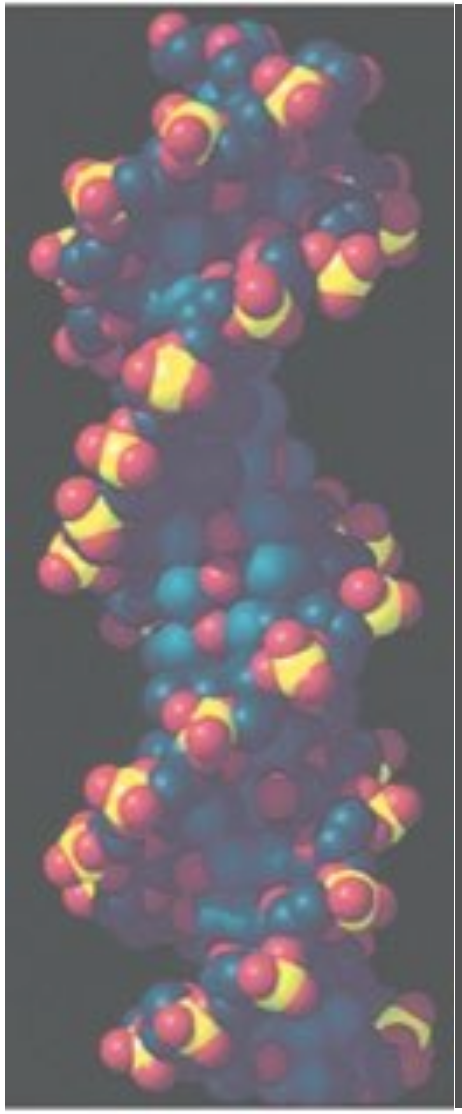
Einführung in die Molekulargenetik

Folien -> unsere homepage, Passwort: Drosophila



„Klassische Genetik“	Was ist ein Gen, Mutation,
Rekombination	Cre/Lox FRT/FLP Anwendung Transgene Organismen
Chromosomen	Aufbau und Modifikationen
Transkription	Regulation, Methoden
Splicing	Beispiele
RNAi/CRISPR	Mechanismen, Anwendungen
Translation	Mechanismus, Regulation
Signalkaskaden	Notch, RTK, Wnt, Hh, TGFß
Nervensystementwicklung von Drosophila	

Einführung in die Molekulargenetik

Bücher:

Knippers	Molekulargenetik (49,95 €)
Janning/Knust	Genetik (39,95 €)
Alberts et al.,	Molecular Biology of the Cell (82,50 €)
Squire et al.,	**Fundamental Neuroscience (80,00 €)**

Gene kontrollieren den Phänotyp



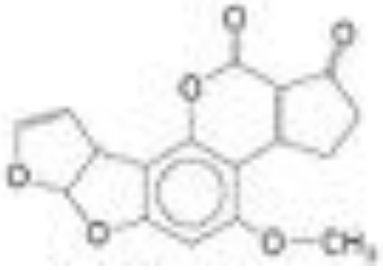
Hypertrichose

Um Gene zu „sehen“ brauchen wir Mutationen !

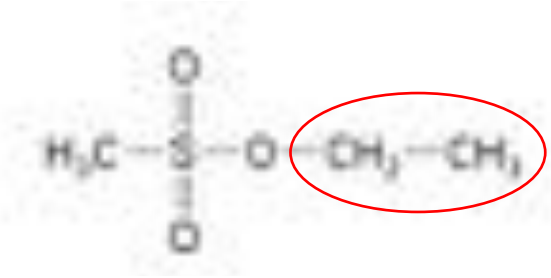
Mutagene Stoffe wirken direkt oder indirekt



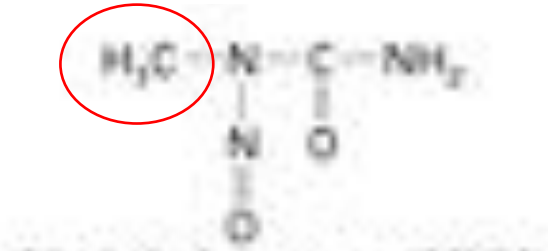
3,4 Benzpyren



Aflatoxin B_1



Ethylmethansulfonat (EMS)

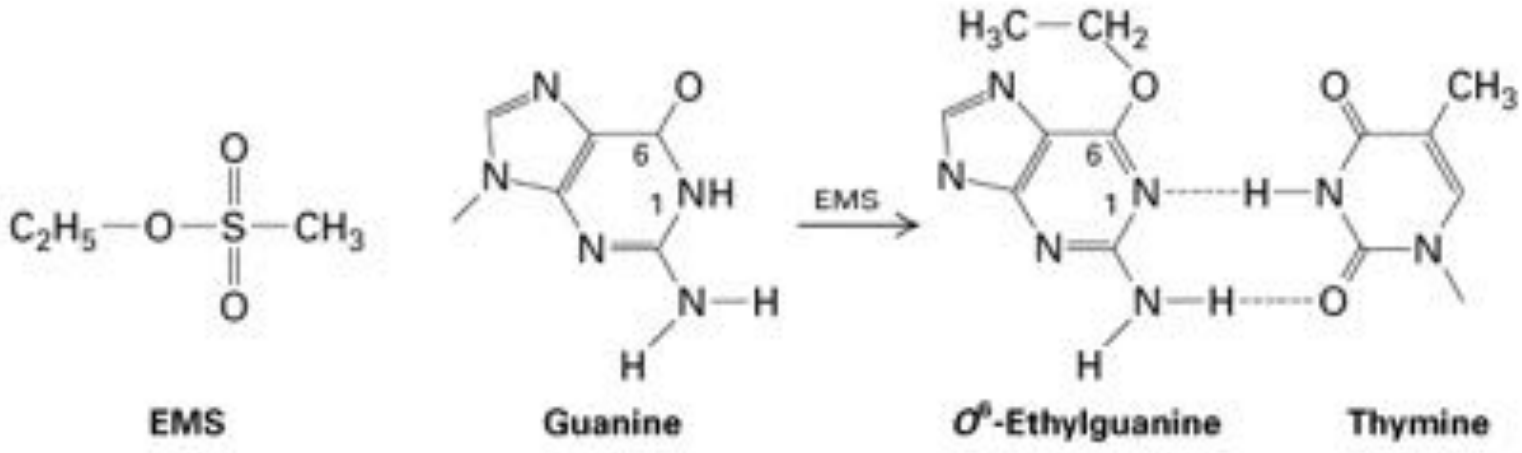


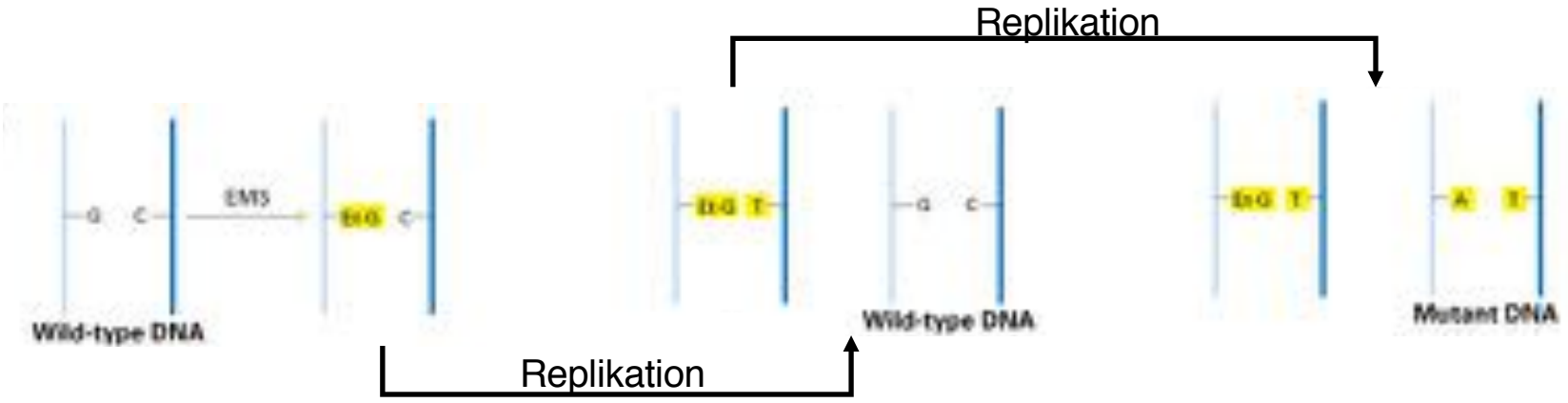
Methyl-nitroharnstoff
Methyl-nitrosourea (MNU)
Ethyl-nitrosourea (ENU)

UV Licht ➡
Thymindimere

Röntgen-Strahlung ➡
Chromosomale Abberationen

EMS induziert G ->A Austausche





C->T Mutation nach (spontaner) Desaminierung von Cytosin



Reparatur zu Thymidin

RNA editing

Antiviral wirkende Enzymaktivität !

Weitere Möglichkeiten der Mutagenese

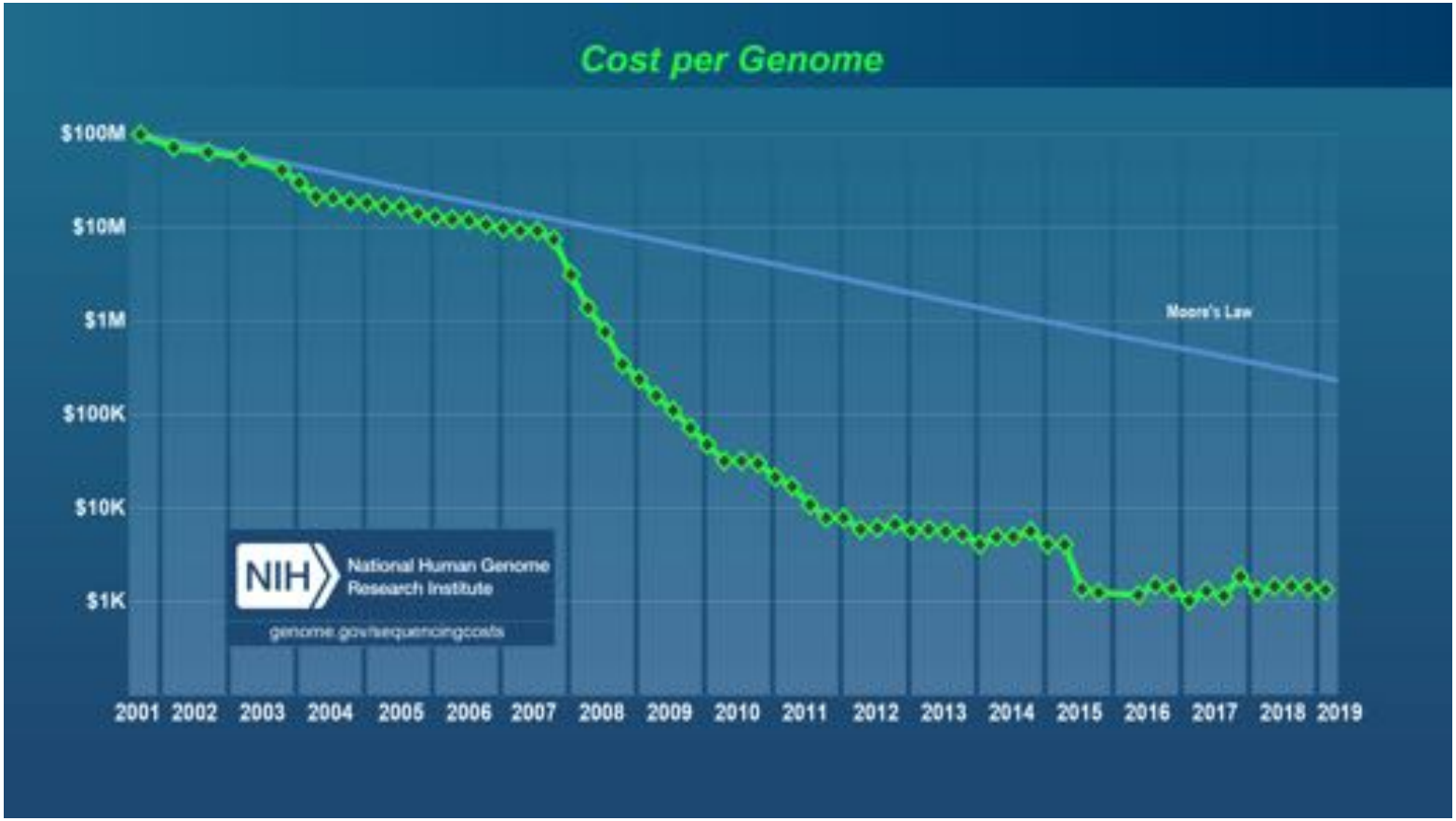
Transposons, mobile DNA

TALEN, CRISPR

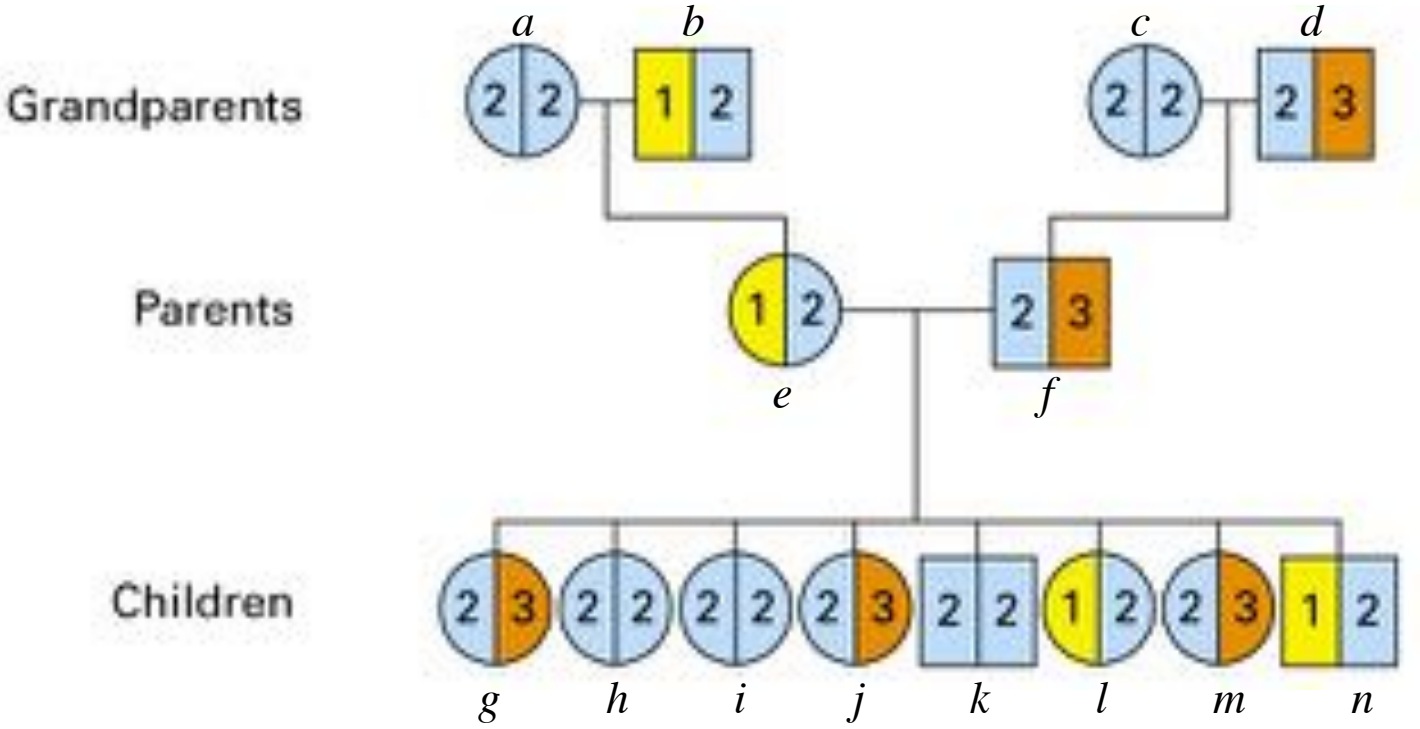
Gene targeting -> homologe Rekombination

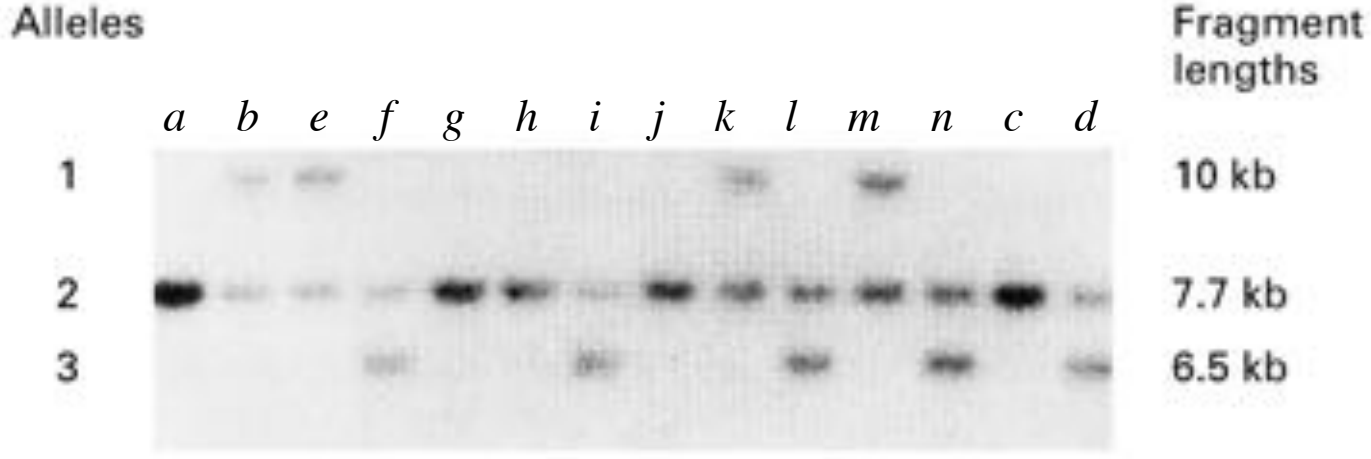
Detektion von Mutationen:

Sequenzanalyse

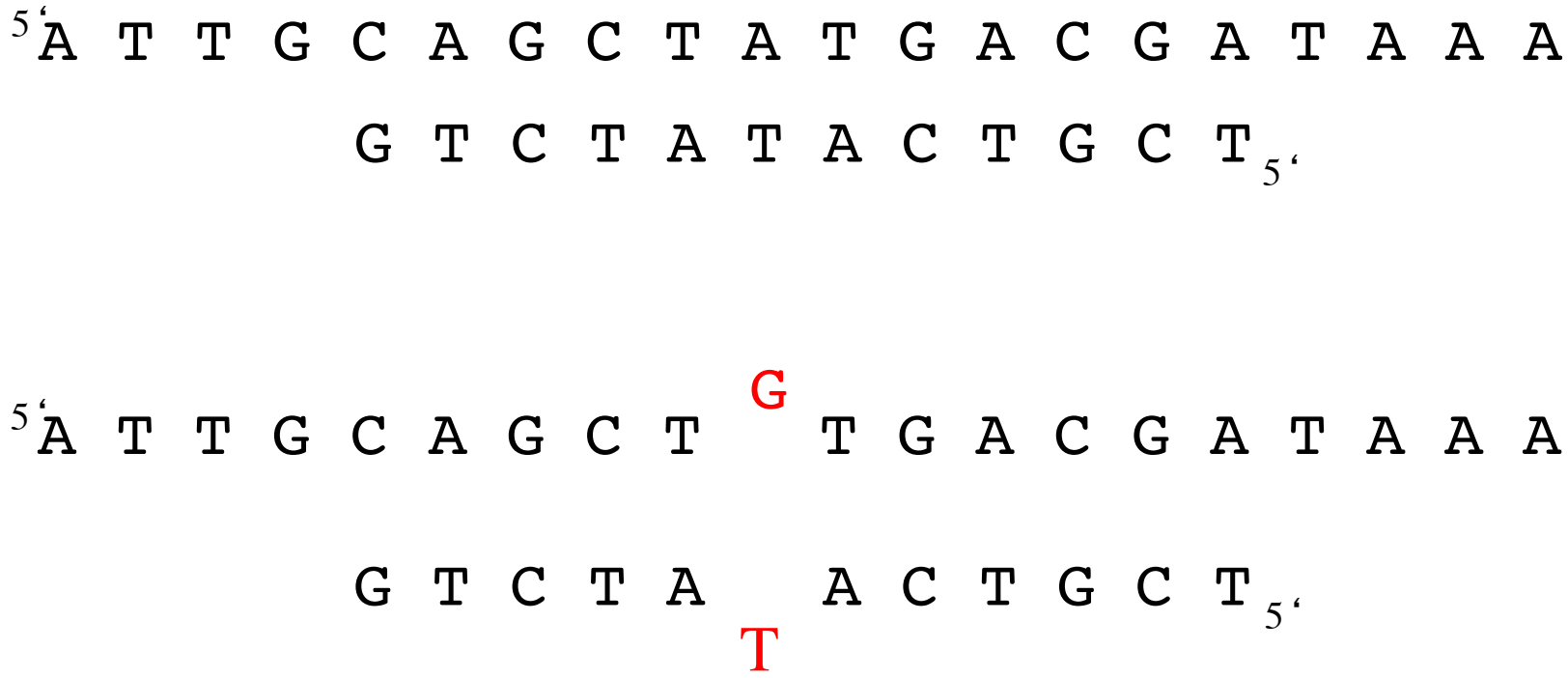


Restriktionslängenpolymorphismus (RFLP)-Analyse

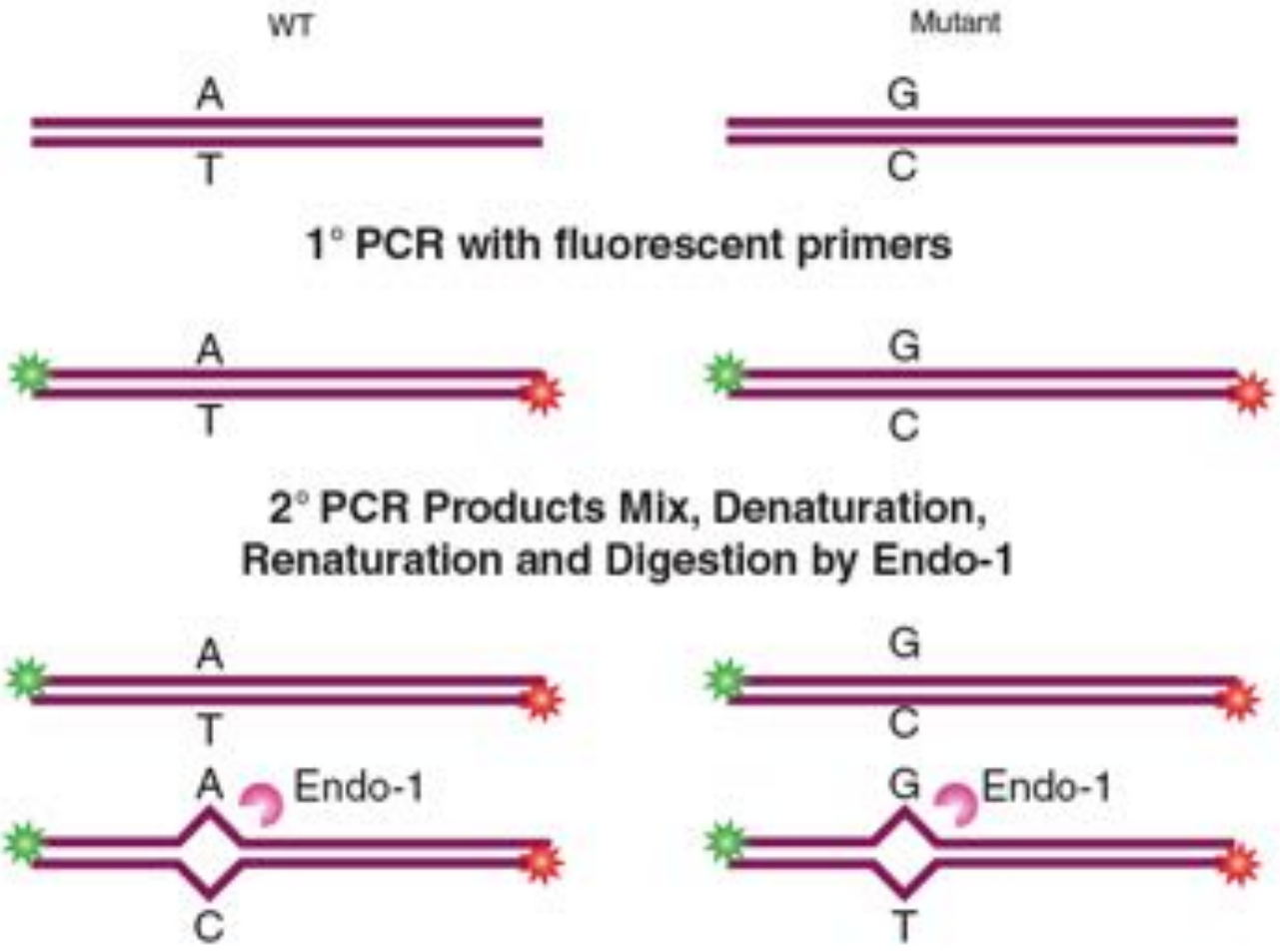




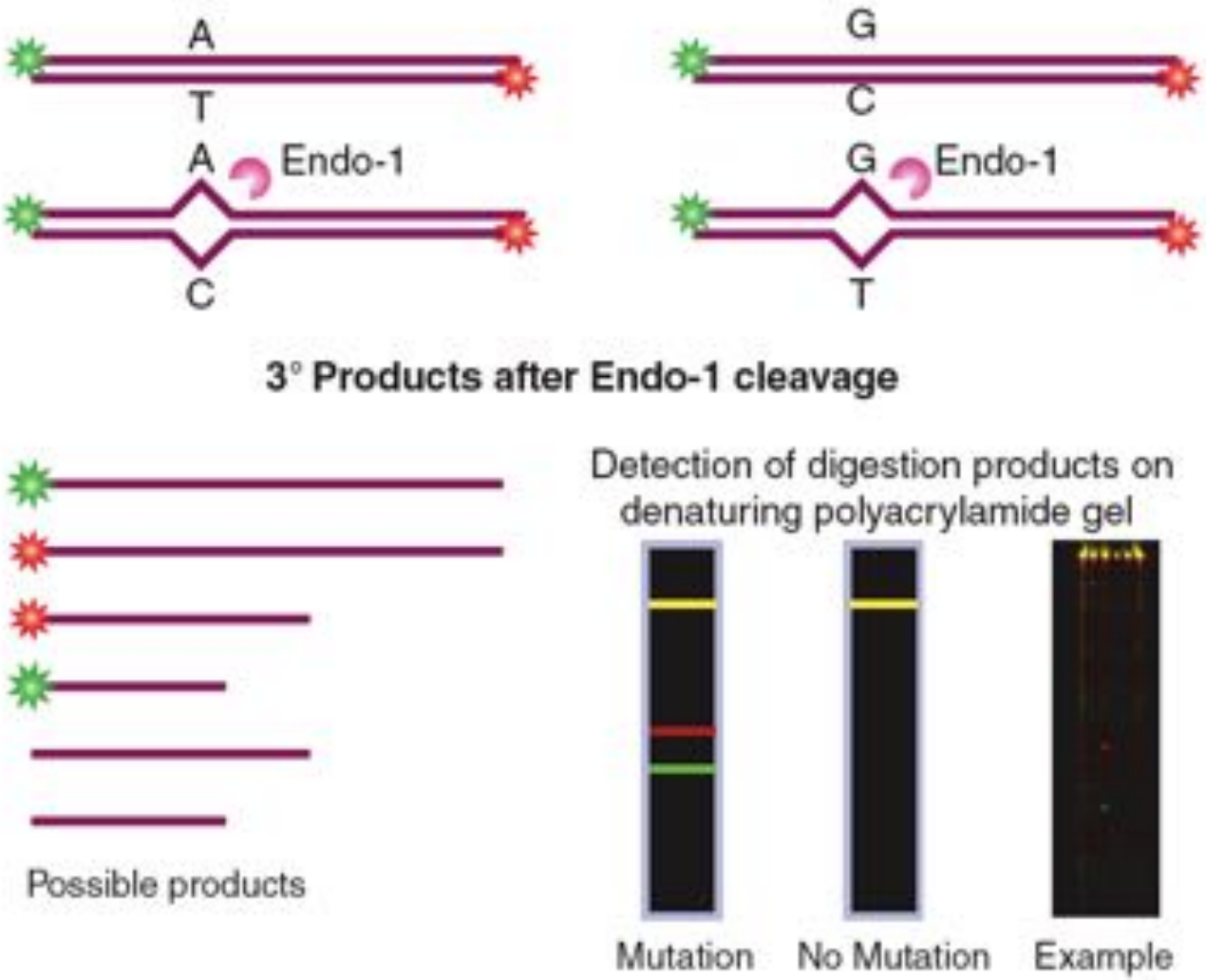
Single nucleotide polymorphisms
SNPs



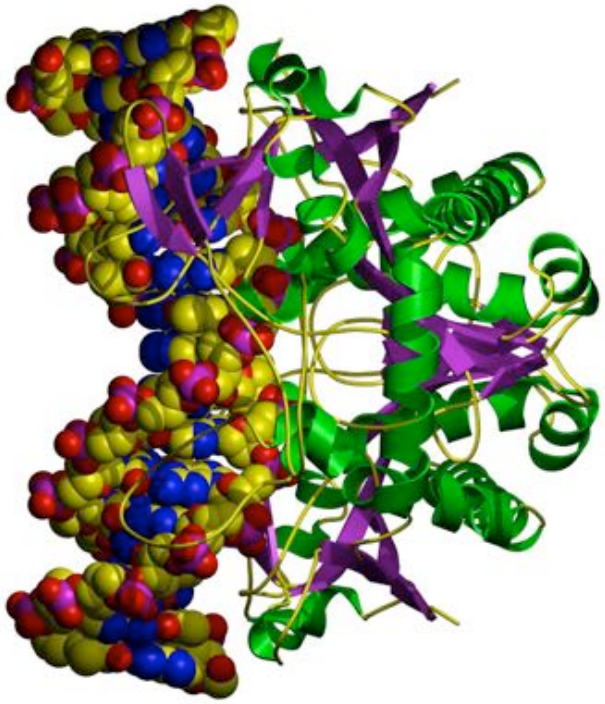
Tilling, Detektion von Punktmutationen



Tilling, Detektion von Punktmutationen

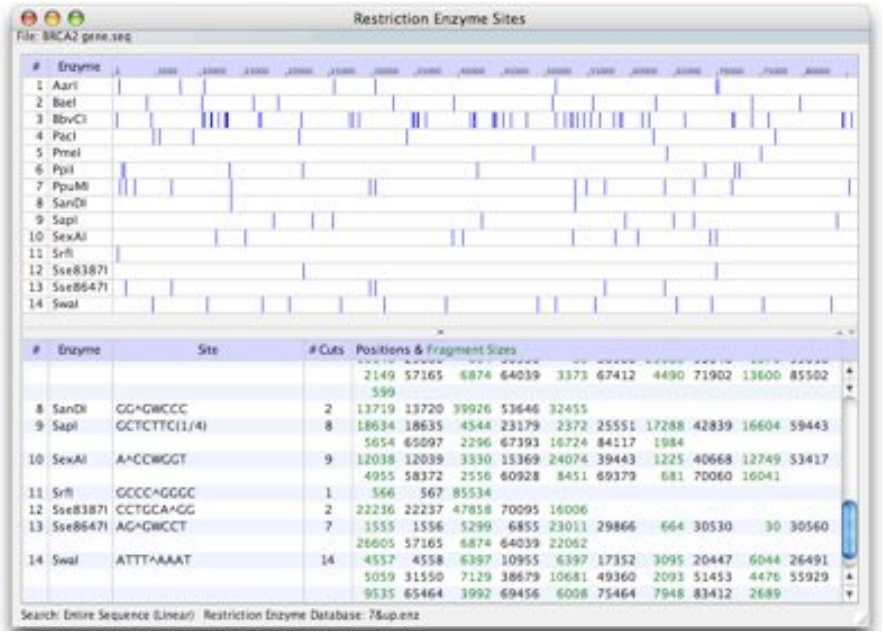


Restriktionsenzyme



5‘-GAATTC-3‘

EcoRI



Abwehrsystem von Prokaryoten. Restriktionsenzyme sind Endonukleasen. Schutz der endogenen DNA durch Methylasen.

Typ I- Typ IV Restriktionsenzyme.
In der Molekulargenetik werden Typ II Enzyme verwendet. Erkennungssequenz 4-6 (8)
Homing Endonukleasen: I-Sce I
Erkennungssequenz: 18 bp ($1/7x10^{10}$)

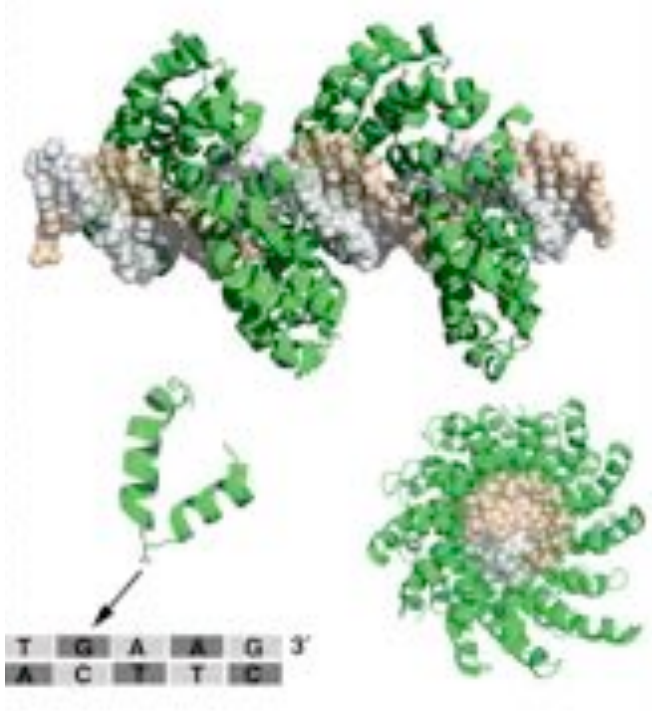
TALEN (Transcription Activator-Like Effector Nuclease)



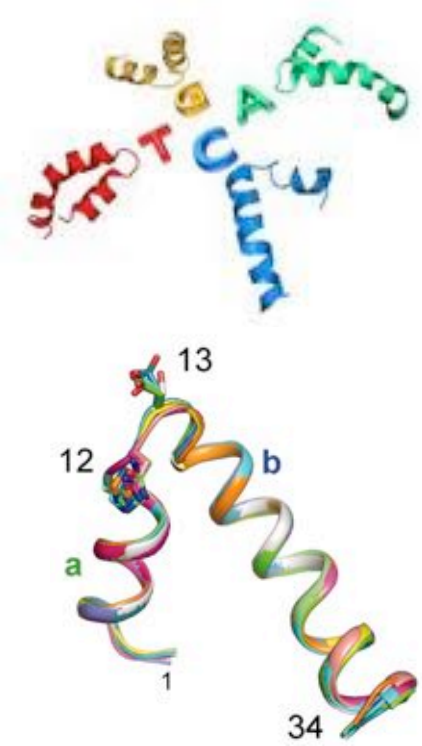


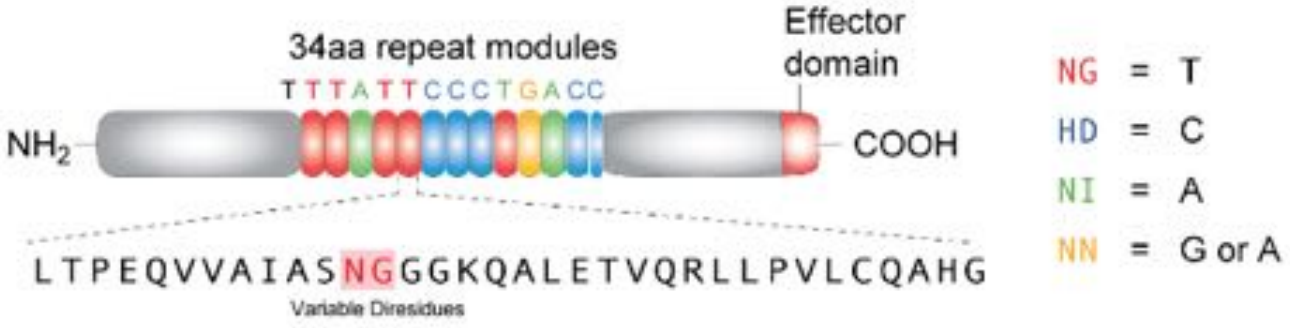
TAL (transcription activator-like) Effektoren sind Proteine die von *Xanthomonas* Bakterien sezerniert werden um pflanzliche Wirtszellen zu infizieren. TAL Proteine binden in den Pflanzenzellen Promotoren und aktivieren die Transkription.

TAL Effektoren erkennen die DNA durch eine ca 34 Aminosäuren große Proteindomäne in der nur zwei Aminosäuren die Bindungsspezifität vermitteln.

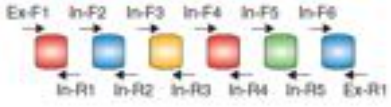


Herstellung eines Proteins mit einer definierten DNA Bindungssequenz





Golden Gate cloning

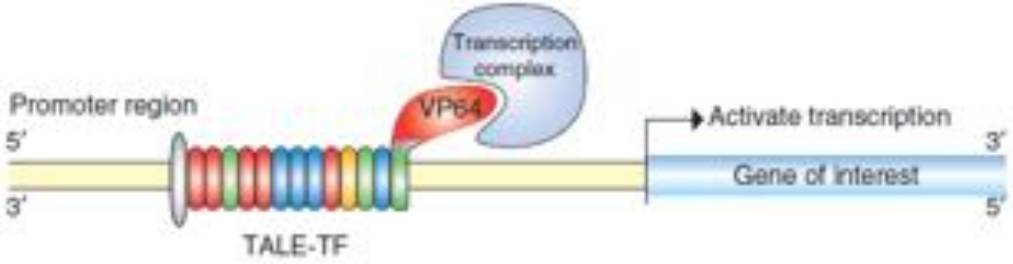


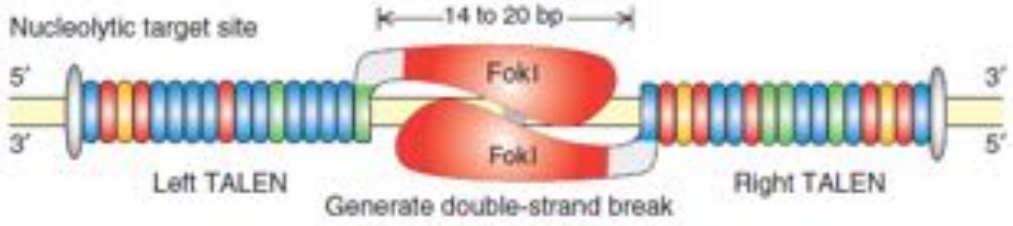




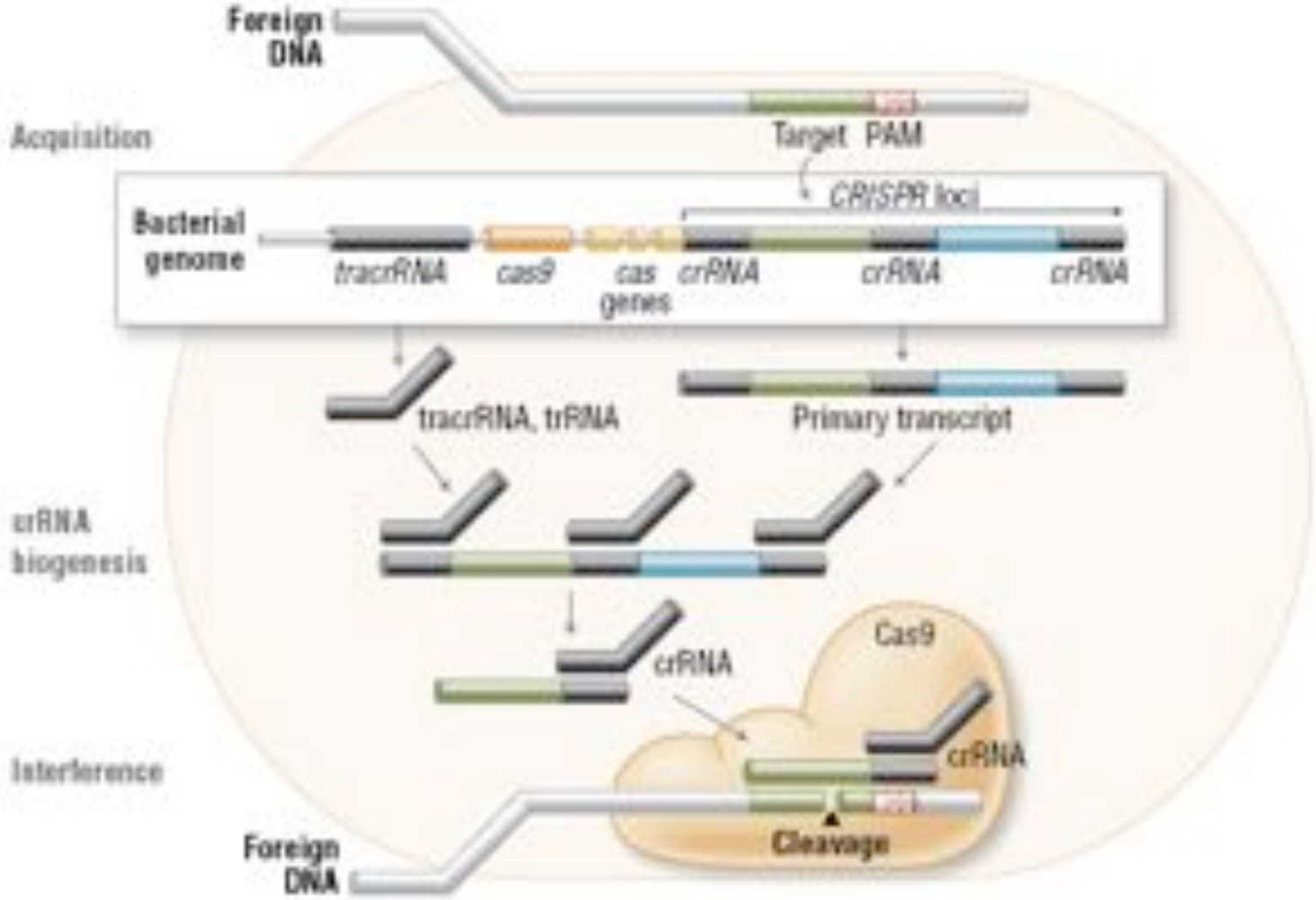




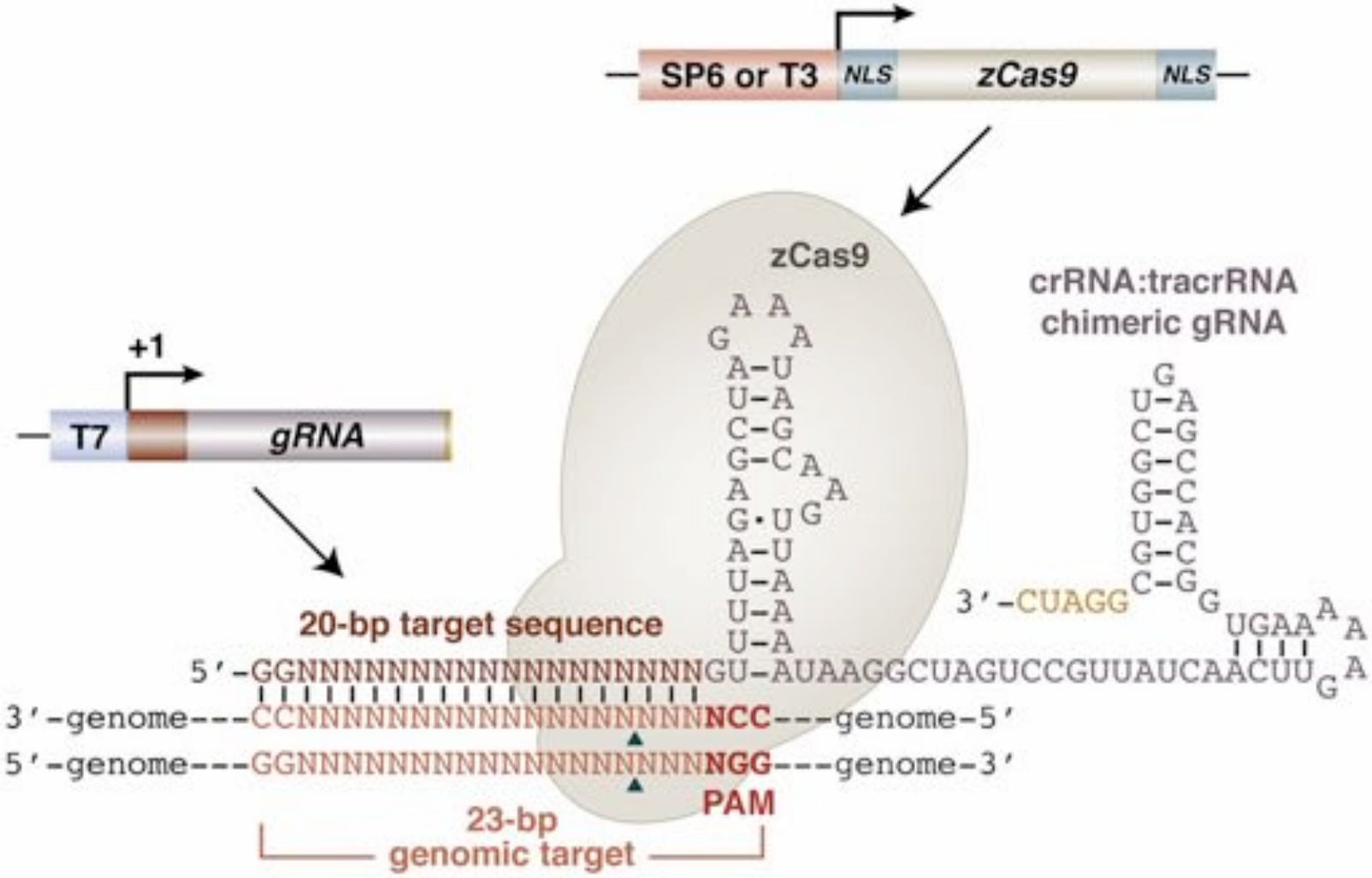




CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)



CRISPR associated (Cas)



Was für Mutationstypen gibt es eigentlich?

Amorphe Allele: totaler Funktionsverlust

Hypomorphe Allele: teilweiser Funktionsverlust

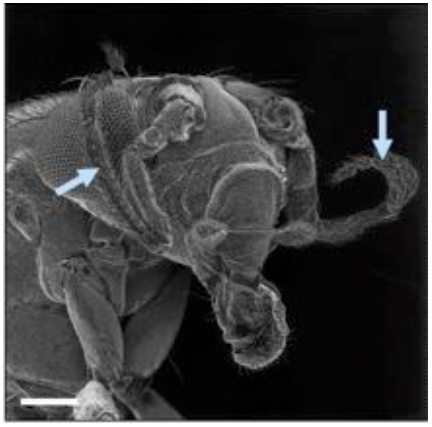
loss of function

Hypermophe Allele: Das Gen wird aktiver

Neomorphe Allele: Neue Funktion

Antimorphe Allele: „Vergiftende Allele“

gain of function



Die Möglichkeiten der Genetik



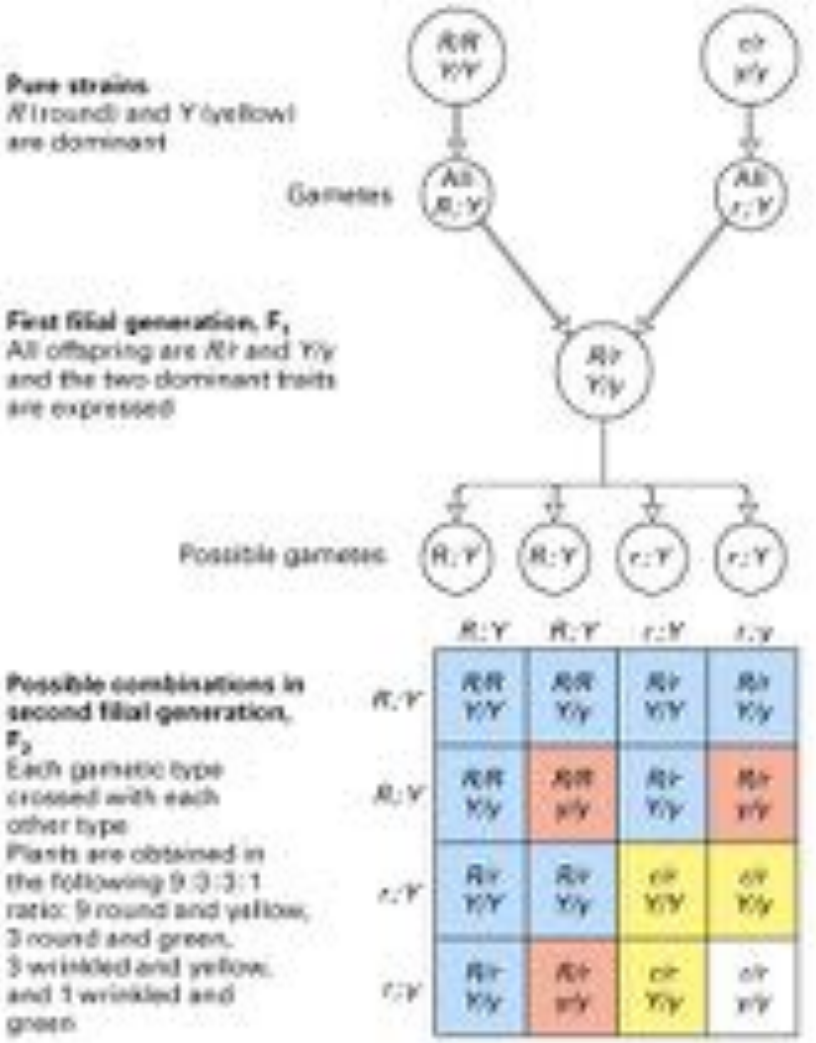


G. Mendel

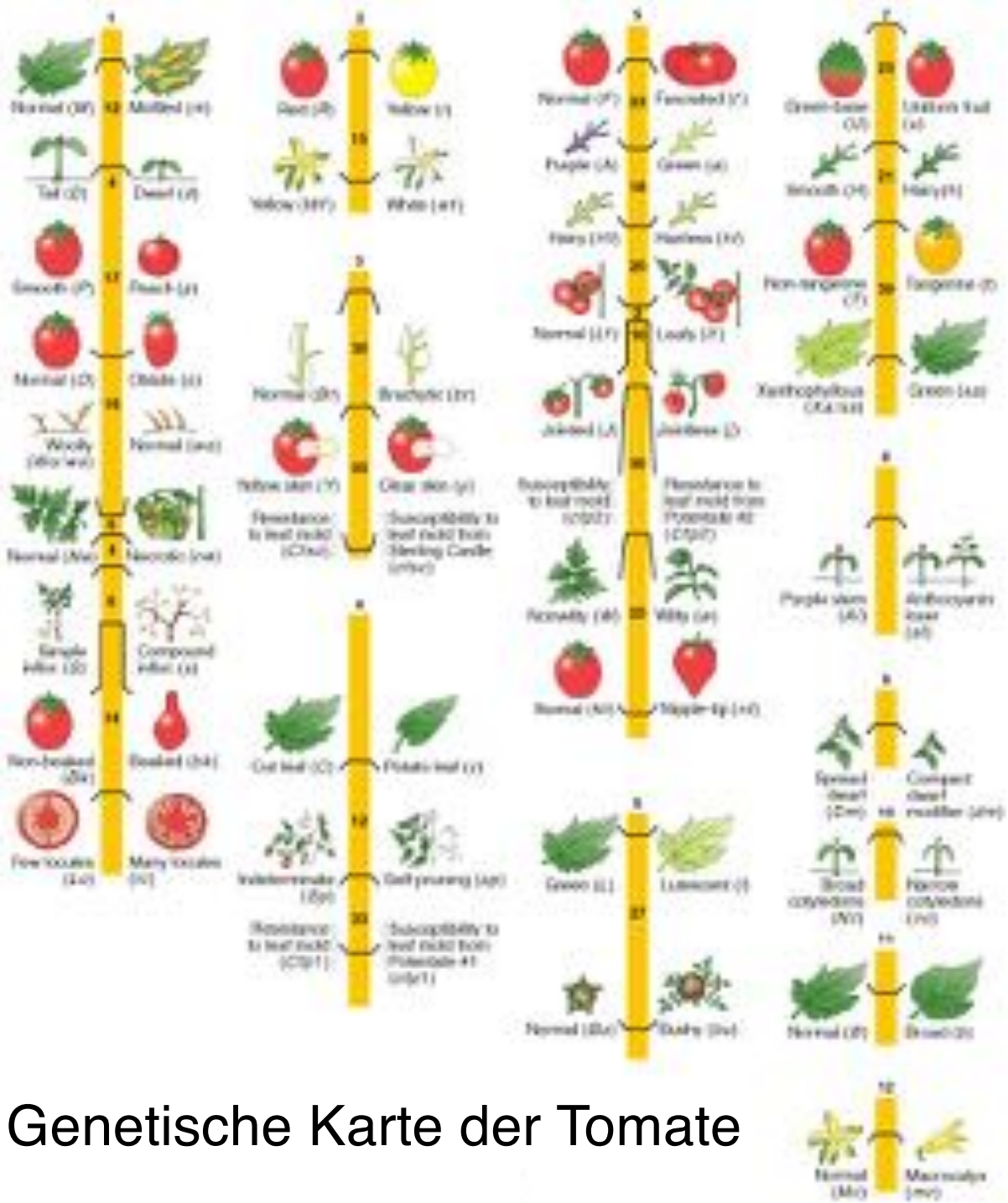
Rezessiv

Dominant

Ungekoppelte Allele



9:3:3:1

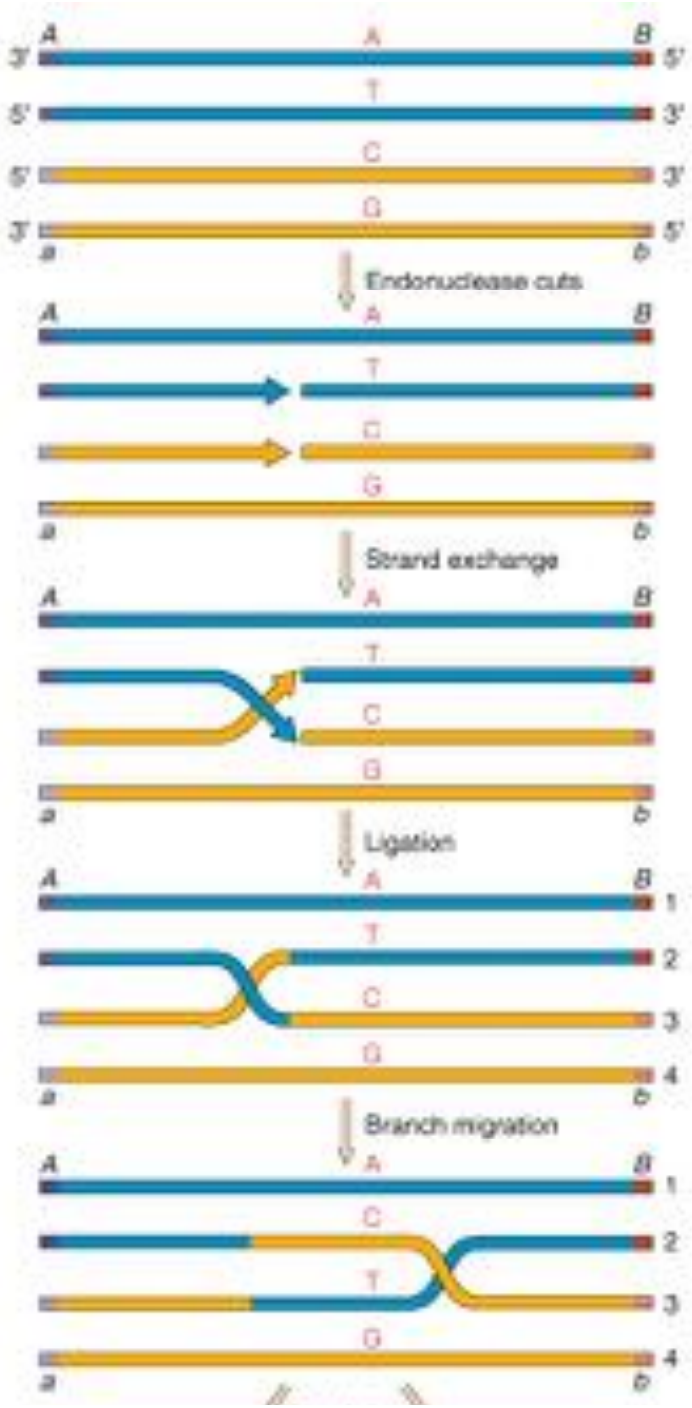


Genetische Karte der Tomate

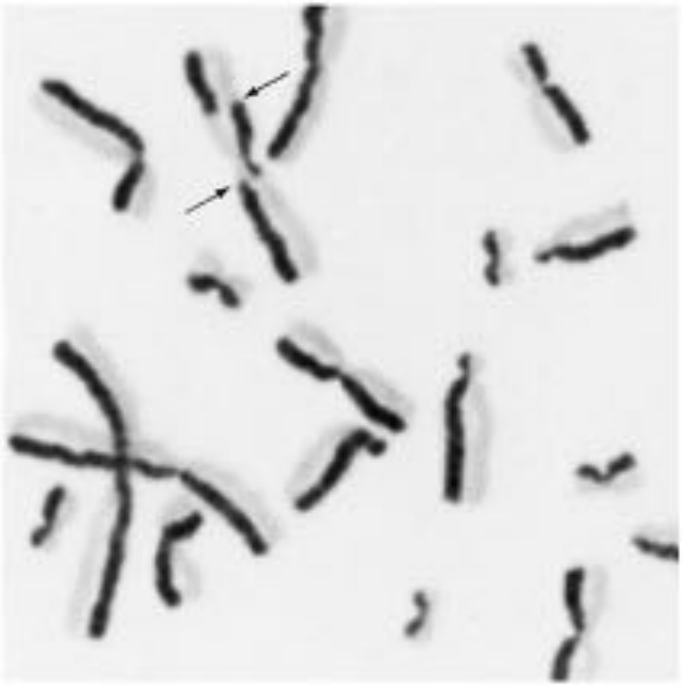
Gekoppelte Allele
auf einem Chromosom!

werden gemeinsam
vererbt, es sei denn,
es tritt Rekombination
auf

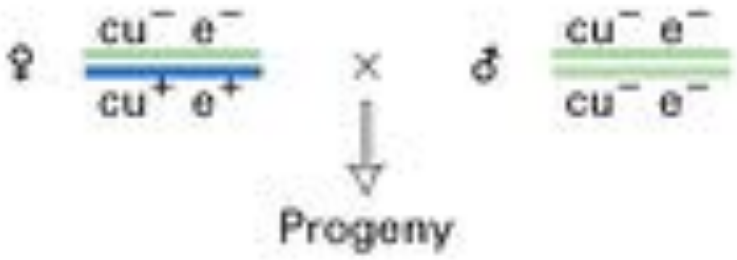




Homologe Rekombination erfordert ein Cross Over



Genetische Kartierung durch Rekombination

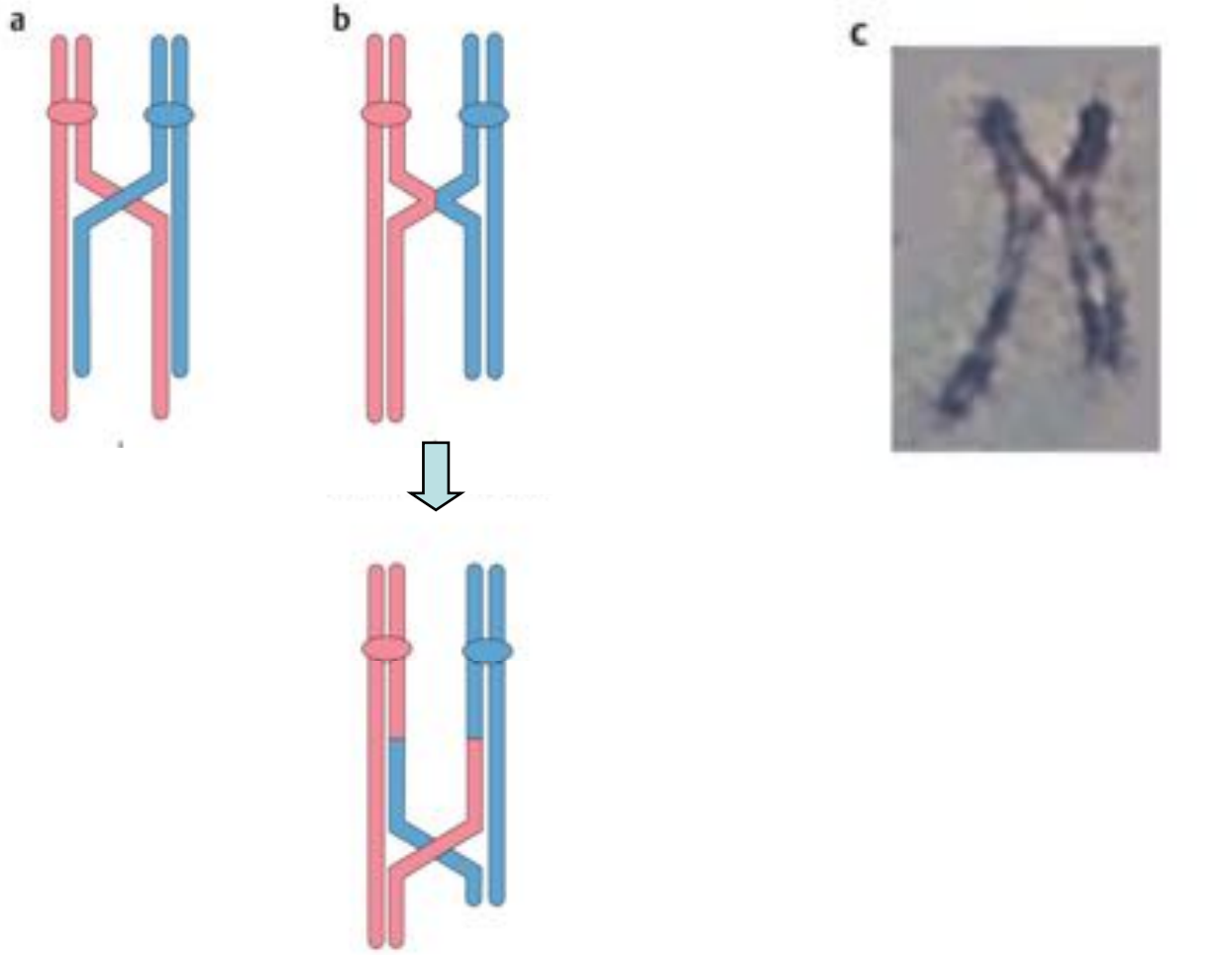


Phenotype	Genotype From ♀	Genotype From ♂	Total
Parental: curled wings, dark body	$cu^- e^-$	$cu^- e^-$	389
normal wings, normal body	$cu^+ e^+$	$cu^- e^-$	414
Recombinant: curled wings, normal body	$cu^- e^+$	$cu^- e^-$	104
normal wings, dark body	$cu^+ e^-$	$cu^- e^-$	93
			1000

$$\text{Genetischer Abstand} = \frac{\sum \text{Rekombinanten}}{\sum \text{Rekombinanten} + \sum \text{Parentalgenotyp}} \times 100$$

$$= \frac{104 + 93}{1000} \times 100 = 19{,}7 \text{ centimorgan (cM)}$$

Erst Cross Over dann Strangtausch



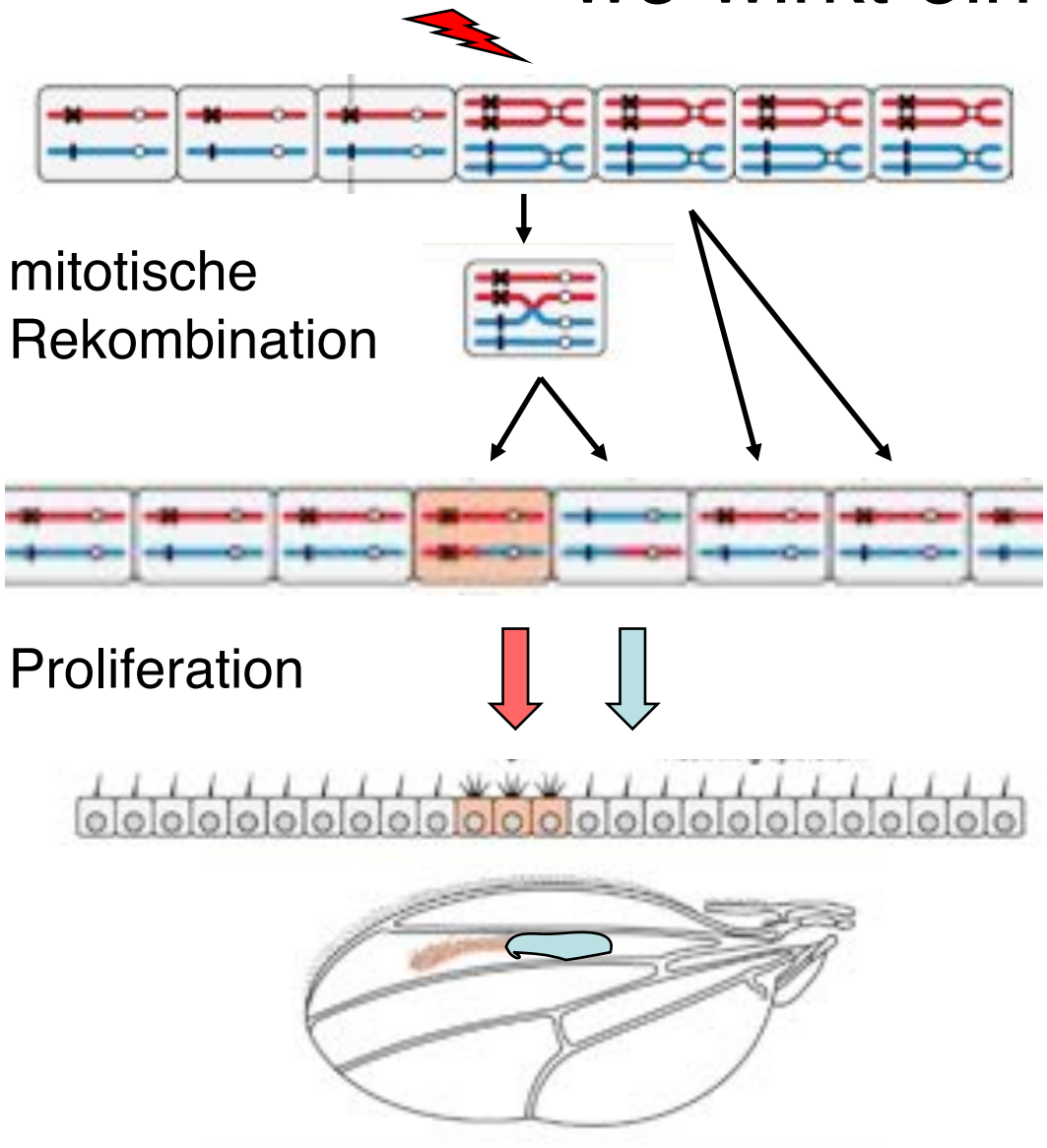
Rekombination

Homologe Rekombination
Rekombination zwischen identischen DNA Sequenzen (in trans, zwei Chromosomen).
In der Meiose aber auch in der Mitose.....

Illegitime Rekombination
Rekombination ohne Sequenzübereinstimmung
Kurze Sequenzidentitäten **Rekombinase**

Rekombination in Cis und Trans möglich

Rekombination während der Mitose, wo wirkt ein Gen?



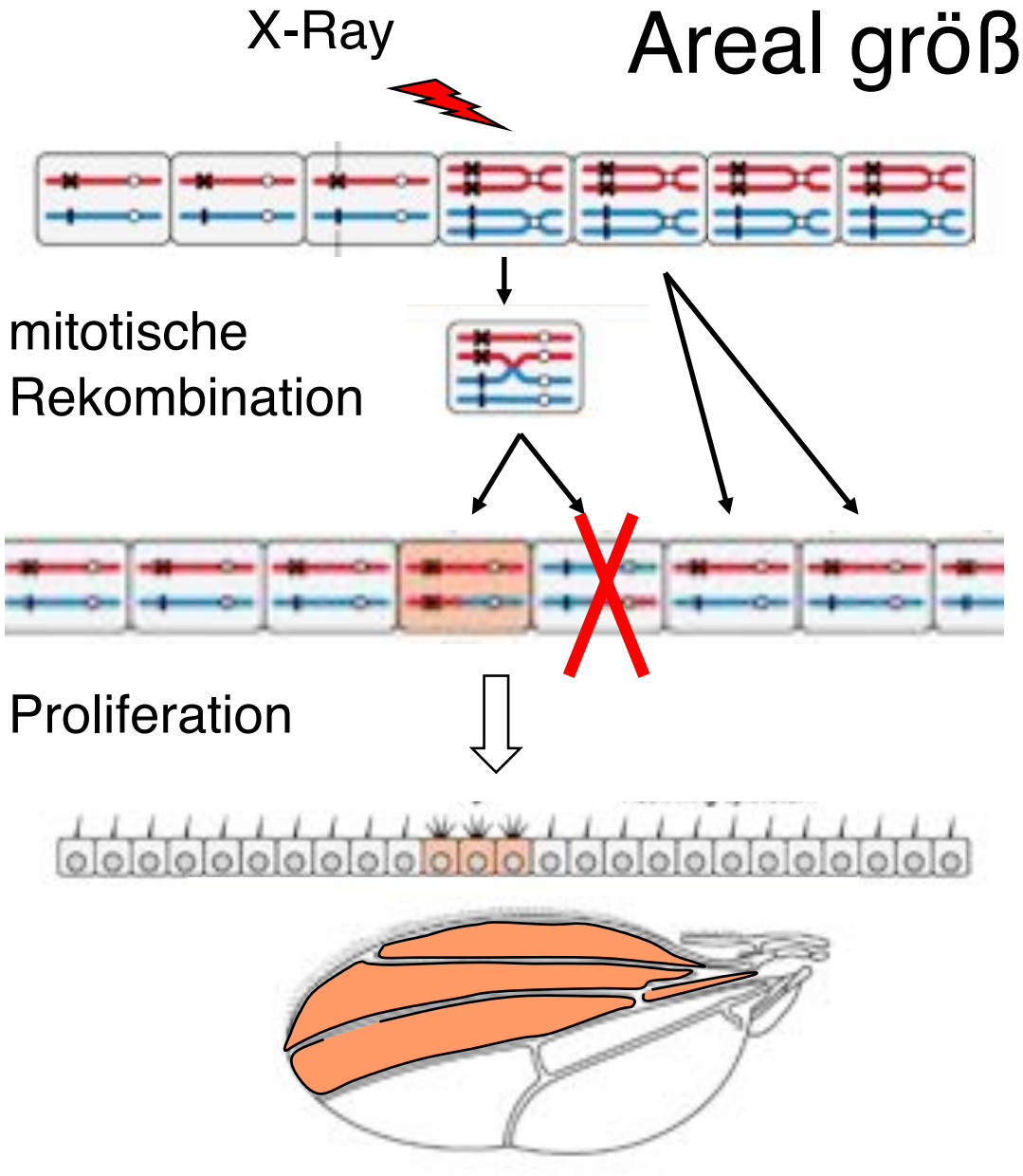
Um in einem heterozygoten Tier einen Teil homozygot zu machen verwendet man die **Mitotische Rekombination**.

Twin spots; Zwillingsfleck

Diese sogenannte **klonale Analyse** hilft Fragen nach der **Zellautonomie** einer Genfunktion zu klären.

Nachteil: Geringe Frequenz

Wie bekommt man das mutante Areal größer?

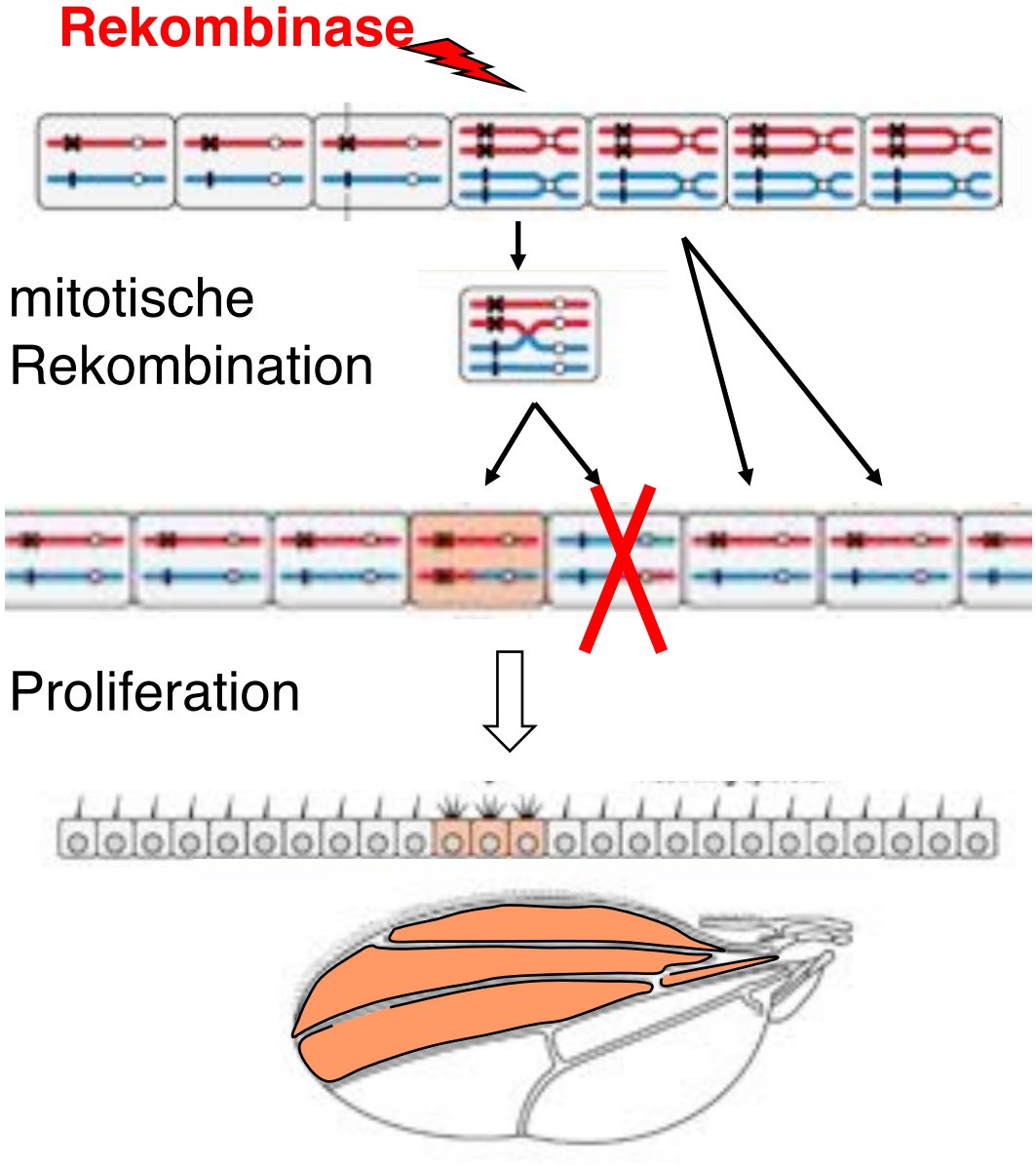


Durch Einführen eines „zell-letal“ Markers wird der wildtypische Klon zum Absterben gebracht.

***Minute* Technik**

Nachteil:
Geringe Frequenz

Wie bekommt man mehr Klone?

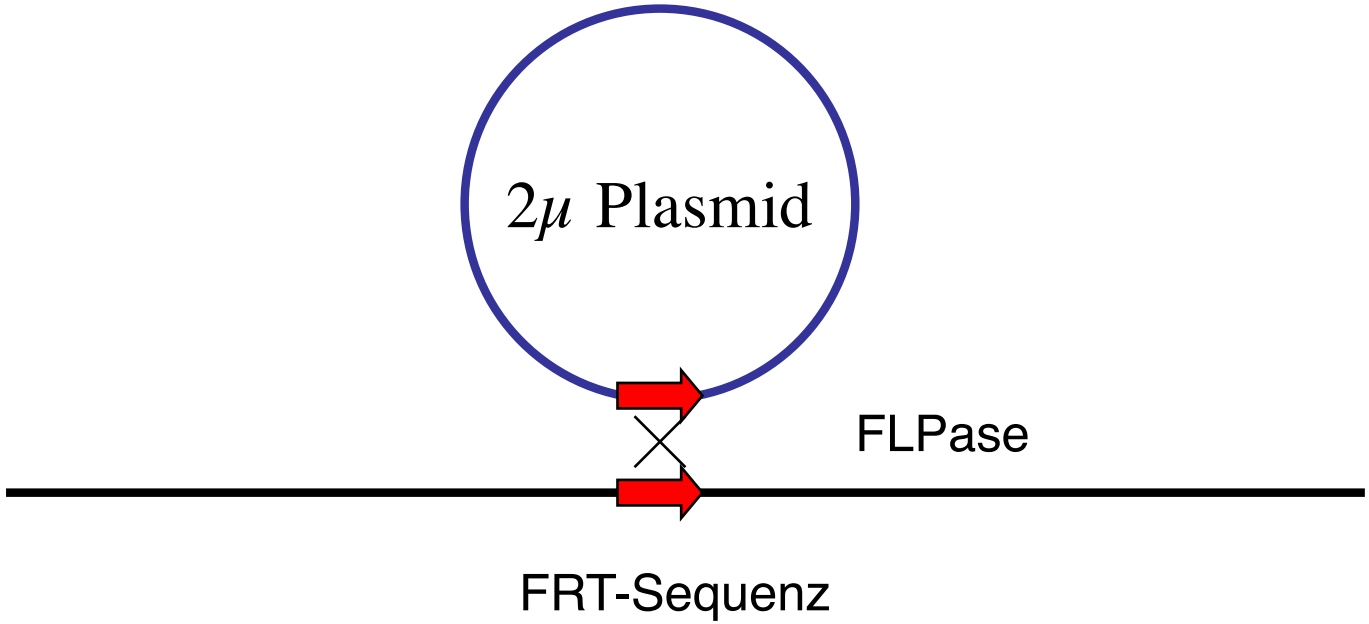


Der Einsatz einer **Rekombinase** und der entsprechenden Zielsequenzen führt zu einer sehr hohen Klonausbeute
Flp (Hefe) -> **FRT**
Cre (Phage) -> **LoxP**.

Minute Technik

Rekombinationssysteme

1) Rekombination des Hefe 2*μ* Plasmids
50-100 Kopien / *Saccharomyces cerevisiae* Zelle
6.318 bp groß; kodiert 4 Proteine,
darunter die sequenzspezifische Rekombinase **FLP**, die eine 14 bp lange **FRT** Rekombinationssequenz erkennt.

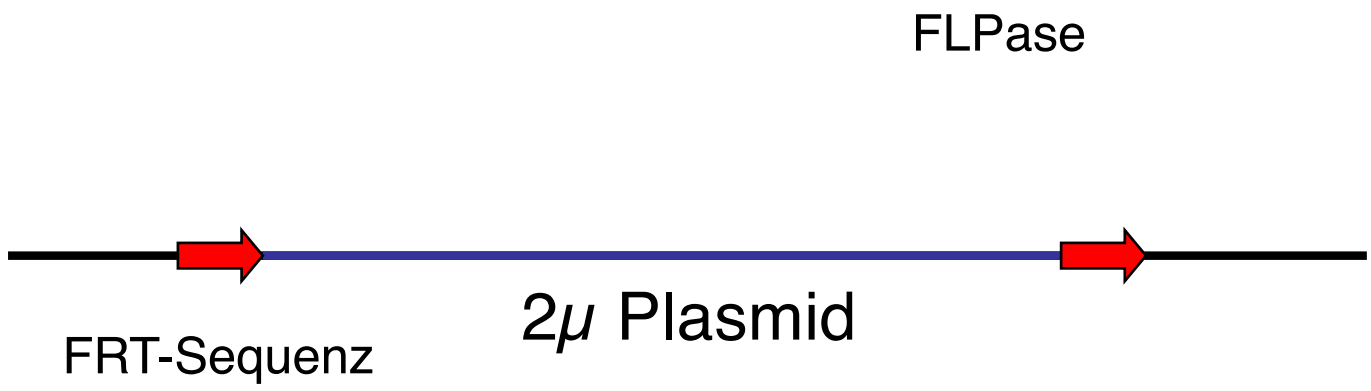


Rekombinationssysteme

1) Rekombination des Hefe 2*μ* Plasmids

Die sequenzspezifische Rekombinase **FLP** induziert einen DNA Strangbruch (Rekombination) und bewirkt die Integration des Plasmids.

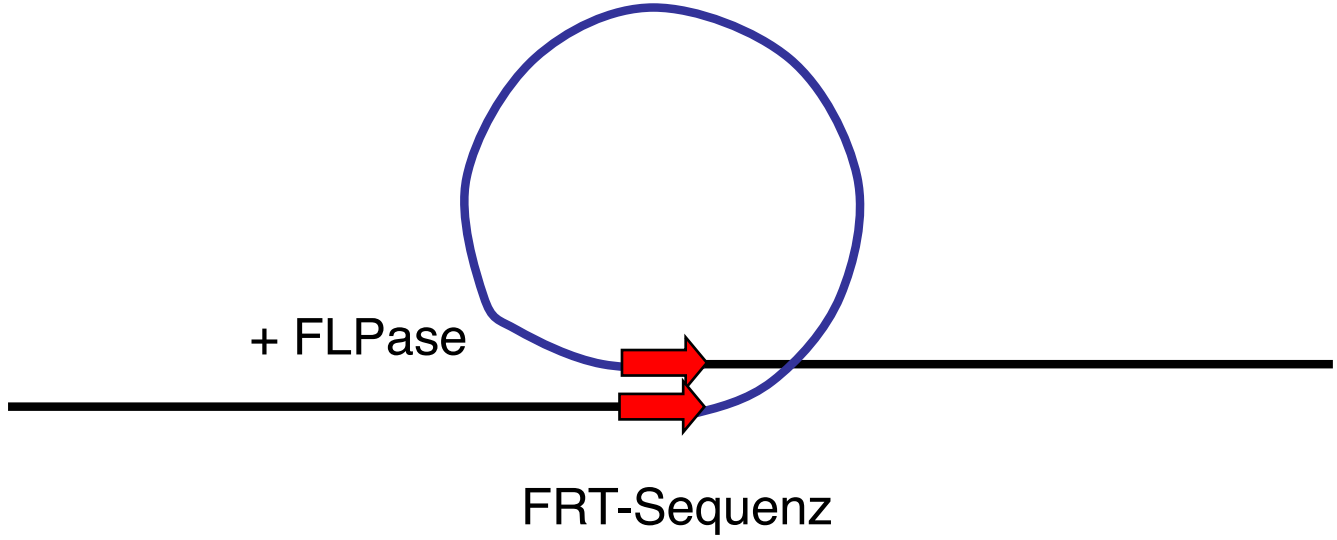
Die Integration ins Chromosom ist reversibel.



Rekombinationssysteme

1) Rekombination des Hefe 2*μ* Plasmids

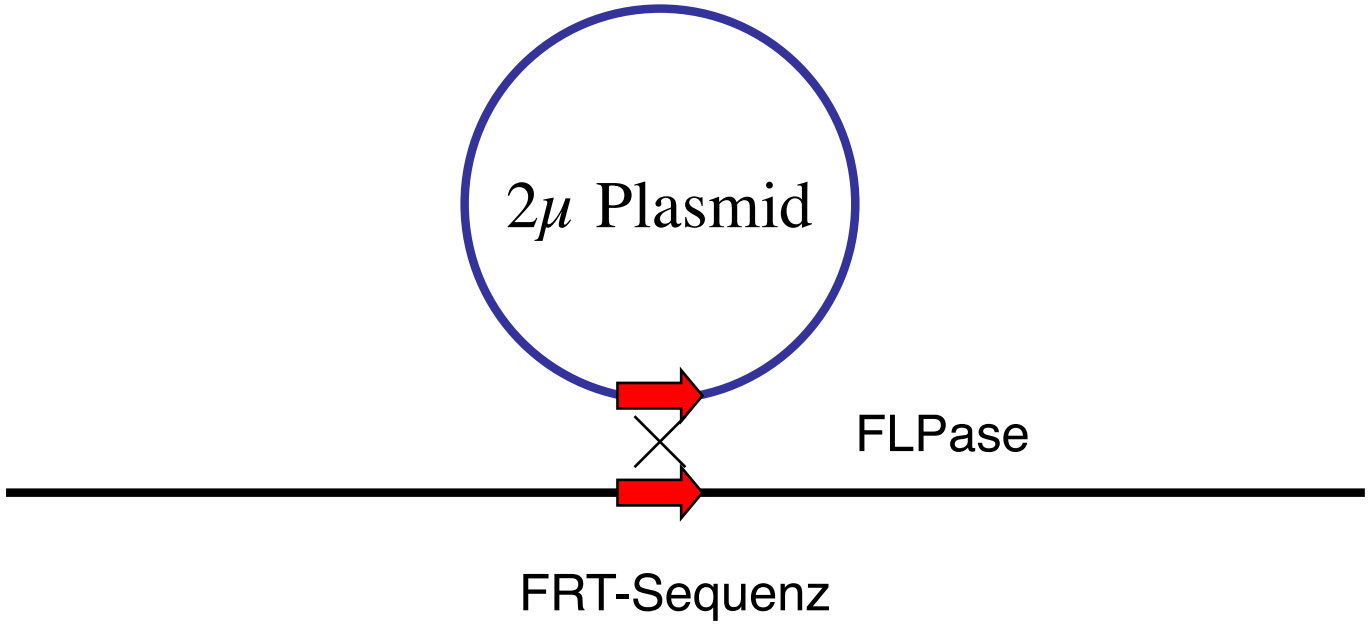
Wenn die FRT-Sequenzen in Tandem angeordnet sind kommt es zu einer Deletion des 2*μ* Plasmids.
Wenn die FRT-Sequenzen invertiert vorliegen, wird der eingeschlossene DNA Bereich umgedreht.



Rekombinationssysteme

1) Rekombination des Hefe 2μ Plasmids

Wenn die FRT-Sequenzen in Tandem angeordnet sind kommt es zu einer Deletion des 2μ Plasmids.
Wenn die FRT-Sequenzen invertiert vorliegen, wird der eingeschlossene DNA Bereich umgedreht.

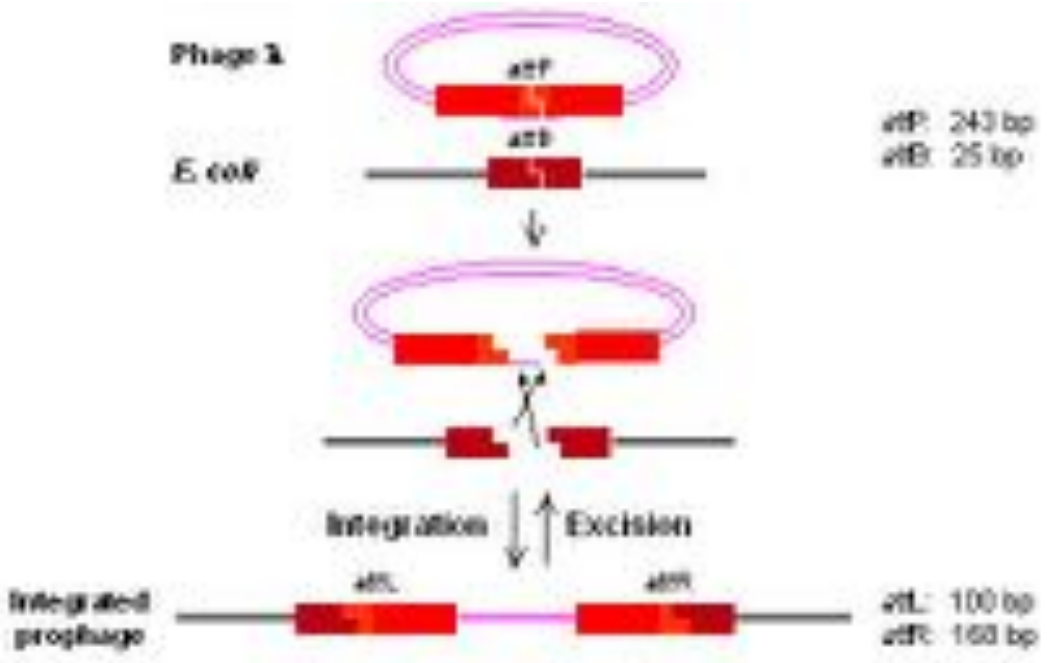


Rekombinationssysteme

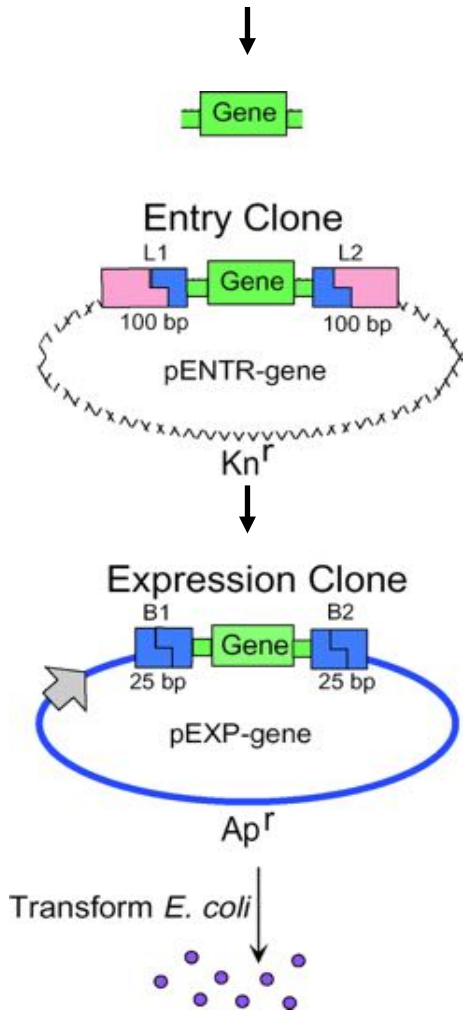
2) Rekombination bei Prokaryoten

Integration temperenter Phagen z.B. Lambda

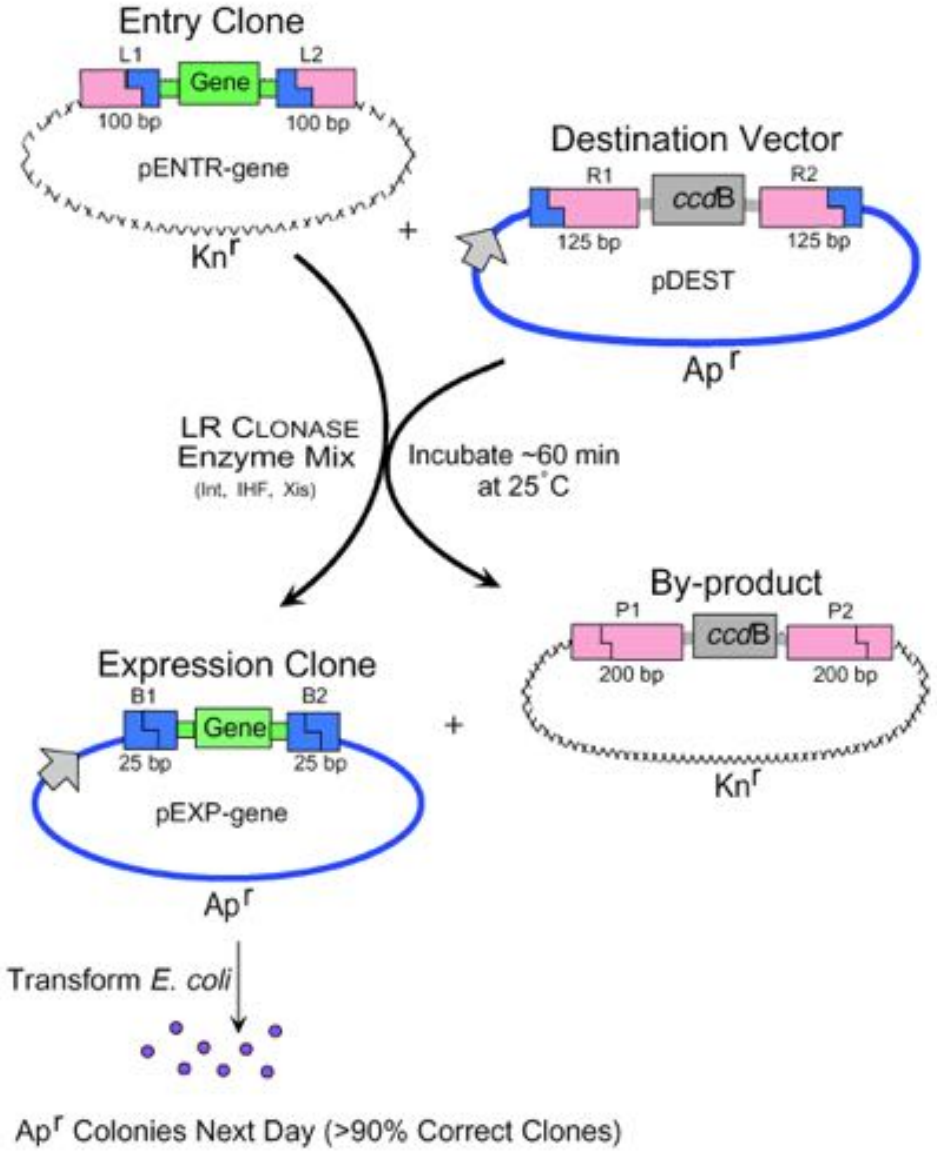
Integrase vom *int* Gen kodiert, benötigt den integration host factor (IHF), der aus zwei Proteinen zusammengesetzt ist. Erkennt die *attP* und *attB* sites. Öffnung des DNA Doppelstrangs über eine 3‘ Phosphodiesterbindung an einem Tyr-Rest.



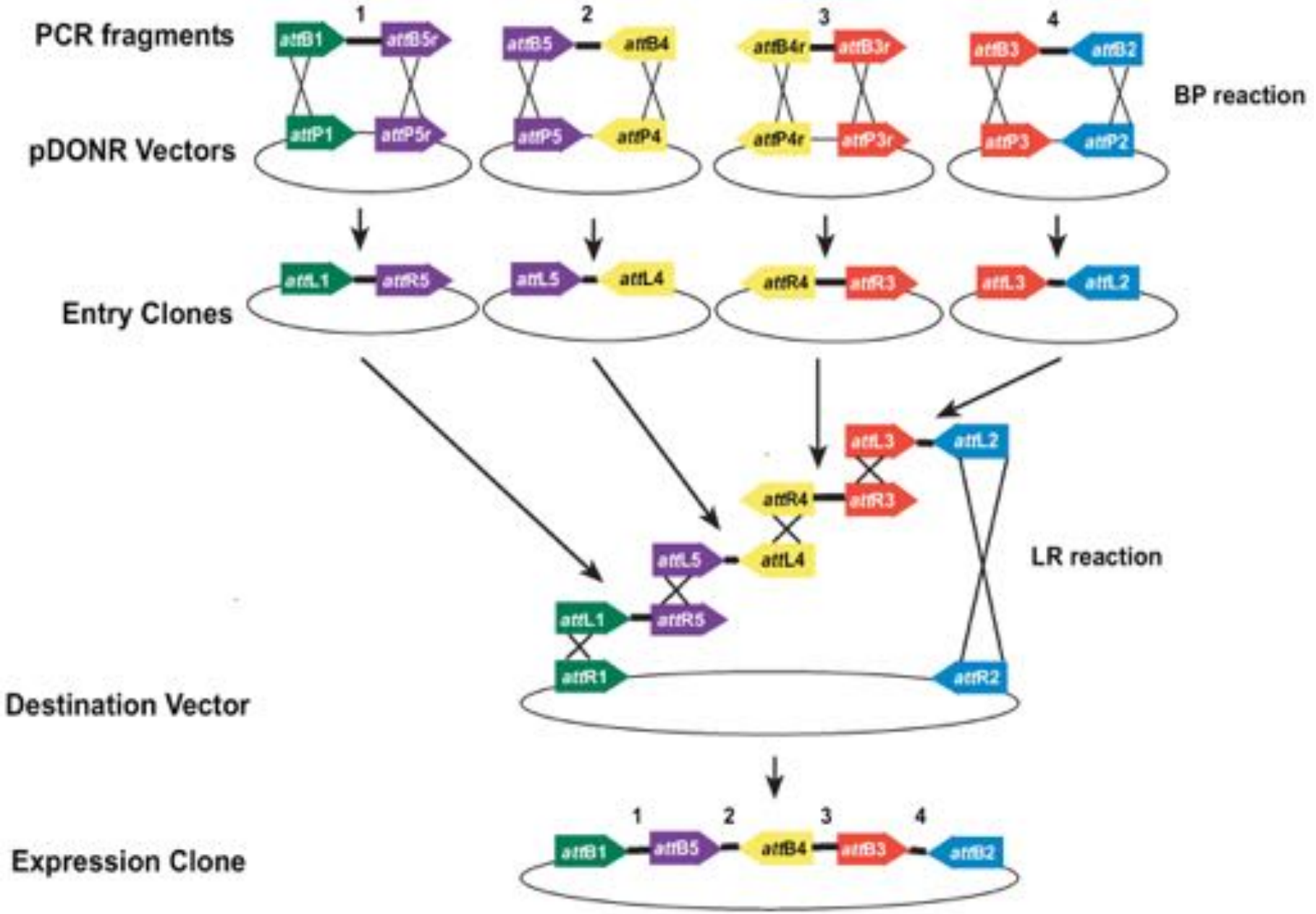
Gateway-System



Umklonieren leicht gemacht



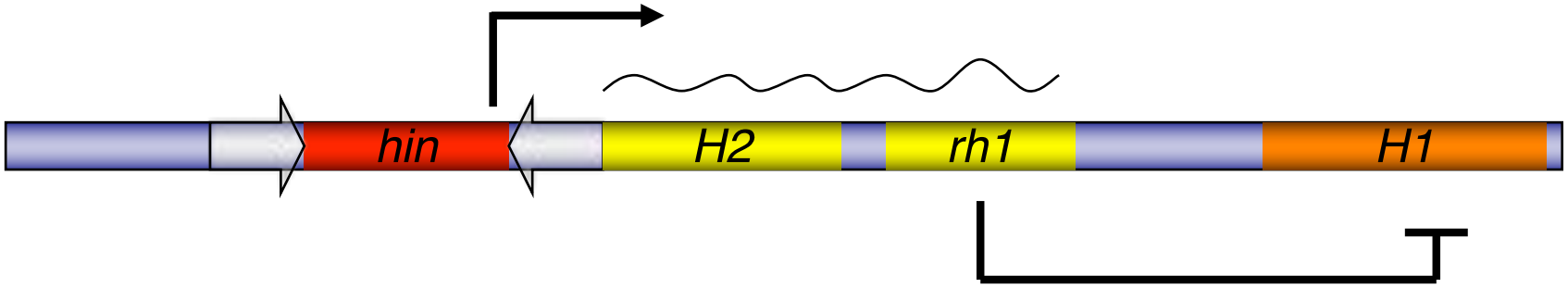
MultiSite Gateway



Rekombinationssysteme

2) Rekombination bei Prokaryoten

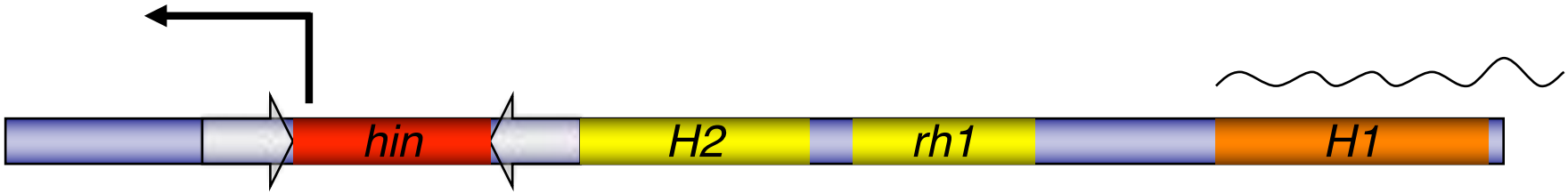
Invertasen: Änderung des Phänotyps durch Inversion eines DNA Fragmentes. Bis auf Cre und FLP Enzyme benötigen diese Enzyme supercoil DNA zur Bindung.
z.B. Invertase Hin bei *Salmonella (*oder Gin Page Mu).



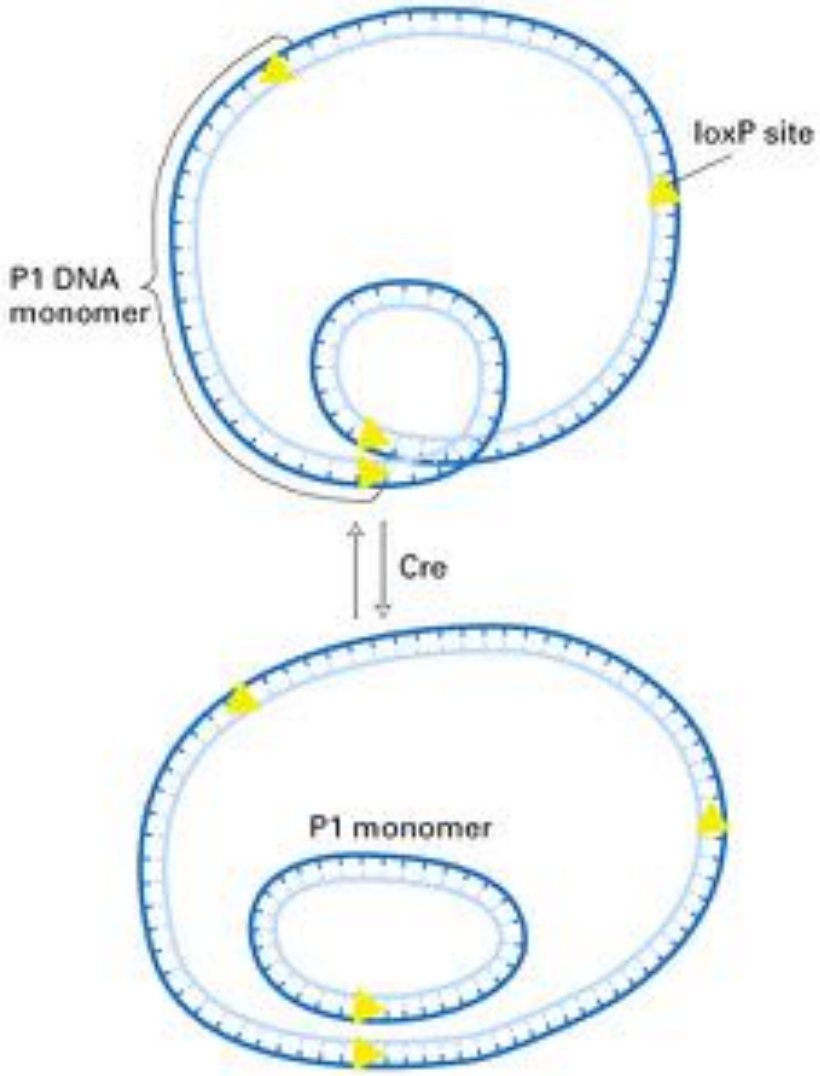
Rekombinationssysteme

2) Rekombination bei Prokaryoten

Invertasen: Änderung des Phänotyps durch Inversion eines DNA Fragmentes. Bis auf Cre und FLP Enzyme benötigen diese Enzyme supercoil DNA zur Bindung.
z.B. Invertase Hin bei *Salmonella (*oder Gin Page Mu).



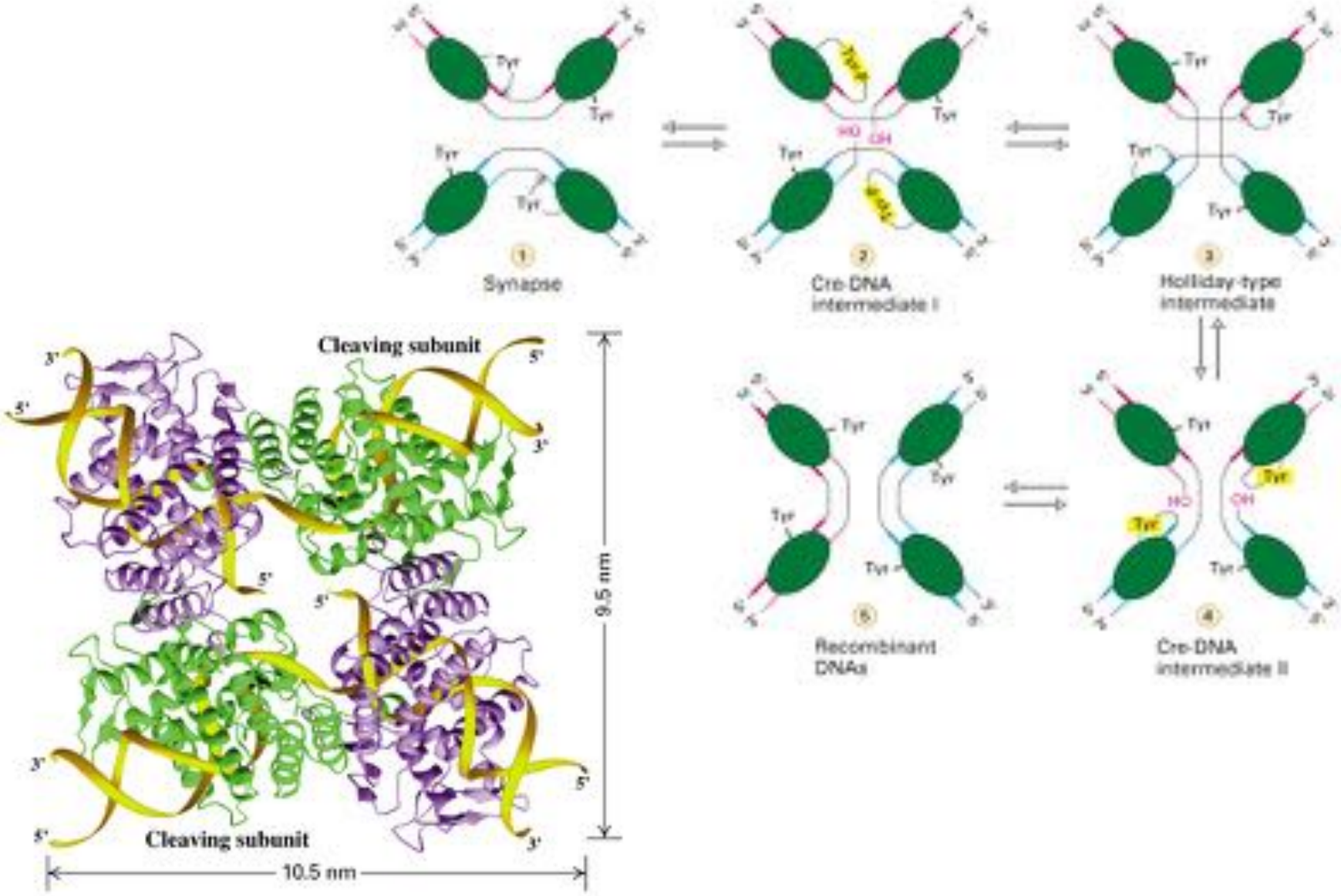
Rekombinationssysteme



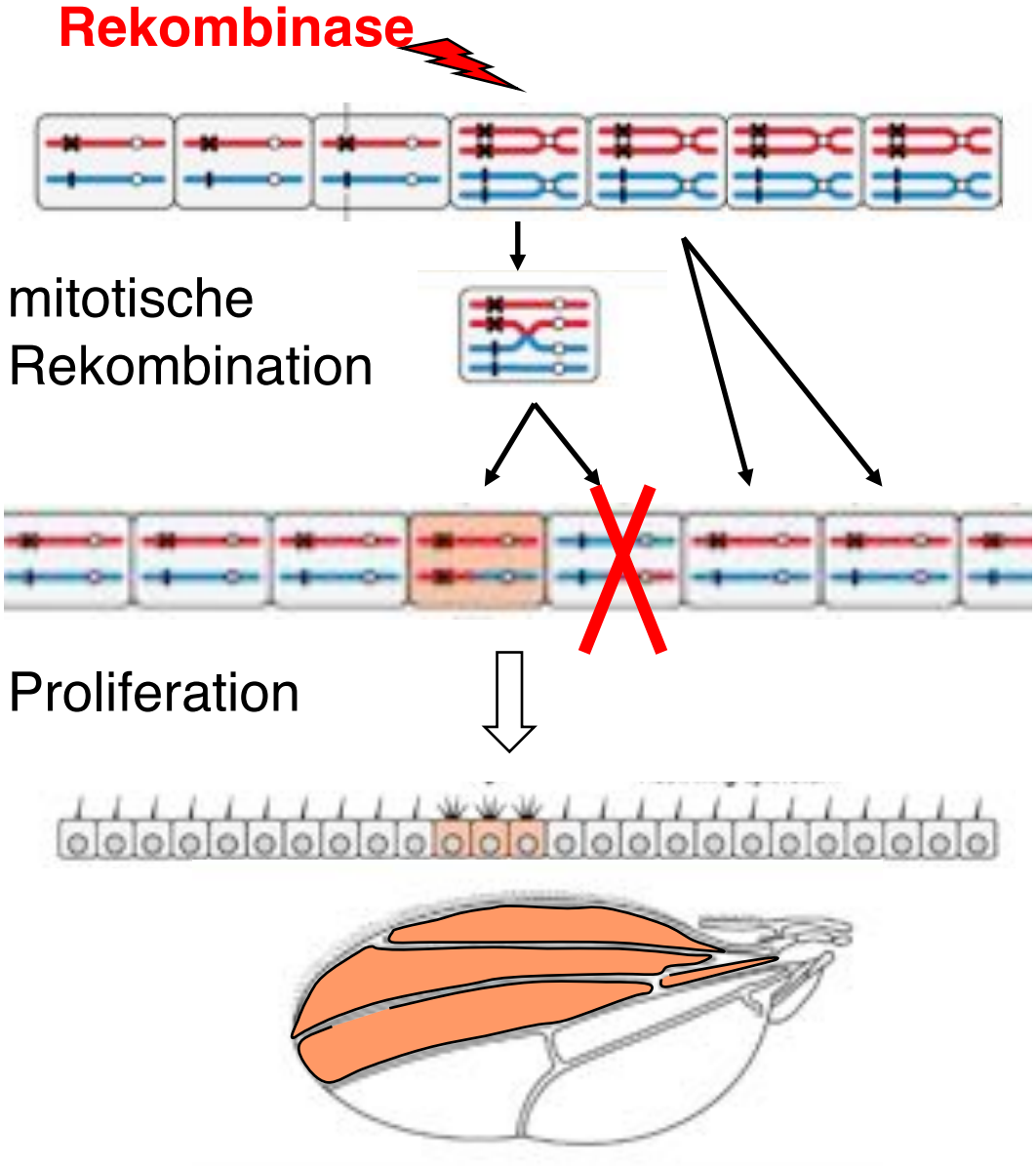
Phage P1

Auflösung der *rolling cricle* Replikationsstruktur durch **Cre Rekombinase** die an die **loxP** site bindet.

Mechanismus der Cre Rekombinase



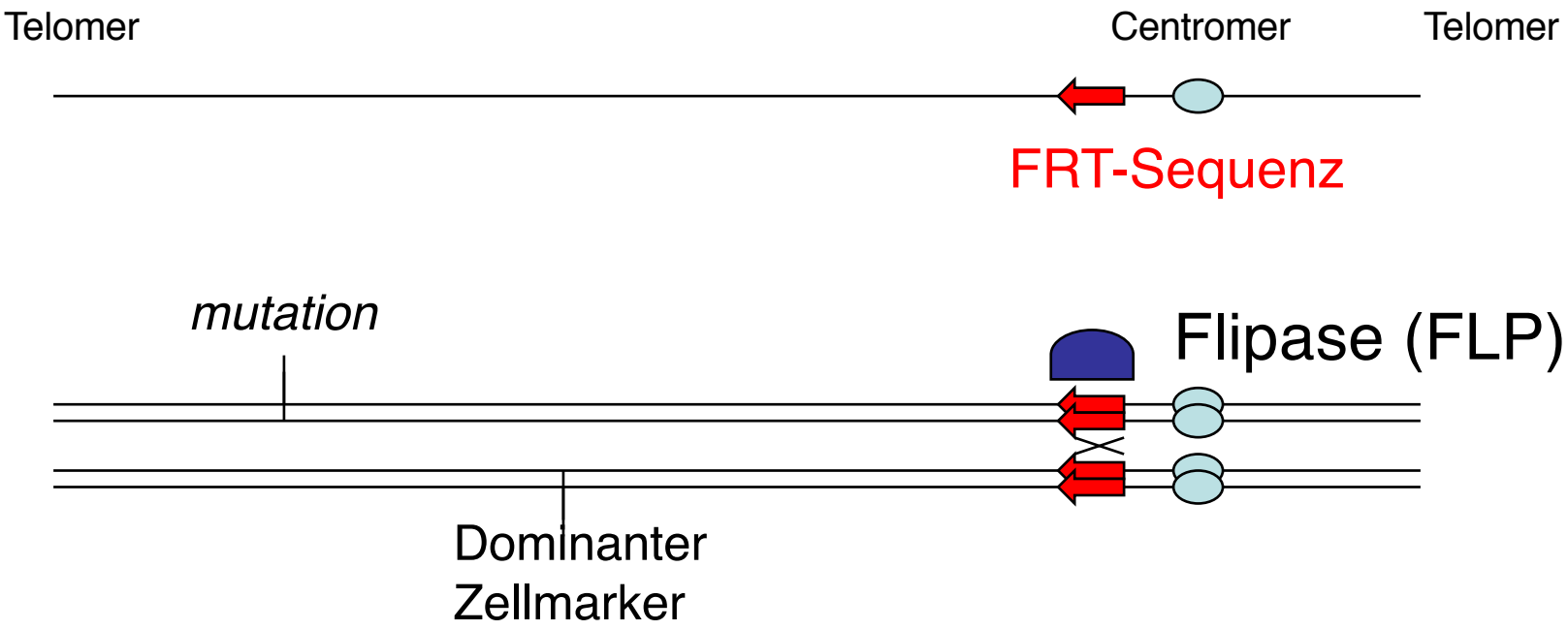
Wie bekommt man mehr Klone?



Der Einsatz einer **Rekombinase** und der entsprechenden Zielsequenzen führt zu einer sehr hohen Klonausbeute
Flp (Hefe) -> **FRT**
Cre (Phage) -> **LoxP**.

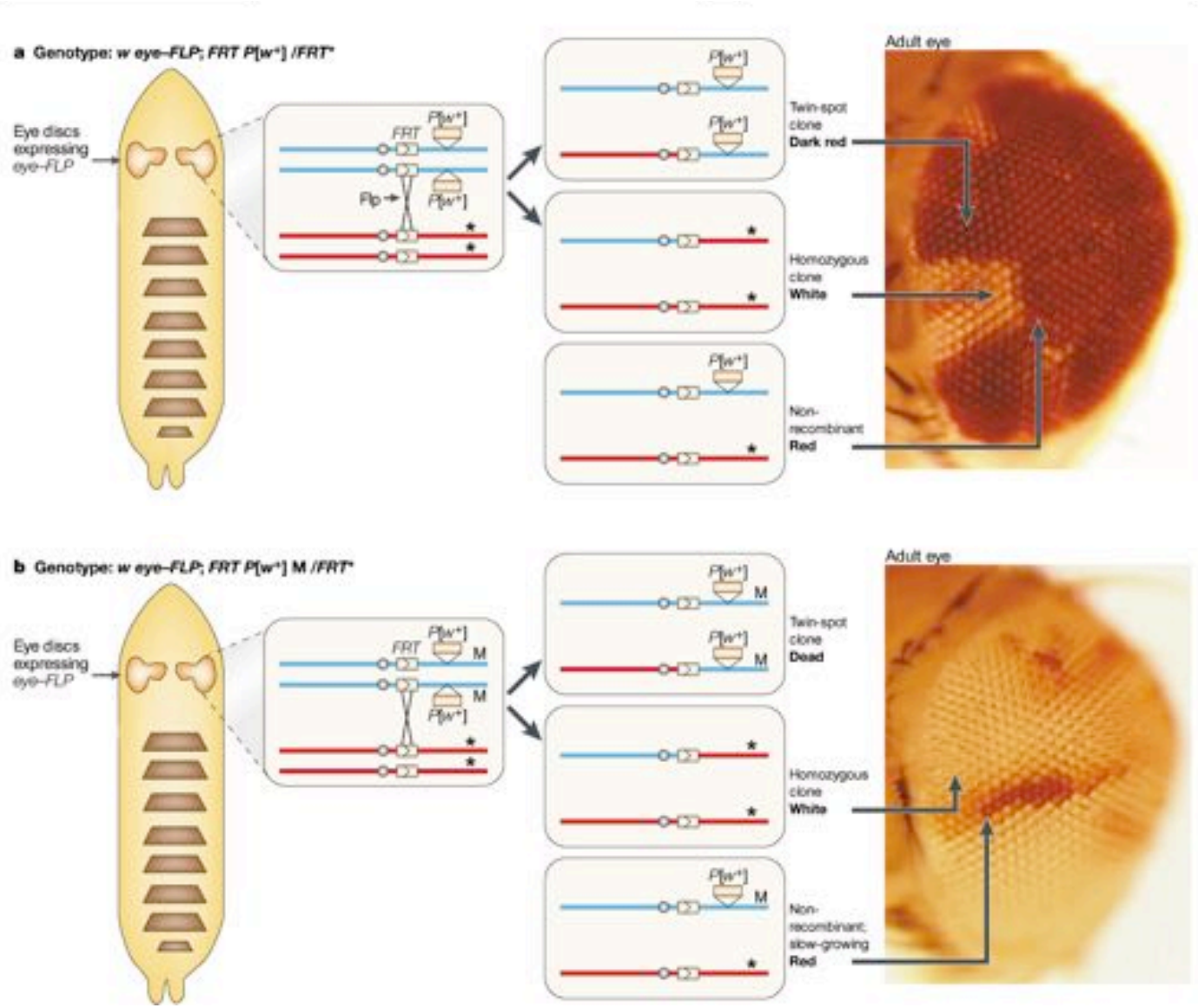
Minute Technik

Sequenzspezifische, mitotische Rekombination in *Drosophila*



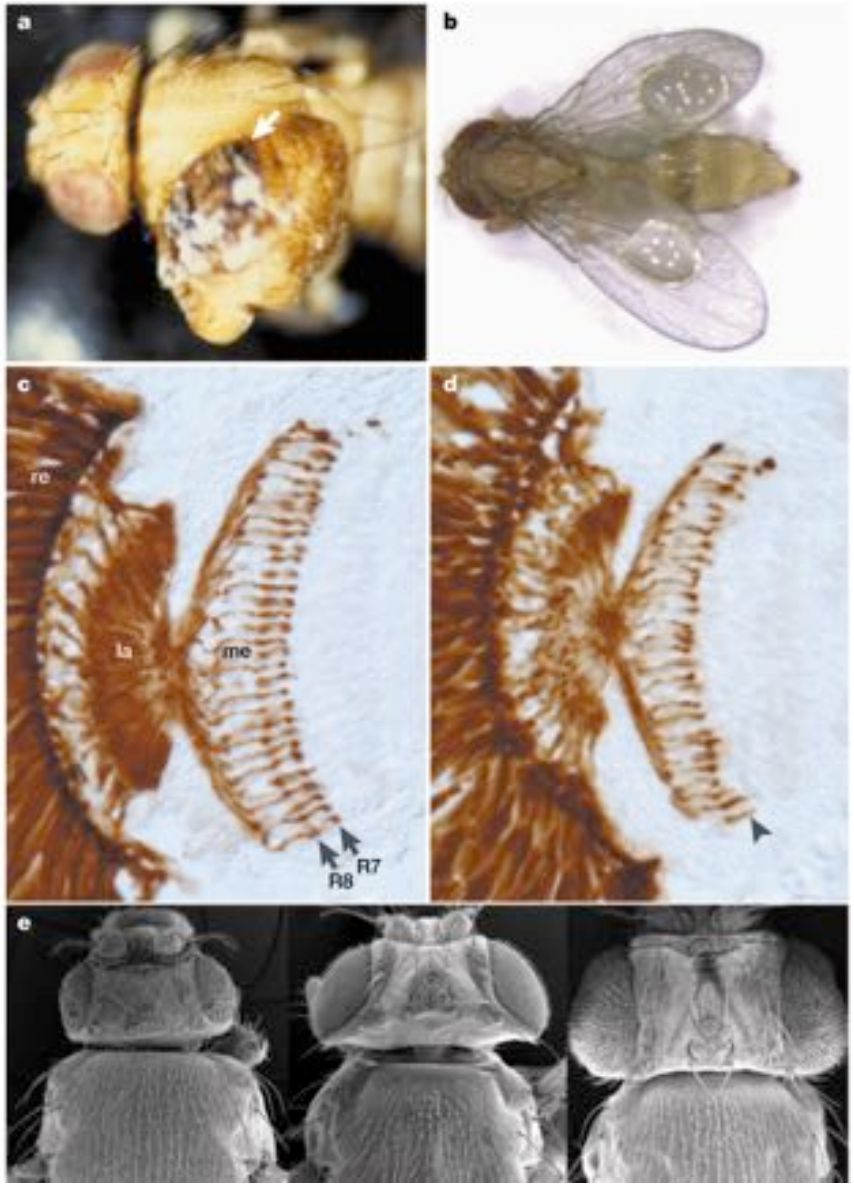
Während der Mitose kommt es zur Verdopplung der Chromatiden.
Anschließend kann FLP eine mitotische Rekombination induzieren.

Das FRT/FLP System in Drosophila



Die Verwendung der Minute-Technik erlaubt die Erzeugung großer mutanter Klone.

Das FRT/FLP System, Beispiele



Mitotische Rekombination: MARCM Technik

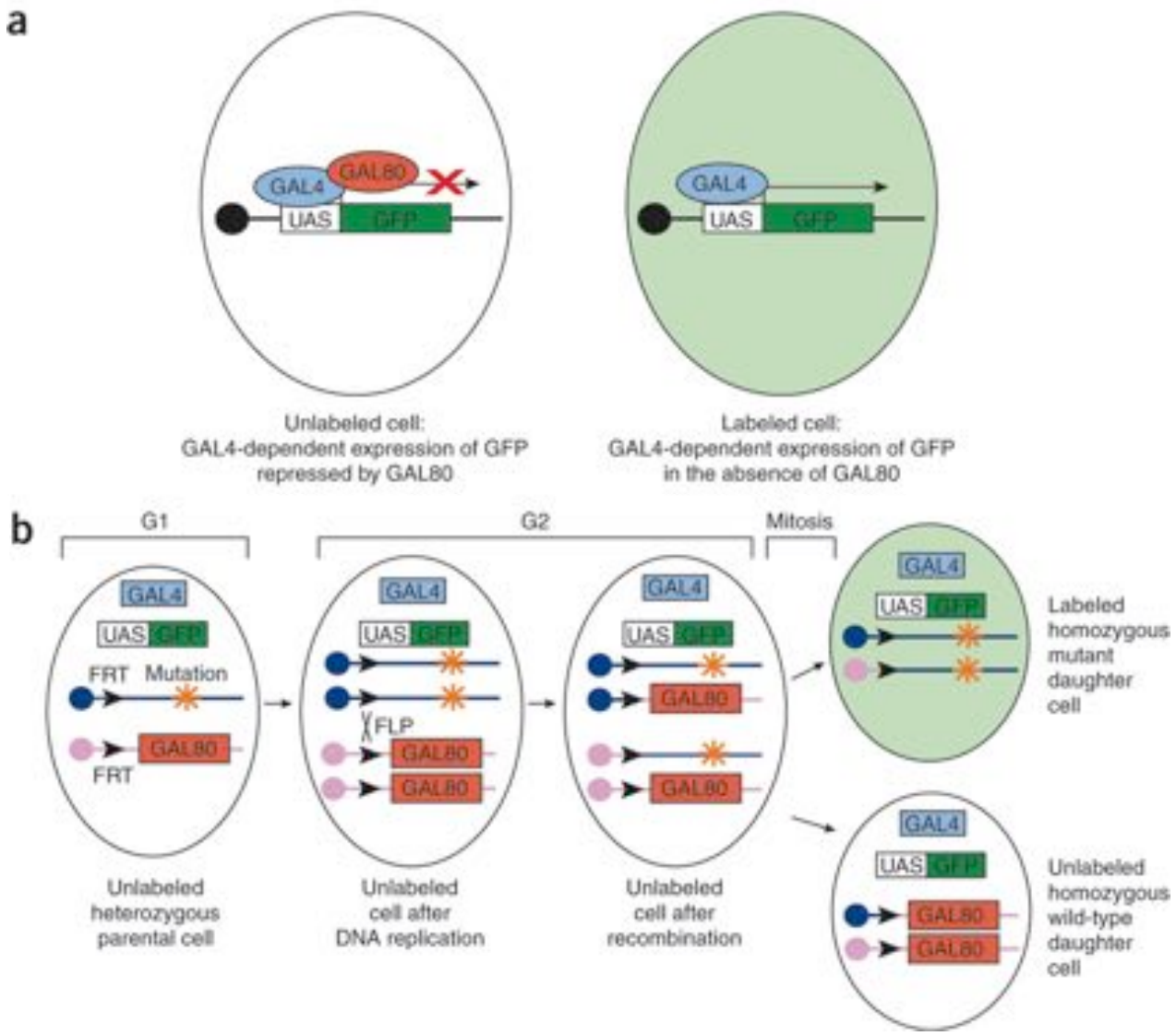
MARCM: Mosaic analysis with a repressible cell marker

Ermöglicht die spezifische Markierung der mutanten Zellklone mit Hilfe von Gal4/Gal80

Gal4 ist ein Transkriptionsfaktor aus der Hefe

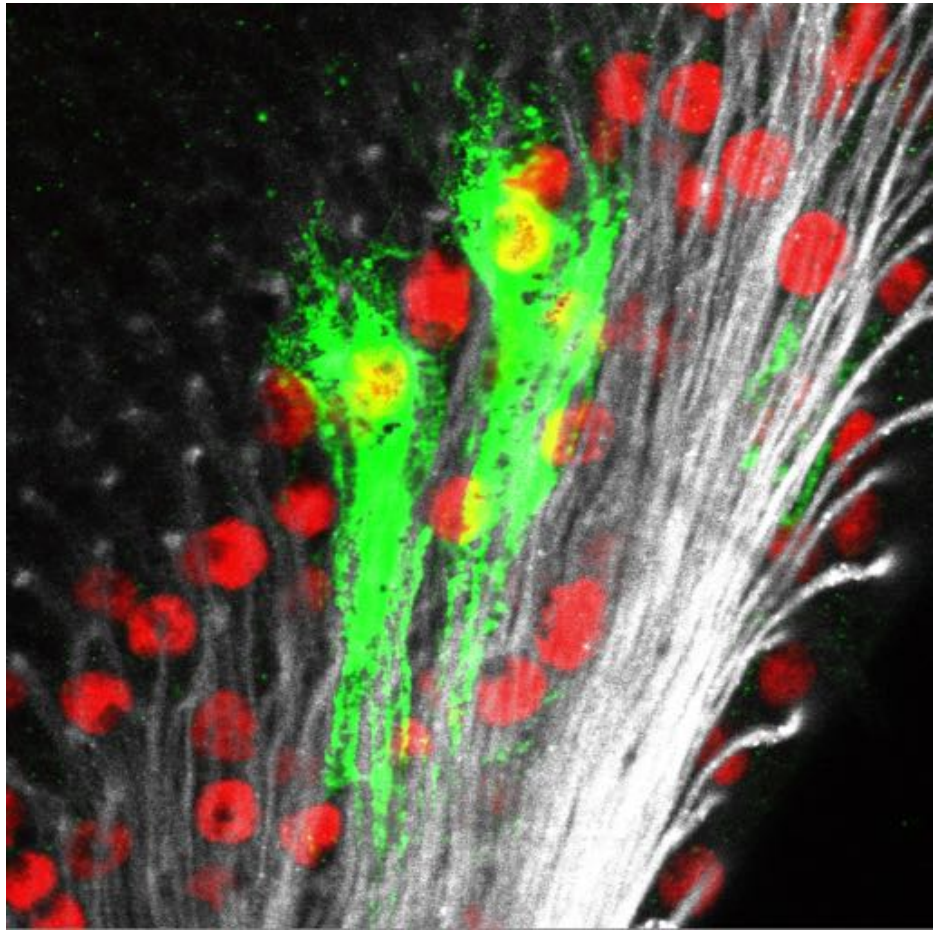
Gal80 ist ein Inhibitor dieses Transkriptionsfktors

Mitotische Rekombination: MARCM Technik

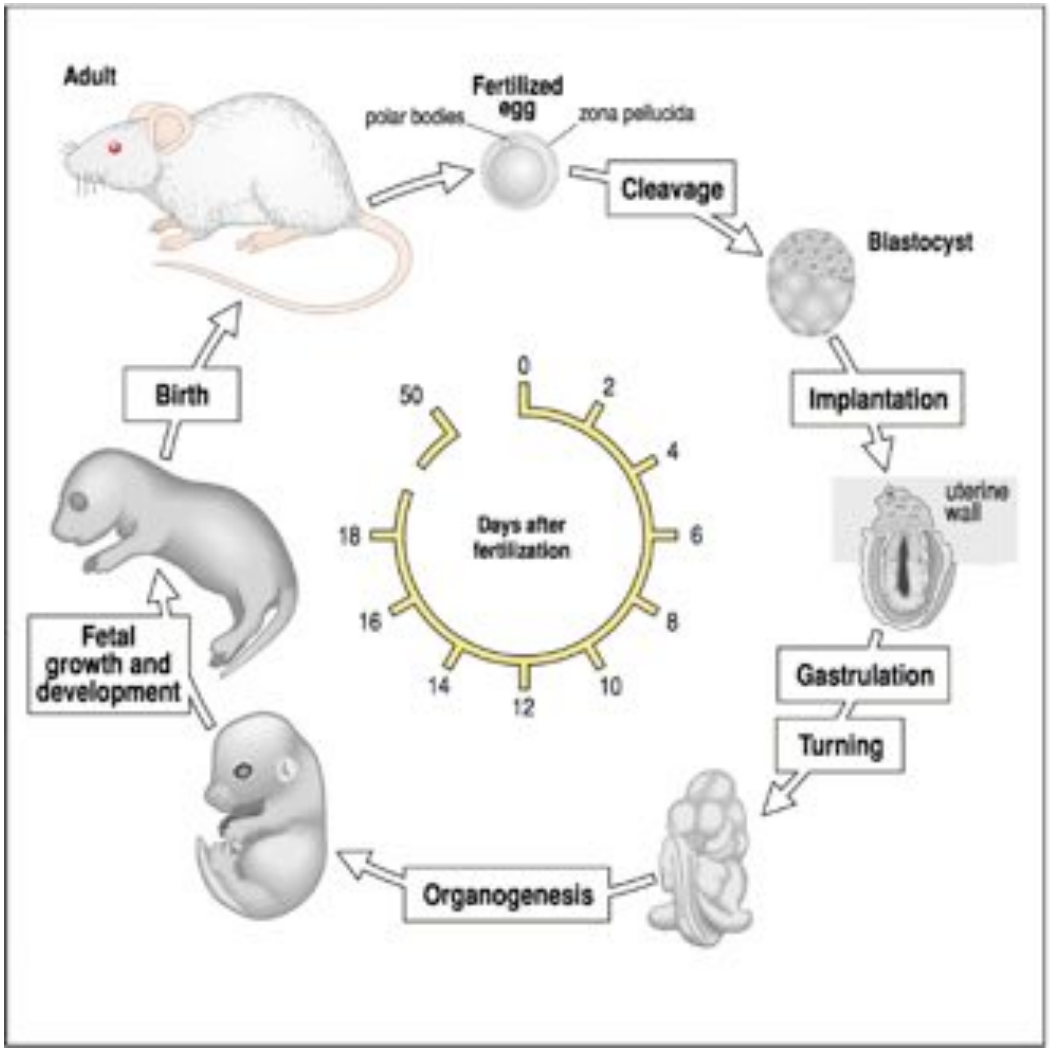


Mitotische Rekombination: MARCM Technik

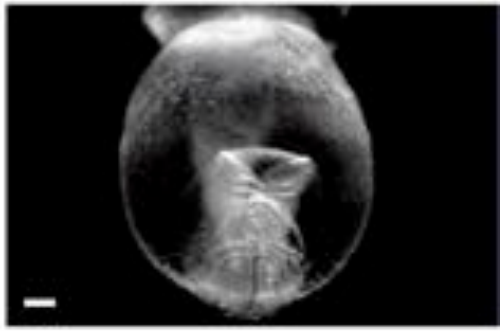
In der F1 kann die phänotypische Konsequenz einer Mutation in Zellklonen untersucht werden.



Rekombination und Mausgenetik

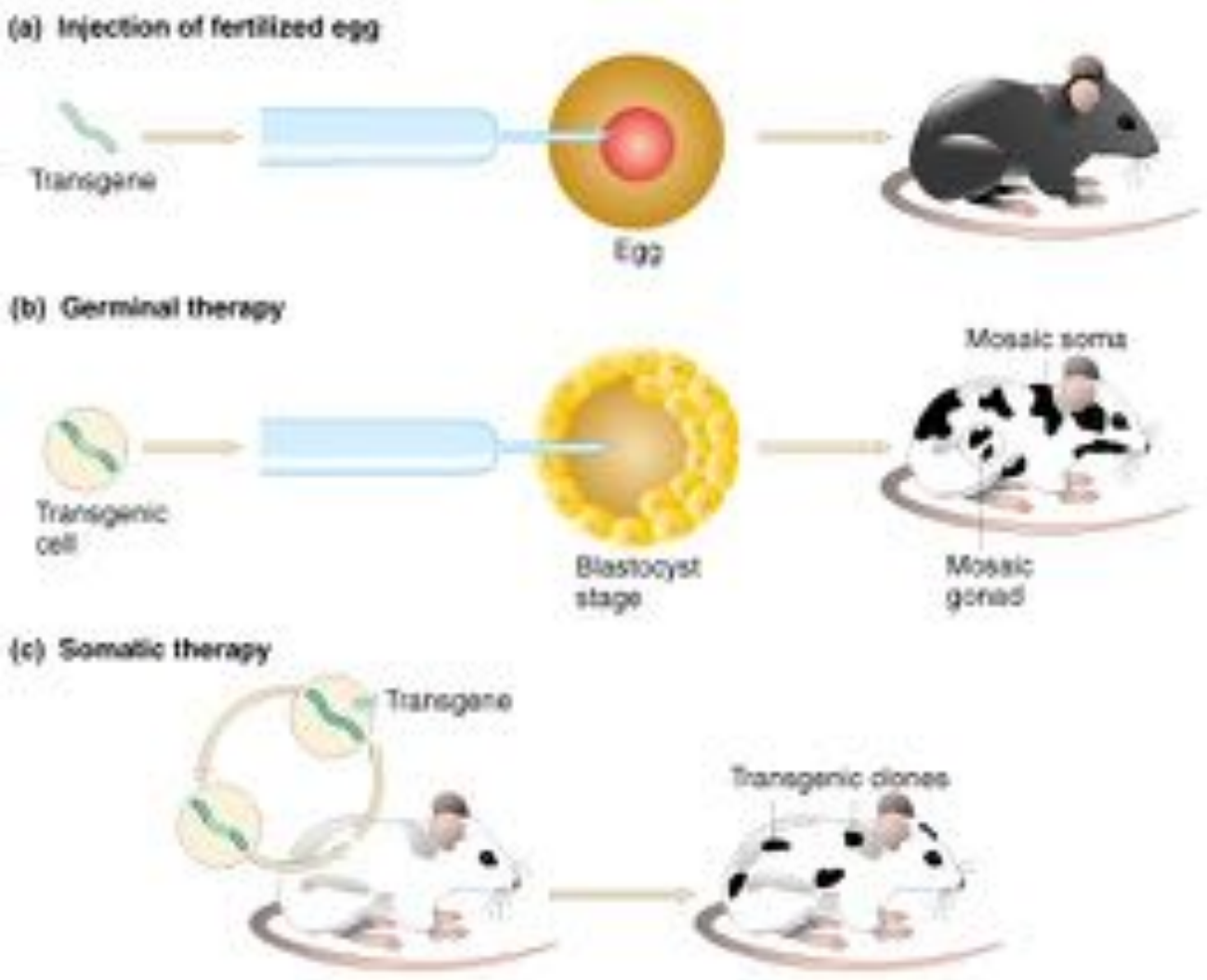






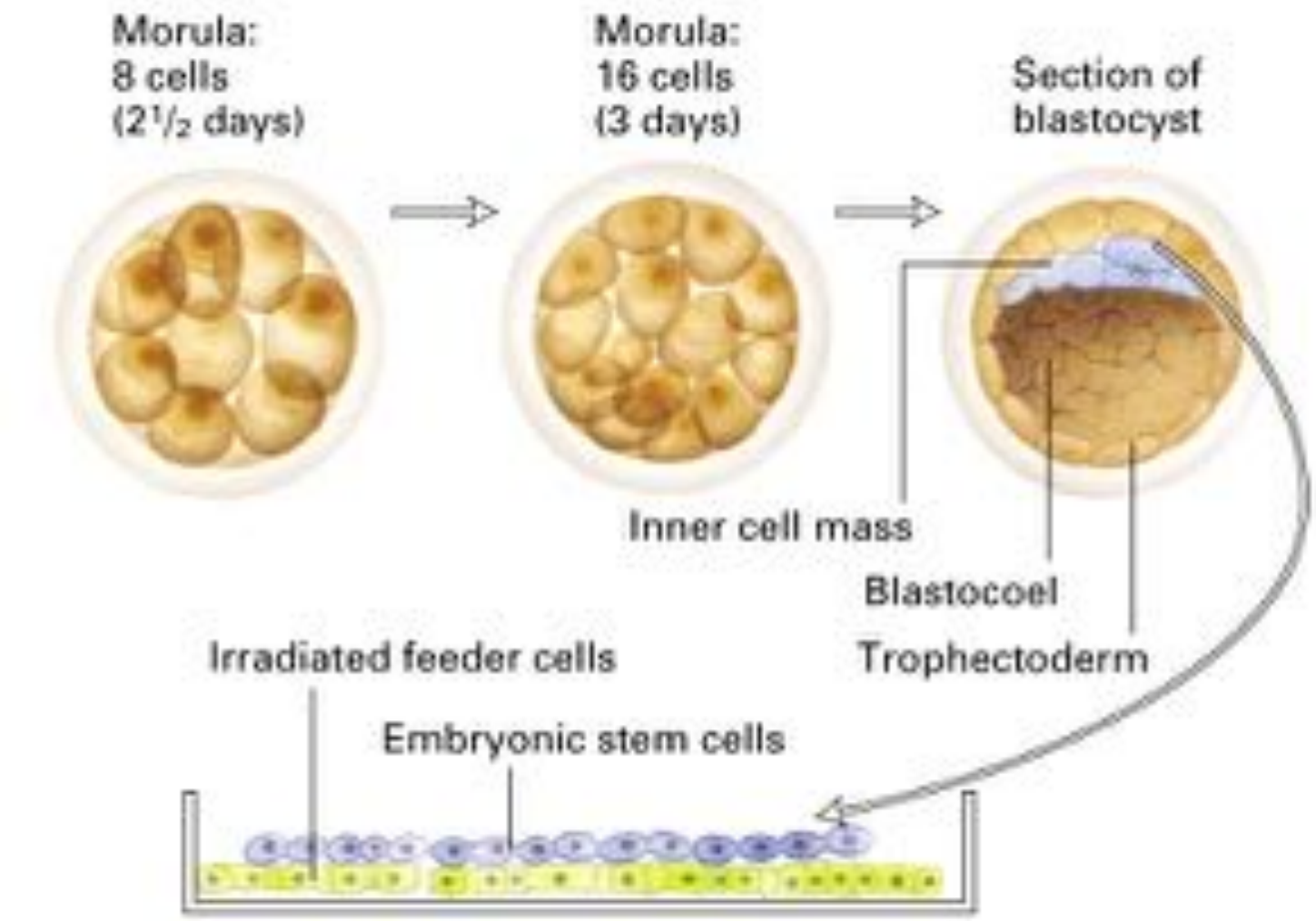


Verschiedene Weg führen zur transgenen Maus, alle benötigen (homologe) Rekombination

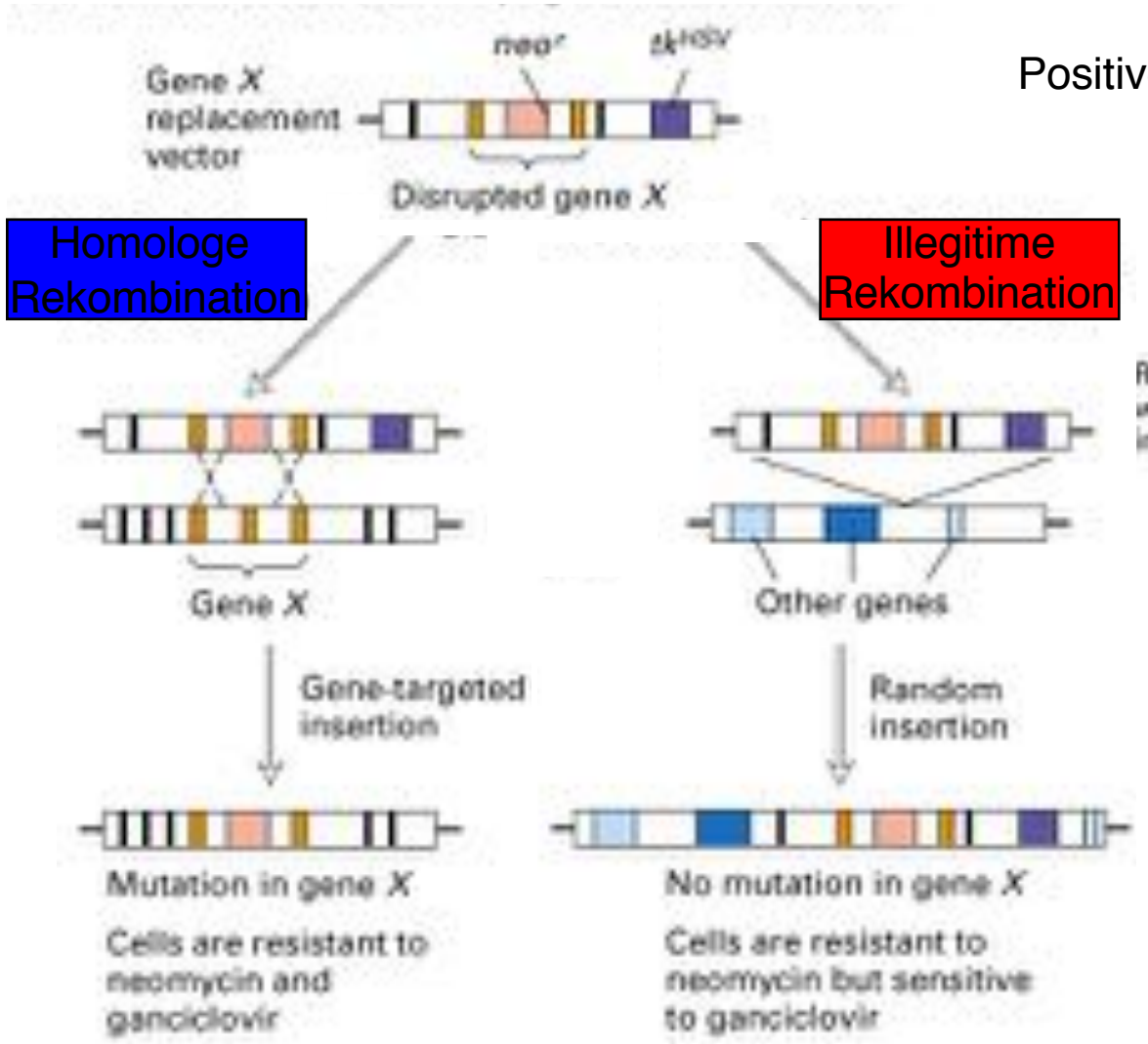




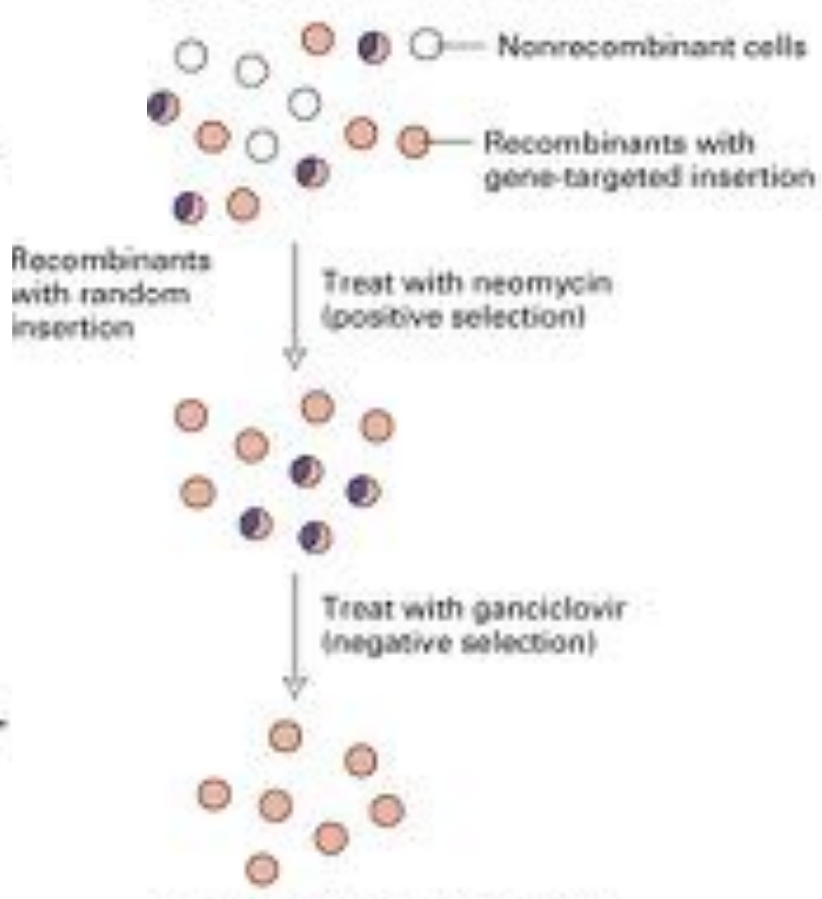
Wie macht man embryonale Stammzellen?

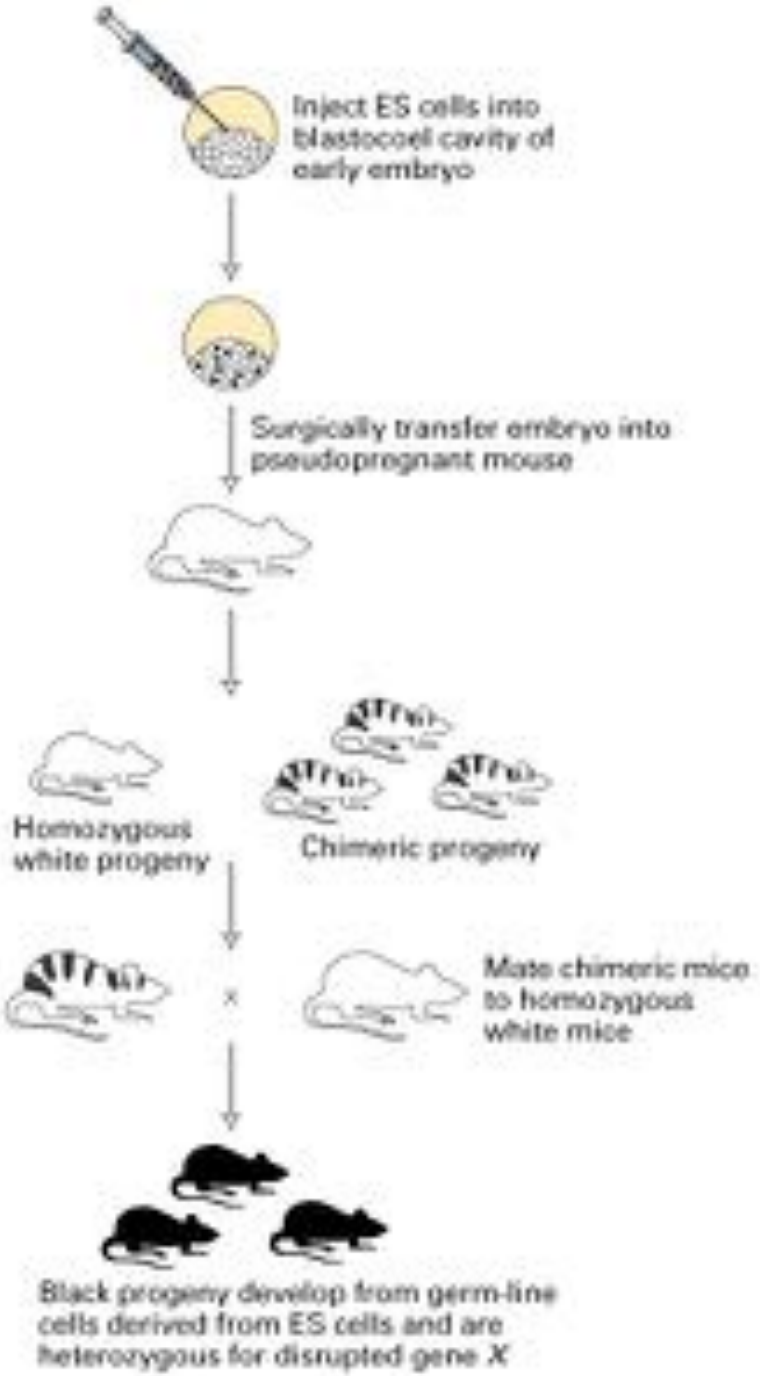


Knockout (KO) Strategie durch homologe Rekombination in ES-Zellen



Positive und negative Selektion der ES-Zellen

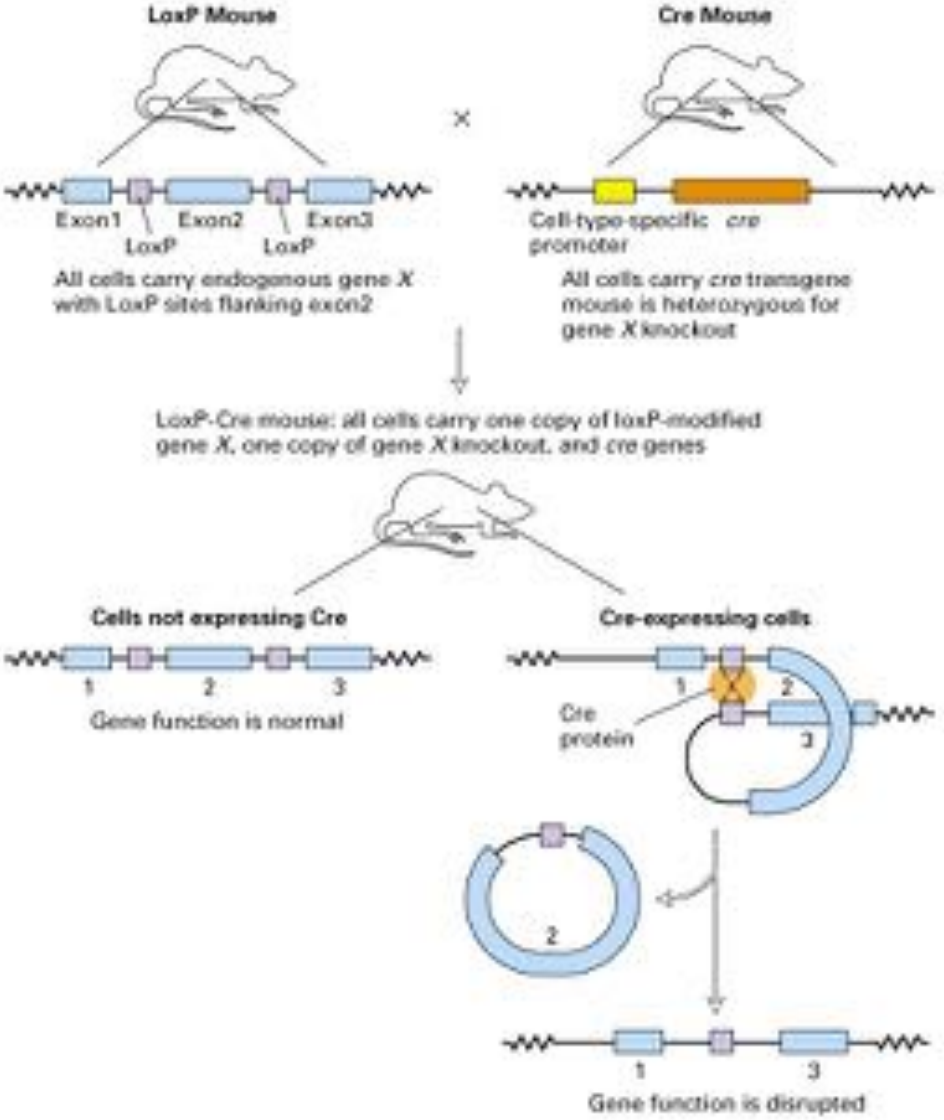




Der Weg zur KO Maus

Die richtigen Genotypen müssen durch PCR (Schwanzspitze) bestimmt werden.

Rekombination in Cis bei der konditionalen Geninaktivierung



Die Keimbahntransformation bei Drosophila über Transposon-Integration und illegitime Rekombination

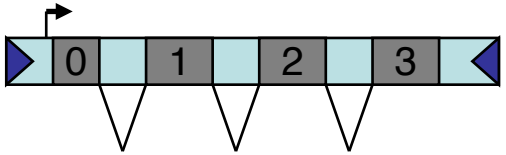


Mikroinjektion P-Element Vektor

Einige der sich entwickelnden Fliegen tragen das Transgen in ihrer Keimbahn

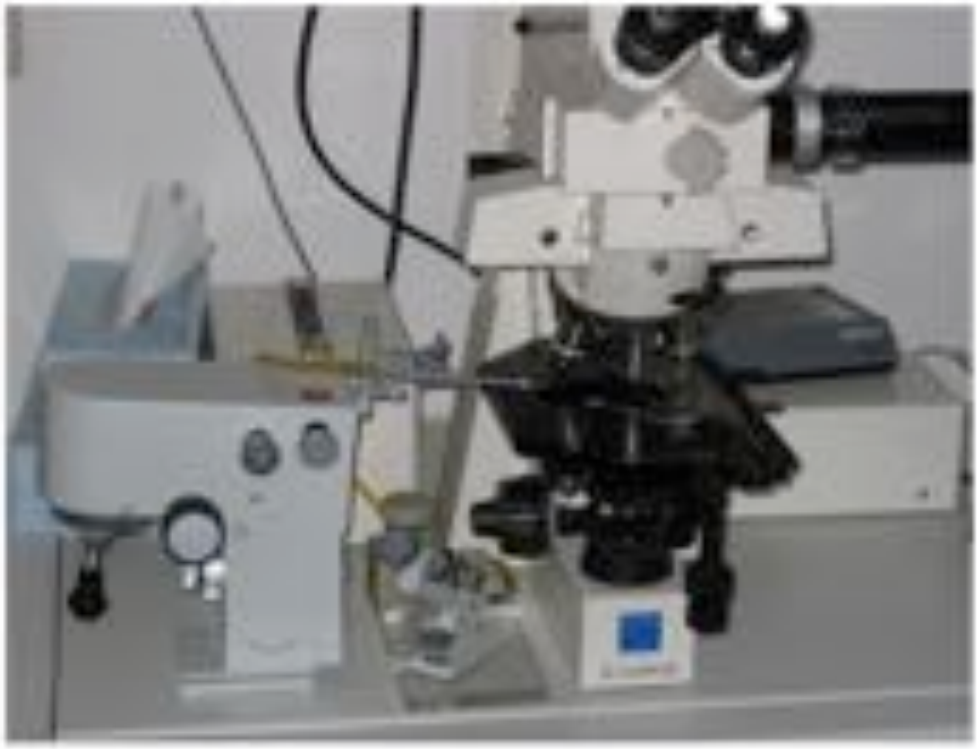
Drosophila

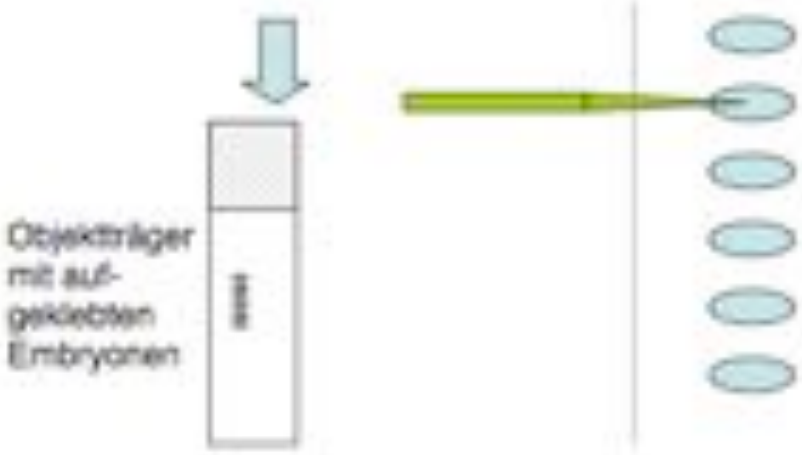
Das P-Element kodiert eine Transposase, die an einem 31 bp langen terminalen *inverted repeat* angreift.



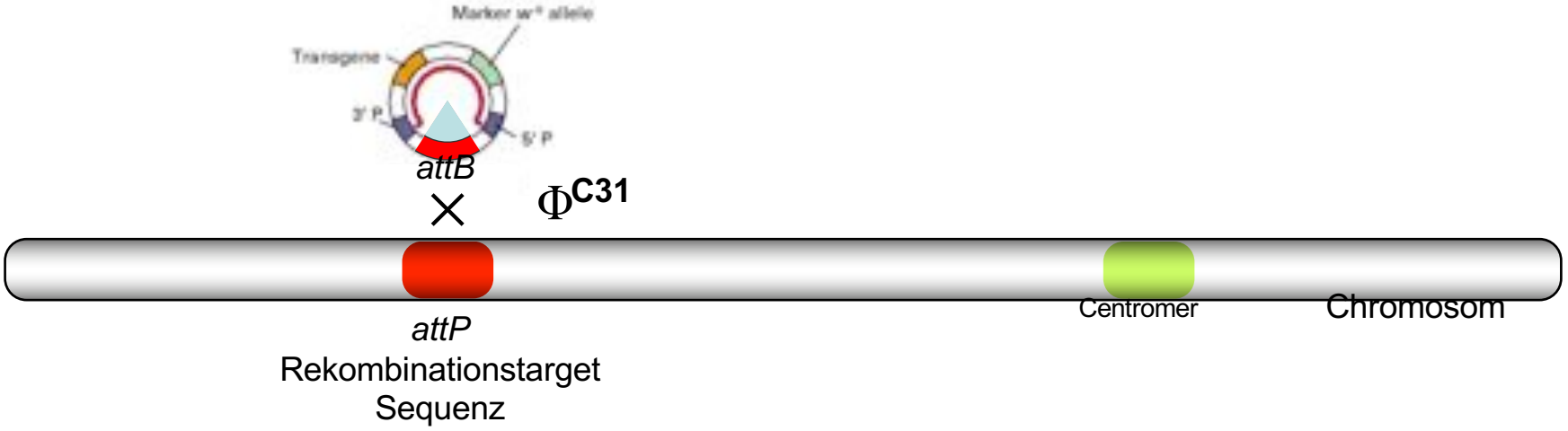
Keimbahntransformation







Rekombinationsvermittelte Transformation der Keimbahn

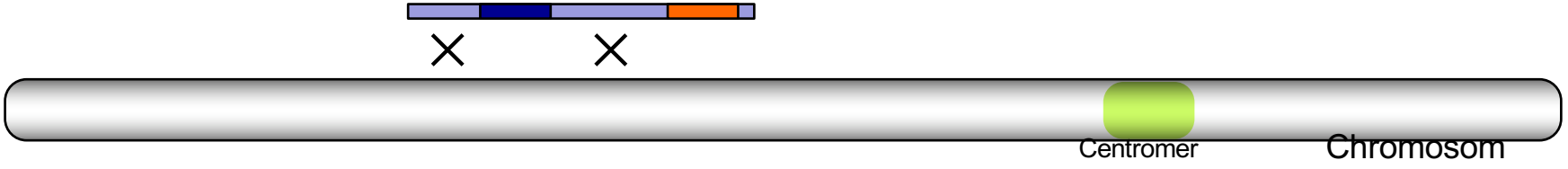


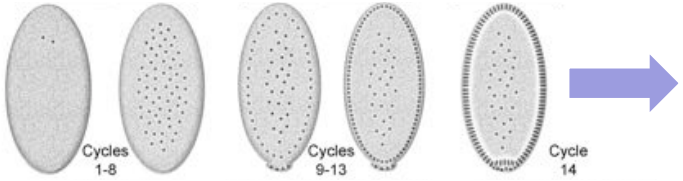




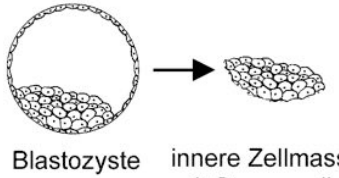


Ist homologe Rekombination in Drosophila möglich?



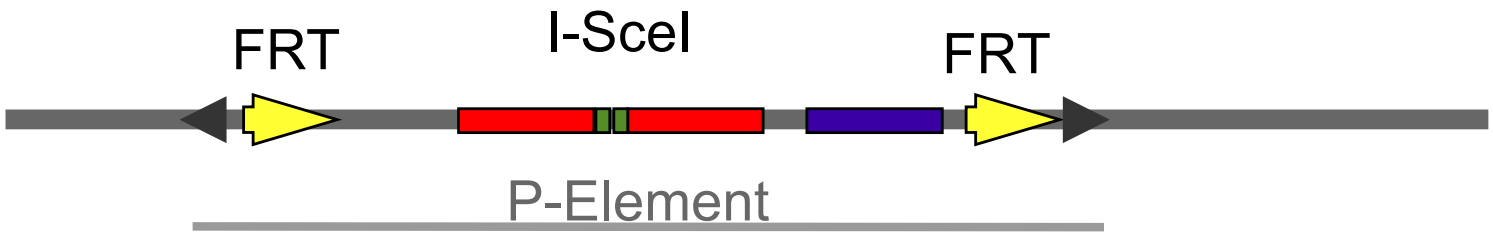








Generelle Strategie zur homologen Rekombination in Drosophila

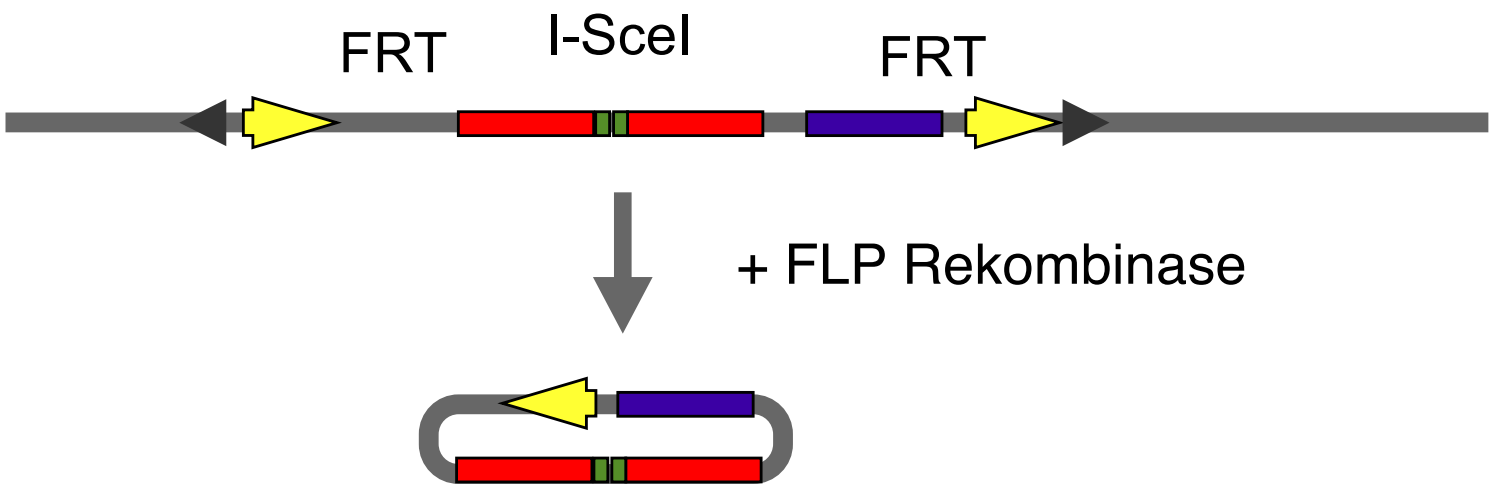


FLP
Rekombinase aus
der Hefe, bindet FRT

I-SceI
Homing endonuclease
18 bp Erkennungs-
sequenz
Hefe Mitochondrium
kodiert

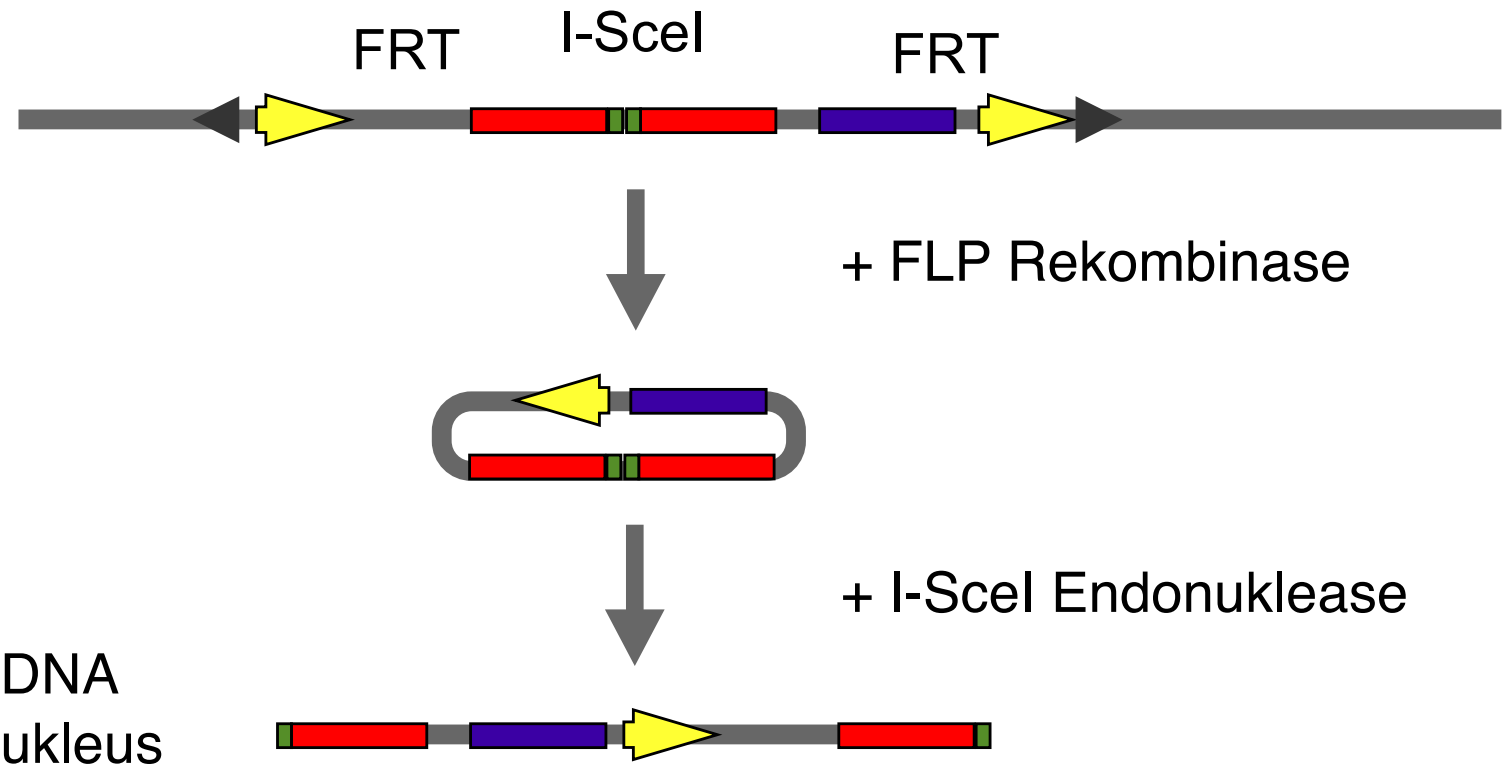
Rong & Golic 2000

Generelle Strategie zur homologen Rekombination in Drosophila



Rong & Golic 2000

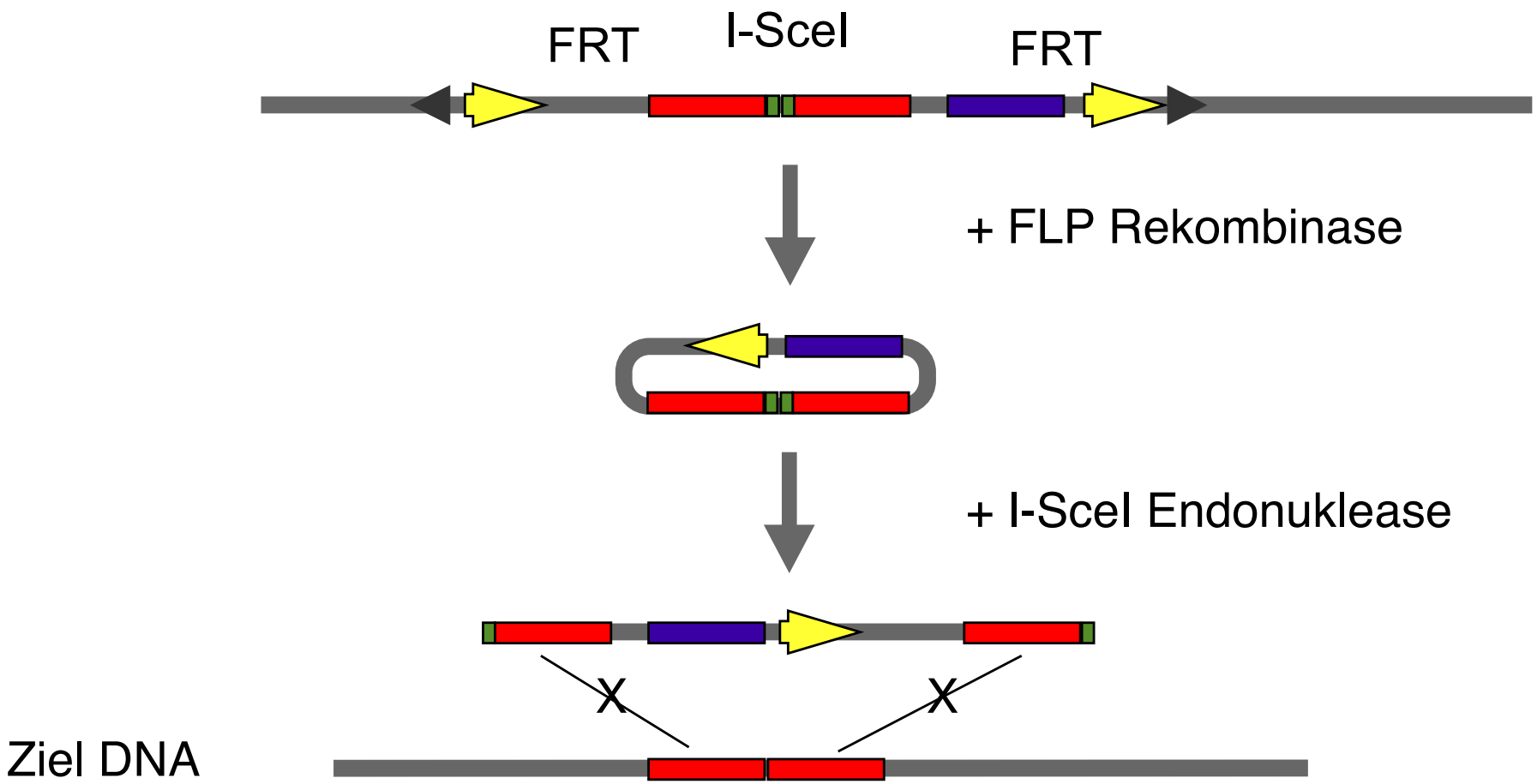
Generelle Strategie zur homologen Rekombination in Drosophila



Ein lineares DNA Molekül je Nukleus

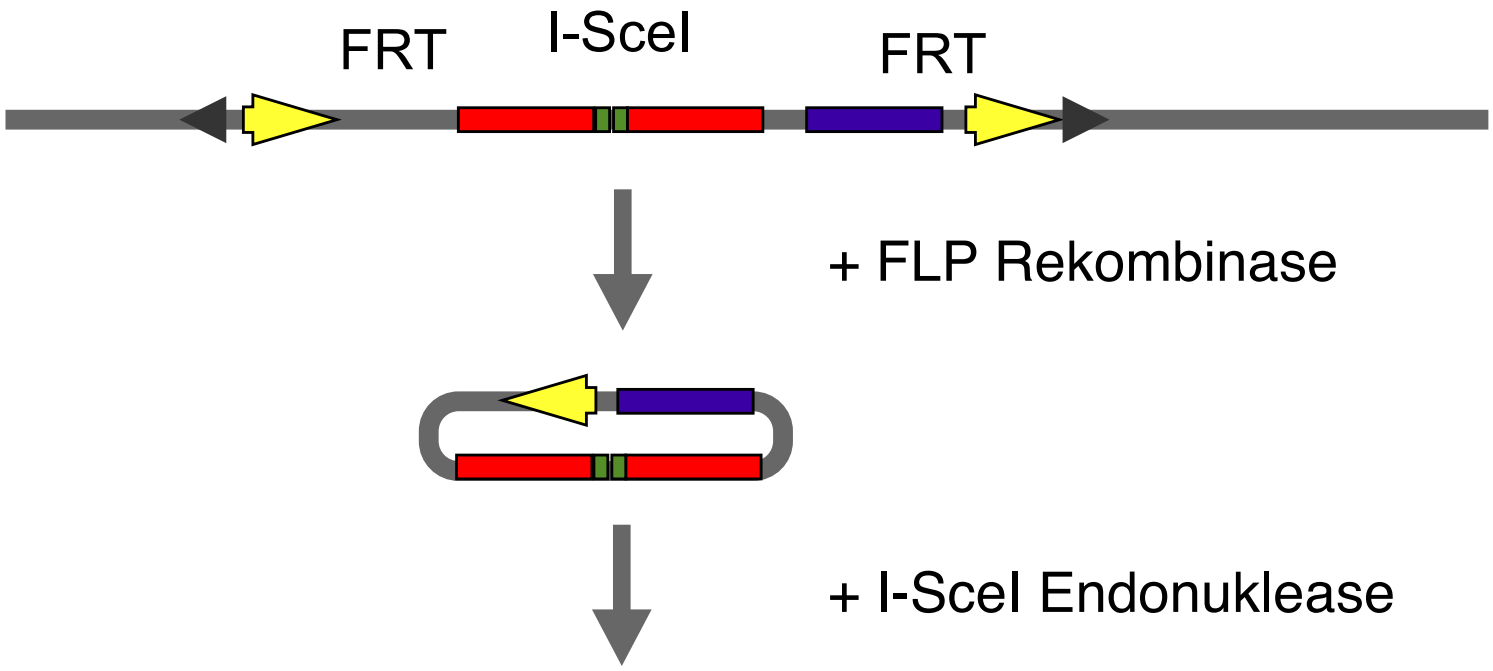
Rong & Golic 2000

Generelle Strategie zur homologen Rekombination in Drosophila



Rong & Golic 2000

Generelle Strategie zur homologen Rekombination in Drosophila

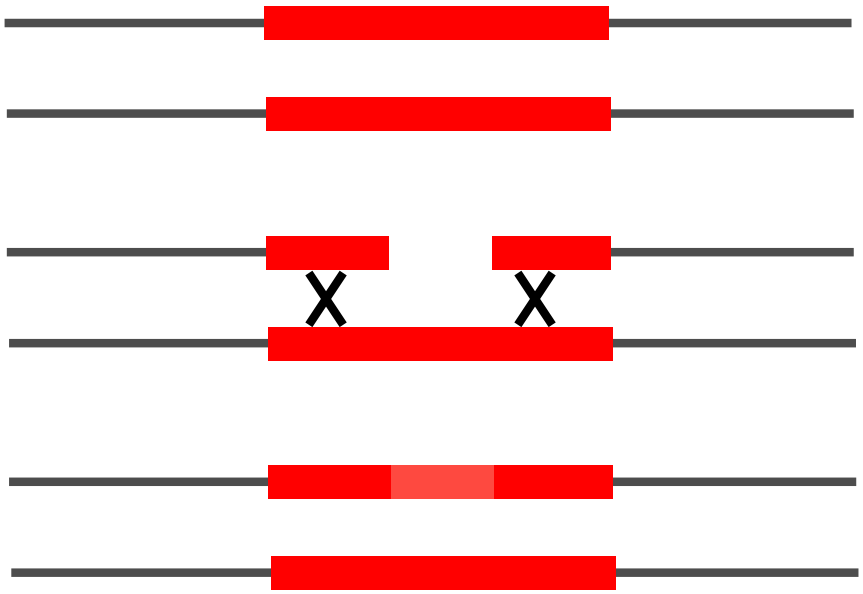


Ziel DNA

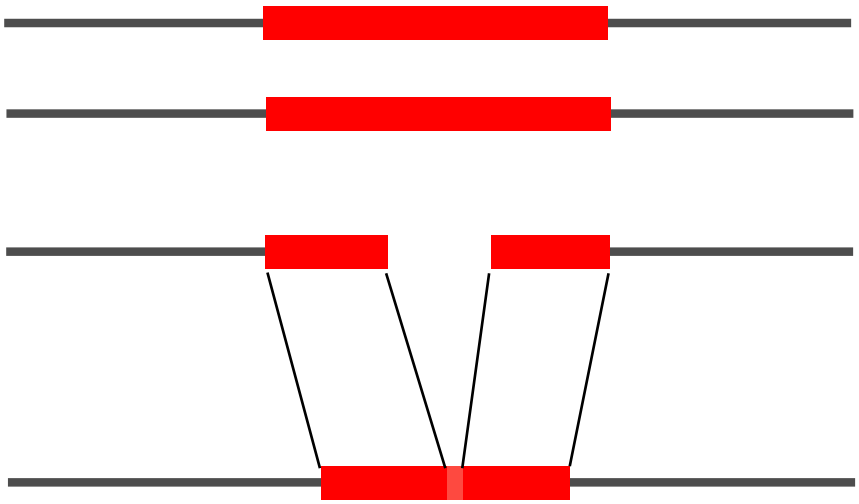


Rong & Golic 2000

Homologe Rekombination ist effektiv und wird auch zur DNA-Reparatur eingesetzt



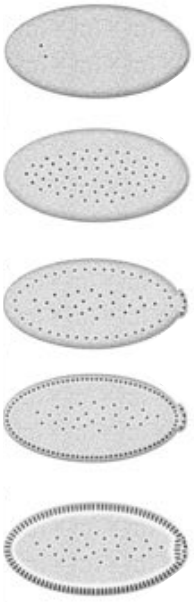
HDR
Homology directed repair



NHEJ
Non-homologous end joining

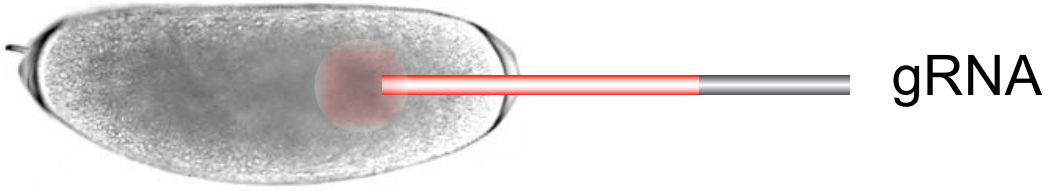
CRISPR / Cas9 im Drosophila System







0,5 mm

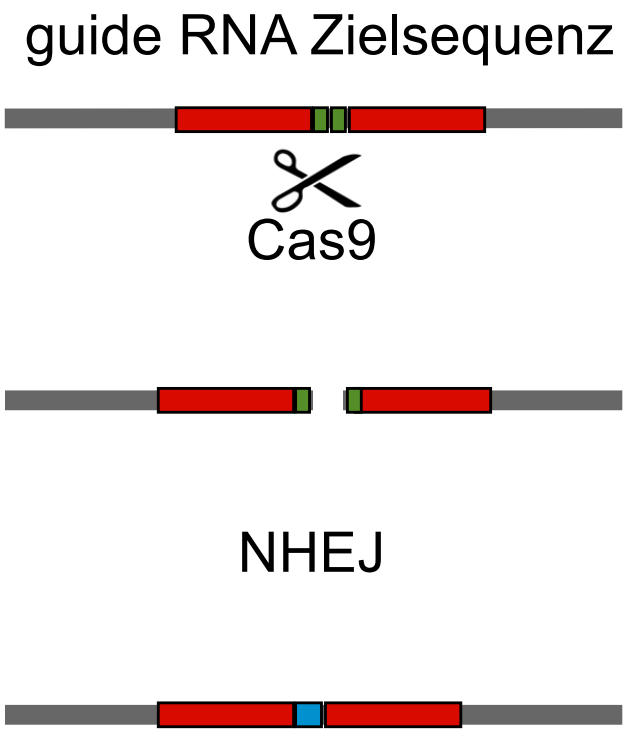


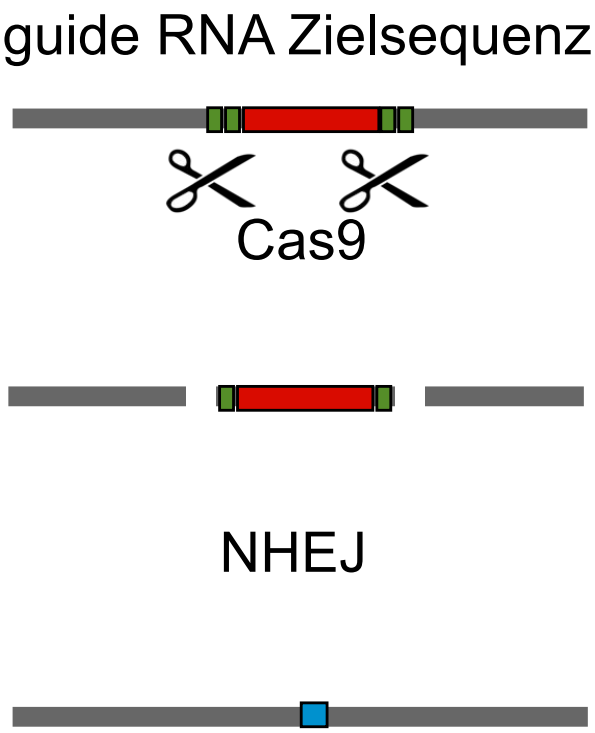
gRNA

act>>Cas9

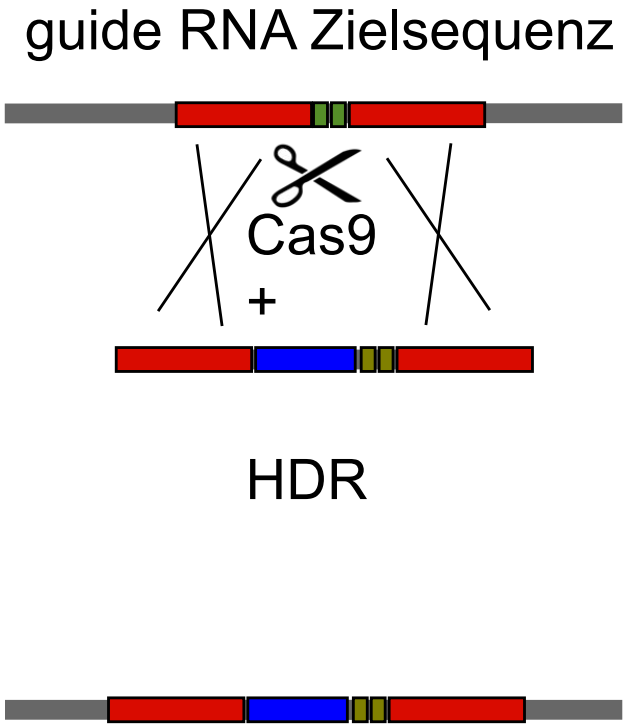
nos>>Cas9

Das CRISPR / Cas9 System ist sehr flexibel





Das CRISPR / Cas9 System ist sehr flexibel



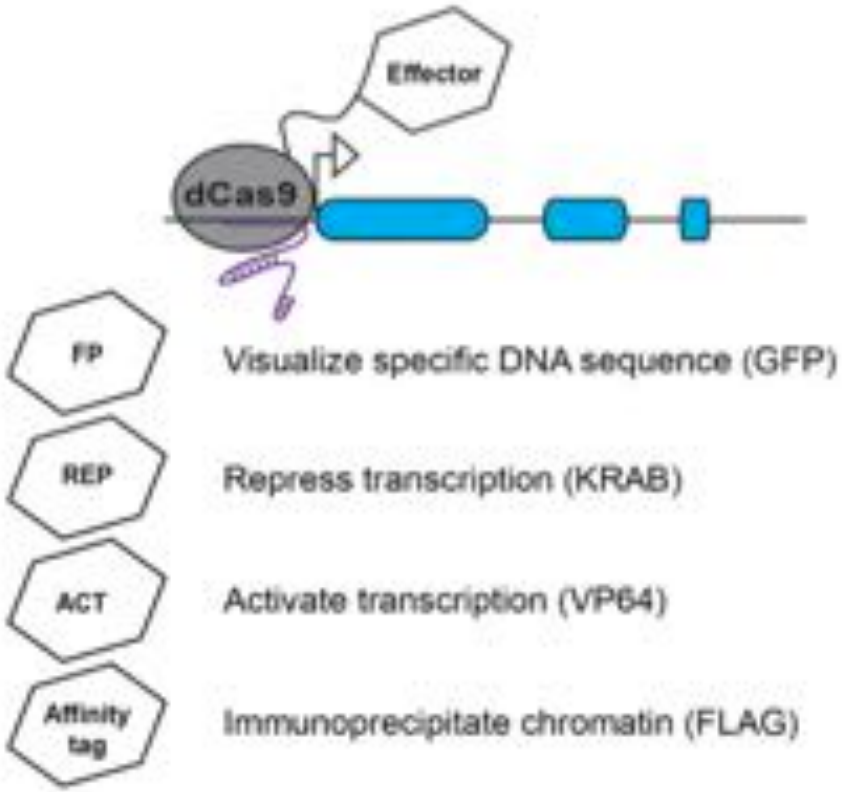
Herstellung von endogen markierten Proteinen (GFP, Dendra, HA)

Insertion einer regulierbaren Promotersequenz

Das CRISPR / Cas System ist sehr flexibel

Cas9, Cas12
DNA Bindung

Cas13
RNA Bindung



Das CRISPR / Cas9 System läßt sich in vielen Organismen anwenden

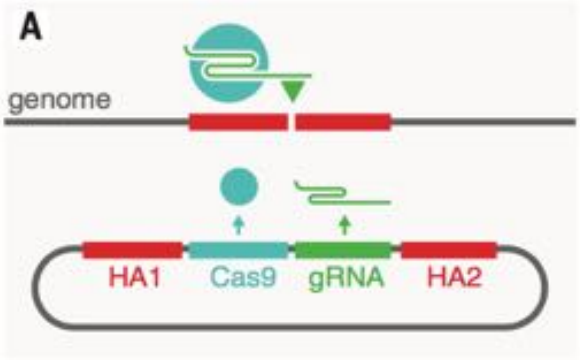
Table 1. *Organisms that have been modified using the CRISPR–Cas9 system*

Organism	Mutations created in: Cultured cells	Mutations created in: Organism (heritable?)	Alleles generated by: NHEJ	Alleles generated by: HDR	References
Vertebrates					
Axolotl		✓	✓		Flowers et al. 2014
Frog		✓ (Yes)	✓		Blitz et al. 2013; Nakayama et al. 2013; Guo et al. 2014
Human	✓		✓	✓	For review, see Sander and Joung 2014
Medaka		✓ (Yes)	✓		Ansai and Kinoshita 2014
Mouse	✓	✓ (Yes)	✓	✓	For review, see Sander and Joung 2014
Monkey		✓	✓		Niu et al. 2014b
Pig	✓	✓ (Yes)	✓		Hai et al. 2014; Sato et al. 2014
Rabbit		✓	✓		Yang et al. 2014
Rat	✓	✓ (Yes)	✓	✓	Li et al. 2013a,b, 2014b; Ma et al. 2014b,c,d
Tilapia		✓ (Yes)	✓		Li et al. 2014a
Zebrafish		✓ (Yes)	✓	✓	For review, see Auer et al. 2014
Invertebrates					
Freshwater flea		✓ (Yes)	✓		Nakanishi et al. 2014
Fruit fly	✓	✓ (Yes)	✓	✓	For review, see Gratz et al. 2013; Bassett and Liu 2014
Roundworm		✓ (Yes)	✓	✓	For review, see Waaijers and Boxem 2014
Silkworm	✓	✓ (Yes)	✓	✓	Wang et al. 2013b; Daimon et al. 2014; Liu et al. 2014b; Ma et al. 2014a; Wei et al. 2014
Plants					
Corn		✓	✓		Liang et al. 2014
Liverwort		✓ (Yes)	✓		Sugano et al. 2014
Rice		✓ (Yes)	✓		For review, see Belhaj et al. 2013
Sorghum		✓	✓		Jiang et al. 2013b
Sweet orange		✓	✓		Jia and Wang 2014
Thale cress		✓ (Yes)	✓	✓	For review, see Belhaj et al. 2013
Tobacco		✓ (Yes)	✓	✓	For review, see Belhaj et al. 2013
Wheat	✓		✓		Upadhyay et al. 2013

We limited our list to those organisms that provide platforms for the study of development (as indicated for some organisms, only cells derived from the organism have been modified to date). Blanks indicate "not tested." For the organisms in which the CRISPR–Cas9 system has been used extensively, see recent reviews. Given the rapid advances in the field, we apologize for any organisms or references that were inadvertently not included.

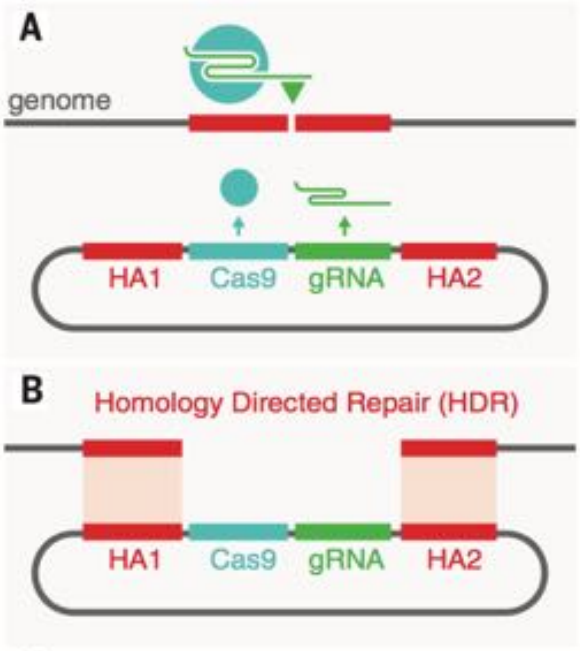
Harrison et al., 2014

CRISPR/Cas und *Gene drive*



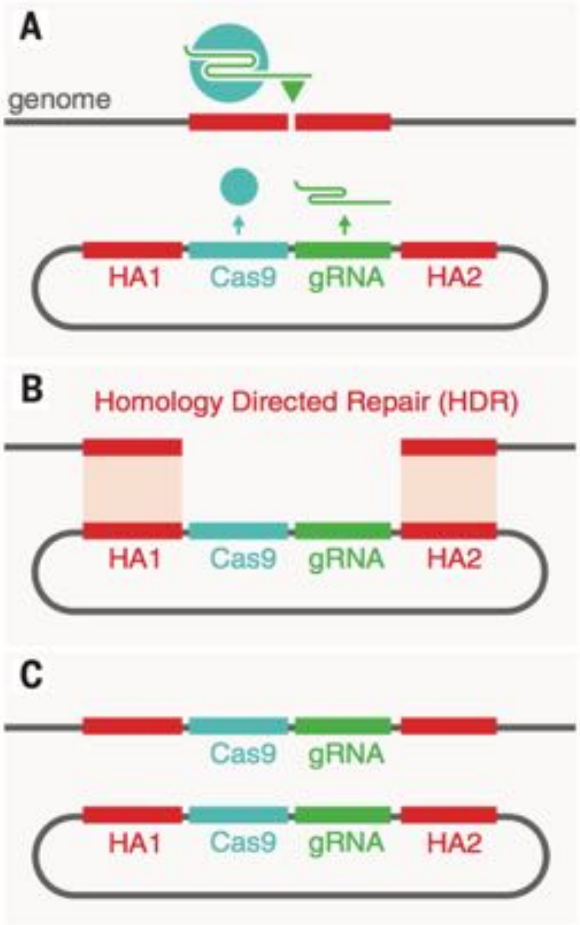
Valentino M Gantz and Ethan Bier, Science 2015

CRISPR/Cas und *Gene drive*



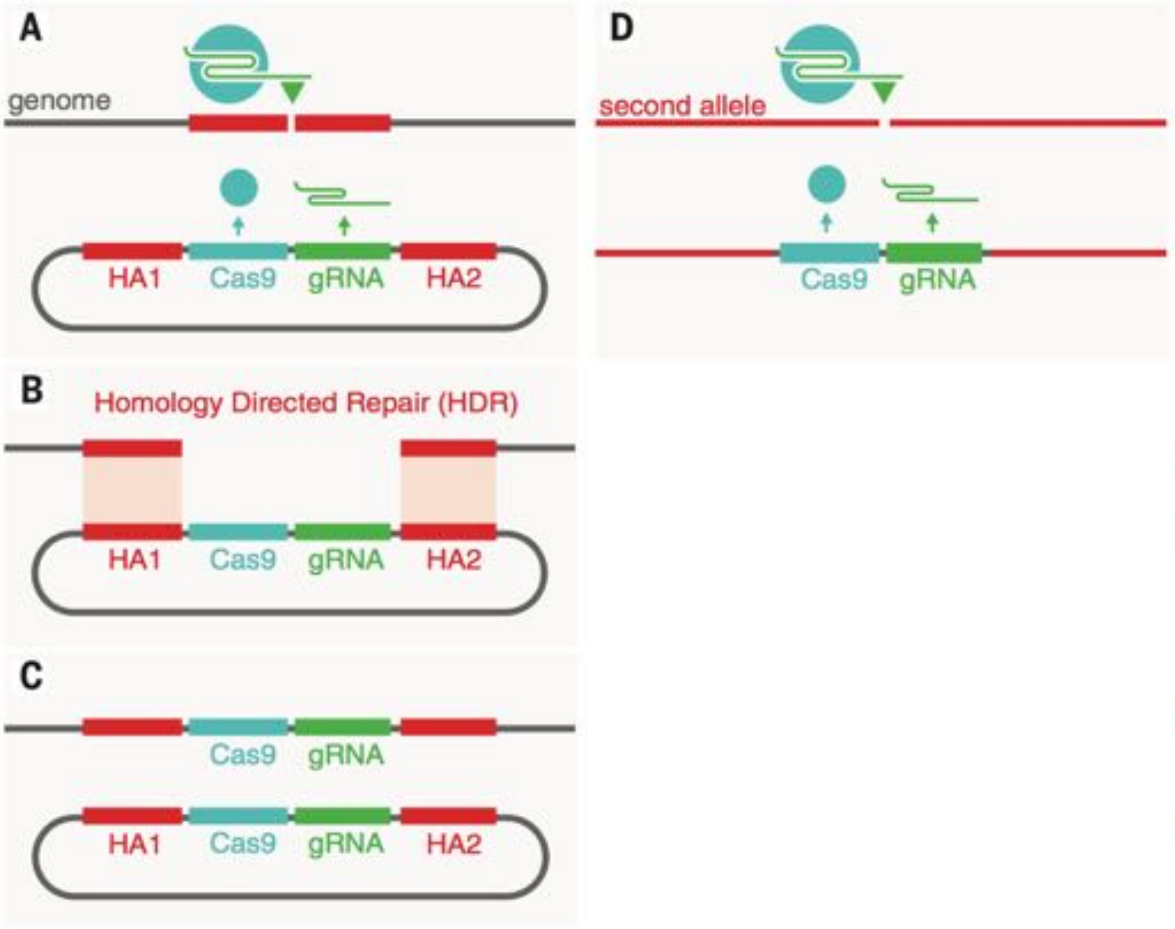
Valentino M Gantz and Ethan Bier, Science 2015

CRISPR/Cas und *Gene drive*



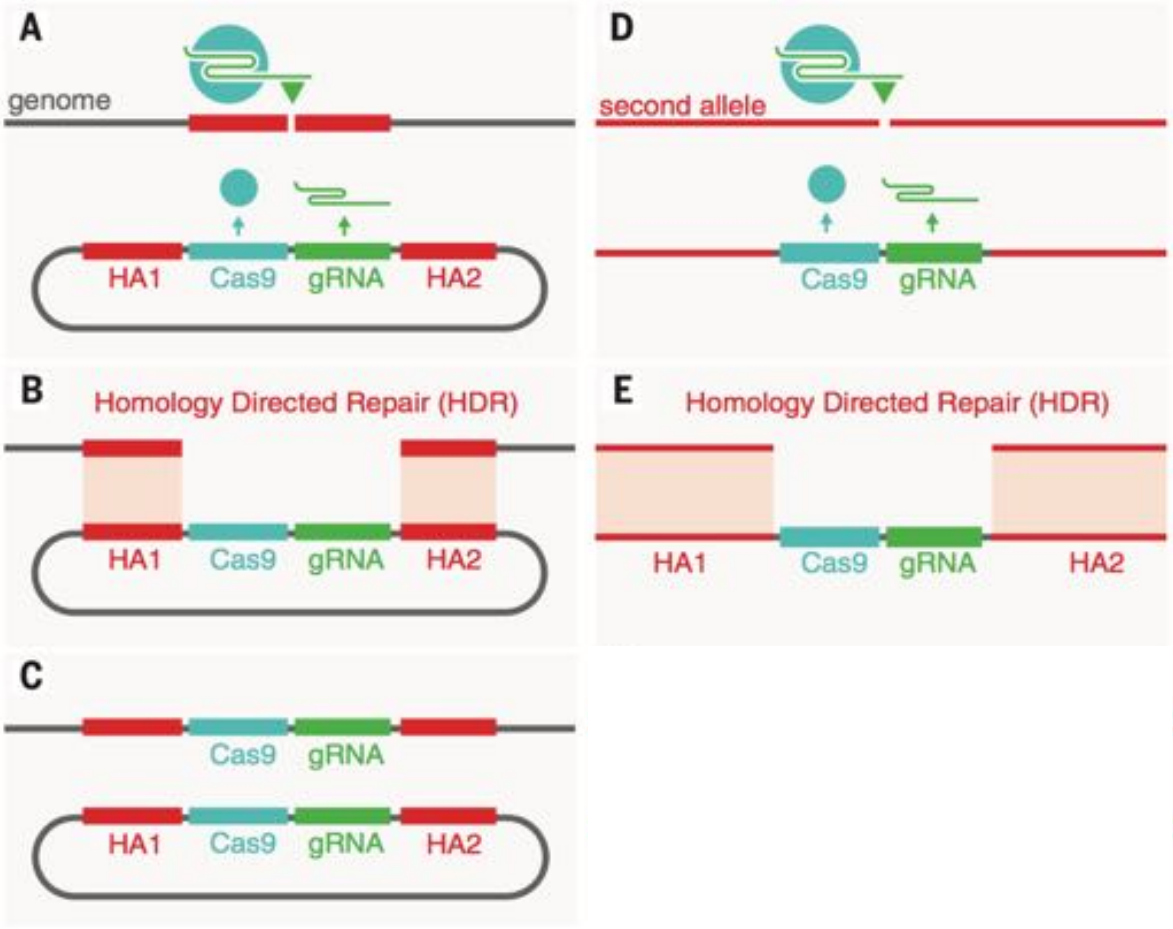
Valentino M Gantz and Ethan Bier, Science 2015

CRISPR/Cas und *Gene drive*



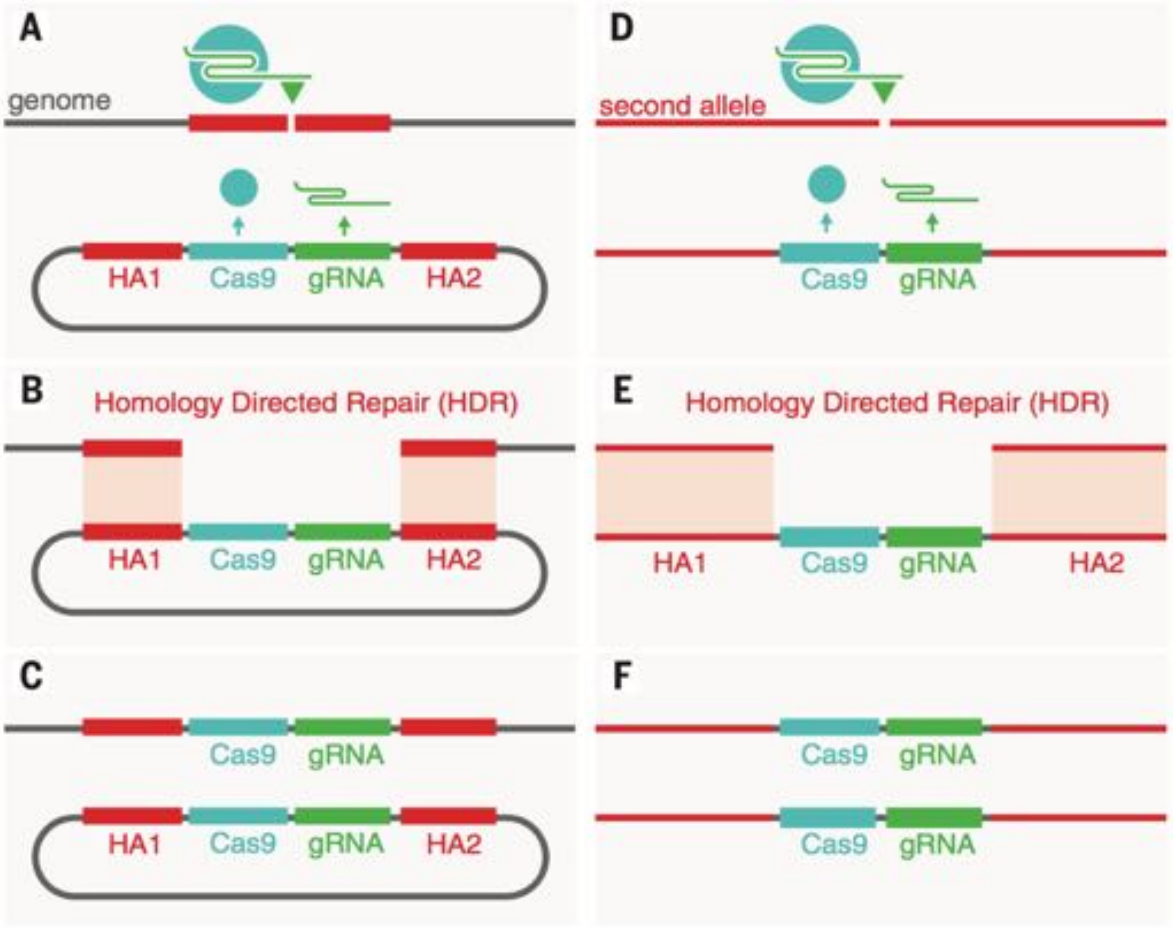
Valentino M Gantz and Ethan Bier, Science 2015

CRISPR/Cas und *Gene drive*



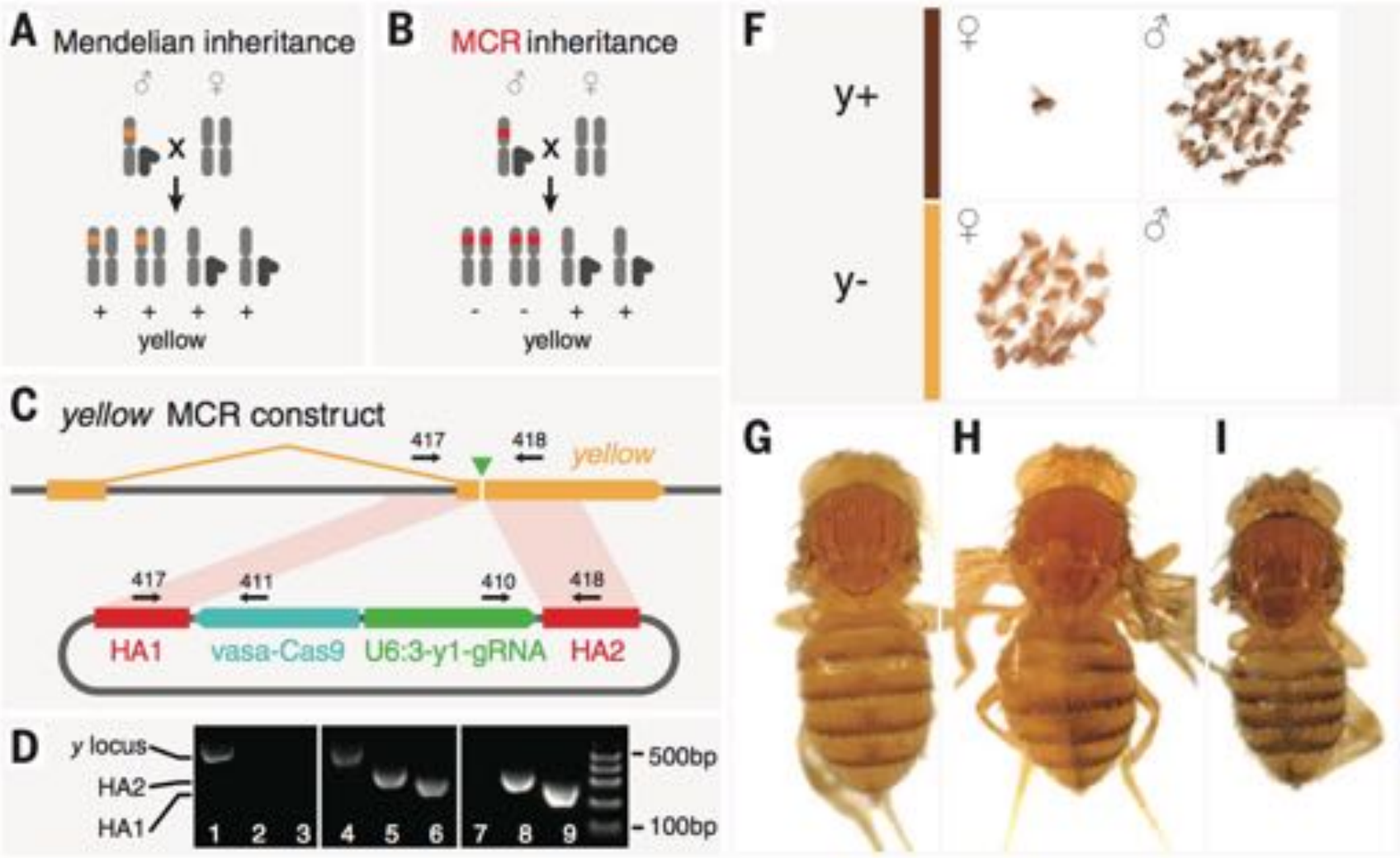
Valentino M Gantz and Ethan Bier, Science 2015

CRISPR/Cas und *gene drive*

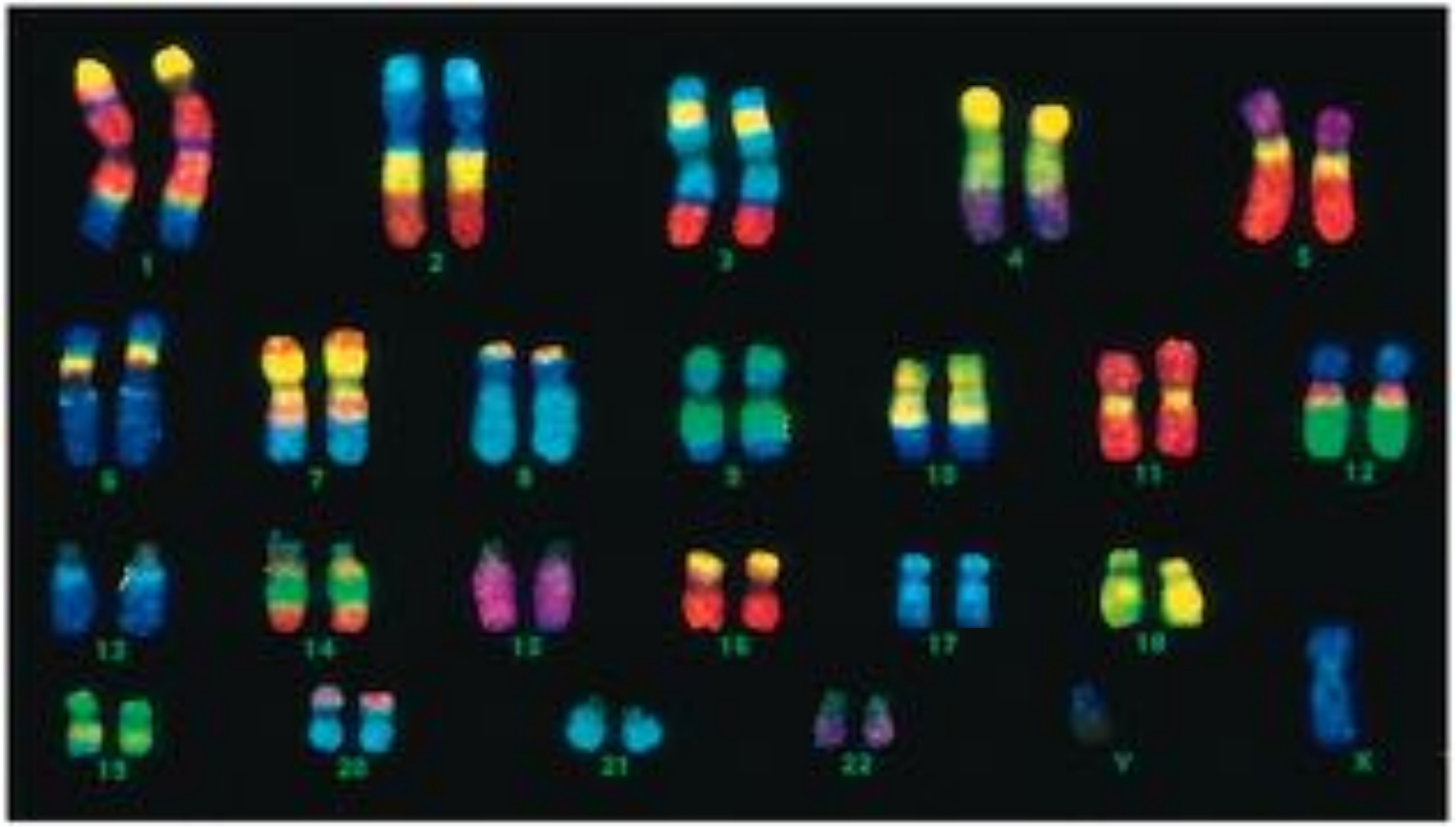


Valentino M Gantz and Ethan Bier, Science 2015

Effizienz des *gene drive*

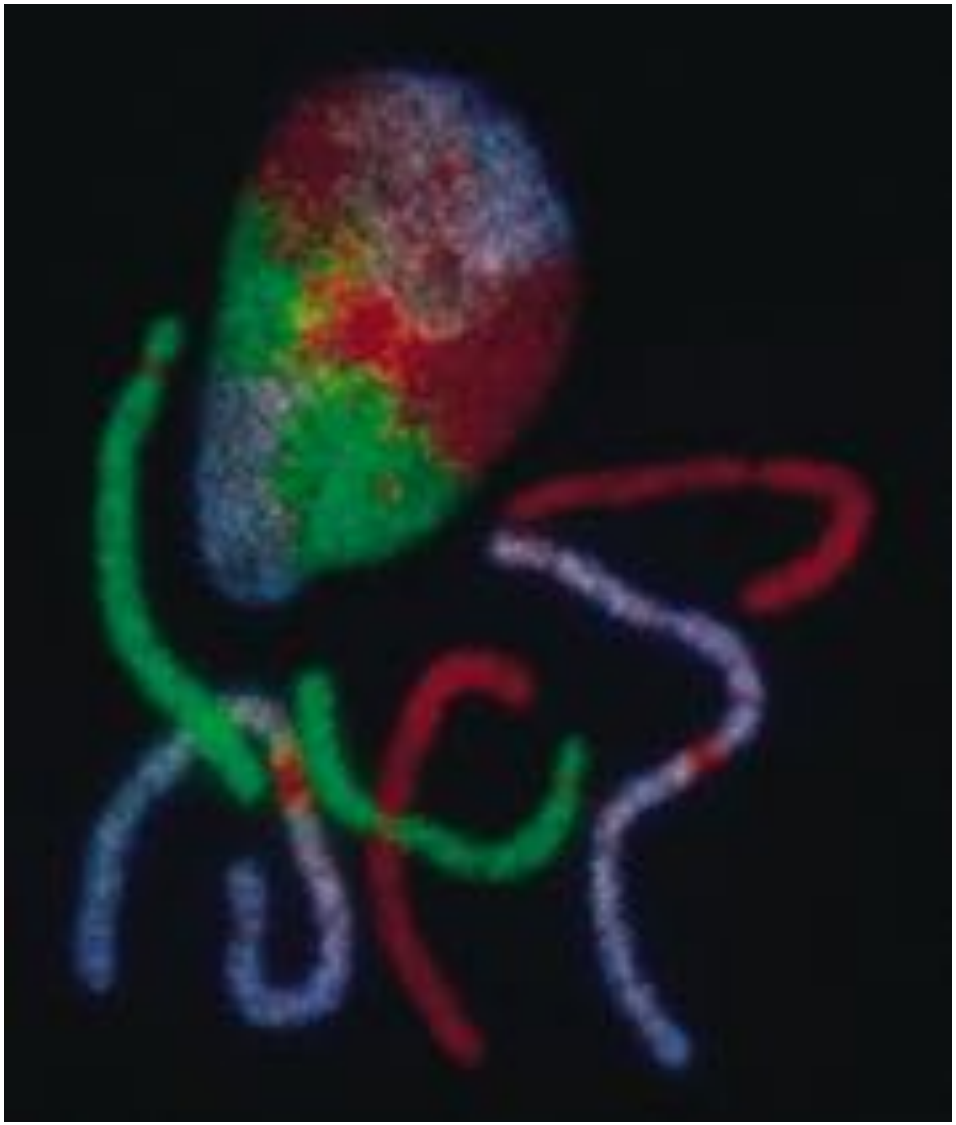


Gene sind auf den Chromosomen in Kopplungsgruppen angeordnet

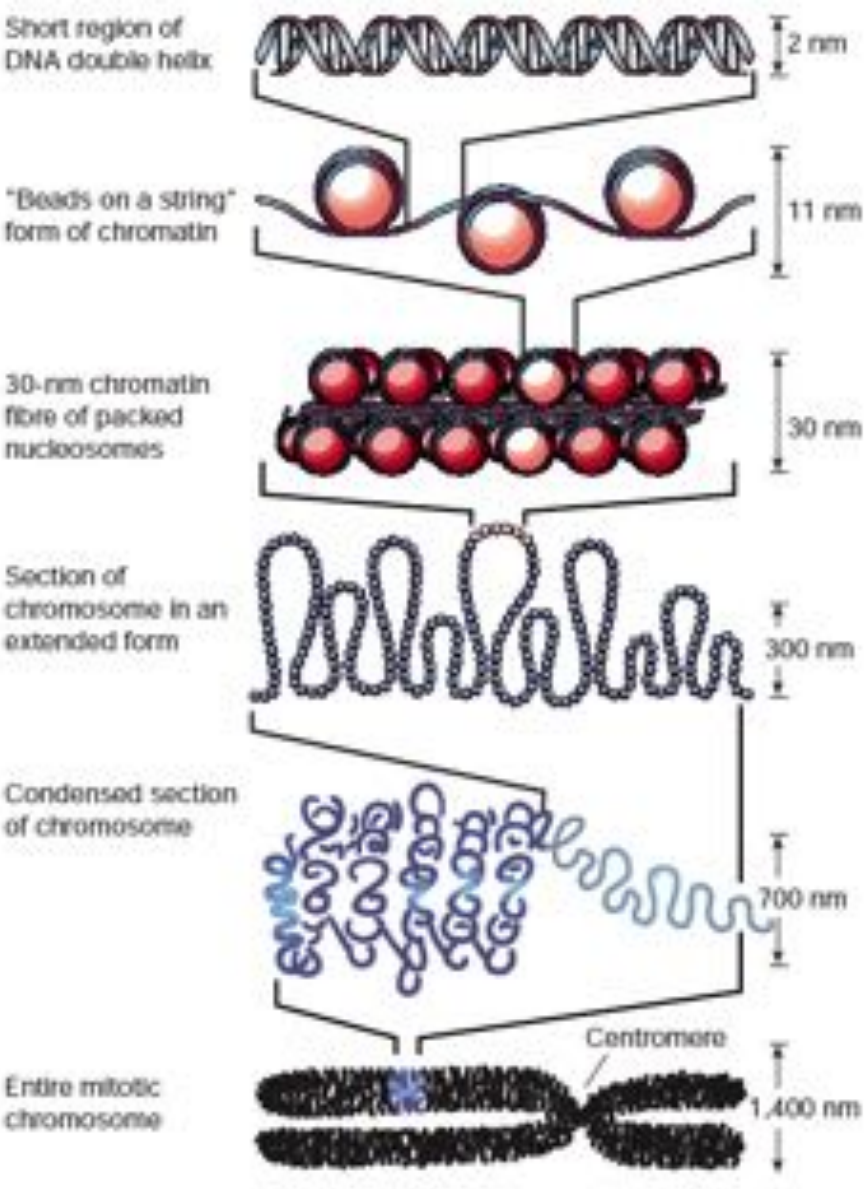


Mensch	ca. 30.000 Gene
Drosophila	ca. 13.000 Gene
C. elegans	ca. 19.000 Gene

Interphasenchromosomen liegen geordnet im Kern vor



Verpackung der DNA



Wie bringe ich etwa 2m DNA in einen Kern mit einem ø10μm ?

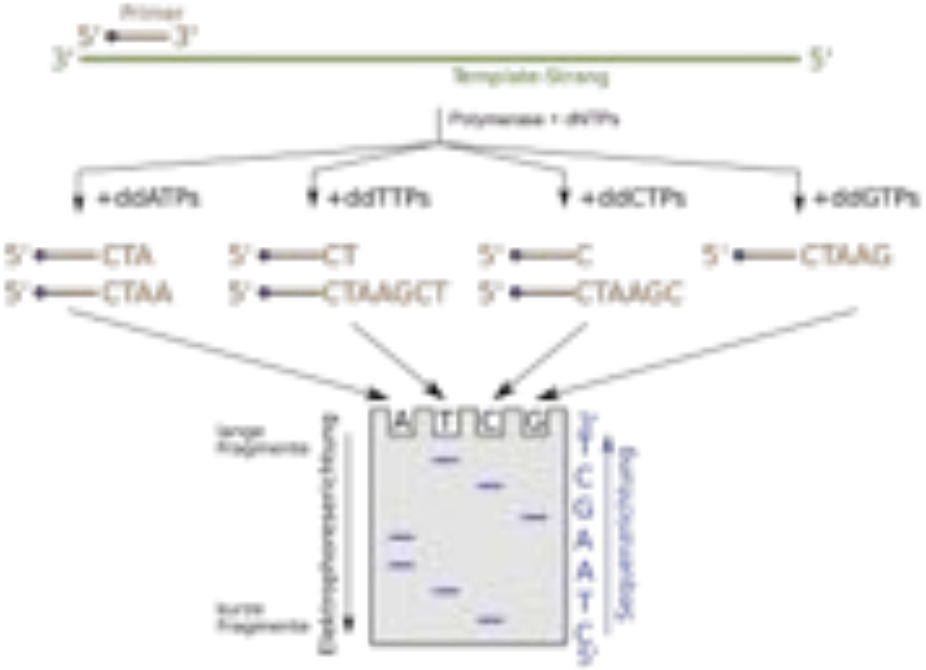
Euchromatin

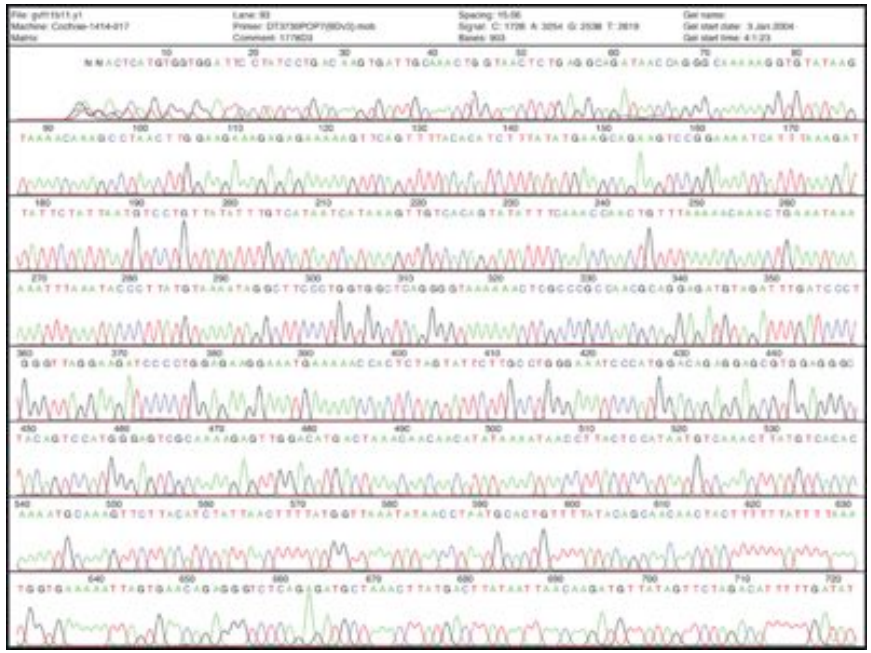
Heterochromatin

konstitutives

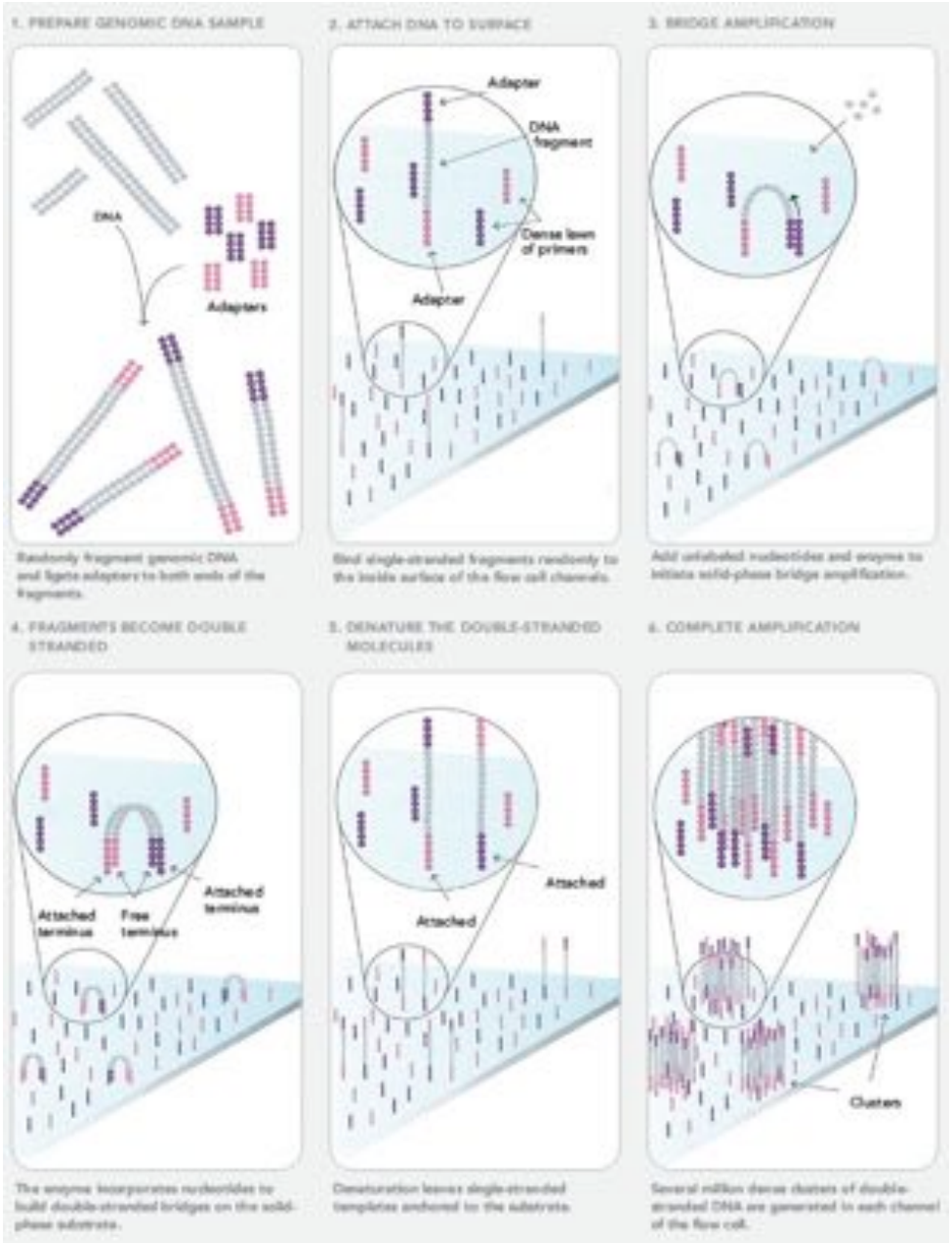
fakultatives

Sequenzierung nach Sanger

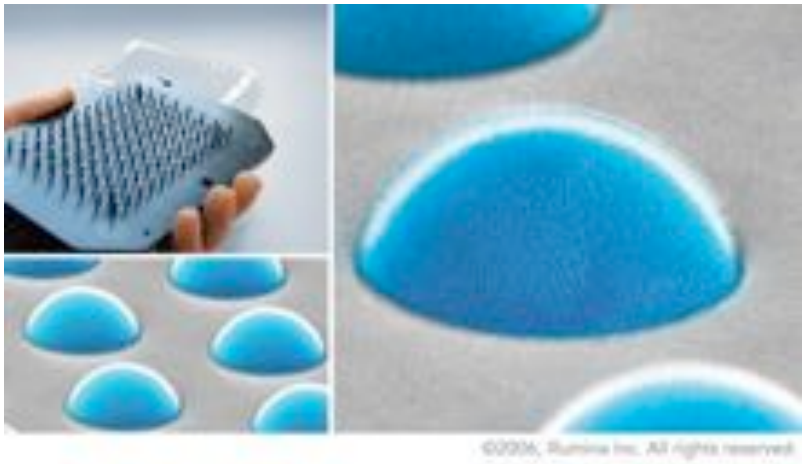




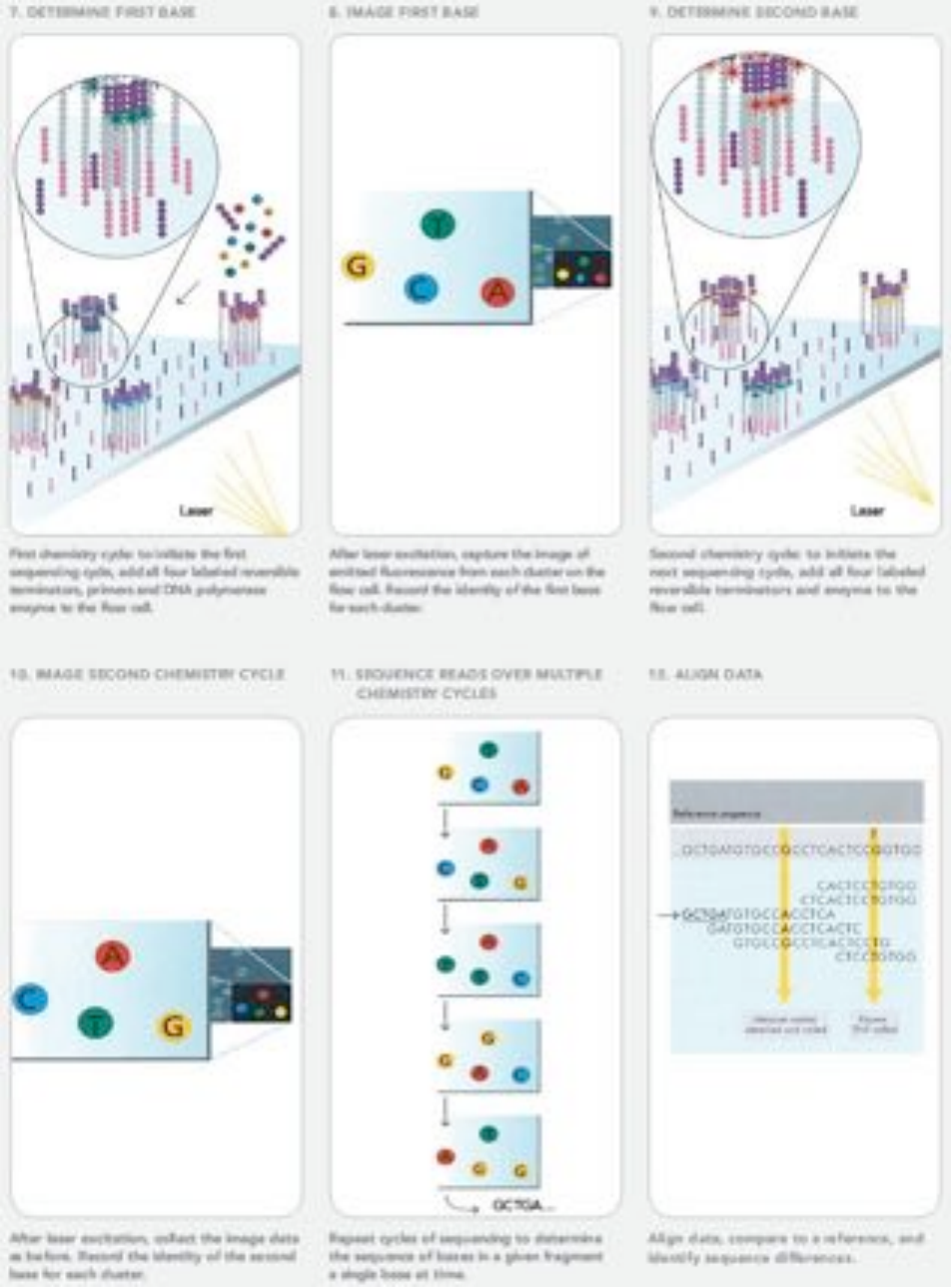
Illumina Sequenzierung



- Die DNA-Chip-Technologie der Firma Illumina verwendet kleine Silica-Kügelchen. Jedes Kügelchen hat einen Durchmesser von 3 Mikrometern und trägt mehr als 100 000 kurze DNA-Stränge.
- Im unteren Bild ist eines der Silica-Kügelchen so stark vergrößert, dass man die DNA-Stränge, die es trägt, als weißen Flaum erkennen kann.

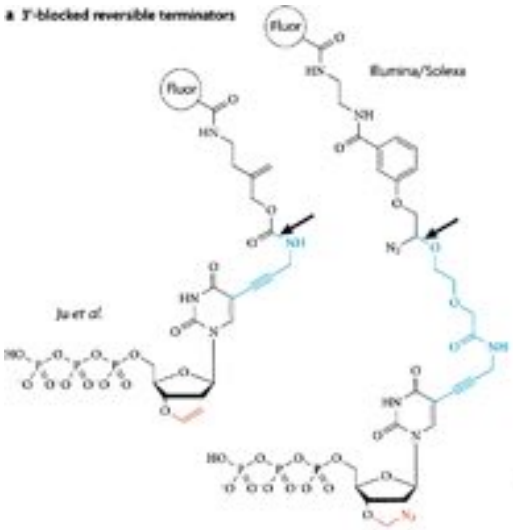


Illumina Sequenzierung

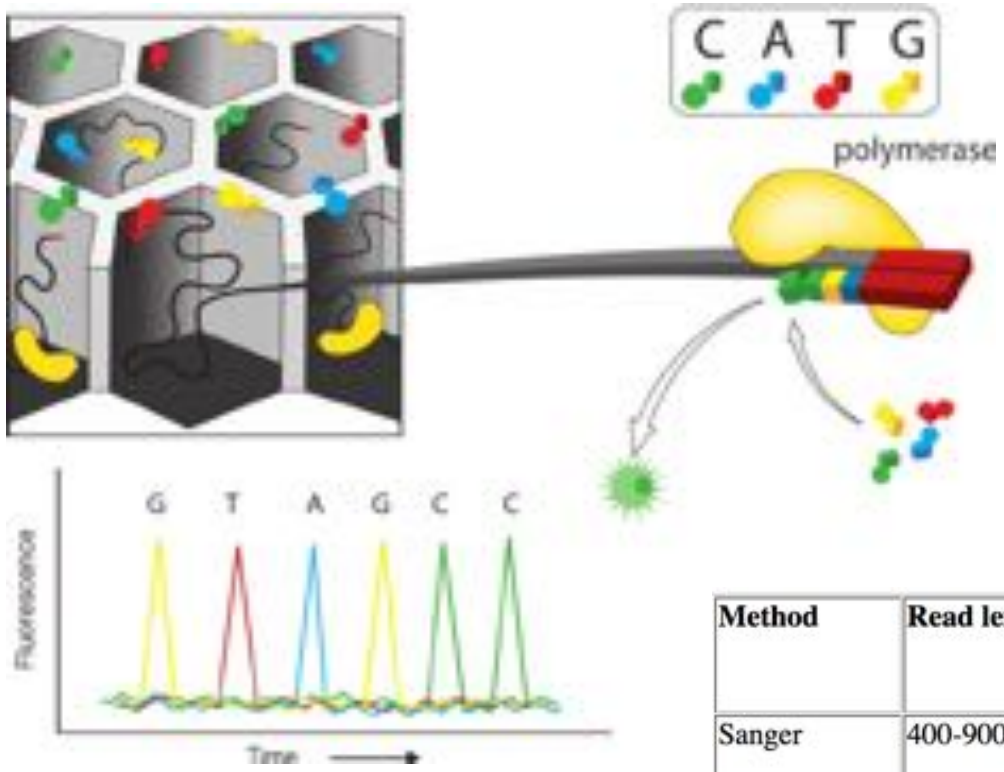


Mit der Illumina Technik werden kurze ca. 50-100 Basen Sequenzen bestimmt. Mehere 100 Millionen „reads“ werden pro Lauf über Nacht generiert. Das menschliche Genom hat ca 3 Milliarden Basenpaare.

Man kann z.B. auch nur die mRNA sequenzieren...



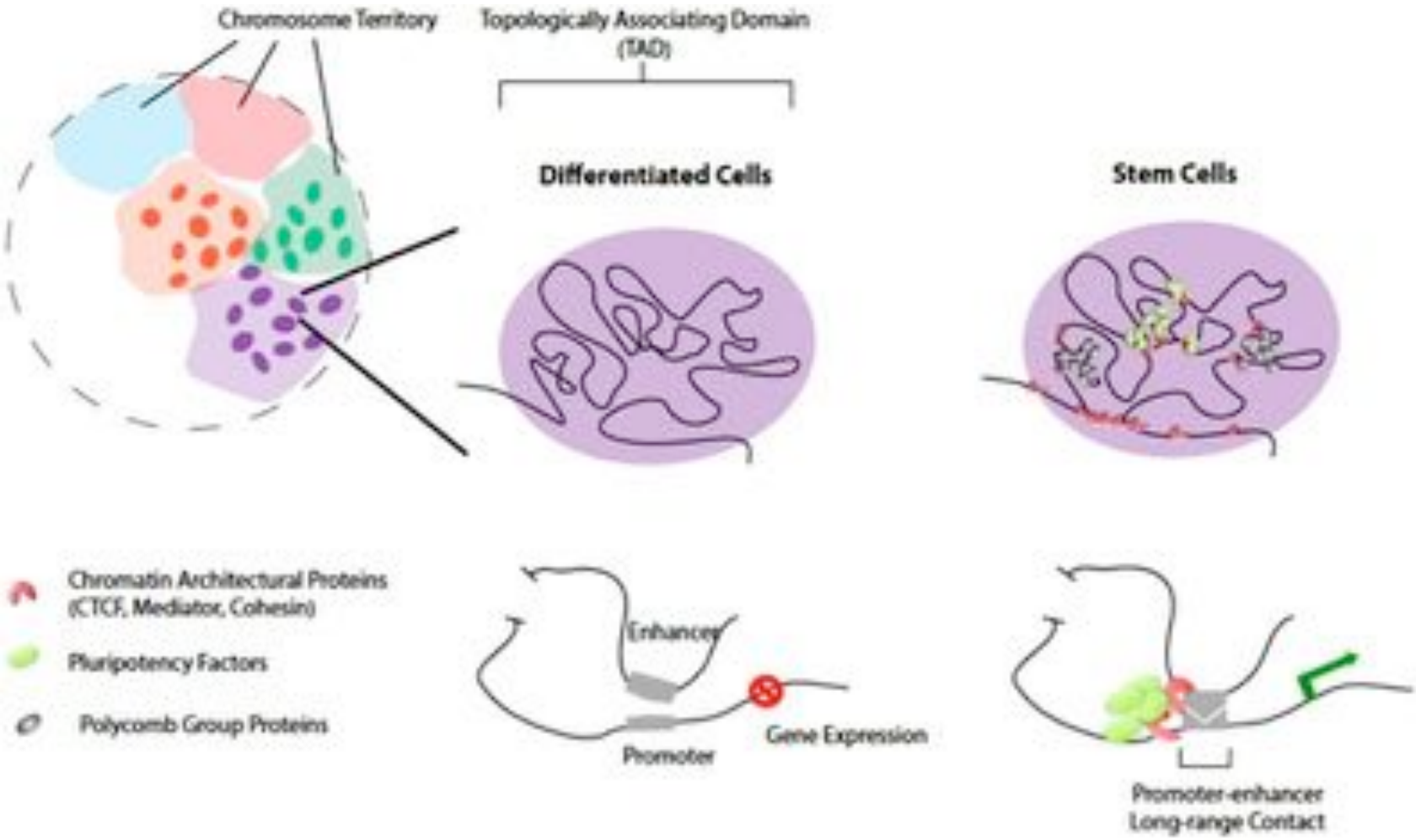
Pac-Bio Sequenzierung



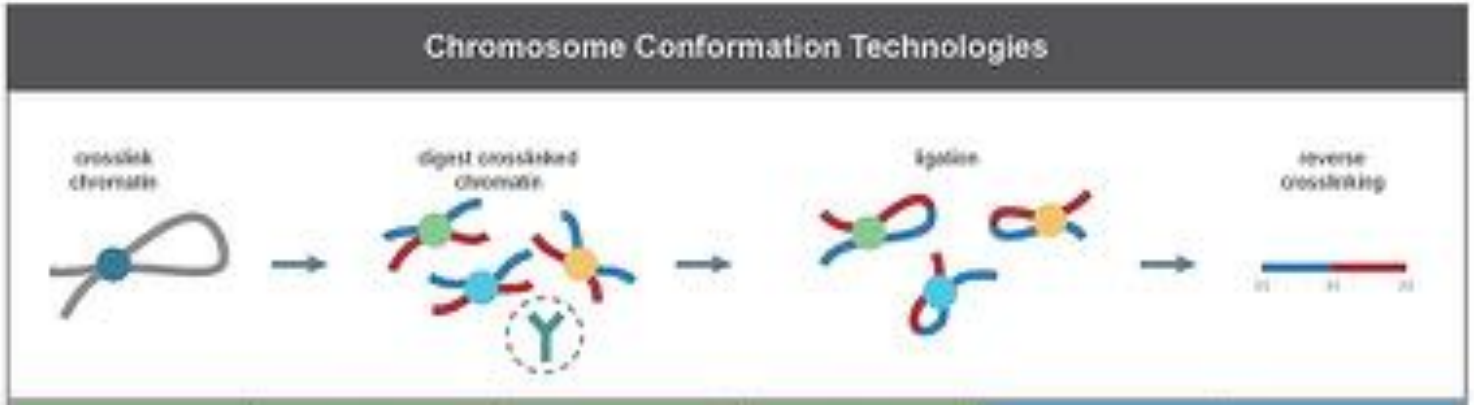
Mit der PacBio: Single molecule real time sequencing (SMRT)

Method	Read length	Accuracy	Reads per run	Time per run	Cost per 1million bases (in US$)
Sanger	400-900 bp	99.9%	N/A	20 minutes to 3 hours	$ 2400
454	700 bp	99.9%	1 million	24 hours	$ 10
Illumina	50-300 bp	99.9%	Up to 6 billion	1 to 11 days	$ 0.05-$ 0.15
ABI/ SOLiD	35- 50 bp	99.9%	1.2-1.4 billion	1-2 weeks	$ 0.13
SMRT (PacBio)	10,000 bp to 15,000 bp	85.0%	50,000 per SMRT cell	30 minutes to 4 hours	$ 0.13 - $ 0.60

Topologisch assoziierte Domänen: TAD



Verpackung der DNA

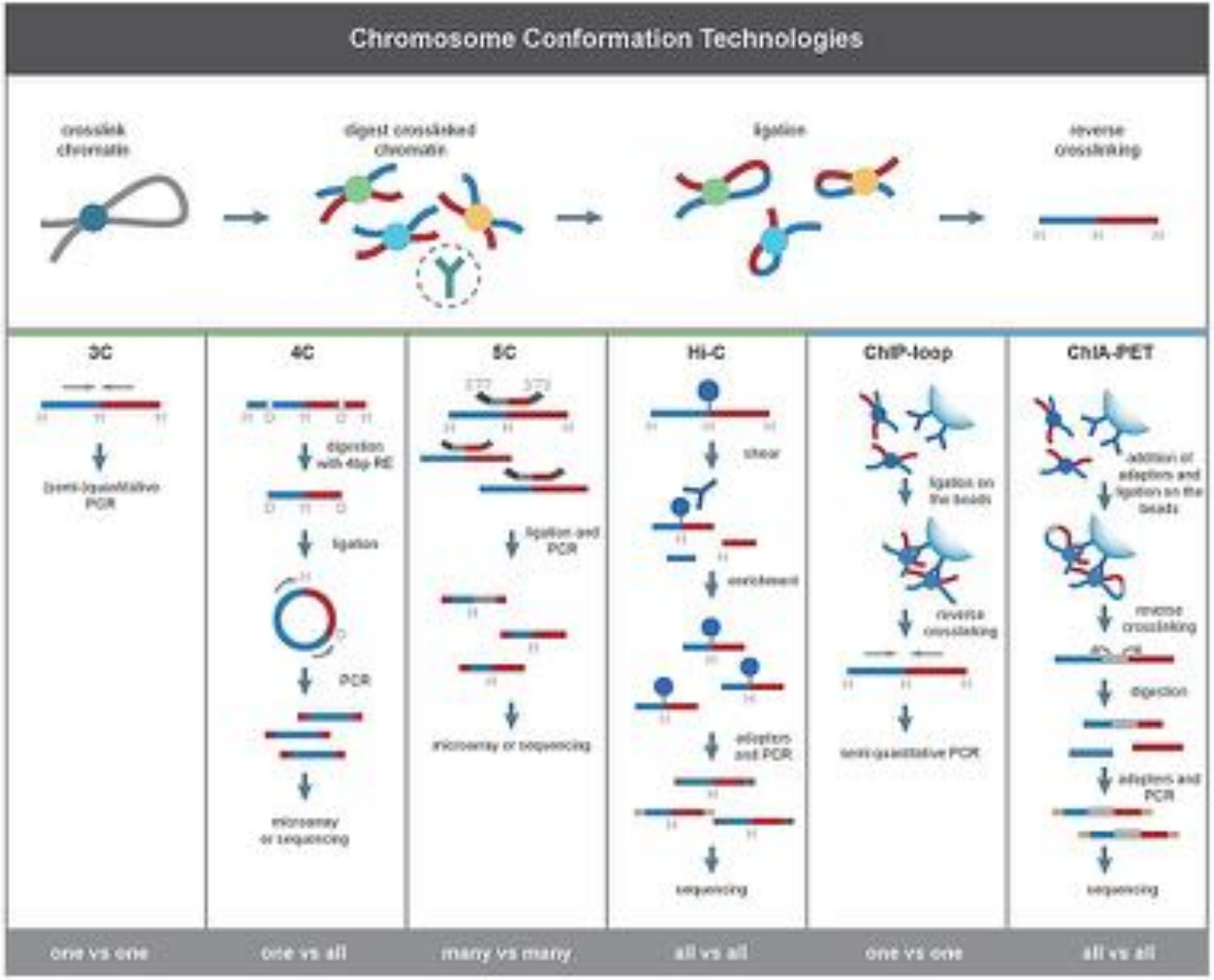


Chromosome confirmation capture **3C**

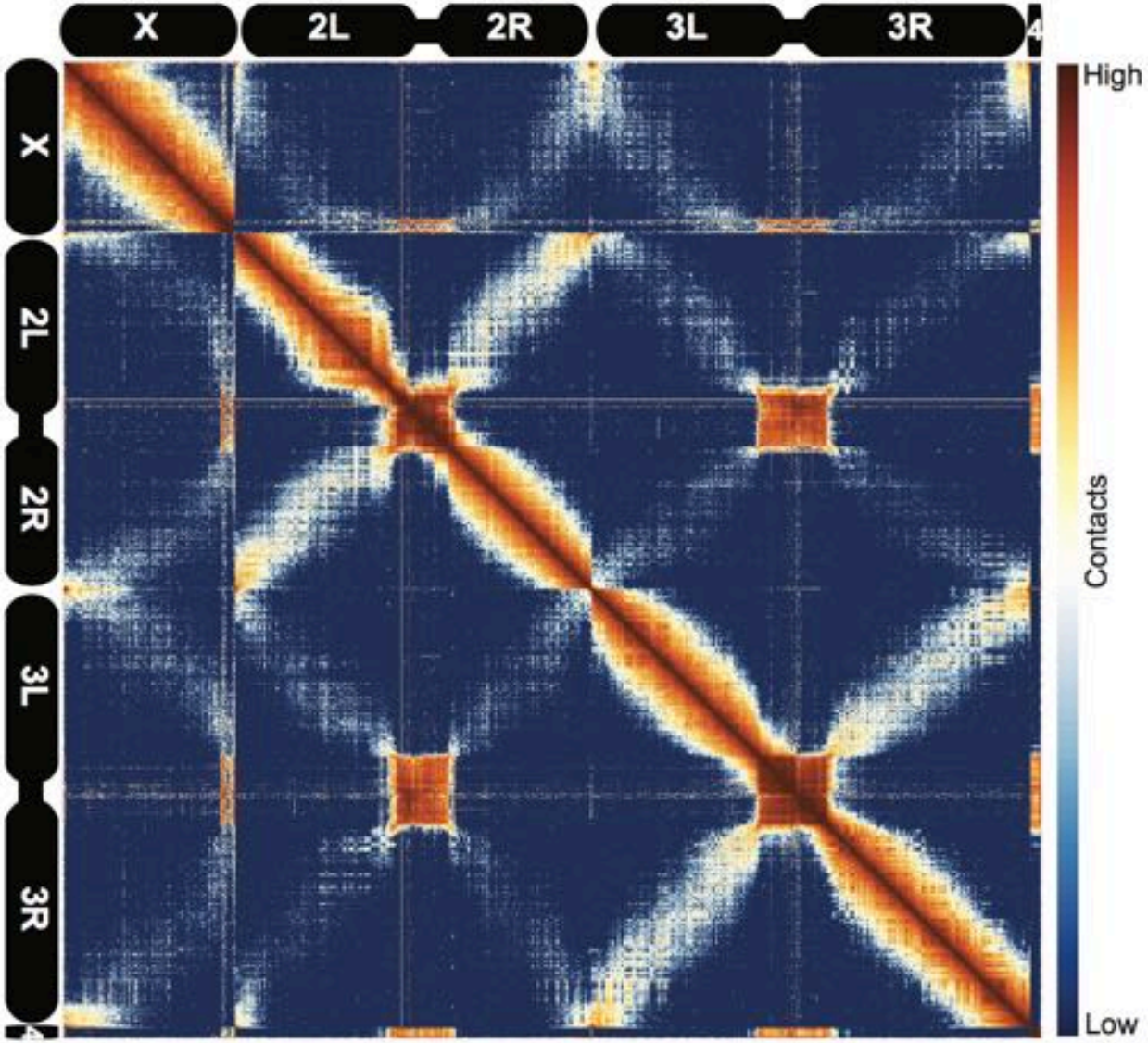
High-throughput sequencing on 3C **Hi-C**
Chromosome conformation capture-on-chip **4C**
Chromosome conformation capture carbon copy **5C**

ChIP-loop 3C und ChIP-seq
ChIA-PET (chromatin interaction analysis by paired-end tag) Hi-C und ChIP-seq

Verpackung der DNA

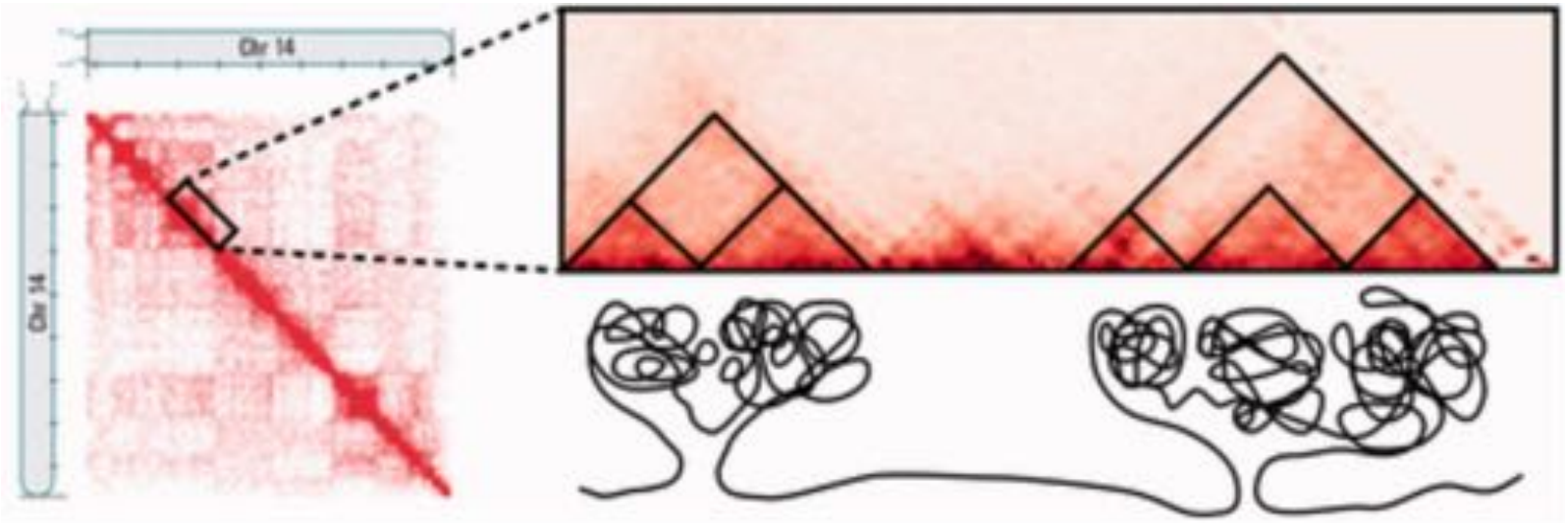


Verpackung der DNA

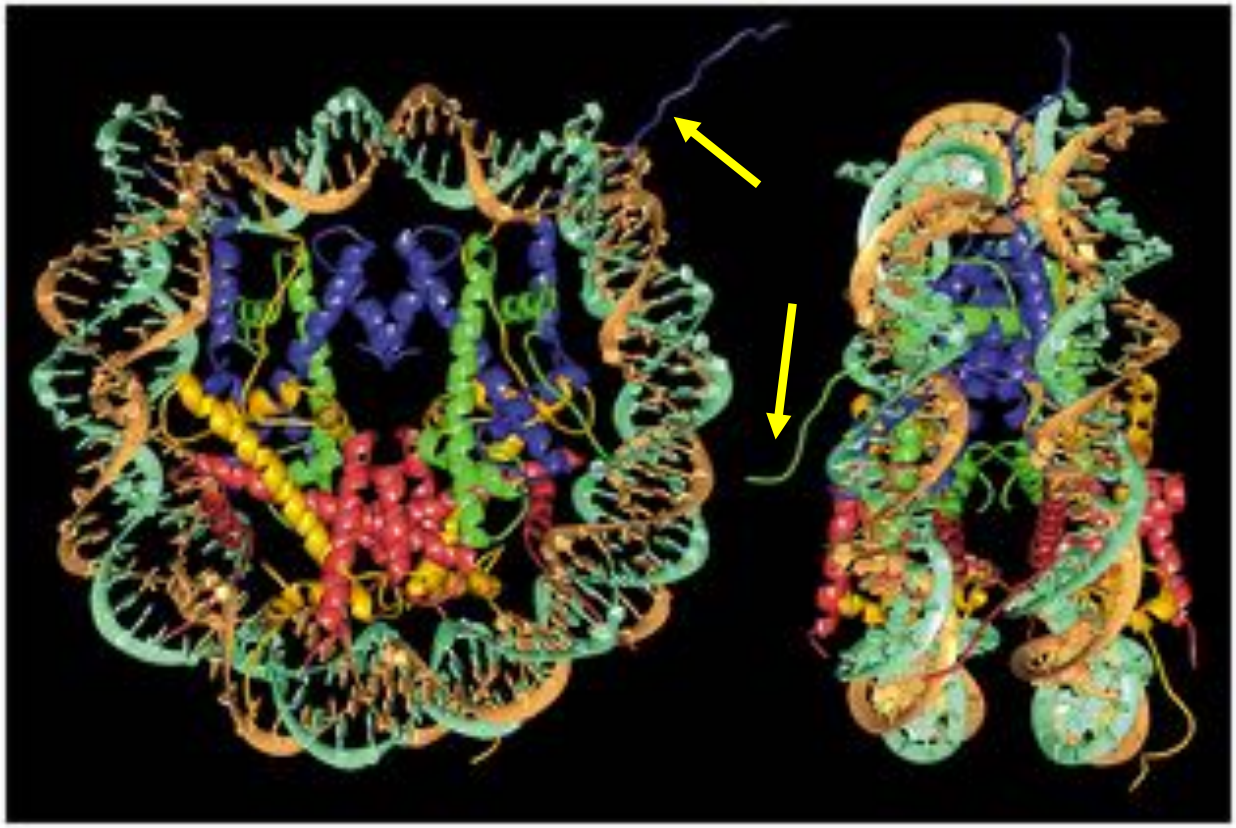


Hi-C Karte der Drosophila Chromosomen mit einer Auflösung von 100kb

Verpackung der DNA



Aufbau der Nucleosomen
Histon-Oktamer 2x (H2A, H2B, H3, H4)



Sehr stark konserviert.
Erbse Kuh 2/102 AS des H4 sind unterschiedlich.

N-terminale Bereiche sind draußen!

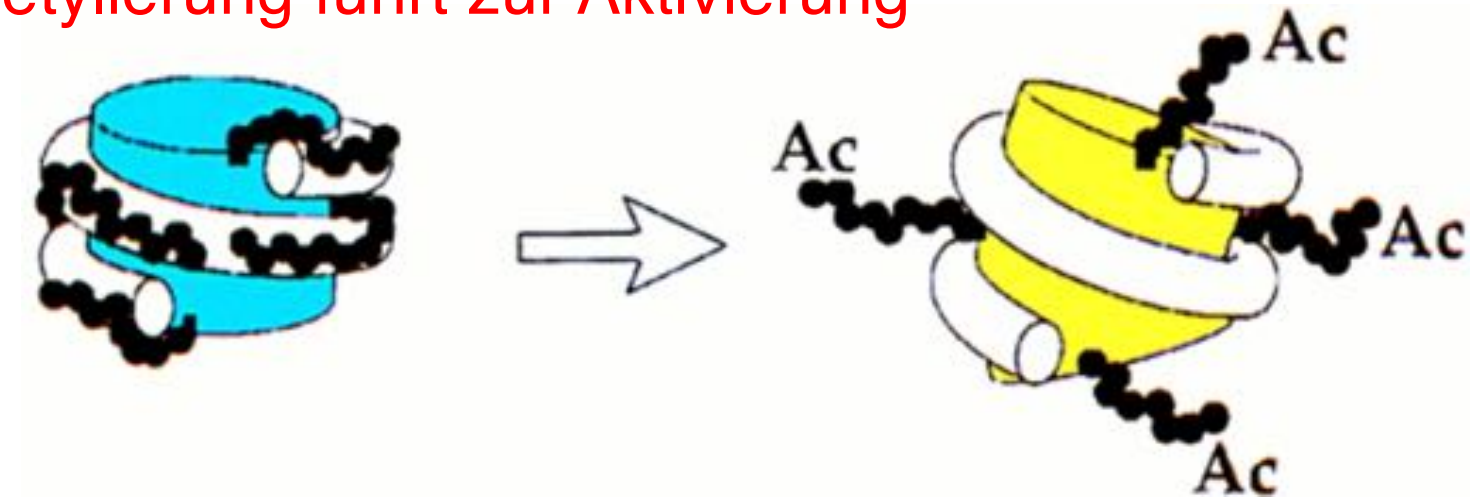
146 bp DNA,
variabler Spacer 20-55 bp

H1 verbindet Nukleosomen

Histonvarianten mit besonderer Funktion: CENP-A (Centromer), macroH2A (Inaktivierung des X-Chromosoms)

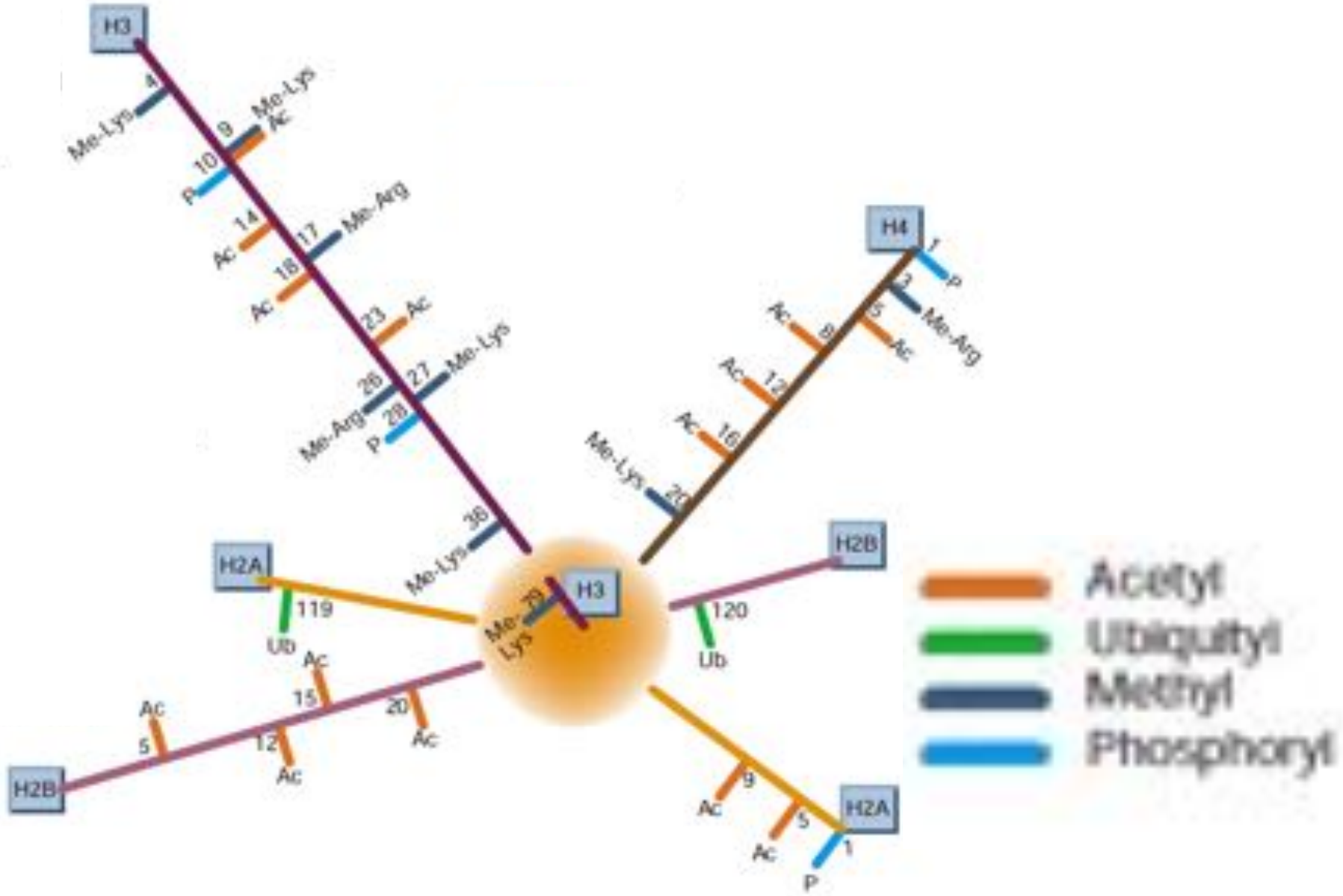
Modifizierung von Histonen führt zur Öffnung oder Kondensation des Chromatins

Acetylierung führt zur Aktivierung

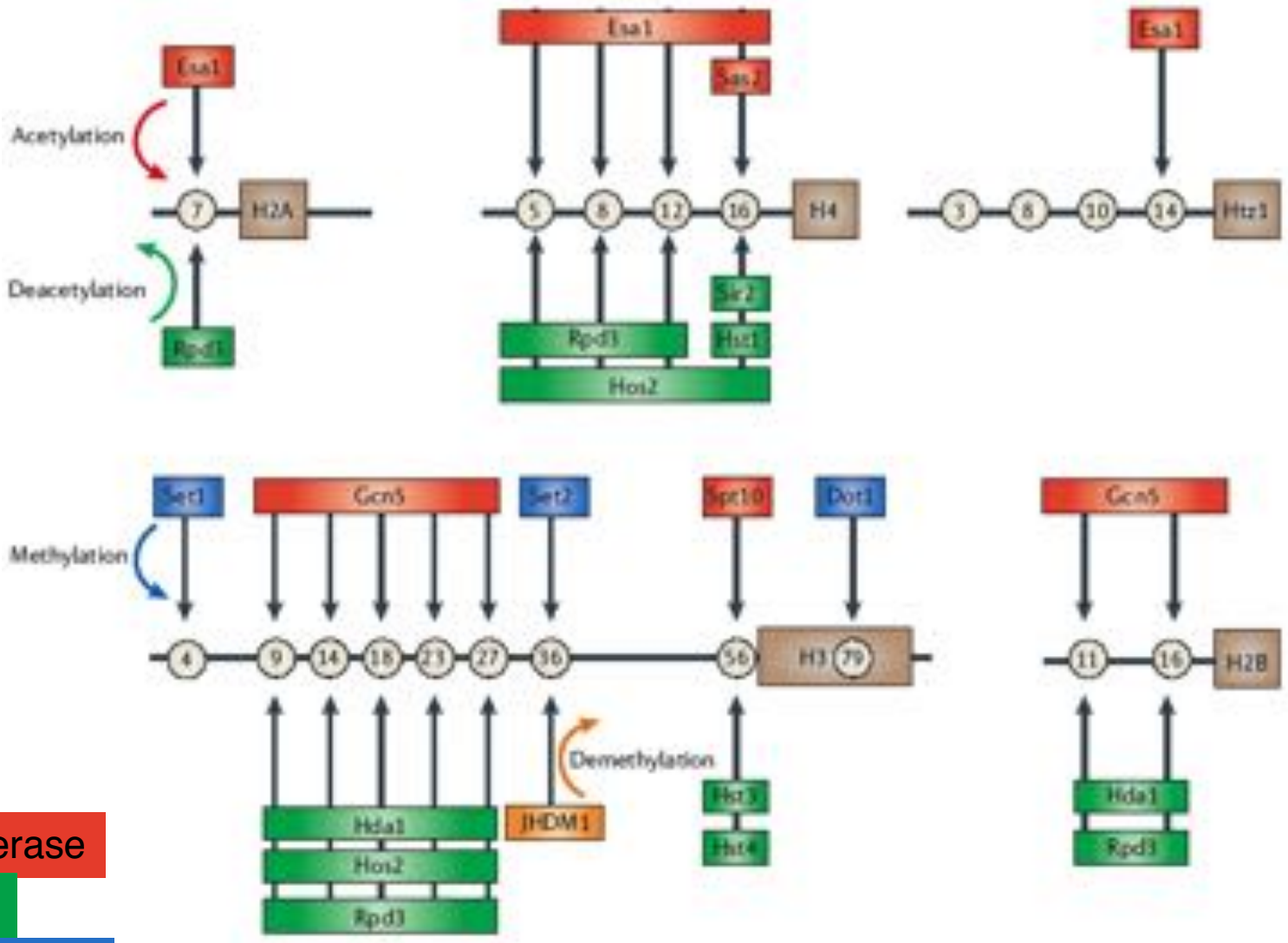


Histon-Acetyltransferase (HAT)	Aktivierung
Histon-Methyltransferase (HMT)	Repression/Aktivierung
Histon-Deacetylase (HDAC)	Repression
Phosphorylierung, Ubiquitinierung	

Der Histon-Code



Der Histon-Code



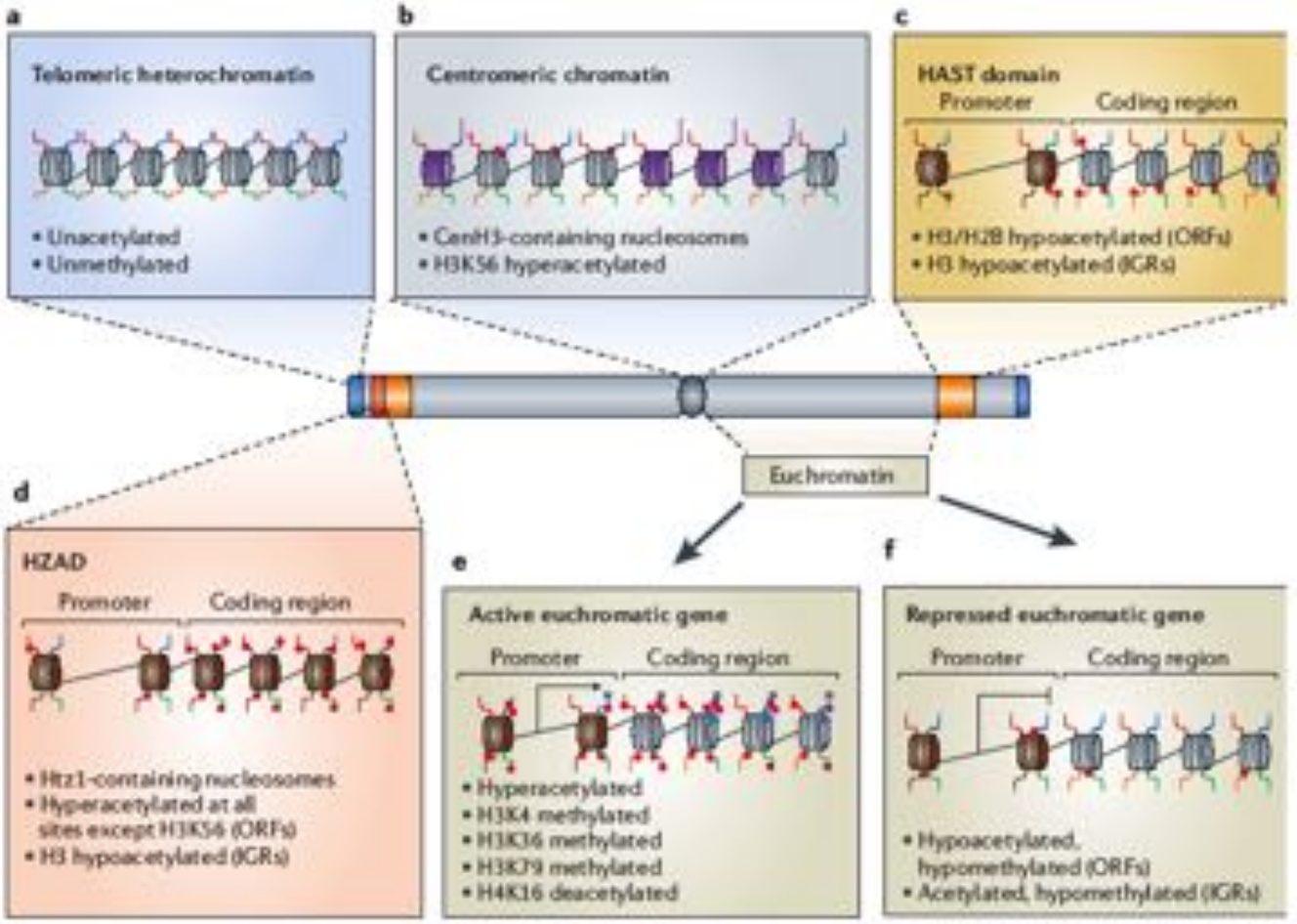
Acetyltransferase

Deacetylase

Methyltransferase

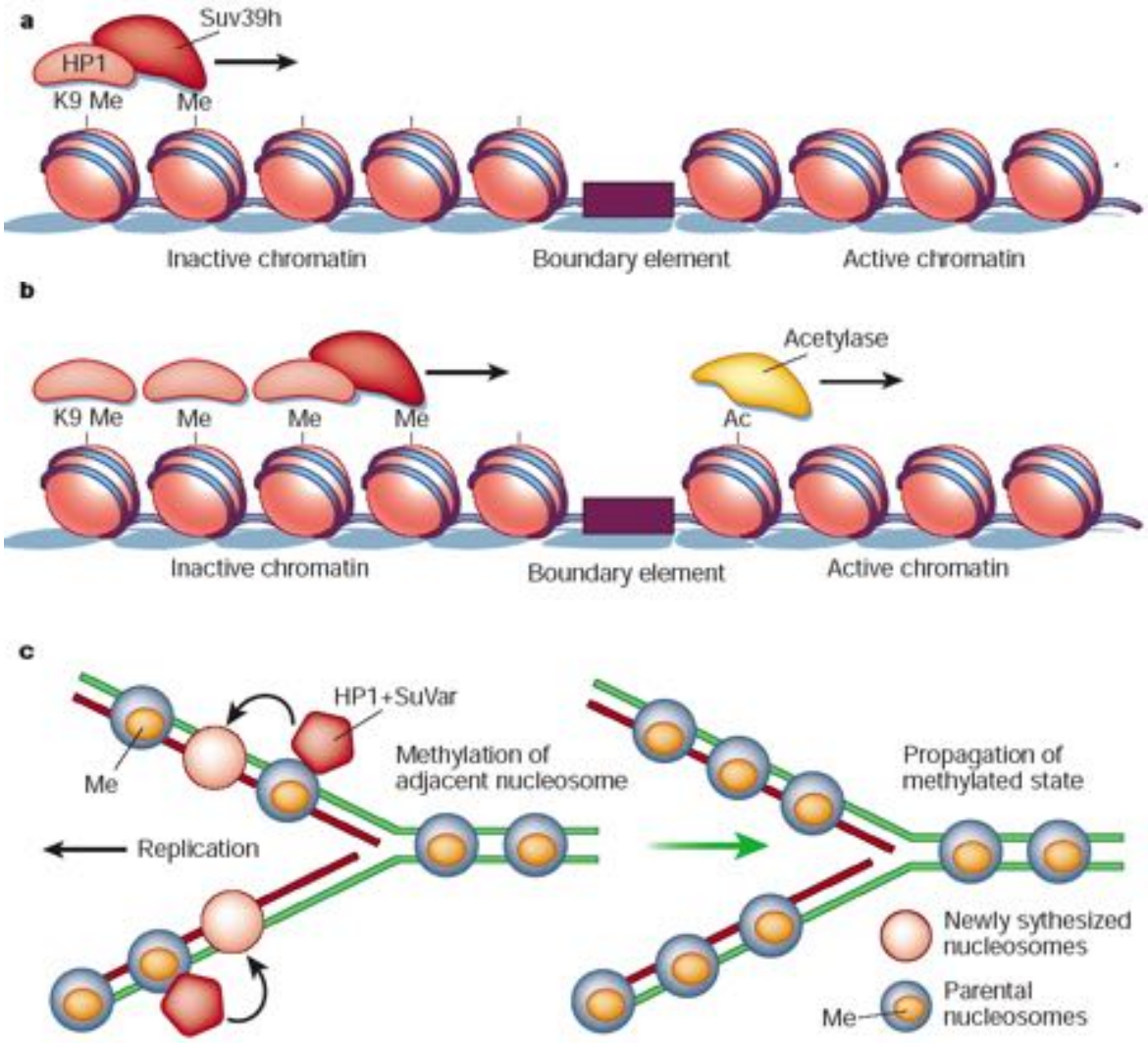
Demethylase

Unterschiedliche Regionen des Chromosoms haben unterschiedliche Histon-Codes

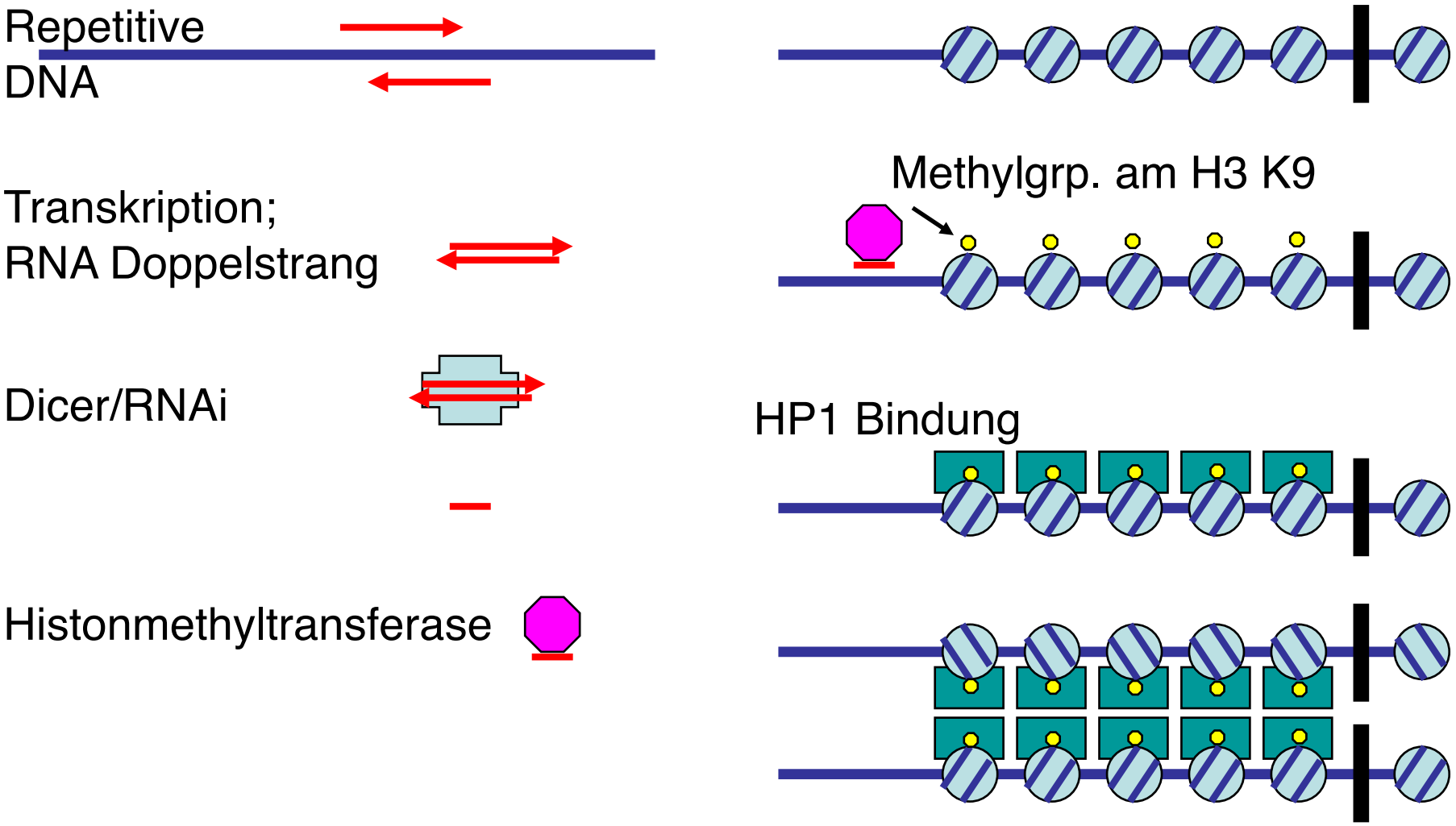


Spezifische Antikörper gegen modifizierte Histone, Chromatin Immunoprecipitation, microarrays

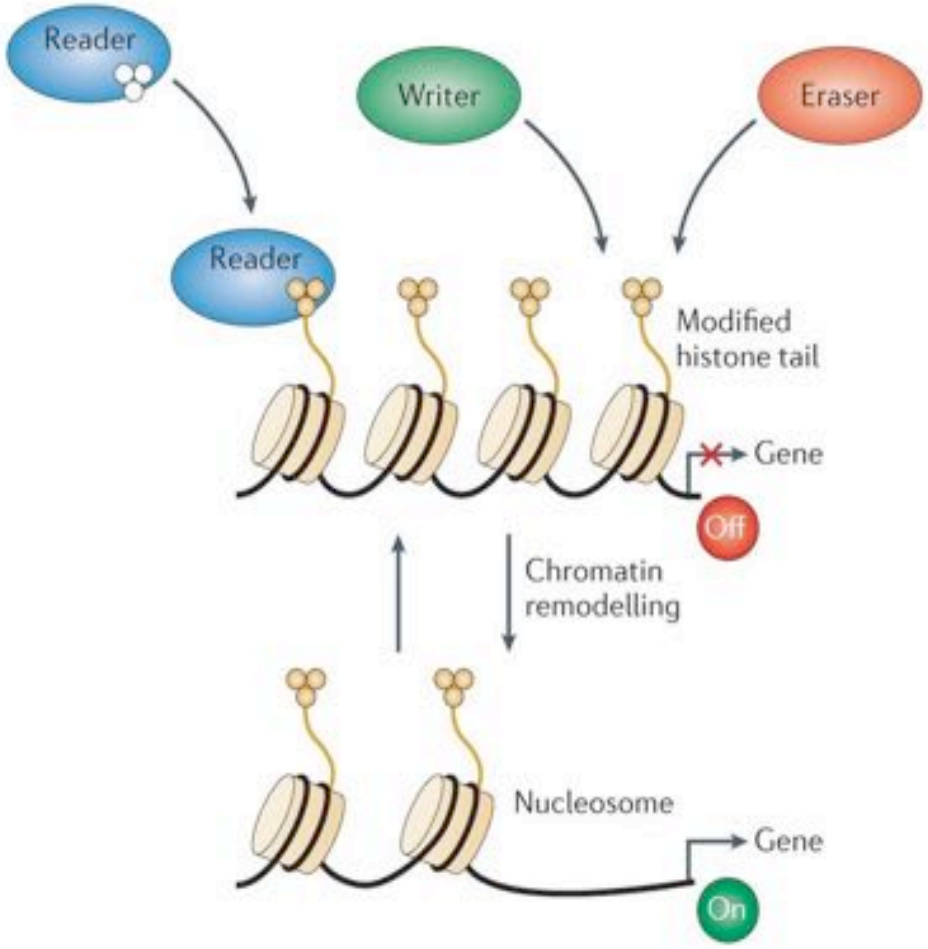
Weitergabe von methylierten bzw. acetylierten Nucleosomen



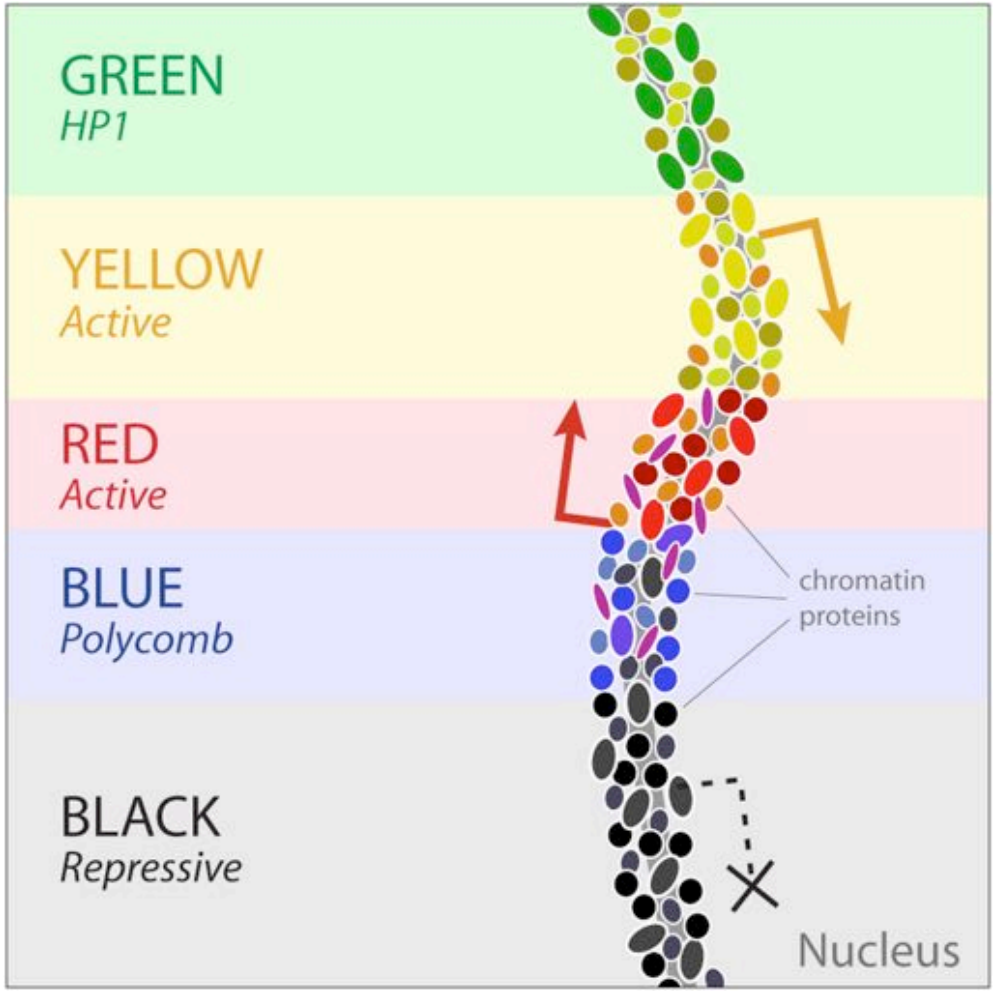
Histon Modifikationen
Induktion der Heterochromatisierung



Drei verschiedene Chromatin – assoziierte Proteine „Reader, Writer und Eraser“

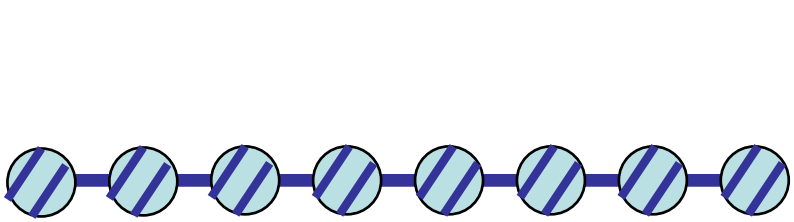


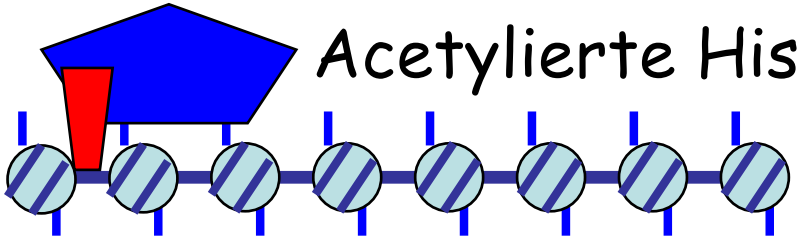
Fünf prinzipiell verschiedene Chromatinarten in Drosophila

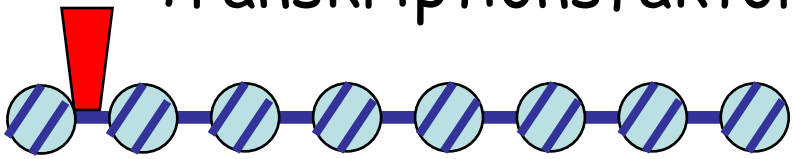


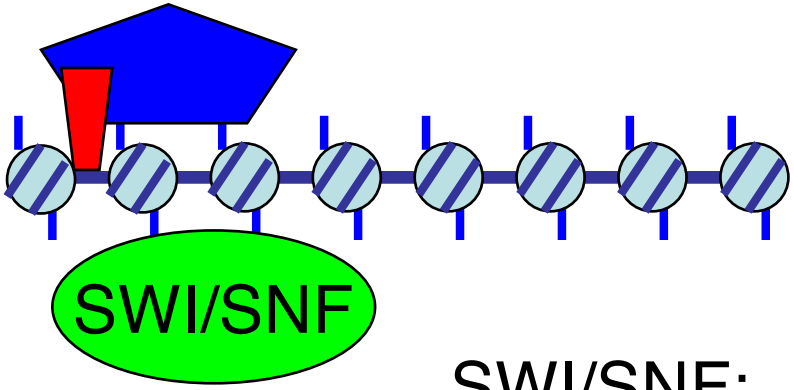
Filion *et al*. Cell 2010

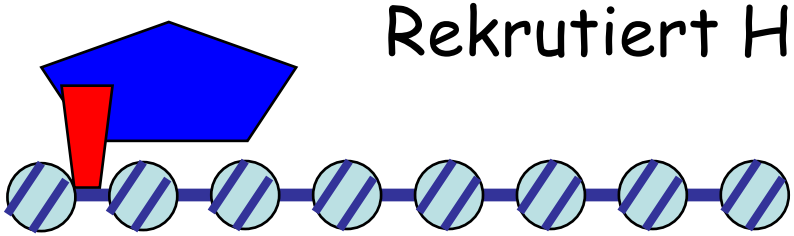
Histon Modifikationen am Promotor







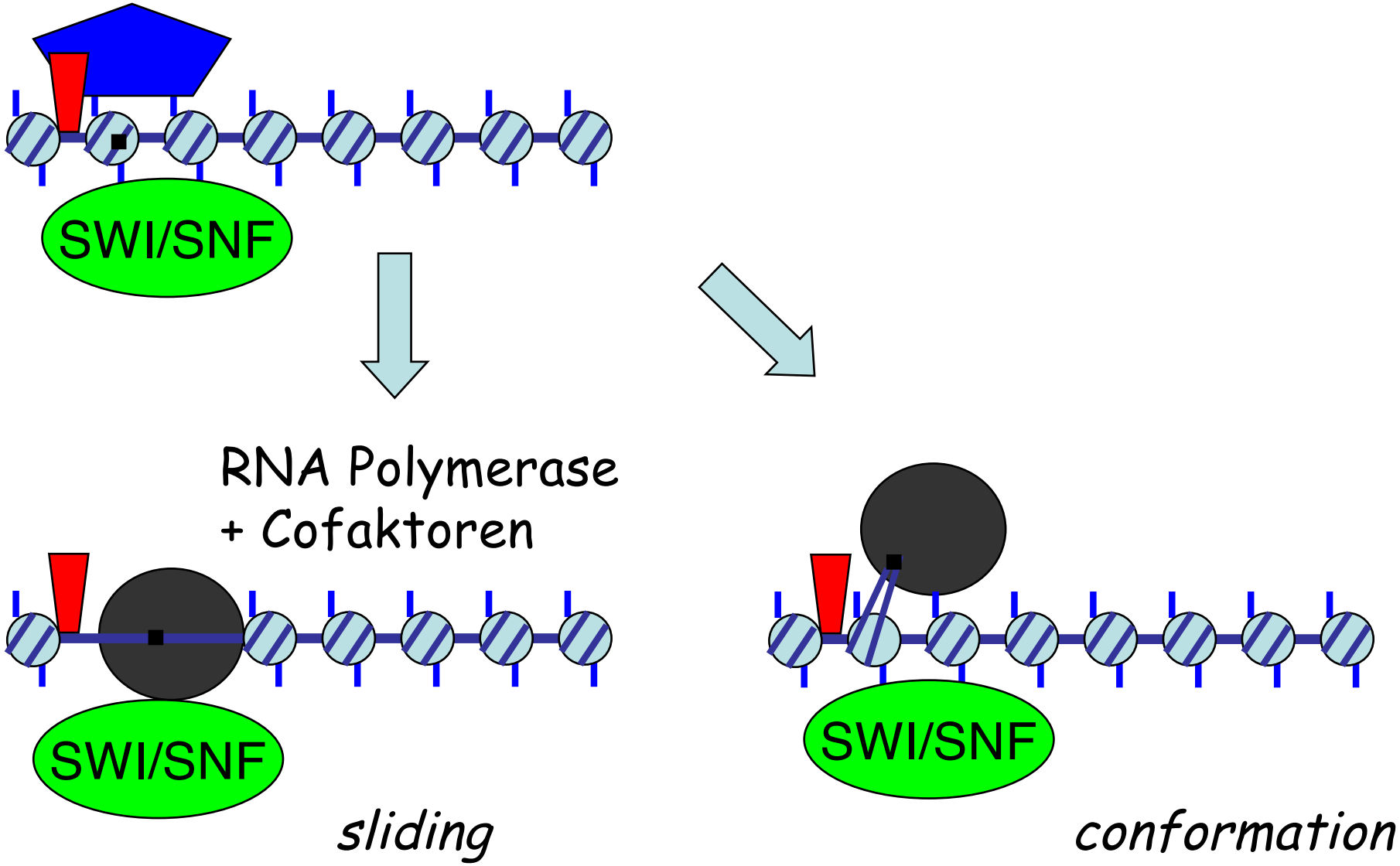




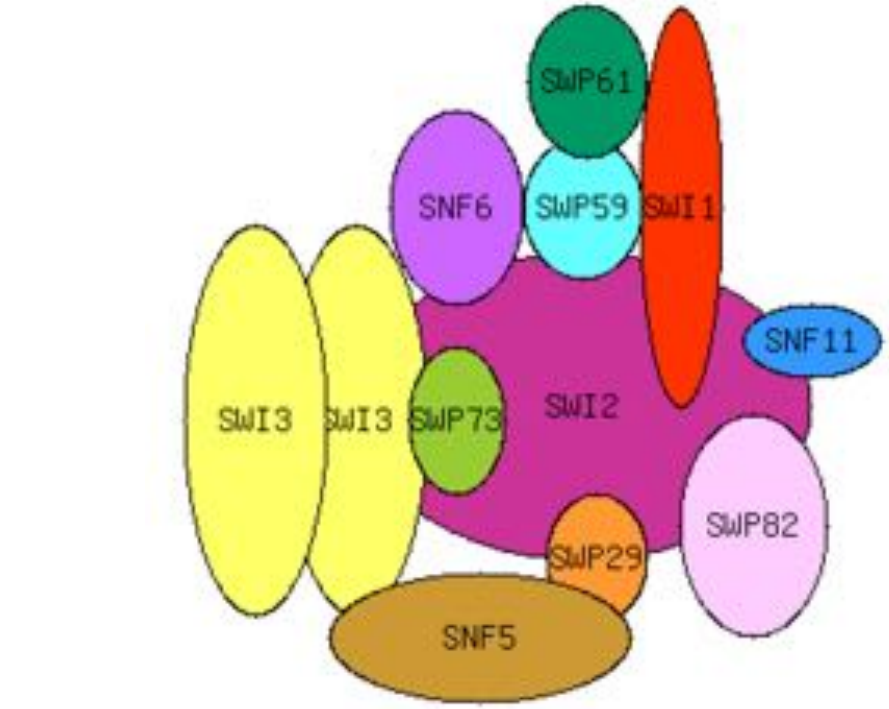
SWI/SNF:
Chromatin remodeling complex

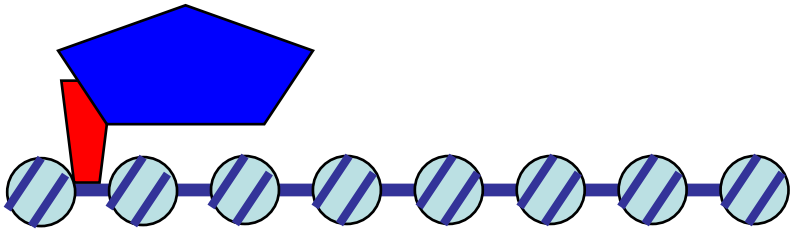
SWI/SNF (SWItch/Sucrose NonFermentable)

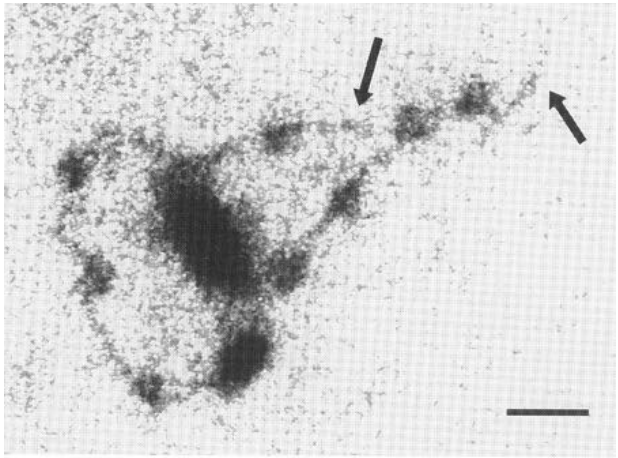
Histon Modifikationen am Promotor II



Der SWI/SNF Komplex besteht aus >12 Untereinheiten



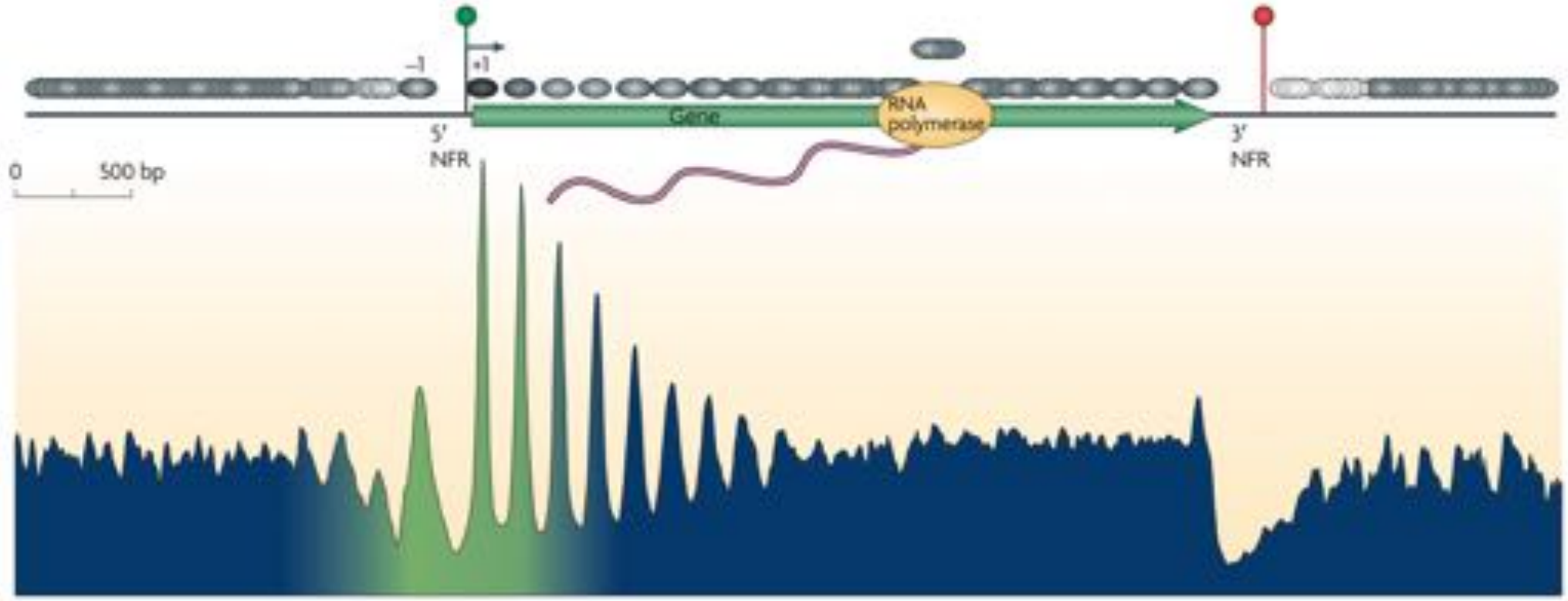




EM-Bild eines SWI/SNF Komplexes an DNA mit Nucleosomen

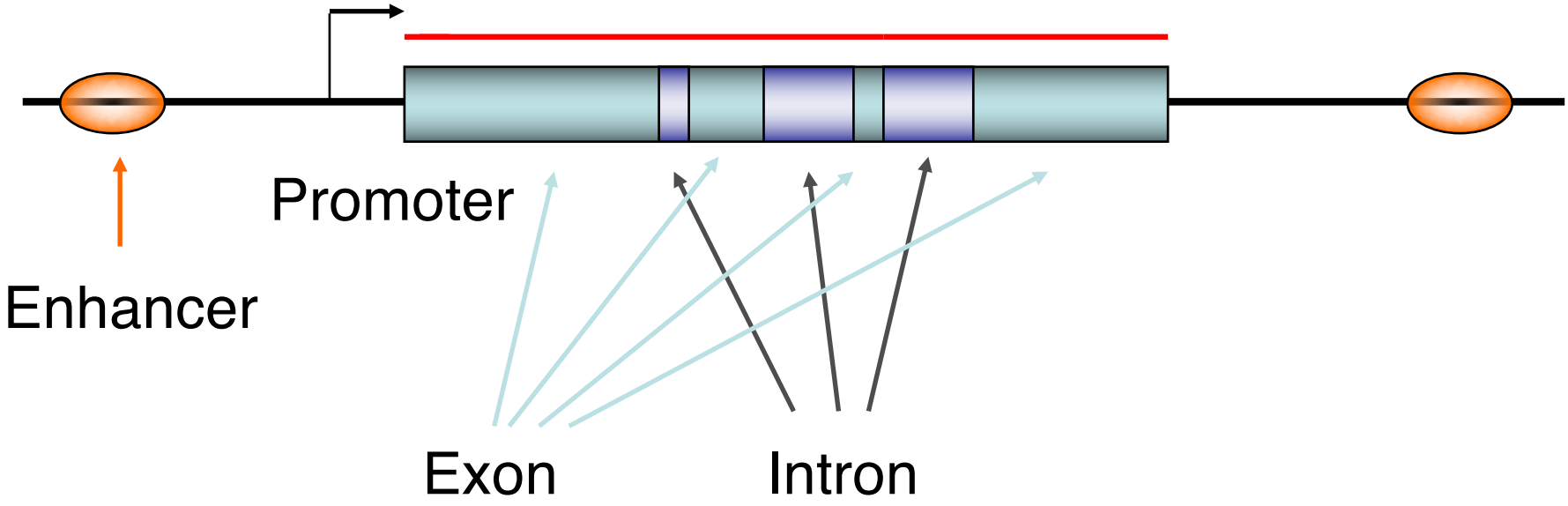
SWItch/Sucrose NonFermentable

Aufbau der Nucleosomen
Histon-Oktamer 2x (H2A, H2B, H3, H4)



CHIP seq

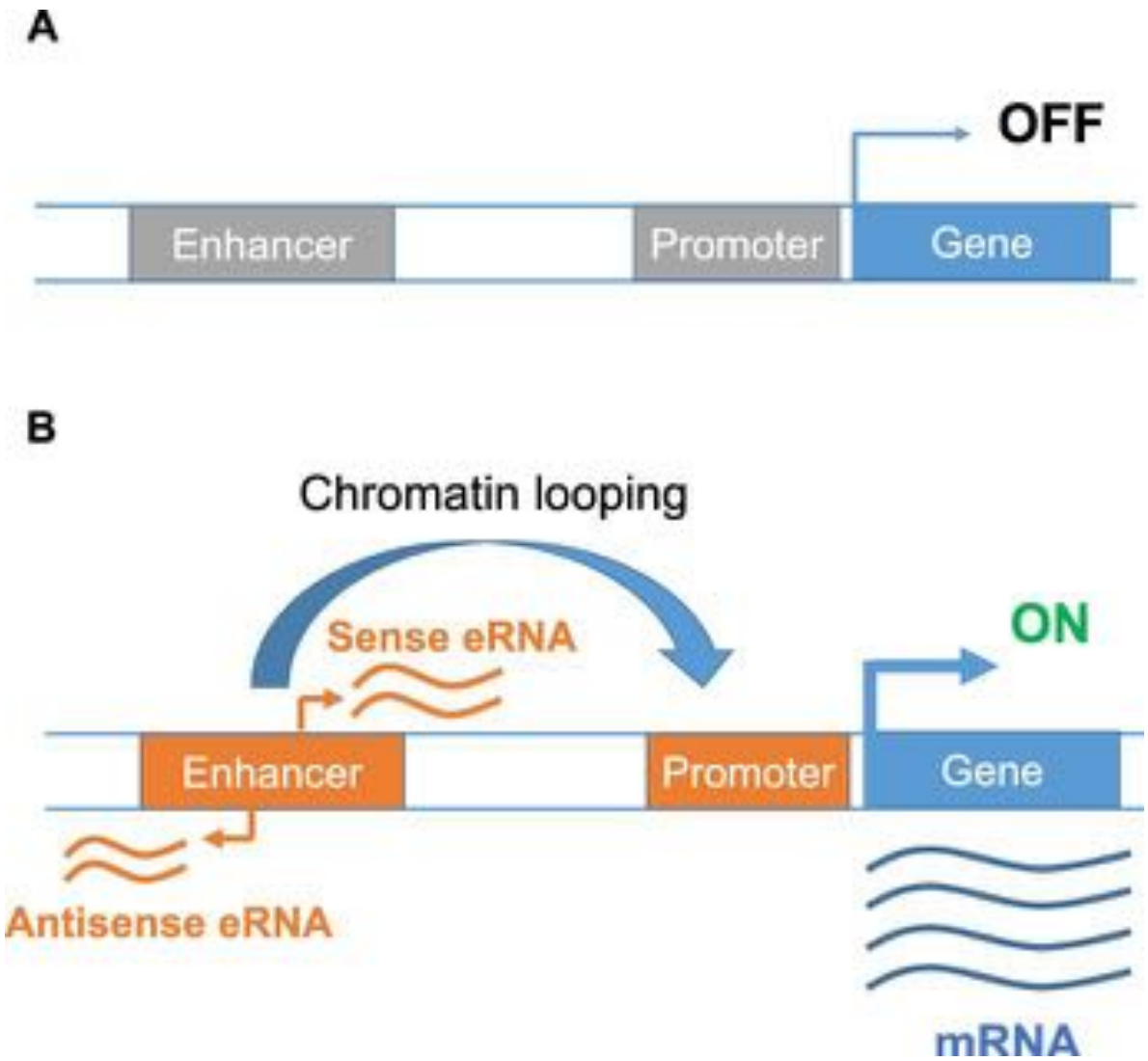
Ein typisches Gen



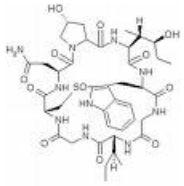
Transkription

Initiation 5‘ cap
Elongation splicing
Termination 3‘ polyA

Ein typisches Gen
aber eRNA!



RNA Polymerasen (Eukaryoten)



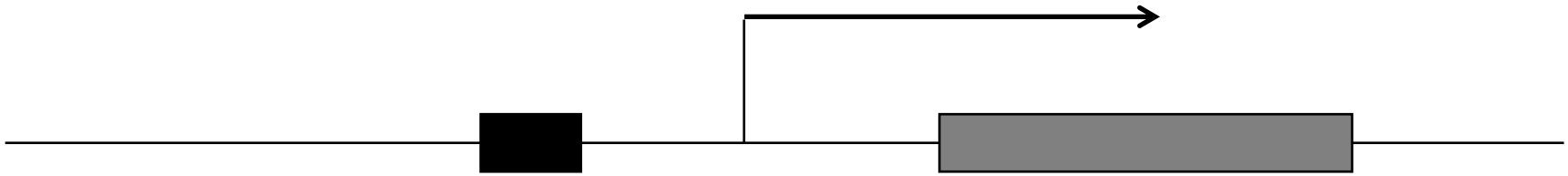
σα-Amanitin

RNA Polymerase I	Im Nucleolus aktiv rRNA (28S-, 18S- und 5,8S)	
RNA Polymerase II	proteinkodierende Gene	++++
RNA Polymerase III	Gene für: 5S-rRNA, tRNAs und andere "kleine" RNAs	++

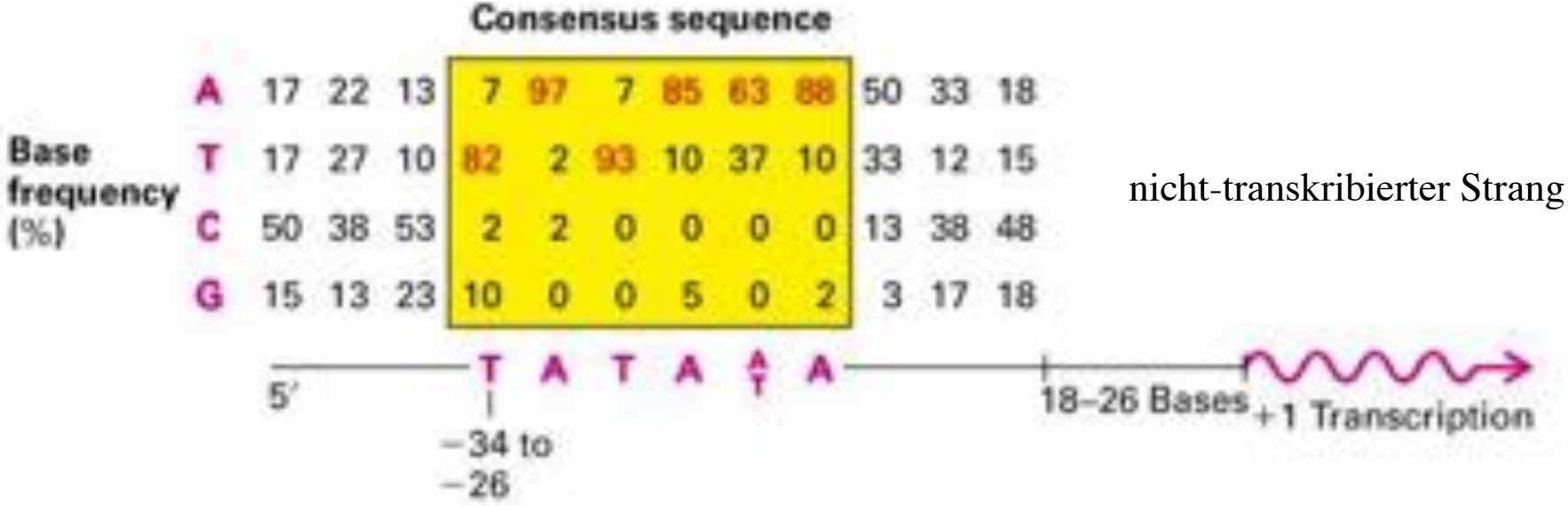
RNAP II ist ein großer Proteinkomplex aus 12 Untereinheiten.

RNAP II kann den Promoter der Zielgene nicht erkennen!

Struktur von RNA Polymerase II Promotoren



TATA-Box



nicht-transkribierter Strang

Cofaktoren der RNA Polymerase II

General transcription factors GTF

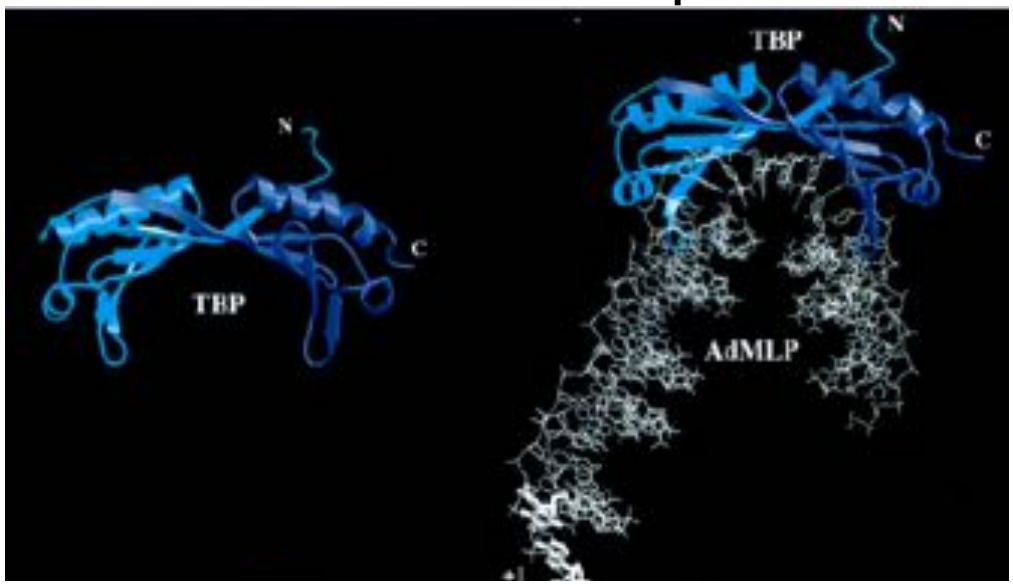
Bildung des Preinitiationskomplexes
DNA, RNAP II und 5 GTFs

Cofaktoren der RNA Polymerase II

General transcription factors GTF

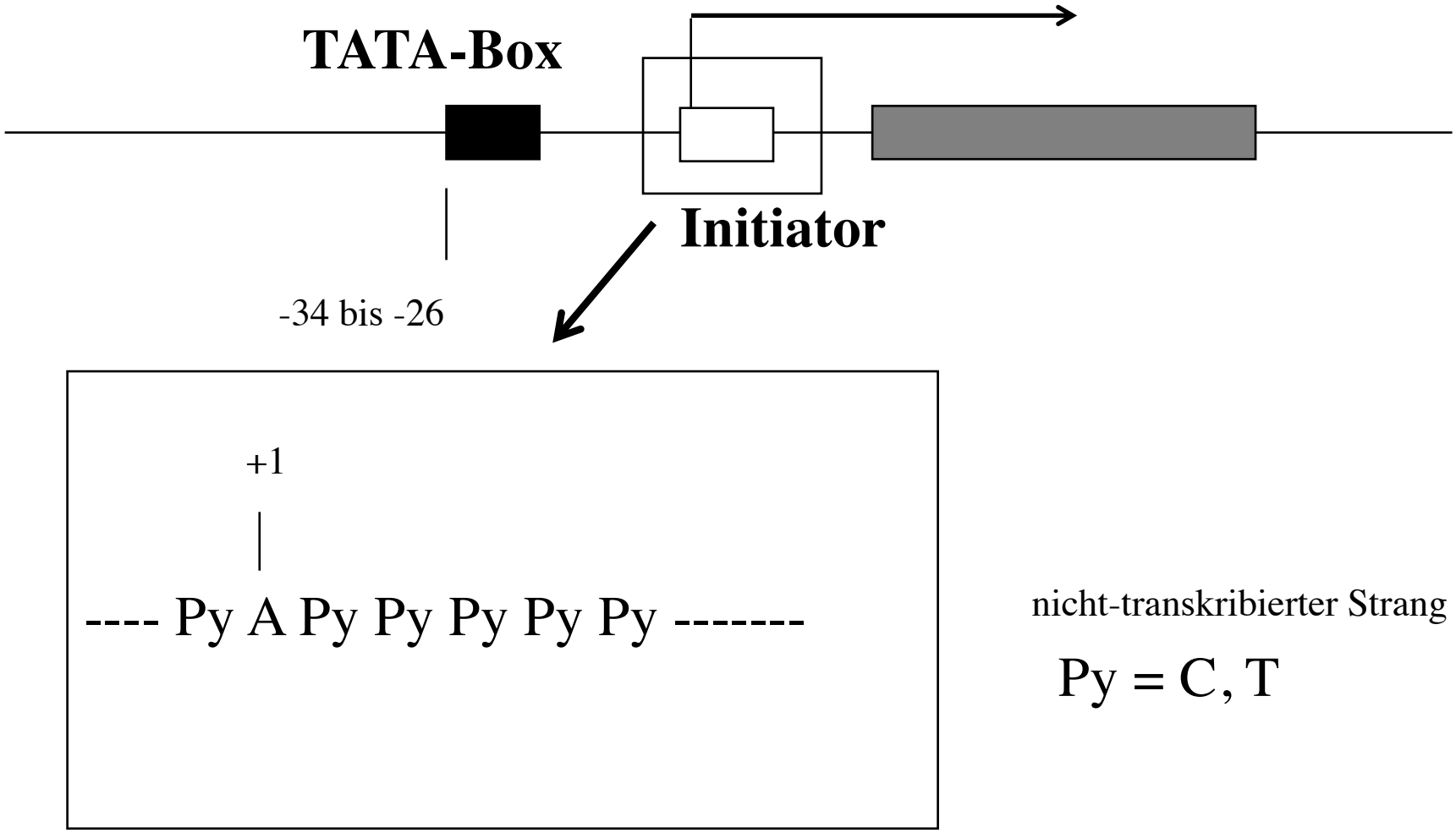
Bildung des Preinitiationskomplexes
DNA, RNAP II und 5 GTFs

Start ist Bindung von TBP des TFIID Komplexes an die TATA Box.



DNA kann „geknickt“ werden.

Struktur von RNA Polymerase II Promotoren

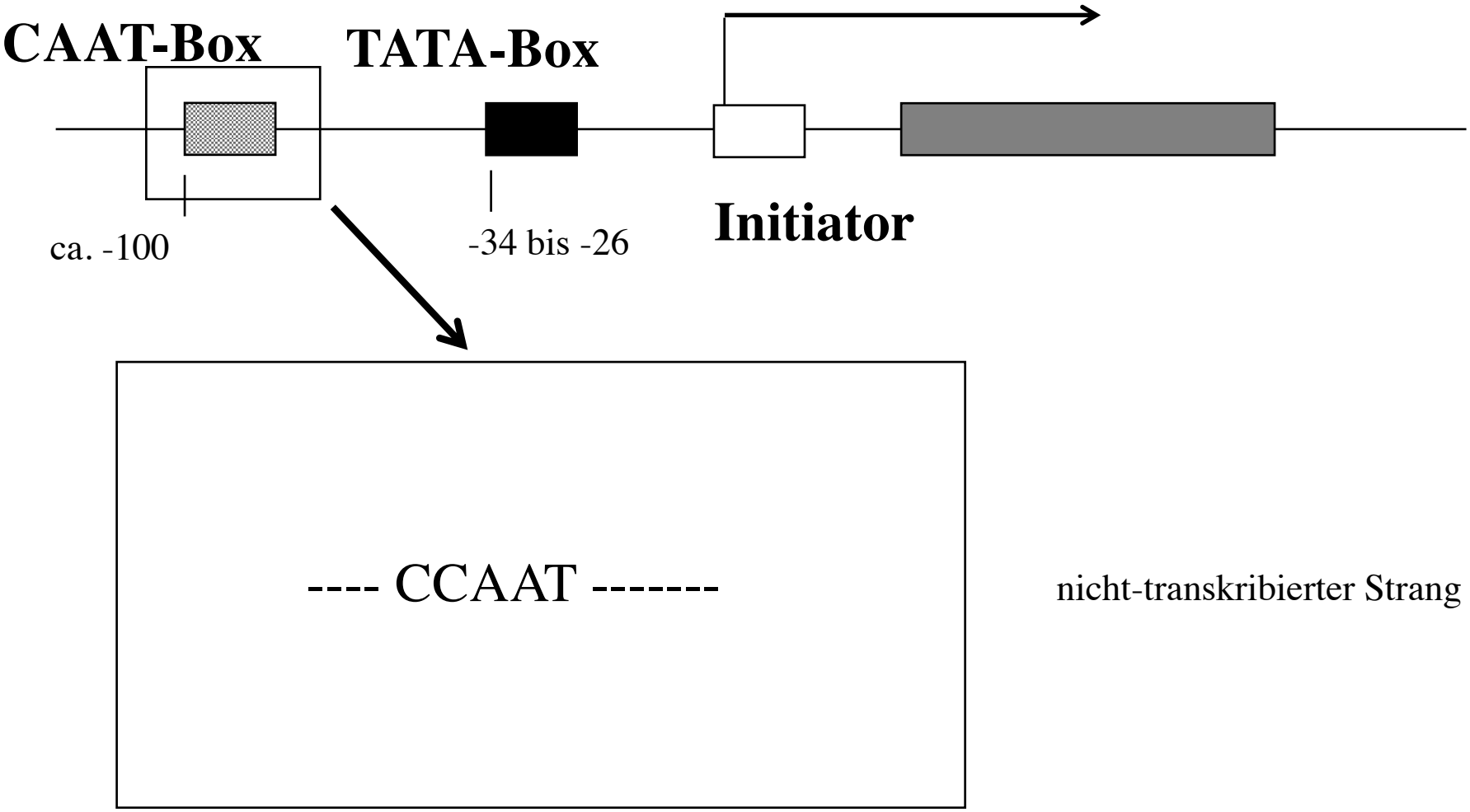


nicht-transkribierter Strang

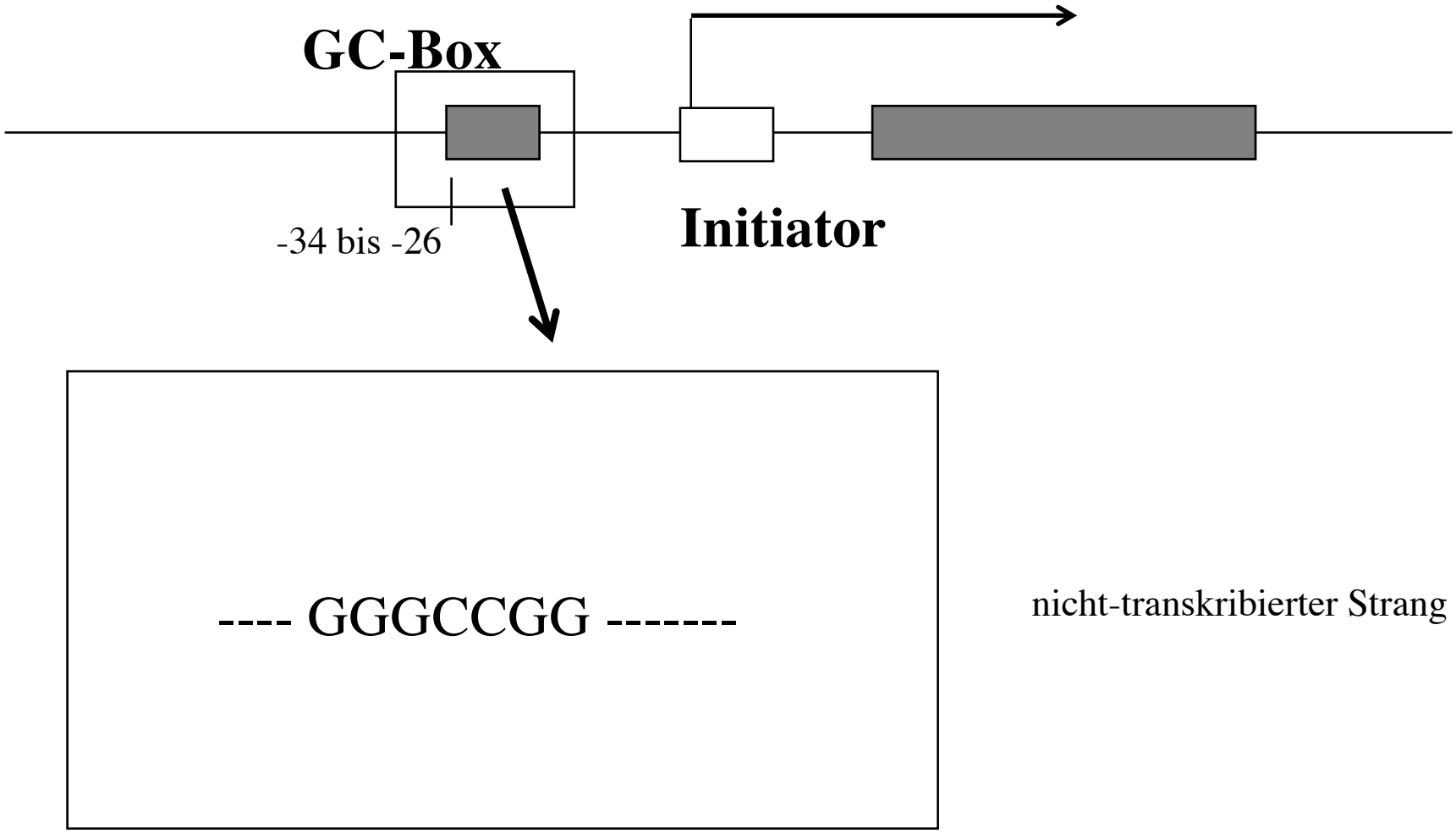
Py = C, T

Diese Elemente finden sich bei **<u>regulierten Genen</u>**

Struktur von RNA Polymerase II Promotoren



Struktur von RNA Polymerase II Promotoren



Haushaltsgene (konstitutiv aktive Gene) enhalten andere Sequenzelemente, wie z.B. eine GC-Box

Cofaktoren der RNA Polymerase II

General transcription factors GTF

Bildung des Preinitiationskomplexes
DNA, RNAP II und 5 GTFs

Start ist Bindung von TBP des TFIID Komplexes an die TATA Box.

Rekrutierung von TBIIB

Unphosphorylierte RNAP II, TFIIF, TFIIE und TFIIH

Aufbau der GTFs

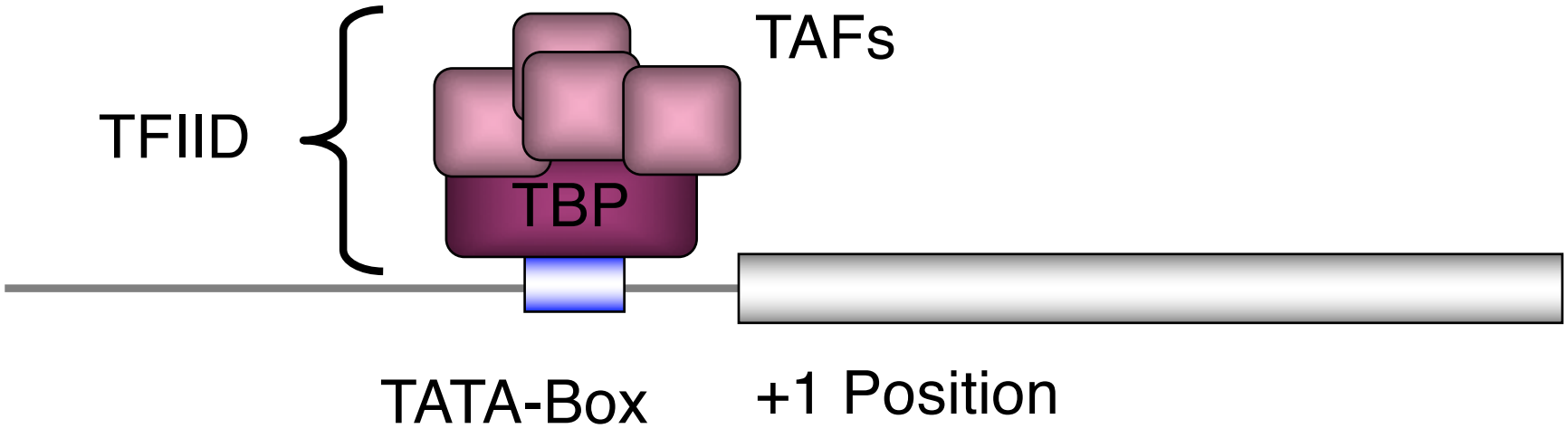
TFII D	=	TBP plus 10 TBP-associated proteins (TAFs)
TFII B	=	1 Protein
TFII F	=	Heterotetramer, 2 große (TFII Fa) und 2 kleine (TFII Fb) SU
TFII E	=	Heterotetramer ähnlich zu TFII F
TFII H	=	größter GTF, 9 Untereinheiten, MW entspricht RNAP II Enzymatische Aktivität (2 Helikasen, 1 Kinase)

Initiation der Transkription durch Polymerase II

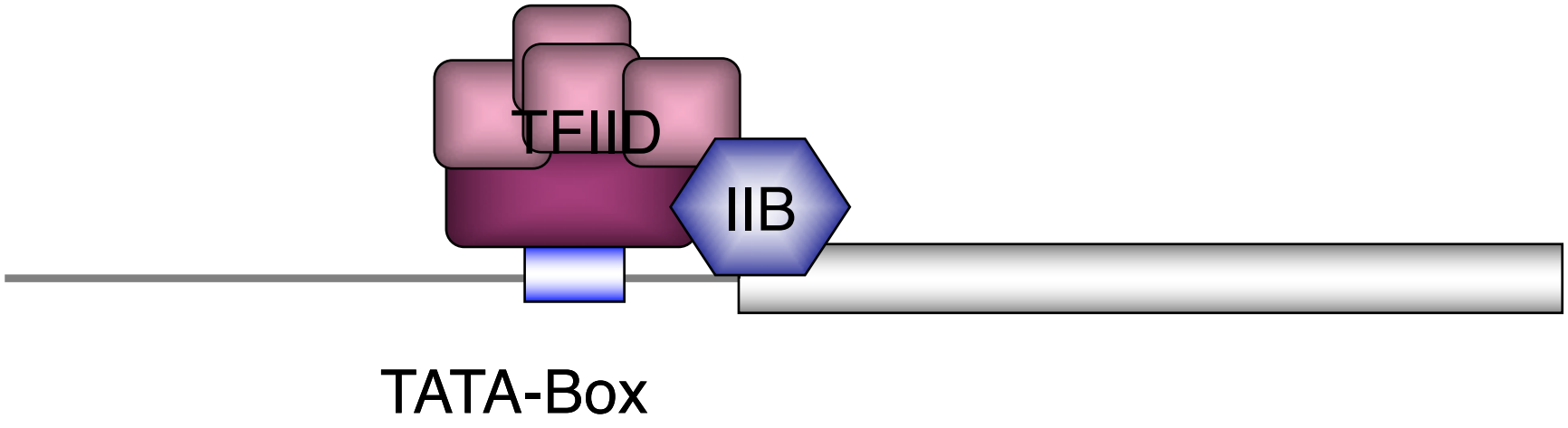
Zunächst wird schrittweise ein Initiationskomplex aufgebaut:

Das TATA-Bindeprotein (TBP) bindet an die TATA-Box

TFIID besteht aus TBP und den
TBP-assoziierten Faktoren (TAFs)

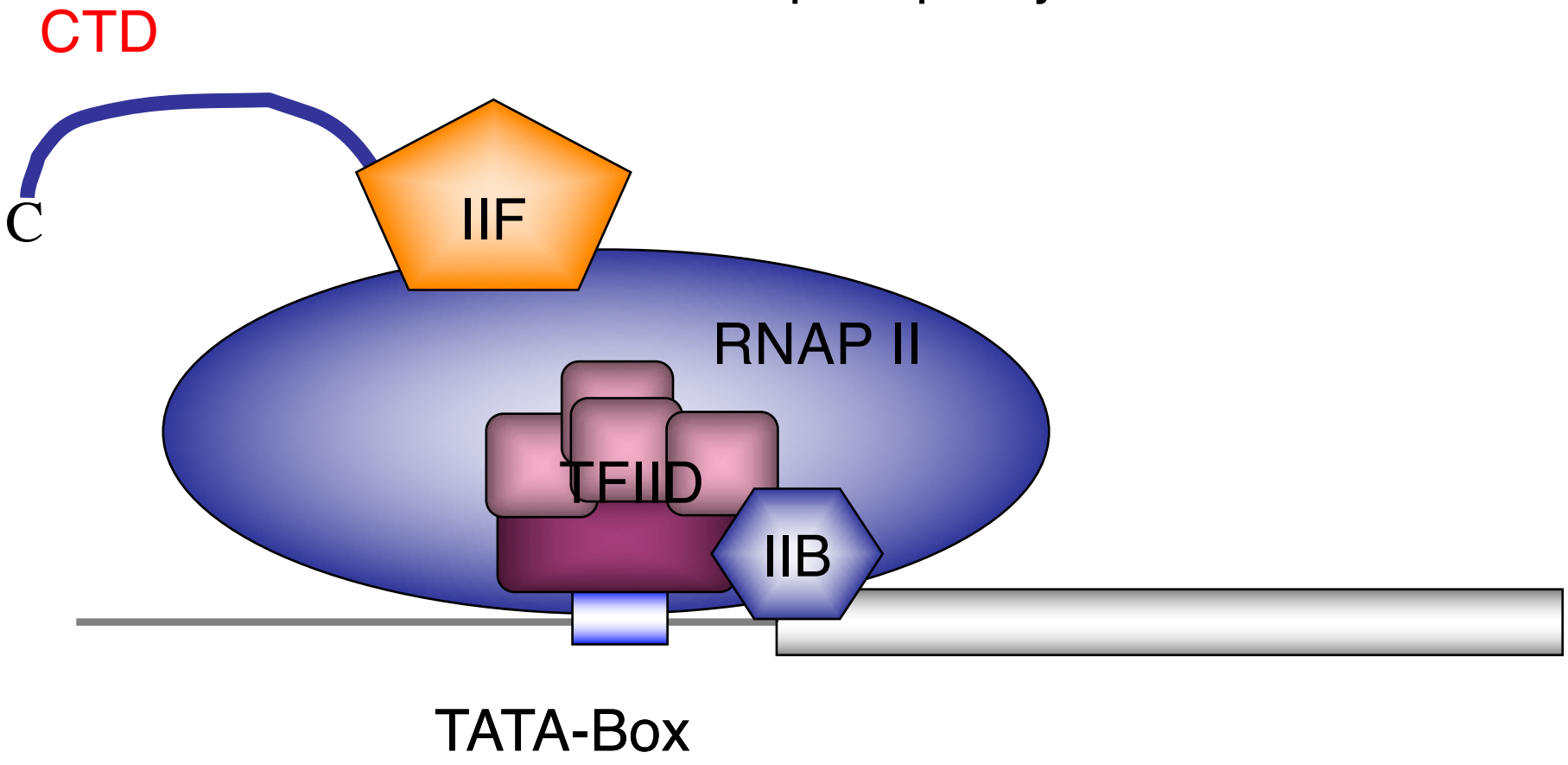


TFIIB kommt hinzu

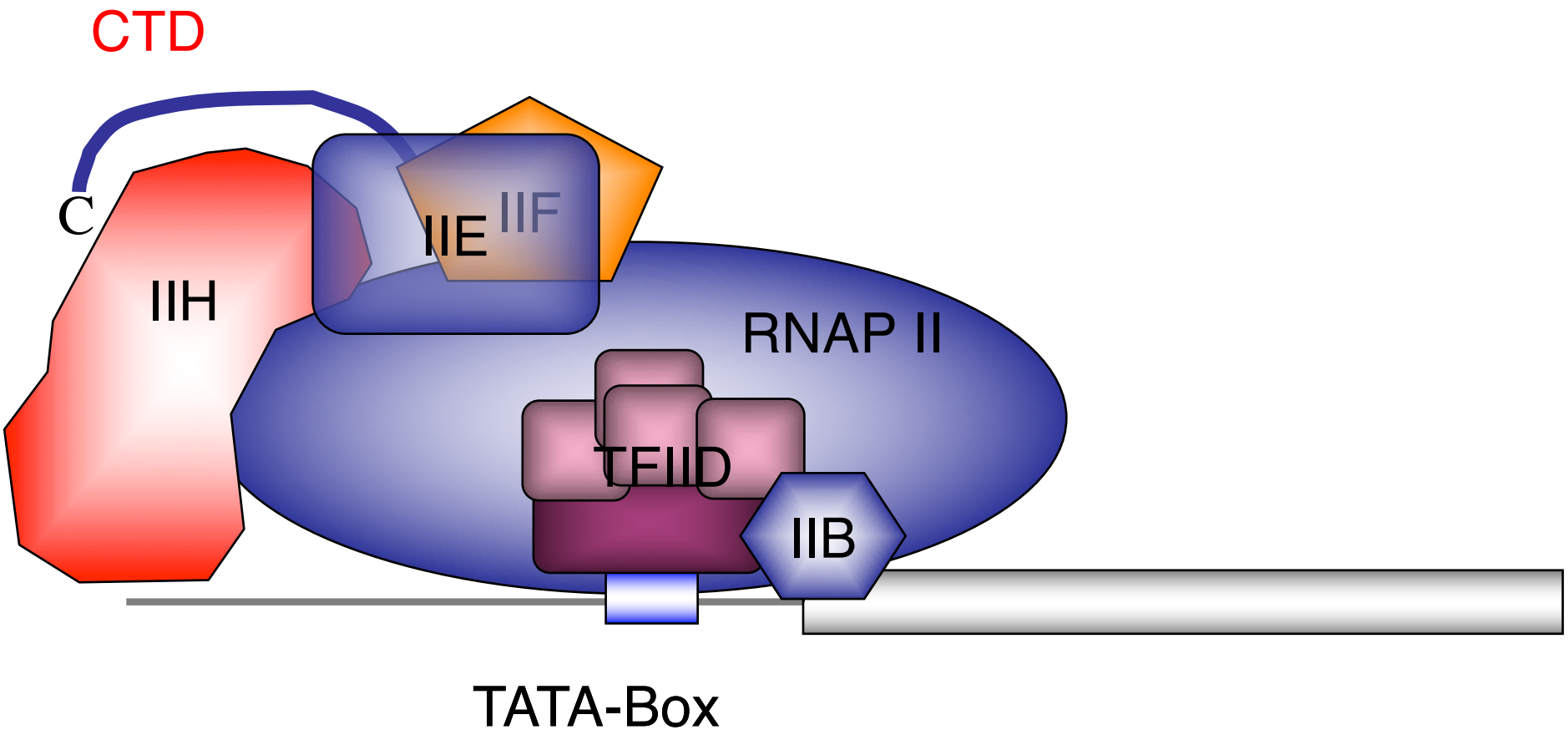


Assoziation weiterer TFIIs:

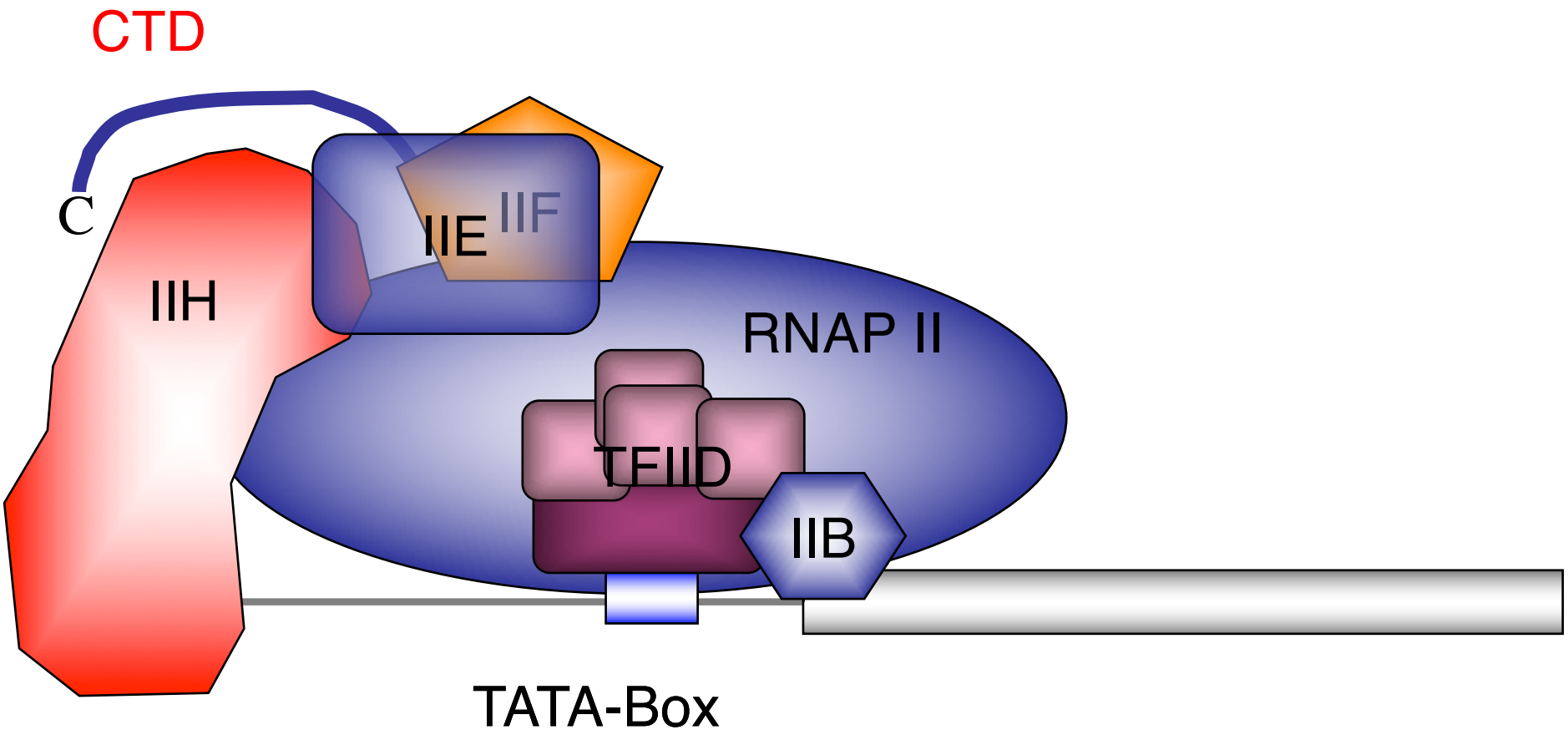
- TFIIF
- TFIIF rekrutiert schließlich die RNAP II
  Der CTD ist nicht phosphoryliert



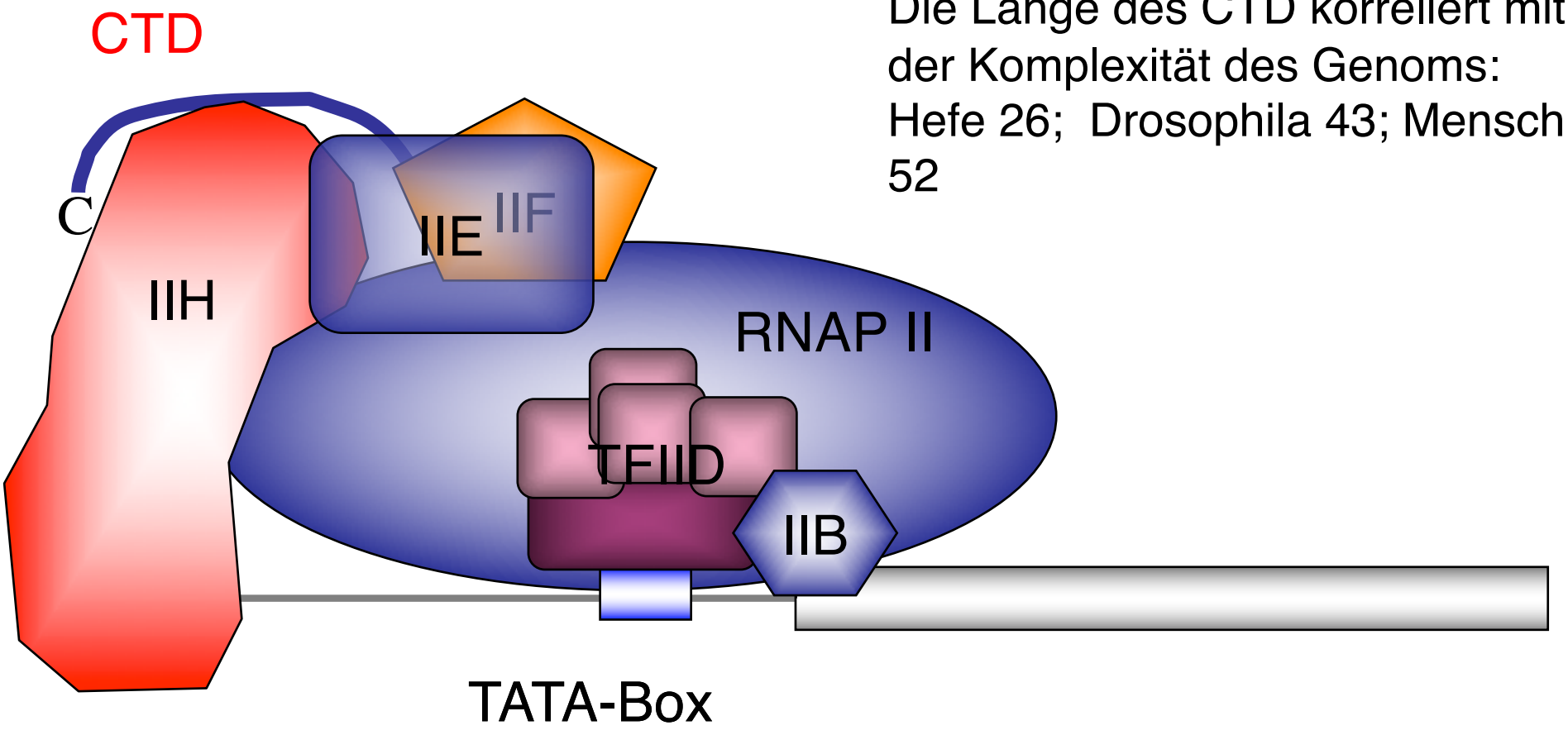
Zuletzt bindet ein Komplex aus TFIIE und TFIIH



TFIIH hat DNA-Helicase Aktivität,
die die DNA des Promotors entwindet.
Es bildet sich ein offener Initiationskomplex

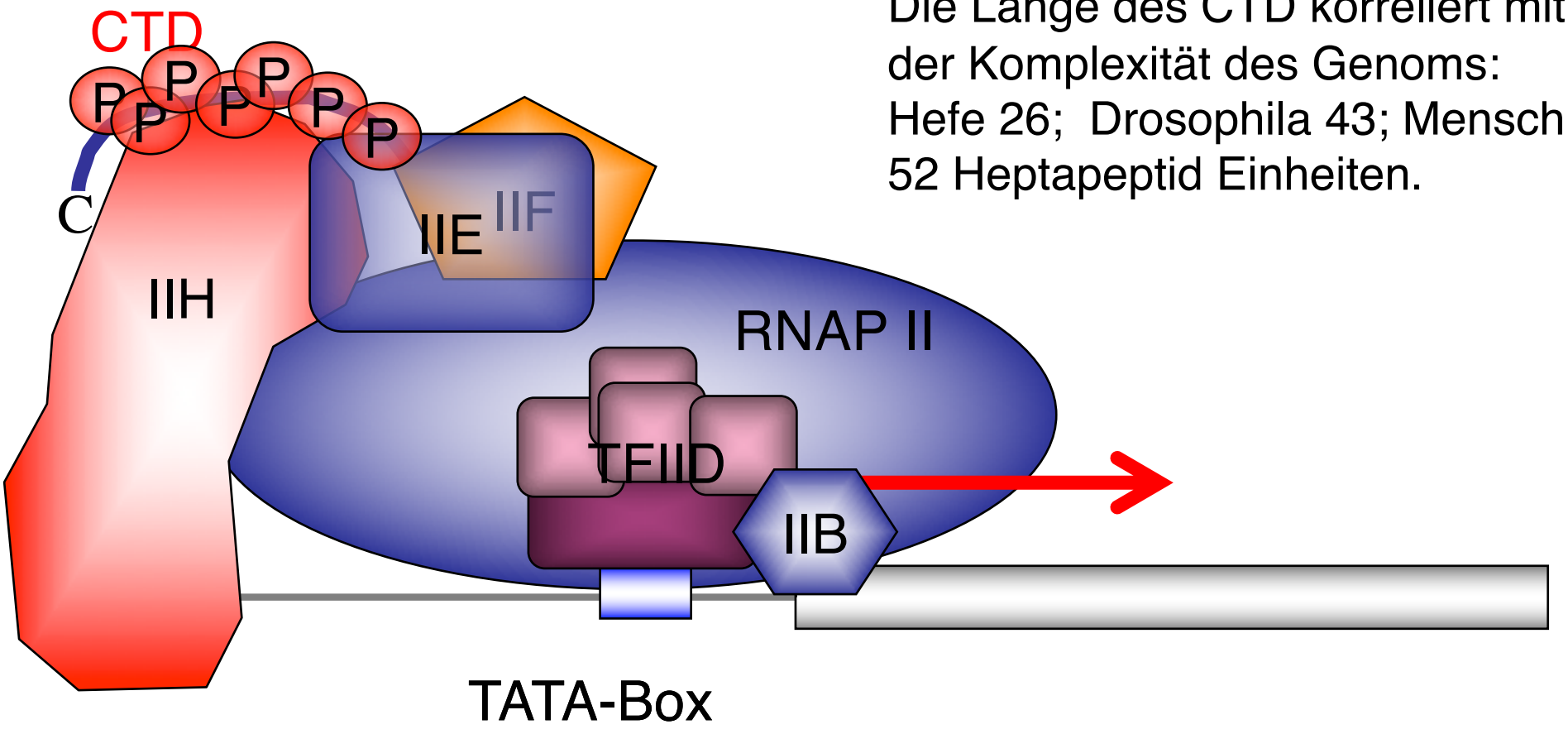


TFIIH hat eine Protein-Kinase Aktivität: Phosphorylierung des C-Terminus der RNA-Polymerase (CTD)



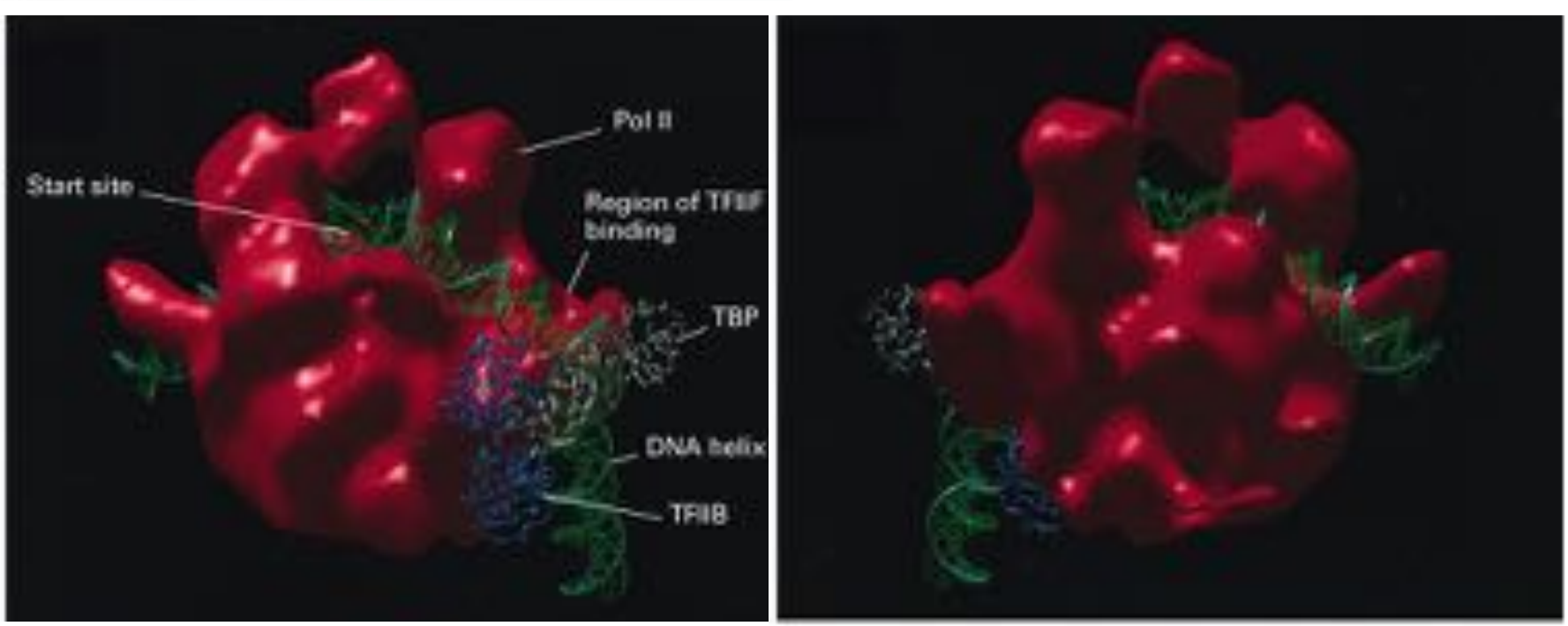
Die Länge des CTD korreliert mit der Komplexität des Genoms: Hefe 26; Drosophila 43; Mensch 52

TFIIH hat eine Protein-Kinase Aktivität: Phosphorylierung des C-Terminus der RNA-Polymerase (CTD)



Die Länge des CTD korreliert mit der Komplexität des Genoms: Hefe 26; Drosophila 43; Mensch 52 Heptapeptid Einheiten.

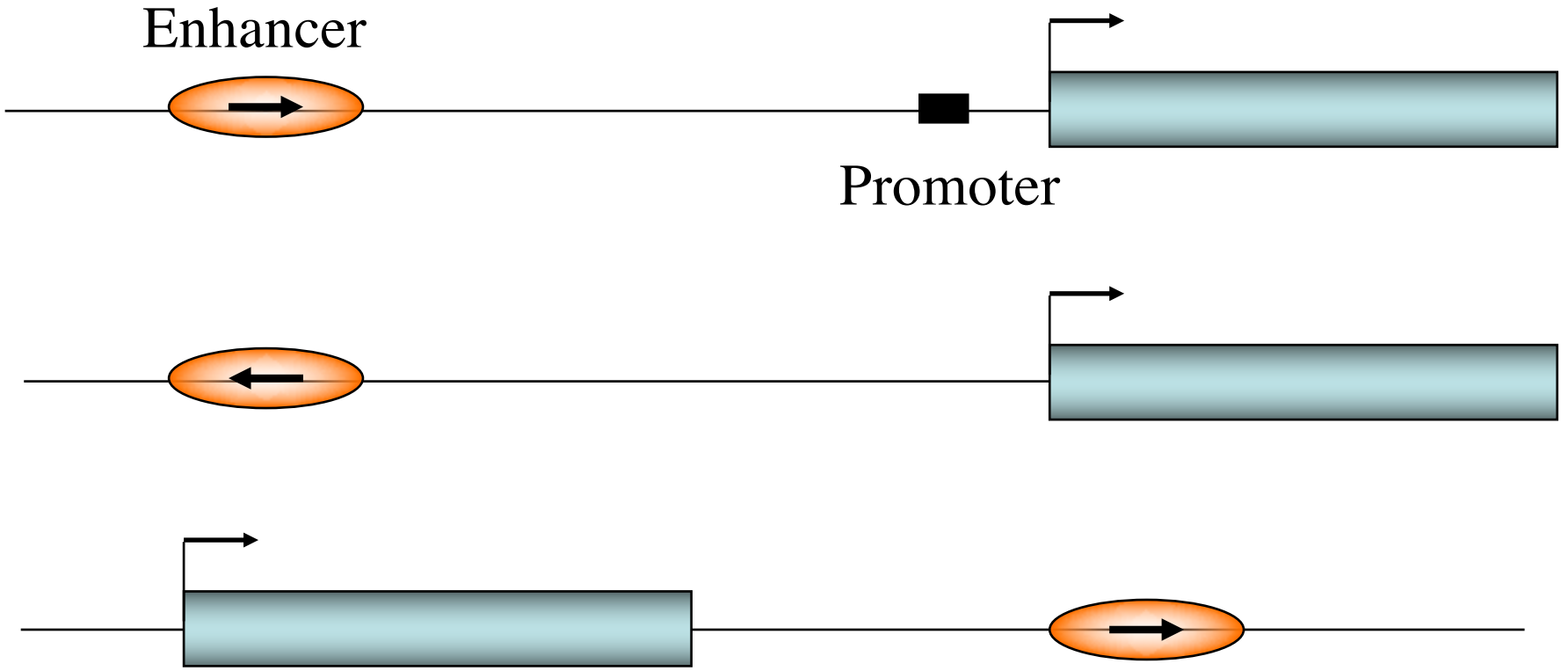
Polymerase II Modell



Nobel-Preise: (1959, 2006)
Kornberg Familie:
Arthur Kornberg (1959)
DNA Polymerase
Roger Kornberg (2006)
RNA Polymerase II

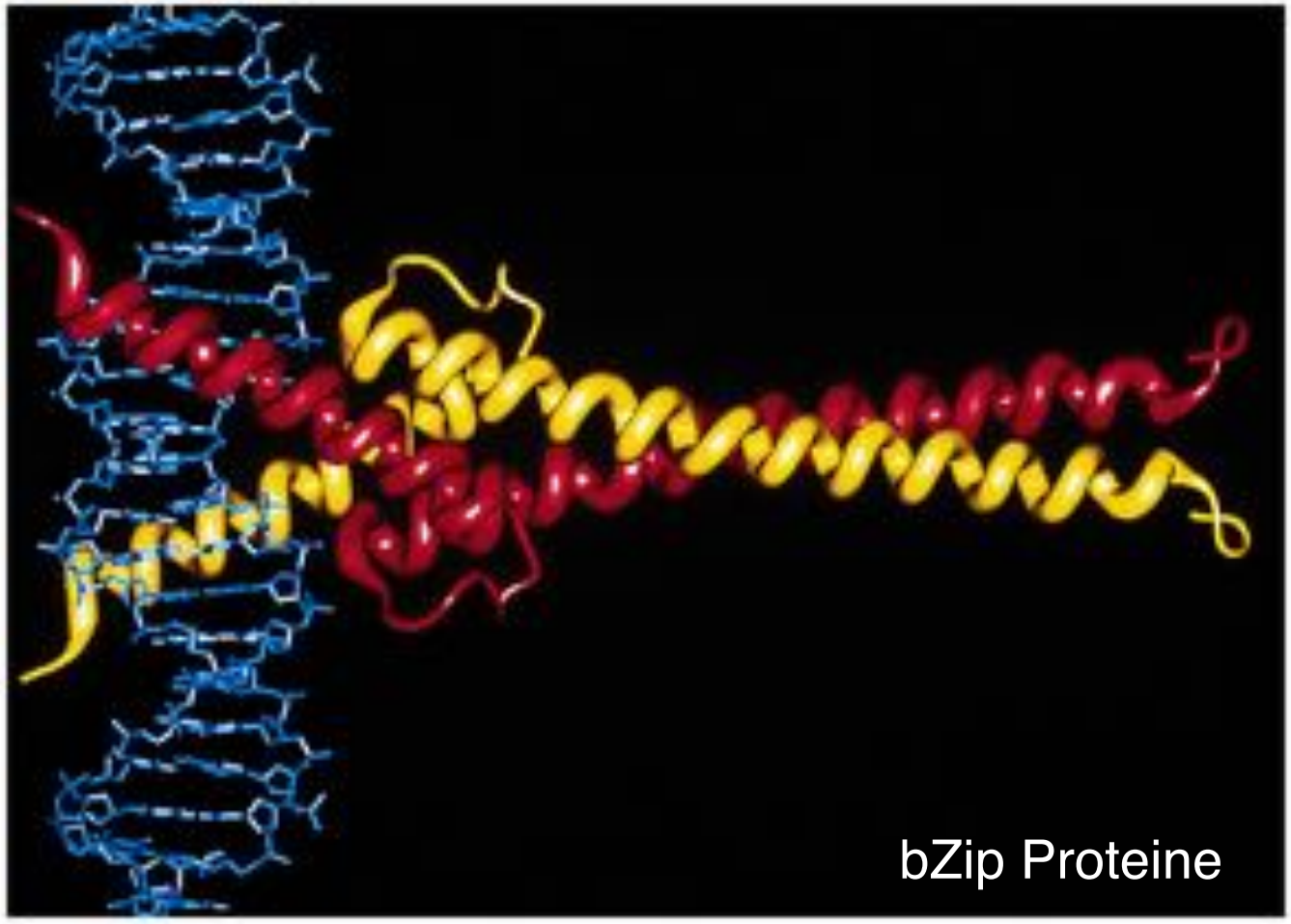
Tom Kornberg: Entwicklungsbiologe
Ken Kornberg: Architekt

Enhancer steuern die räumliche und zeitliche Expresssion von Genen



Enhancer stimulieren die Transkription von einem Promoter unabhängig von ihrer Position und Orientierung.
Sie binden Transkriptionsfaktoren. Silencer

Transkriptionsfaktoren



Anatomie eines Transkriptionsfaktors



DNA-Bindungsdomäne

bHLH; Zn-Finger; Homoedomäne
ETS-Domäne

Interaktionsdomäne

mit Polymerase (Aktivierung, Repression),
Histon Modifikatoren

Regulatorische Domäne

Dimerisierungsdomäne

Transkriptionsfaktoren

Reinigung der Transkriptionsfaktoren über Chromatographie (sequenzspezifische DNA-Affinitätschromatographie). 5% aller Proteine sind TFs!

Klassifizierung der Transkriptionsfaktoren erfolgt über ihre DNA bindende Domäne.

Man unterscheidet zwischen aktivierenden und reprimierenden TFs.

Repression ist in der Regel indirekt und erfolgt über eine Modulation des Chromatins -> HDACs

BTB/POZ Domäne, Engrailed Domäne, WRPW -> Groucho

Krüppel associated box (KRAB).

Beispielgruppen

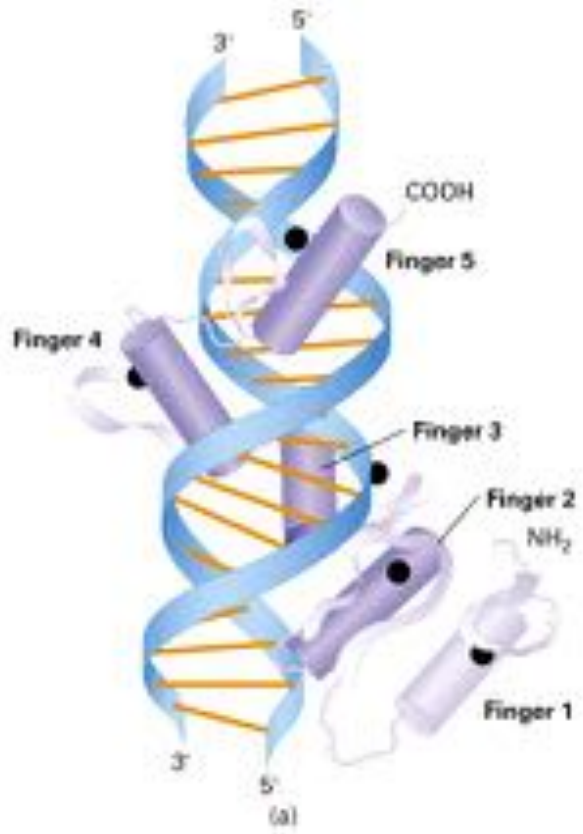
- **Homeodomänen** Proteine (Segmentierung) HTH
- **bHLH** Proteine (neurale Kompetenz, Spezifizierung)
- **ETS** Domänen Proteine (RTK-Signalkaskaden) winged Helix Proteine
- **Zink-Finger** Proteine 3 Untergruppen

  C2H2 häufigster TF (ca.1000) Monomer;

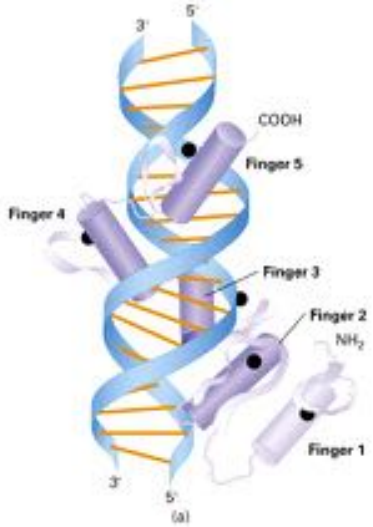
  C4 Zinkfinger 100 TFs z.B. (Hetero)Dimer Steroidrezeptoren

  C6 Zinkfinger z.B. Gal4, Homdimer

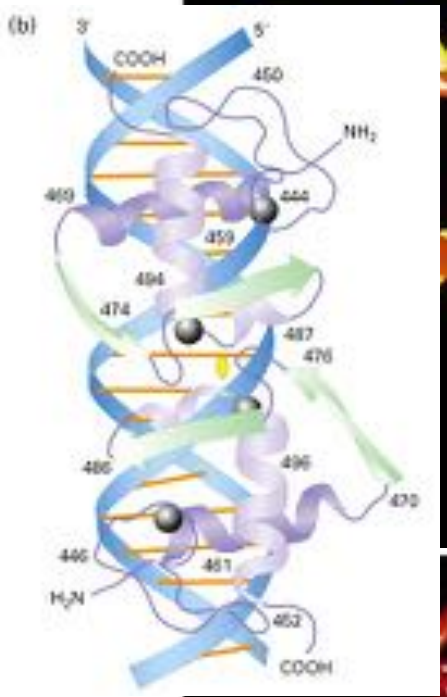
- **STAT** (Signal transducer and activator of transcription) z.B. Interferon-Antwort
- **Leucin Zipper** Proteine (bZip)
- **Membran-gebundene TFs** (z.B. Notch).

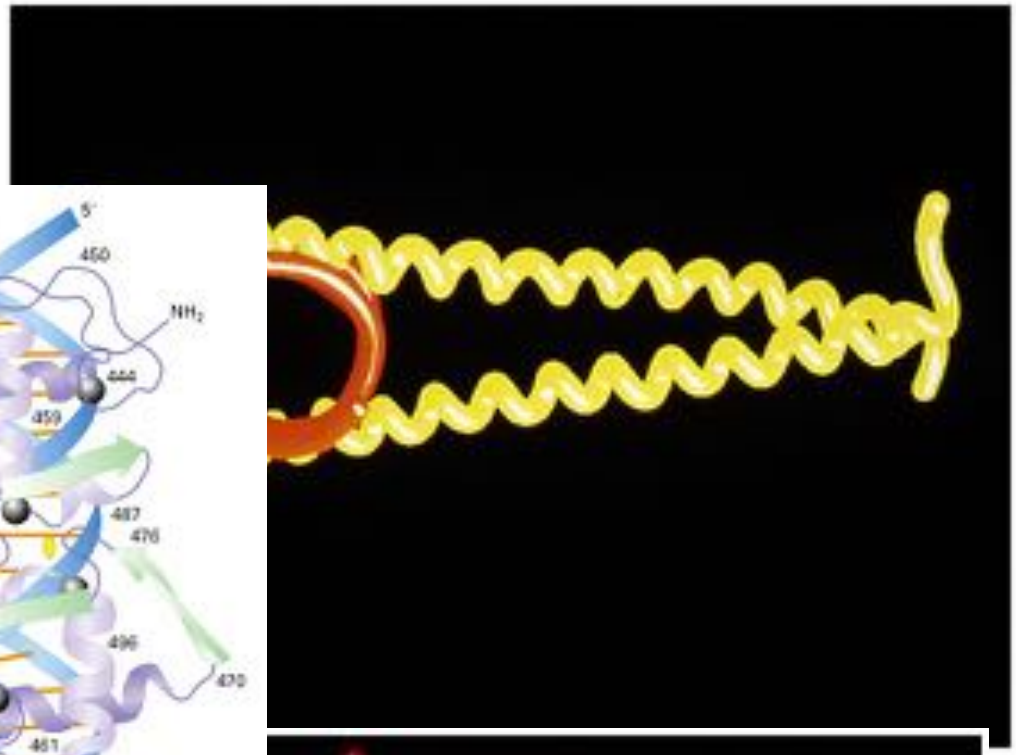


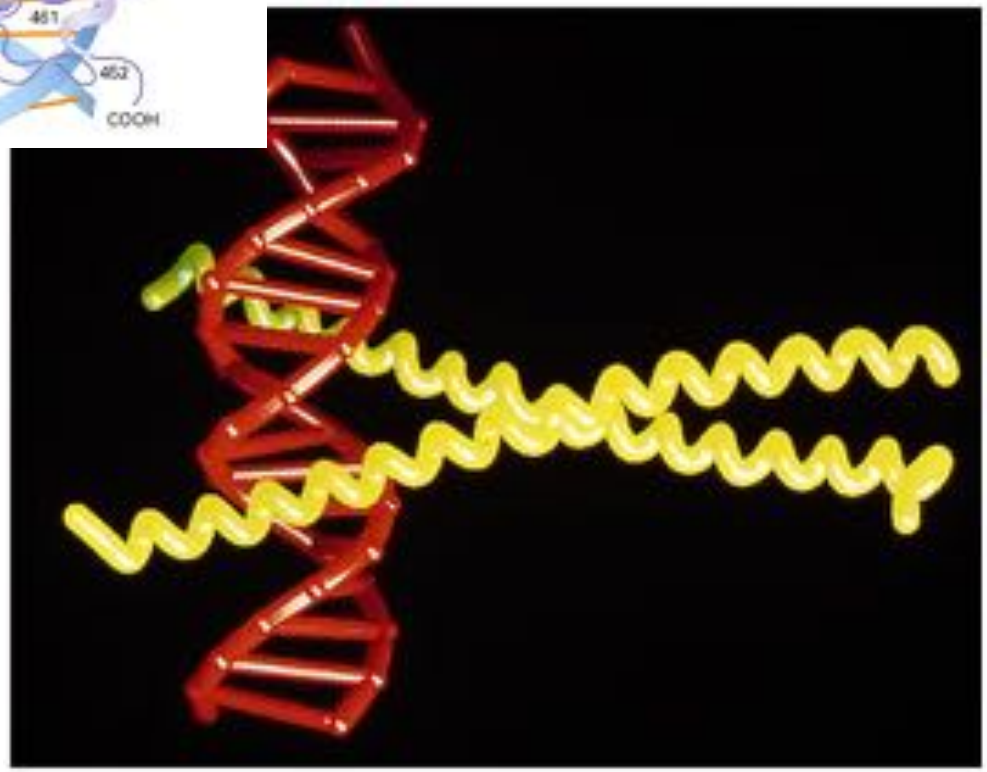
(a)


3'
5'
COOH
Finger 5
Finger 4
Finger 3
Finger 2
NH_2
Finger 1
3'
5'
(a)


40
60
40
8'
7'
8'
5'
3'

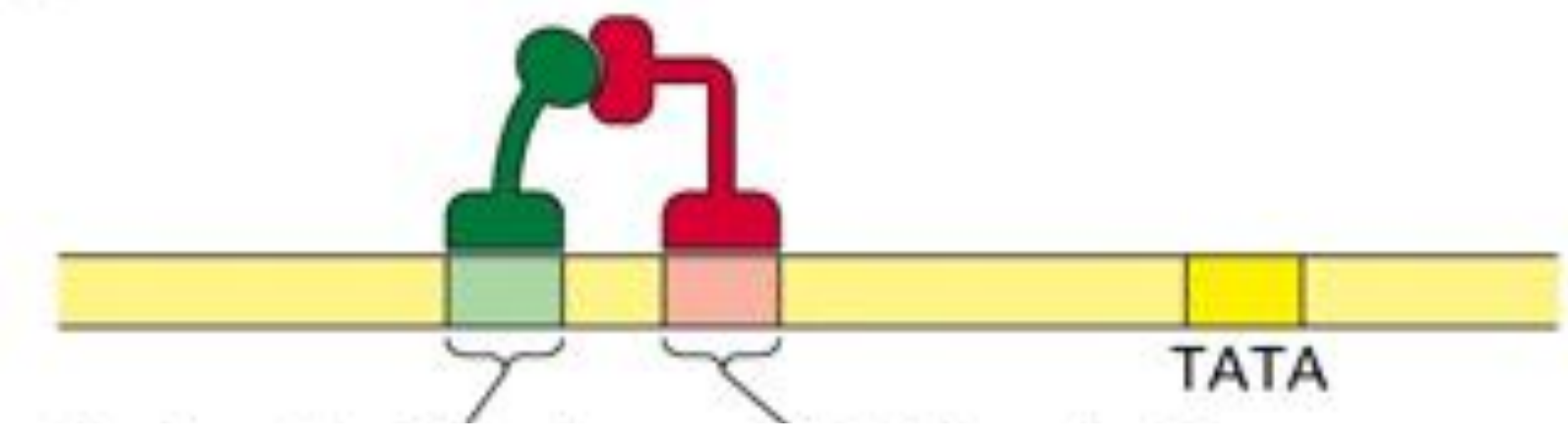

(b)
3'
5'
COOH
450
NH_2
469
444
459
494
474
487
476
496
486
470
466
461
H_2N
452
COOH





Modelle zur Wirkung von Transkriptionsfaktoren

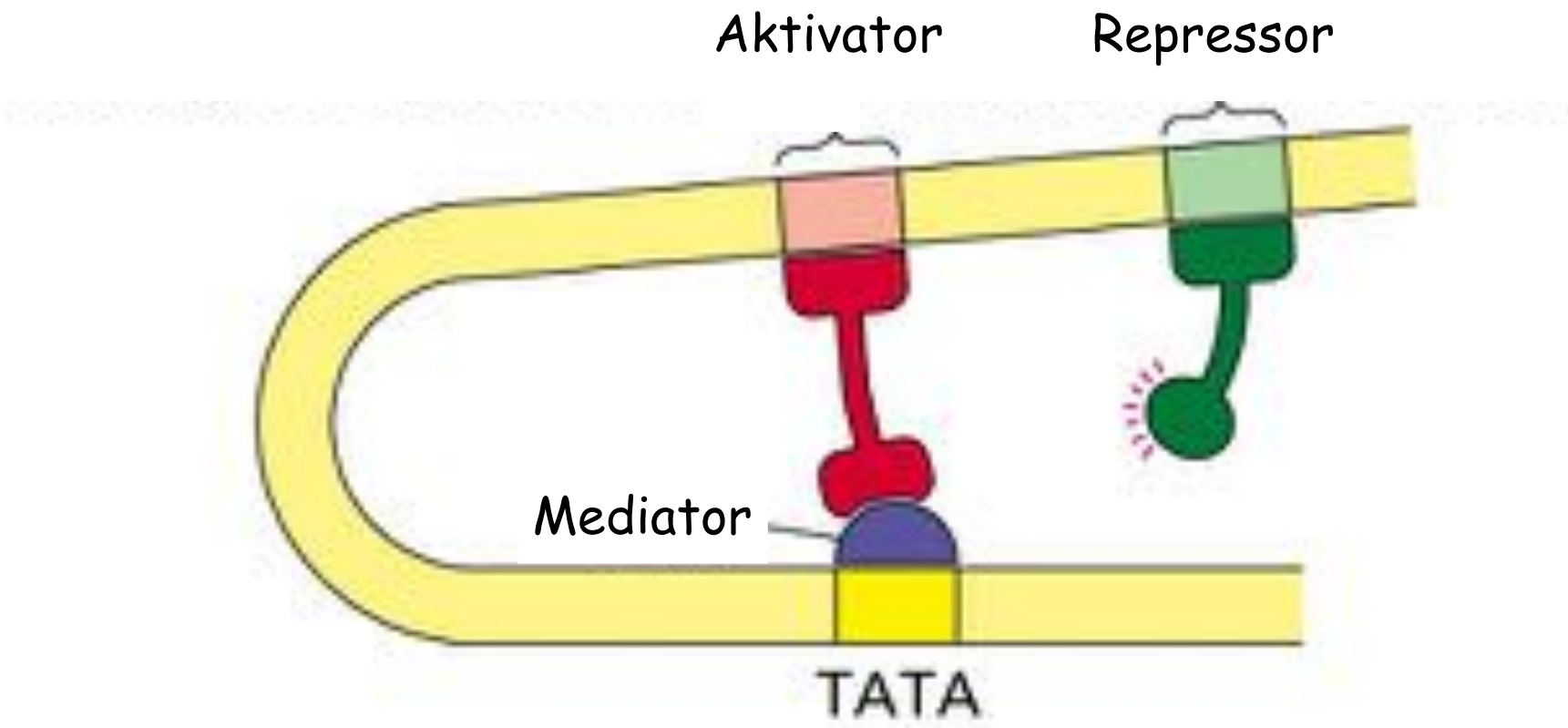
Interaktion von Transkriptionsfaktoren



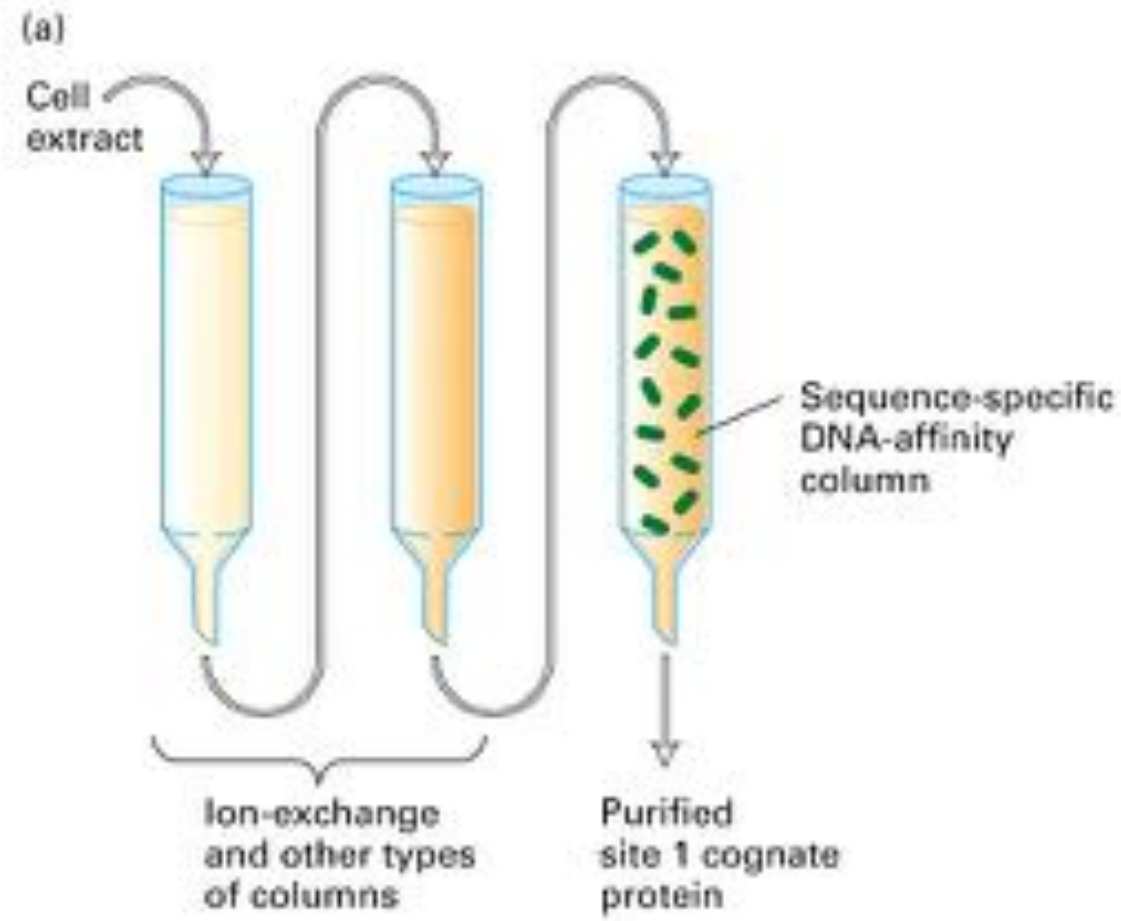
Aktivator Repressor

Modelle zur Wirkung von Transkriptionsfaktoren

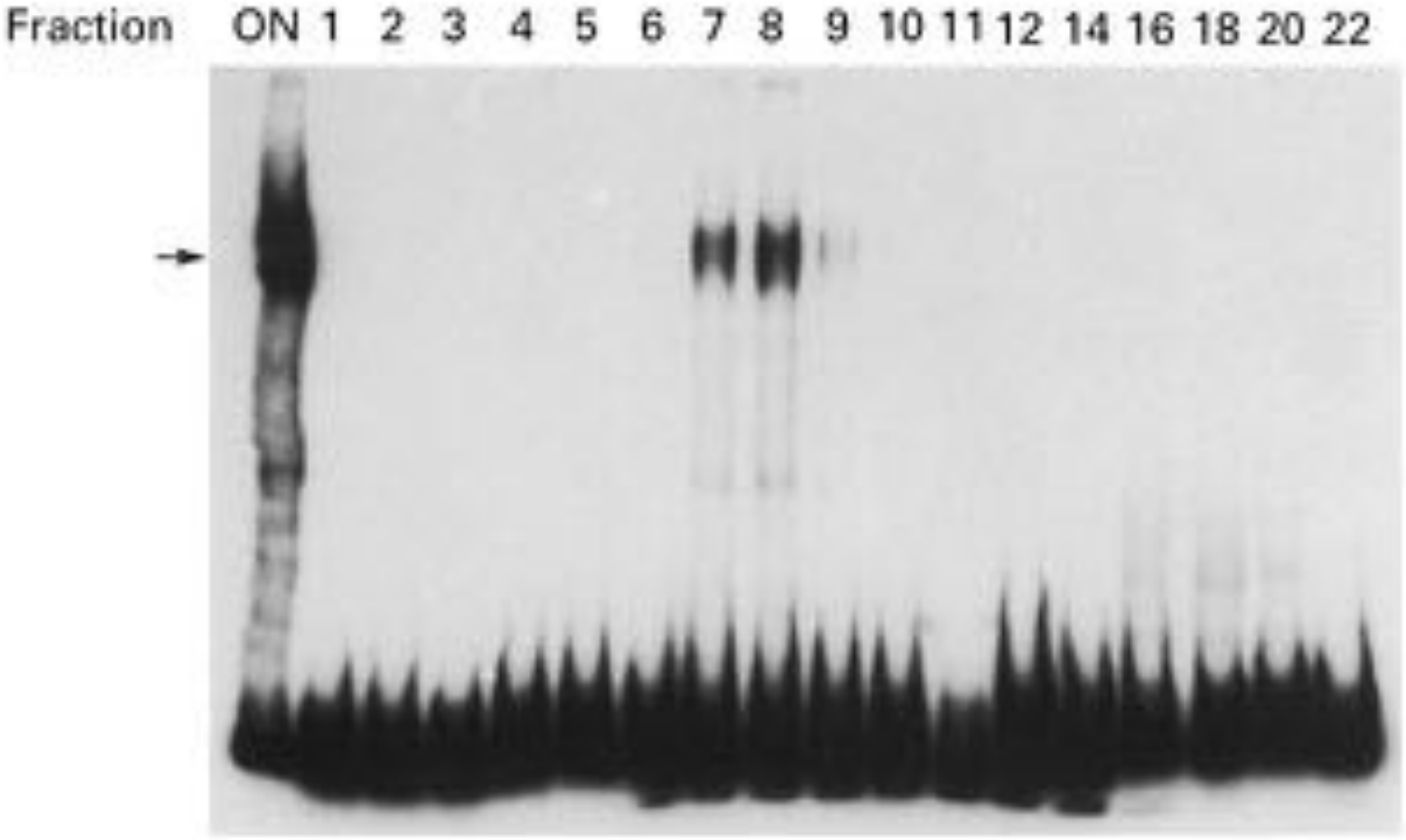
Die Interaktion mit dem Promotor erfolgt indirekt



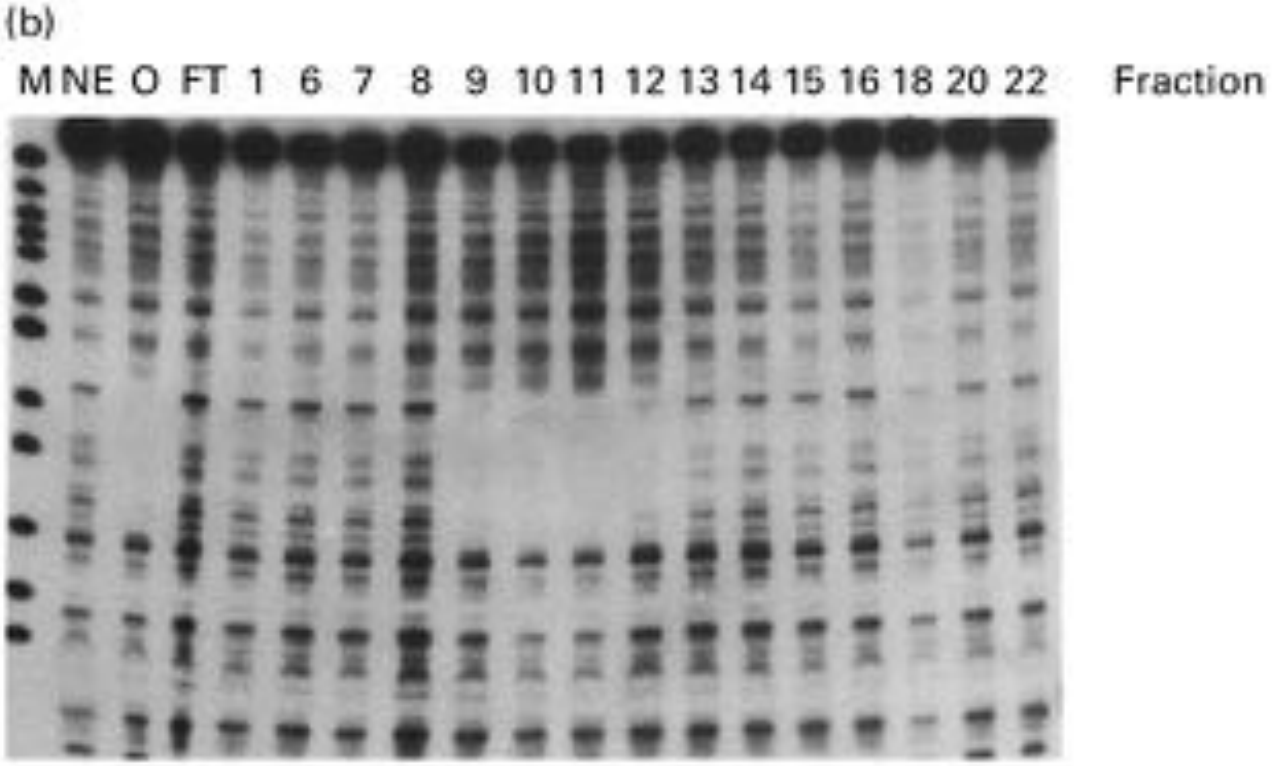
Säulenchromatographische Verfahren zur Reinigung von Transkriptionsfaktoren

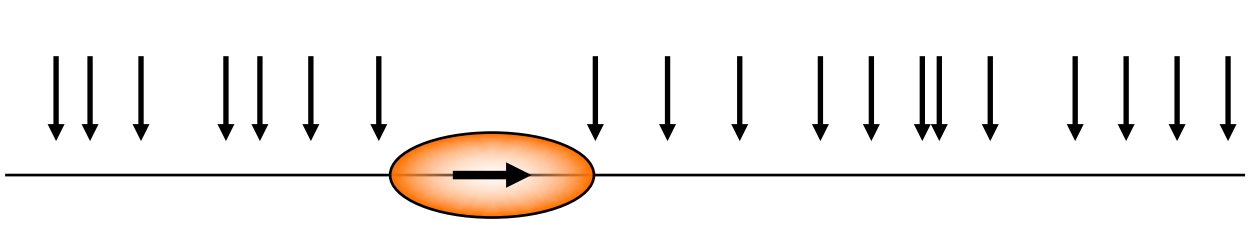


Wie finde ich die DNA-Bindungssequenz?
Electrophoretic Mobility Shift Assay (EMSA)
oder Band shift assay



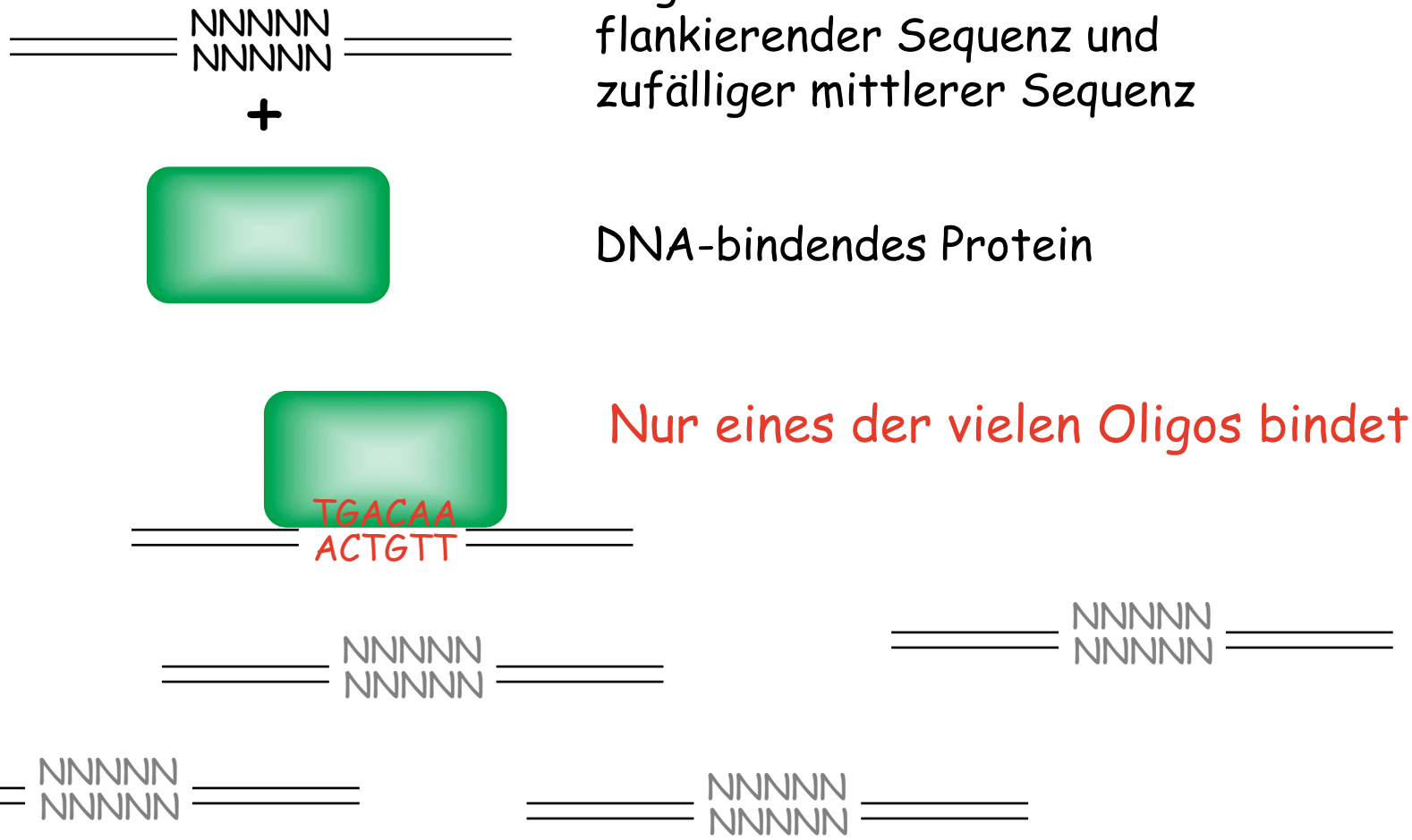
Wie finde ich die DNA-Bindungssequenz?
Früher: Der „foot print“





Mögliche DNAase Schnittstellen

Wie finde ich die DNA-Bindungssequenz?
Heute: PCR-select



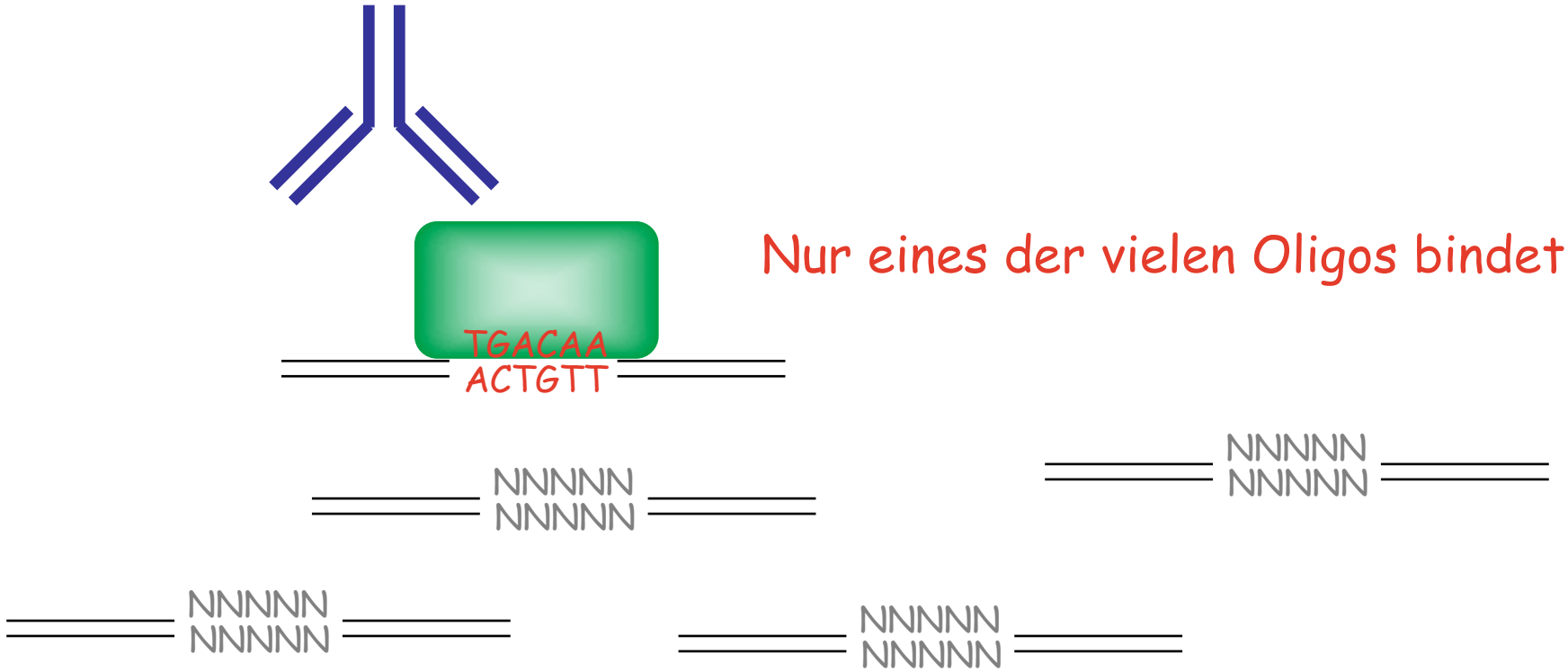
Oligonukleotide mit bekannter flankierender Sequenz und zufälliger mittlerer Sequenz

DNA-bindendes Protein

Nur eines der vielen Oligos bindet

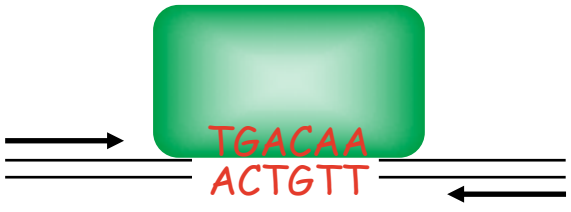
Wie finde ich die DNA-Bindungssequenz?
Heute: PCR-select

+ Antikörper, die spezifisch den Transkriptionsfaktor binden. Immunpräzipitation.



Nur eines der vielen Oligos bindet

Wie finde ich die DNA-Bindungssequenz?
Heute: PCR-select



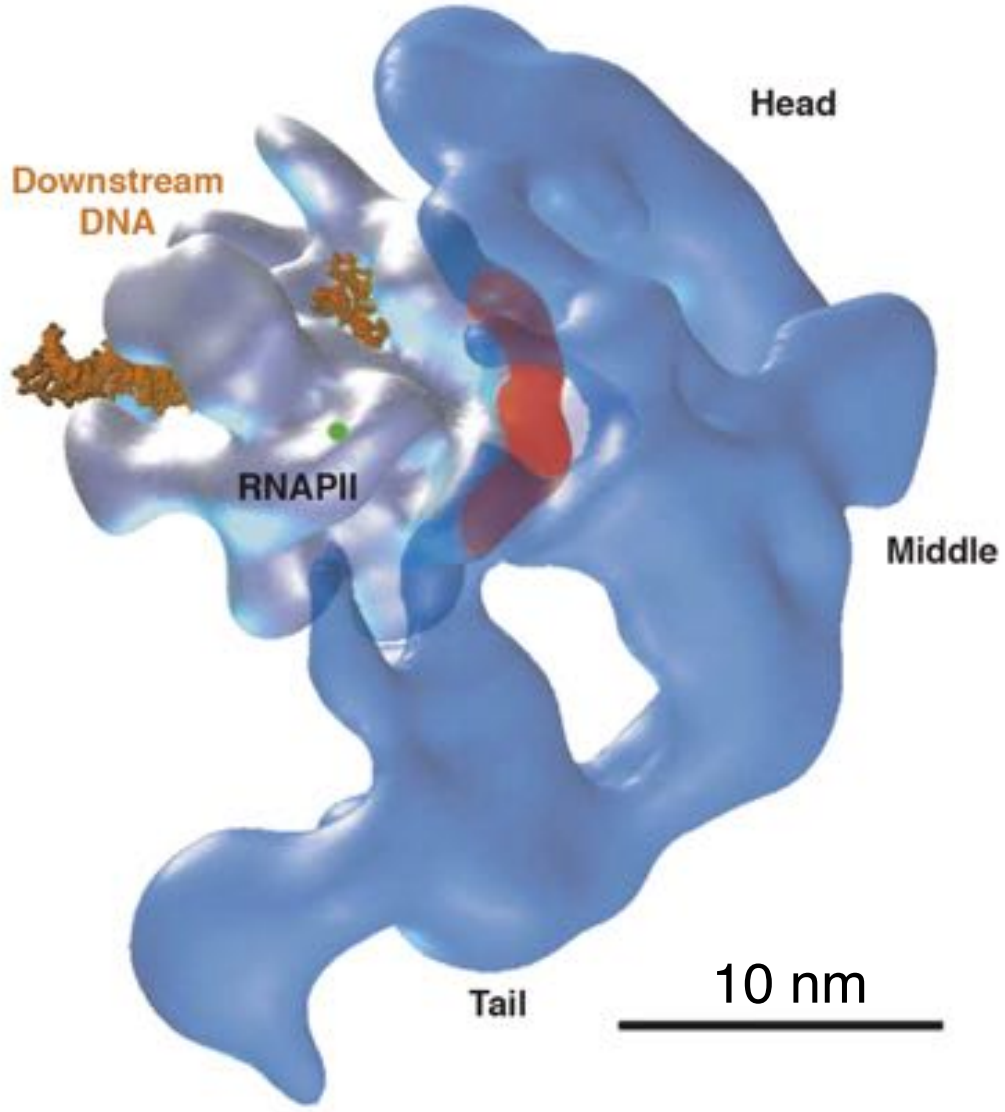
PCR, um die Target Sequenzen zu amplifizieren.

Klonieren der DNA Fragmente.

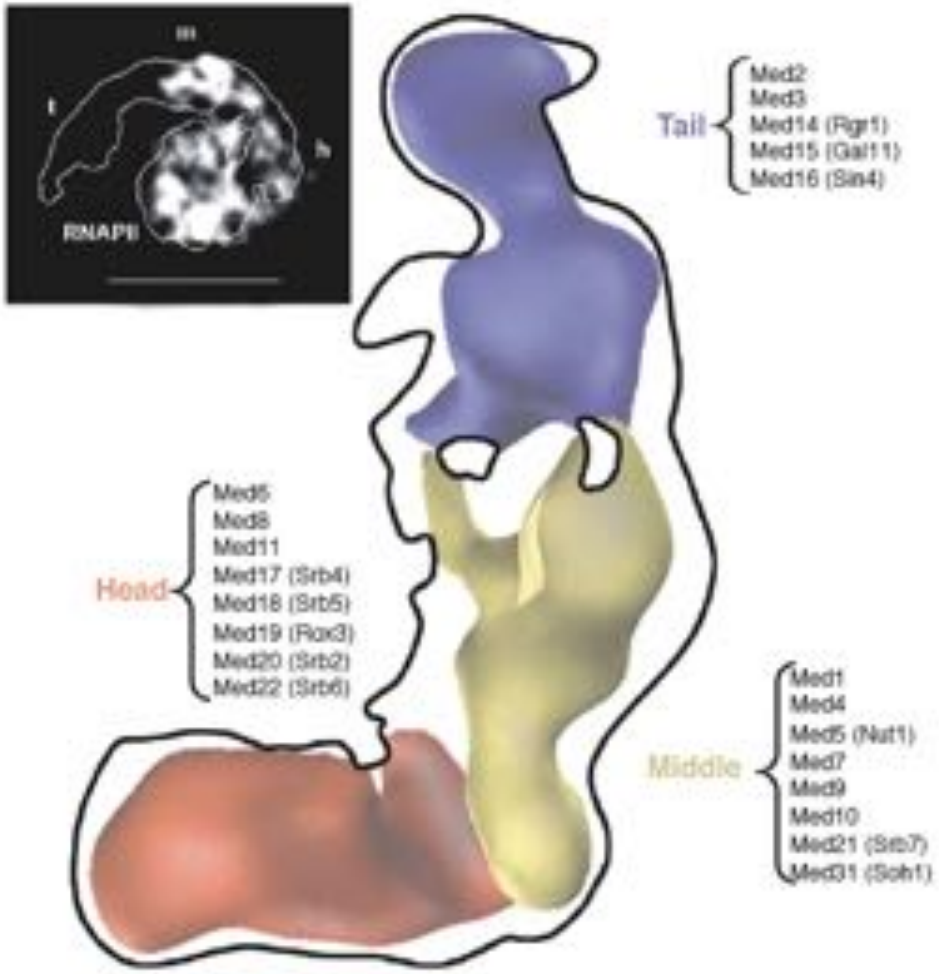
Sequenzanalyse.

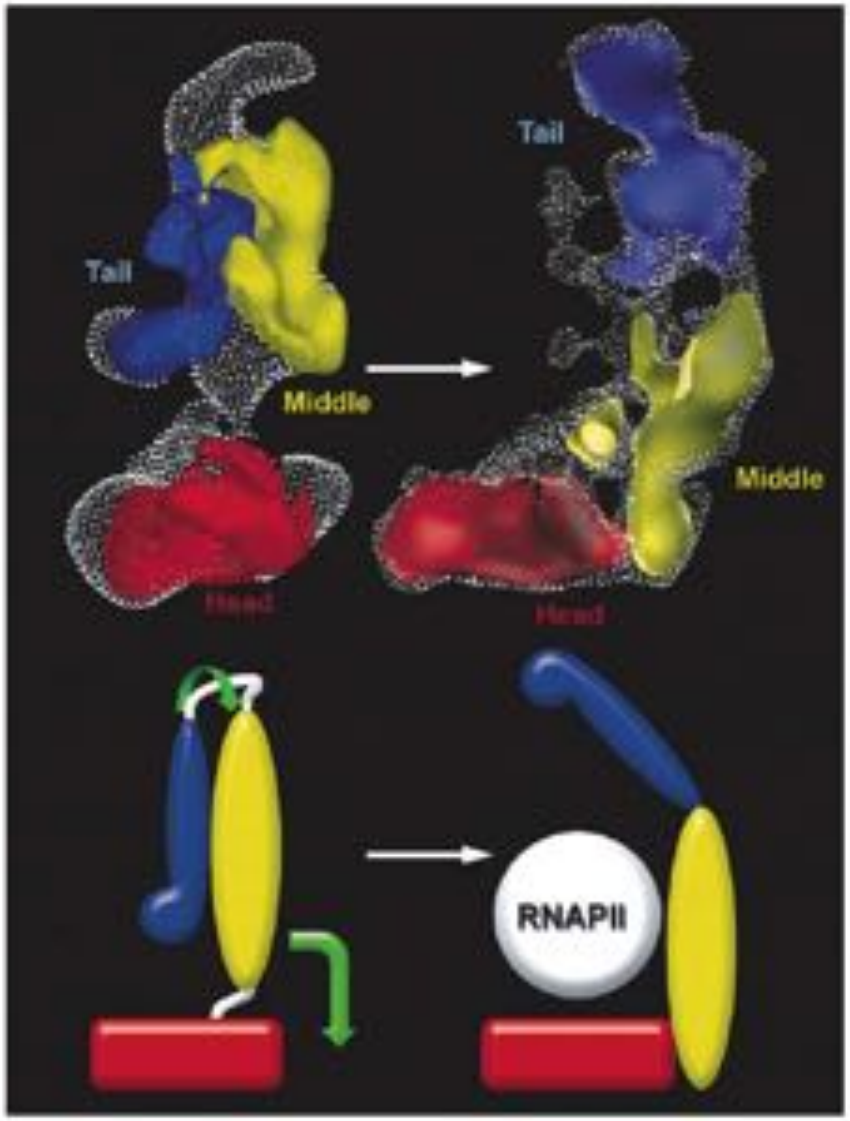
Zielsequenz!

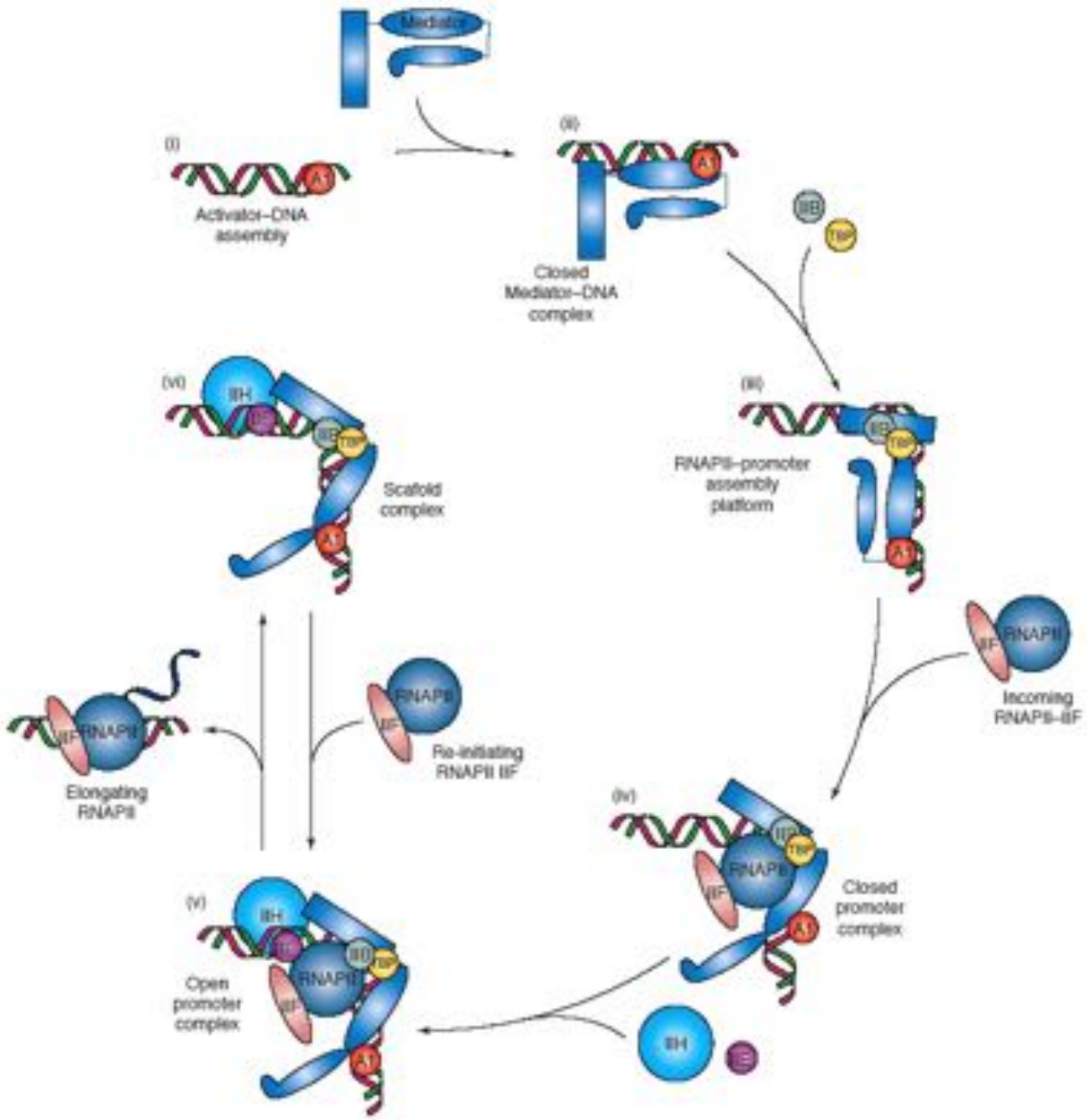
Interaktion Mediator - RNA PII



Der Mediator-Komplex (>30 UE)





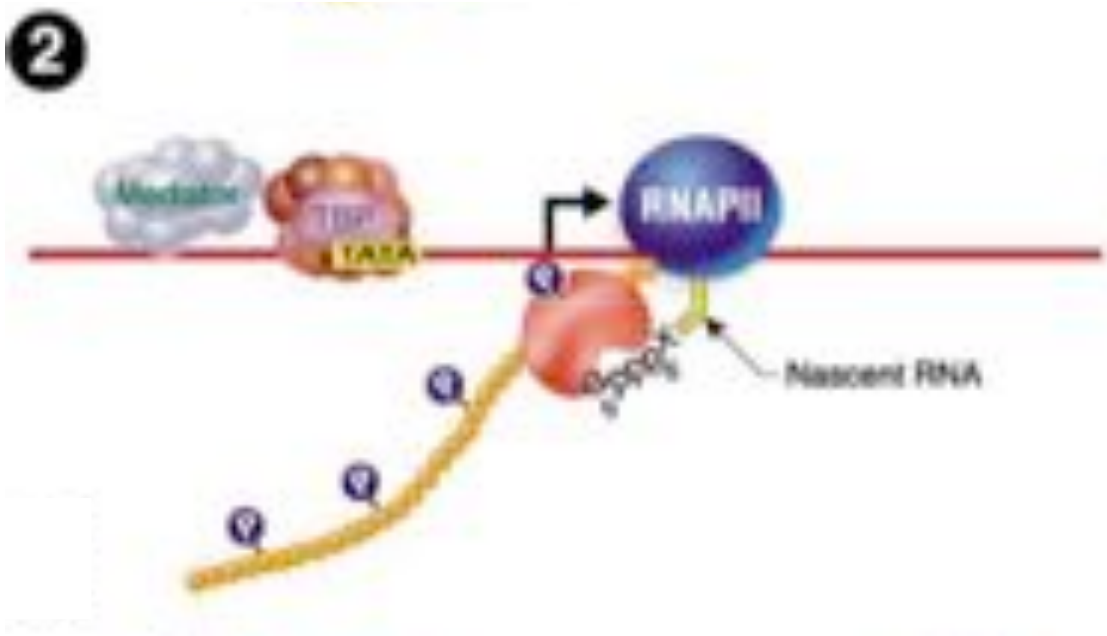


Regulation der Transkripition durch den Mediator-Komplex

Die C-terminale Domäne der Pol II koordiniert Transkription und Processing



Vor der Inititation der Transkription ist CTD nicht phosphoryliert und ist mit den Initiationskomplex assoziiert

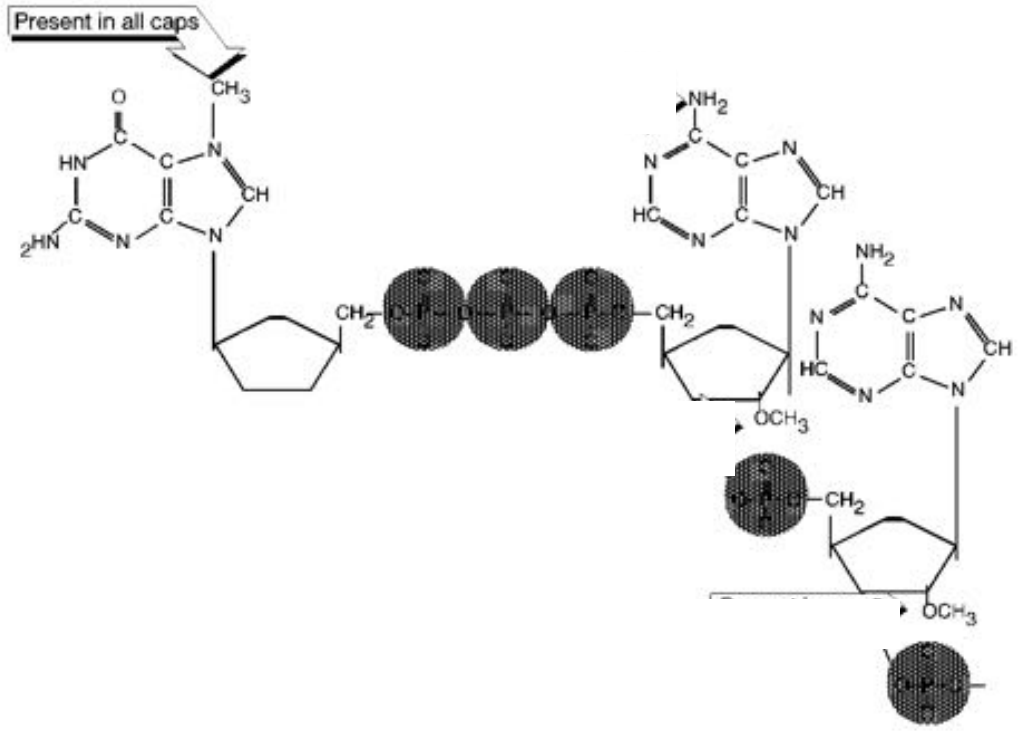


Die C-terminale Domäne der Pol II (CTD) trägt 52 Einheiten (Mensch) der Konsensussequenz
Y S P T S P S die phosphoryliert wird.
Promoter clearance,
5‘ Cap

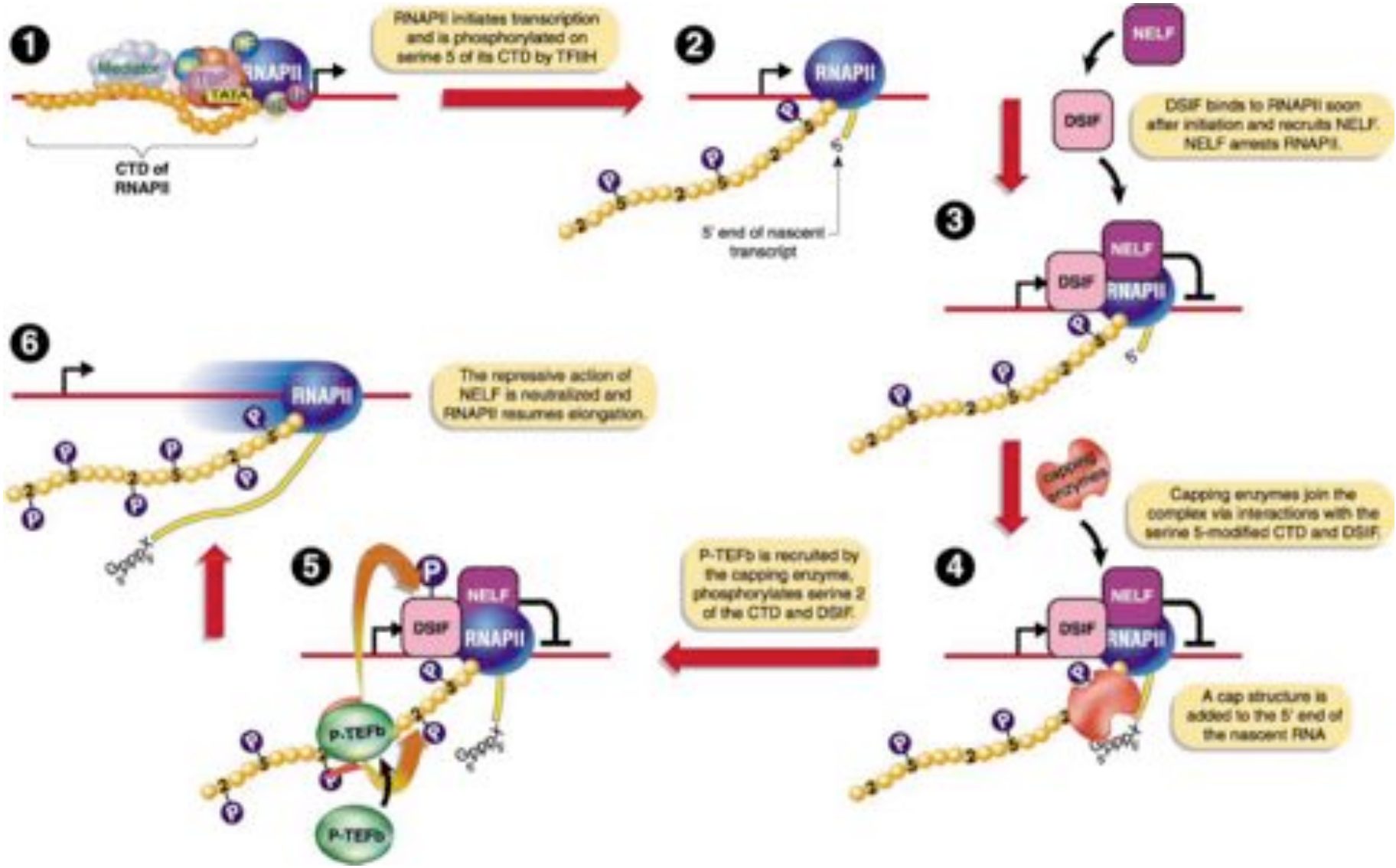
Processivität RNA Pol II: 50-90 Nukleotide / Sekunde

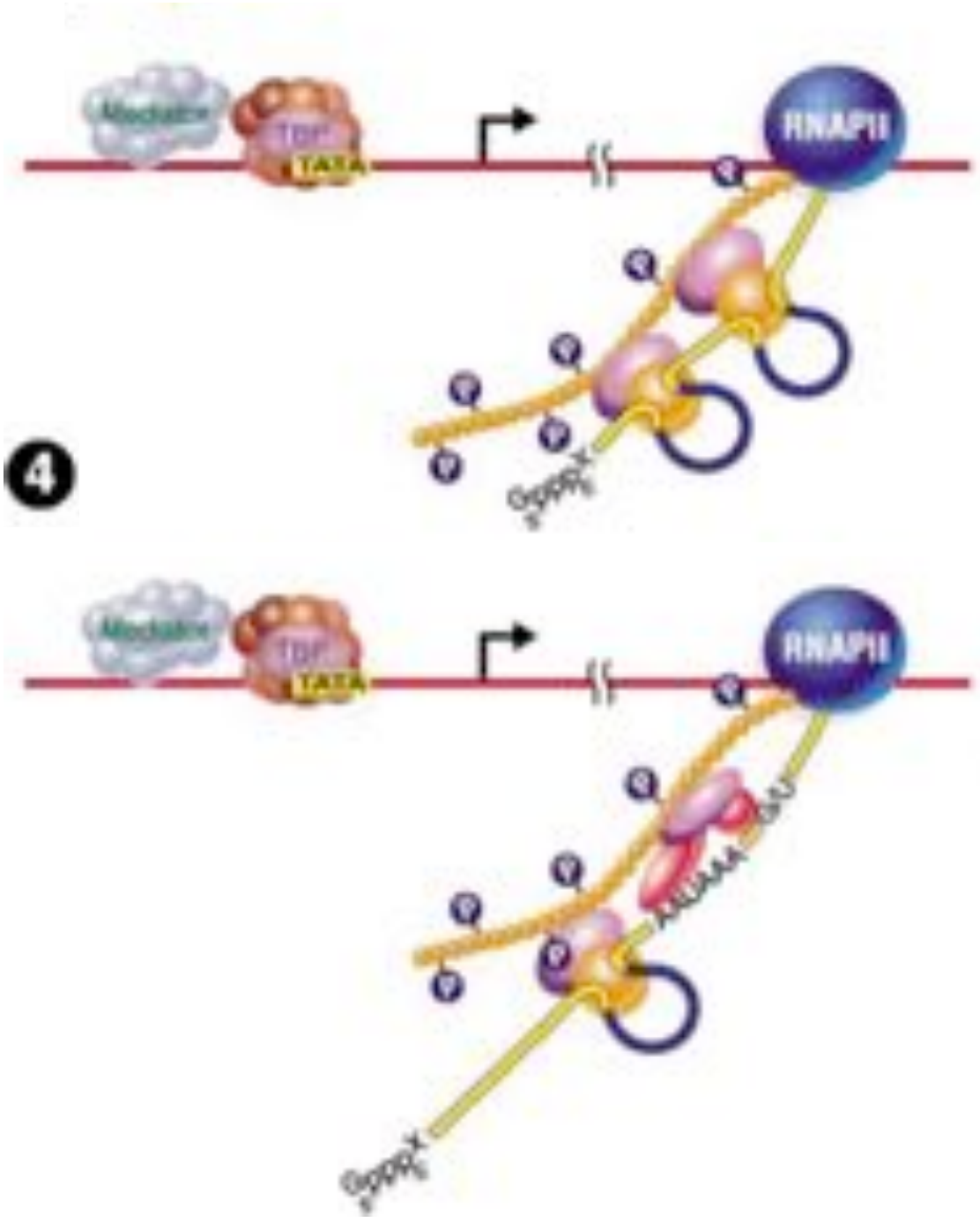
Capping

Cap: 7-Methylguanosin über eine 5'-5'-Triphosphat-Brücke



Checkpoint Modell der Cap Bildung





Die phosphorylierte CTD rekrutiert die Spliceosomen

AG	GU		A AG	

und ist an der Termination der Transkription beteiligt.
CstF cleavage stimulation factor F
CPSF cleavage and polyadenylation specificity factor
AAUAAA 10-30 nt, cleavage
Poly-A-Polymerase
PABP
An die naszente RNA binden Transportproteine die den Export aus dem Kern regulieren.

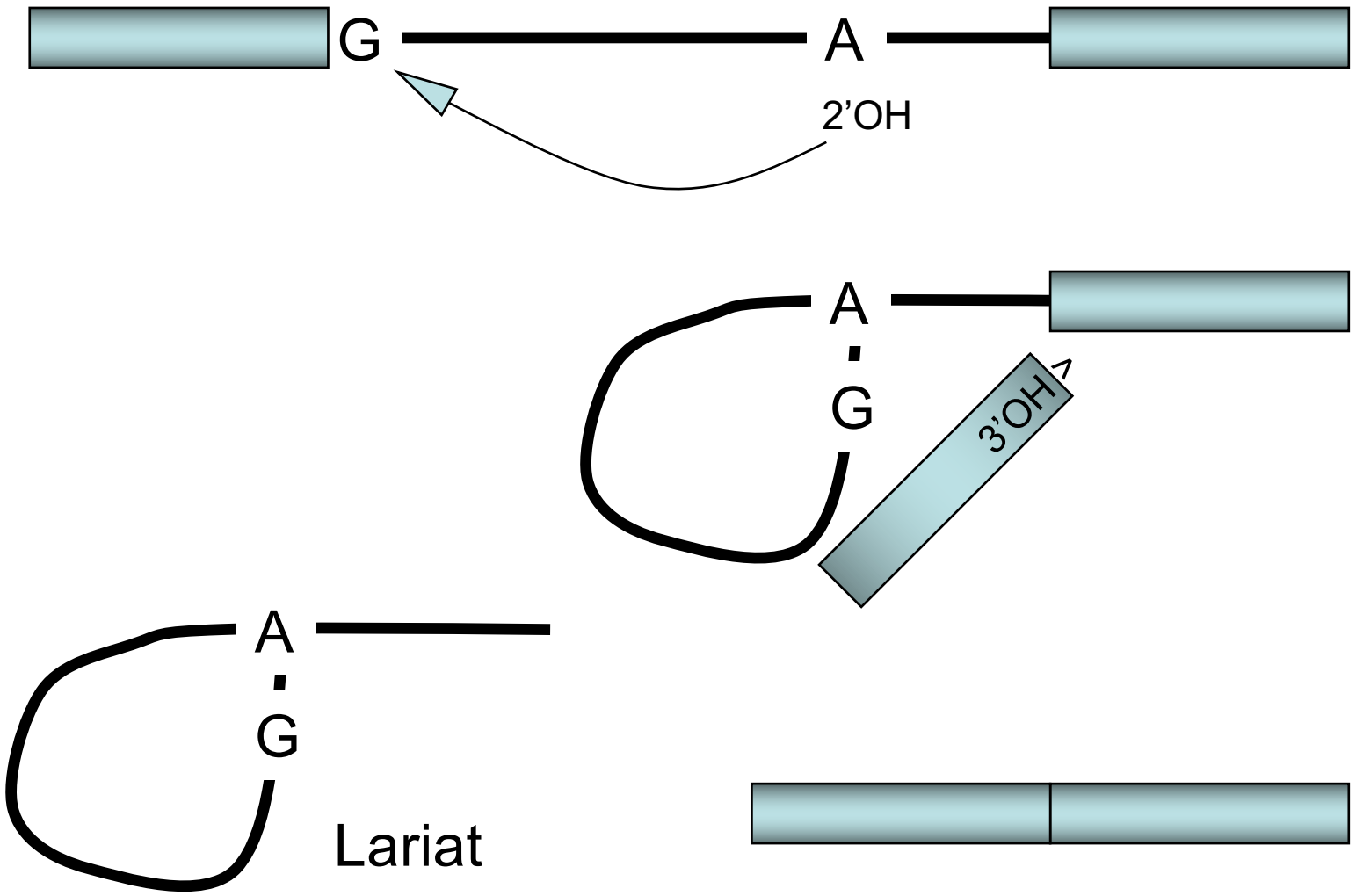
Splicing

Introns = Relikte der RNA Welt; Ribozymaktivität

Selbstsplicende Introns -> Typ I Einbau eines zusätzlichen Guanin

-> Typ II Lariatbildung

Selbstsplicing Typ II



Splicing, was brauchen wir?



GU/AG Regel konserviert von Hefe bis Mensch.

Ca. 1% der Introns AT/AC -> Dann anderes Spliceosome

Splicing, was brauchen wir?

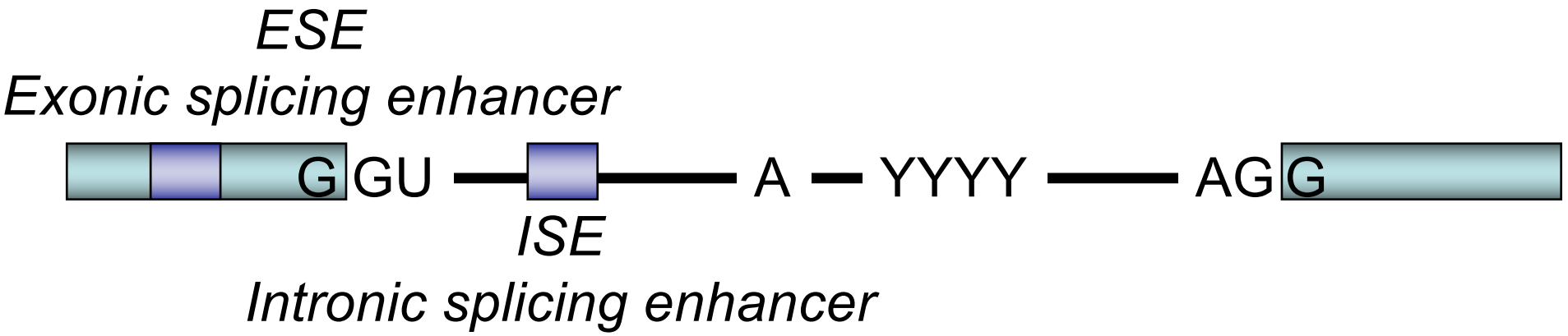


GU/AG Regel konserviert von Hefe bis Mensch.

Ca. 1% der Introns AT/AC -> Dann anderes Spliceosome

Branchpoint -> Lariat Bildung und Polypyrimidin Element (3')

Splicing, was brauchen wir?



GU/AG Regel konserviert von Hefe bis Mensch.

Ca. 1% der Introns AT/AC -> Dann anderes Spliceosome

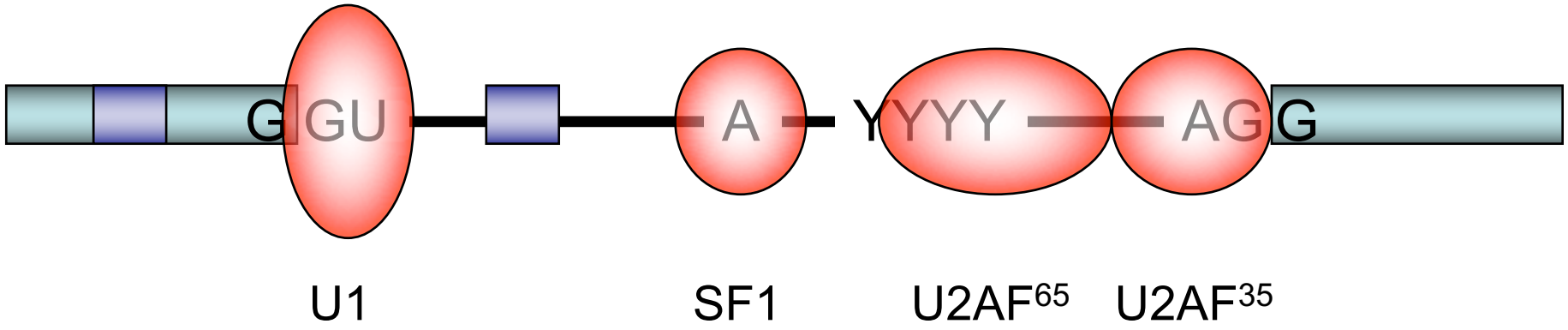
Branchpoint -> Lariat Bildung und Polypyrimidin Element (3')

ESE und ISE Elemente. Exon definition über ESE.

Introns oft wesentlich größer als Exons

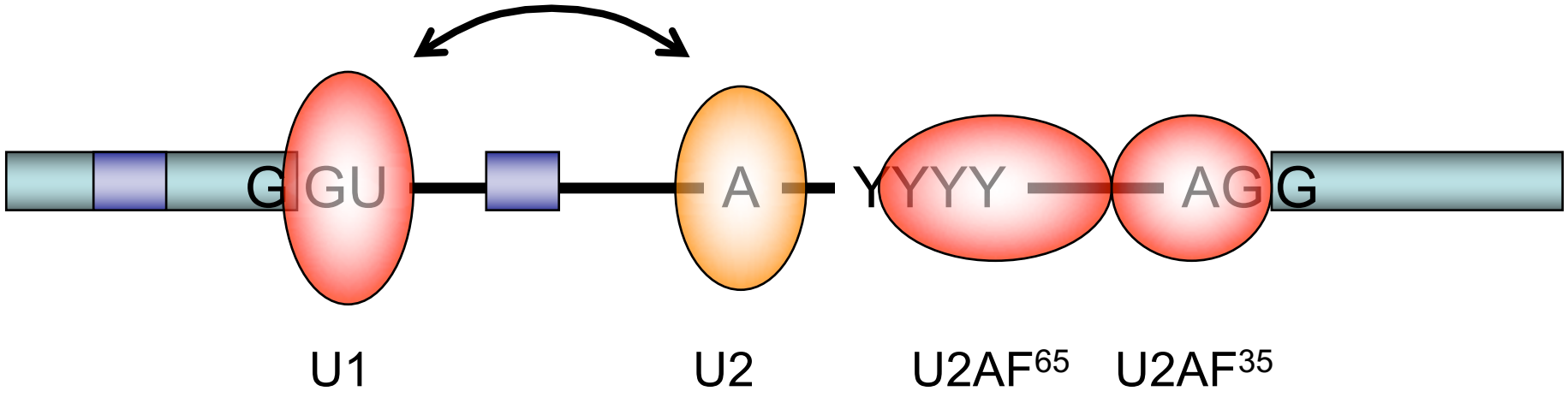
Inhibitorische Elemente: ESI und ISI

Splicing, der E Complex



snRNA: U1

Splicing, der A Complex



snRNA: U1 U2 **B Complex: U5 U4/U6 ; hnRNP**

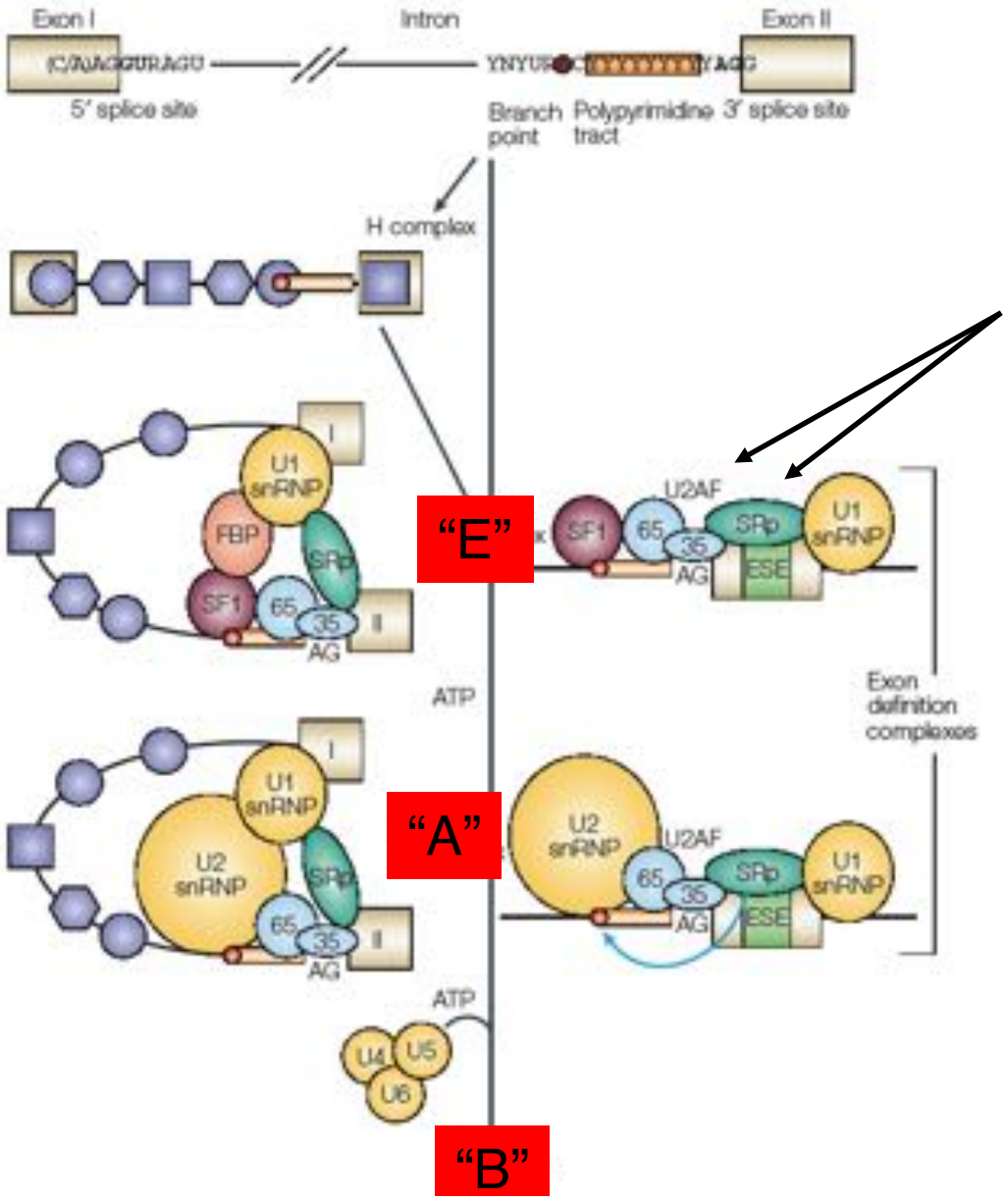
snRNP: "*snurps*" mit jeweils >10 Proteinen assoziiert
U1-snRNP U2-snRNP usw.

Sm Proteine kommen in allen *snurps* vor.

Lupus erythematodes, Autoimmunerkrankung

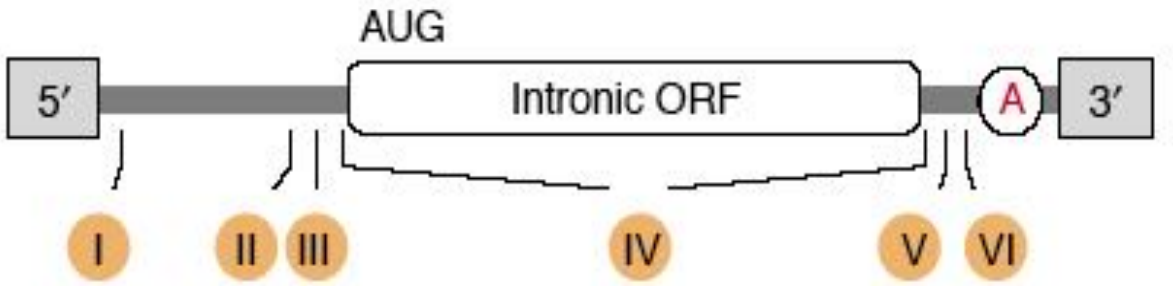


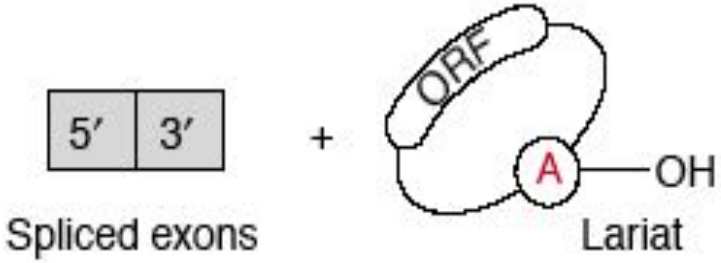
Spliceosome Assembly geschieht über den Exon definition complex



SR Proteine binden am ESE und initiieren die Bildung eines Spliceosoms

Ursprung des Splicens ?
Typ II Introns enthalten ORFs

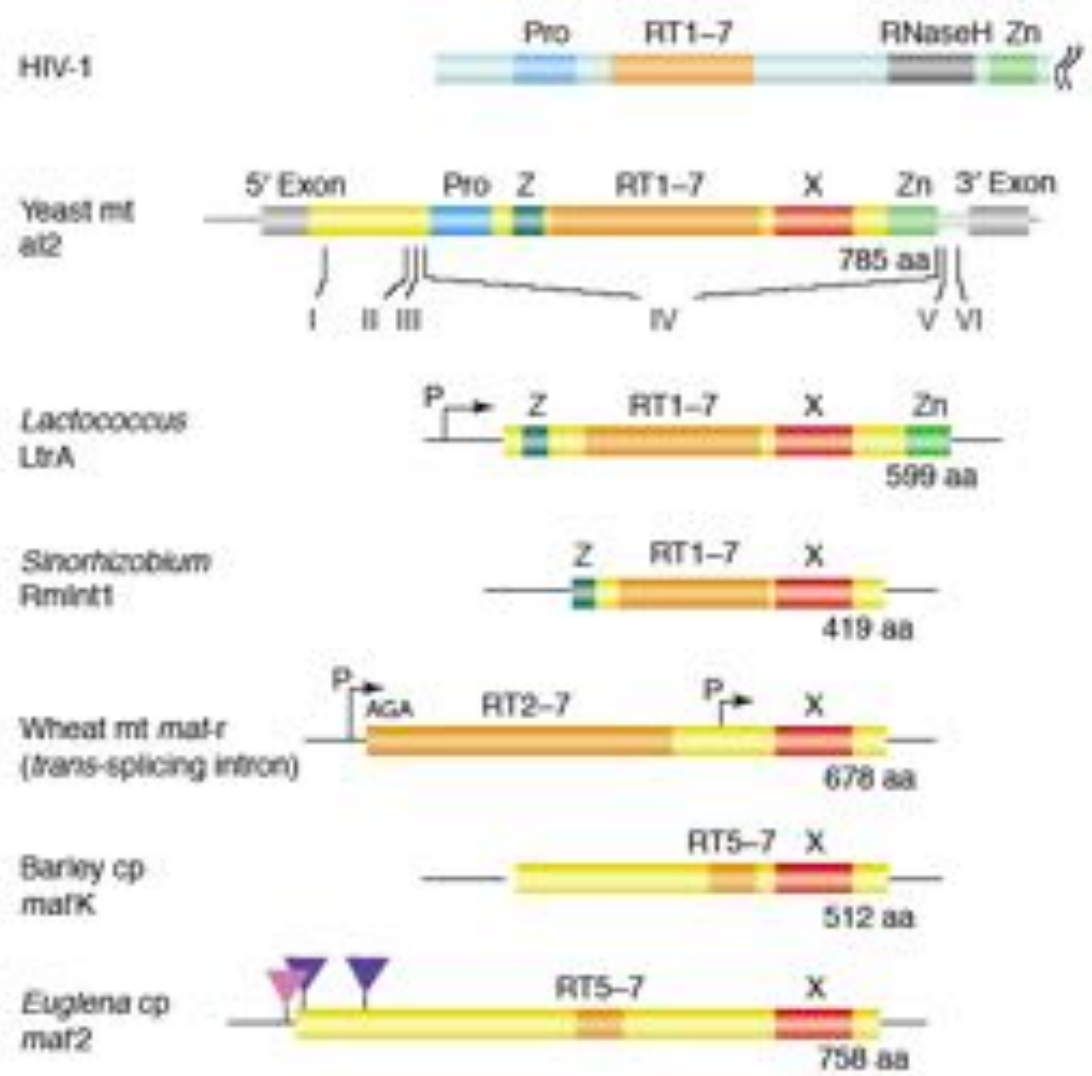




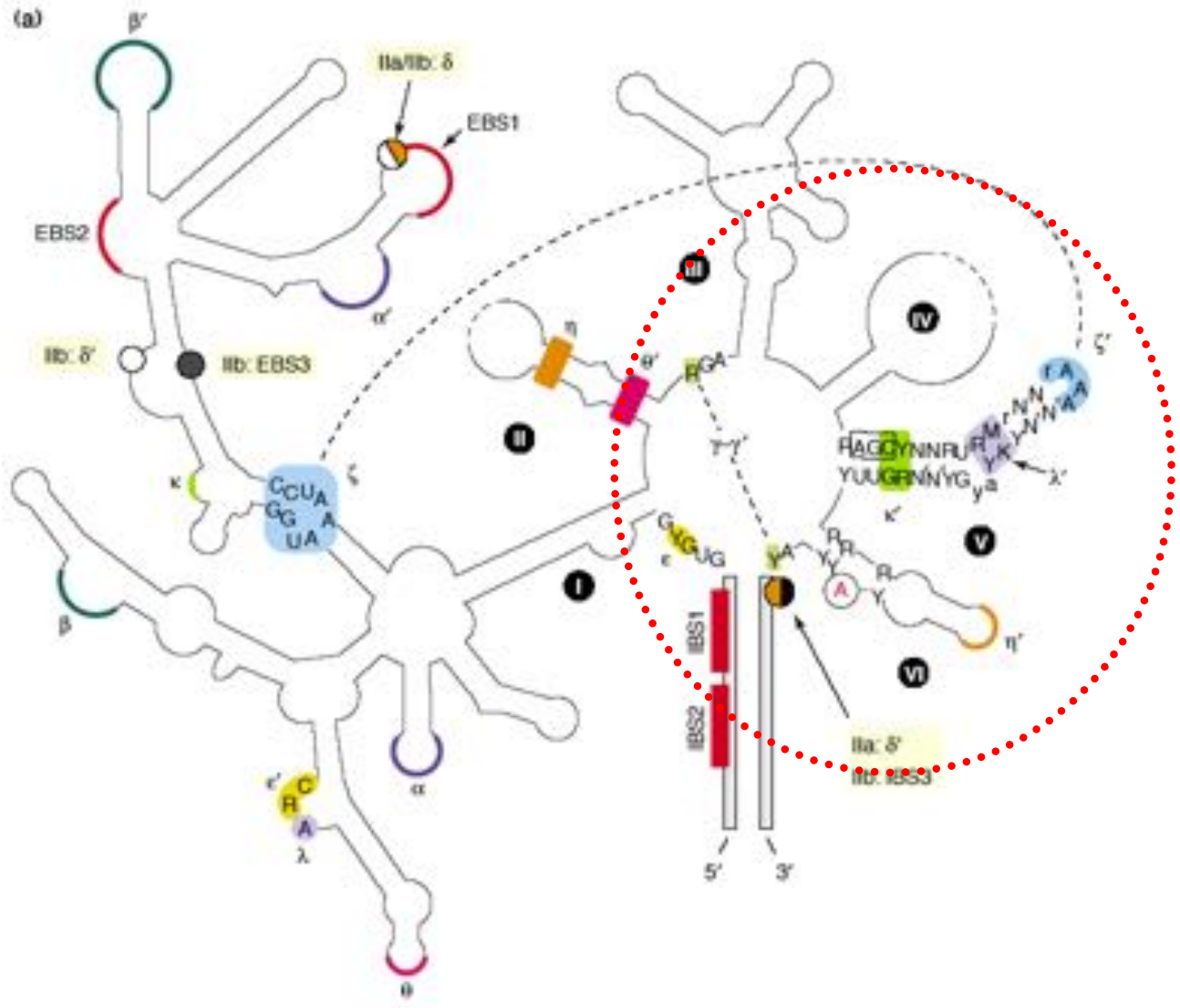
Ursprung des Splicens ?
Typ II Introns enthalten ORFs

Virus

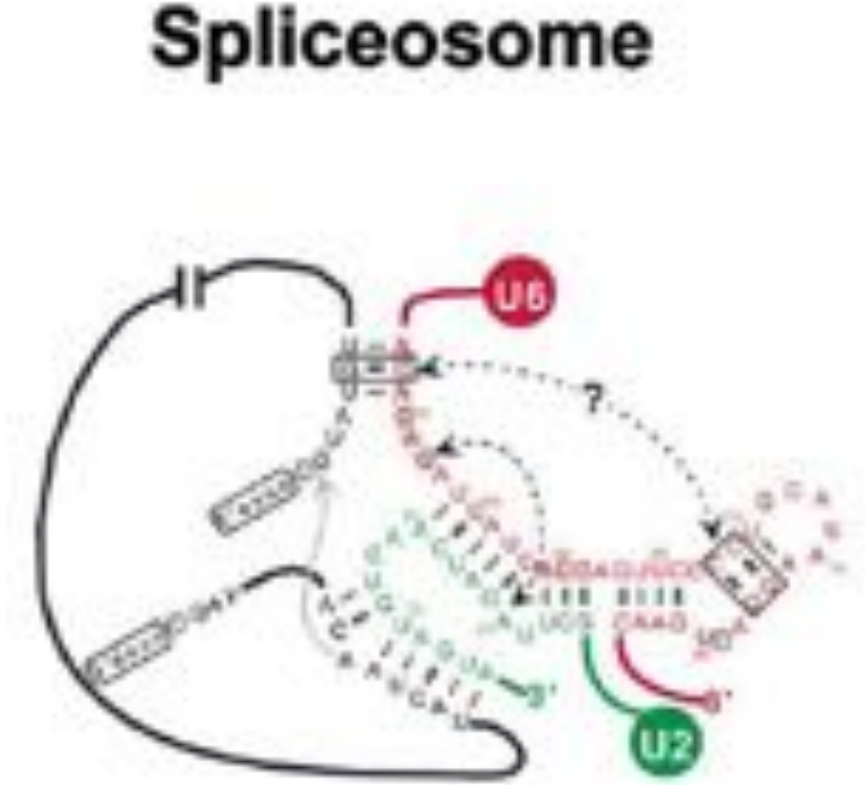
Typ II Introns



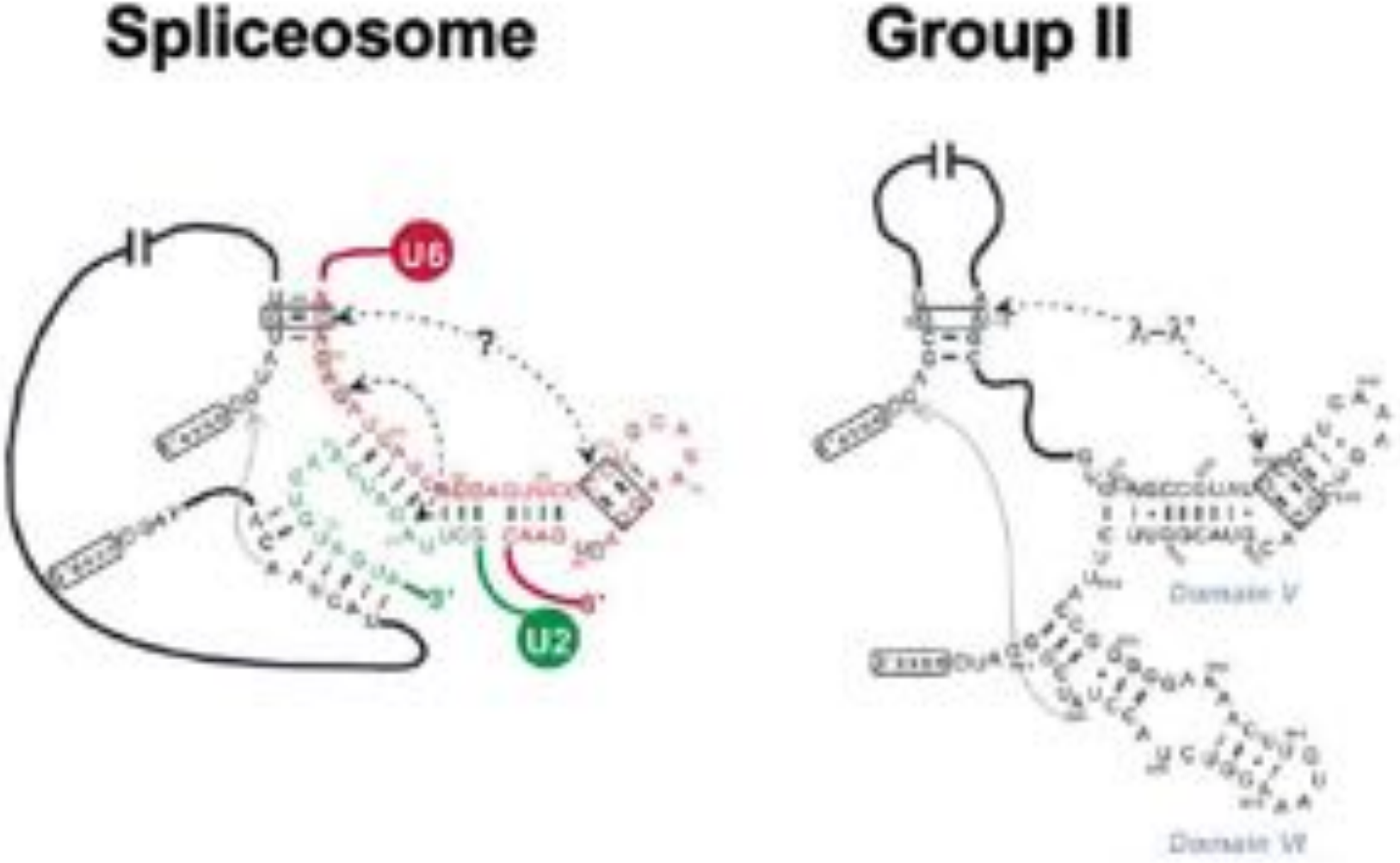
Ursprung des Splicens ?
Komplexe Faltung des Typ II Introns



Ähnlichkeiten zwischen Typ II Introns und Spliceosomen



Ähnlichkeiten zwischen Typ II Introns und Spliceosomen



Spliceosome-Assembly

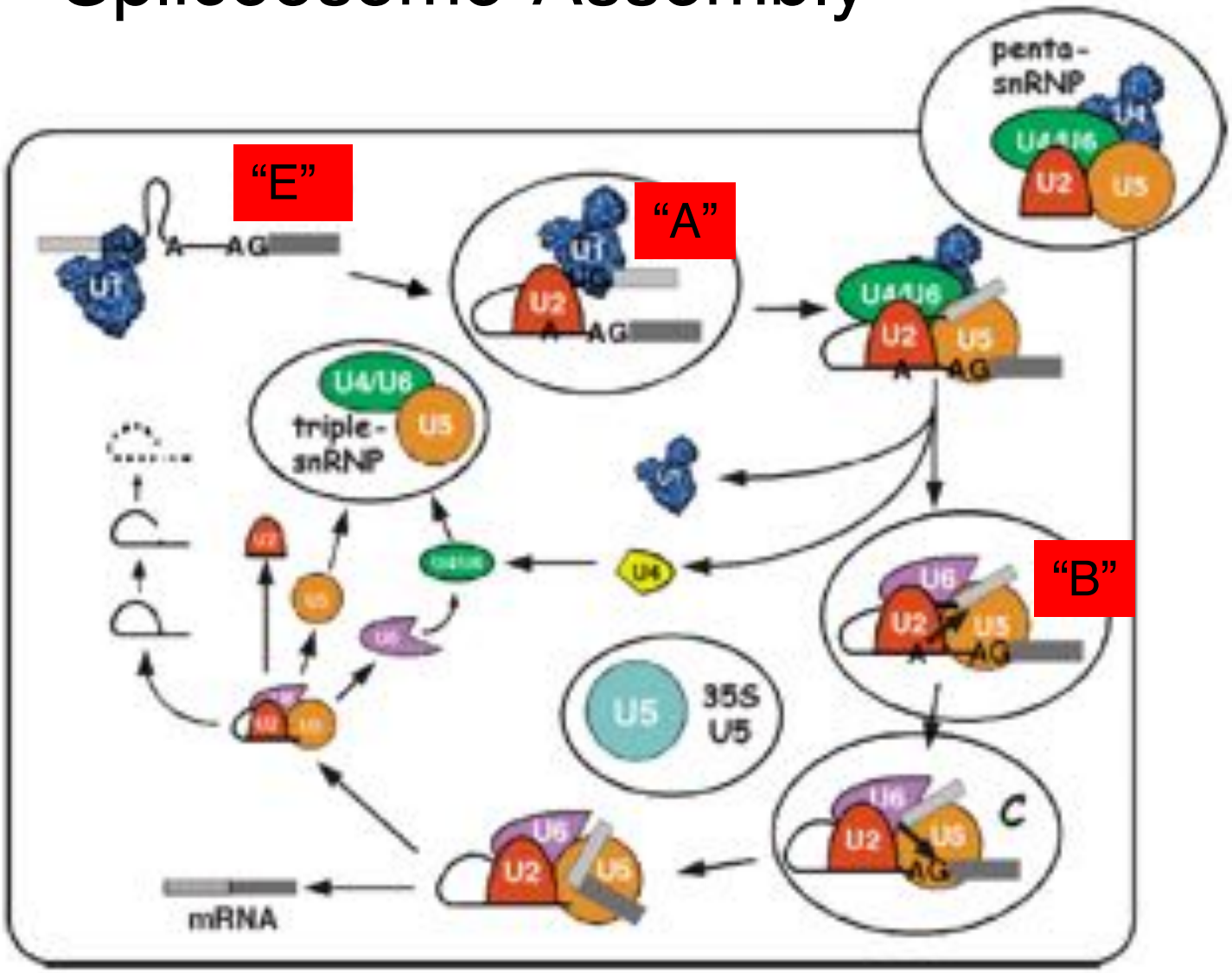
Pre-mRNA

U1

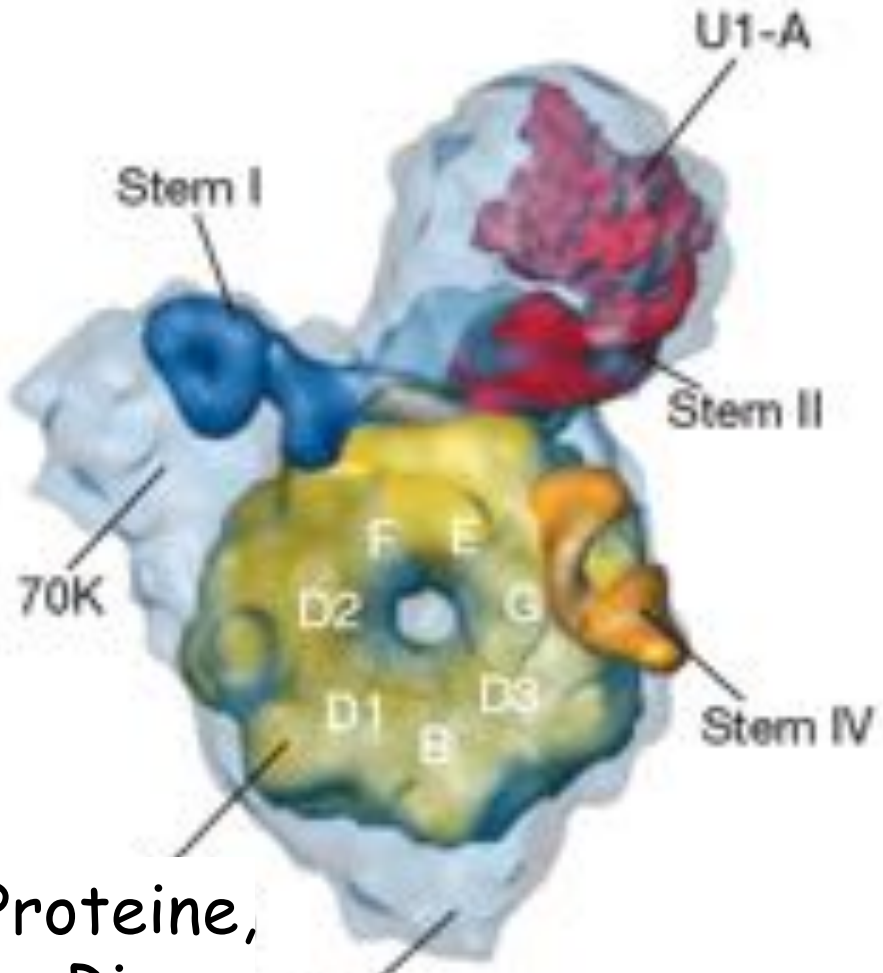
U2

U5
U4/U6

mRNA



Aufbau der U1-snRNP

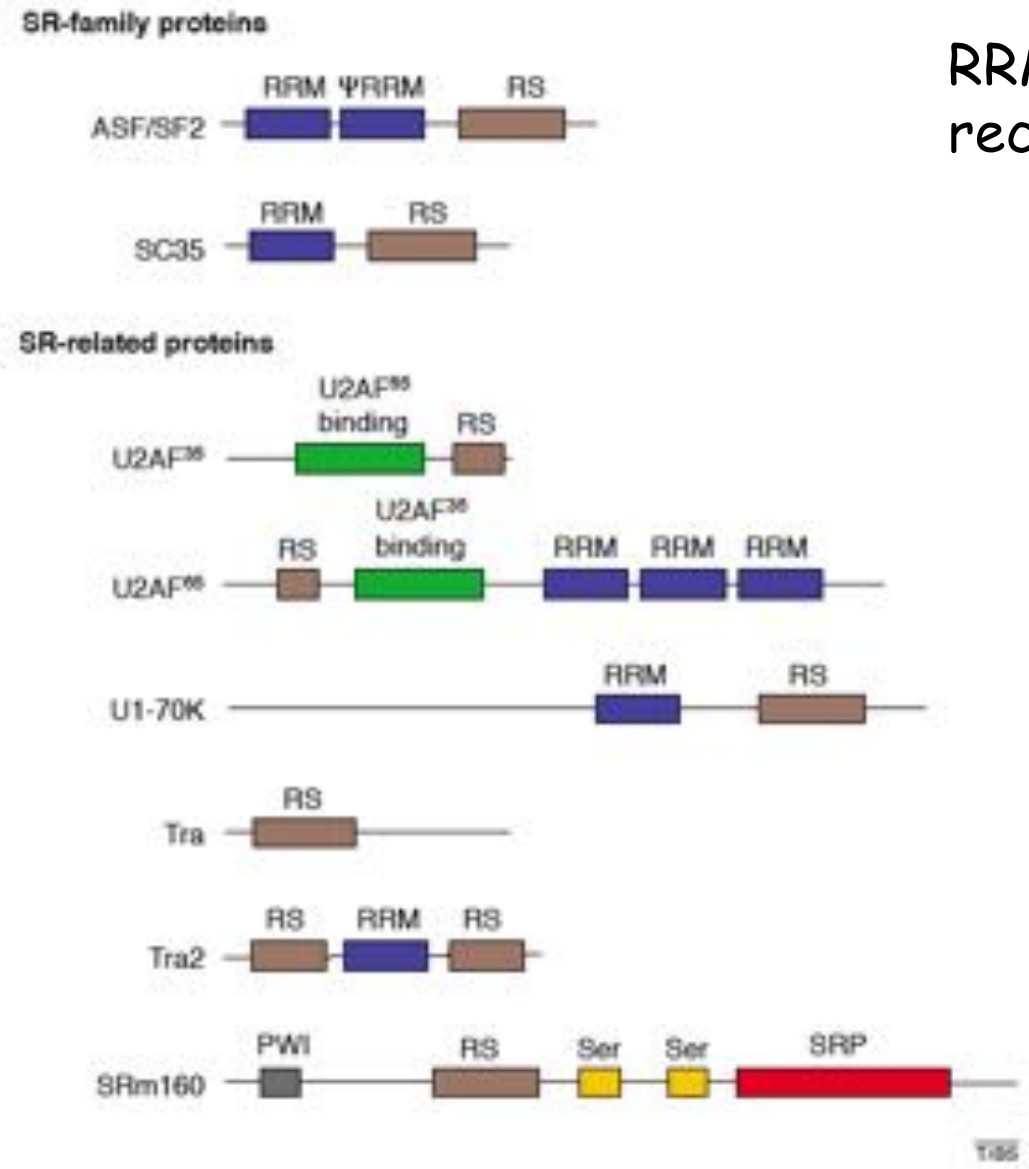


Sm Proteine,
7er Ring



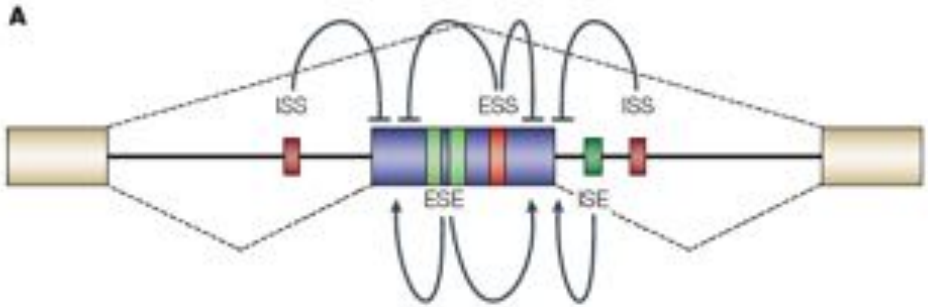
U1 RNA

Die SR-Proteine binden RNA und sind wichtige Splicing Co-Faktoren



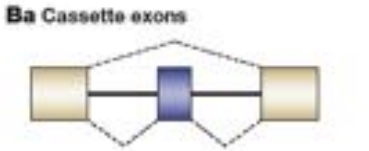
RRM = RNA recognition motif

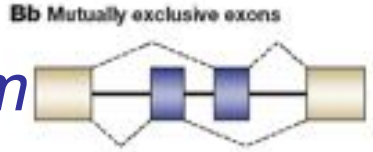
Prinzipielle Möglichkeiten des alternativen Splicings



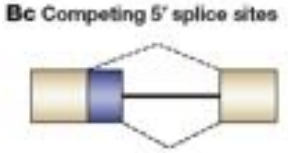
Erhöhung der Anzahl von Proteinformen, die von einem Gen generiert werden können.

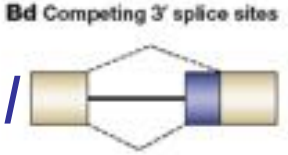
Regulatorische Funktion.



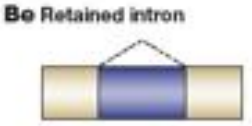


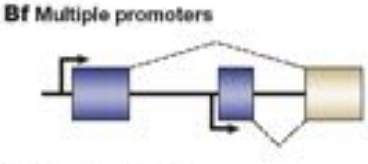
Dscam





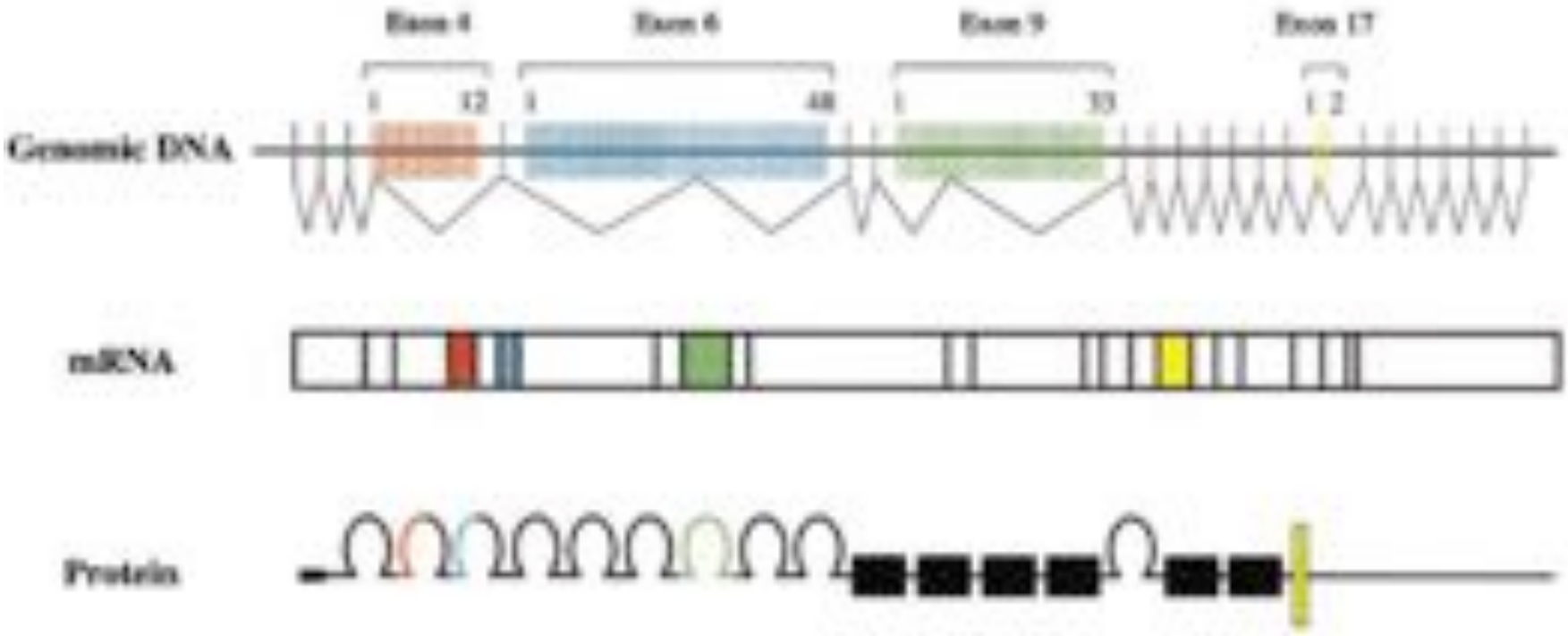
Sex lethal





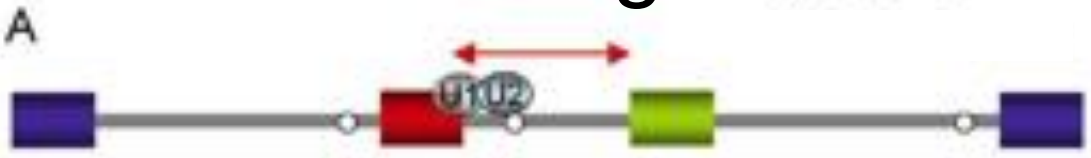
Beispiel für Splicing I

Dscam Erhöhung der Anzahl von Proteintypen.
Ein Gen > 38.000 Isoformen.

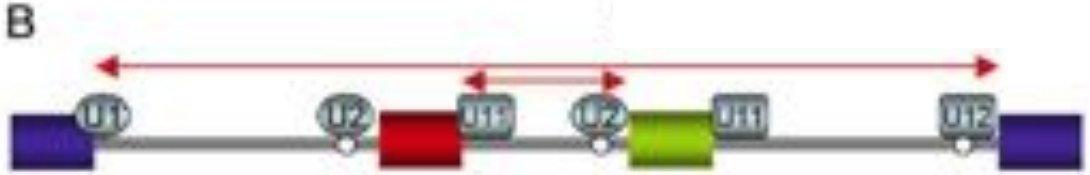


Wie wird das alternative Splicing kontrolliert? Erklärungsmodelle

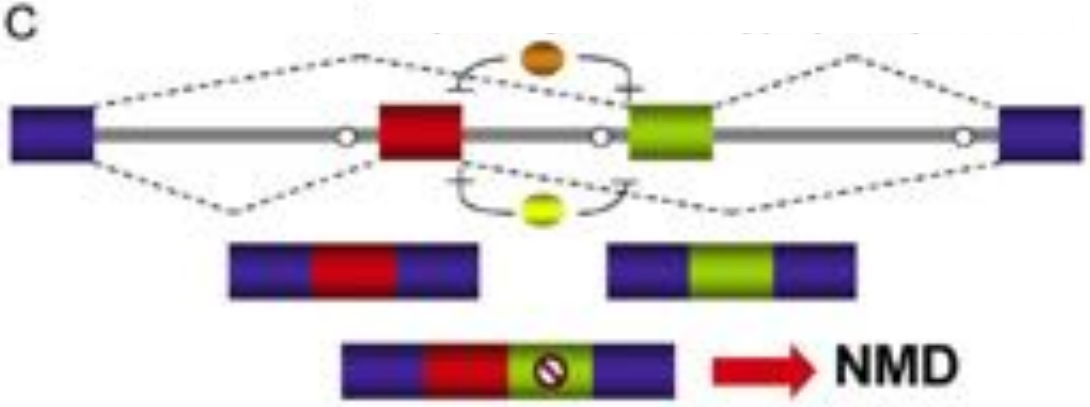
Sterische Interferenz



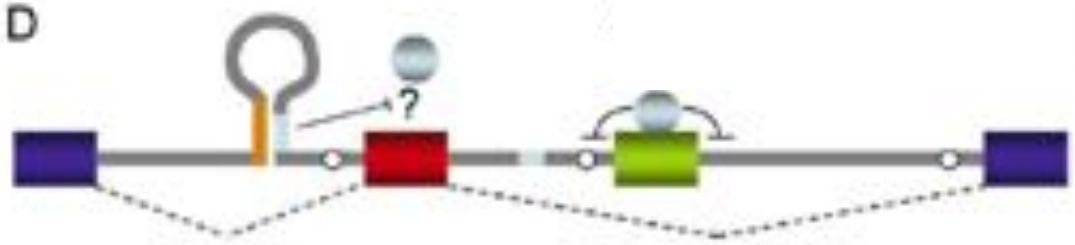
Unterschiedliche Spliceosomen



NMD



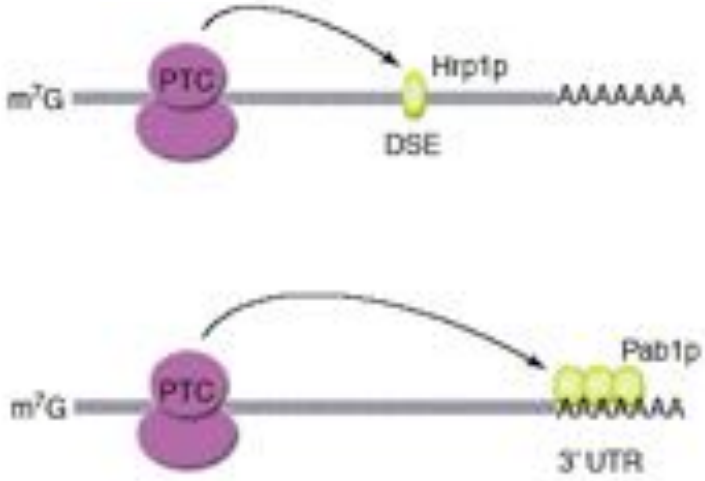
Sequenz Interaktion Dscam



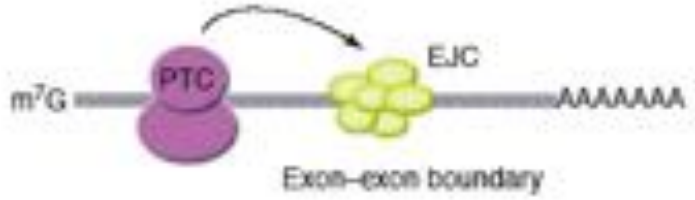
Unterschiedliche Arten der PTC-Erkennung bei der NMD

Erkennung des PTC
(premature termination codon)

Hefe, Drosophila

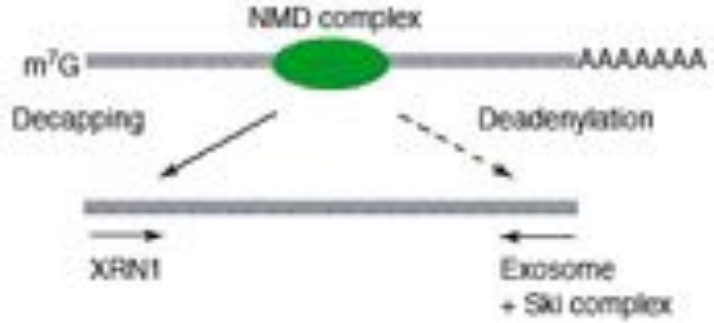


Säuger

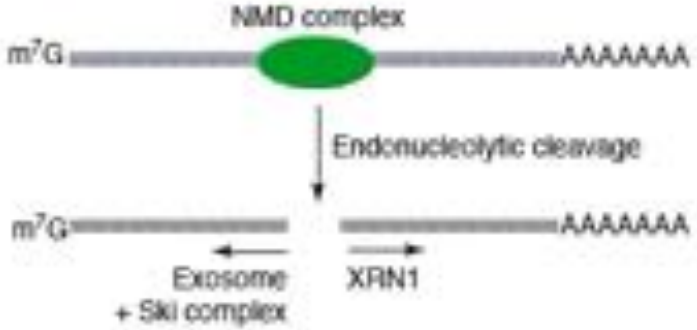


Abbau der RNA

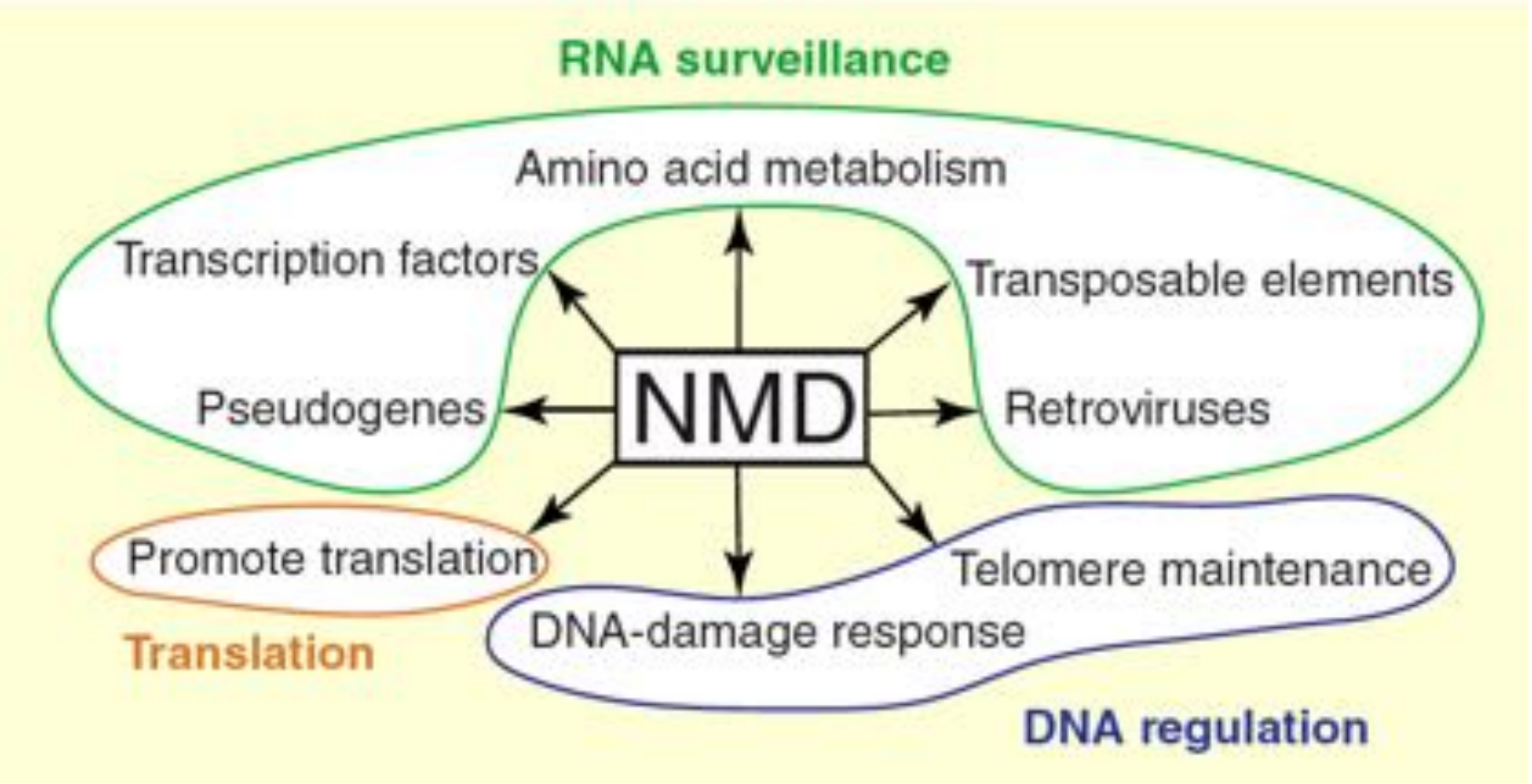
Hefe, Säuger



Drosophila

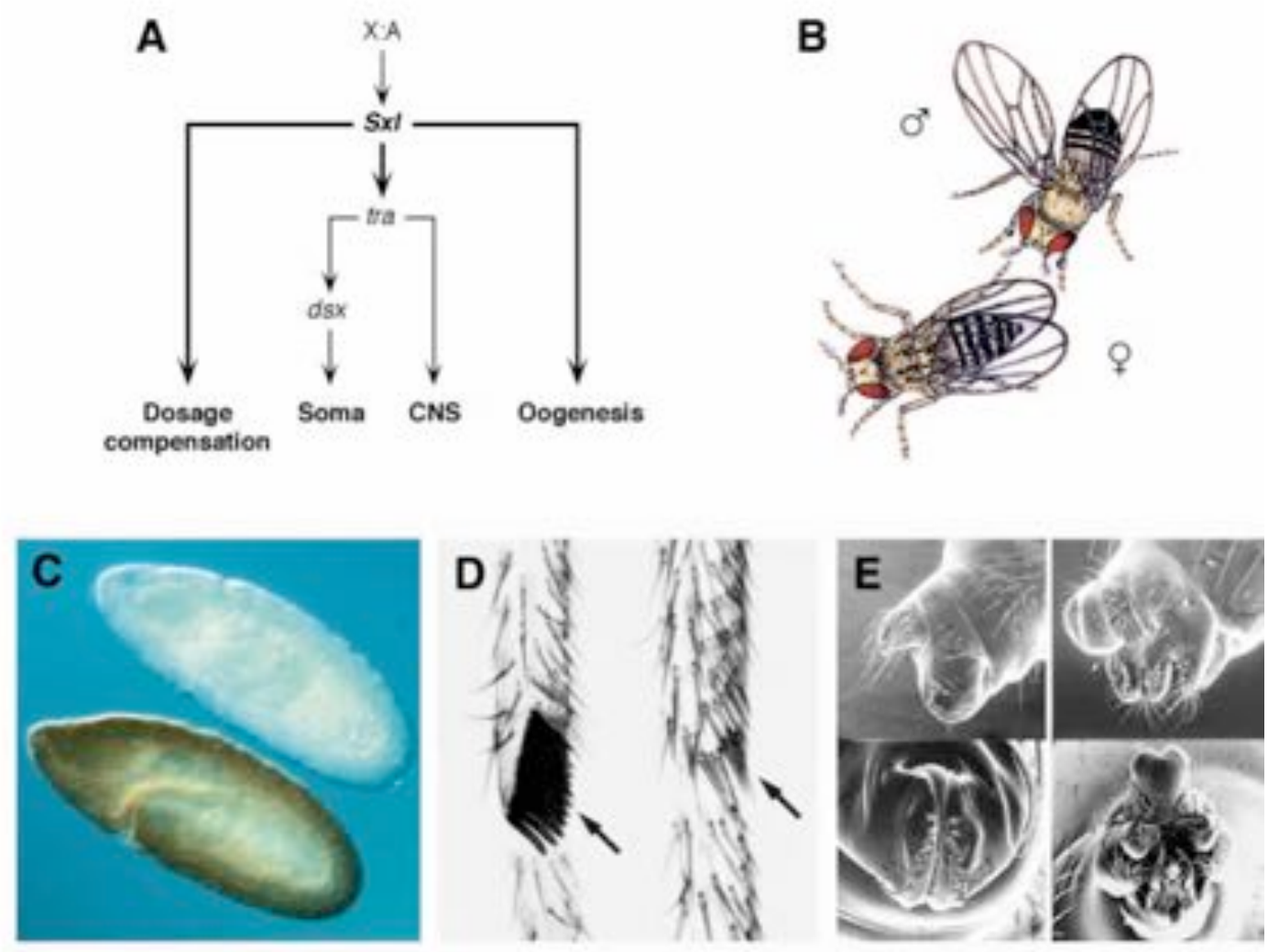


Vielfältige Funktionen der NMD



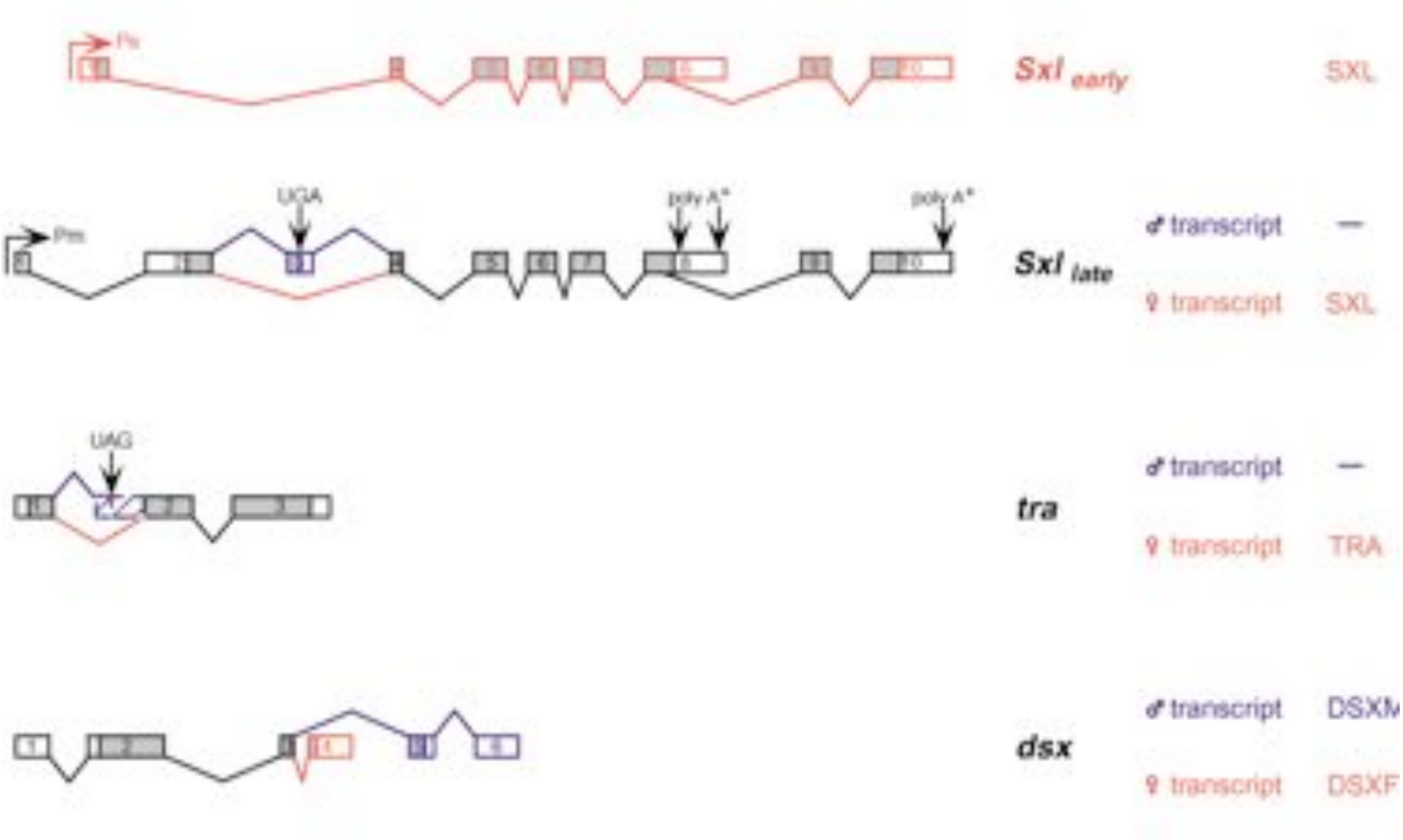
Beispiel für Splicing II

Sex lethal Splicing als Regulatorische Funktion bei der Geschlechtsbestimmtung von Drosophila

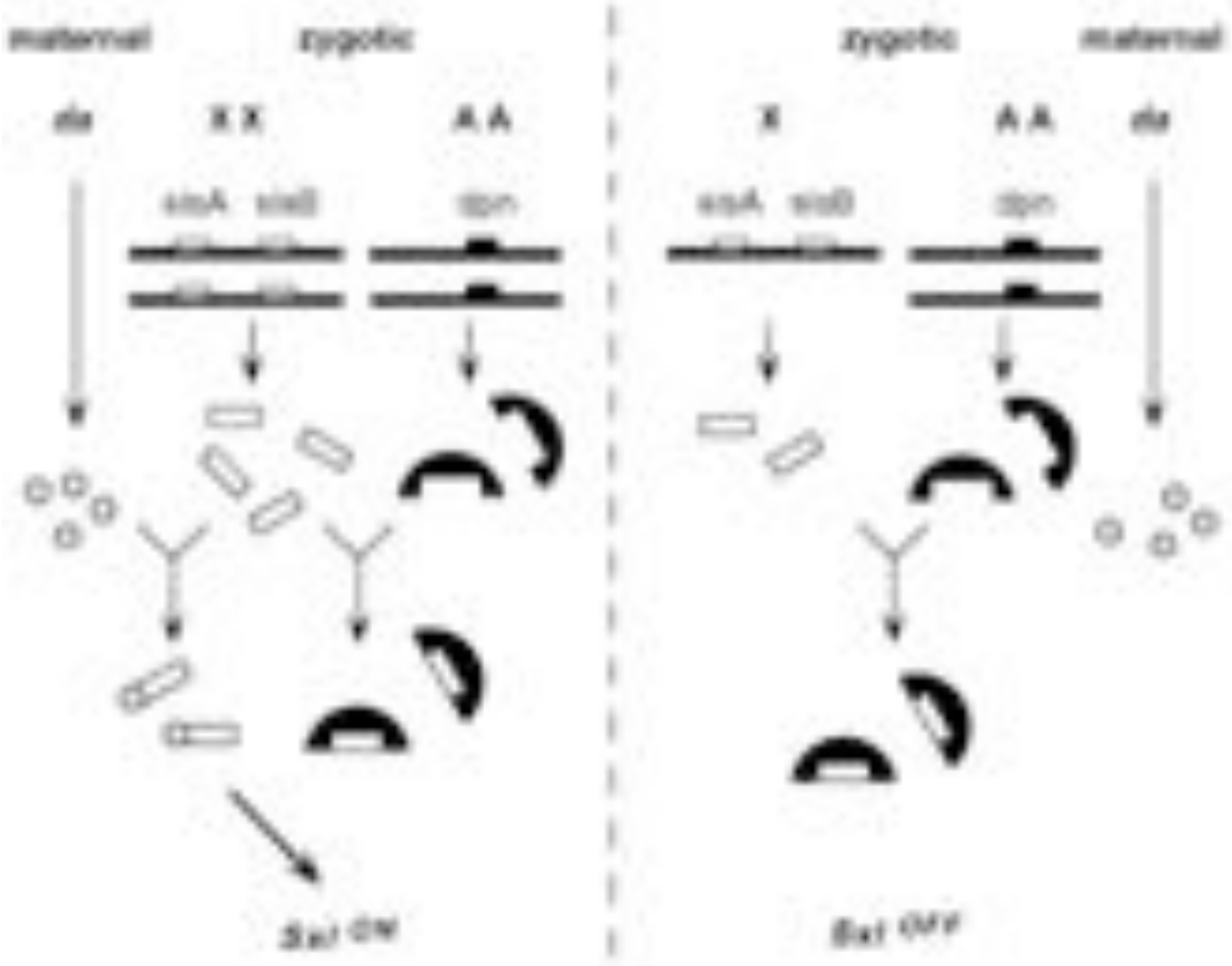


Beispiel für Splicing II

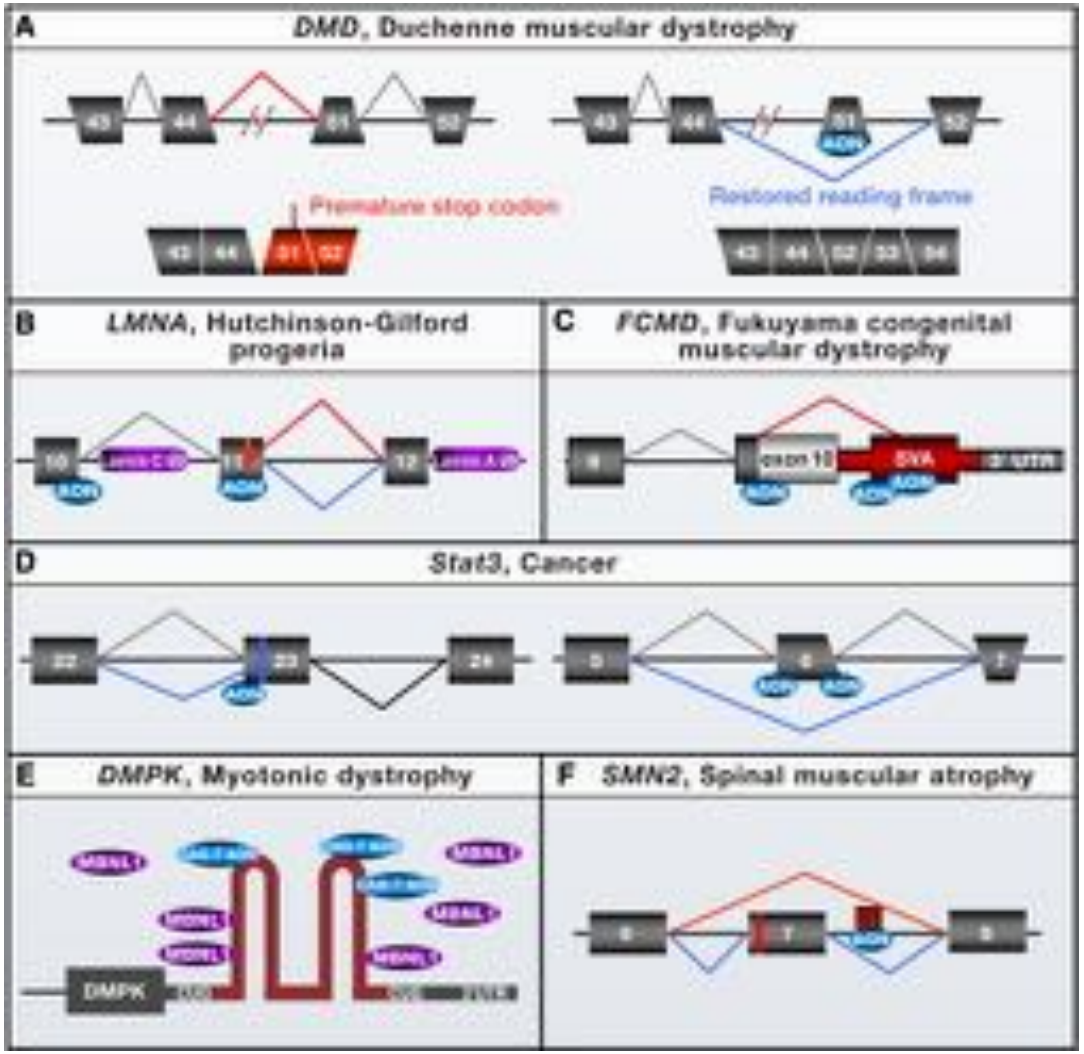
Sex lethal Splicing als Regulatorische Funktion bei der Geschlechtsbestimmtung von Drosophila



Sex lethal II Zählen der X-Chromosomen



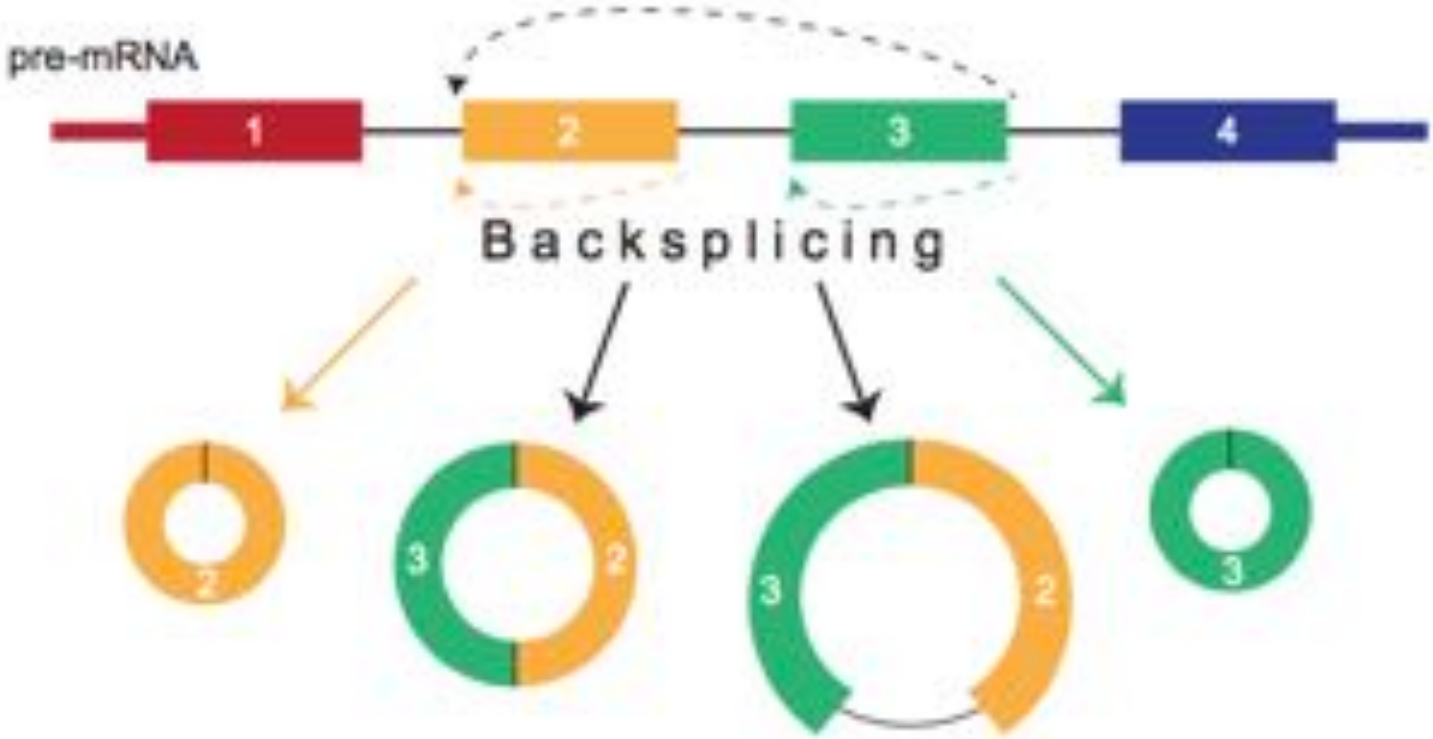
Splicing und Krankheiten



Quaking (Maus) KH-Protein, Splicing, Transport, Stabilität

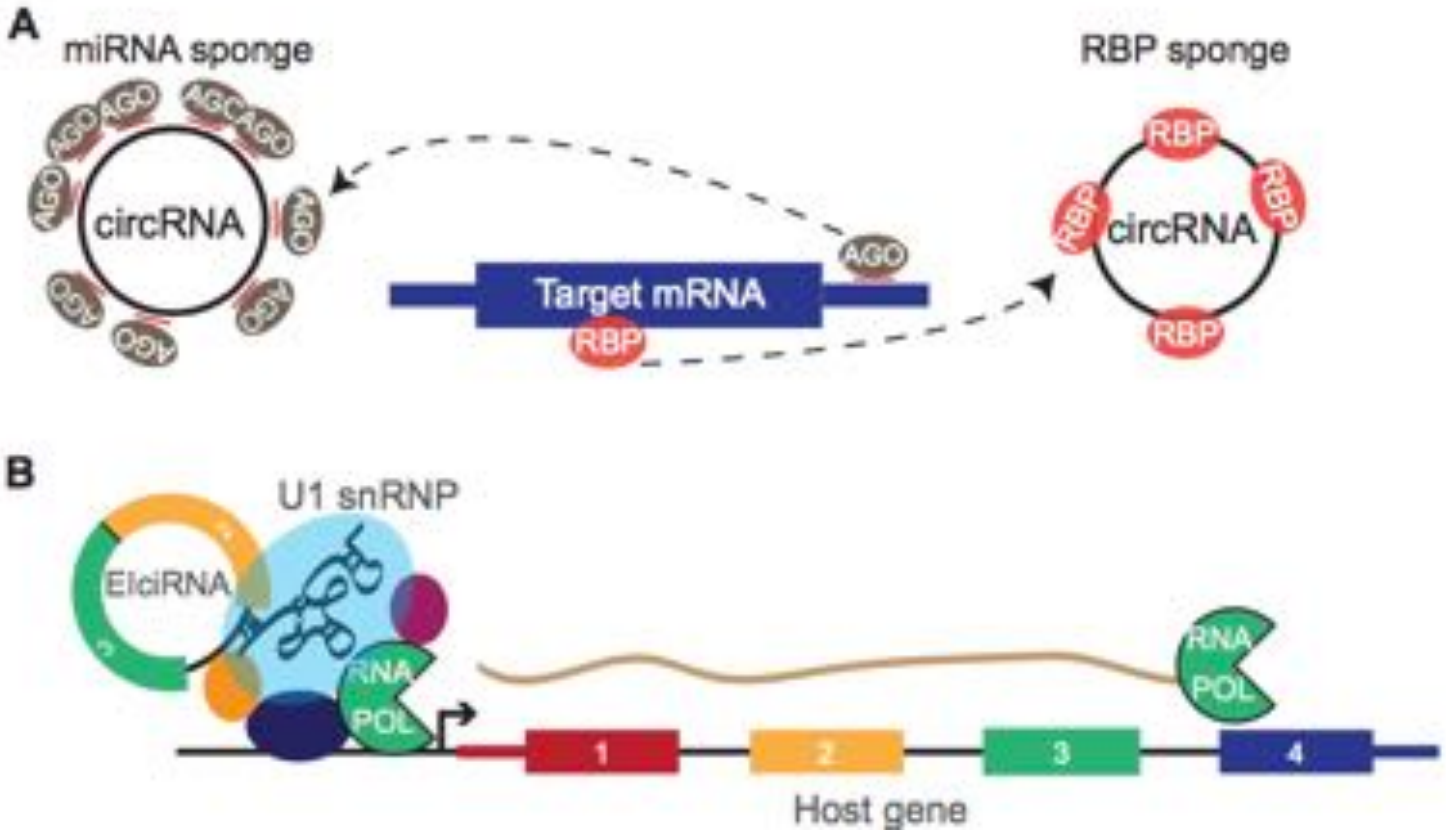
-> Demyelinisierungsdefekt

Splicing und circuläre RNA (circRNA)



{Patop:2018fu, Barrett:2016eo, Holdt:2018ft}

Funktionen der circRNA



{Patop:2018fu, Barrett:2016eo, Holdt:2018ft}

Formen der RNA Qualitätskontrolle

mRNA

NMD Nonsense-mediated decay (zu frühe Termination der Translation; Rekrutierung von Upf Proteinen zum Terminationskomplex Decapping; 5'->3' Abbau; Endonuklease)

NSD Nonstop decay (Kein Stop Codon; Ski7p Rekrutierung; 3'->5' Abbau; Endonuklease)

NGD No-Go decay (Starke Translationsverzögerung; Rekrutierung von Hbs1p und Dom34p; Endonuklease; Exonuklease)

REMD Ribosome extension-mediated decay (Translation über normales Stop-Codon; Entfernung von 3' UTR bindenden Proteinen)

tRNA

RTD rapid tRNA decay

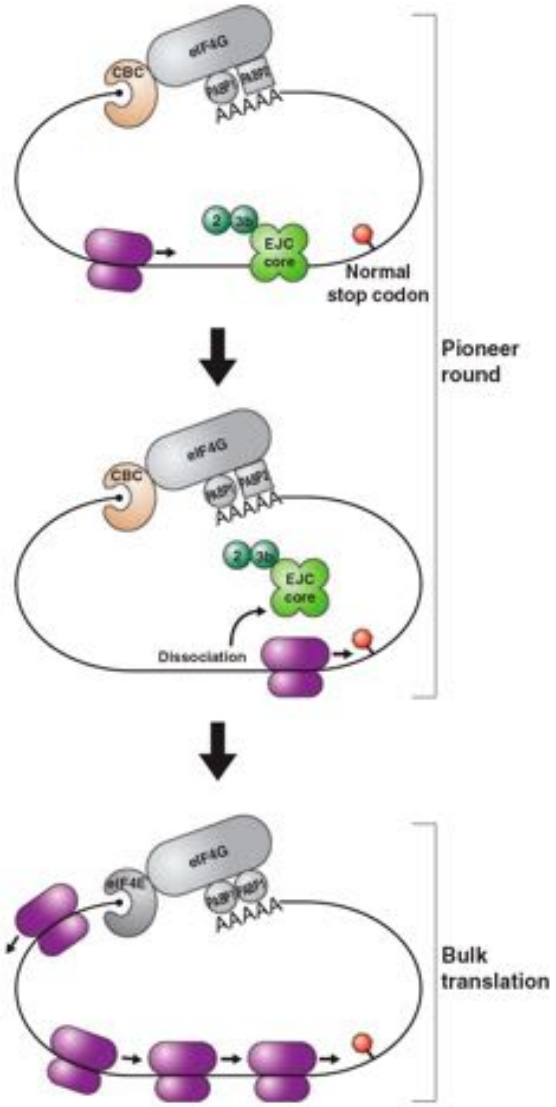
rRNA

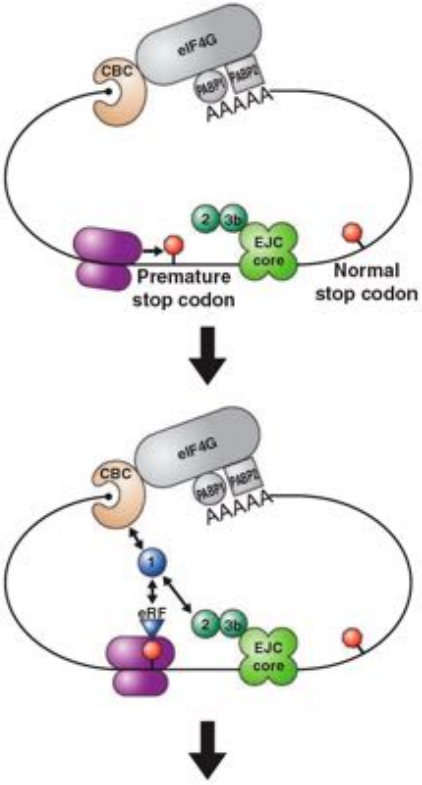
NRD Non functional rRNA decay

NMD: Mechanismus der *premature stop codon (PTC)* Erkennung bei Säugern

Normale mRNA

Defekte mRNA

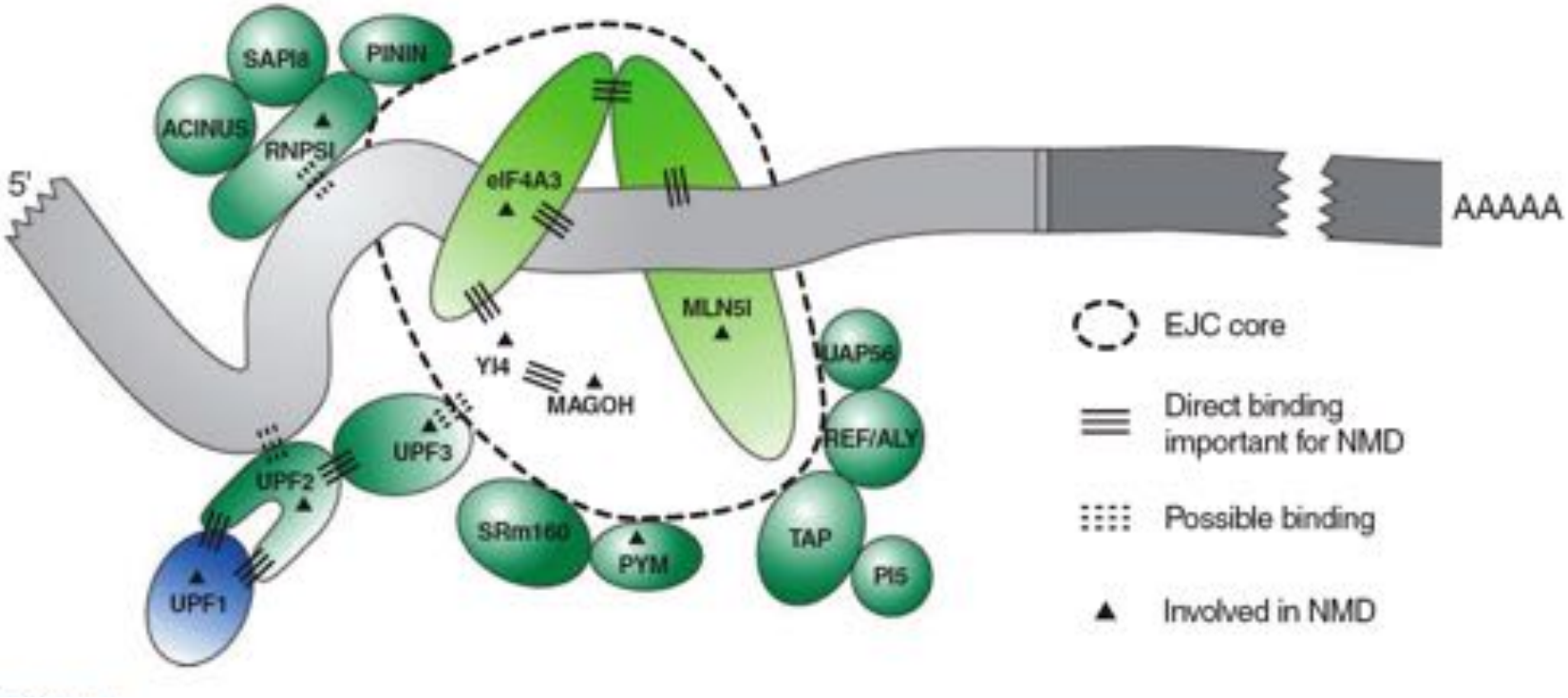




mRNA Abbau

Der Trigger des NMD ist die Bindung des *up-frameshift protein 1* (UPF1) an UPF-2.

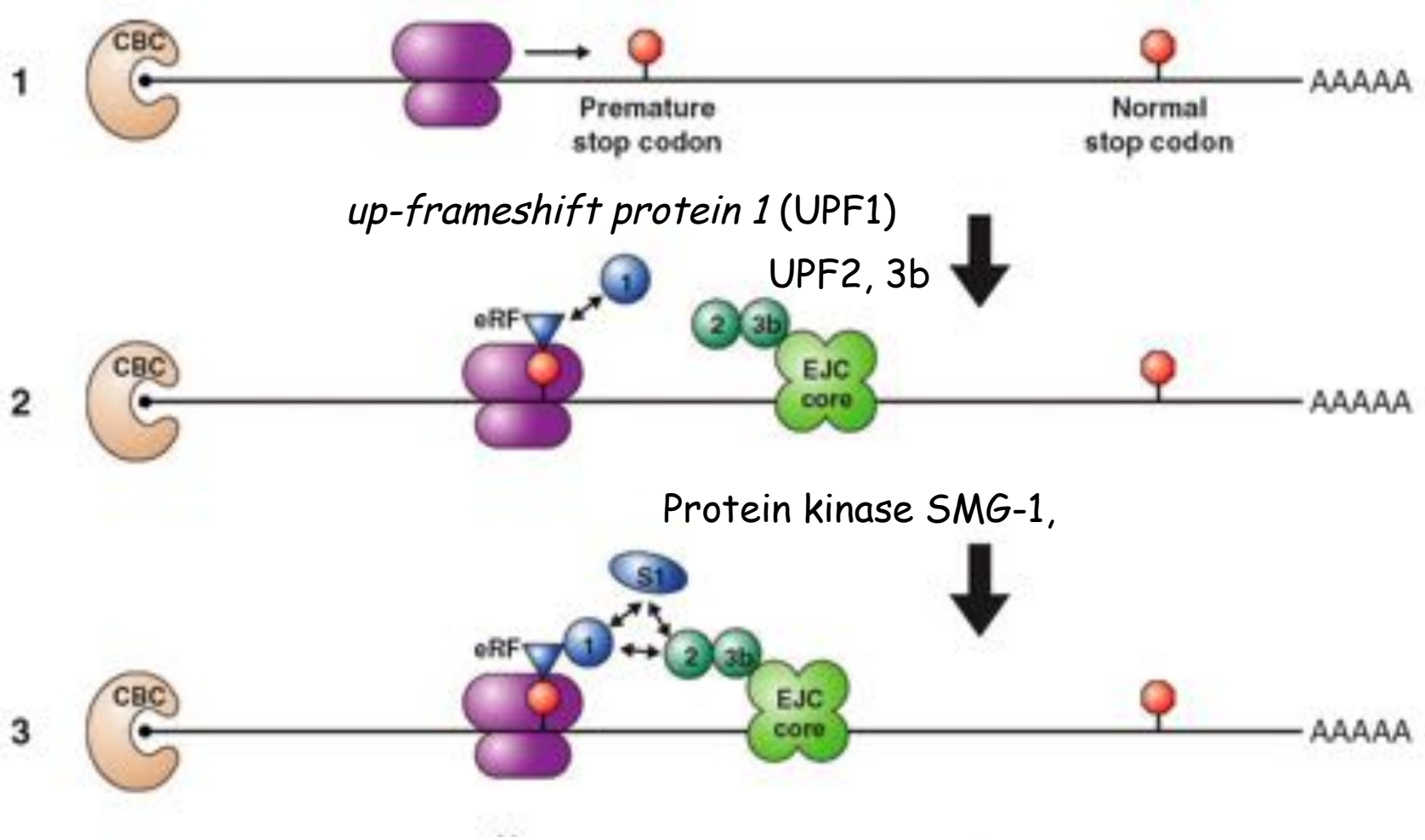
Modell des *Exon-Junction* Komplexes (EJC)



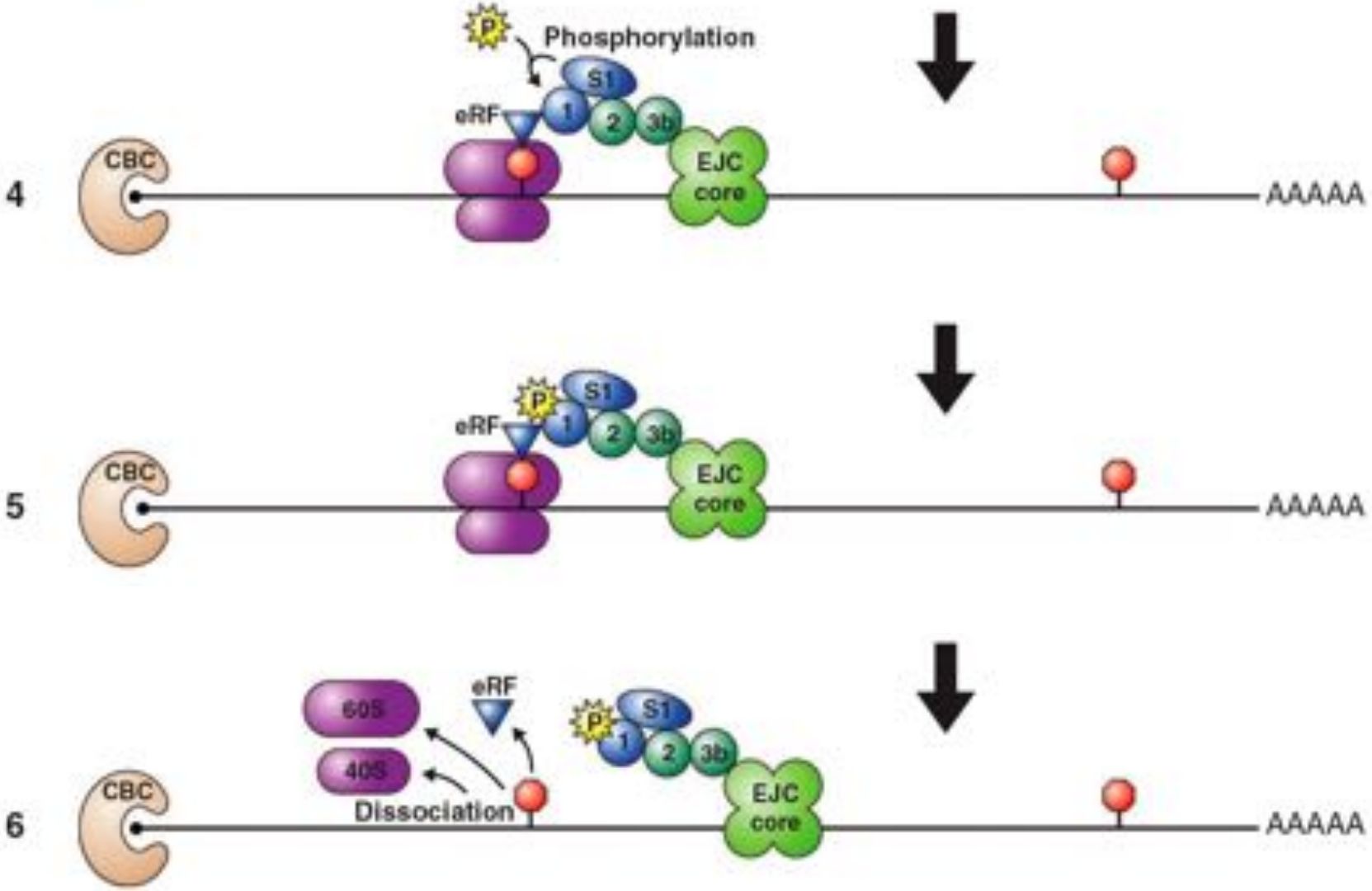
1. Funktion des EJC beim Transport der mRNA vom Kern in das Cytoplasma, 2. NMD, 3. Translationskontrolle

Vorbereitung der mRNA für den Abbau nonsense mediated mRNA decay (NMD)

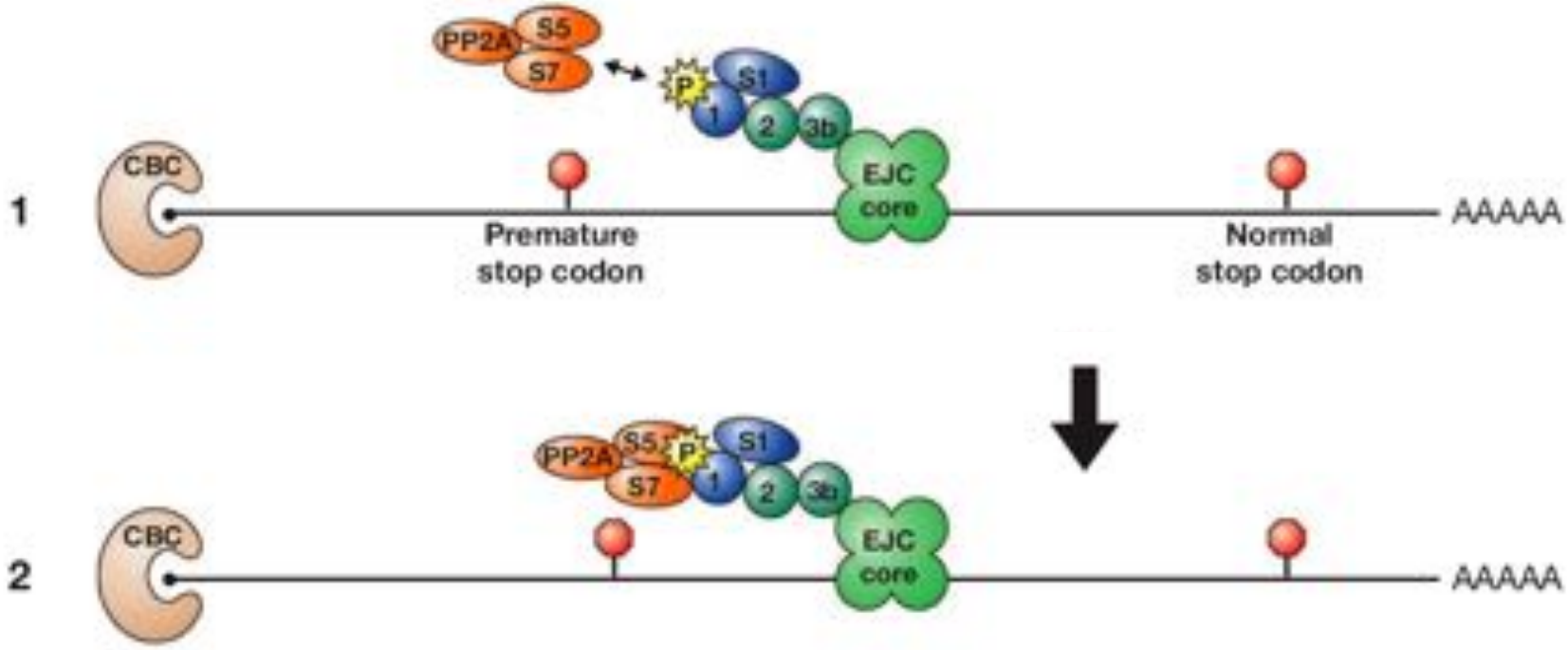
CBC: *C*ap-binding complex



Vorbereitung der mRNA für den Abbau nonsense mediated mRNA decay (NMD)

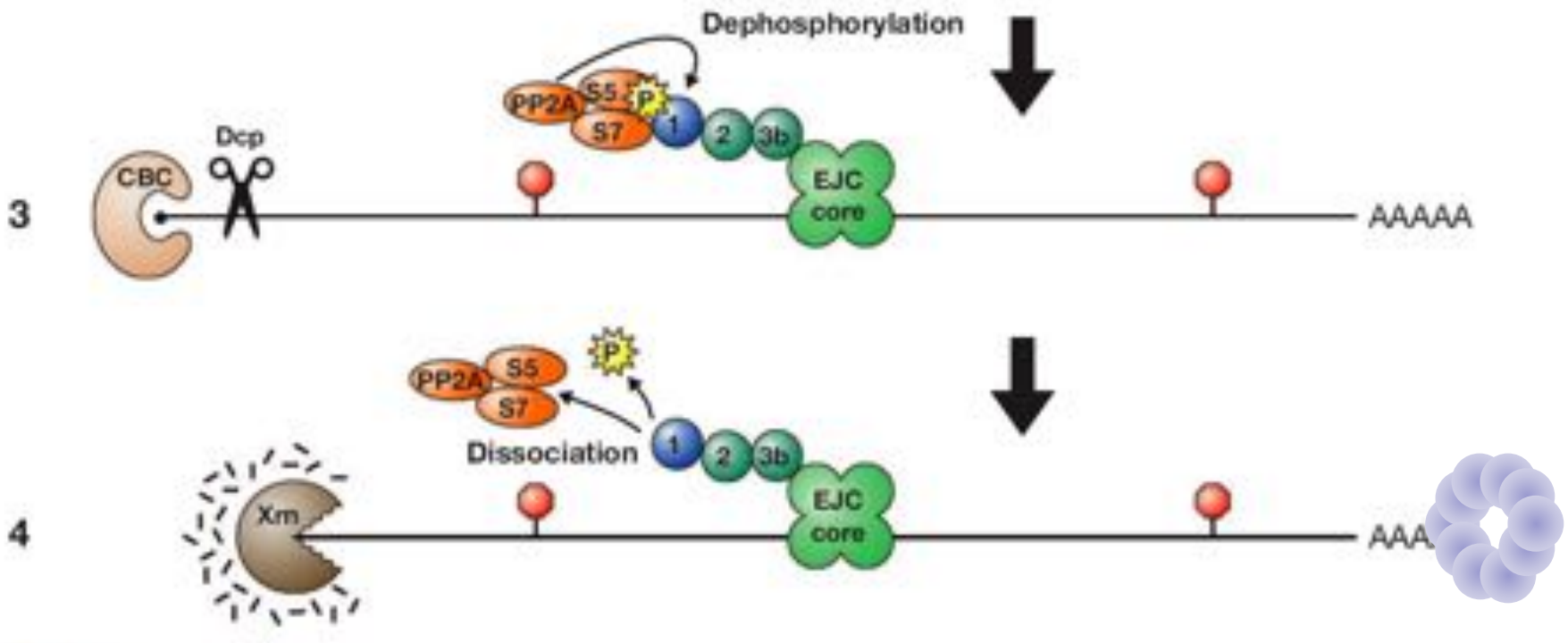


Vorbereitung der mRNA für den Abbau nonsense mediated mRNA decay (NMD)



SMG5, 7
PP2A: Protein phosphatase 2A

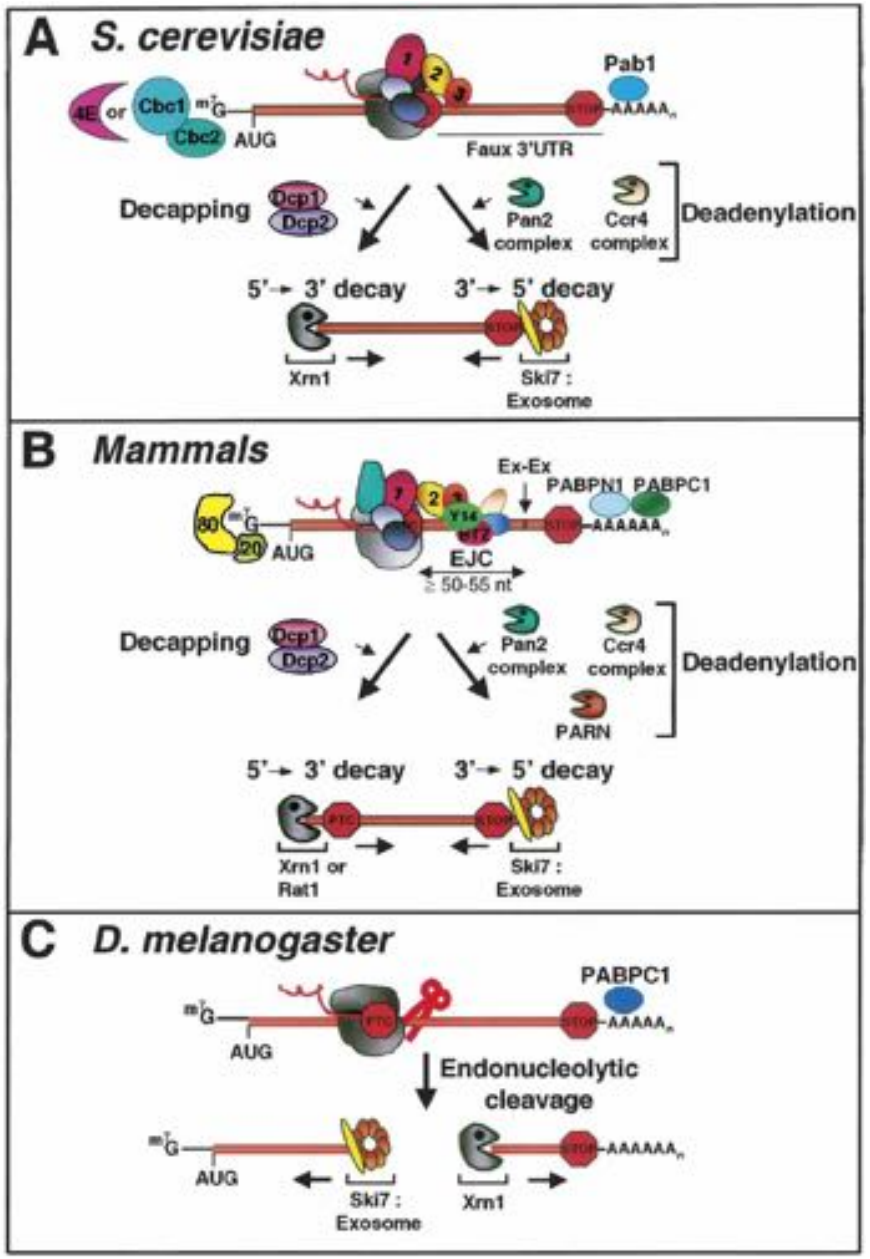
NMD



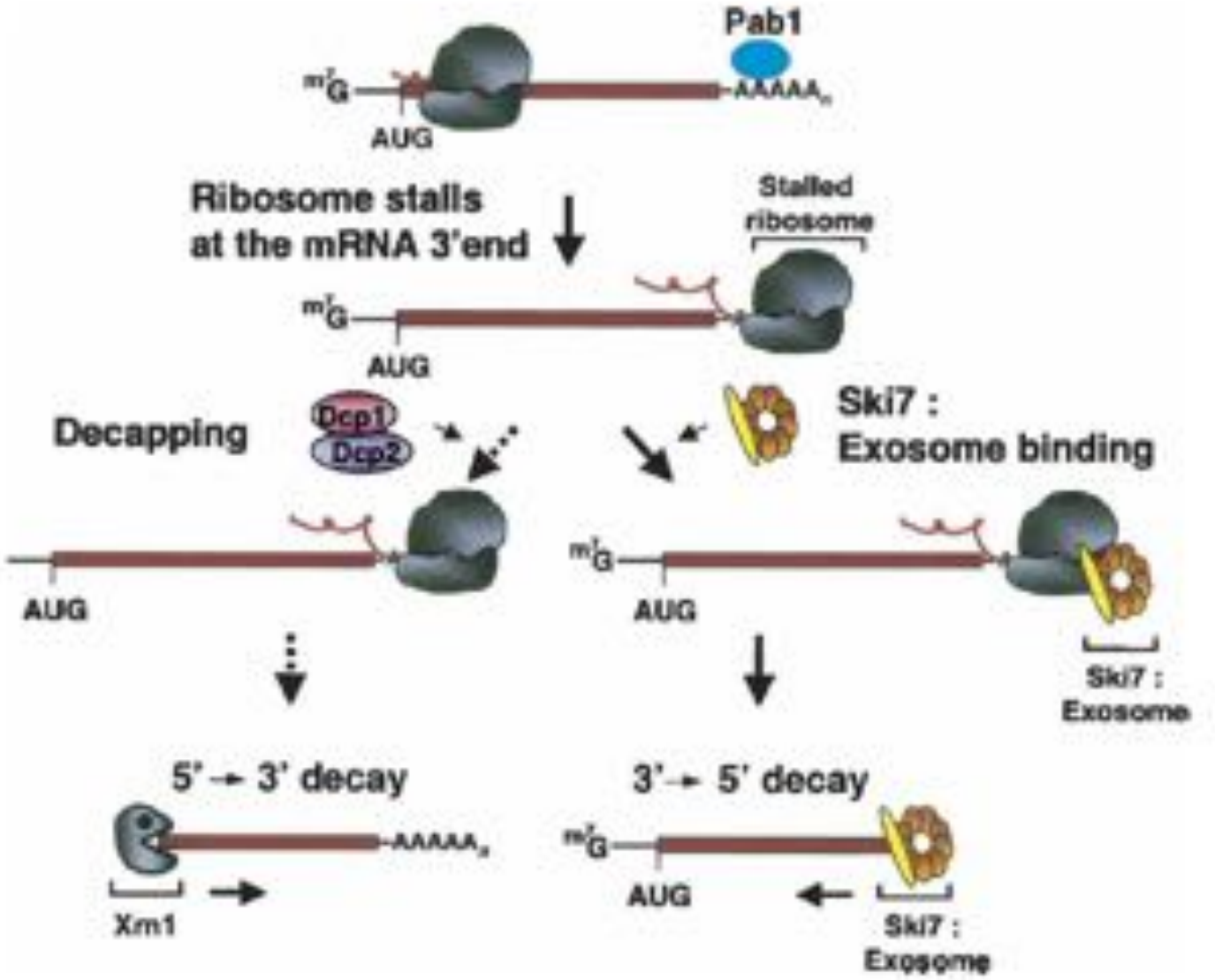
Exosome: 10 SU Komplex mit 3'->5' Exonuklease Aktivität. (Ski7)

Xrn1p 5'->3' Exonuklease. Erfordert uncapped mRNAs

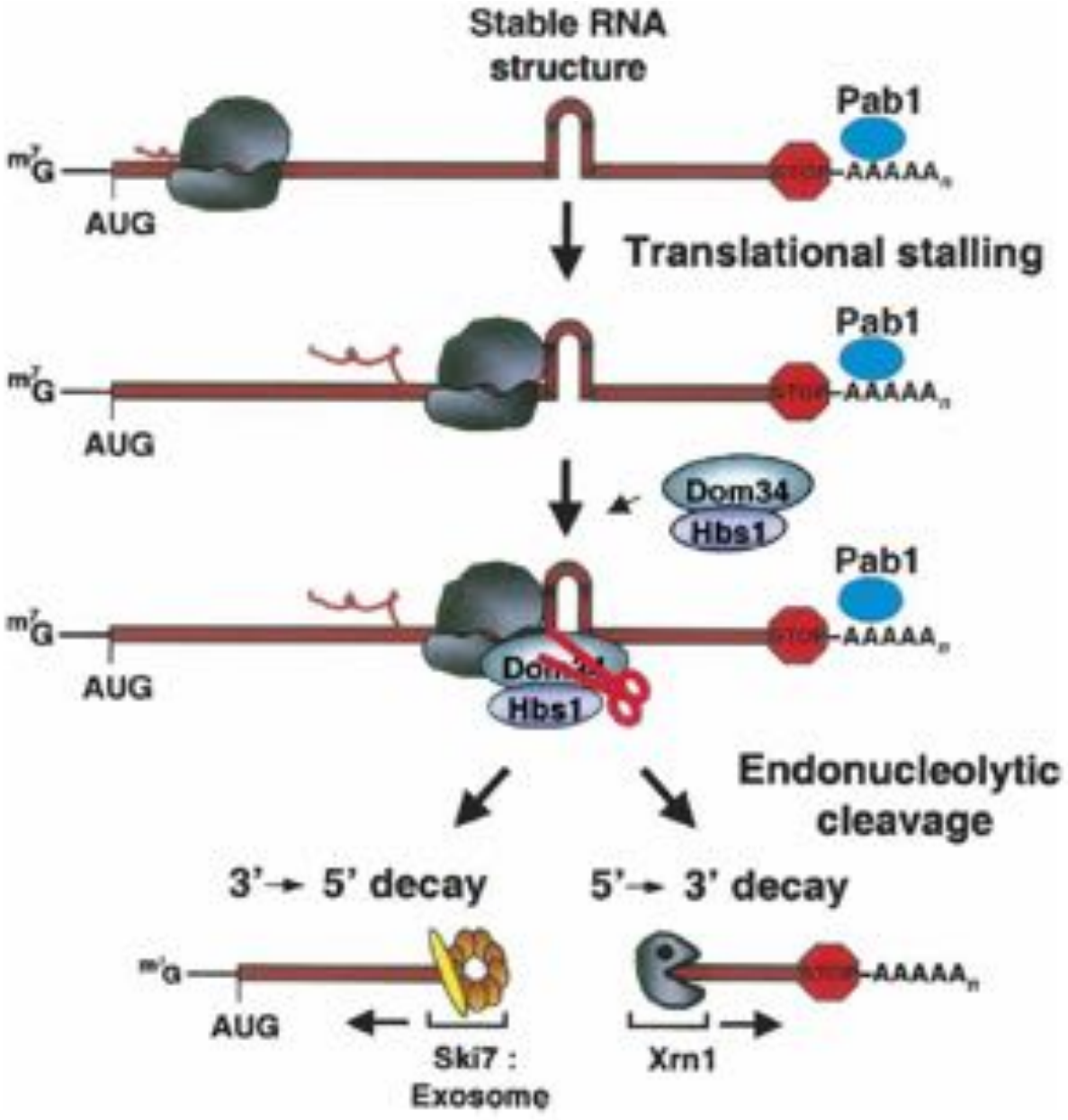
NMD Aktivitäten in verschiedenen Systemen

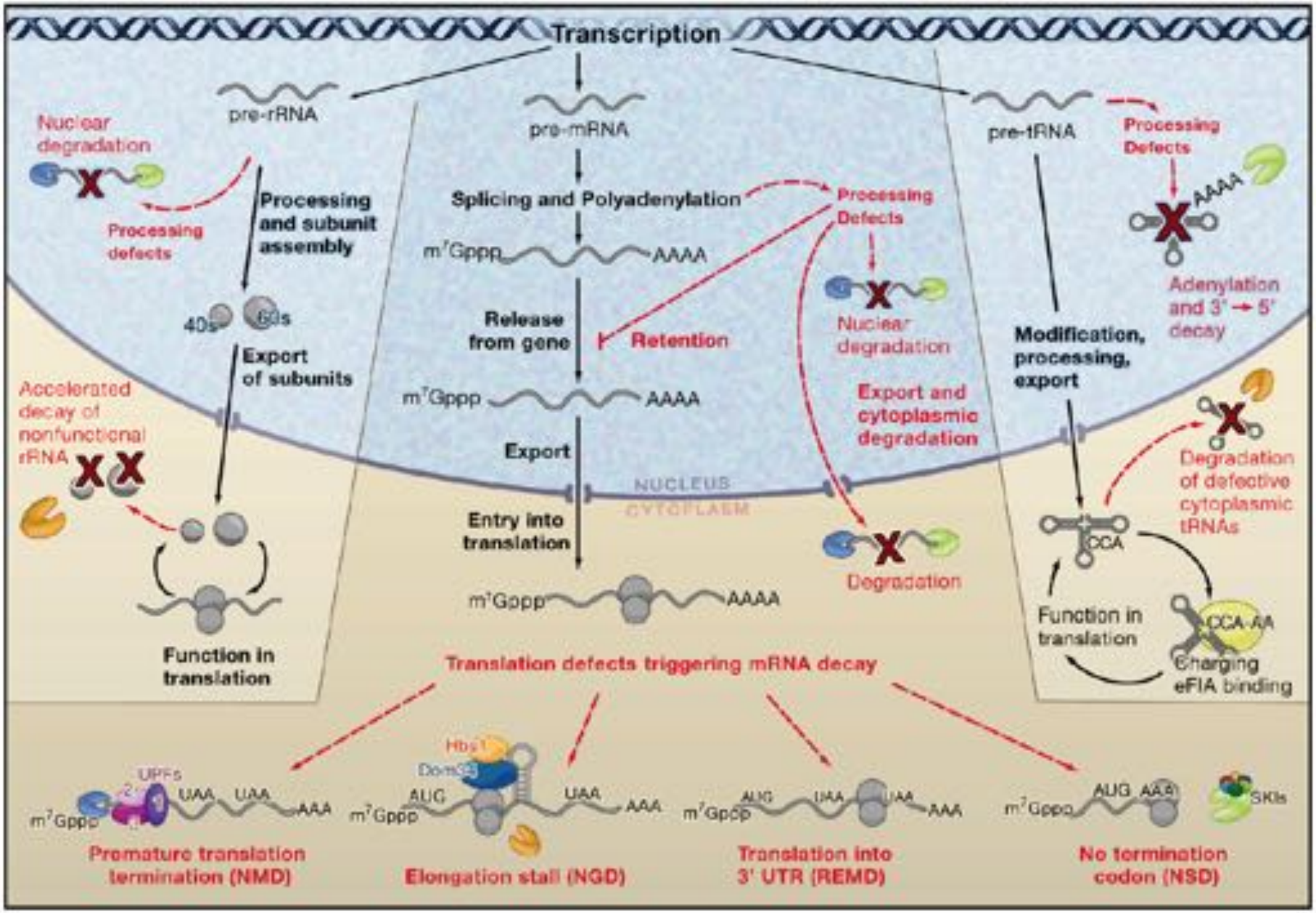


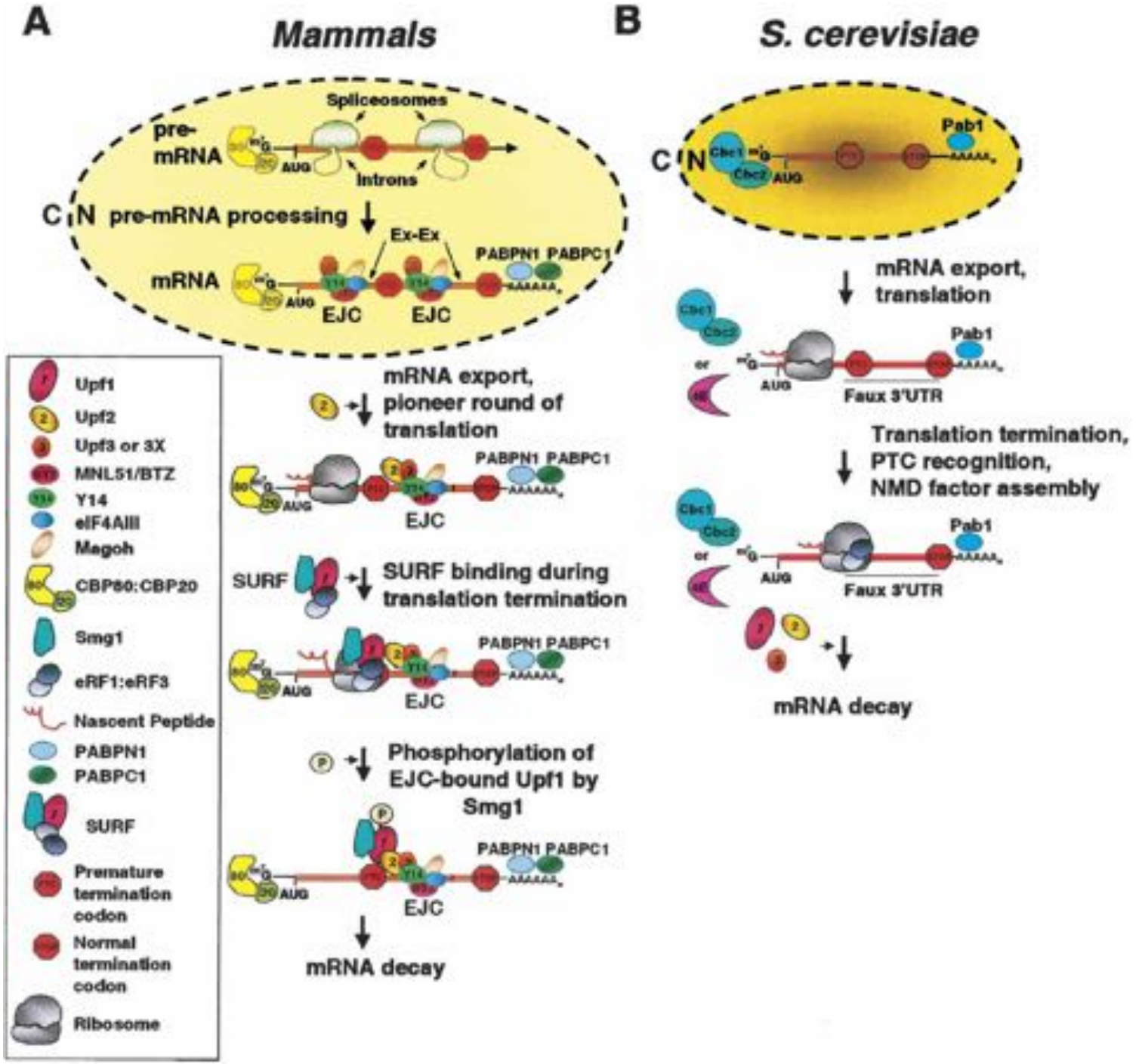
NSD: Nonstop decay



NGDNo-Go decay




Transcription
pre-rRNA
pre-mRNA
pre-tRNA
Nuclear degradation
Processing defects
Processing and subunit assembly
40s
60s
Export of subunits
Splicing and Polyadenylation
m7Gppp
AAAA
Release from gene
Retention
Export
Entry into translation
NUCLEUS
CYTOPLASM
Processing Defects
Nuclear degradation
Export and cytoplasmic degradation
Degradation
Processing Defects
Adenylation and 3' → 5' decay
Modification, processing, export
Accelerated decay of nonfunctional rRNA
Function in translation
CCA
Degradation of defective cytoplasmic tRNAs
Function in translation
CCA-AA
Charging eFIA binding
Translation defects triggering mRNA decay
UPFs
UAA
AAA
Premature translation termination (NMD)
Hbs1
Dom34
AUG
Elongation stall (NGD)
Translation into 3' UTR (REMD)
No termination codon (NSD)
Ski


A
Mammals
Spliceosomes
pre-mRNA
AUG
Introns
C N pre-mRNA processing
Ex-Ex
PABPN1 PABPC1
mRNA
EJC
EJC
Upf1
Upf2
Upf3 or 3X
MNL51/BTZ
Y14
eIF4AIII
Magoh
CBP80:CBP20
Smg1
eRF1:eRF3
Nascent Peptide
PABPN1
PABPC1
SURF
Premature termination codon
Normal termination codon
Ribosome
mRNA export, pioneer round of translation
EJC
SURF
SURF binding during translation termination
EJC
Phosphorylation of EJC-bound Upf1 by Smg1
EJC
mRNA decay
B
S. cerevisiae
Pab1
C N
Cbc1
Cbc2
AUG
mRNA export, translation
Faux 3'UTR
Translation termination, PTC recognition, NMD factor assembly
mRNA decay

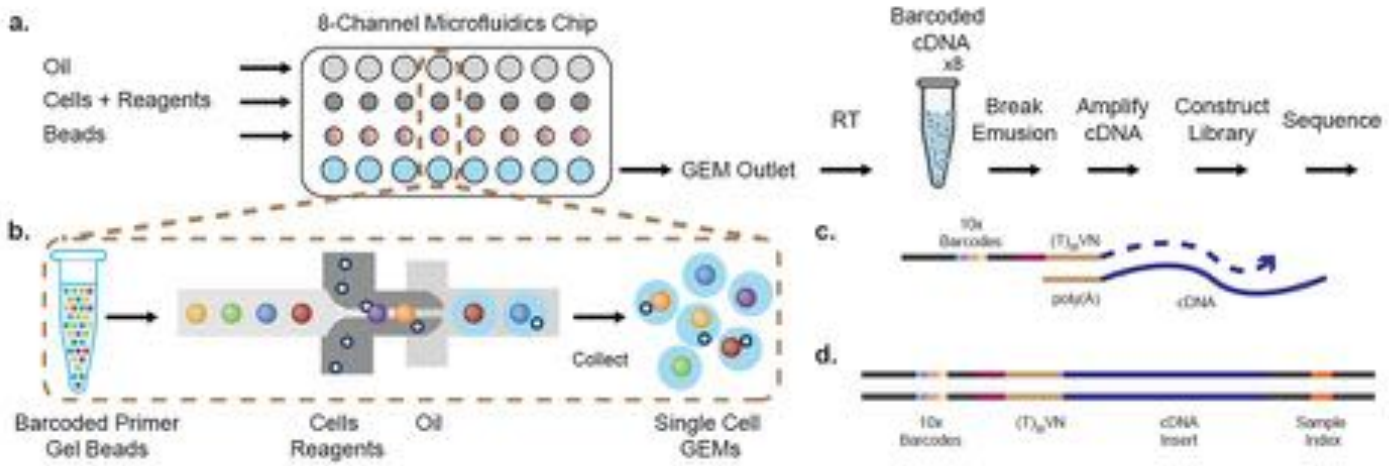
RNA Verteilung

RNA wird zeitlich und räumlich spezifisch exprimiert

RNA wird spezifisch in der Zelle lokalisiert
-> Kompartimentierung

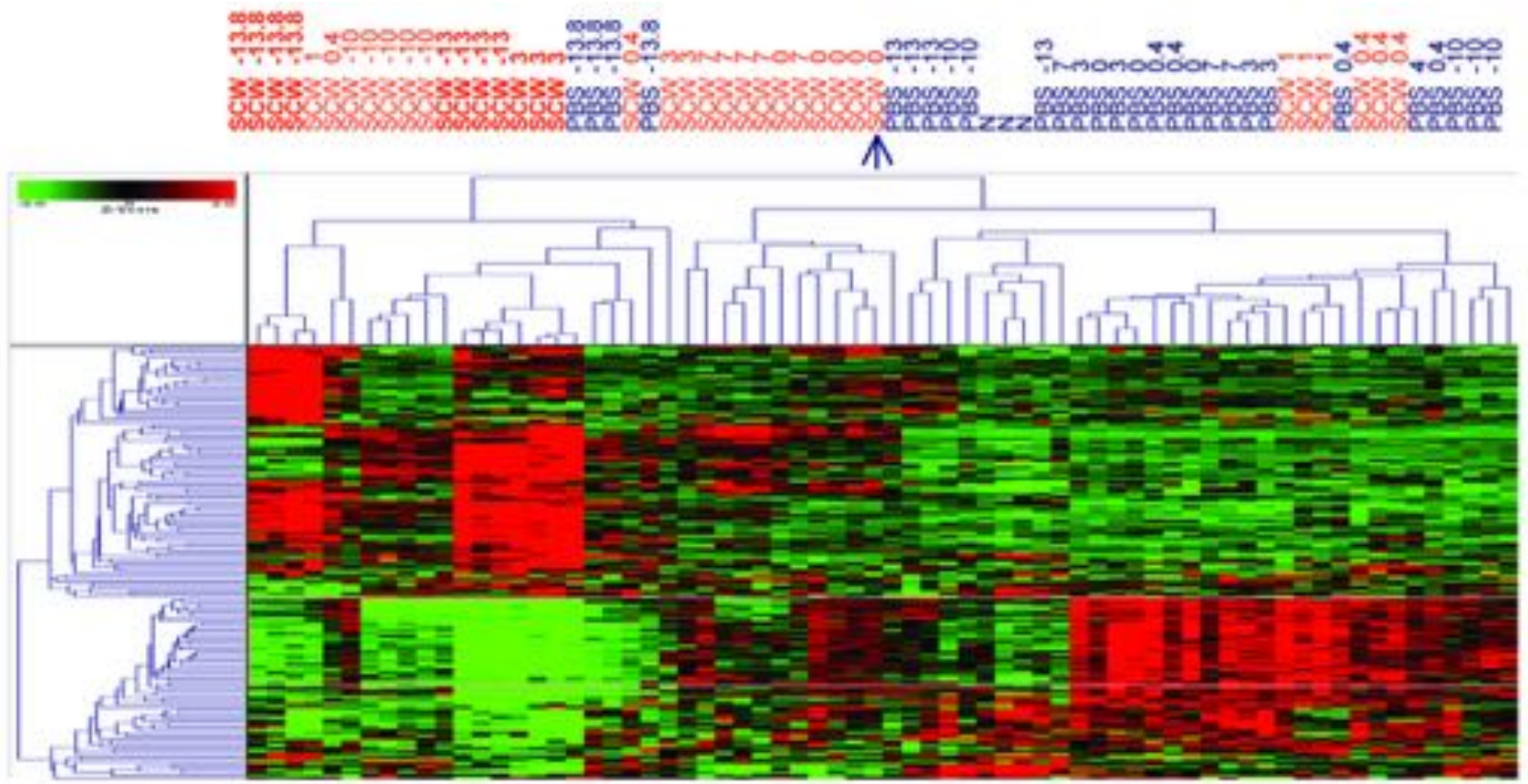
Single cell RNA Sequenzierung

10X Genomics droplet-based protocol



- Encapsulates up to 48,000 cells in 10 minutes

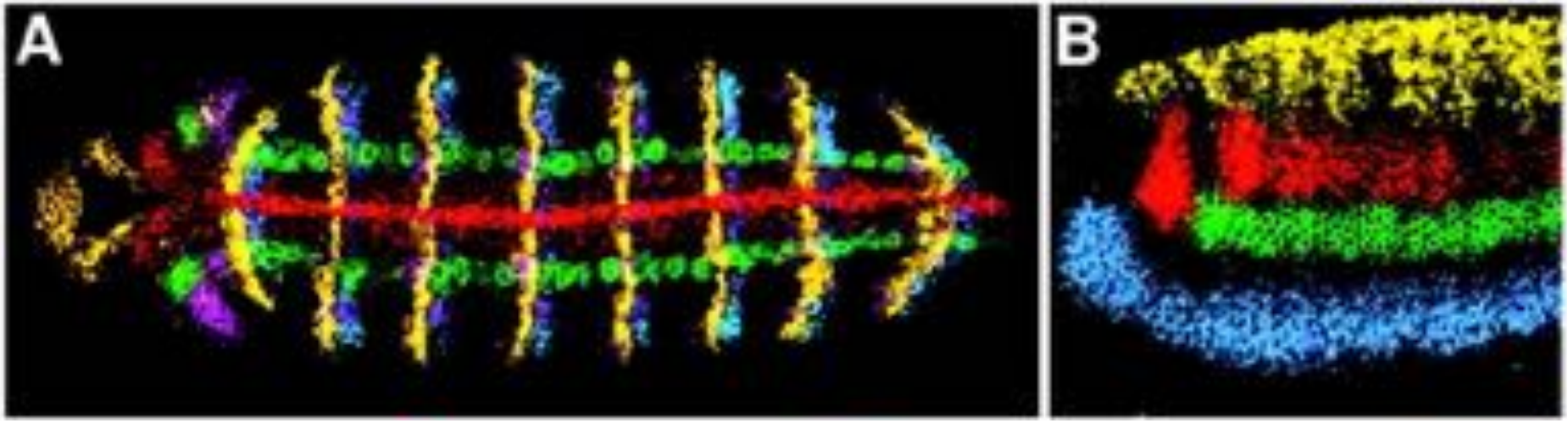
Heat maps zeigen die globale Änderung der Transkription



Die globale Detektion der Transkription wird zunehmend in der Diagnostik eingesetzt.

RNA Verteilung

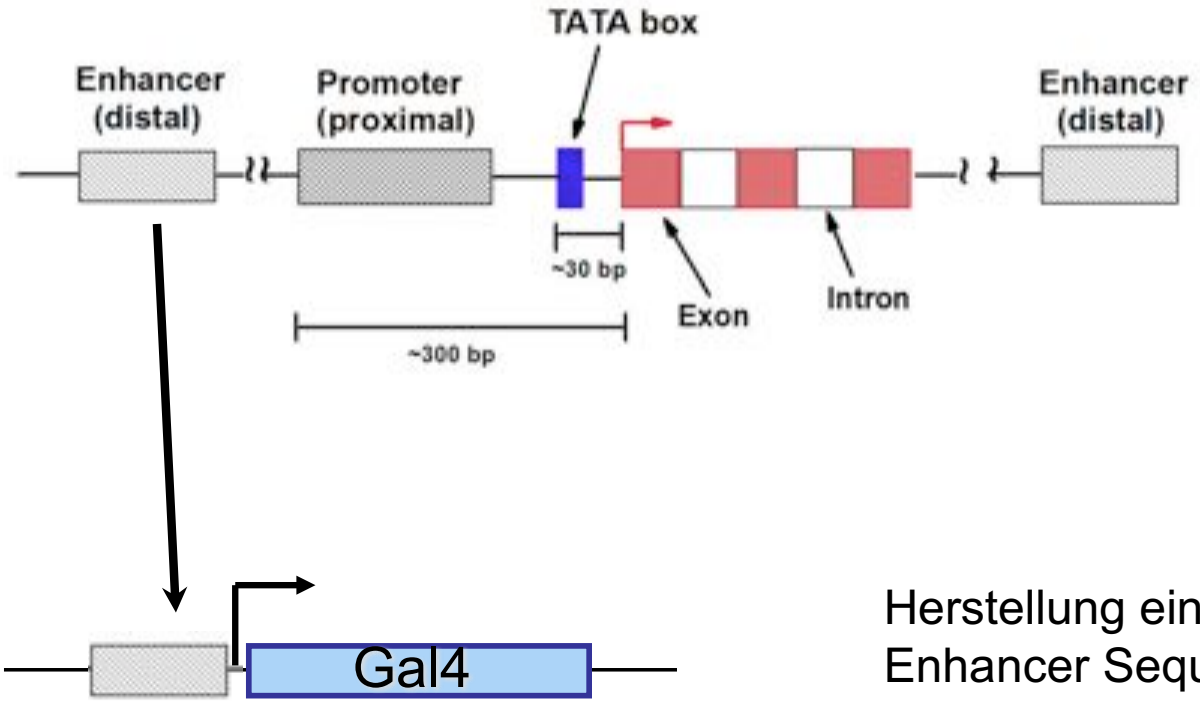
RNA wird zeitlich und räumlich spezifisch exprimiert



Fluoreszenz-markierte RNA Sonden von 4 Genen

-> in situ hybridisierung

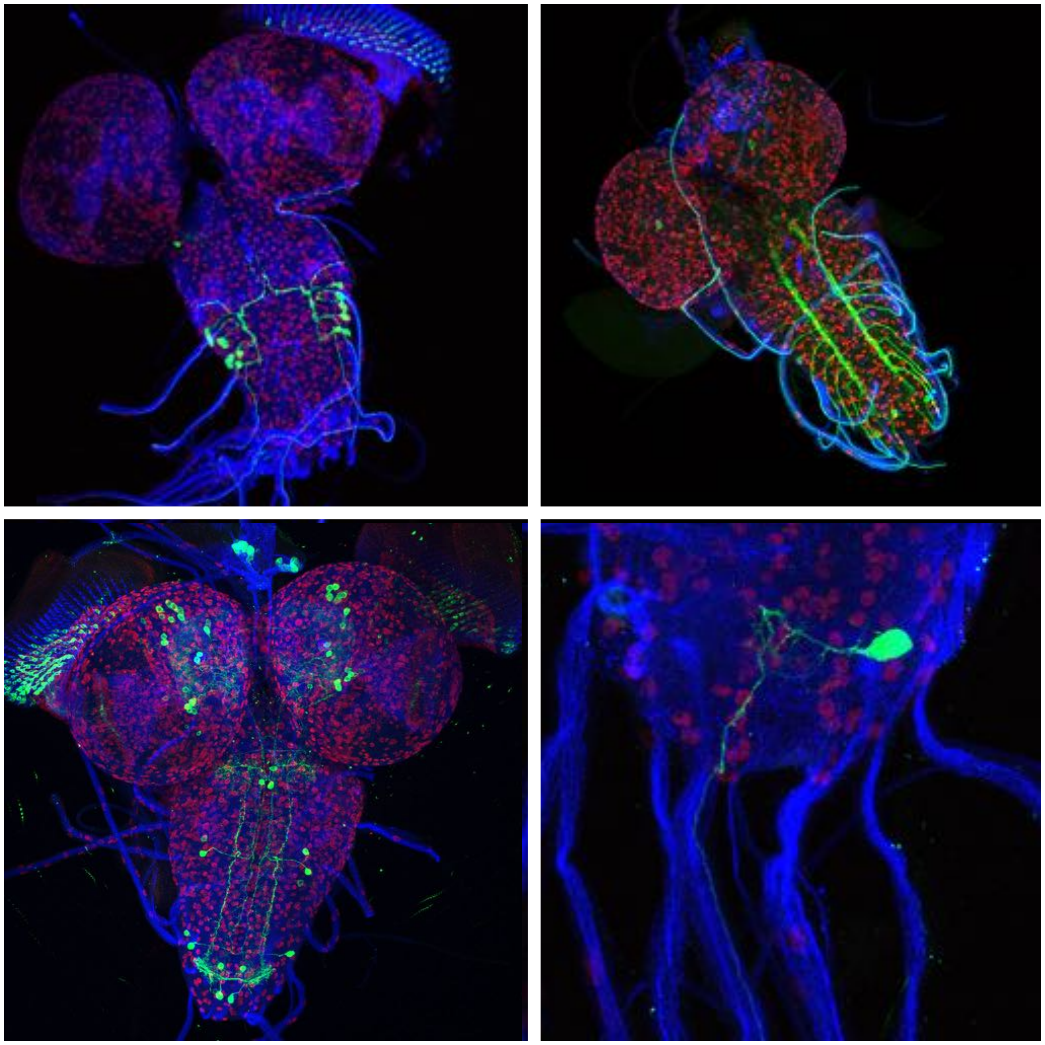
Identifizierung von regulatorischen Sequenzen



- Struktur eines typischen Gens

Herstellung eines Transgens mit einer Enhancer Sequenz vor z.B. dem Gal4 Gen. Expression von UAS-gekoppelten Transgenen

Identifizierung von regulatorischen Sequenzen

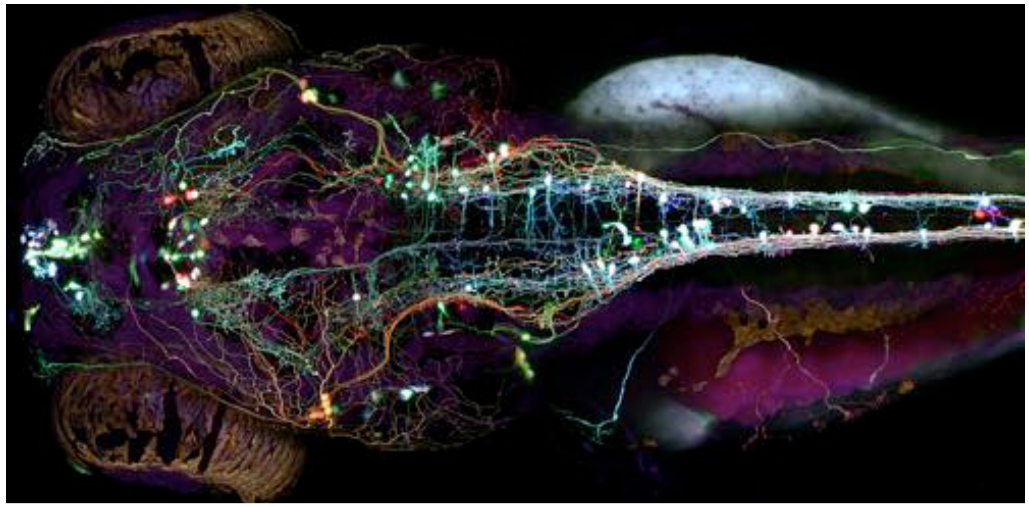


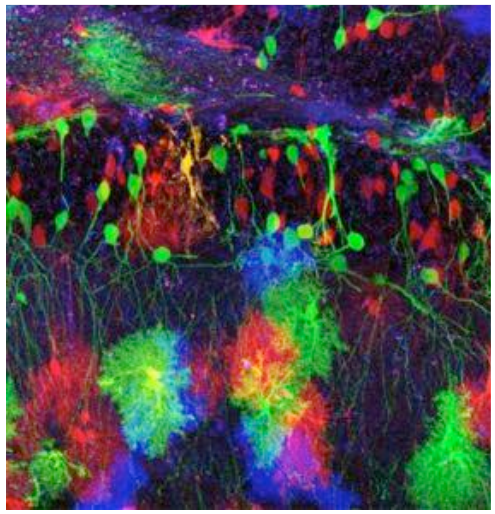
Ansteuerung einzelner Nervenzellen

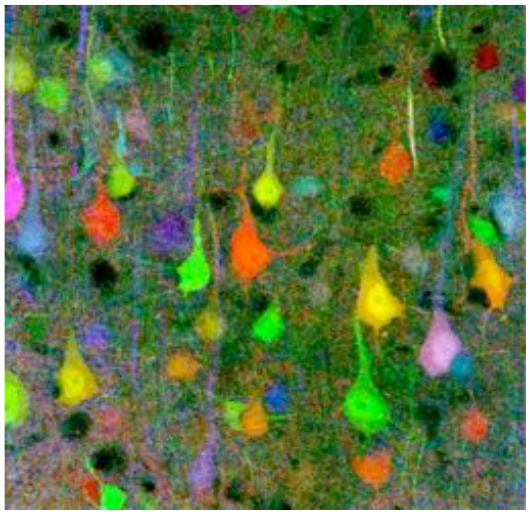
im Gehirn von Drosophila Larven.

Verhaltensanalyse!

Identifizierung von regulatorischen Sequenzen



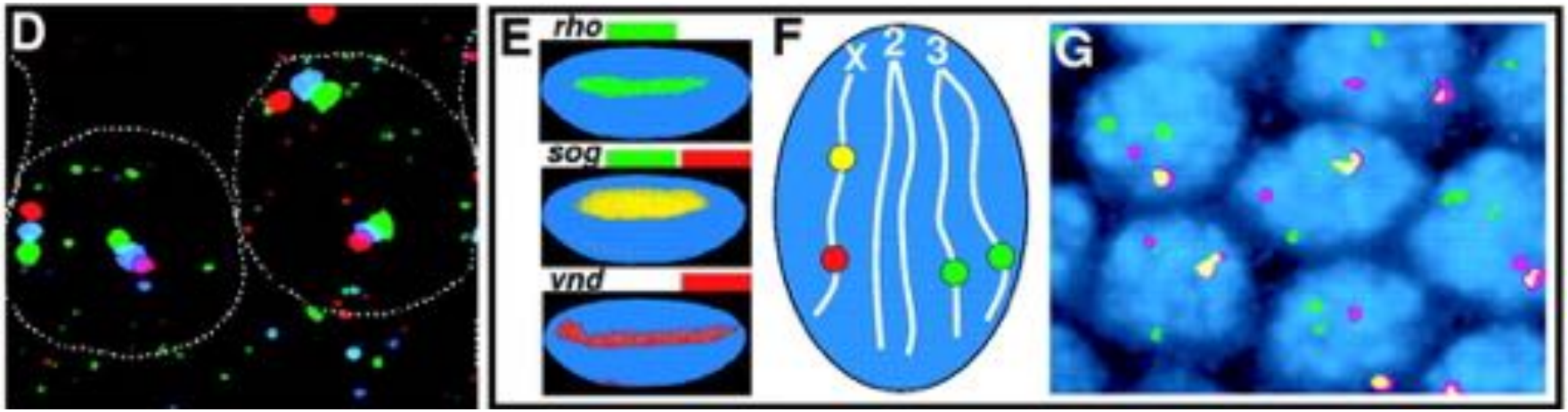




Ansteuerung einzelner Nervenzellen im Gehirn eines Zebrafisches oder der Maus.

RNA Verteilung

Nachweis von nascenten Transkripten im Zellkern

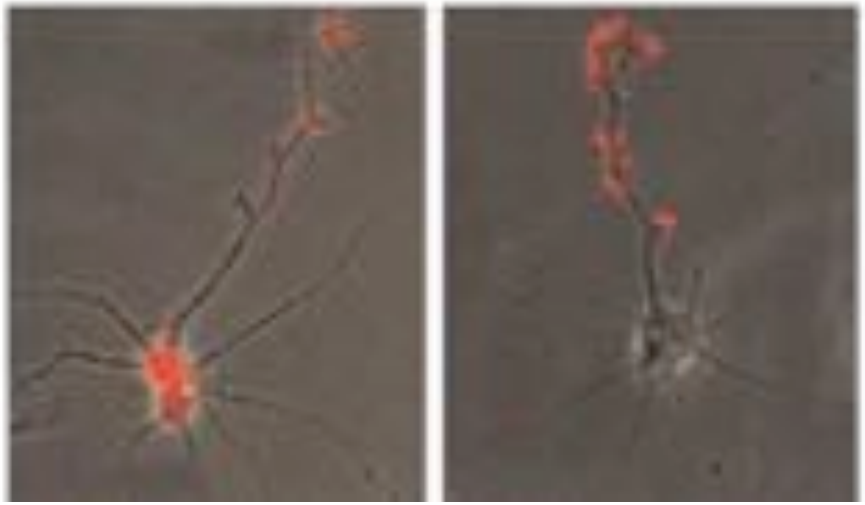


Subzellulär lokalisierte RNA

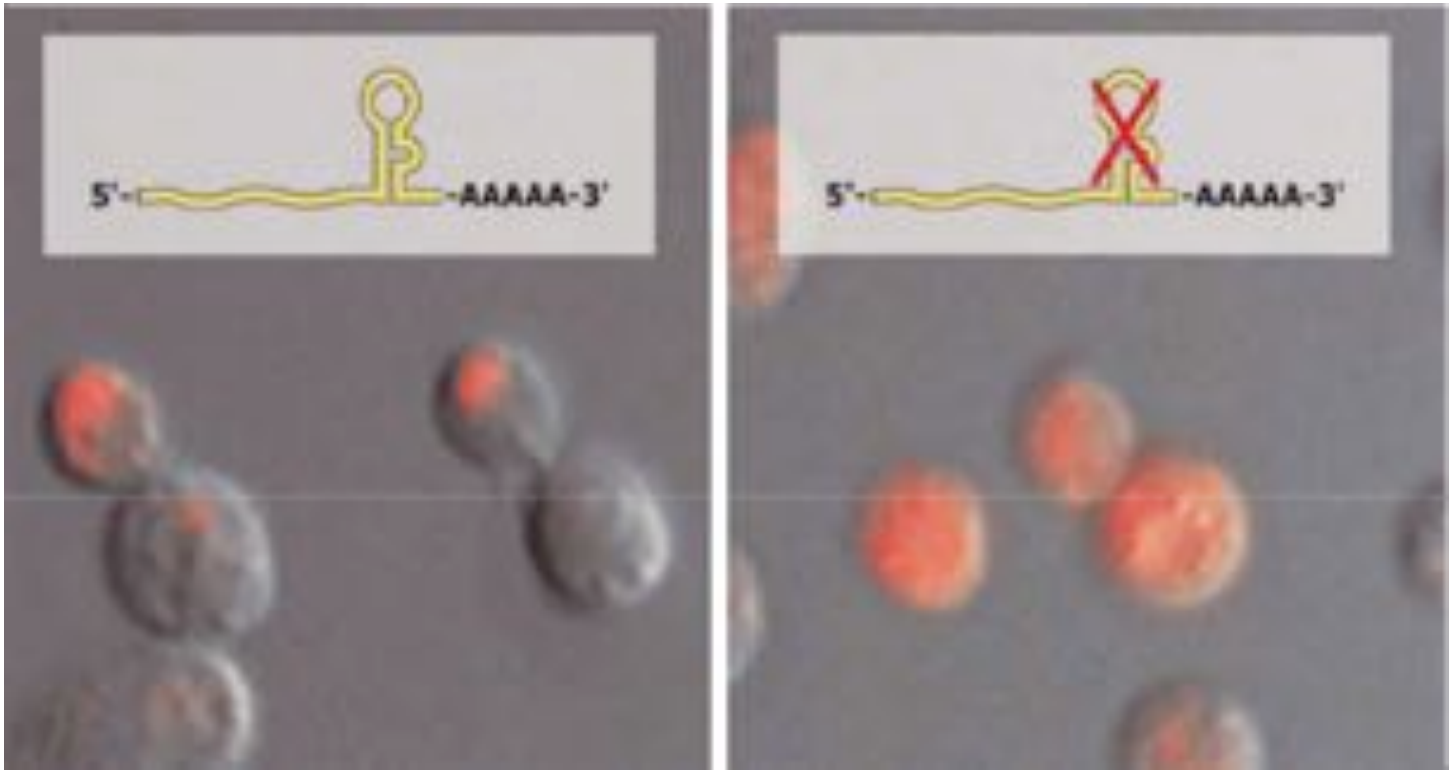
RNA wird spezifisch in der Zelle lokalisiert
-> Kompartimentierung, maternale Faktoren



nanos

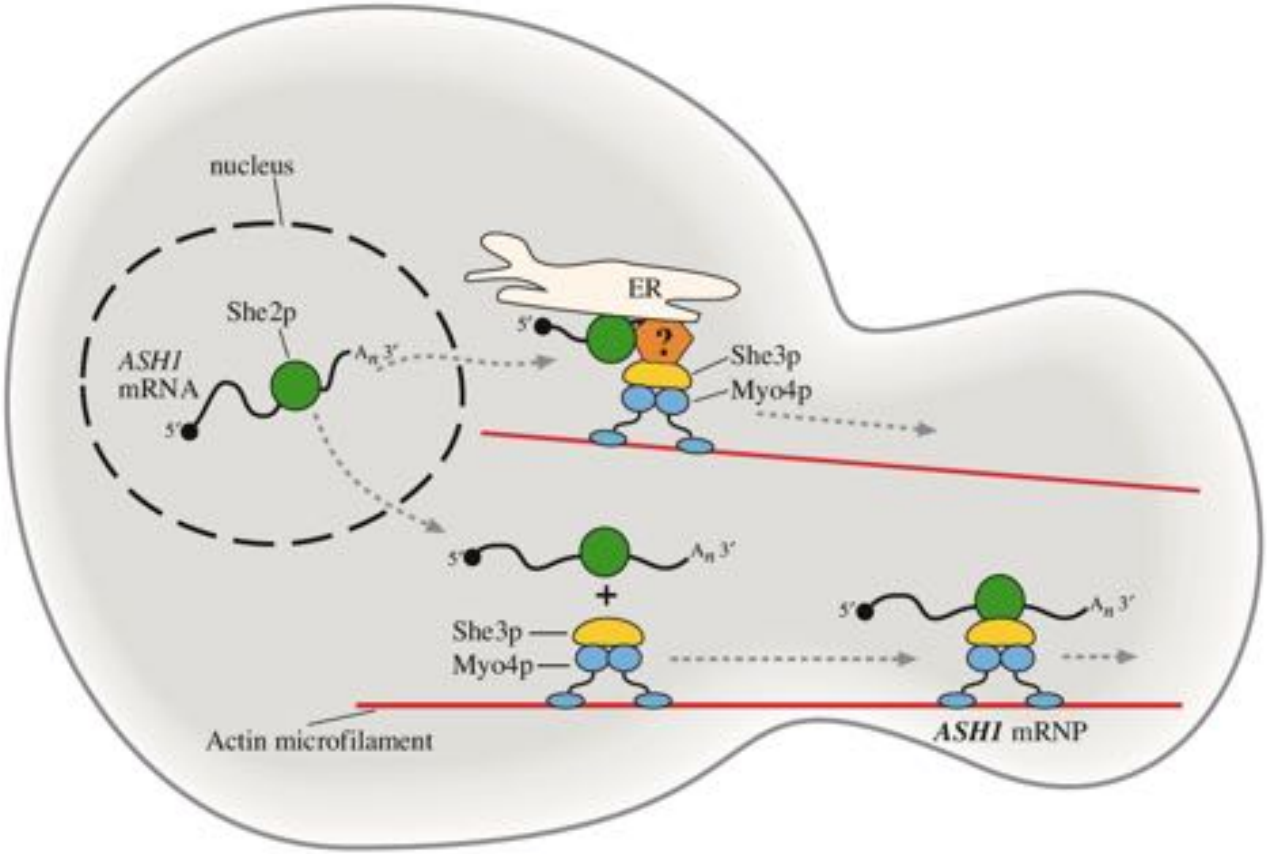


Subzelluläre RNA Verteilung hängt von RNA-Sekundärstrukturen ab



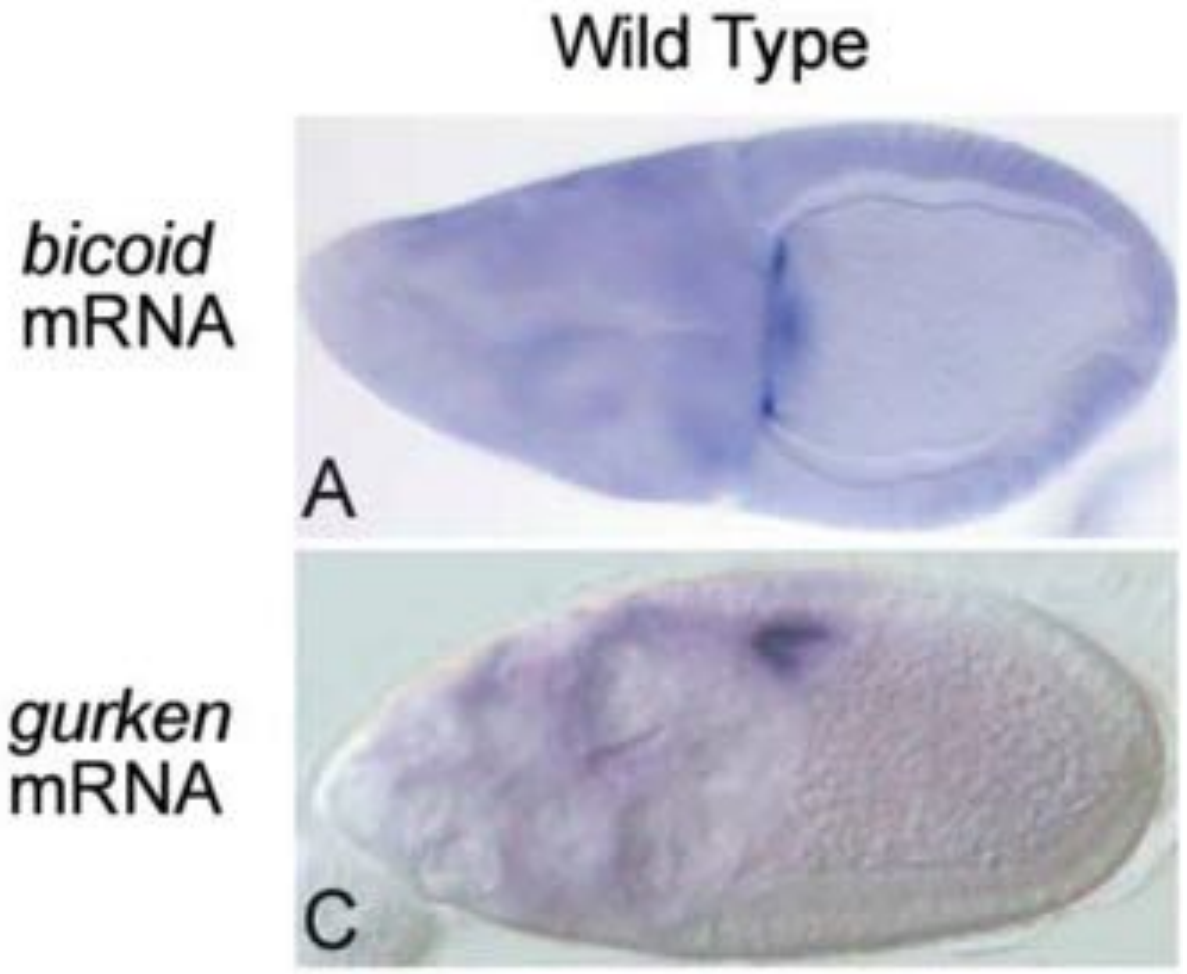
Ash1 mRNA

Subzelluläre RNA Verteilung hängt von RNA-Sekundärstrukturen ab



Ash1 mRNA. Transport entlang von Aktin, Typ IV Myosin

Lokalisierte Transkripte in der Drosophila Oozyte



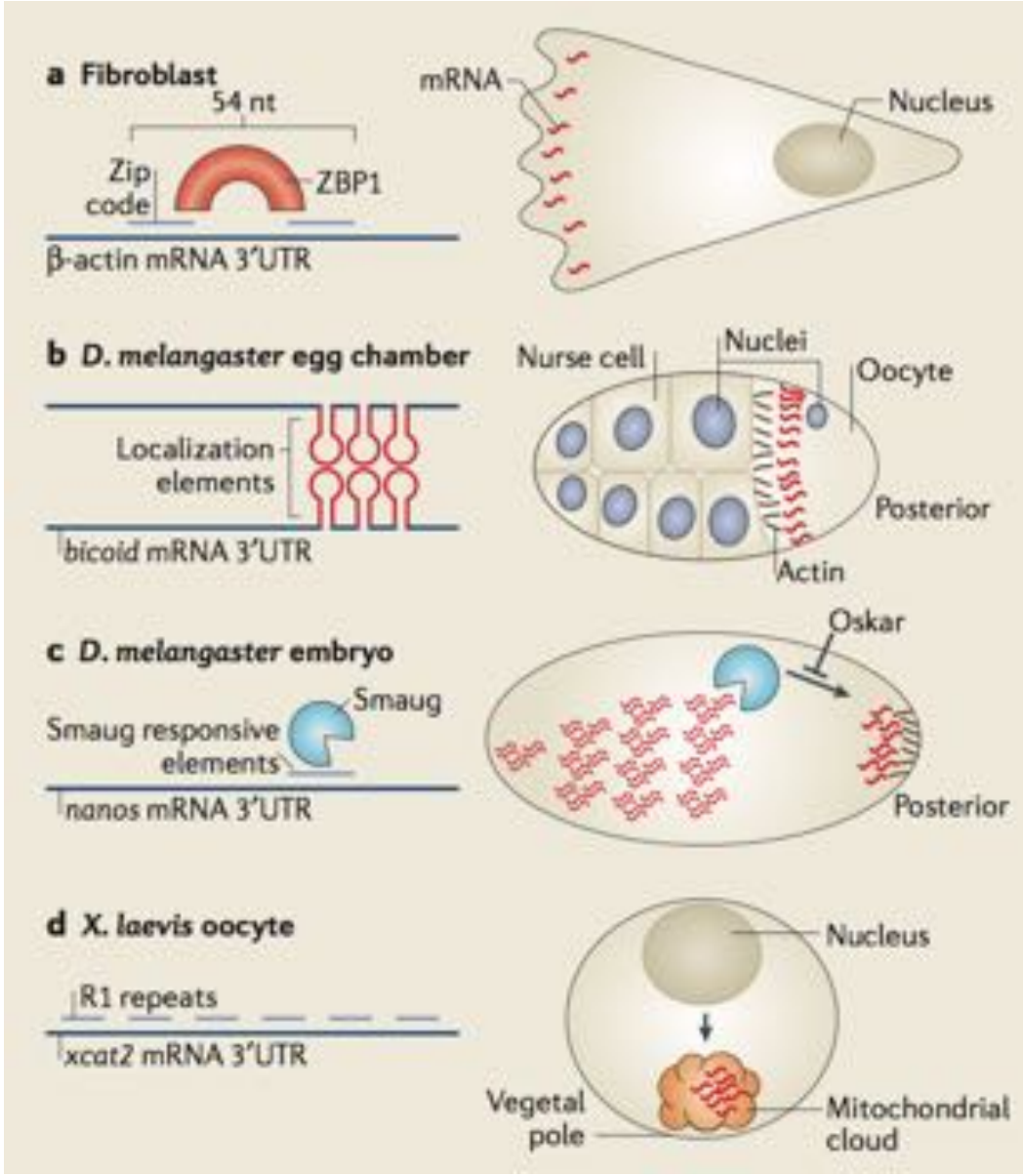
Cis-Elemente die die mRNA Lokalisation steuern

Transport

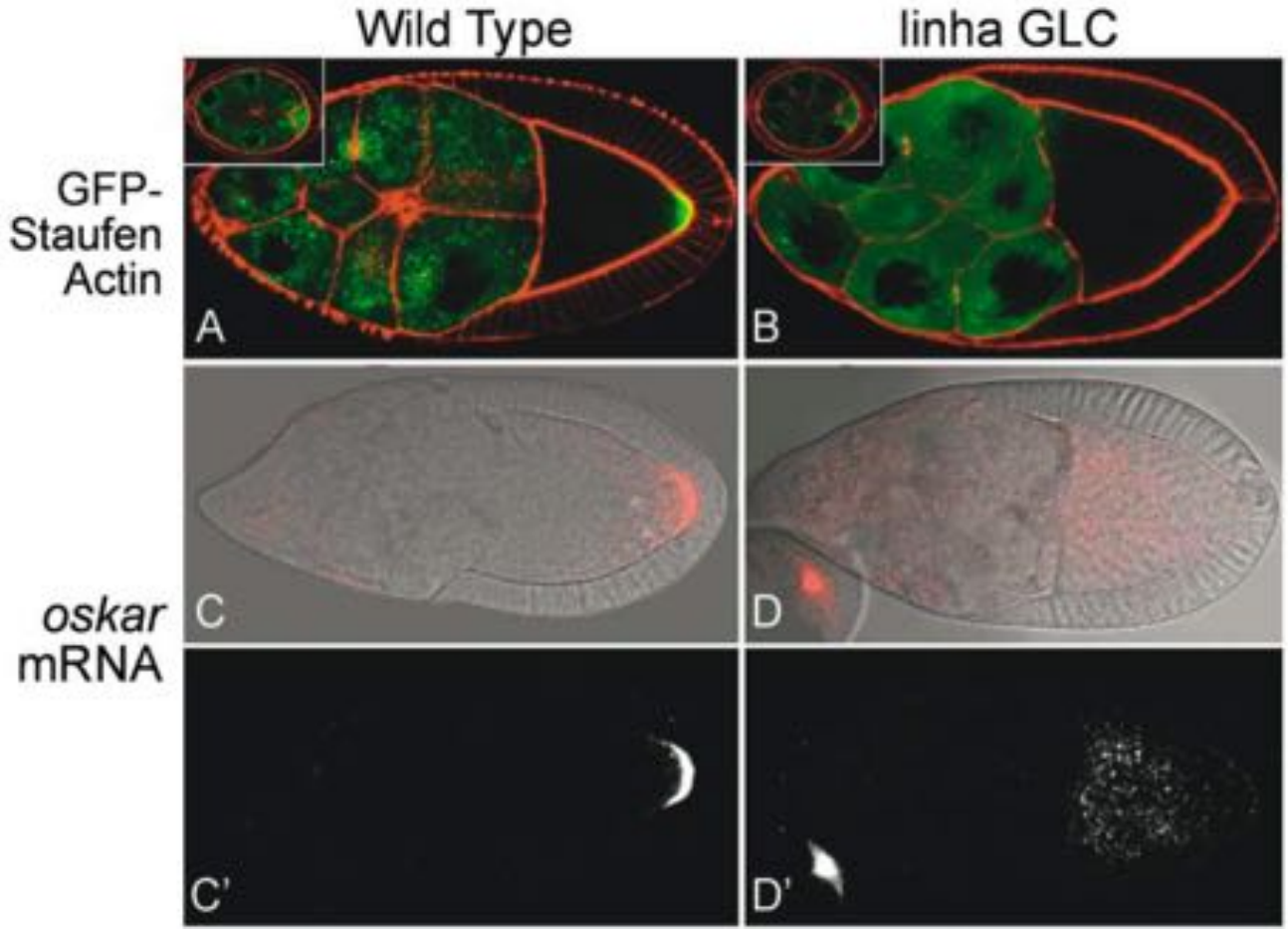
Transport

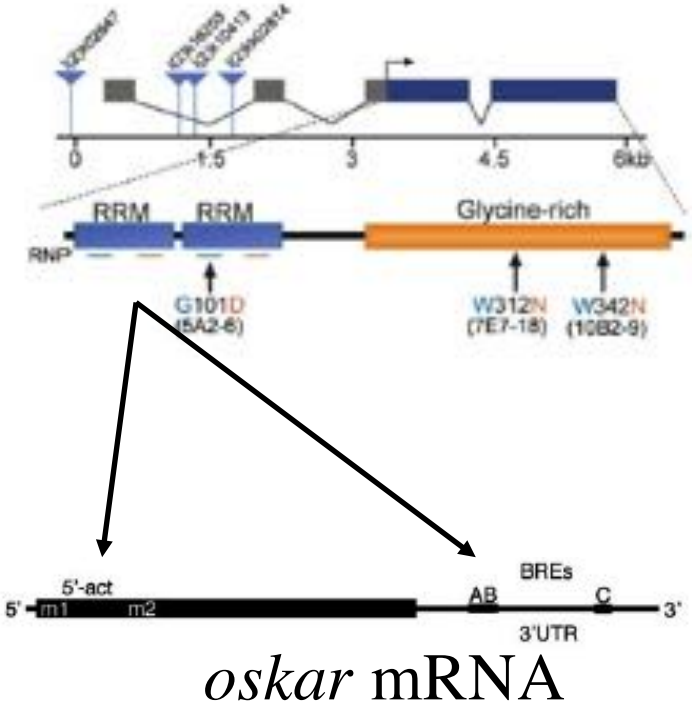
Abbau

Diffusion + Binden



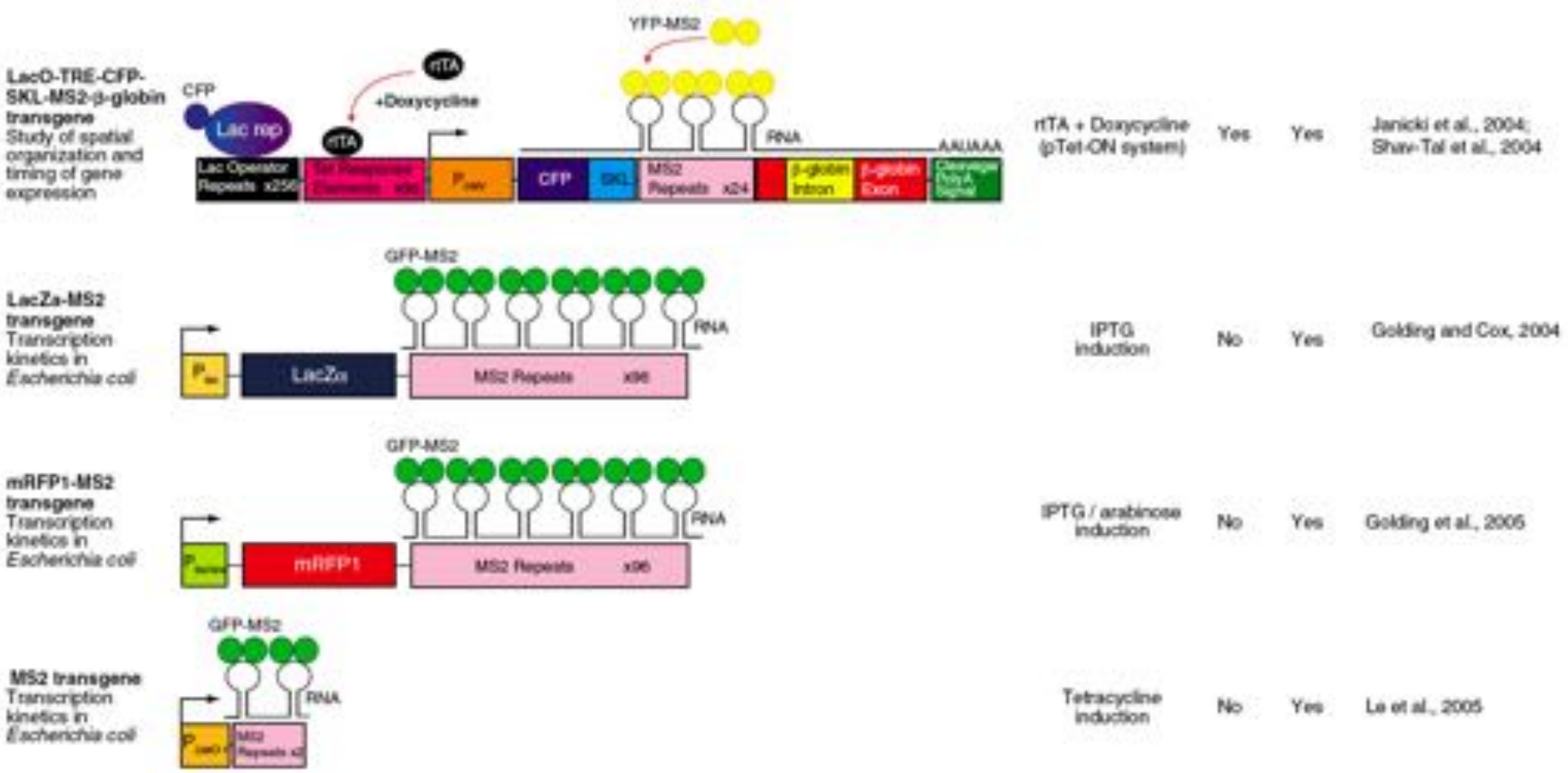
Das RRM-Protein Hrp48 (hnRNPA/B Homolog) kontrolliert die Lokalisation von Oskar (MT-abhängig, Kinesin-Motor)





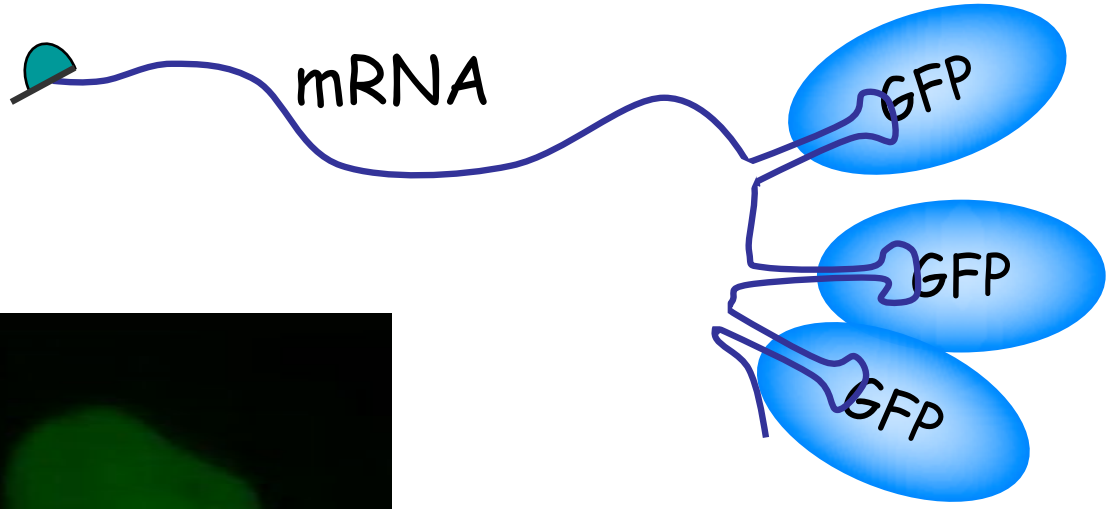
oskar mRNA

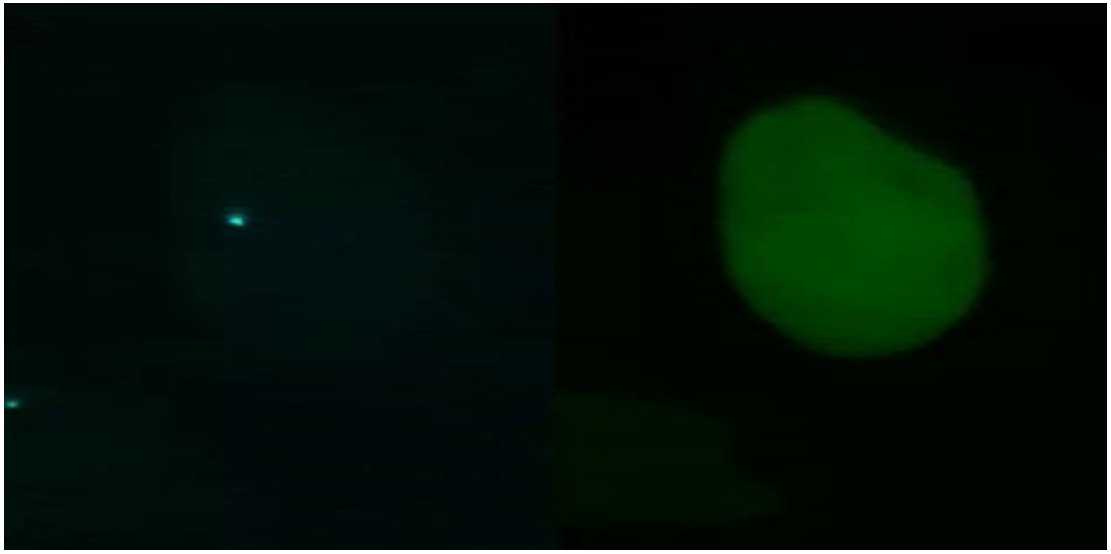
Transkriptionsreporter



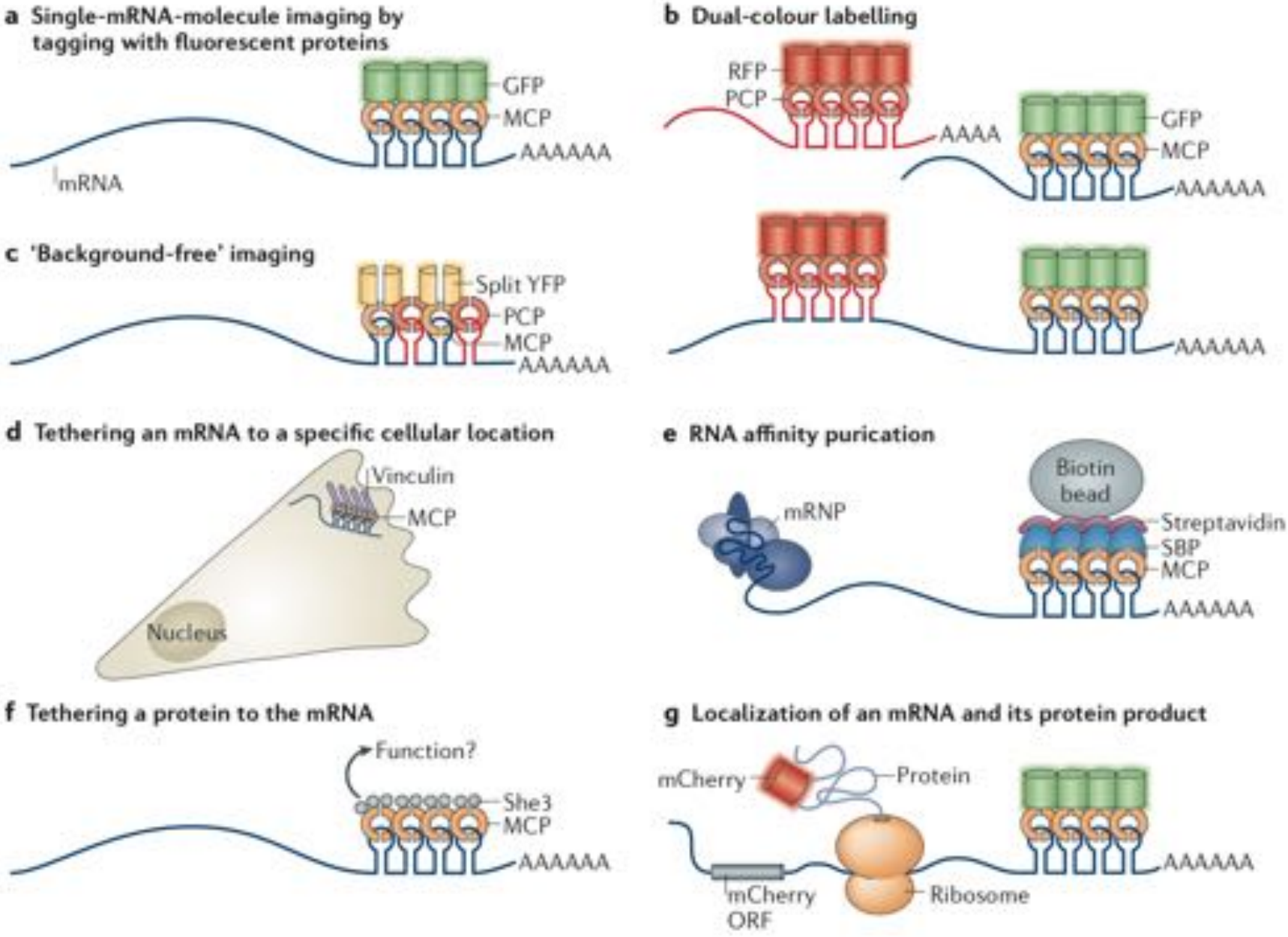
Kann man Transkription sehen? MS2





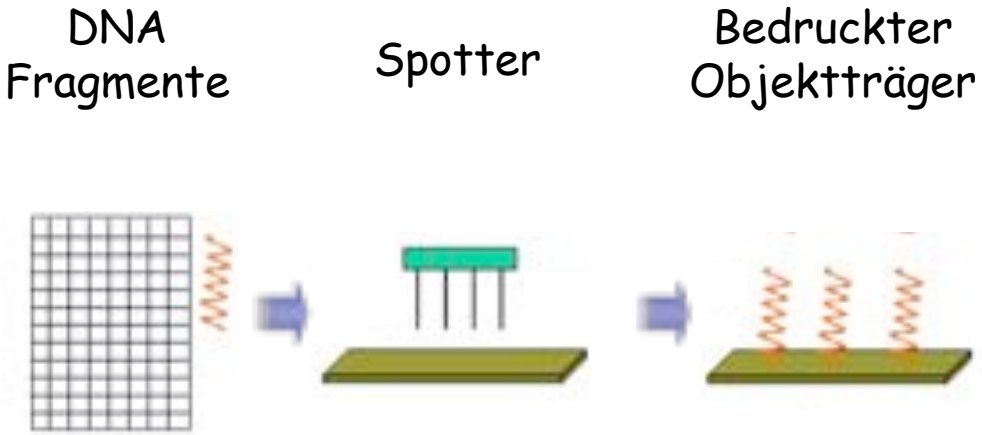


Möglichkeiten der MS2 Technologie

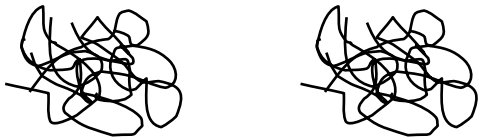


Kann man die Transkription von vielen Genen gleichzeitig messen?

Prinzip von DNA-arrays:



Herstellung der Sonden



Reverse Transkriptase



Fluoreszierende Sonden

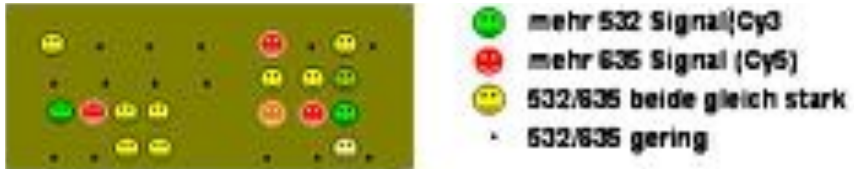
Kann man die Transkription von vielen Genen gleichzeitig messen?

Prinzip von DNA-arrays:

Bedruckter Objektträger + Fluoreszierende Sonden



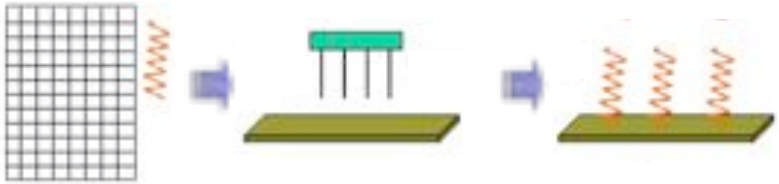
Hybridisierung und Auswertung



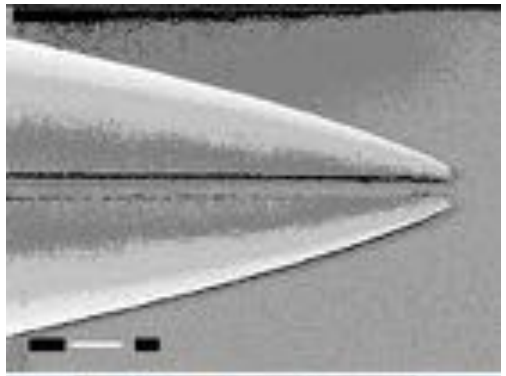


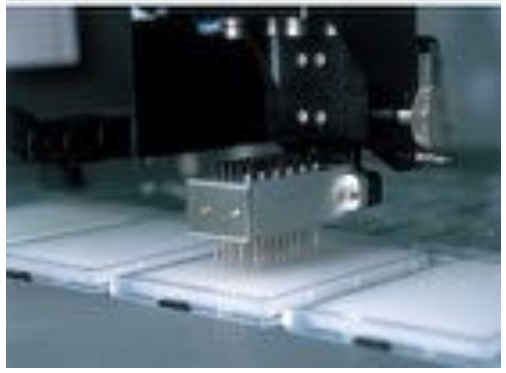
Herstellung von *gene chips*

DNA Fragmente | Spotter | Bedruckter Objektträger







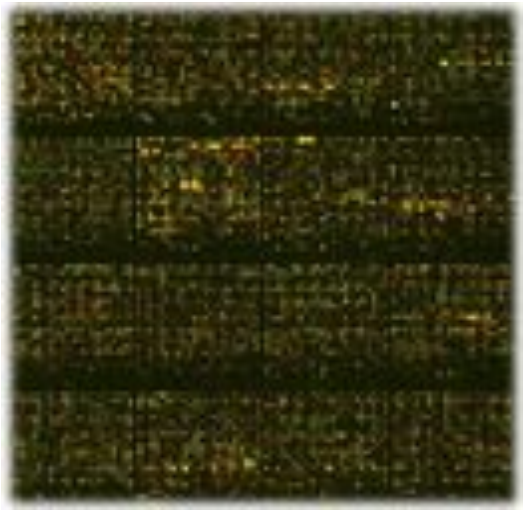


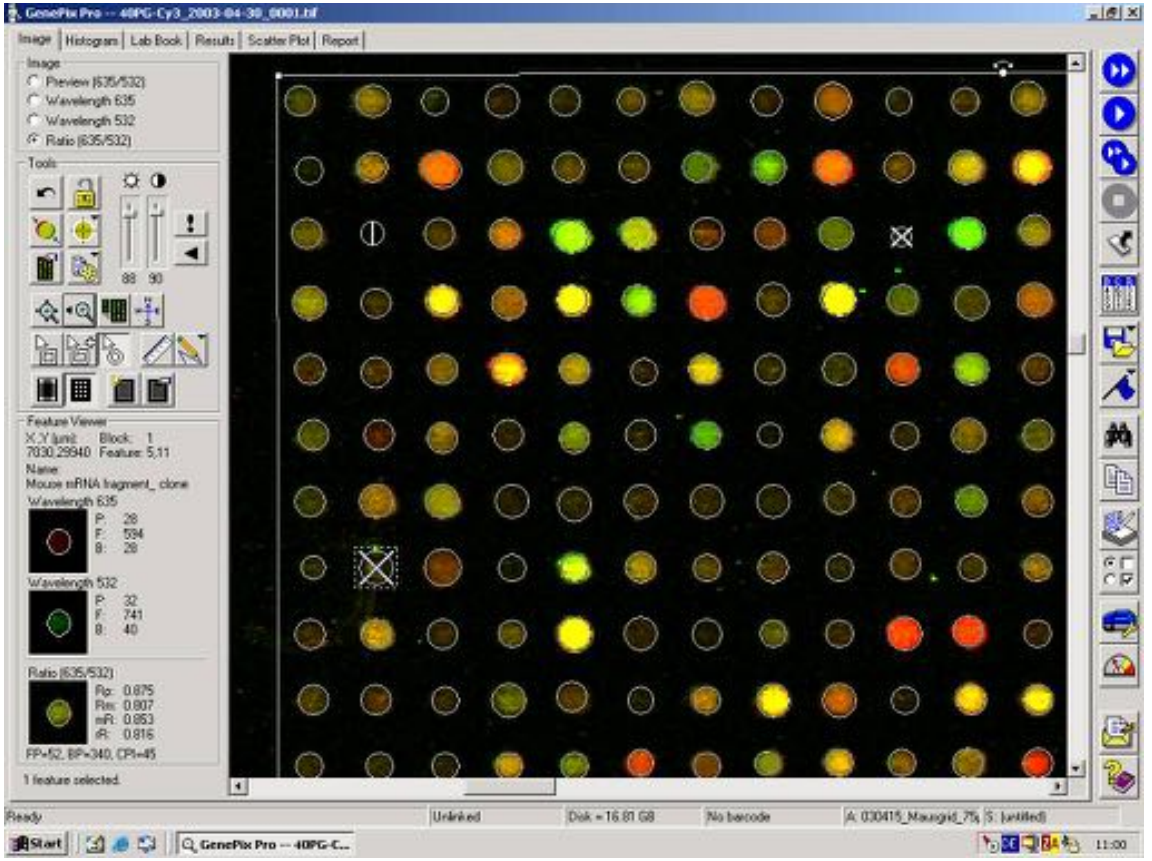
Der Druckkopf verteilt DNA im Abstand von 150 µm. Pro Druck, der derzeit etwa 40 Stunden dauert, entstehen bis zu 120 identische Microarrays.

Ein *Gene Chip* trägt 40.000 verschiedene Sequenzen

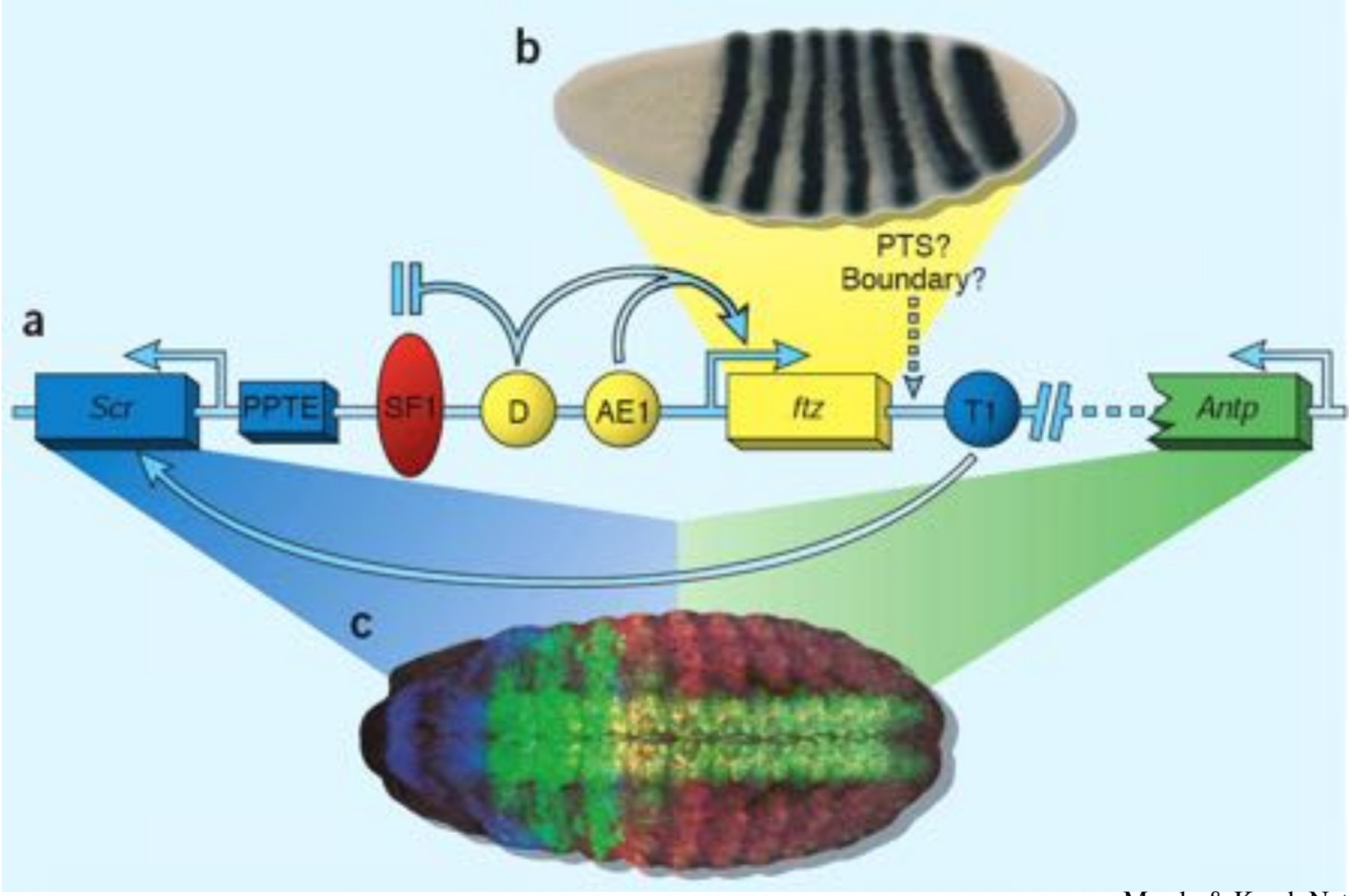


Auswertung nach der Hybridisierung



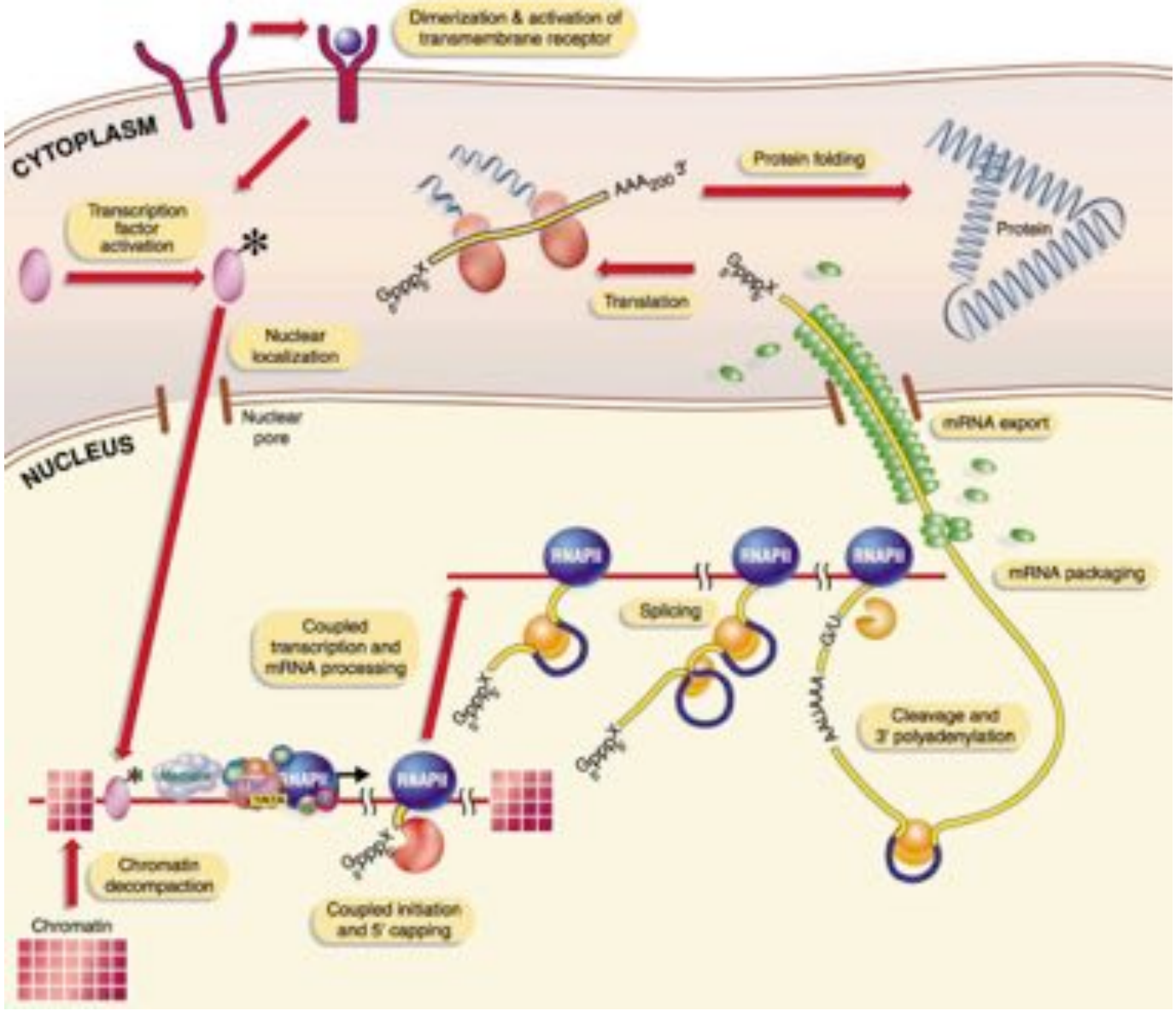


Komplexe Regulation der Transkription

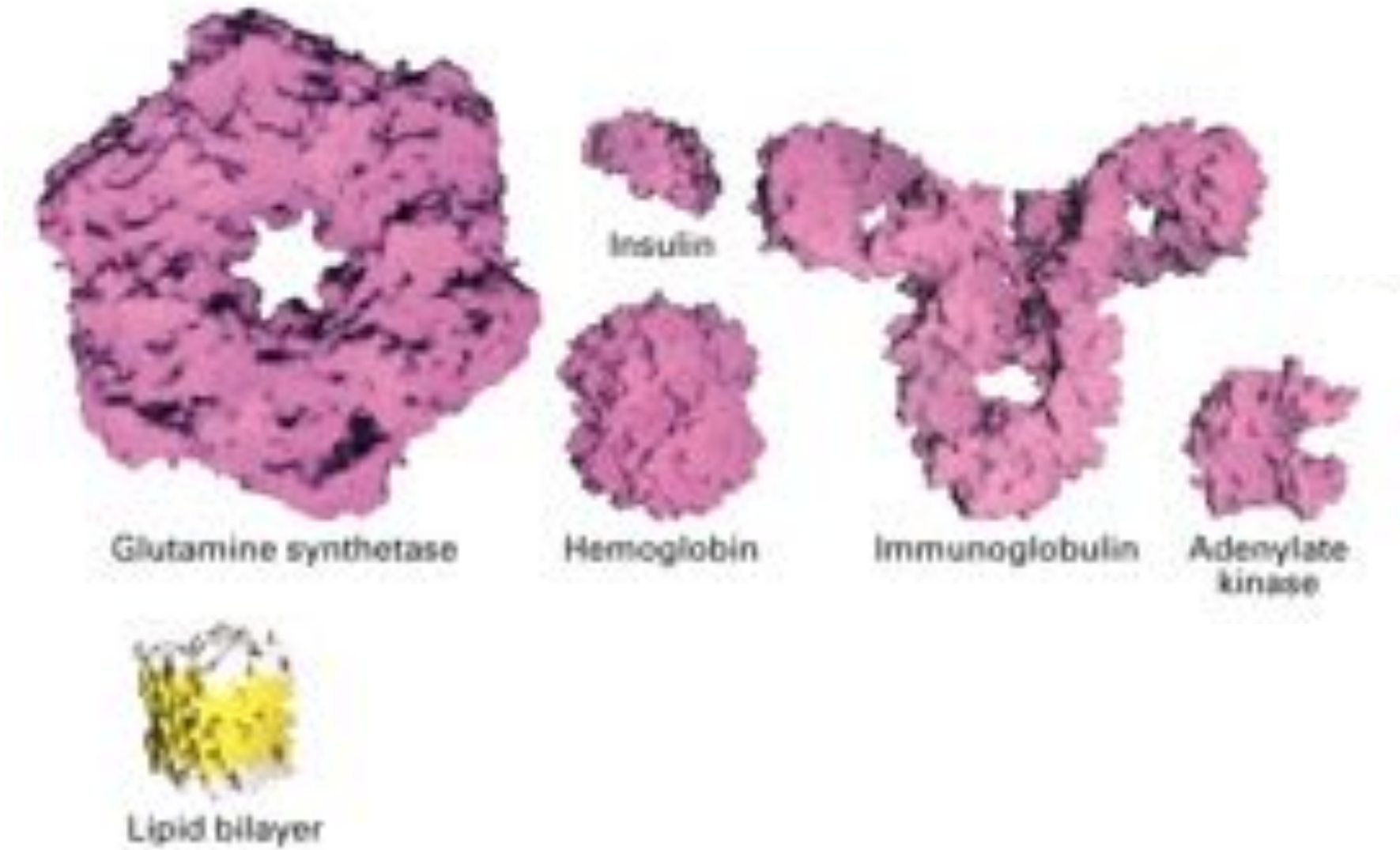


Maeda & Karch Nature 2003

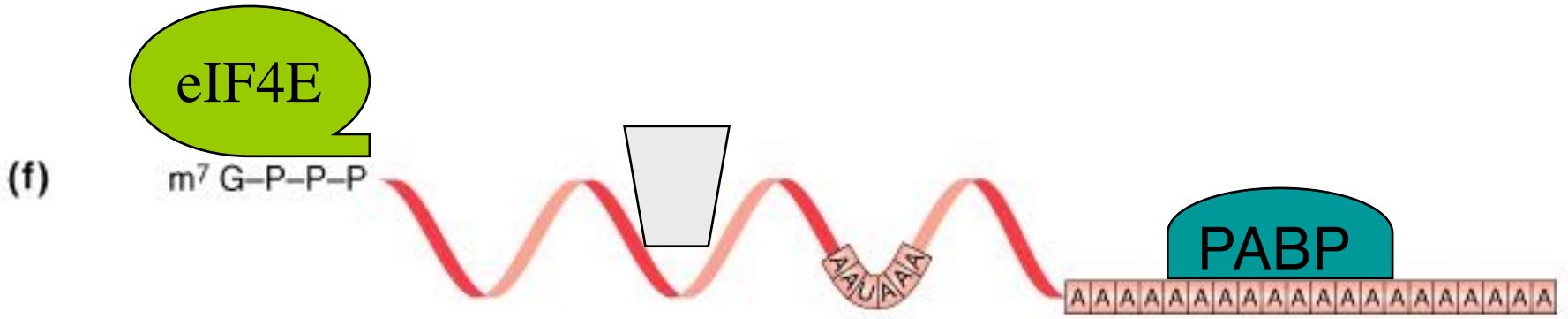
Möglichkeiten der Regulation der Gen Expression



DNA  Proteine



RNA bindende Proteine regulieren die Translation

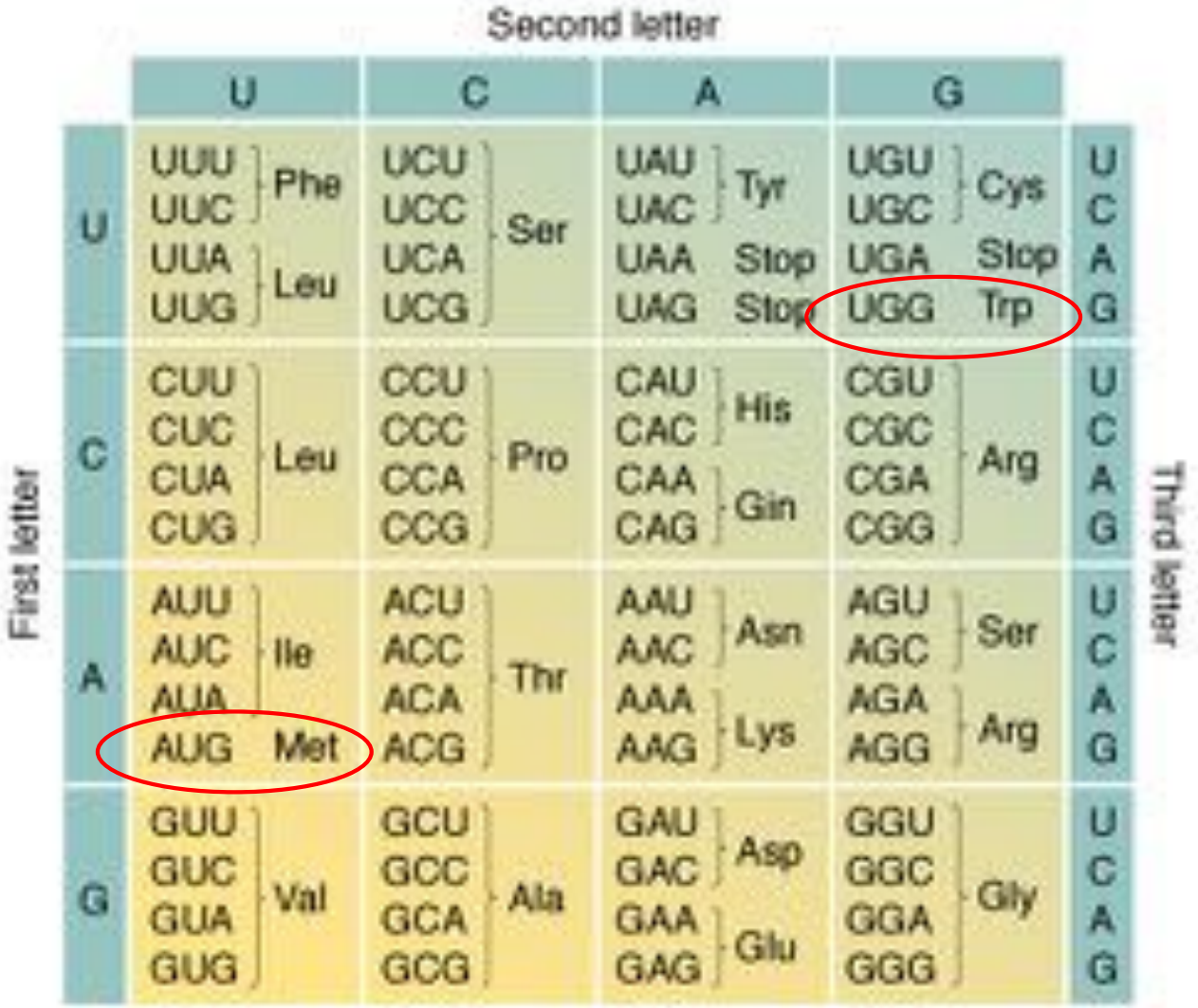


Poly A Binding Protein

Die Translation kann auf mehreren Ebenen reguliert werden:

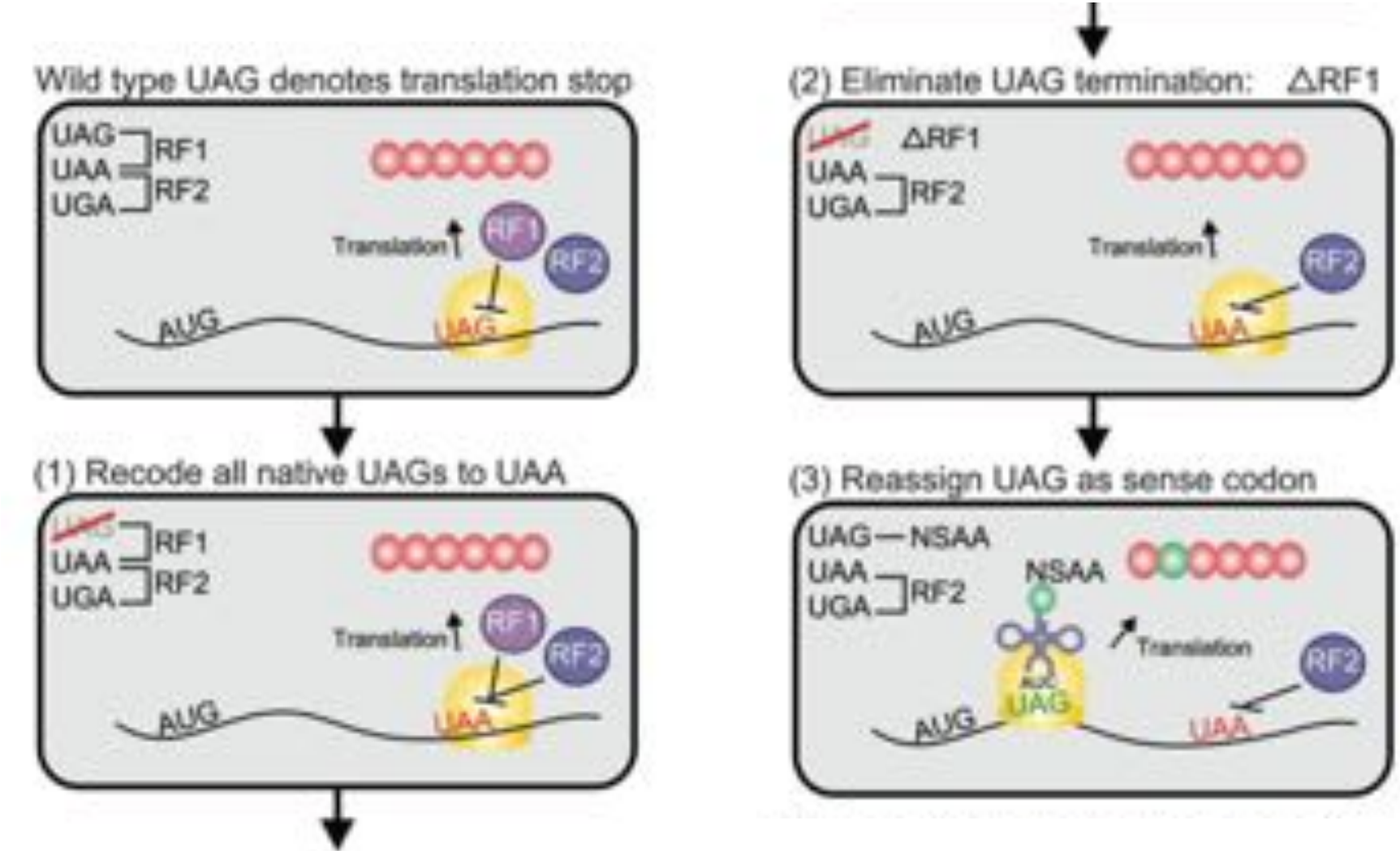
- RNA Stabilität
- Initiation
- Elongation

Der genetische Code ist universell



Codon	Universal Code	Unusual Code	Occurrence*
UGA	Stop	Trp	*Mycoplasma, Spiroplasma,* mitochondria of many species
CUG	Leu	Thr	Mitochondria in yeasts
UAA, UAG	Stop	Gln	*Acetabularia, Tetrahymena,* Paramecium, etc.
UGA	Stop	Cys	*Euplotes*

Genome engineering



Das AUG bestimmt den Leserahmen = ORF

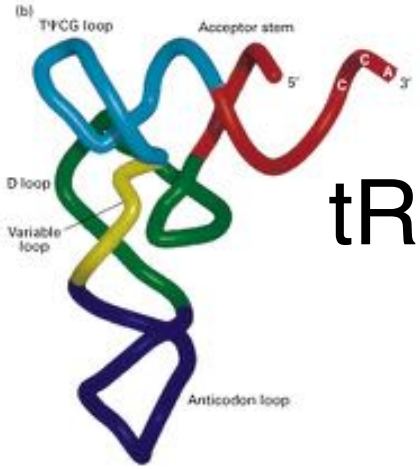


ACGGACCAUGUACCCCCGCAGCUGCA

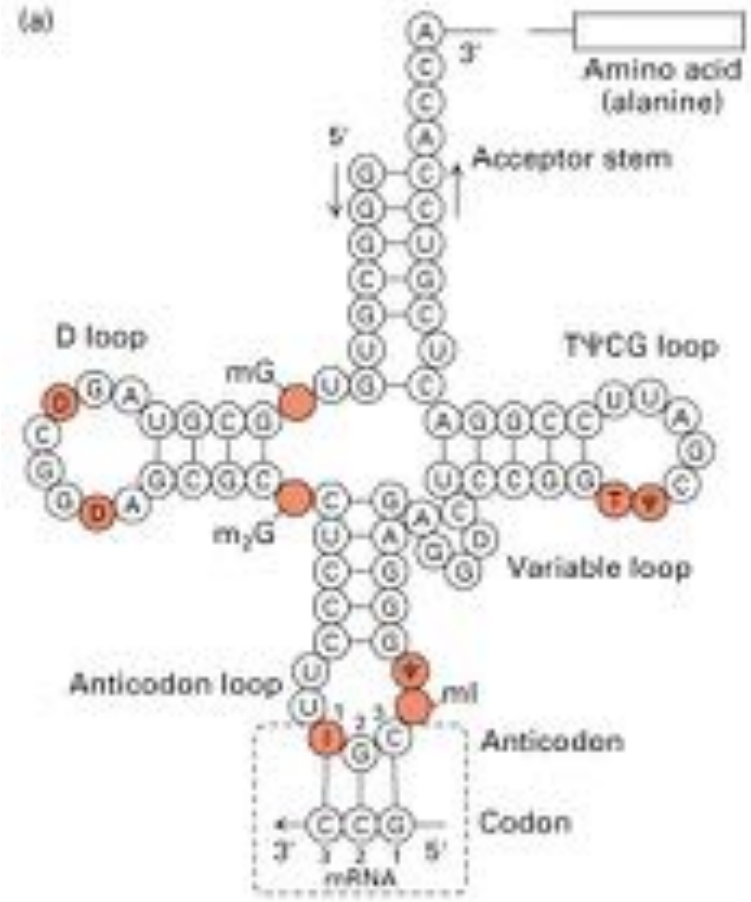
Met Tyr Pro Arg Ser Cys

Second letter

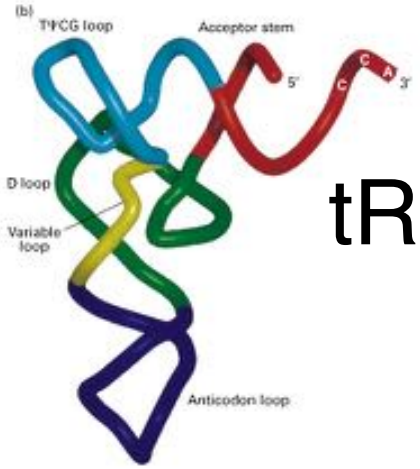
First letter	U	C	A	G	Third letter
U	UUU Phe, UUC Phe, UUA Leu, UUG Leu	UCU Ser, UCC Ser, UCA Ser, UCG Ser	UAU Tyr, UAC Tyr, UAA Stop, UAG Stop	UGU Cys, UGC Cys, UGA Stop, UGG Trp	U C A G
C	CUU Leu, CUC Leu, CUA Leu, CUG Leu	CCU Pro, CCC Pro, CCA Pro, CCG Pro	CAU His, CAC His, CAA Gln, CAG Gln	CGU Arg, CGC Arg, CGA Arg, CGG Arg	U C A G
A	AUU Ile, AUC Ile, AUA Ile, AUG Met	ACU Thr, ACC Thr, ACA Thr, ACG Thr	AAU Asn, AAC Asn, AAA Lys, AAG Lys	AGU Ser, AGC Ser, AGA Arg, AGG Arg	U C A G
G	GUU Val, GUC Val, GUA Val, GUG Val	GCU Ala, GCC Ala, GCA Ala, GCG Ala	GAU Asp, GAC Asp, GAA Glu, GAG Glu	GGU Gly, GGC Gly, GGA Gly, GGG Gly	U C A G



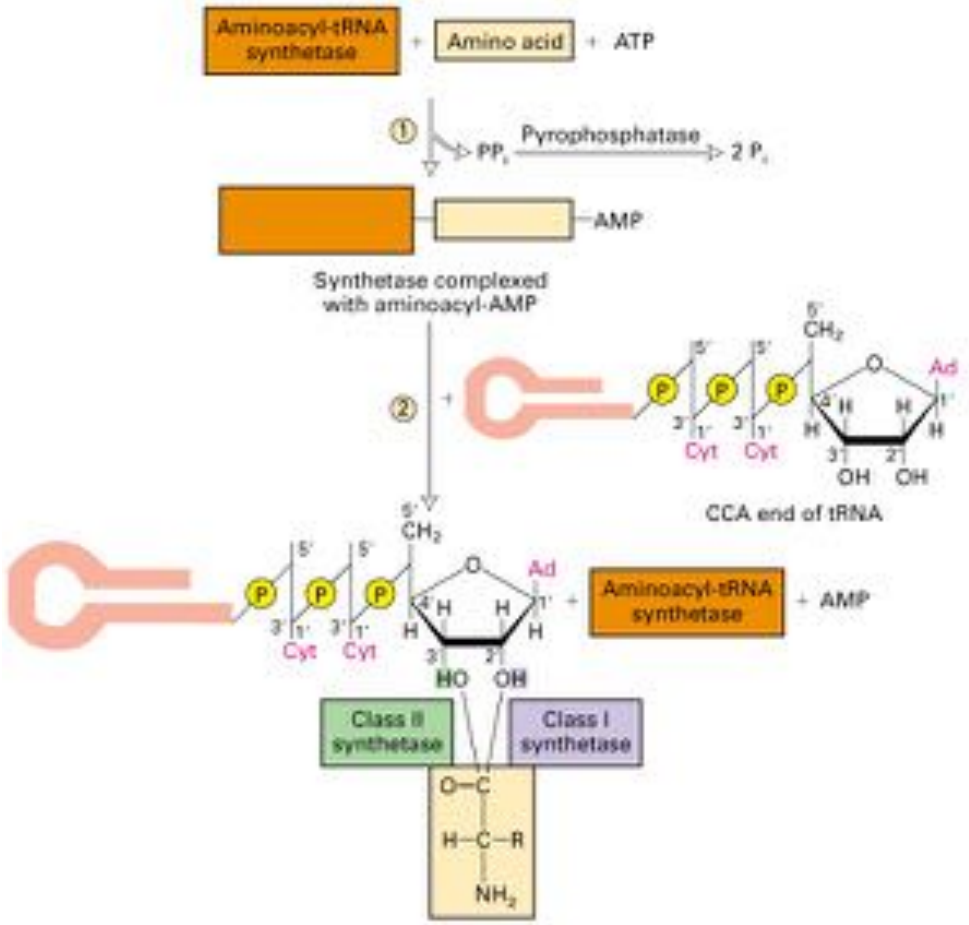
tRNA



Es gibt 20 Aminosäuren.
Es gibt 64 (4^3) Codons.
Prokaryoten haben 30-40 verschiedene tRNAs
Eukaryoten haben 50-100 verschiedene tRNAs.
Jede tRNA wird vielfach modifiziert.
Am 3 ‘ Ende wird eine CCA Sequenz angehängt (nicht Genom-kodiert!).

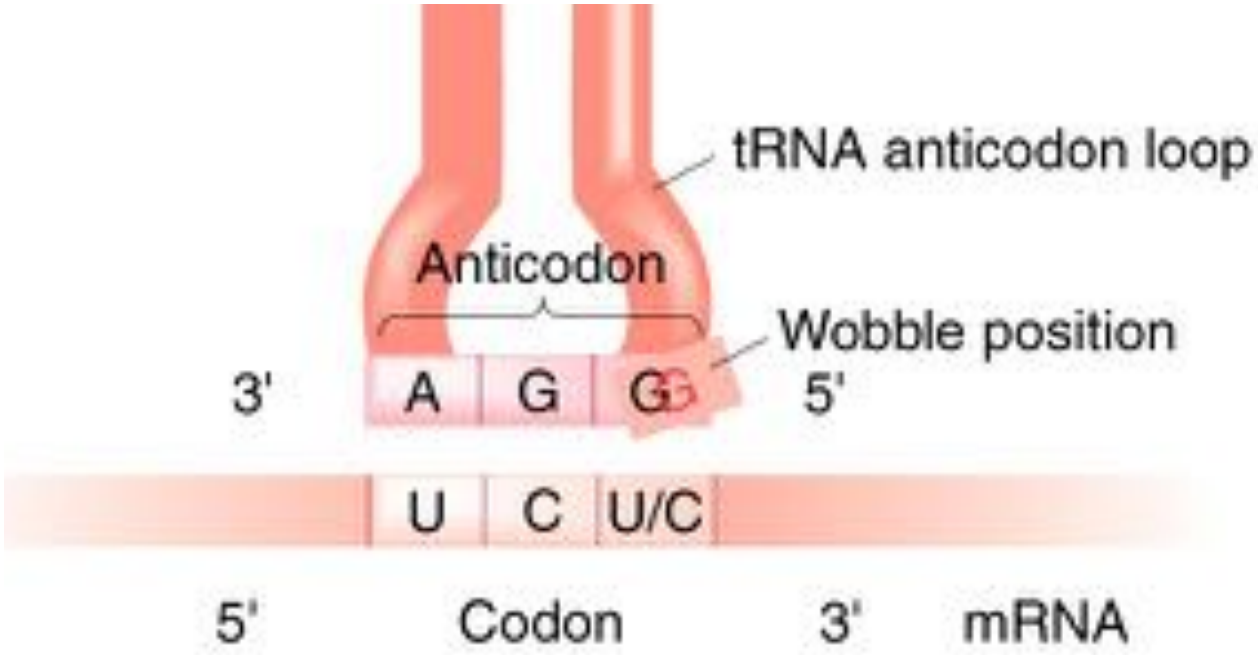


tRNA



Jede tRNA wird von einer spezifischen Aminoacyl-tRNA-Synthetase erkannt.
Nach Beladung erkennt die tRNA ein spezifisches Codon
-> aber Wobble-Position!

Eine tRNA kann mehrere Codons auf der mRNA erkennen





If these bases are in first, or wobble, position of anticodon

C	A	G	U	I	
G	U	C	A	C	then the tRNA may recognize codons in mRNA having these bases in third position
		U	G	A	
				U	

Ribosomen

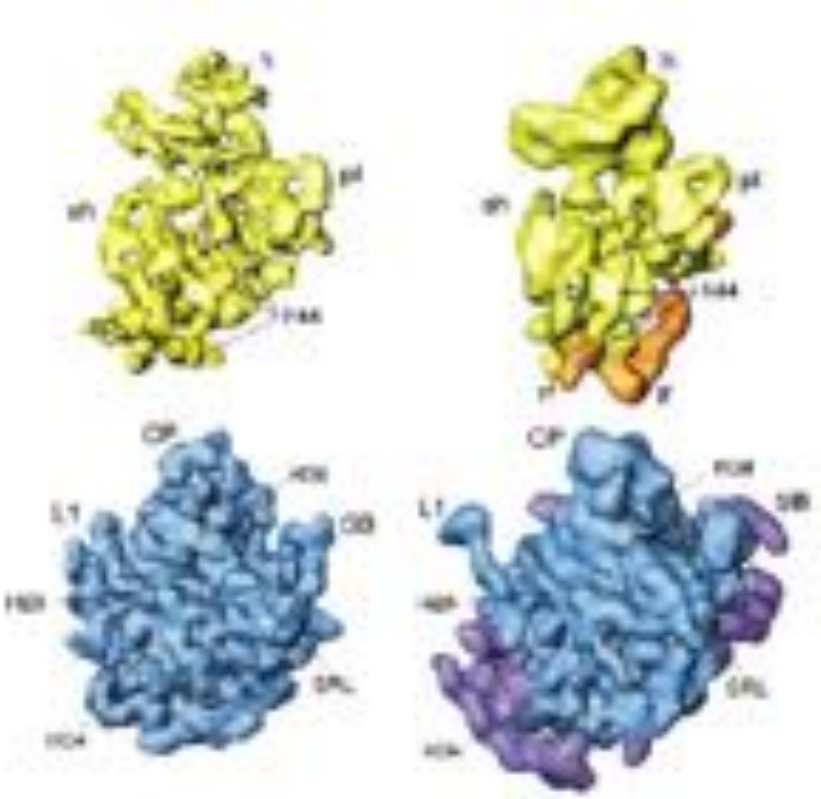
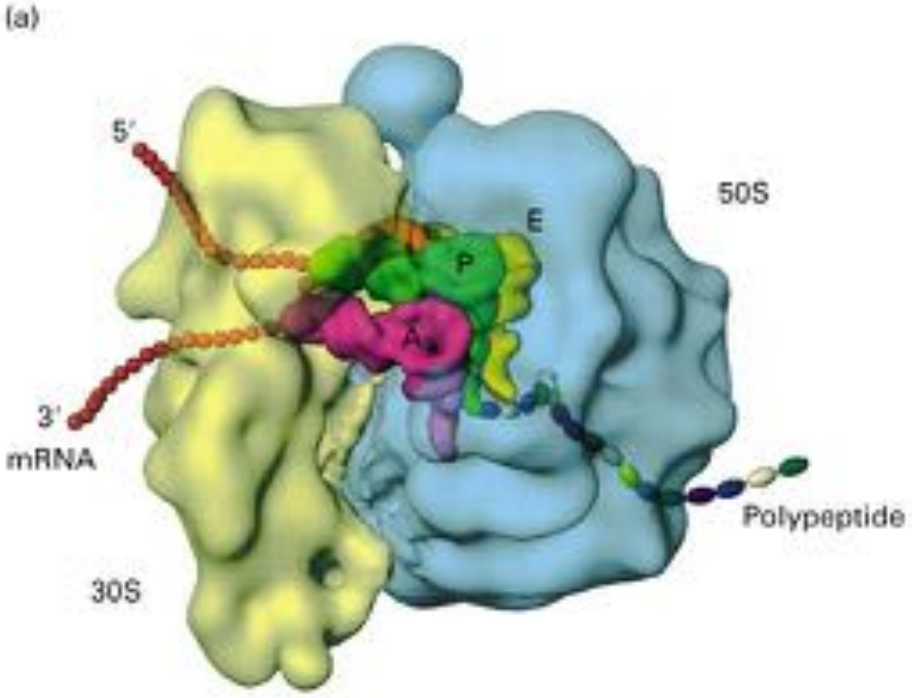
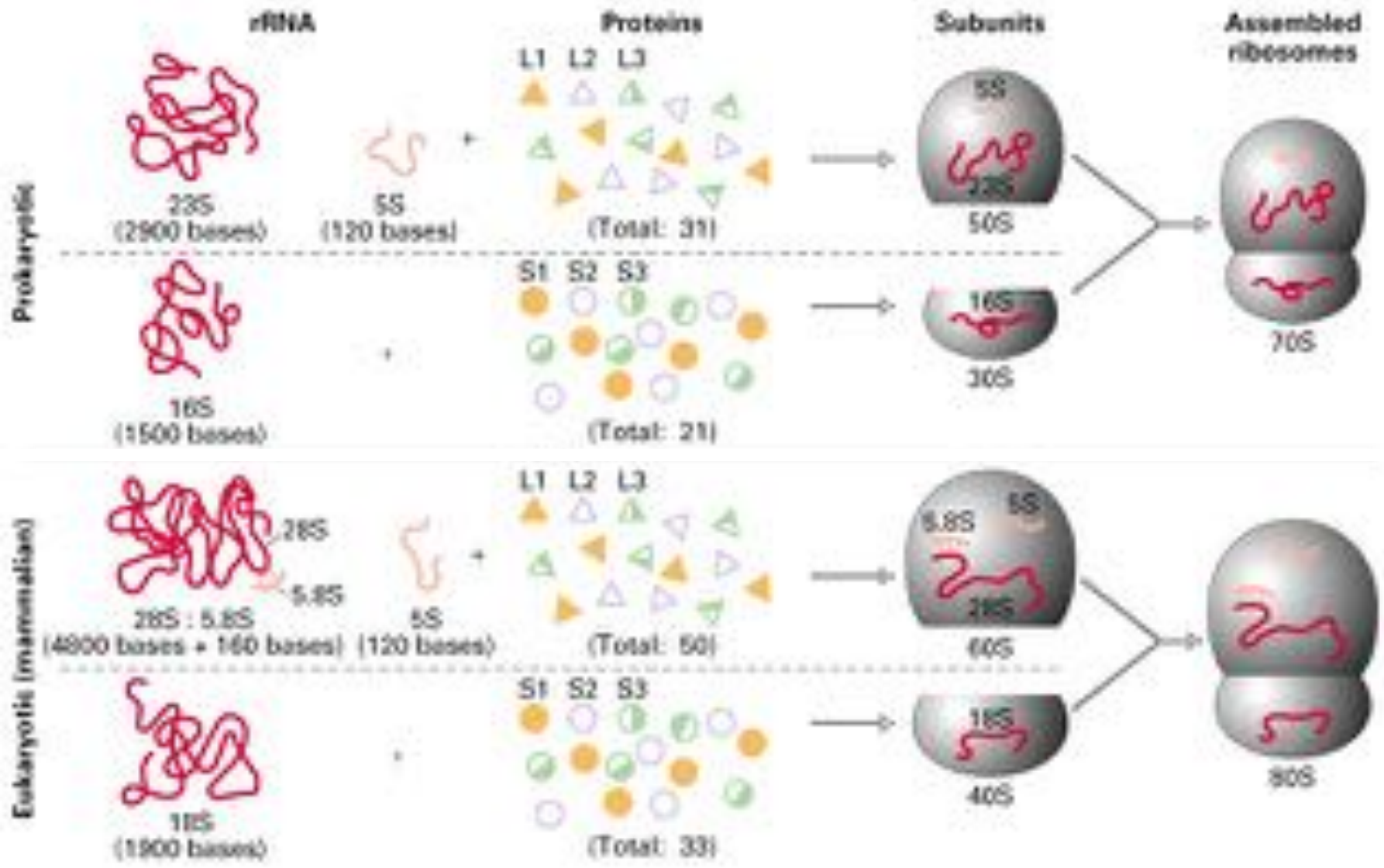


Figure 1. Comparison of EM Density for rRNA from *E. coli* and *S. cerevisiae* Ribosomes

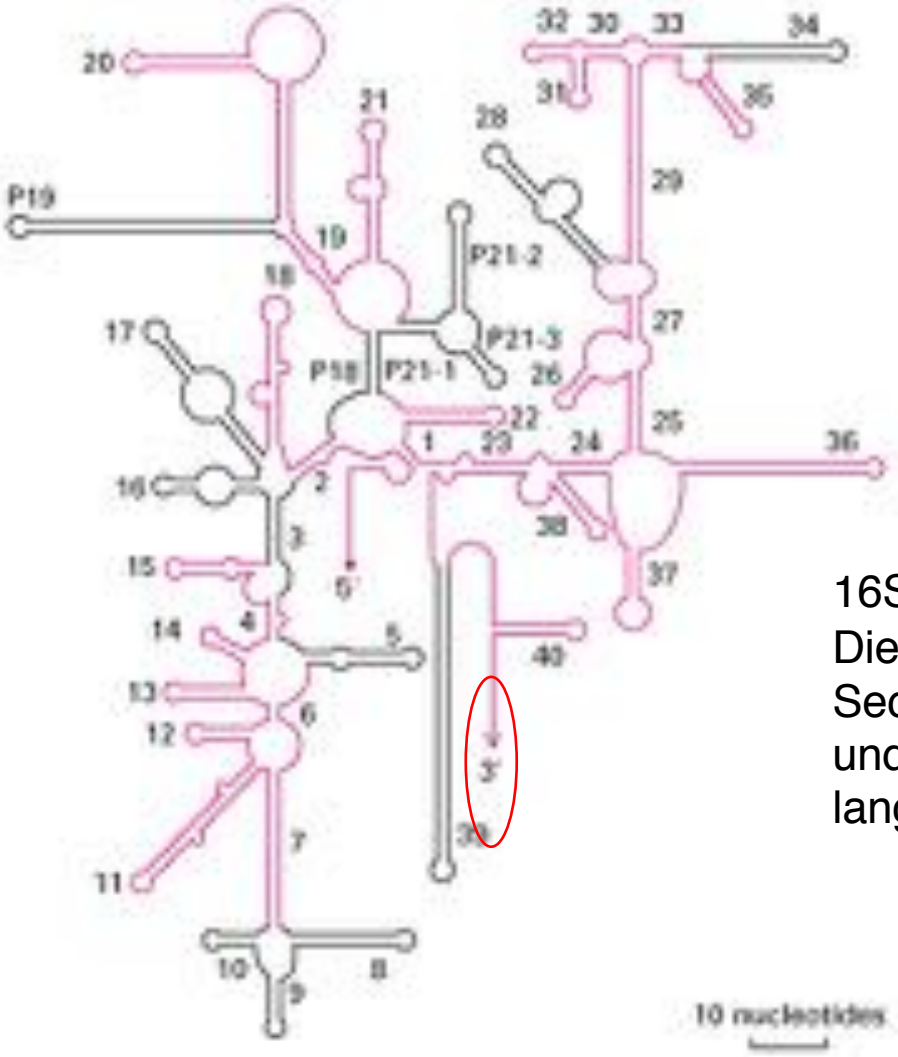


Prokaryoten 30S und 50S -> 70S
Eukaryoten 40S und 60S -> 80S

Ribosomenaufbau

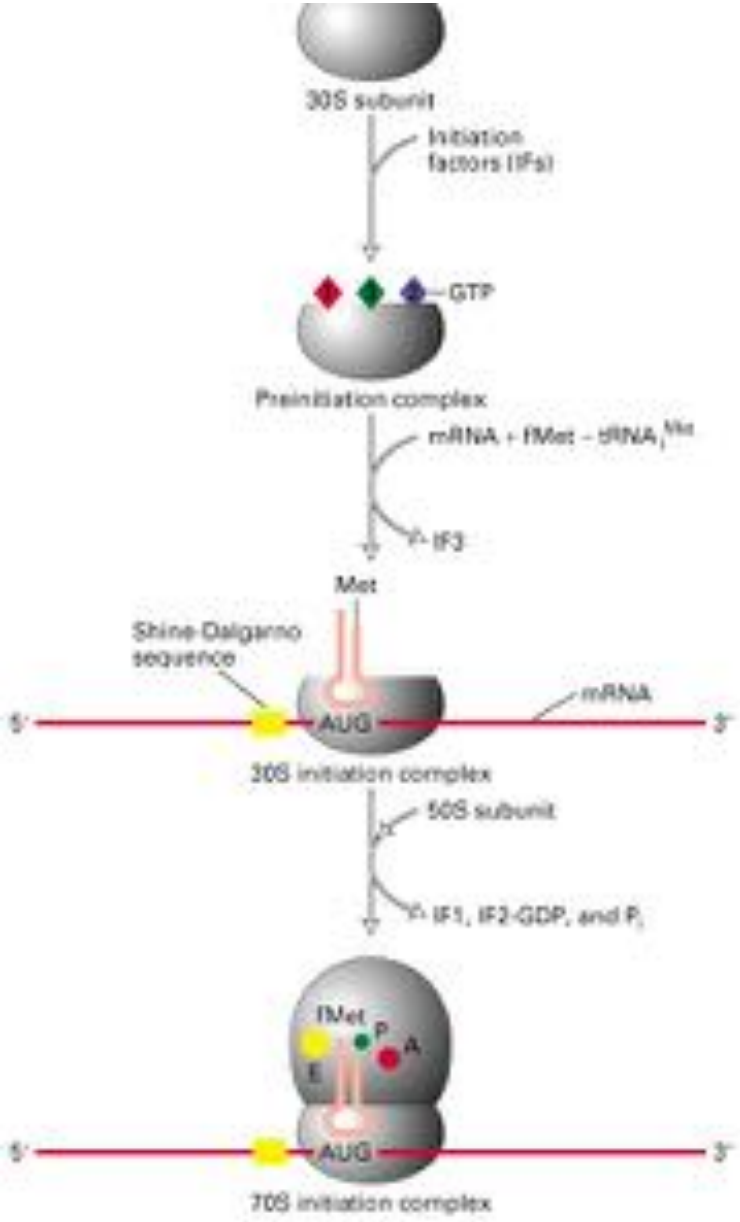


Ribosomale RNAs weisen ausgeprägte Sekundärstrukturen auf



16S rRNA (Prokaryoten)
Die Shine-Dalgarno Sequenz liegt an 3 ‘ Ende und ist 6-8 Nukleotide lang.

Initiation der Proteinsynthese bei Prokaryoten



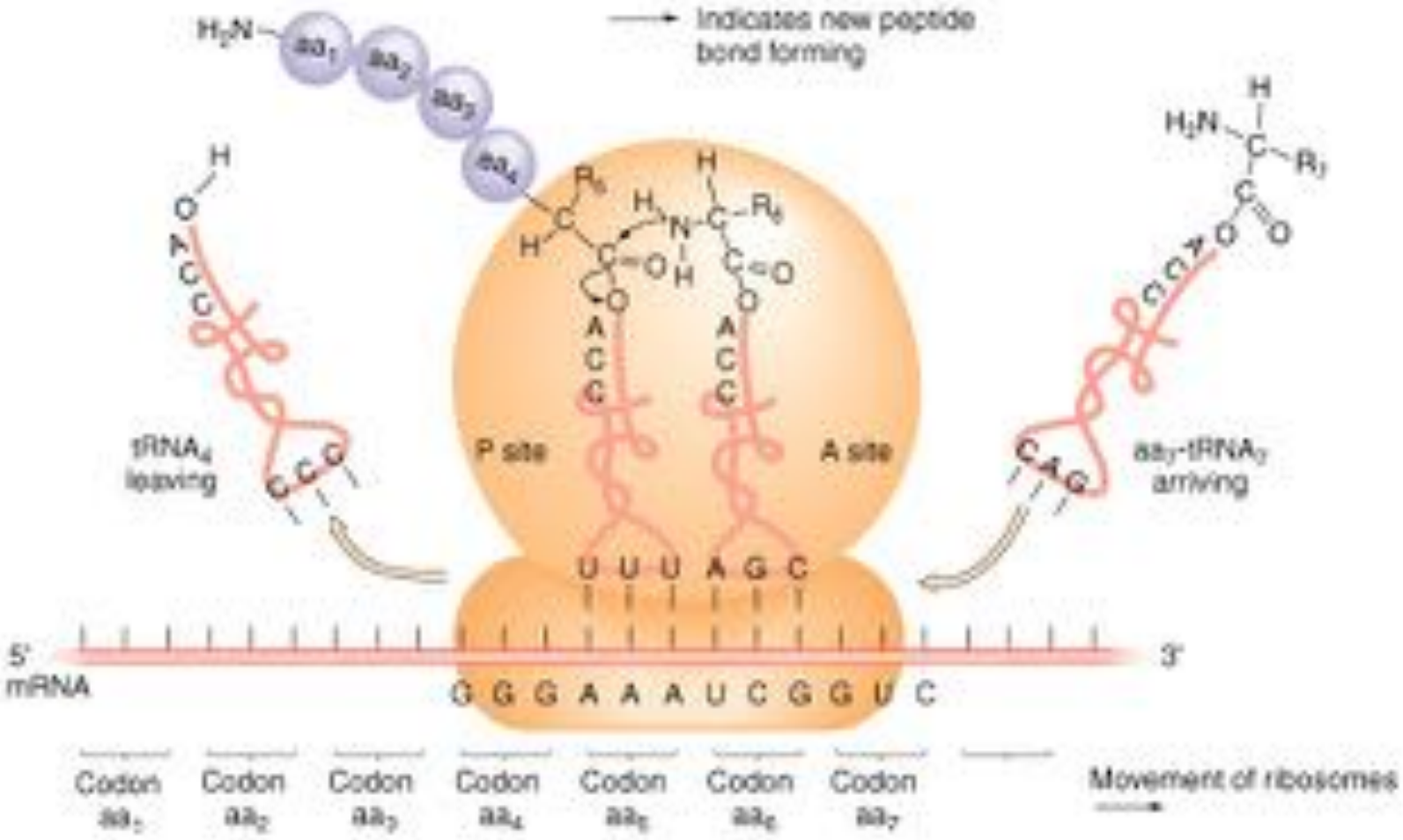
Drei Initiationsfaktoren binden an die 30S Untereinheit.

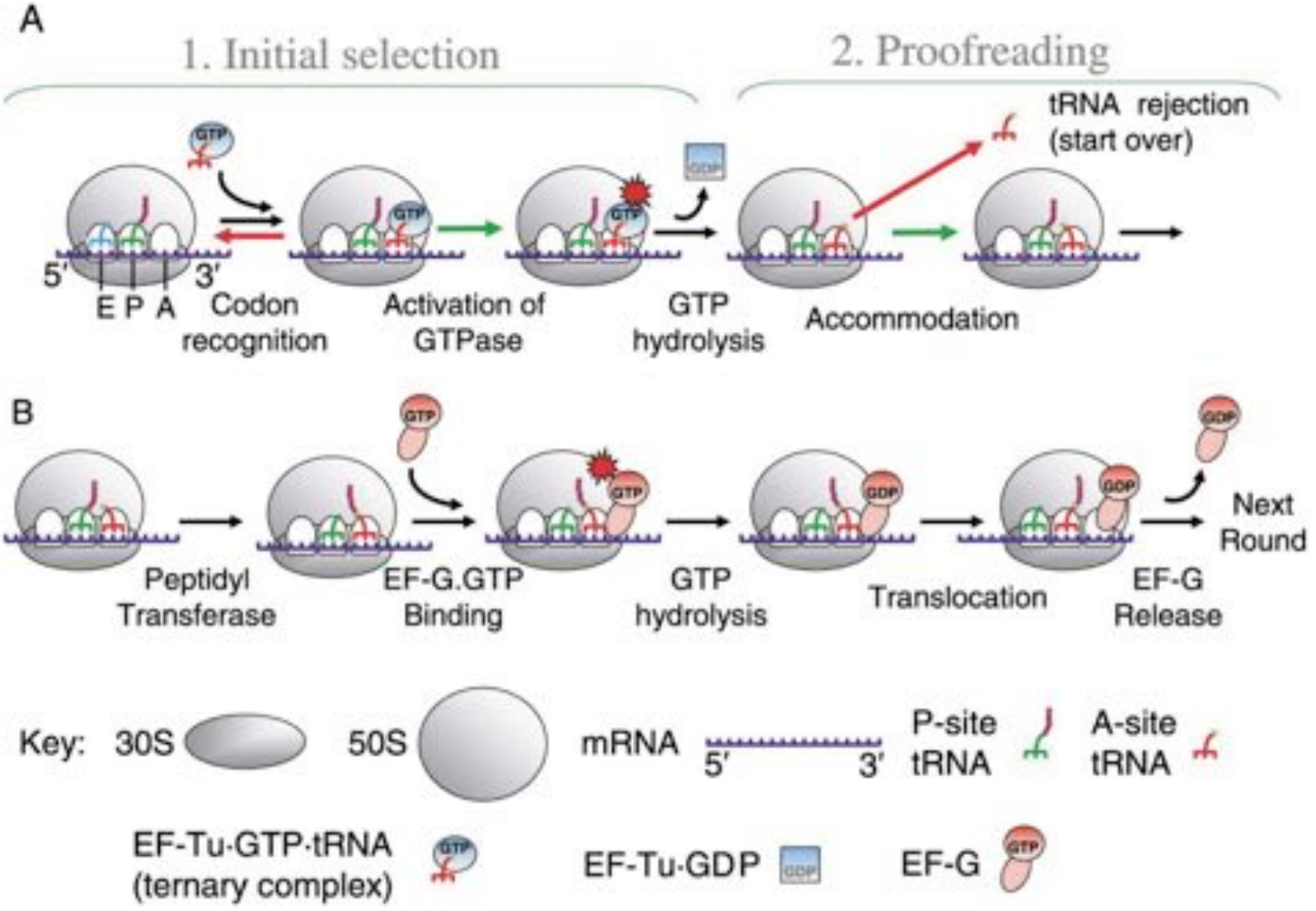
Die Formyl-Met tRNA bindet in Abhängigkeit von der Shine-Dalgarno Sequenz der 16S RNA.

Hydrolyse eines an IF2 gebundenen GTP führt zur Bildung des 70S Initiationskomplexes.

A-Stelle: Bindung der Aminoacyl tRNA
P-Stelle: Peptidyl-transferase
E-Stelle: Exit

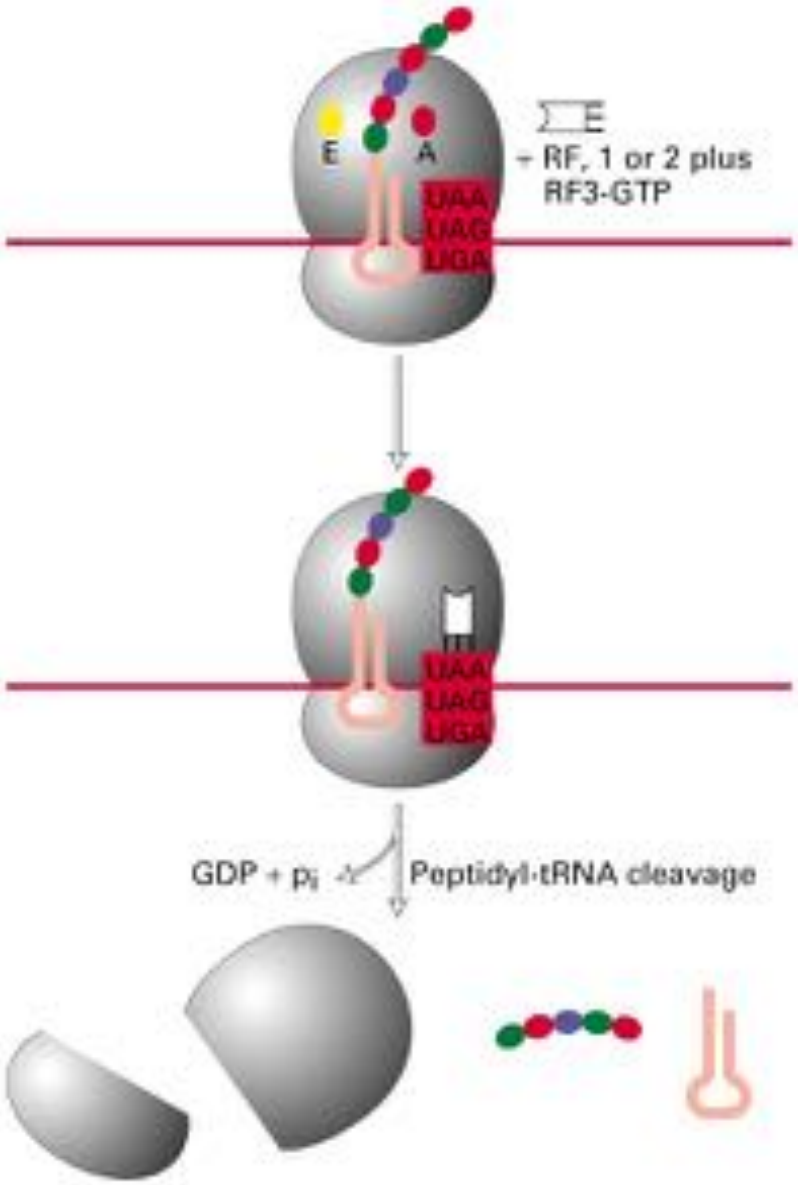
Elongationsphase





Fehlerhäufigkeit bei der Translation: $1/10^4$ ca. 8-10 As/sec

Termination der Translation



Terminationsfaktoren, auch release factors (RF) genannt, erkennen die Stopp-Codone
RF1 -> UAG
RF2 -> UGA UAA wird von beiden erkannt. Molekulare Mimikry

Die anschließende Bindung von RF3-GTP führt zur Kettenfreisetzung und nach GTP-Hydrolyse zum Zerfall des Ribosoms

Initiation der Translation in Eukaryoten

Die Translationseffizienz wird bestimmt über:

die Cap-Struktur am 5‘ Ende der mRNA,

über Sekundärstrukturen am 5‘ Ende,

über kurze **uORFs**,

das PolyA bindende Protein,

RNAs (miRNA) und

über Initiationsfaktoren.

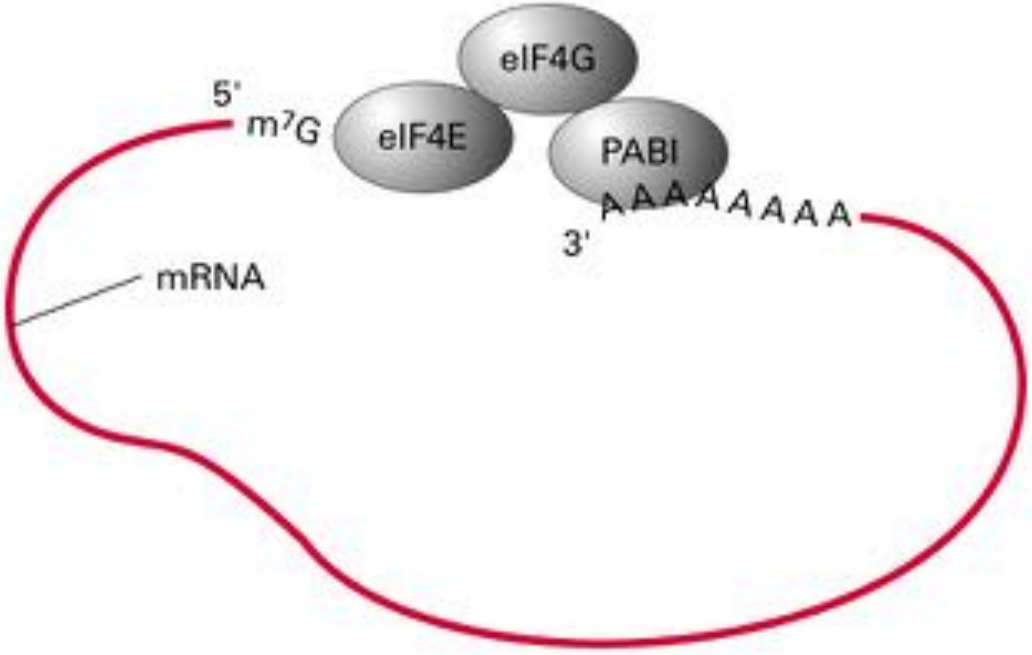
Table 1. Translation Initiation Factors

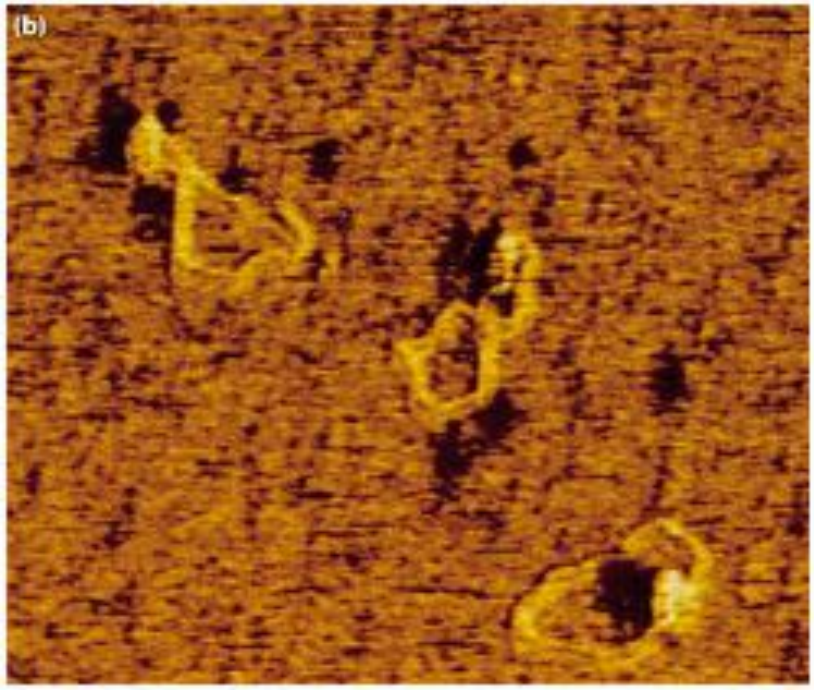
Eukaryotic Factor	Prokaryotic Factor	Archaeal Factor	Function
eIF1	IF3*	a-eIF1	Fidelity of AUG codon recognition
eIF1A	IF1	a-eIF1A	Facilitate Met-tRNA$_i^{Met}$ binding to small subunit
eIF2		a-eIF2	Bind Met-tRNA$_i^{Met}$ to 40S subunit; GTPase
eIF2B			Guanine-nucleotide exchange factor for eIF2
eIF3			Promote Met-tRNA$_i^{Met}$ and mRNA binding to 40S subunit
eIF4A		a-eIF4A	DEAD-box helicase
eIF4B			Promote eIF4A activity
eIF4E			m^{7}GpppX cap binding protein
eIF4F			Cap binding complex of eIFs 4A, 4E, and 4G
eIF4G			Adaptor protein interacts with many other factors
eIF4H			Similar to eIF4B
eIF5			AUG recognition and promote eIF2 GTPase activity
eIF5B	IF2	a-eIF5B	Subunit joining; in prokaryotes Met-tRNA$_i^{Met}$ binding

*The proposed grouping of eIF1 and IF3 is based on their common function to insure accurate Met-tRNA$_i^{Met}$ and AUG codon selection, and structural similarity of eIF1 (Fletcher et al., 1999) to the C-terminal domain of IF3 (Biou et al., 1995).

eIF3 = 5-11 Untereinheiten

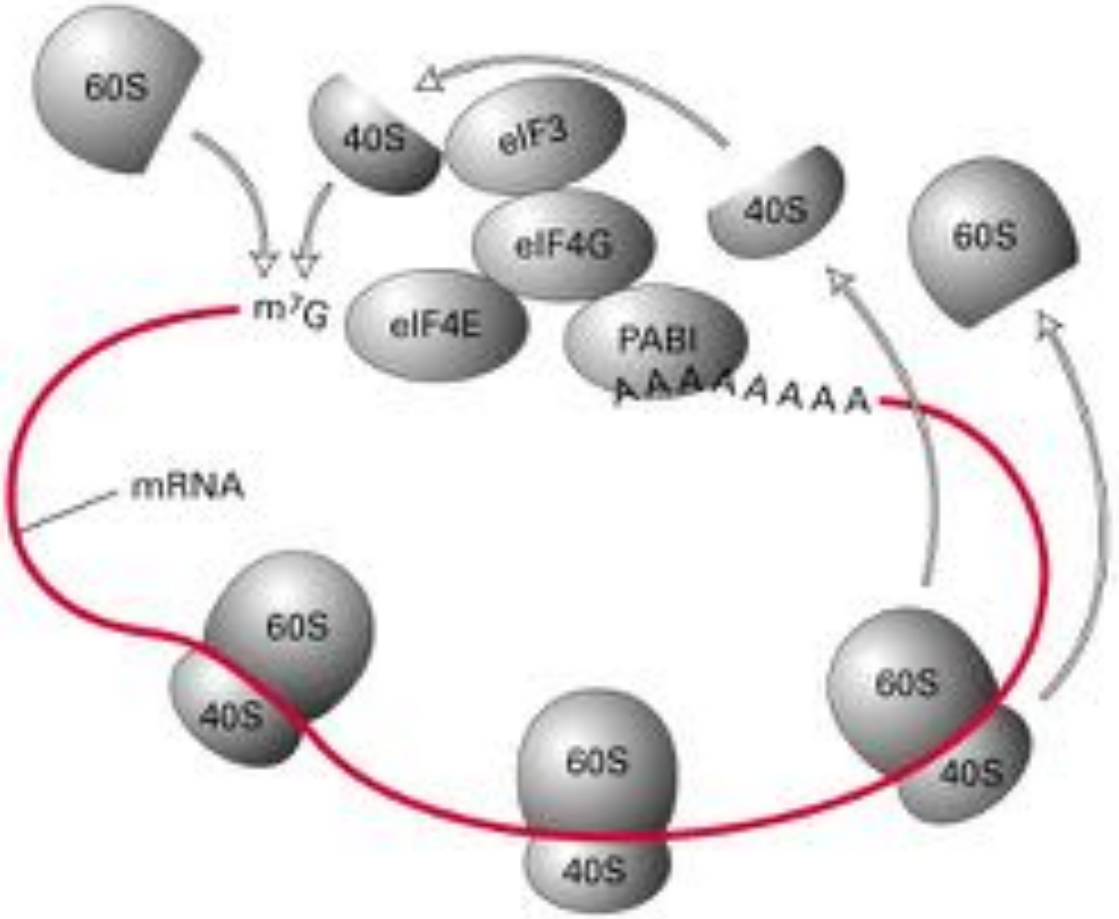
Warum hat das PolyA bindenden Protein einen Einfluß auf die Translation?



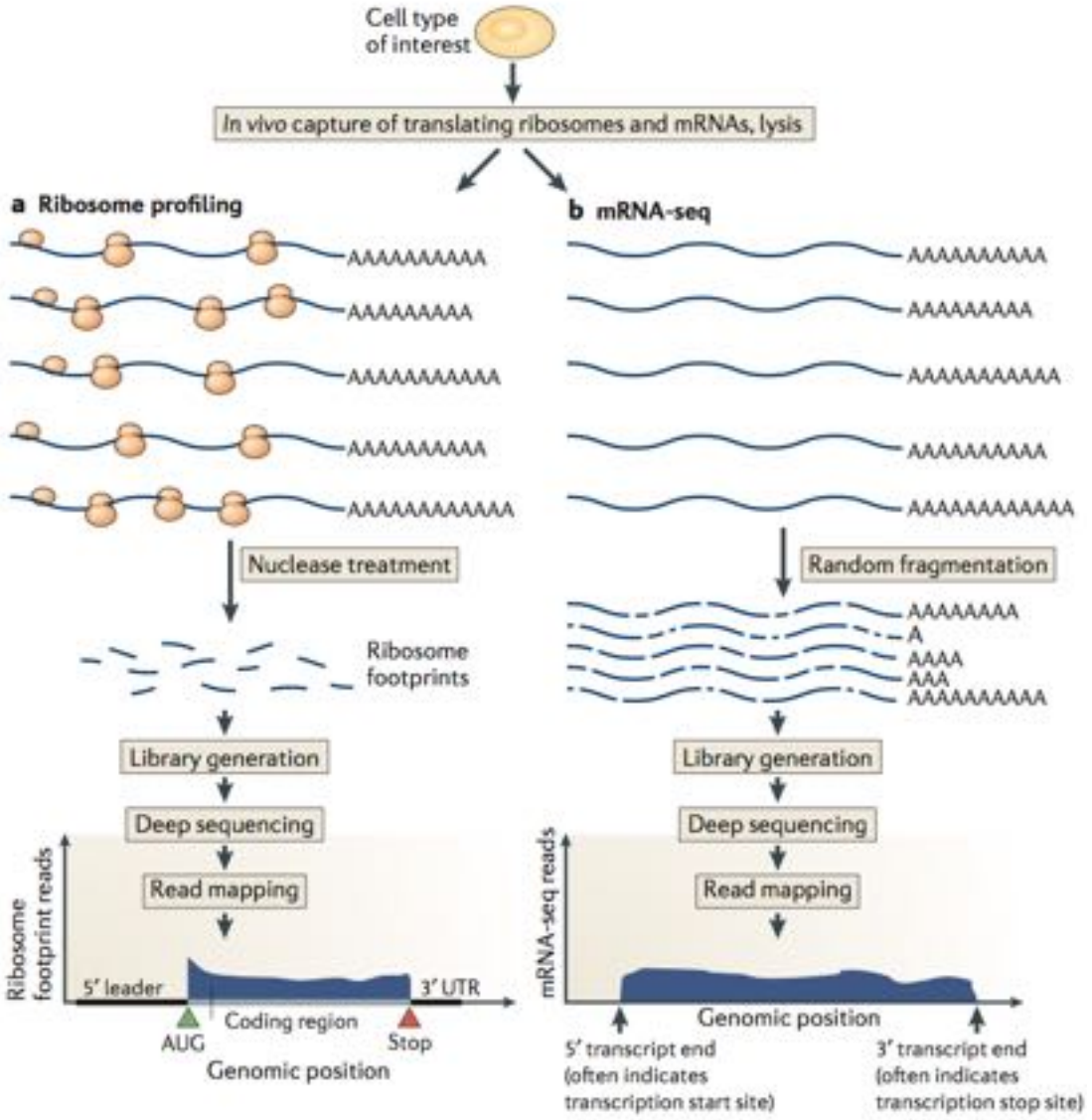


Qualitätskontrolle: Zirkularisierung der mRNA 1996 erstmals beschrieben

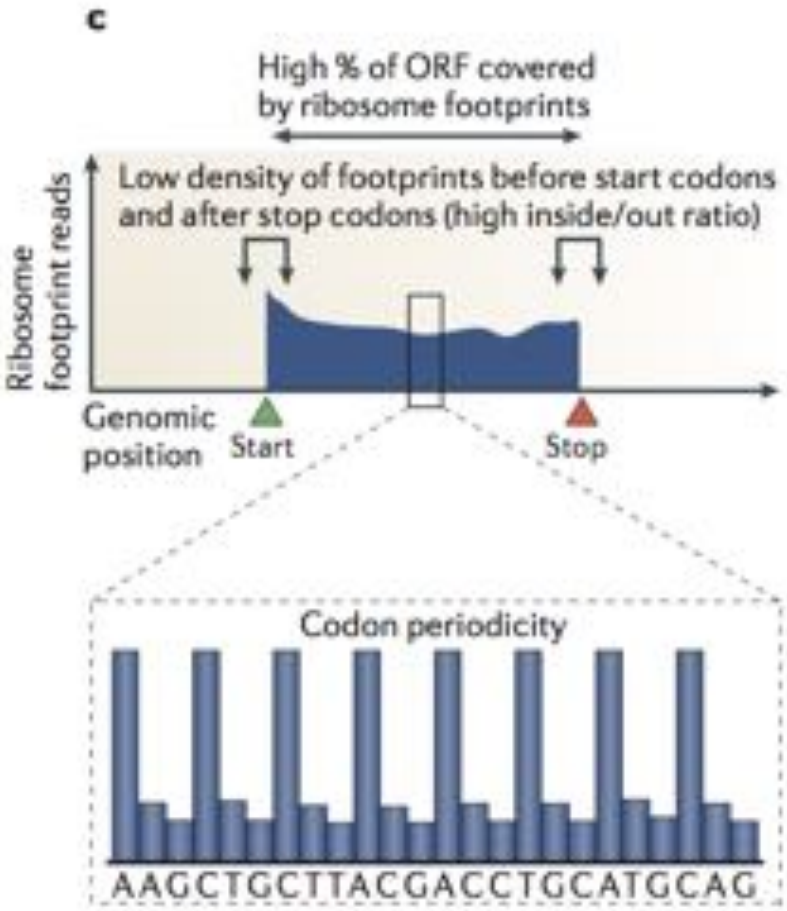
Initiation und Termination sind räumlich benachbart



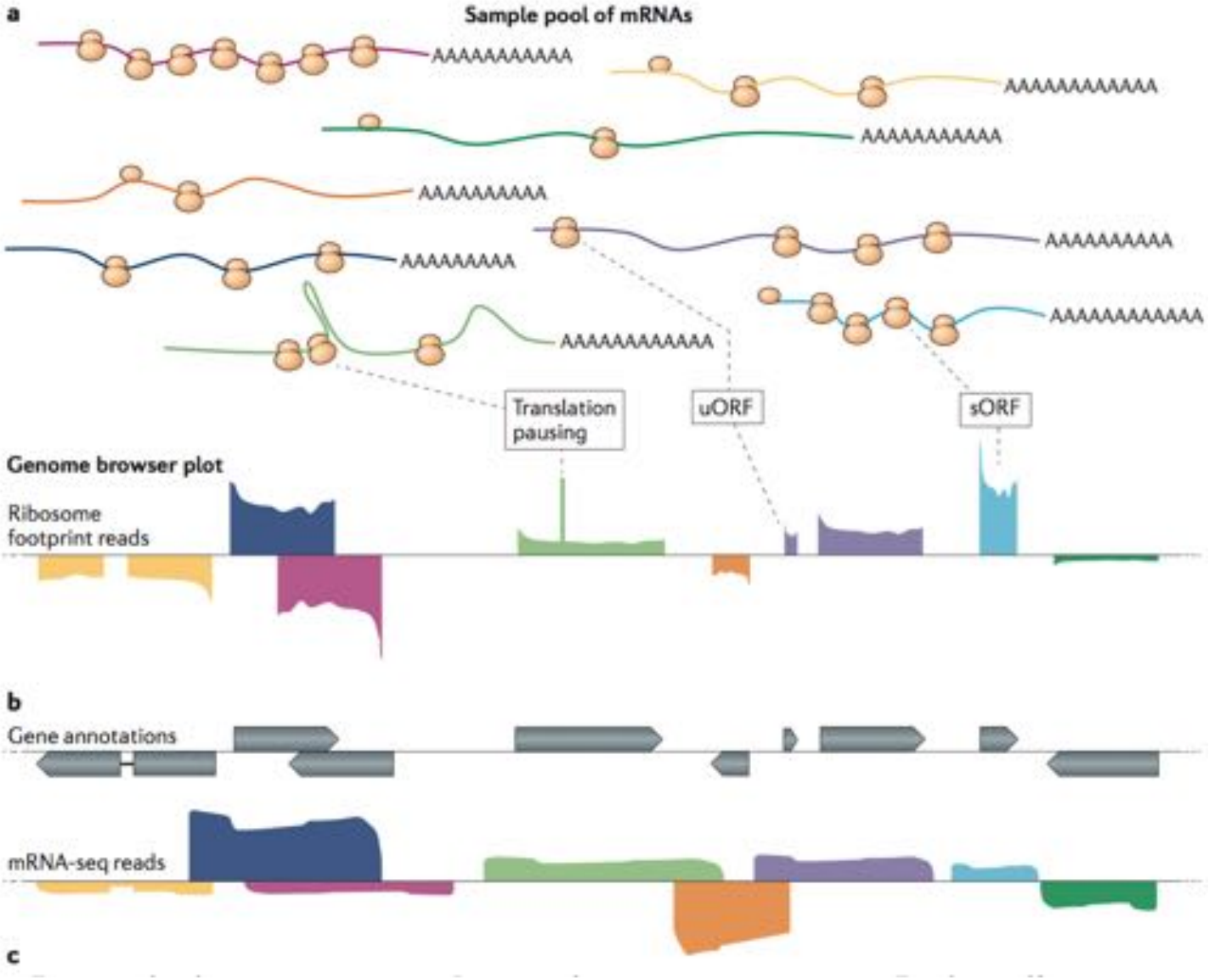
Ribosome profiling



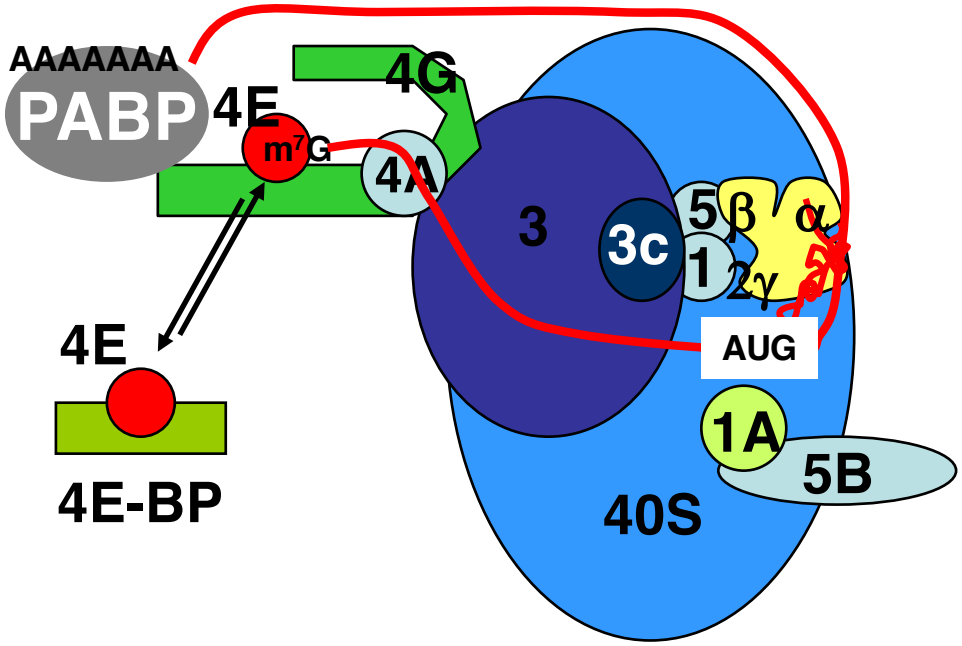
Ribosome profiling



Ribosome profiling



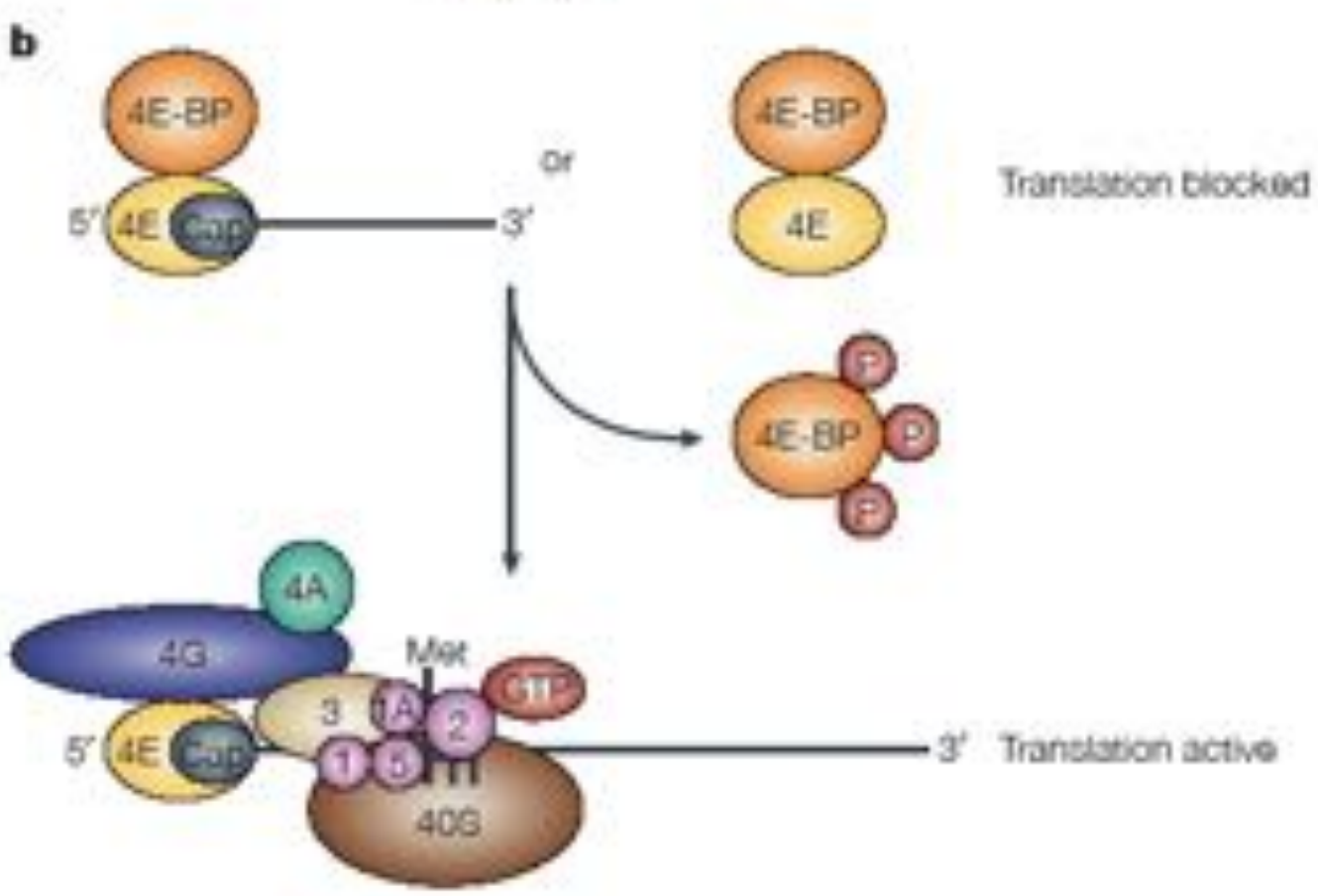
Kontrolle der Initiation



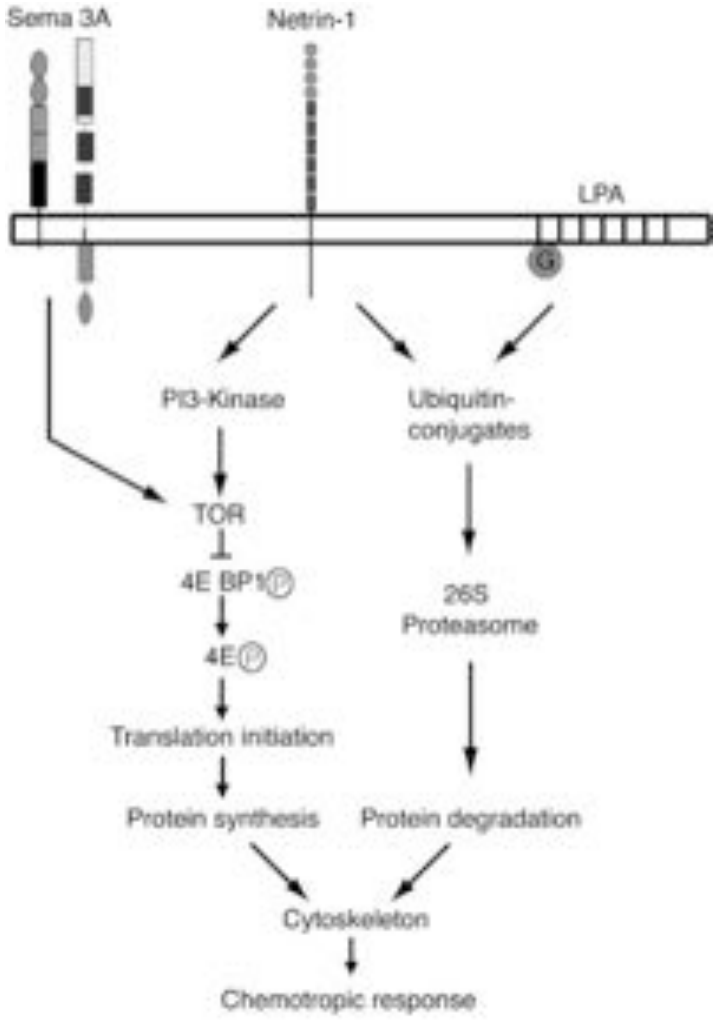
Cap-mRNA + eIF4E rekrutiert eIF4G und dann eIF4A(Helikase Aktivität).
eIF4G ist ein Adapter Protein das eine Interaktion mit dem PolyA Bindungsprotein und dem eIF3 vermittelt.

4E-BP bindet eIF4E.
eIF2 reguliert die Initiation der Translation.

Regulation der Translationsinitiation über das eIF4E Bindungsprotein 4E-BP

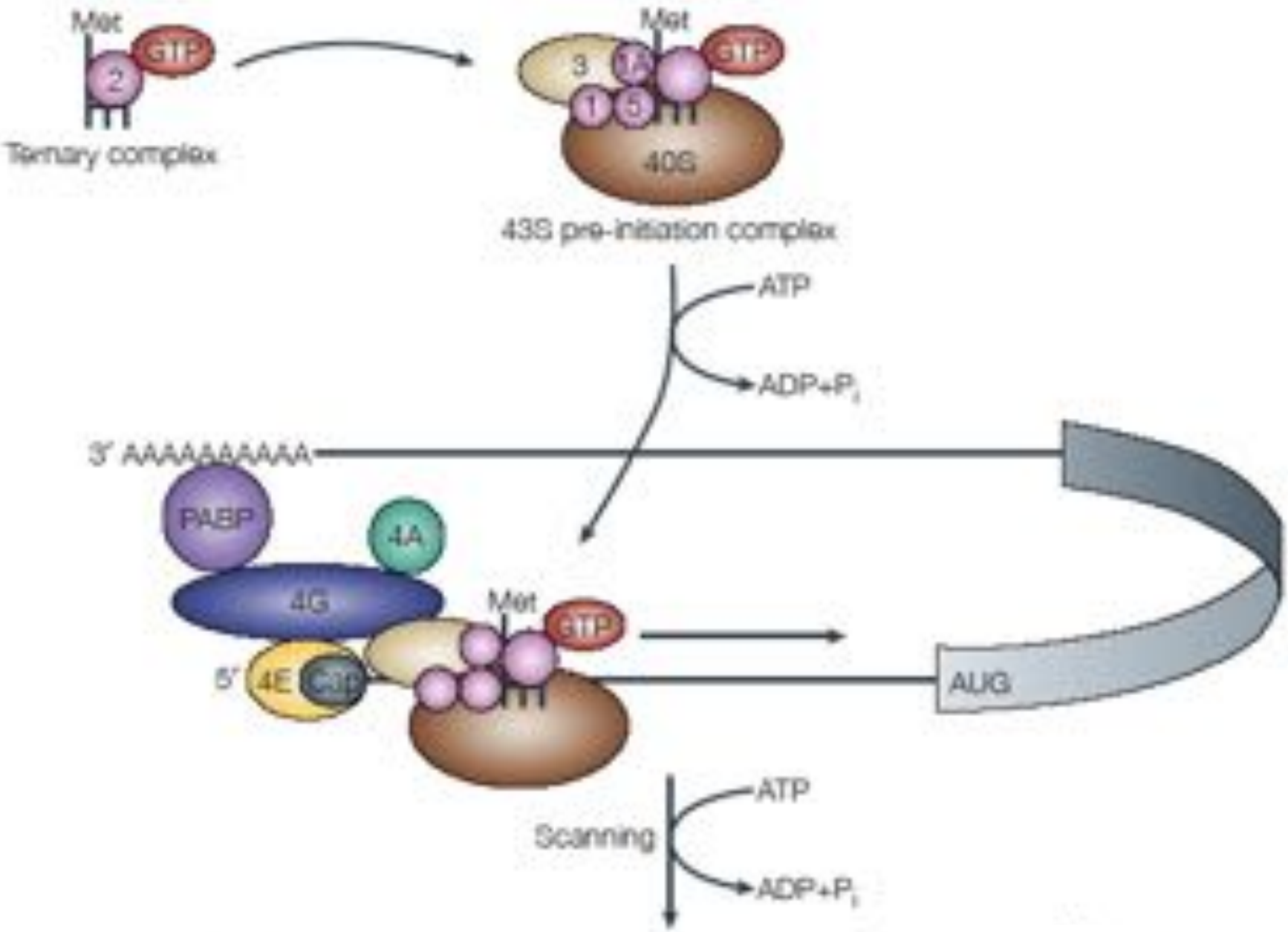


Lokale Translationskontrolle in der Synapse druch 4E-BP

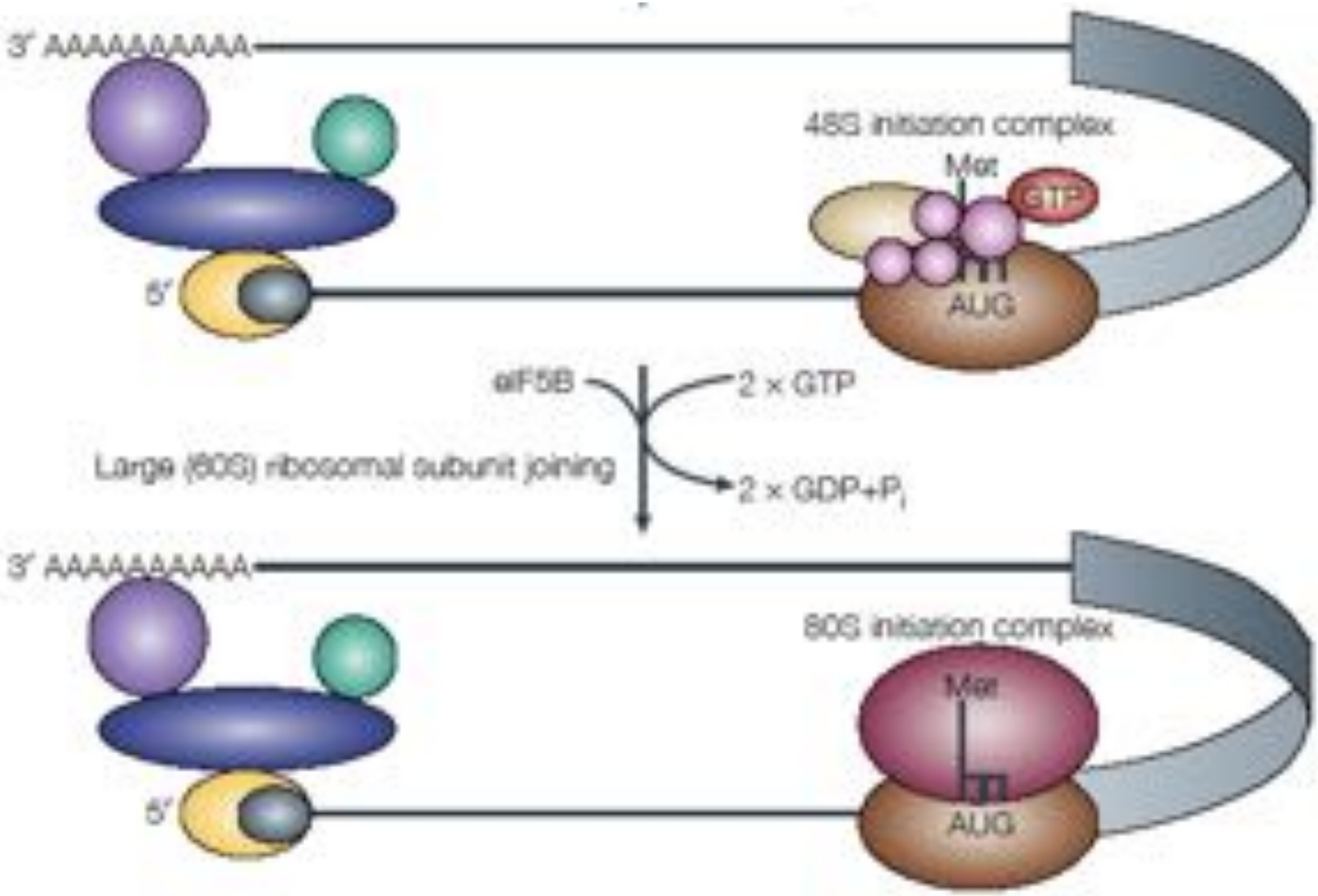


Lokale Translation im Wachstumskegel oder in der Synapse
-> Plastizität.

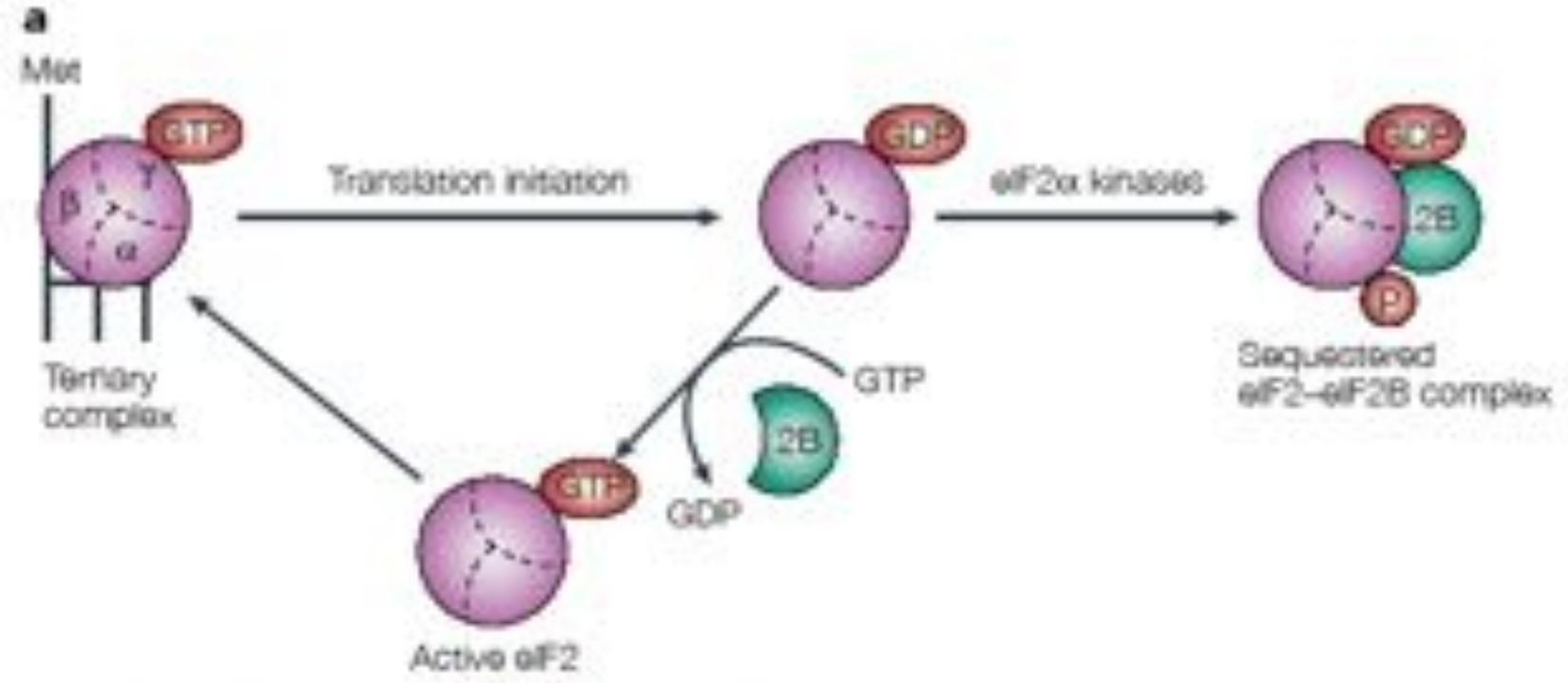
5’-Cap abhängige Initiation der Translation



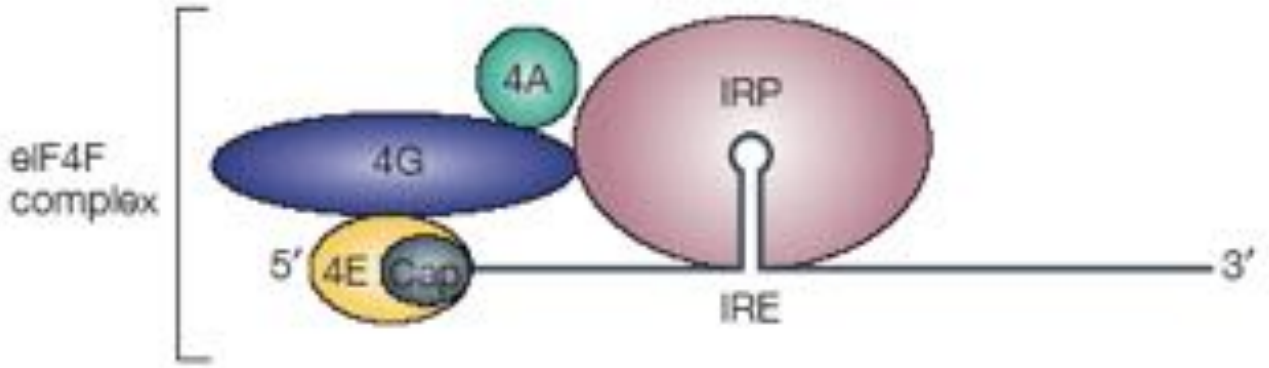
5’-Cap abhängige Initiation der Translation



Globale Kontrolle der Translation -> eIF2 Phosphorylierung

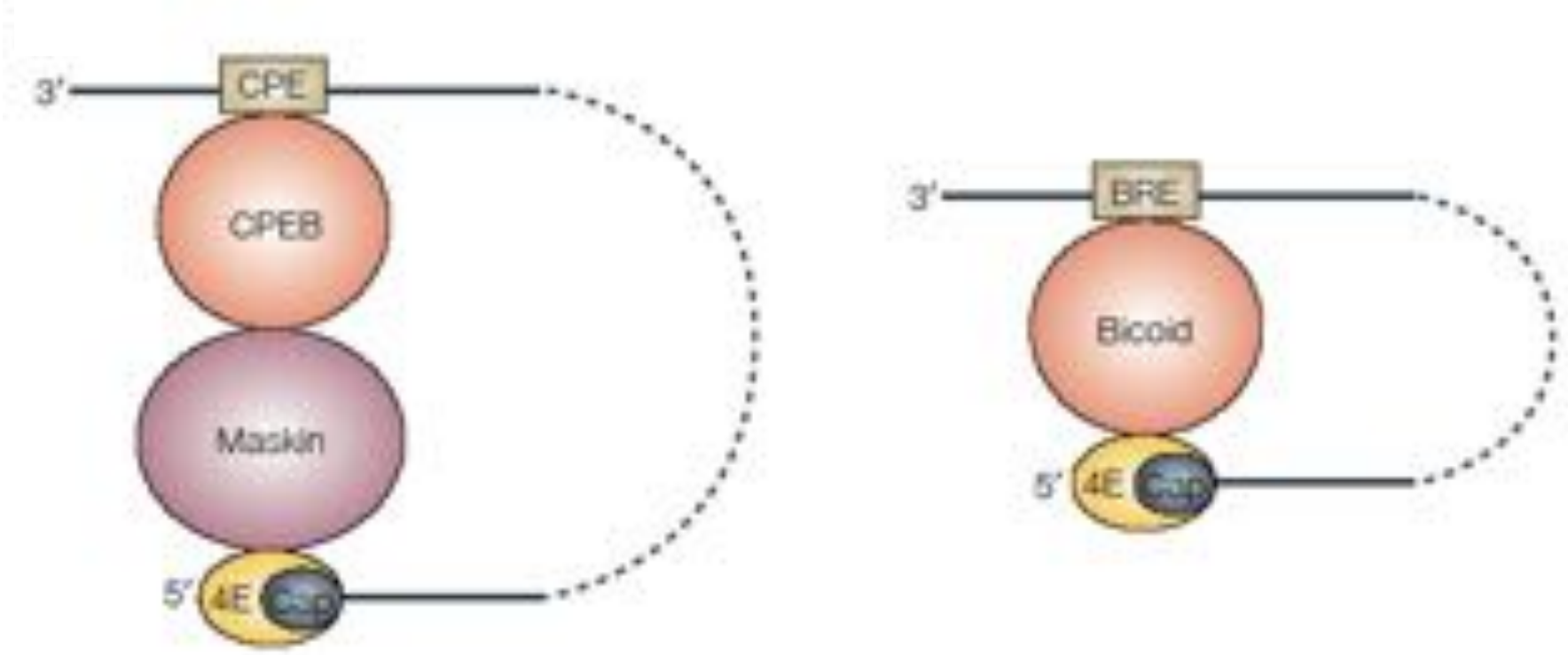


RNA spezifische Translationskontrolle



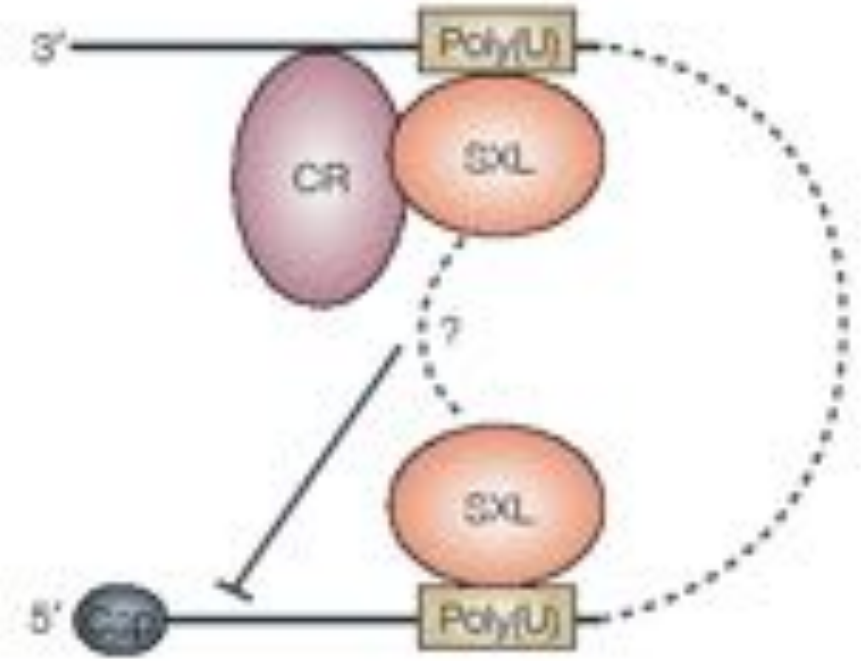
Eisen-abhängige Translation
Das *Iron Regulatory Protein* bindet an das *Iron Responsive Element* und verhindert die Bildung des Prä-Initiationskomplexes.

RNA spezifische Translationskontrolle



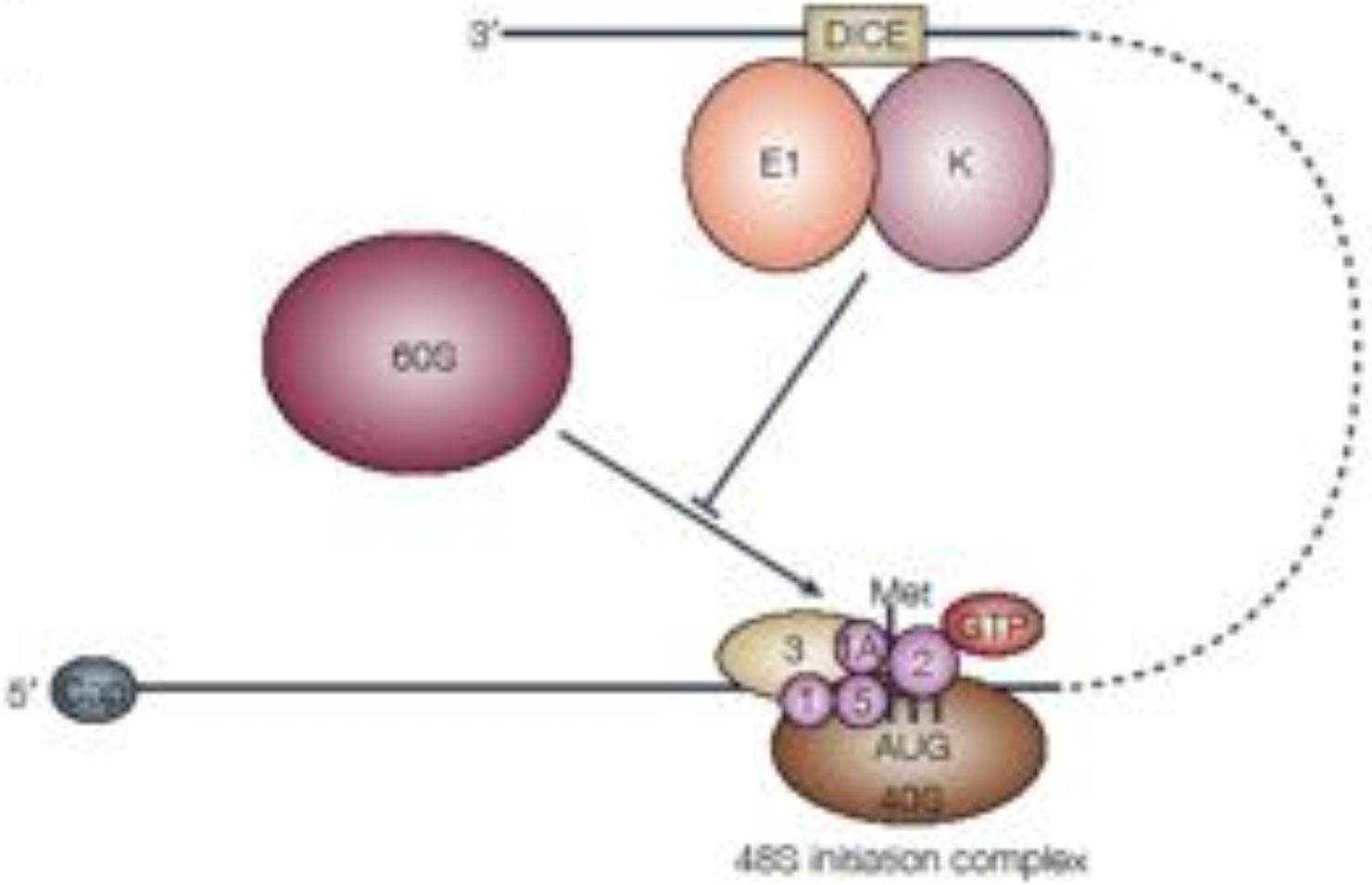
Interferenz der Ausbildung des eIF4E-Komplexes. Duale Rolle von Bicoid als Transkriptionsfaktor und Translationsregulator.
CPE: cytoplasmic polyadenylation element; BRE: Bicoid response element.

RNA spezifische Translationskontrolle



Sex lethal als Translationsinhibitor, 5' - Cap-unabhängig
Suppression der *msl* mRNA Translation bei der Dosiskompensation

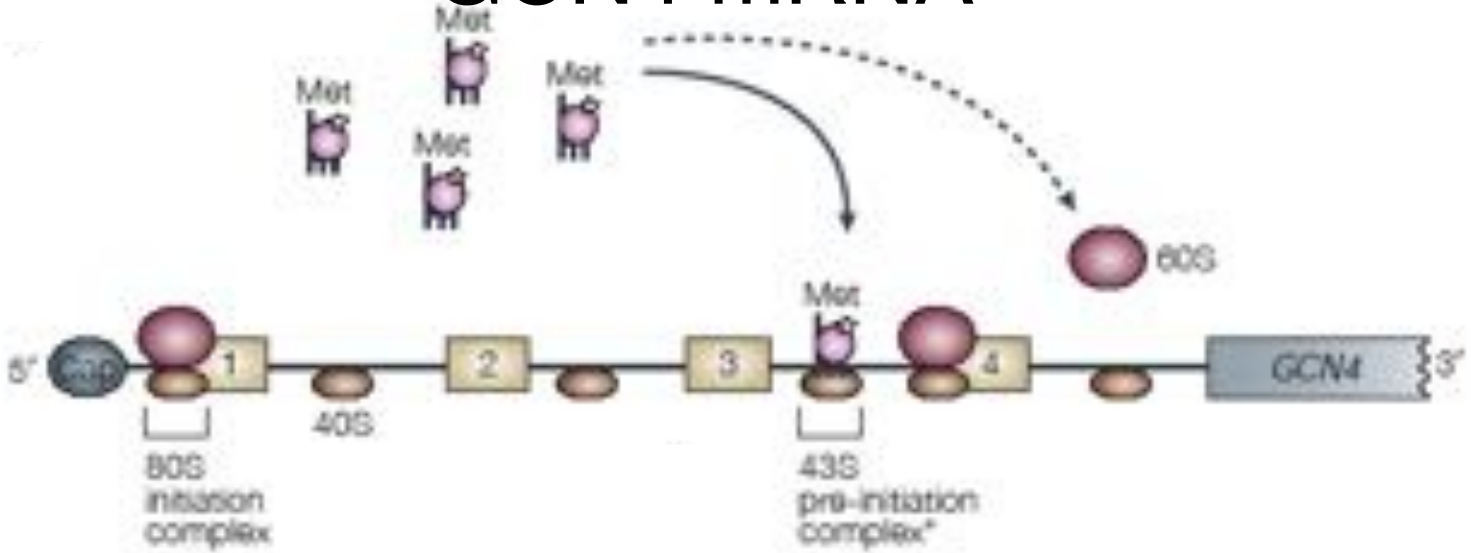
Regulation der Rekrutierung der 60S UE



Bindung der hnRNP K//hnRNP E1 Proteine an das *differentiation control element* verhindert die Rekrutierung der 60S UE.

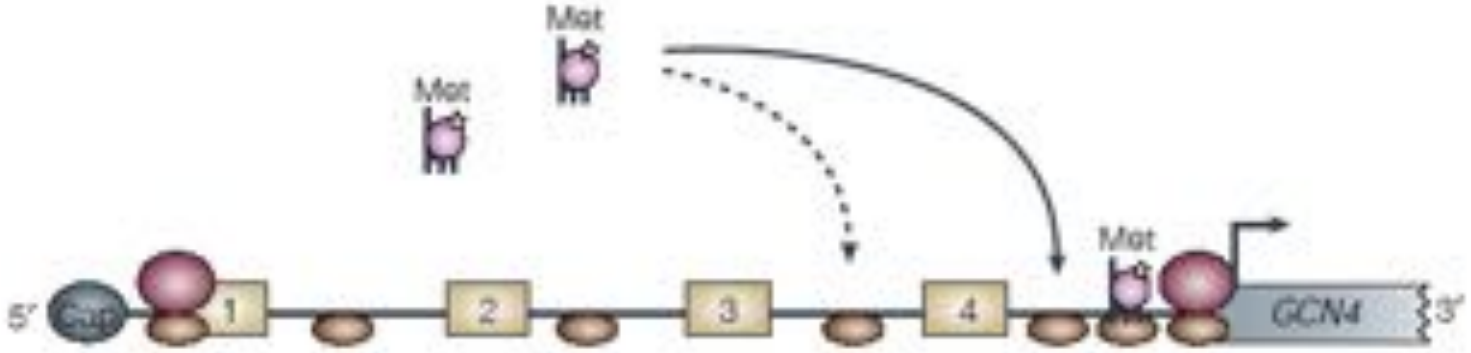
Kontrolle der Aminosäuresynthese in der Hefe durch upstream ORFs (uORFs) in der GCN4 mRNA

Sättigung

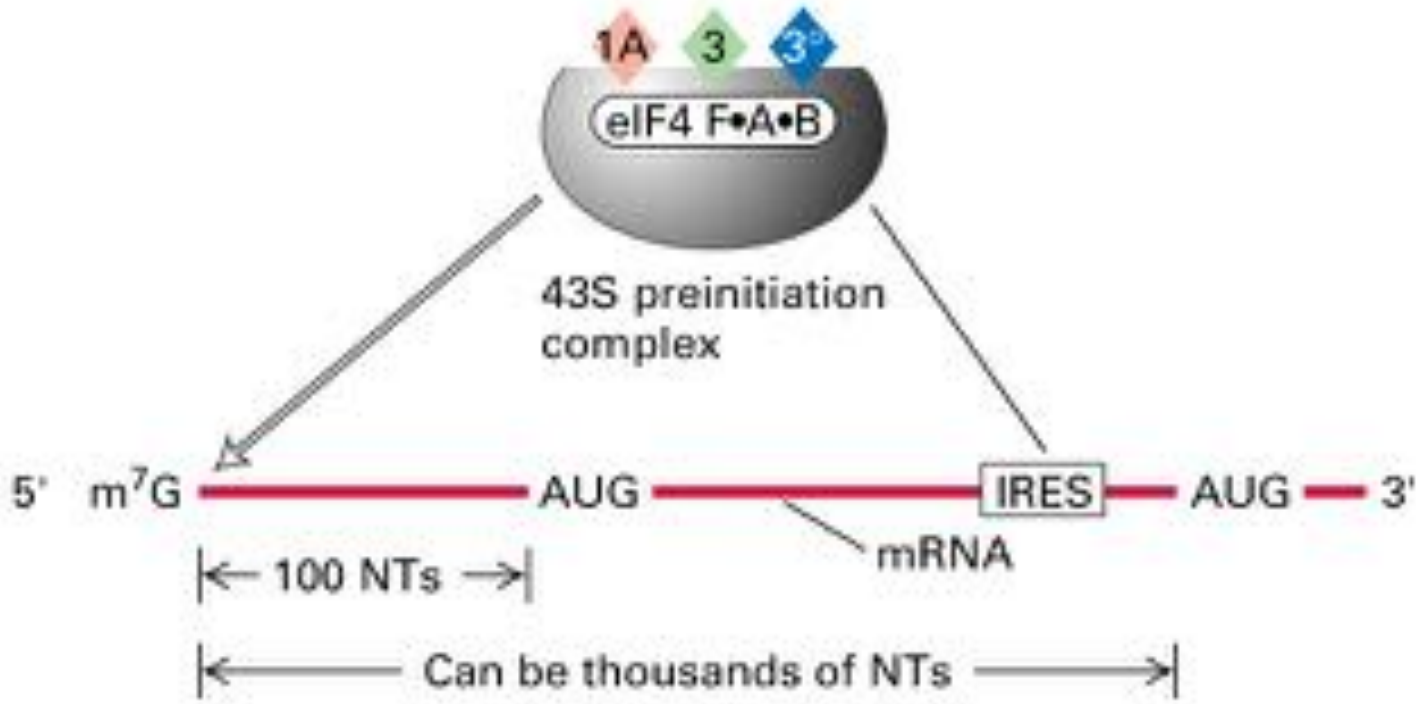


Hunger

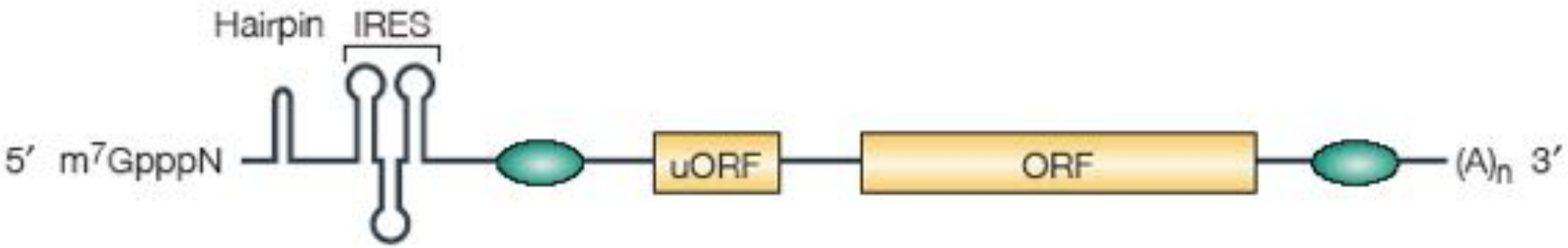
eIF2α Phosphorylierung, geringe Konzentration des 43S-Komplexes



Die Initiation der Translation erfolgt am Cap oder an einer IRES Sequenz



Zusammenfassung: Wie beeinflusst die mRNA die Translation?



Proteine rekrutieren den RNAi Apparat

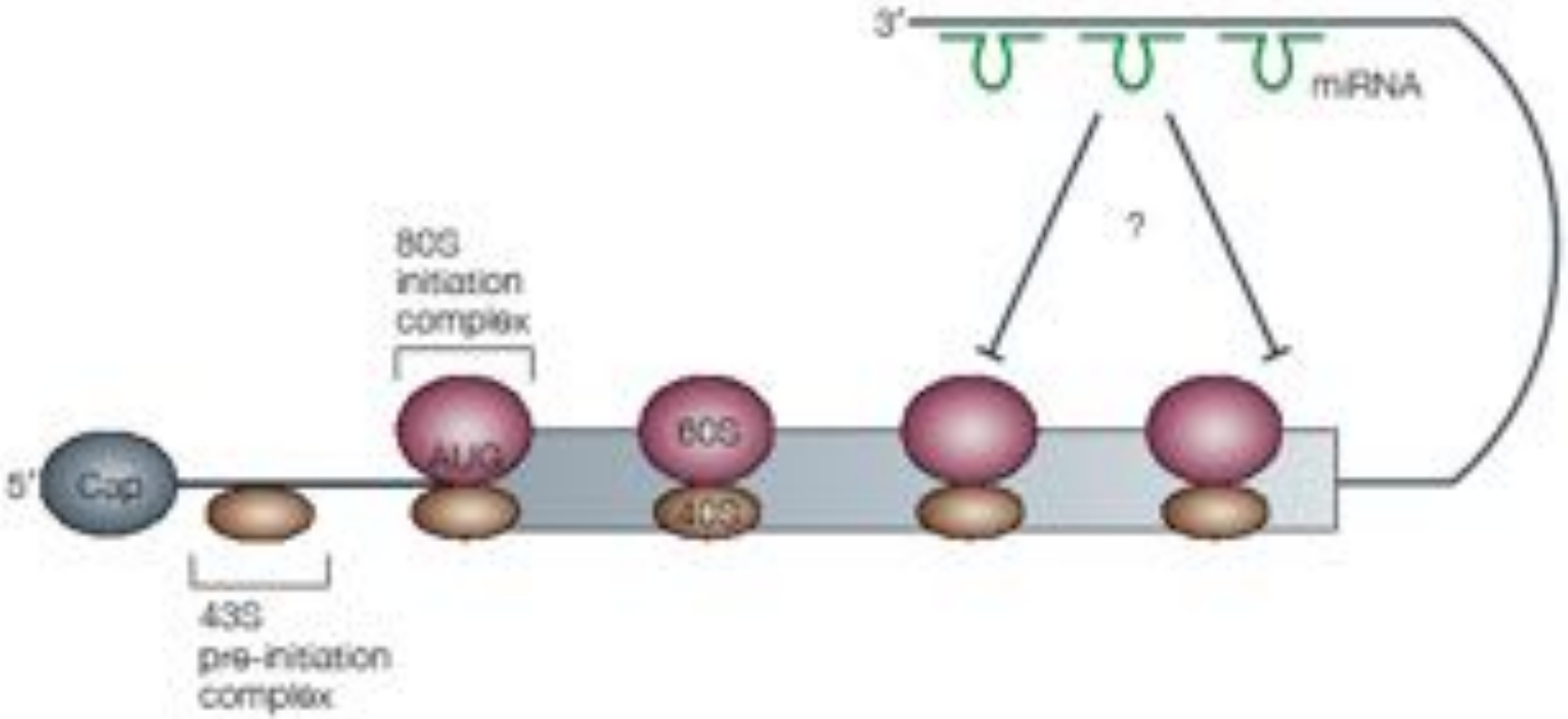
Das Fragile X-Syndrom Protein bindet an die 3‘ Region von vielen RNAs und unterdrückt die Translationseffizienz.

Lokale Translationskontrolle in der Synapse!

Rekrutierung des RNAi Apparates

-> RNA interferenz

Translationskontrolle durch miRNA



Zentrales Dogma der Molekularbiologie

DNA -> RNA-> Protein

Die DNA ist der Ursprung aller Informationen

Die RNA ist das entscheidende Element

DNA <-> RNA -> Protein

Die reverse Transkriptase erzeugt DNA aus RNA

RNA kann enzymatisch wirken ->

Ribozyme, RNA splicing

RNA vermittelt die Umsetzung der genetischen

Information, mRNA, tRNA, rRNA

RNA kontrolliert die Regulation der Transkrition und

Translation miRNA -> RNA Interferenz

RNA kontrolliert die zellulären „Immunantwort“ siRNA ->

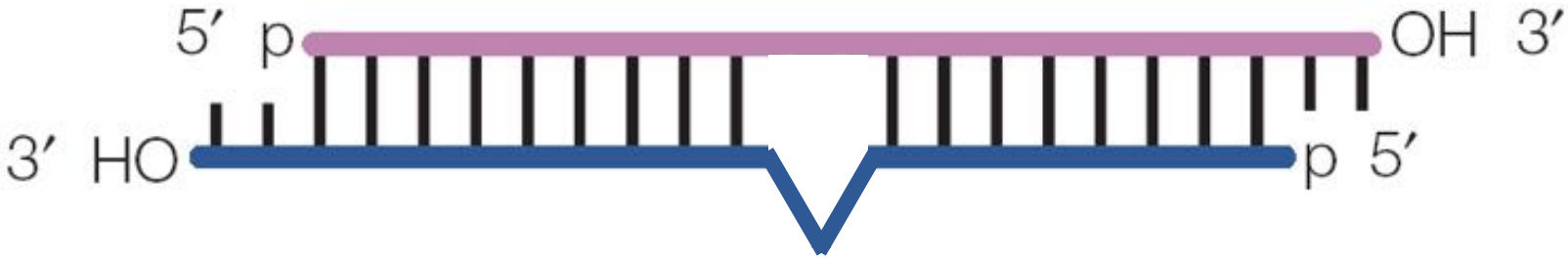
RNA Interferenz

small interfering RNA (siRNA)



- Doppelstrang RNA perfekte Paarung
- 21-23 bp lang
- 3 ‘ OH Terminus mit 2 Nukleotiden Überhang
- RNA induced gene silencing complex (RISC)

micro RNA (miRNA)



- Doppelstrang RNA imperfekte Paarung
- 21-23 bp lang
- 3 ‘ OH Terminus mit 2 Nukleotiden Überhang
- Post translational gene silencing PTGS
- Translational gene silencing TGS

RNA Interferenz (RNAi)

80er Jahre, Co-suppression, jetzt: miRNA, siRNA und piRNA

1993 *C. elegans* kleine komplementäre RNAs bei der Genregulation: *lin-4* reguliert *lin-14* *(miRNA)*

1998 Fire & Mello dsRNA vermittelt *gene silencing*

1999 RNAi wird über kleine RNAs vermittelt *(siRNA)*

2000 RNAi wird über 21/23 bp große RNAs vermittelt
3’ Überhang -> RNAase III Aktivität

RNAse III; Dicer, Drosha dsRBD Doppelstrang RNA Bindungsdomäne

Effektor-Complex: RISC (RNA-induced silencing complex)

Slicer = Argonaute RNAase H

2006 Nobel Preis an A. Fire und G. Mello

Cosupression

Beispiel Petunien



Wildtyp



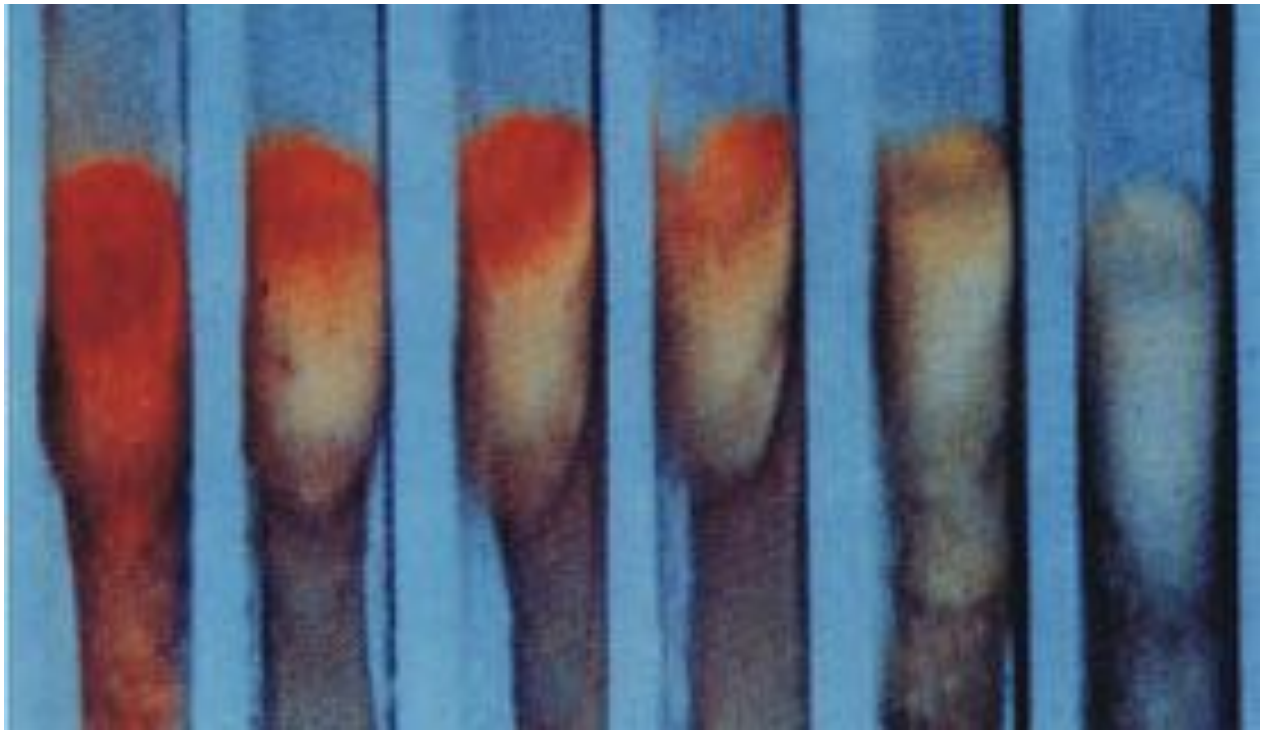
Transgene Pflanze

Chalkonsynthase

Cosupression = Zusätzlich eingebrachtes Gen wird unterdrückt und gleichzeitig alle homologen Gene im Organismus.

Quelling

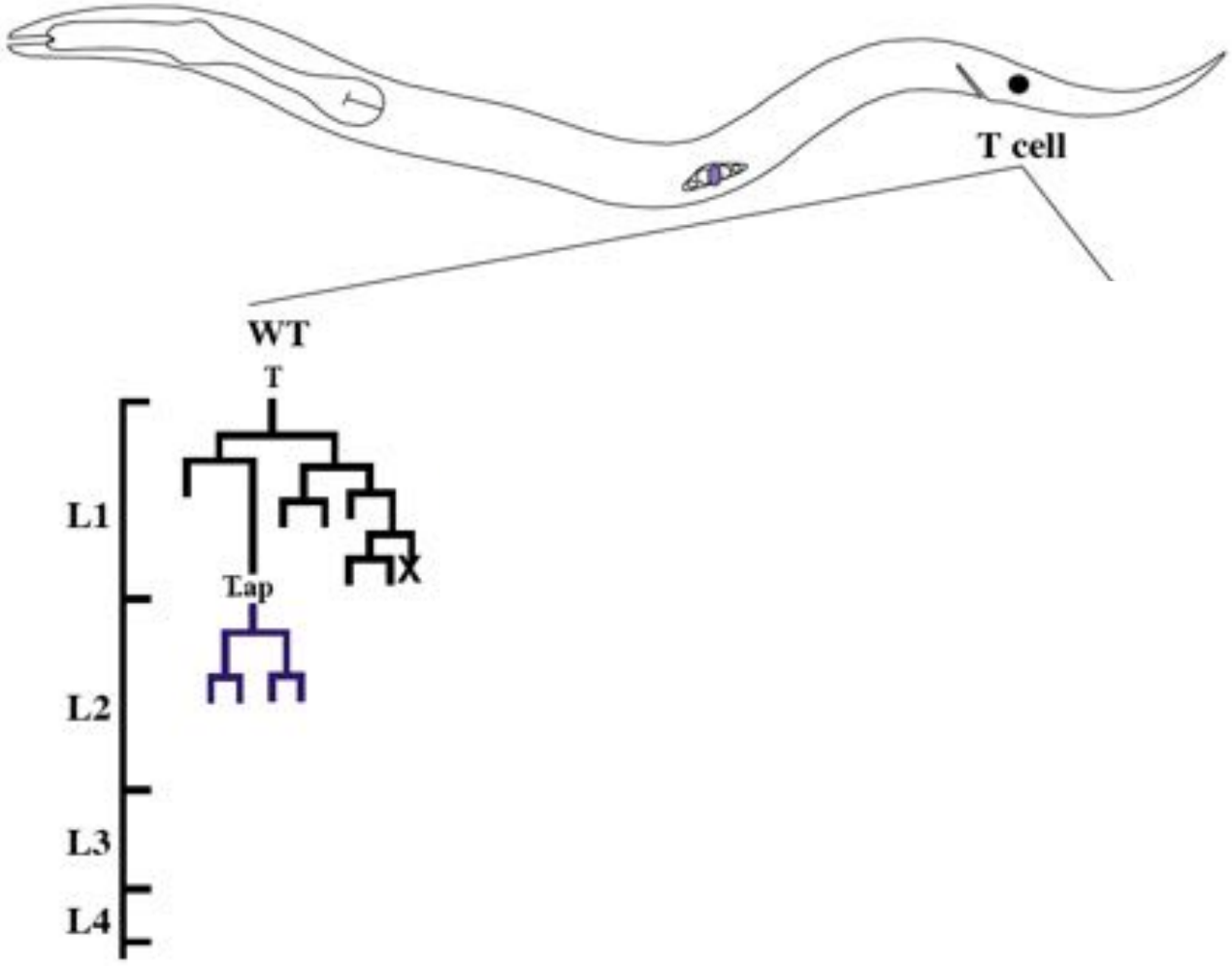
entdeckt bei *Neurospora crassa*



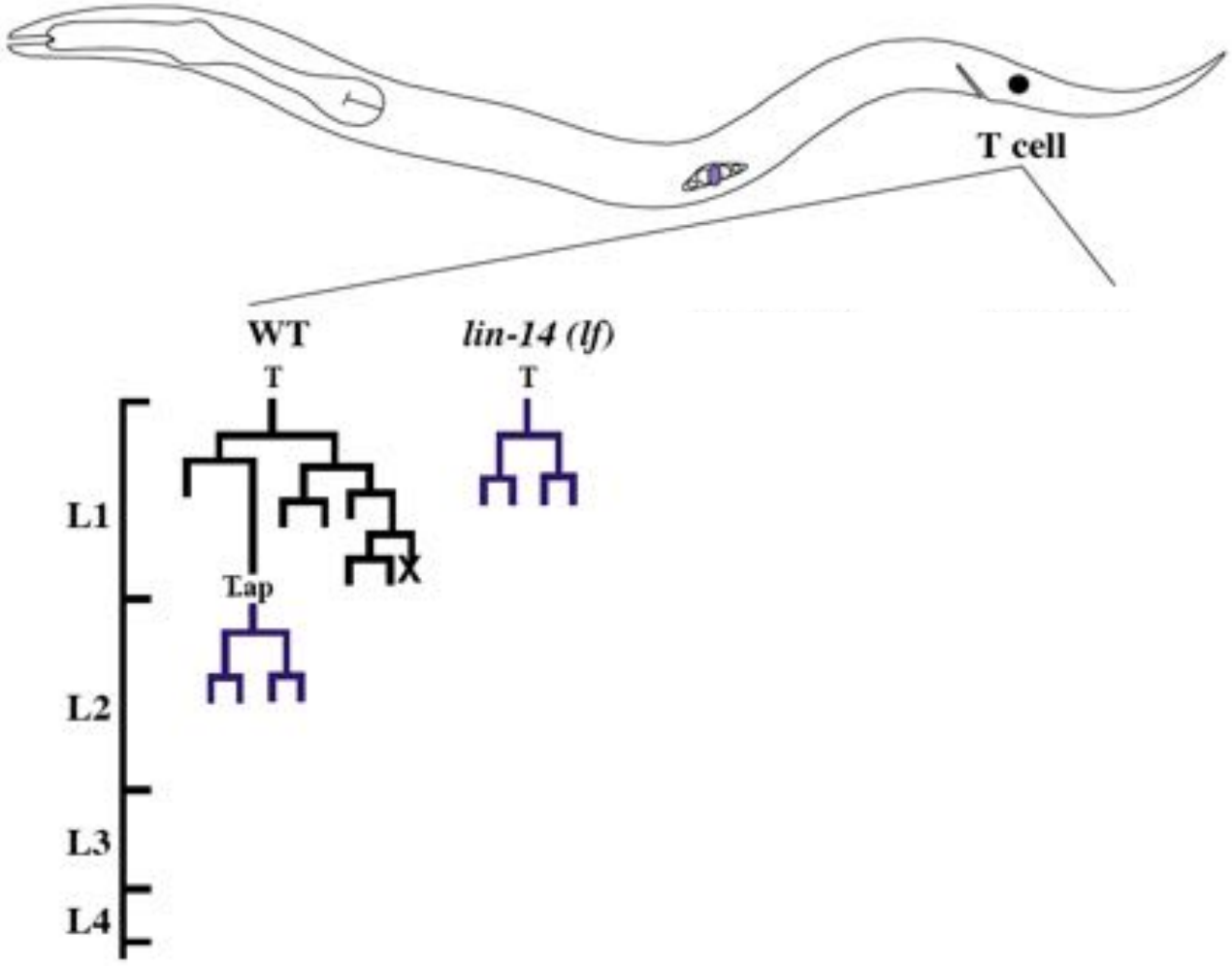
wt Mutantenstämme

Gleiches Phänomen wie bei der Cosupression

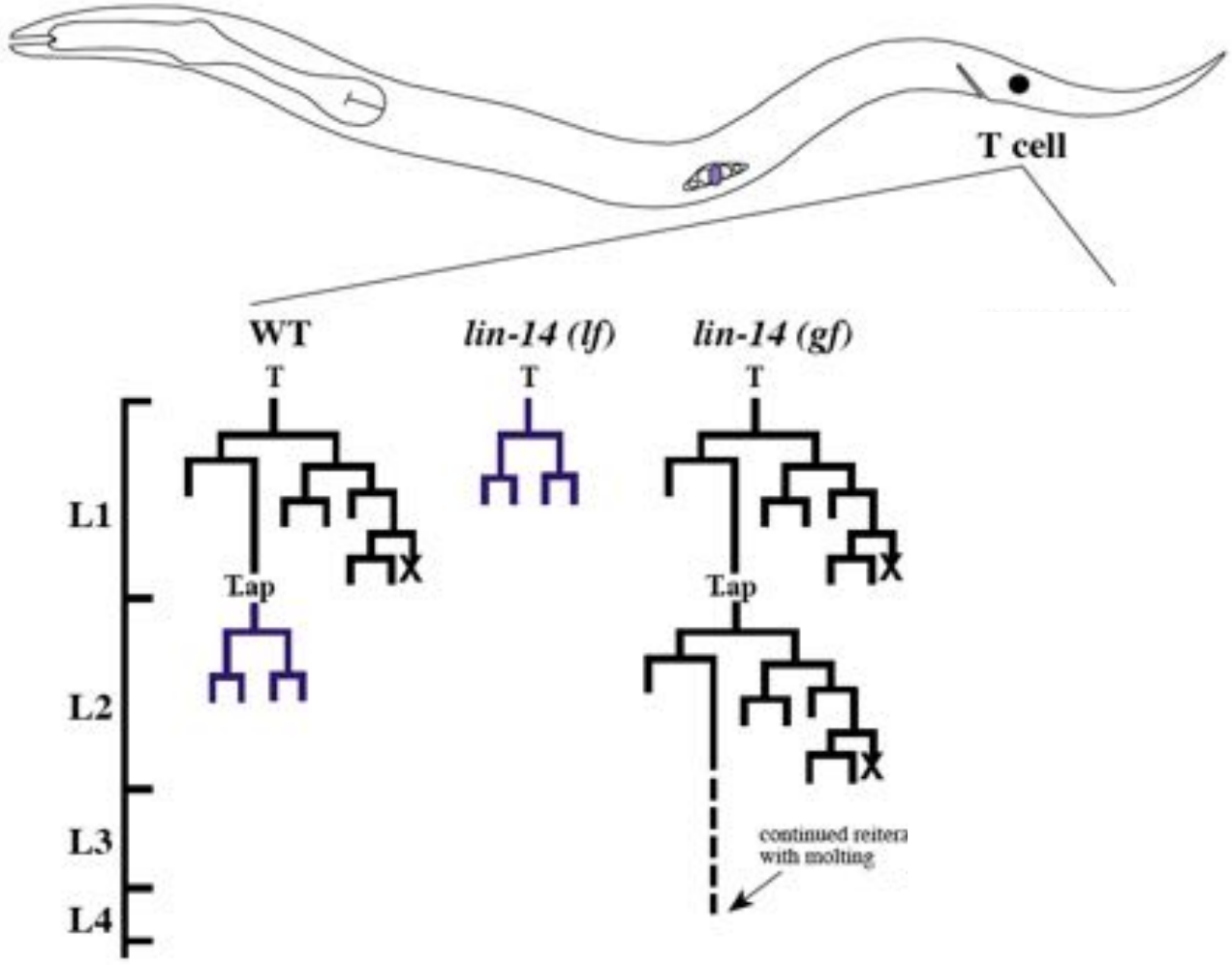
Der Phänotyp von *lin-4* und *lin-14* Mutationen



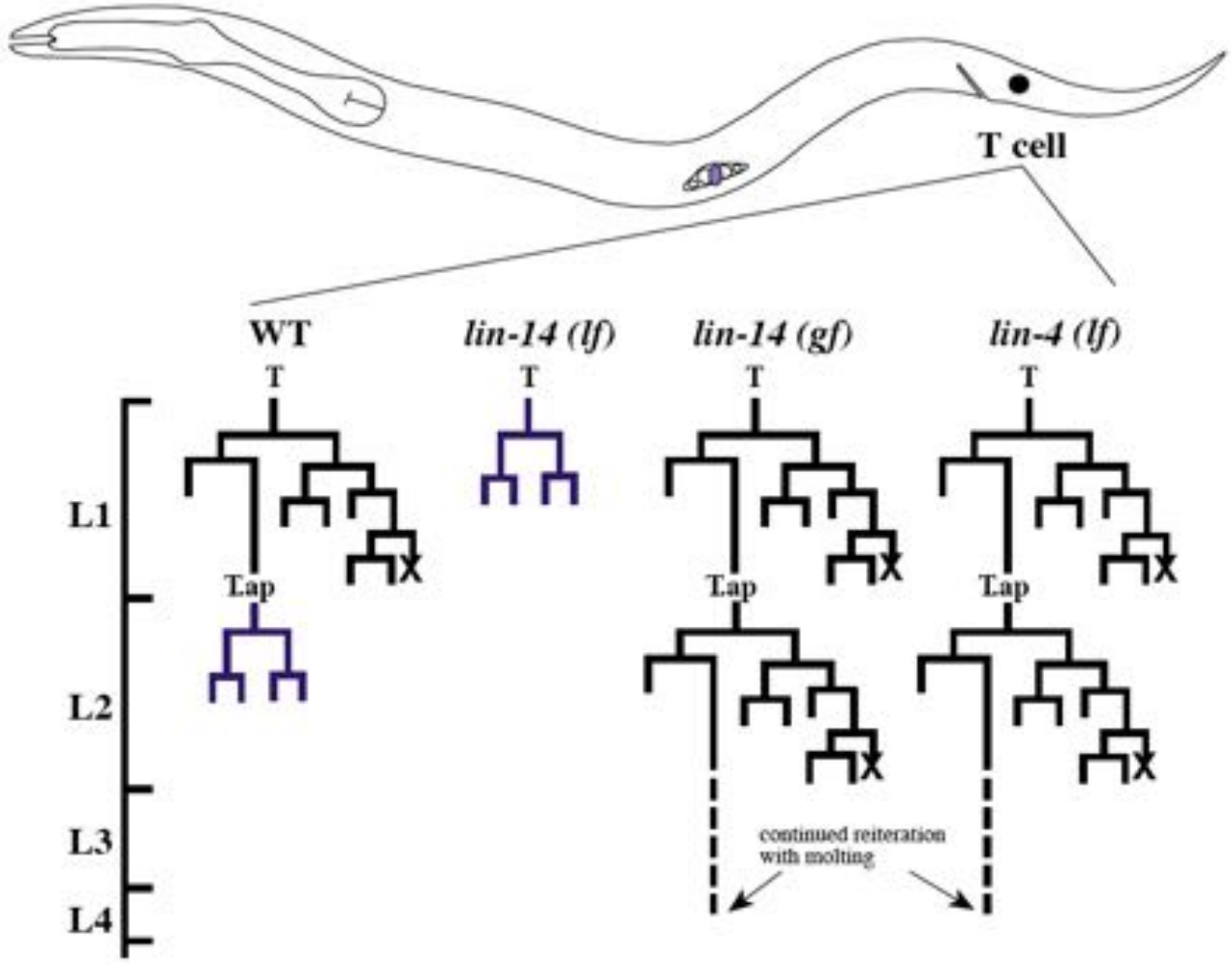
Der Phänotyp von *lin-4* und *lin-14* Mutationen



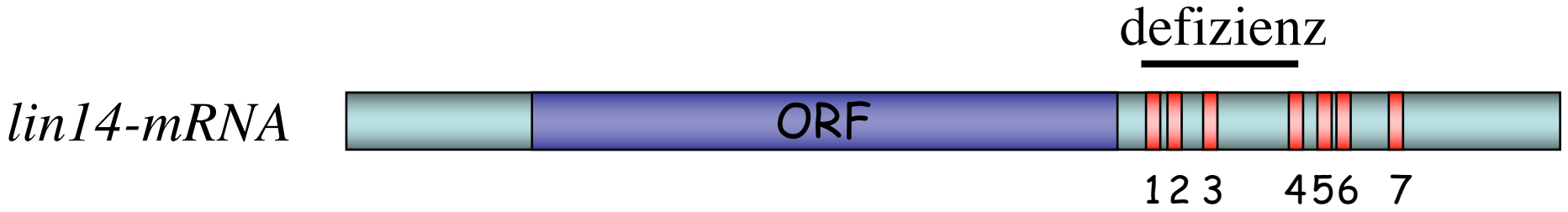
Der Phänotyp von *lin-4* und *lin-14* Mutationen



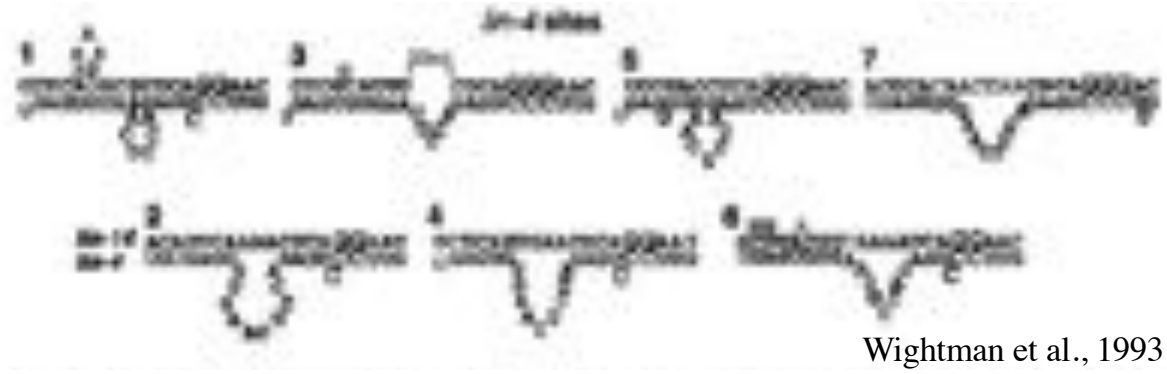
Der Phänotyp von *lin-4* und *lin-14* Mutationen



lin4 reguliert *lin14*



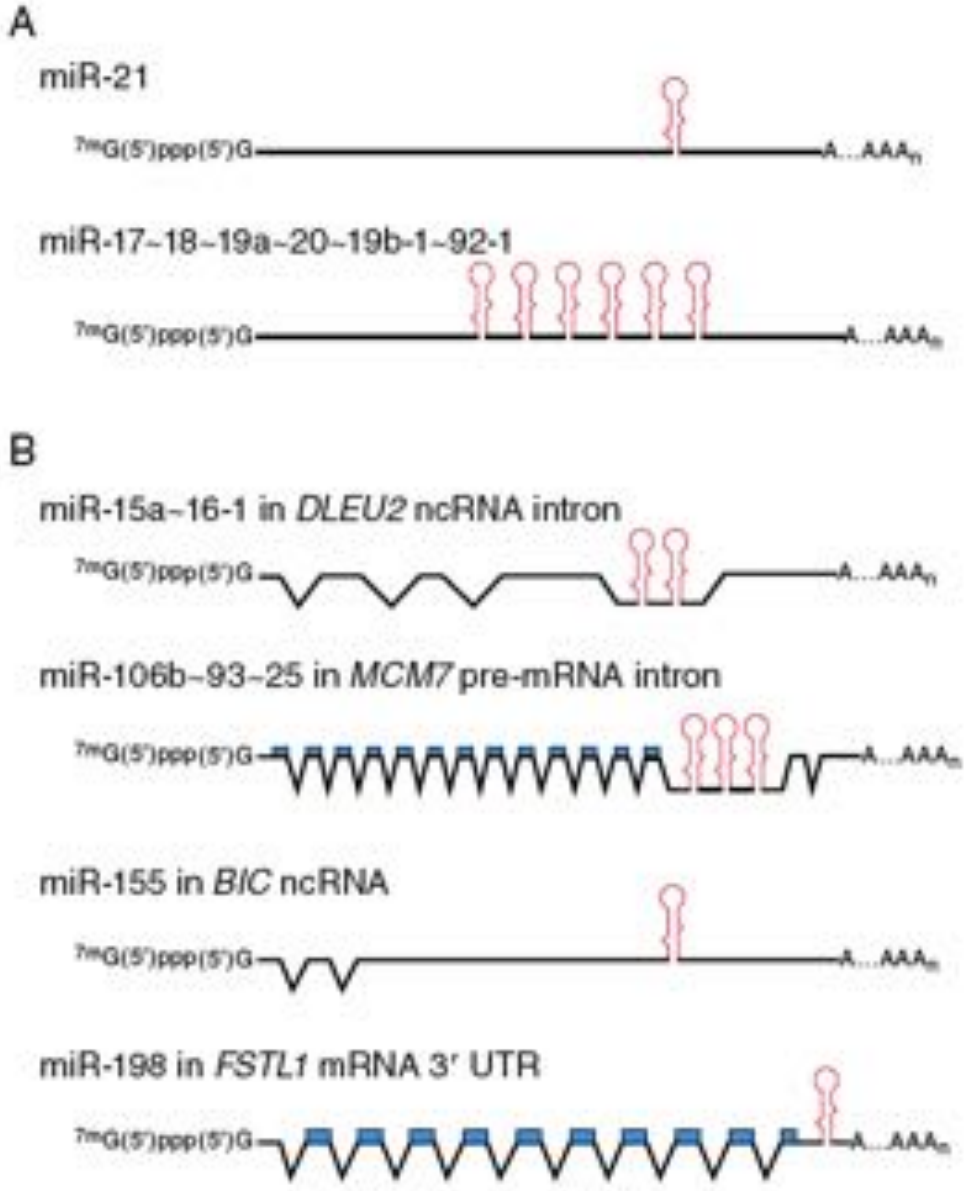
Die lin4 RNA kann die *lin14* mRNA im 3 ‘ UTR binden und die Translation regulieren.



Wightman et al., 1993

Heute etwa viele 1000 miRNAs bekannt (beim Mensch ca. 1000).

Heterogener Ursprung der miRNAs



Eigene Transkriptionseinheit, mono- und polycistronisch

Intron von non-coding RNA

Intron von protein-coding RNA

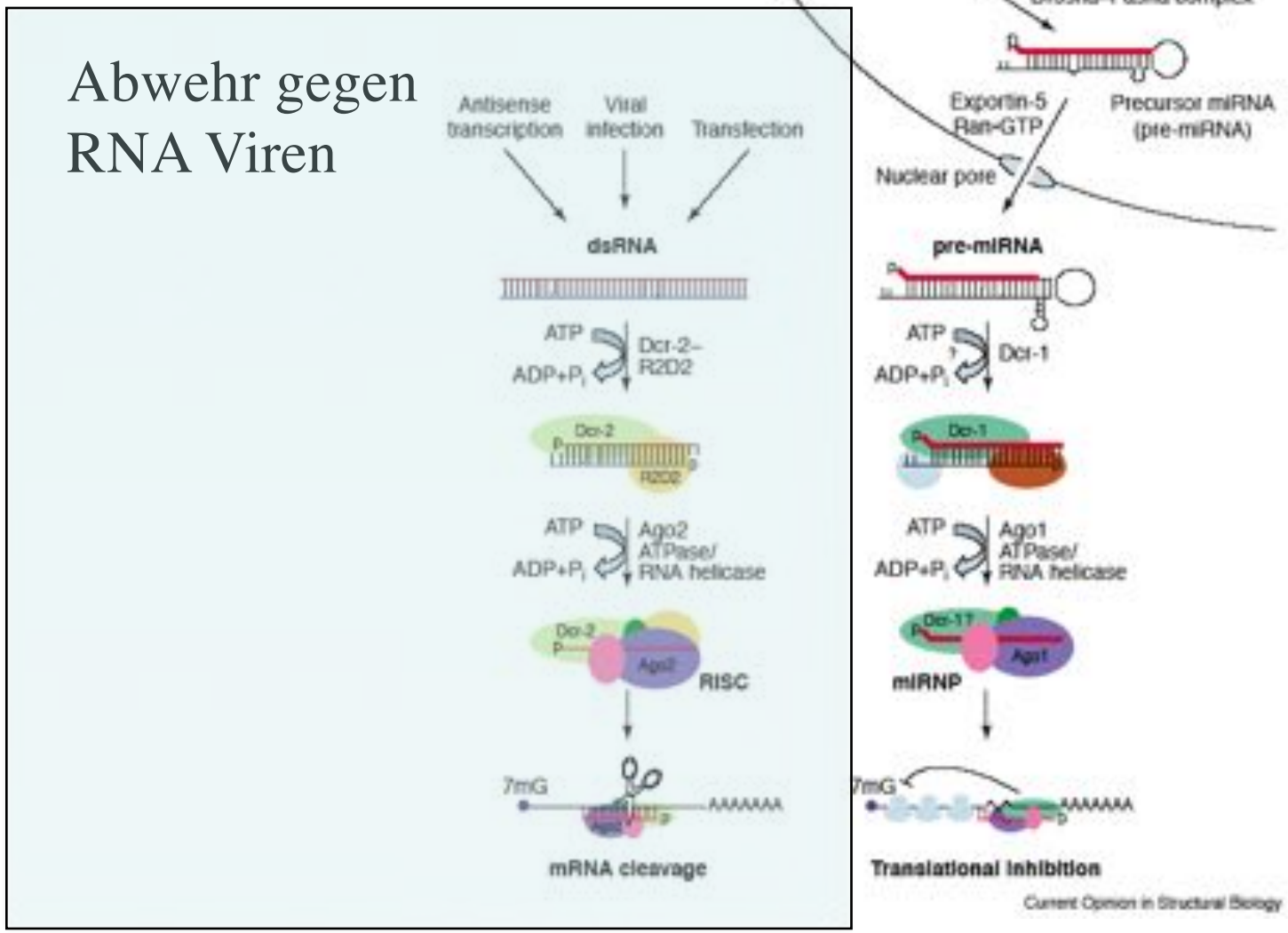
Exon von non-coding RNA

Exon von protein-coding RNA

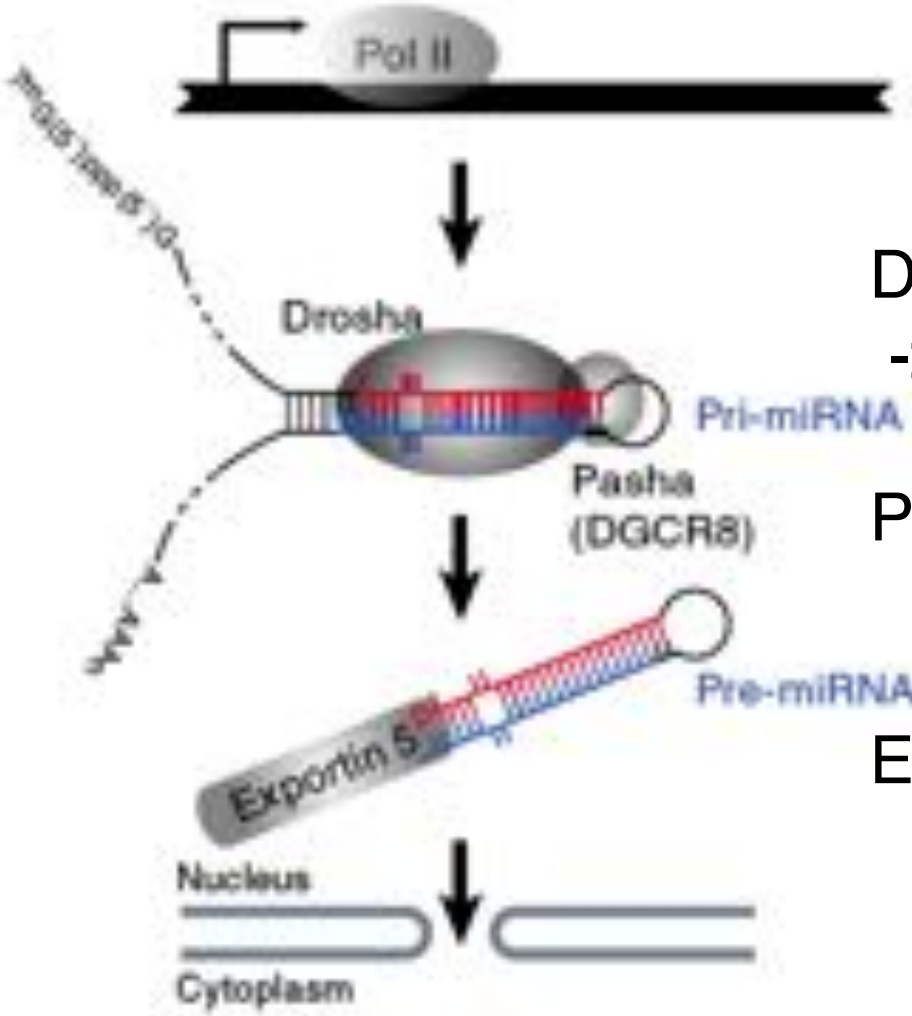
Wege der RNA Interferenz

Kern-kodierte Regulations-mechanismen

Abwehr gegen RNA Viren



Der miRNA Syntheseweg

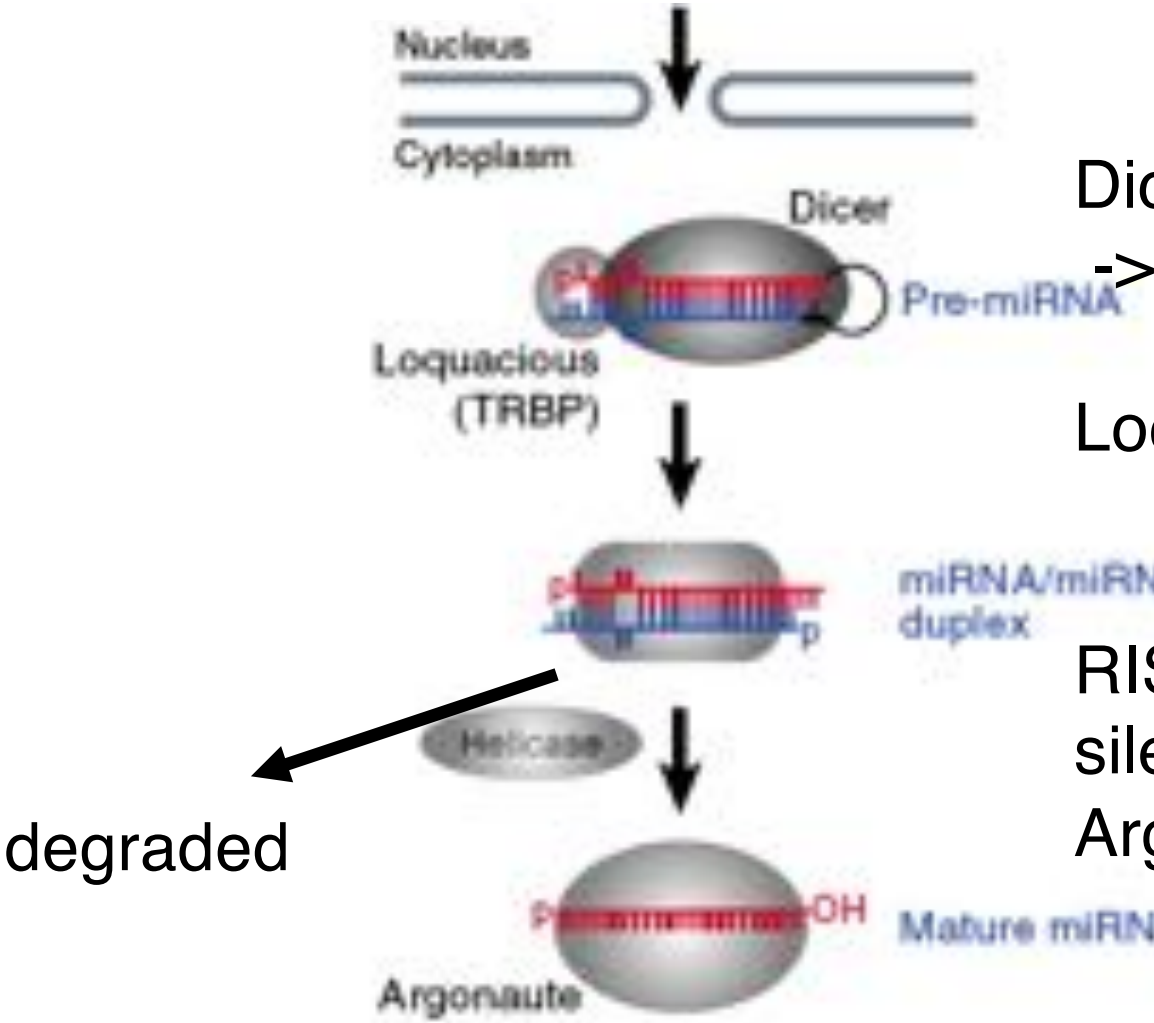


Drosha (RNase III)
-> 70 nt stem loop.

Pasha (dsRBD)

Exportin/RanGTP

Der miRNA Syntheseweg

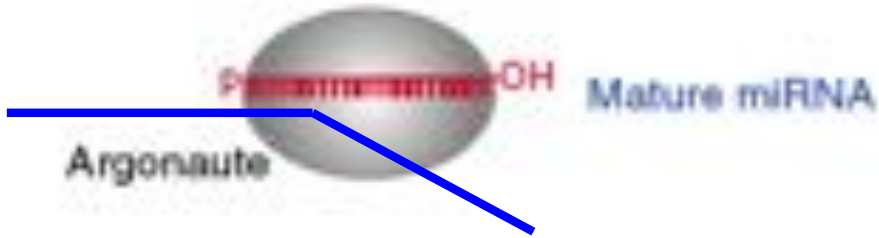


Dicer (RNase III)
-> 21 nt miRNA.

Loquacious (dsRBD)

RISC (RNA induced silencing complex)
Argonaute

Der miRNA Regulationsweg

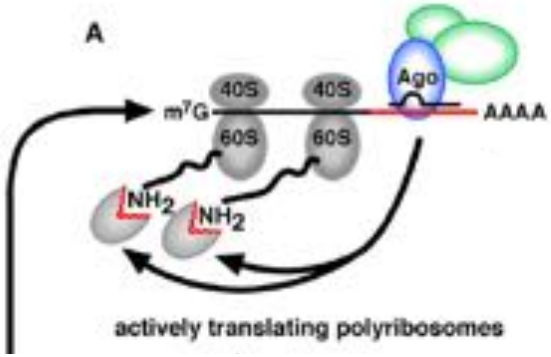


Ziel-RNA Erkennung erfordert die 5’ Region (Nukelotide 6-8) = *seed region*

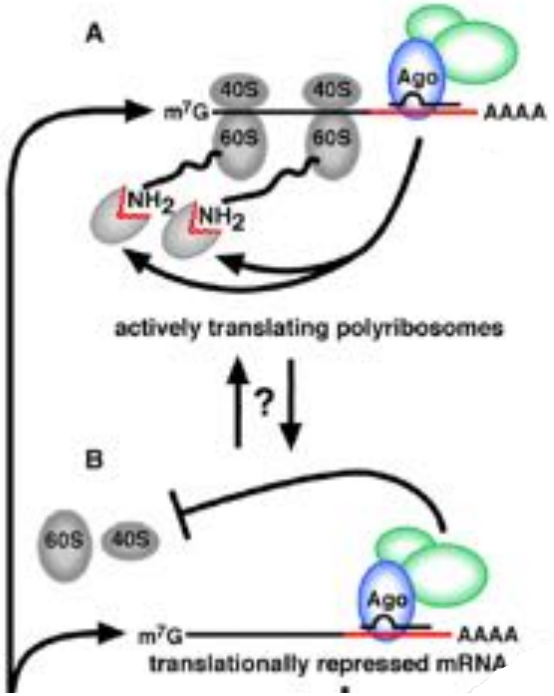
d.h. Regulation einer sehr großen Zahl Gene durch miRNA!!

Ohne intensive Basenpaarung -> Block der Translation.

Aber Degradation in *P-Bodies*

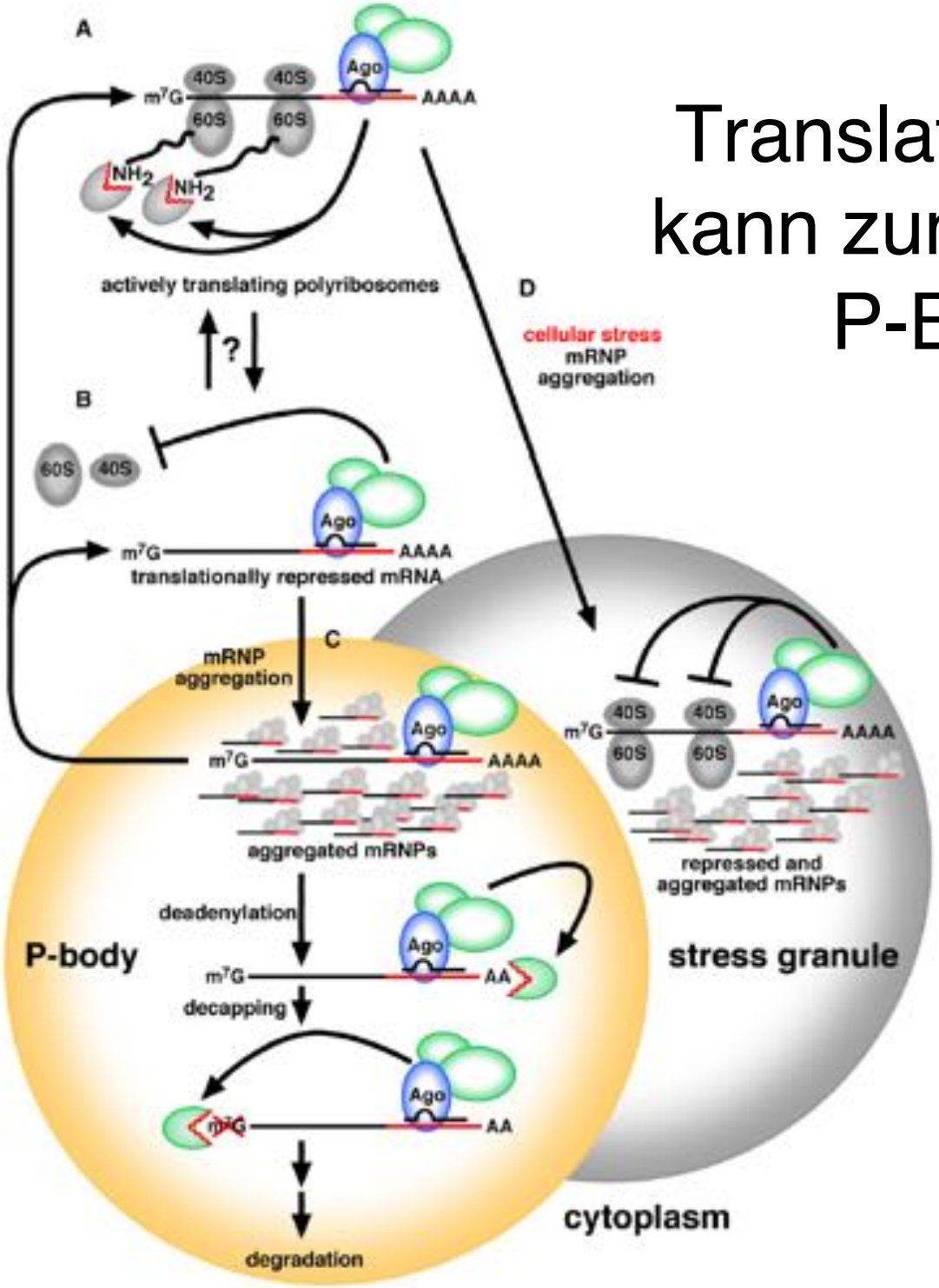


Translationale Hemmung kann zum mRNA Abbau in P-Bodies führen

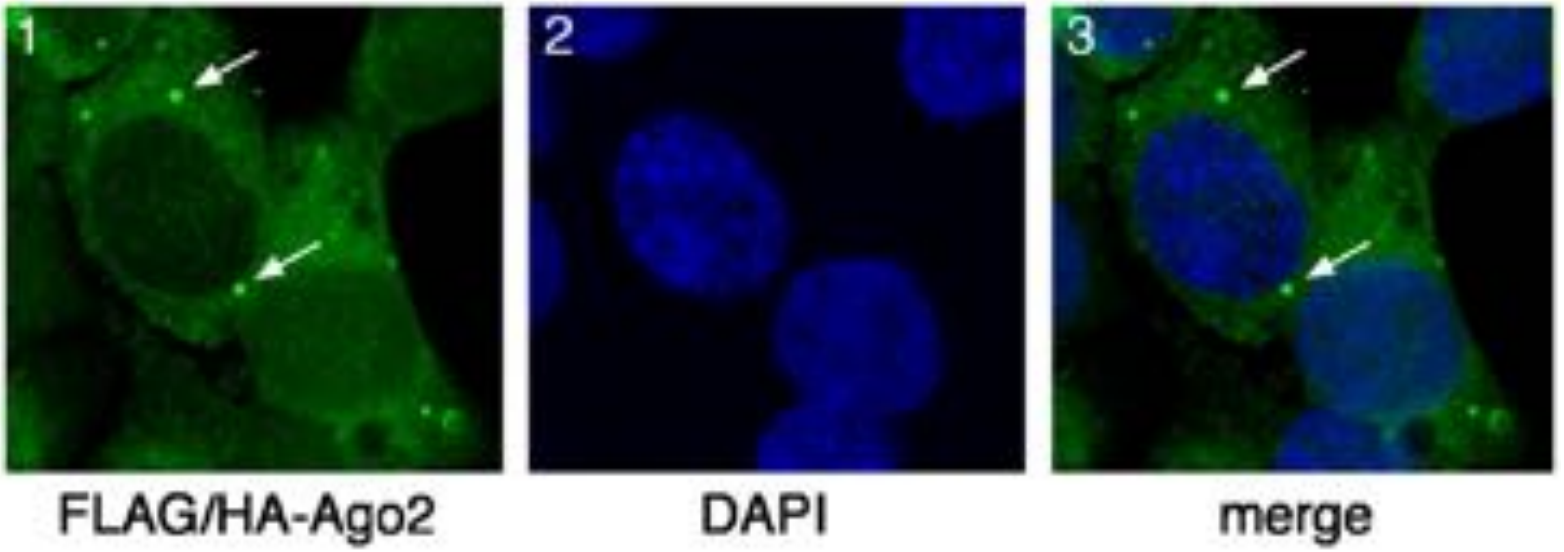


Translationale Hemmung kann zum mRNA Abbau in P-Bodies führen

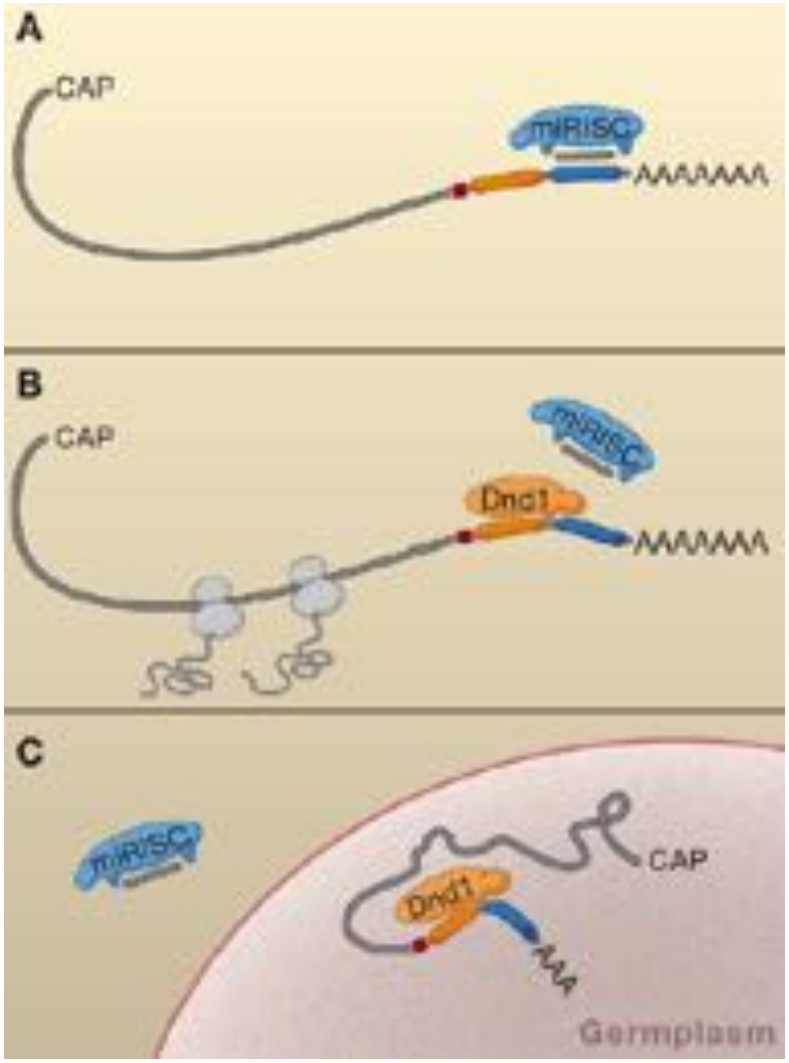
Translationale Hemmung kann zum mRNA Abbau in P-Bodies führen



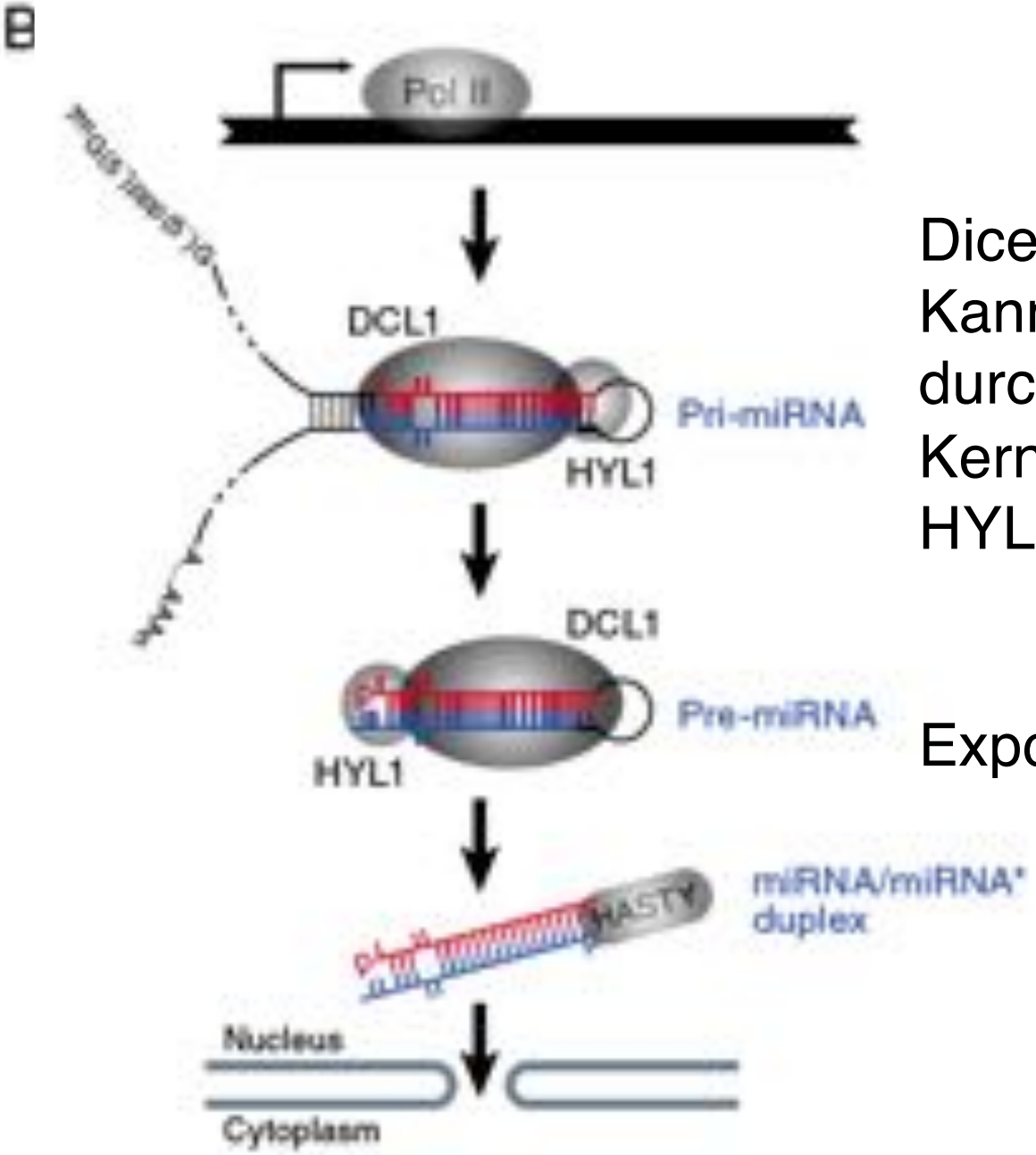
P-Bodies



Das Dead end Protein schützt Transkripte in der Keimbahn vor miRNA vermittelter Suppression



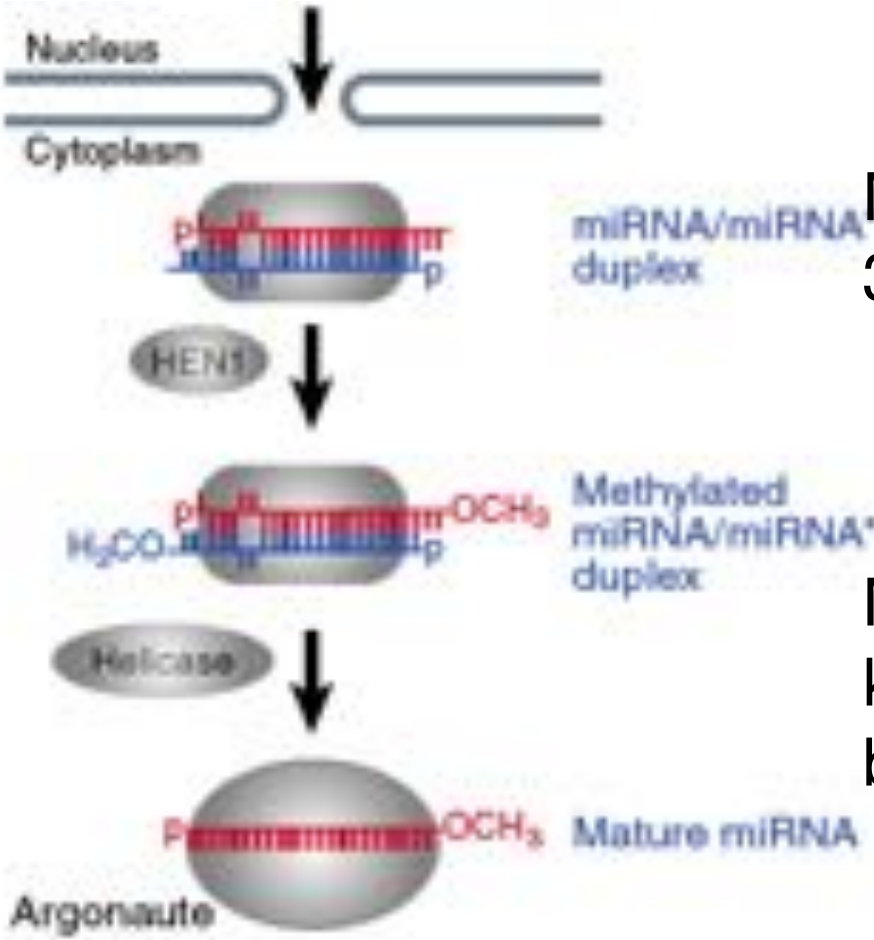
Der miRNA Syntheseweg (Pflanzen)



Dicer like 1
Kann beide Schnitte durchführen (im Kern)
HYL1 (dsRBD)

Exportin/RanGTP

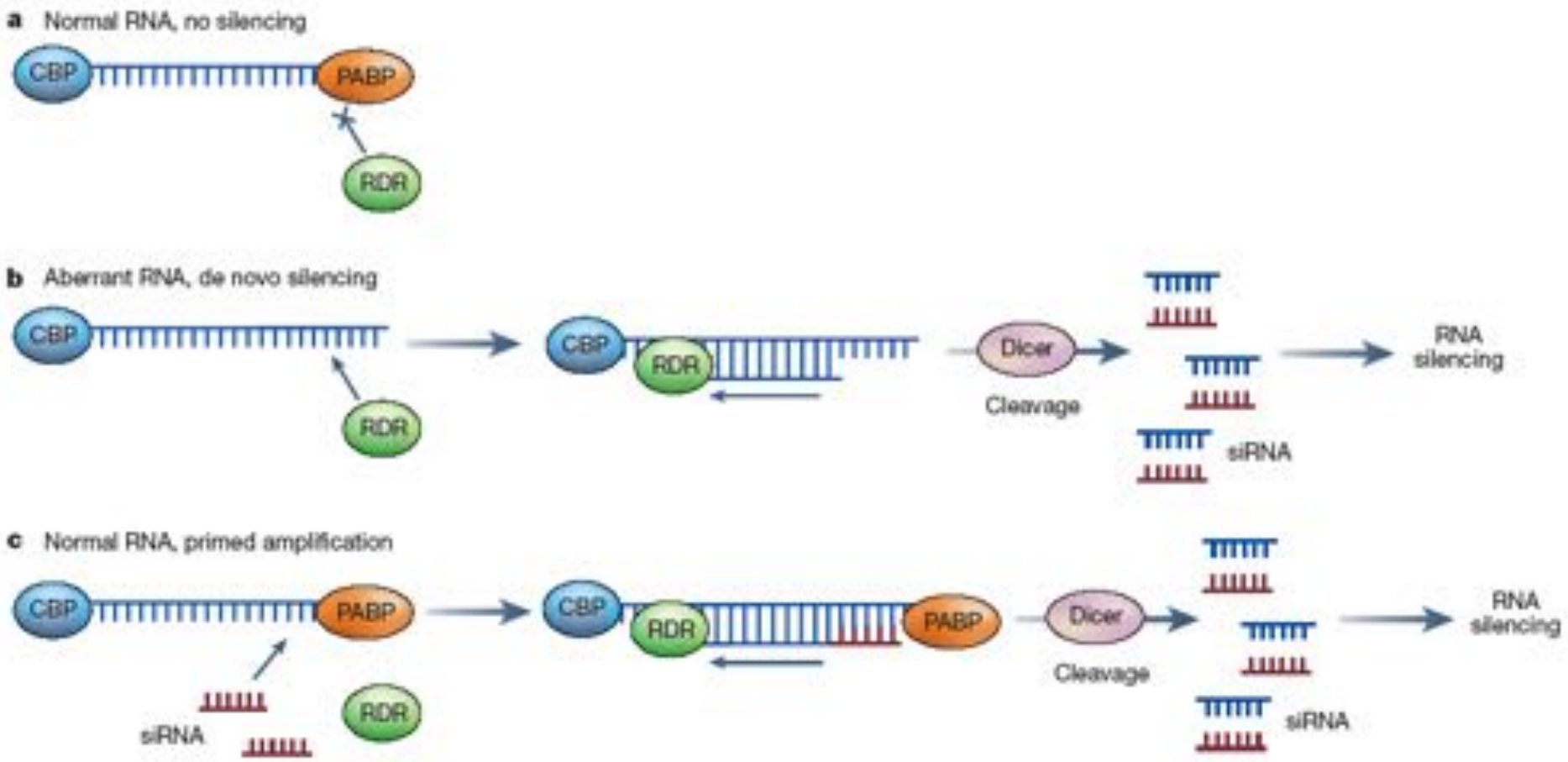
Der miRNA Syntheseweg (Pflanzen)



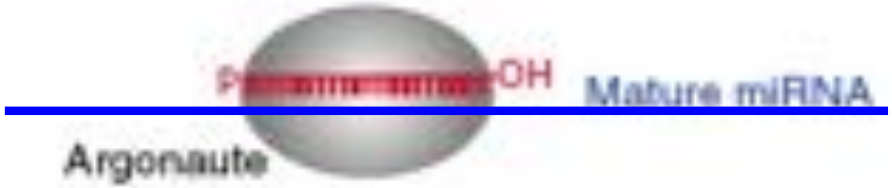
Methylierung des 3’ Endes

Methylierung kann RDR blockieren

RNA dependend RNA-Poylmerase (RDR)

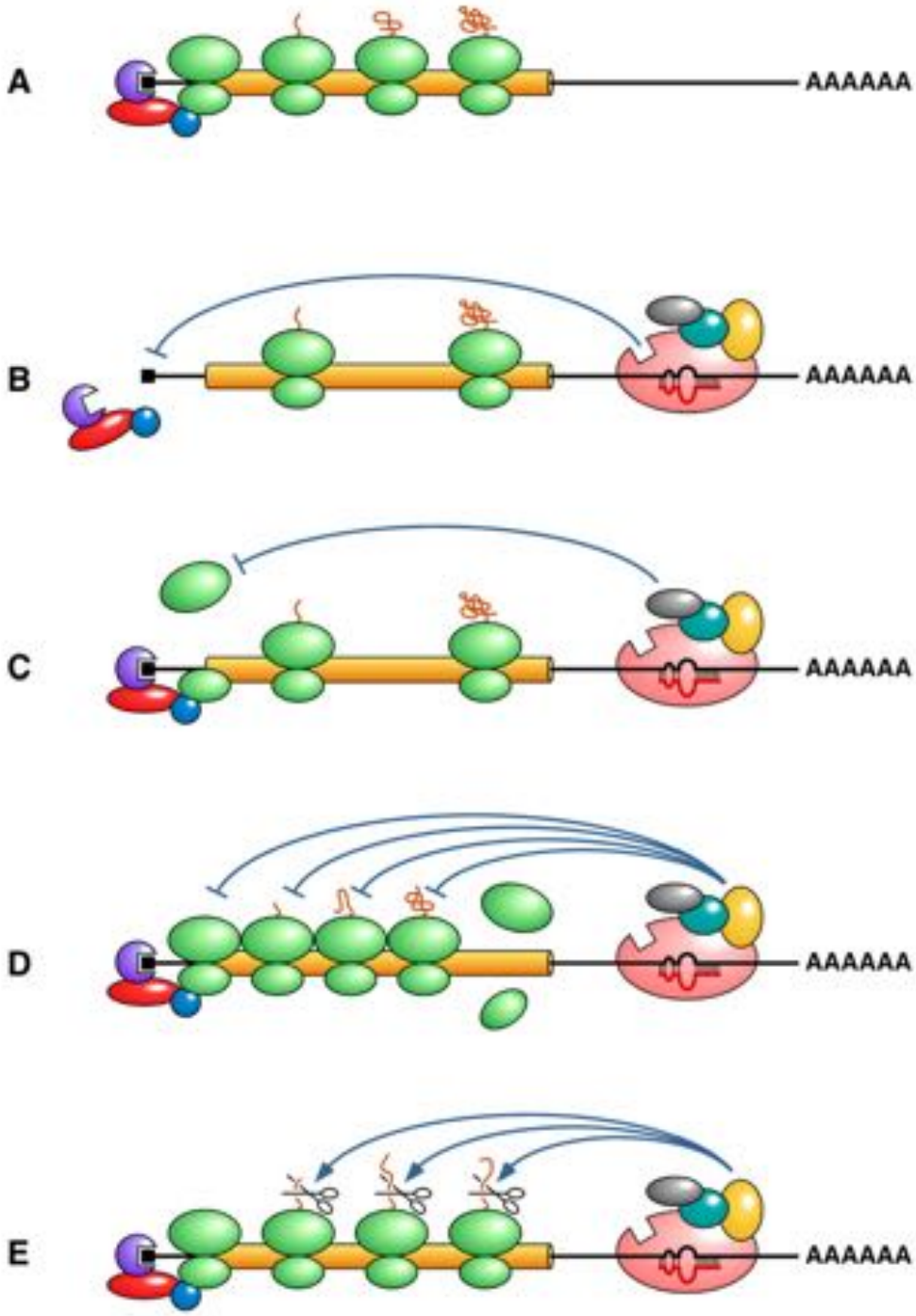


Der miRNA Regulationsweg



Ziel-RNA Erkennung fast komplette Paarung

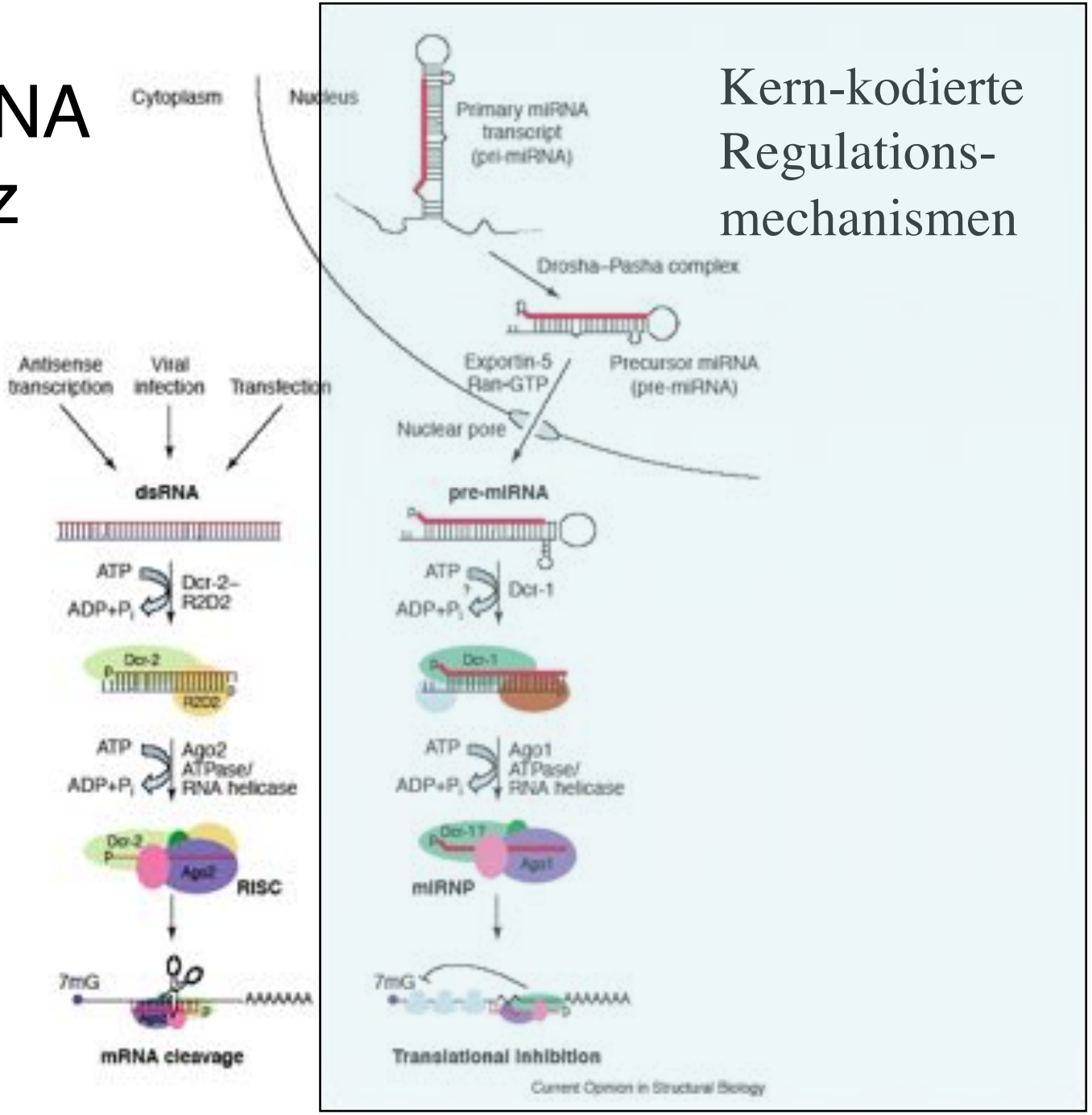
Schnitt der RNA



Wu 2008

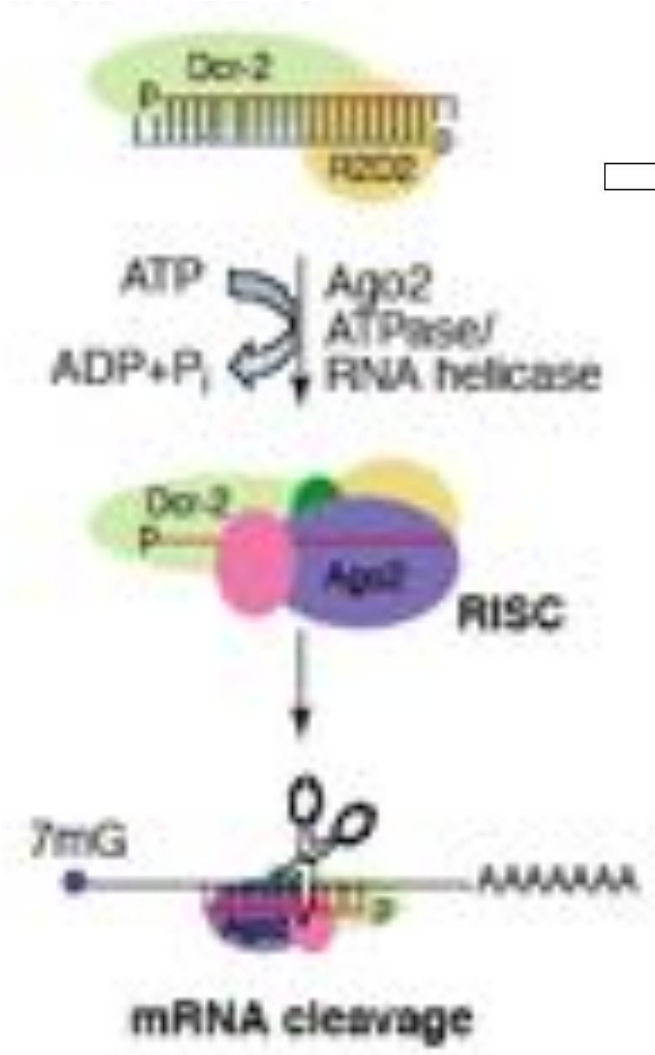
Wege der RNA Interferenz

Abwehr gegen RNA Viren

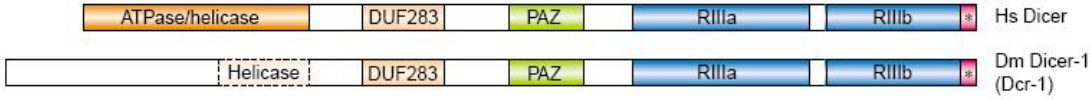


Kern-kodierte Regulations-mechanismen

siRNA-Mechanismus

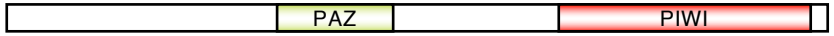


Dicer



PAZ (Piwi Argonaute Zwille) Domäne, bindet 3'Ende der RNA

Argonaute



PIWI Domäne bindet 5'Ende ähnlich zu RNase H, schneidet DNA/RNA Duplex

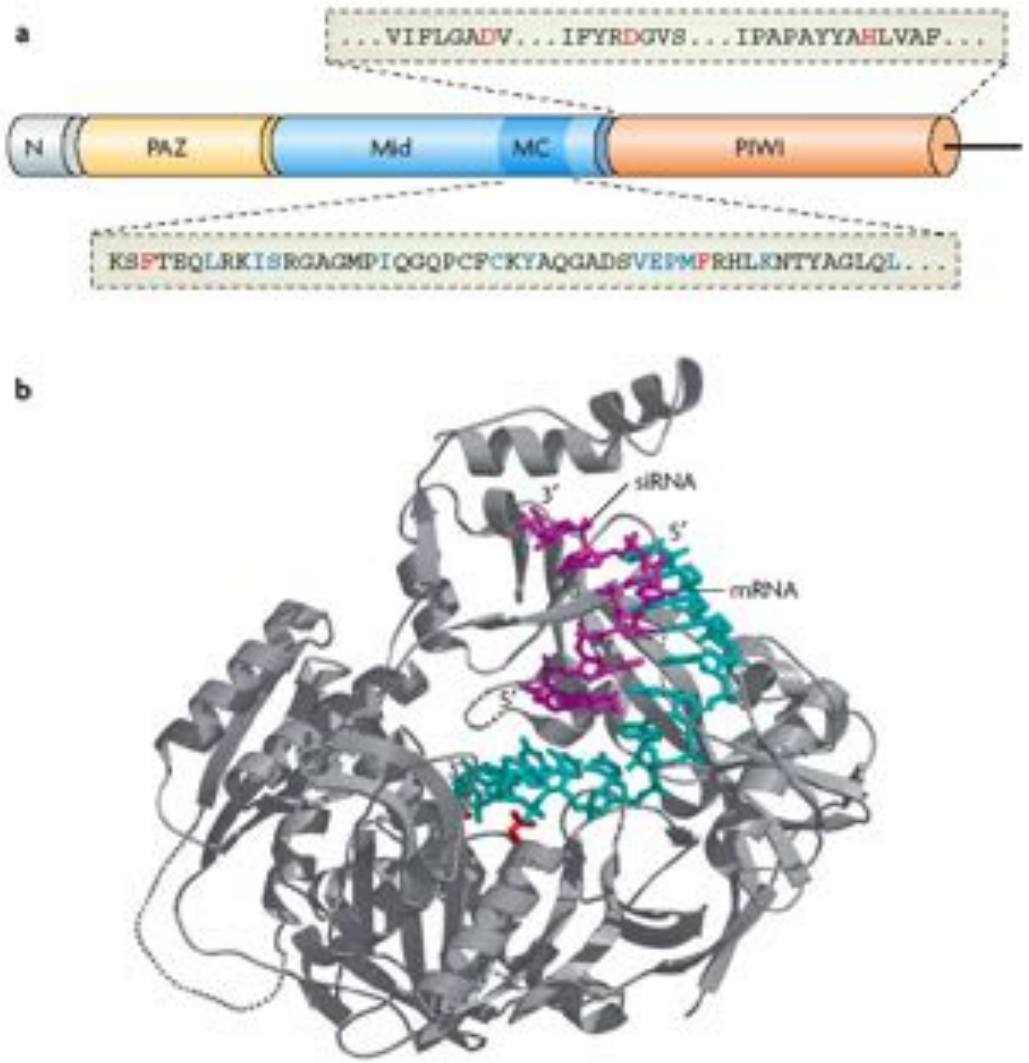
Drosophila: 5 Gene
Mensch: 8 Gene
C. elegans: 27 Gene

Strukturelle Eigenschaften der Argonaute Proteine

PAZ Domäne: bindet 3' Ende der ssRNA

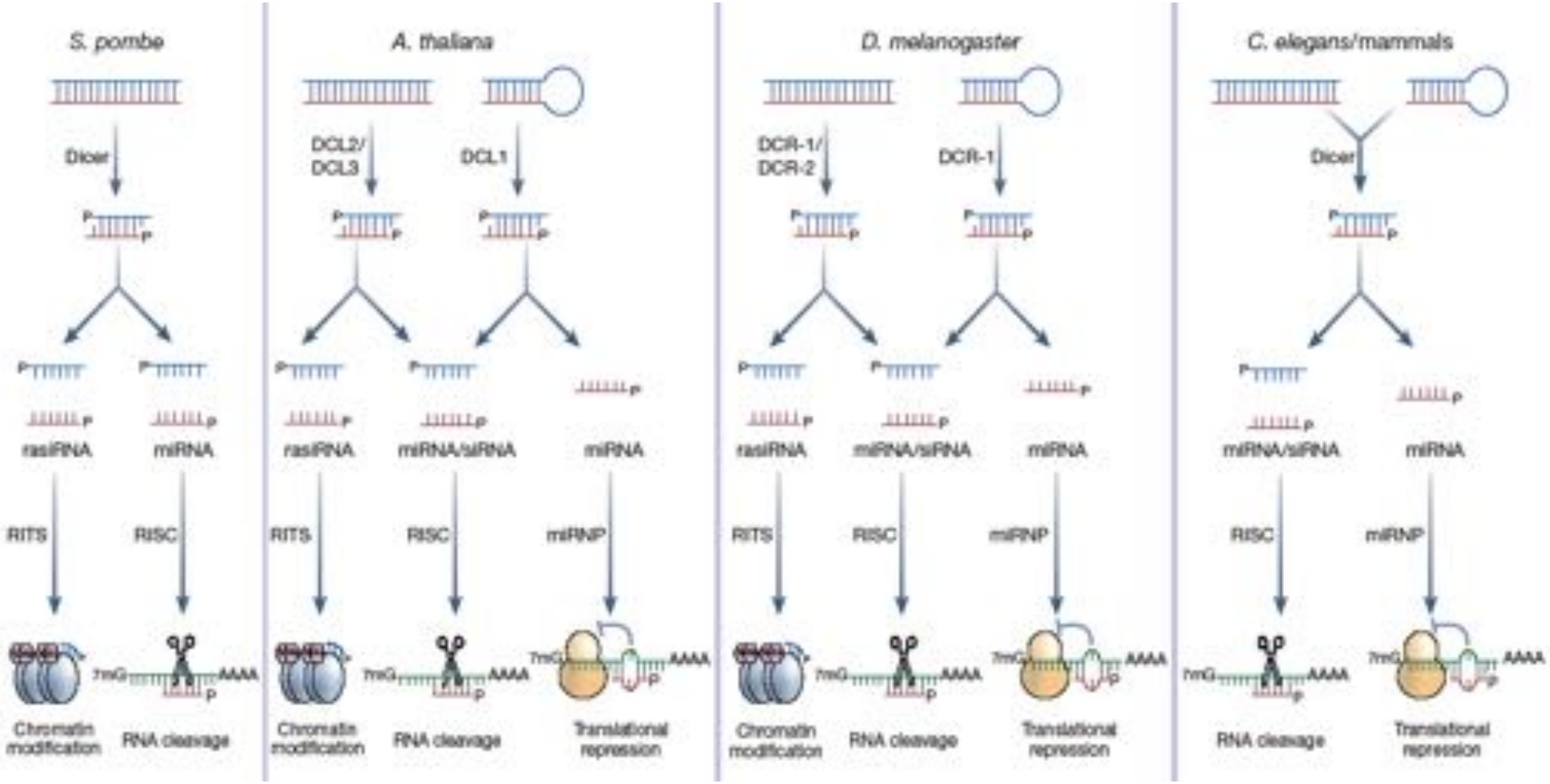
PIWI Domäne: Katalytisch aktiv.

MID Domäne: eIF4E Homologie, Regulation der Translation



Blau: Cap-binding Domäne, konservierte Aminosäuren zu eIF4E

RNAi Wege in unterschiedlichen Organismen



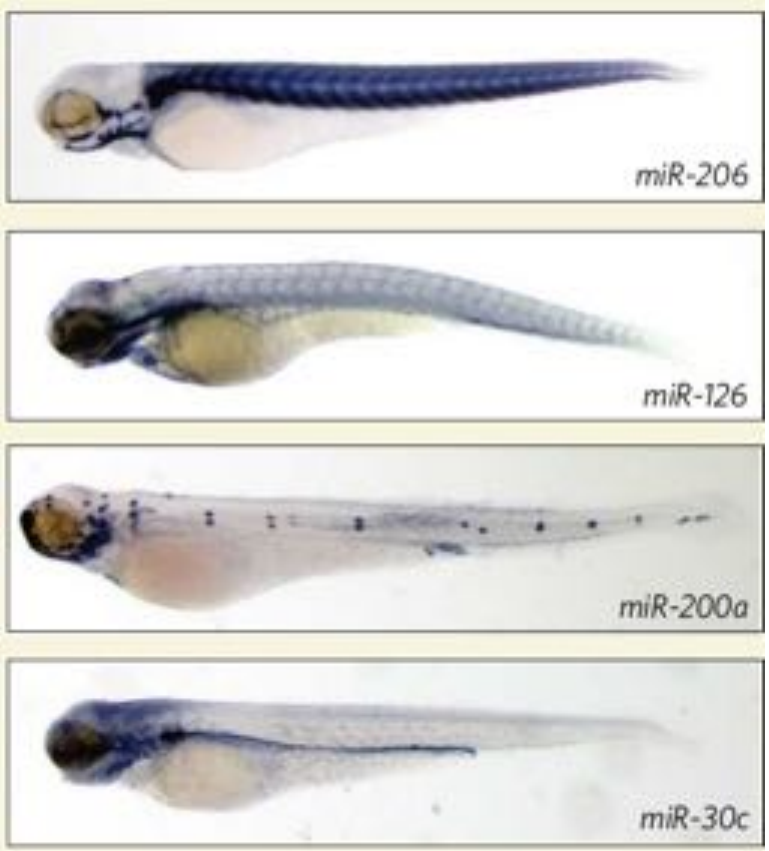
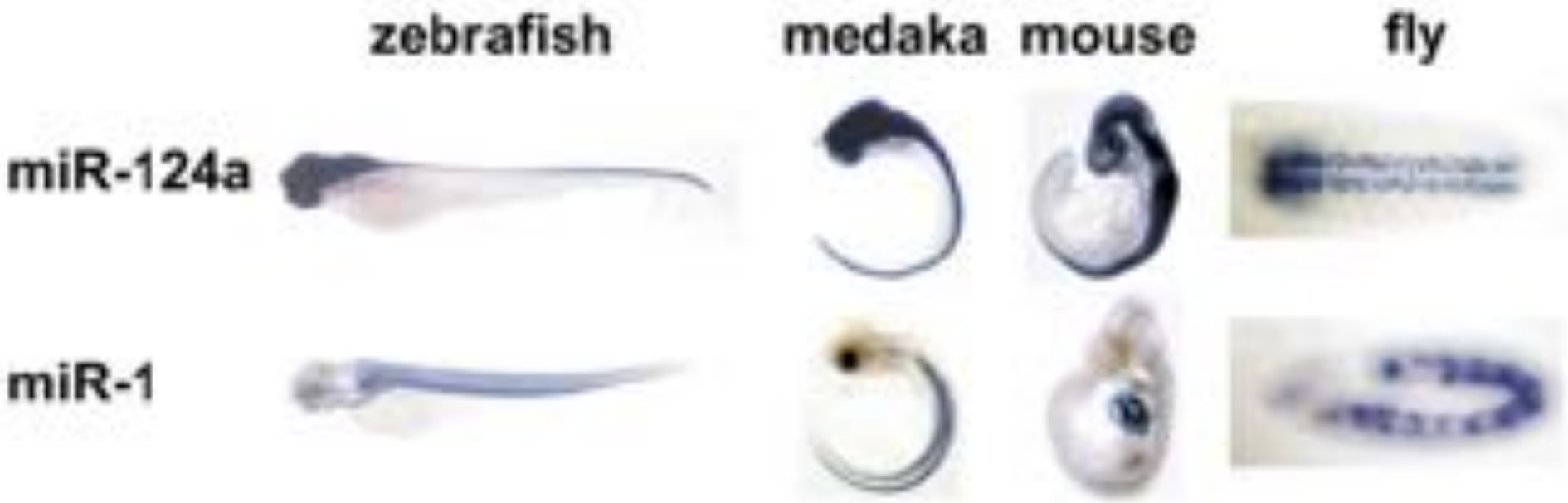
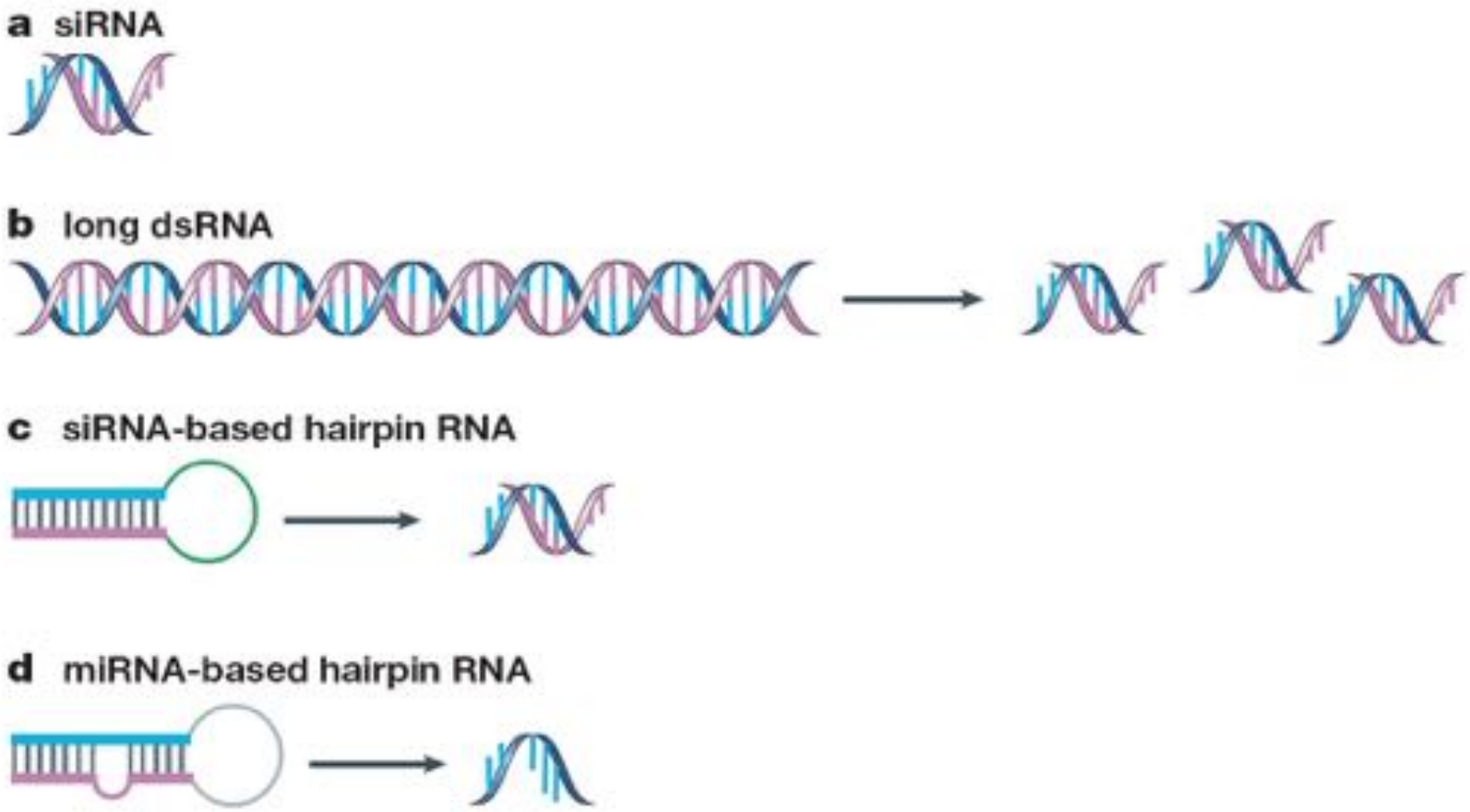


Figure 2 | miRNAs have tissue-specific functions. In zebrafish embryos, localization patterns of individual miRNAs indicate that their activity could be limited to tissues and organs in which they are expressed. As indicated by blue staining, *miR-206* is mainly expressed in the muscle, *miR-126* in the blood vessels and the heart, *miR-200a* in the lateral-line system (a mechanosensory system detecting water motion) and sensory organs, and *miR-30c* in the kidney precursor.

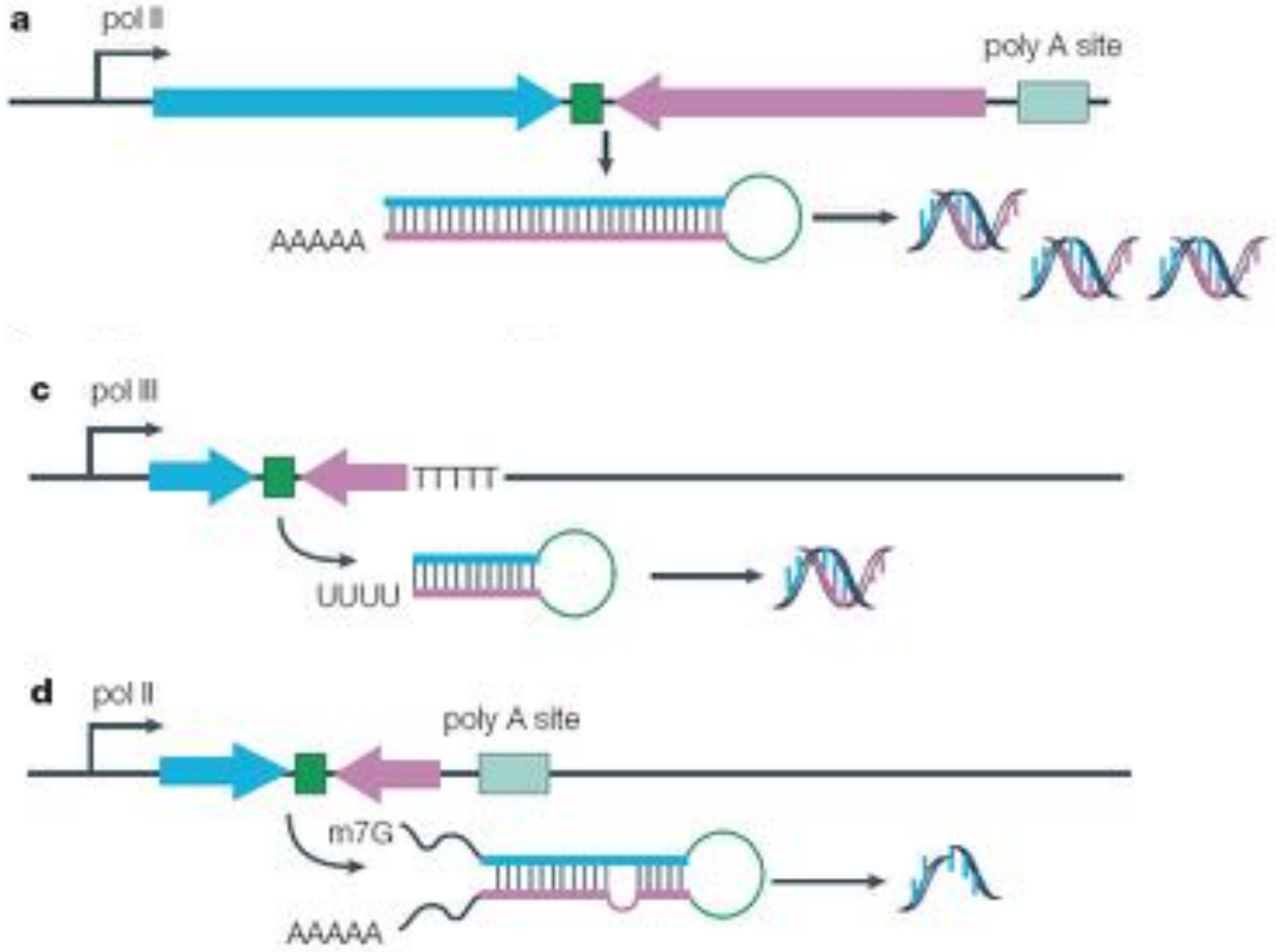
RNAi Gene sind konserviert



Wie kann man die RNAi-Antwort induzieren

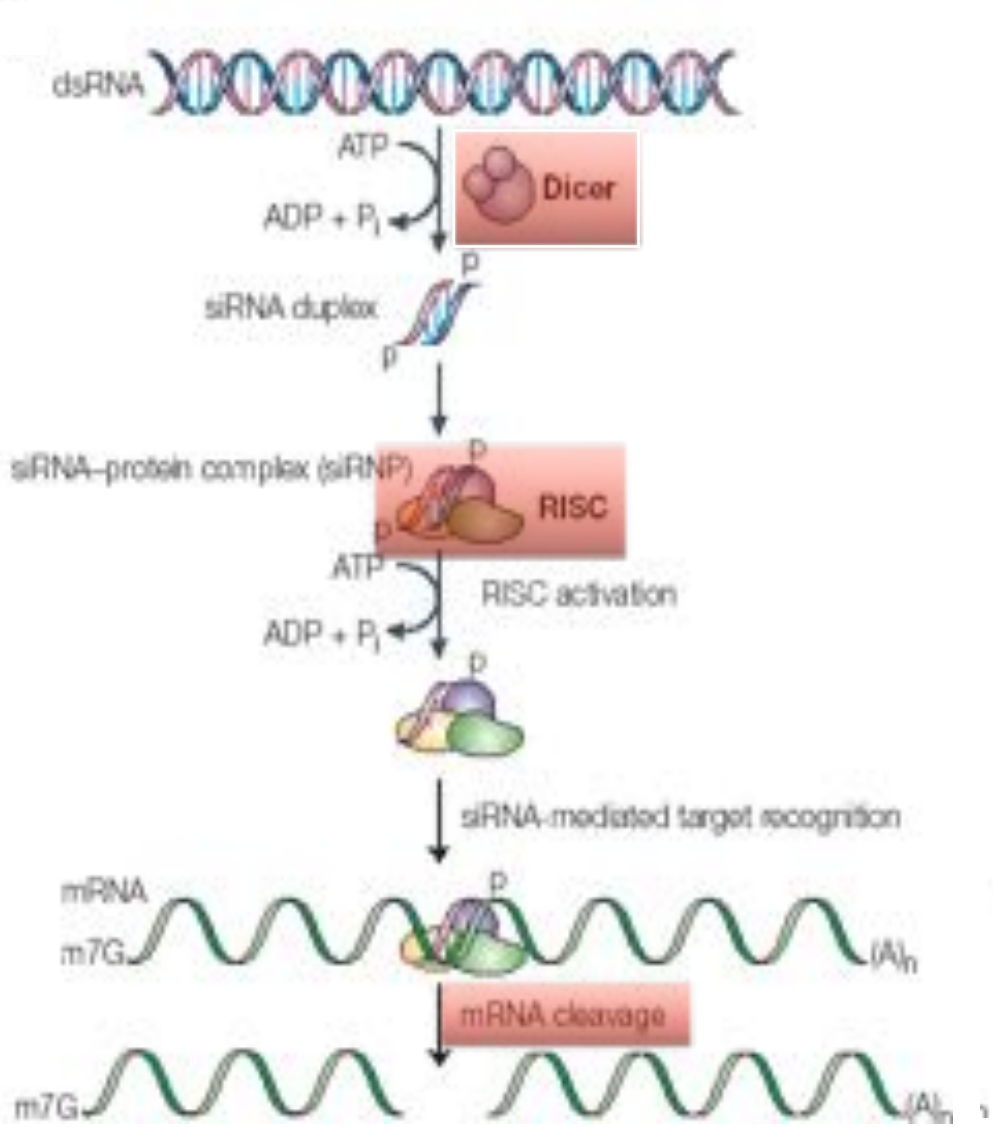


Wie kann man die RNAi-Antwort induzieren

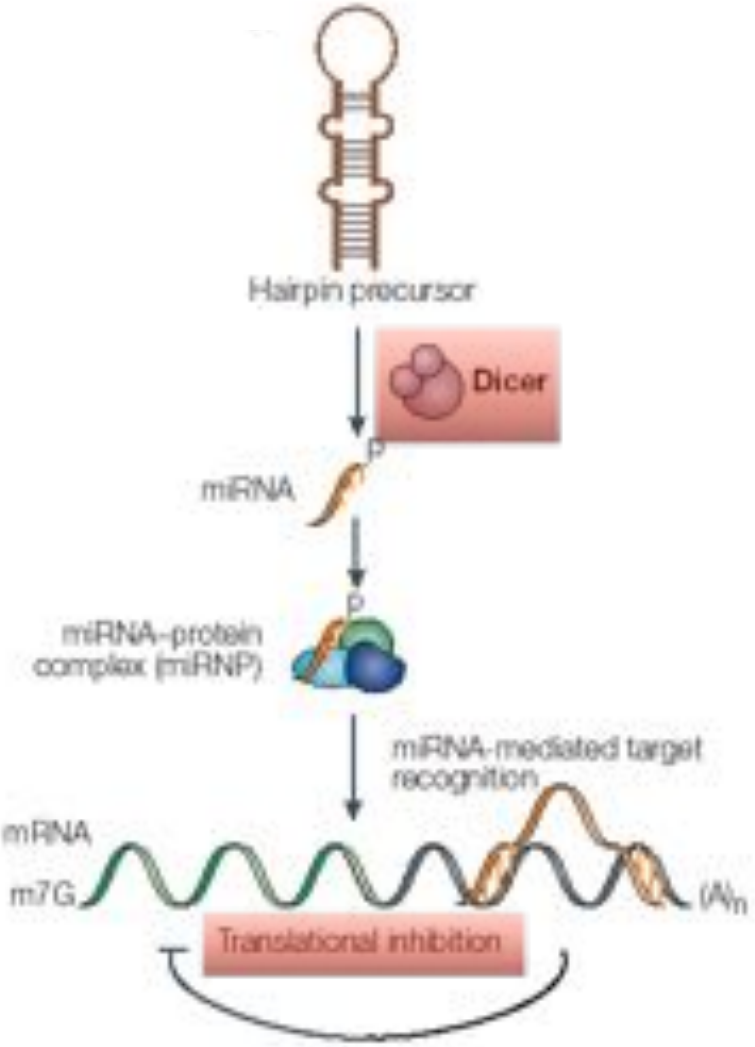


RNAi Wiederholung

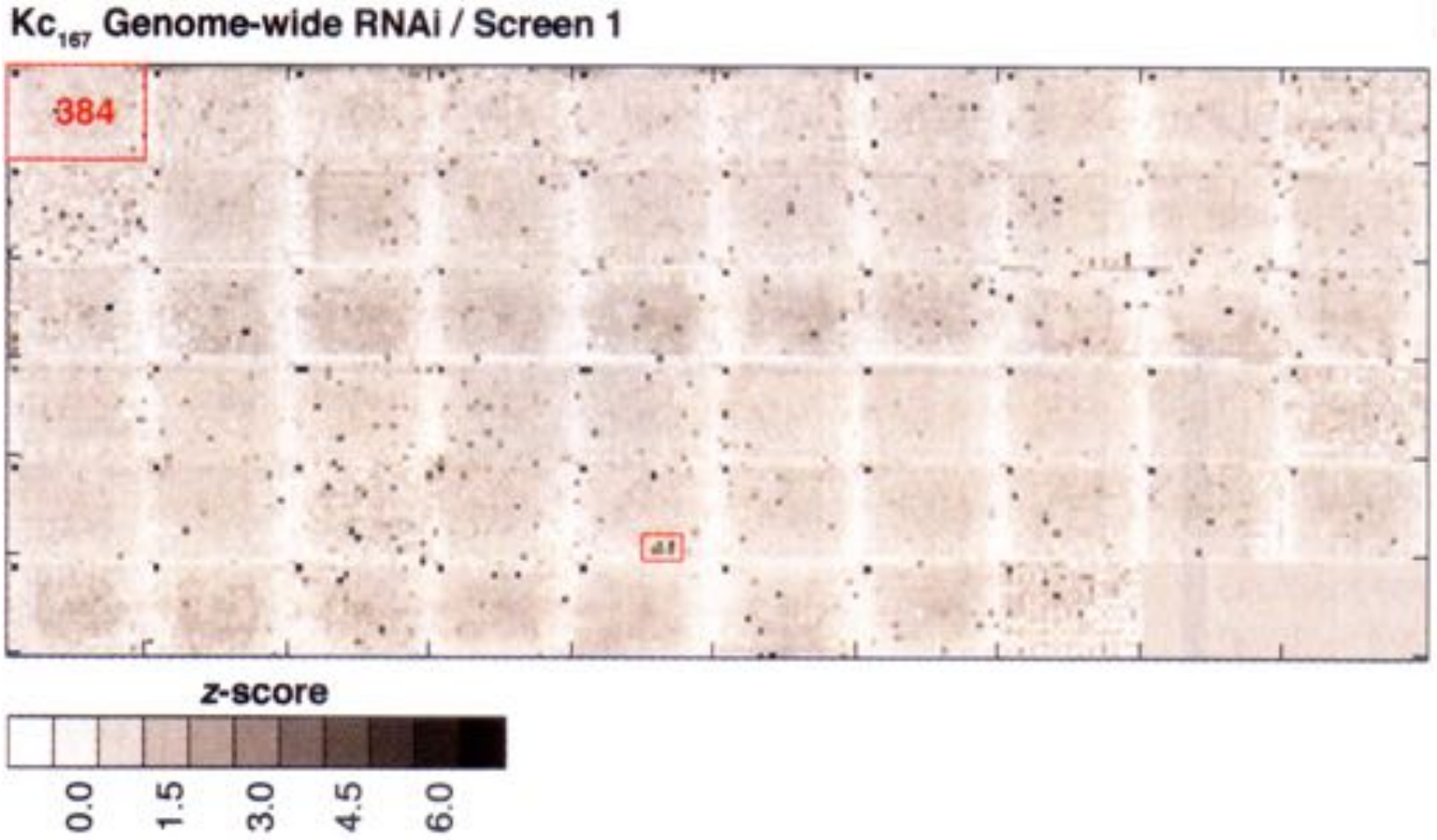
siRNA



miRNA



Genom-weite RNAi Experimente



Zellkulturmodelle bei *Drosophila*

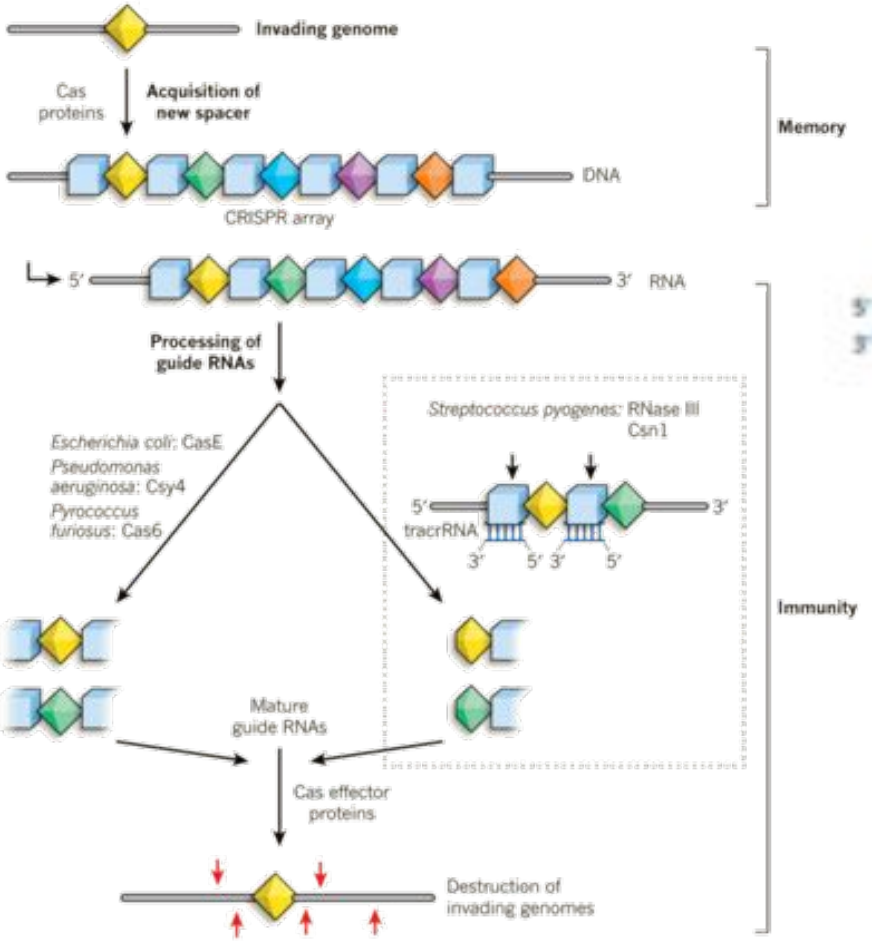
CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats

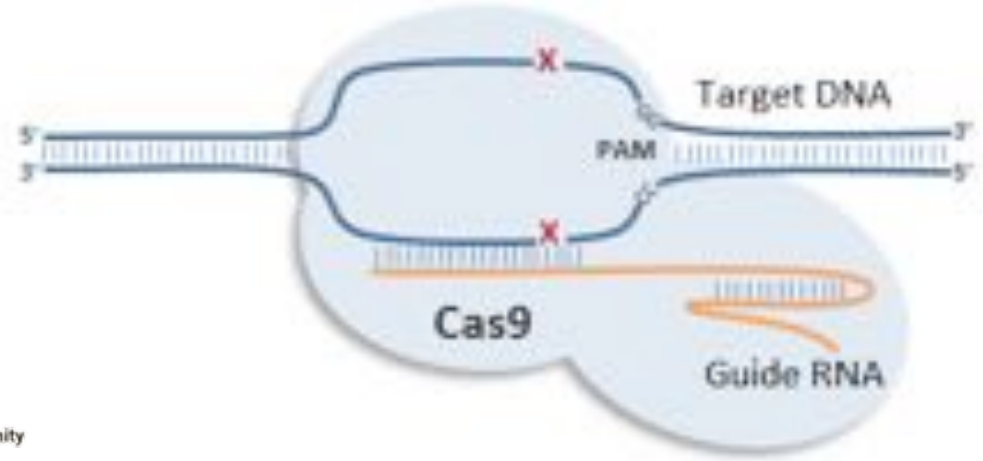
1987 erstmals beschrieben

2005 erste Hinweise das CRISPR an der adaptiven Immunität von Bakterien beteiligt ist.

2010 CRISPR funktioniert in Eukaryoten

CRISPR

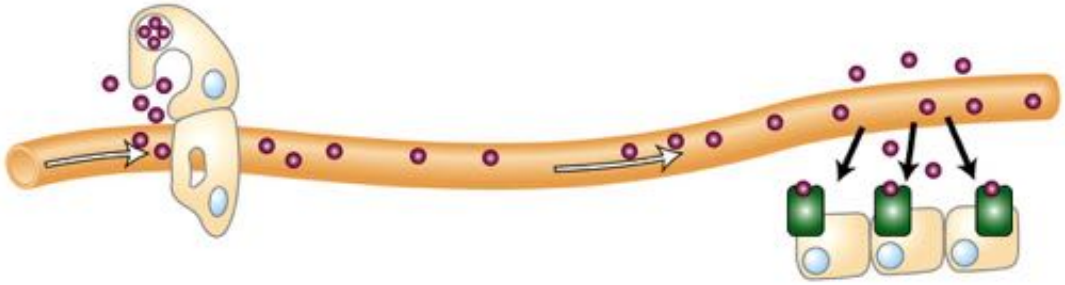




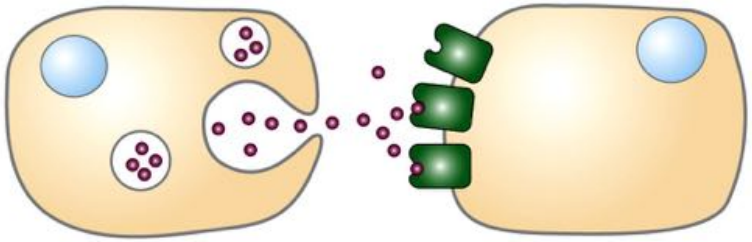
CAS
CRISPR associated nuclease

Signaltransduktion

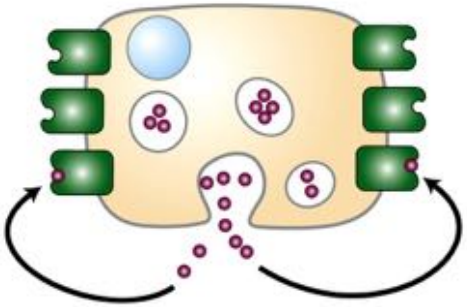
Endokrine Signale



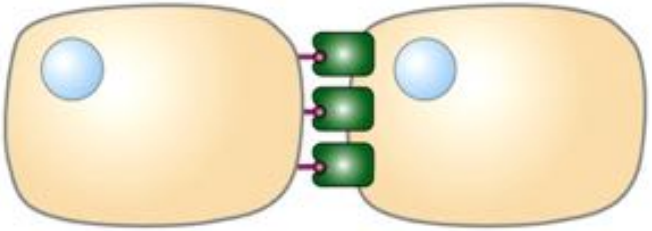
Parakrin



Autokrin



Juxtakrin





Signal

Rezeptor

Signal, Membrangebunden

Signaltransduktion

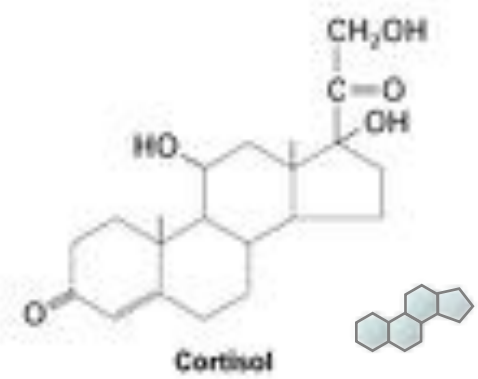
Wie kann eine Zelle äußere Einflüsse wahrnehmen und in eine Änderung der Aktivität umsetzen?

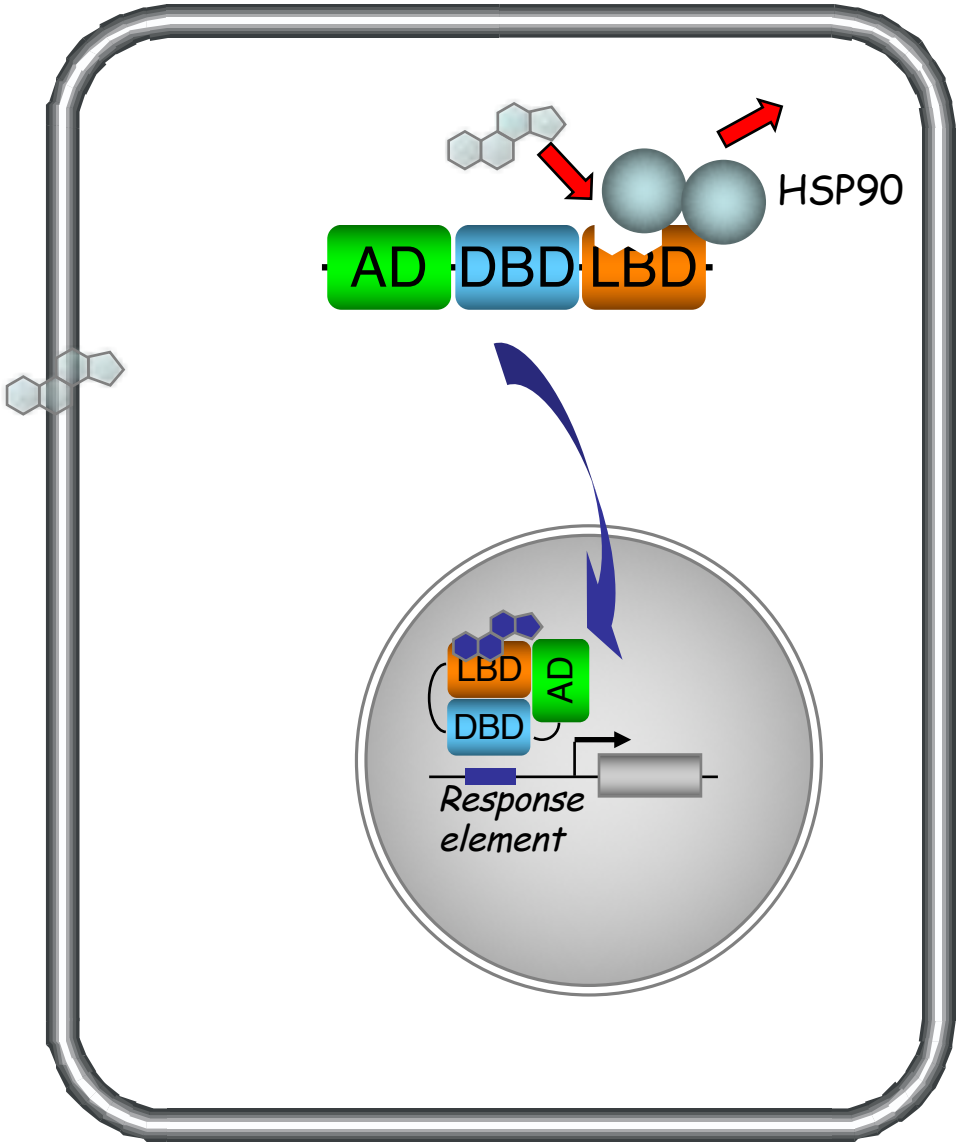
Lokale Wirkung -> second messenger (Ca^{2+}; cAMP; cGMP)

Wirkung auf den Zellkern -> Regulation der Transkription

- Hormon-Rezeptoren
- Notch (Membrangebundene TF)
- EGF-Signalweg
- TGFß-Signalweg
- Wnt-Signalweg
- Hh-Signalweg
- JAK/STAT-Signalweg
- Trimere G-Proteine

Steroide und ihre Rezeptoren (C4 Zn-Finger)

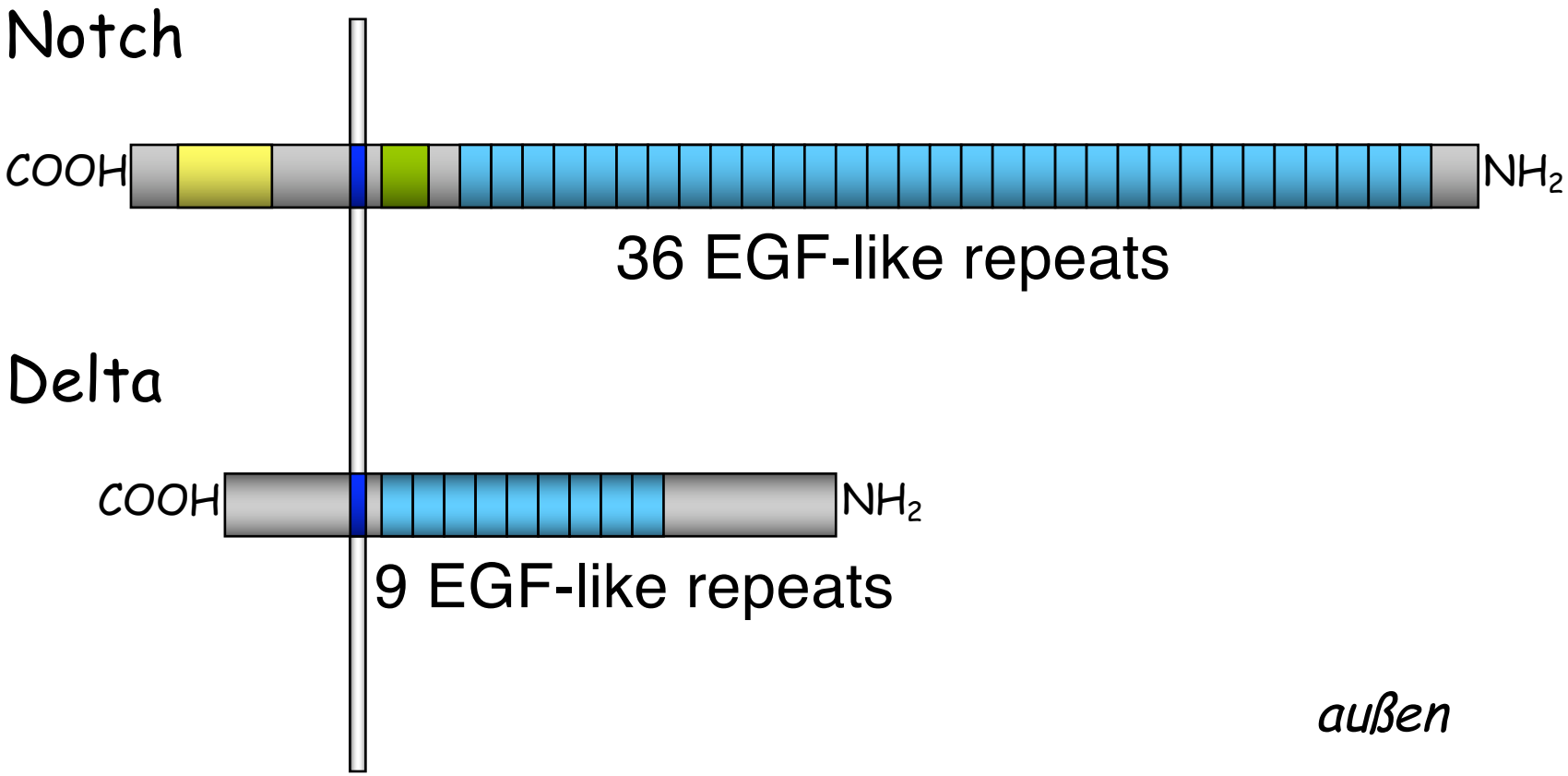




Typ II
Bindung des Liganden im Cytoplasma, Verdrängung von HSP90, Dimerisierung
Typ I
Bindung im Zellkern

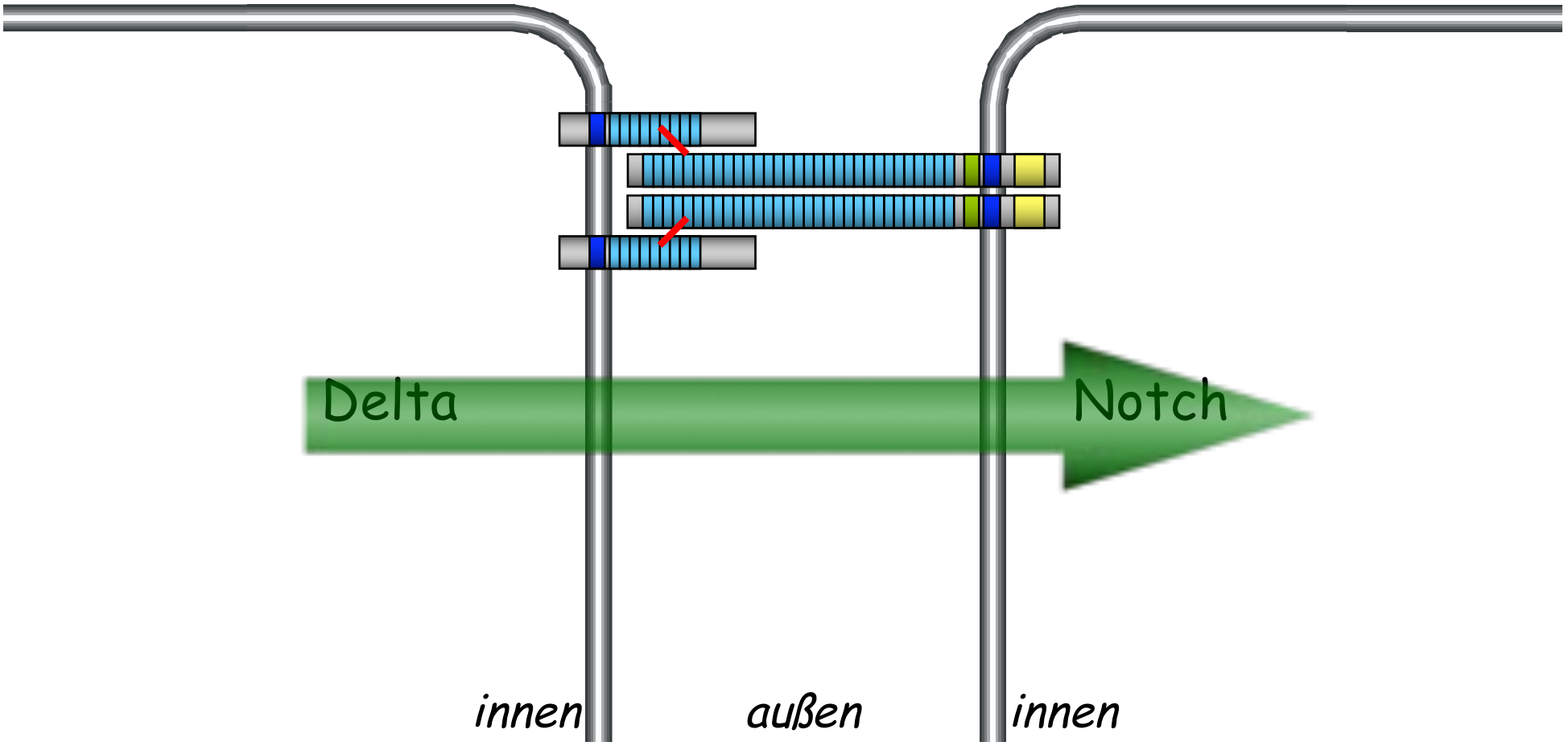
Der Notch-Signalweg

Delta und Notch kodieren Transmembranproteine, die als Signal (Delta) bzw. als Rezeptor (Notch) dienen.



Der Notch-Signalweg

Delta wirkt nicht Zellautonom auf den Notch-Rezeptor

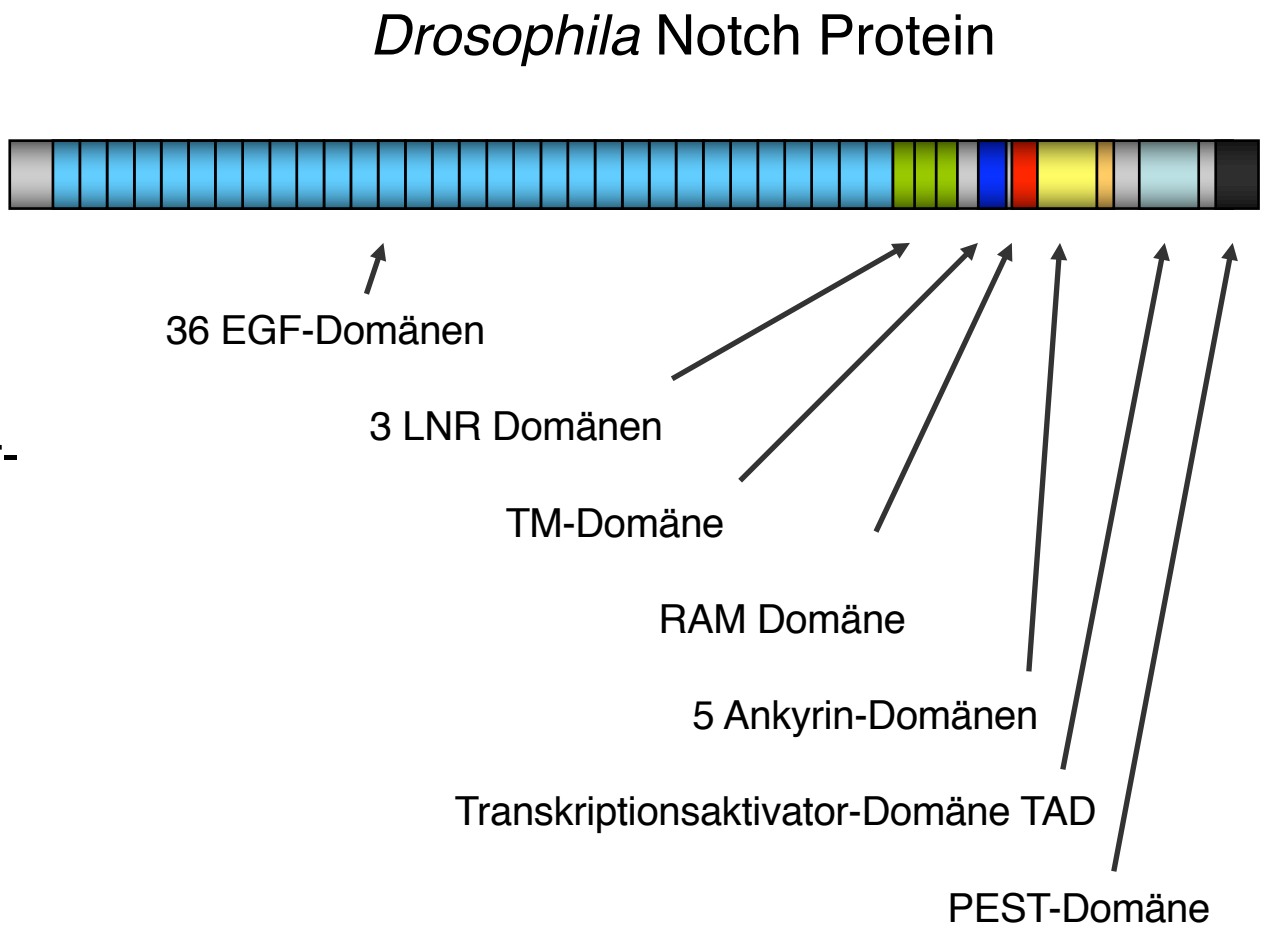


Notch Signaltransduktion

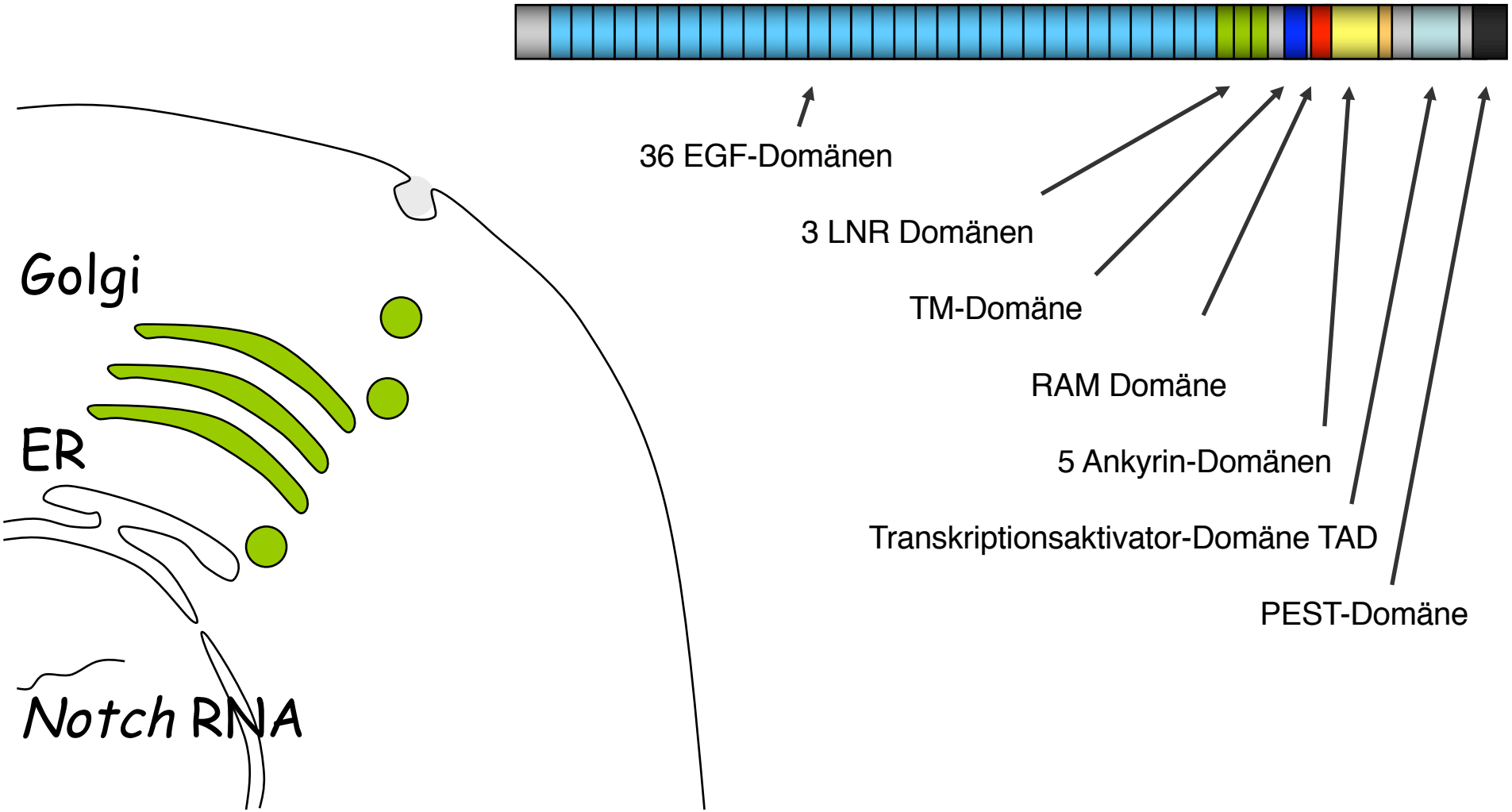
Hier erfolgt die Liganden Bindung.

Verhindern Rezeptor-aktivierung in der Abwesenheit von Ligand.

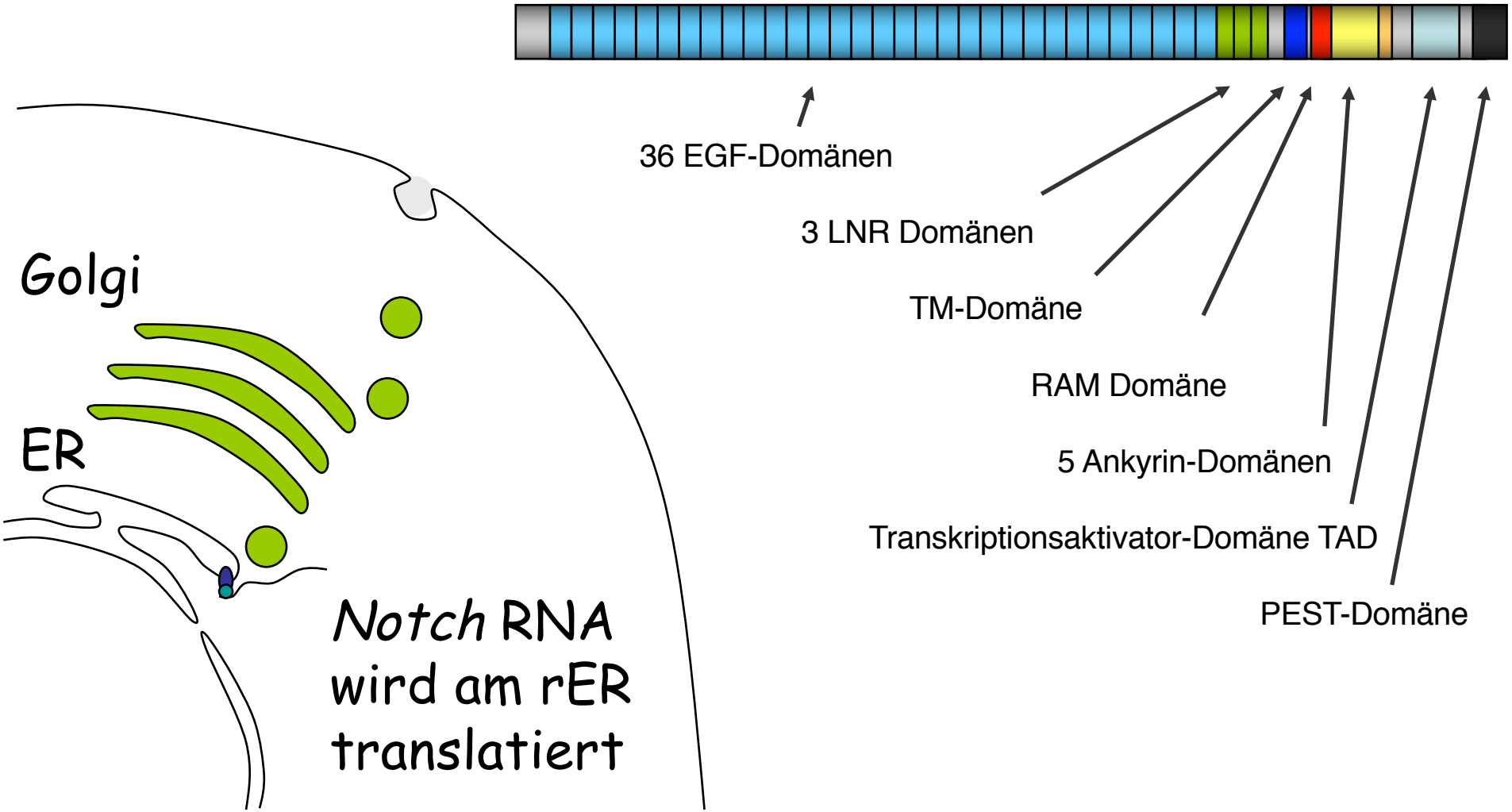
Bindung von Su(H)/CSL.



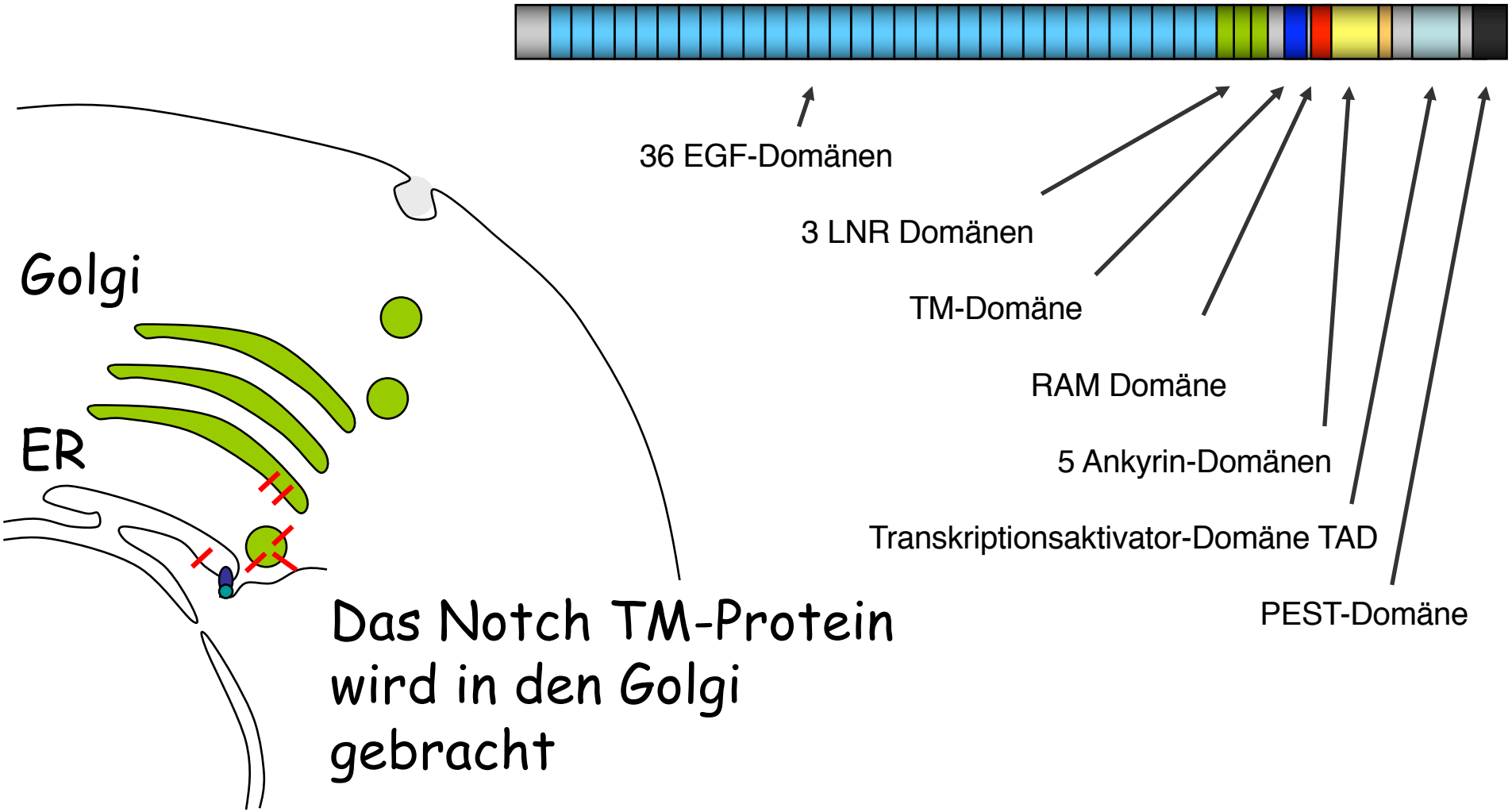
Notch Signaltransduktion



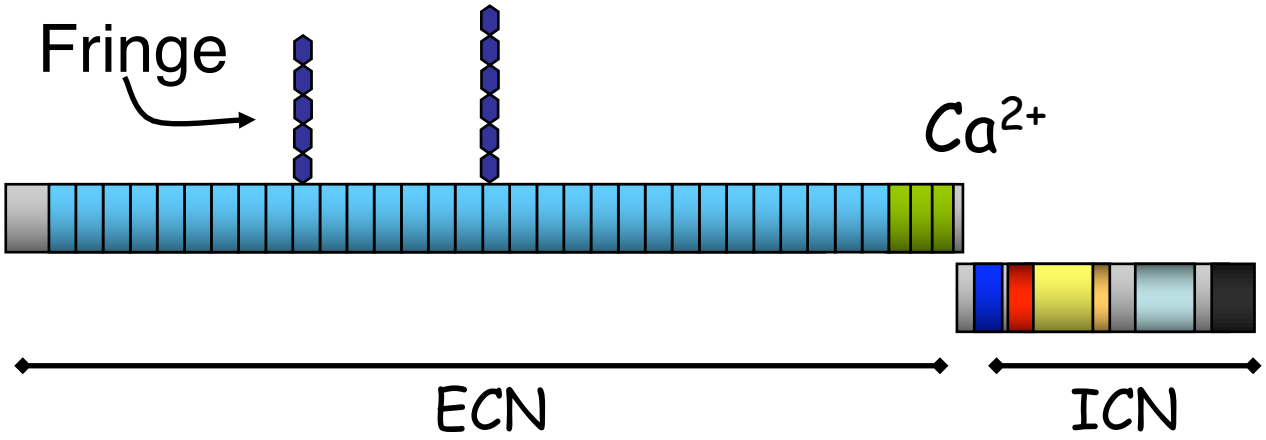
Notch Signaltransduktion

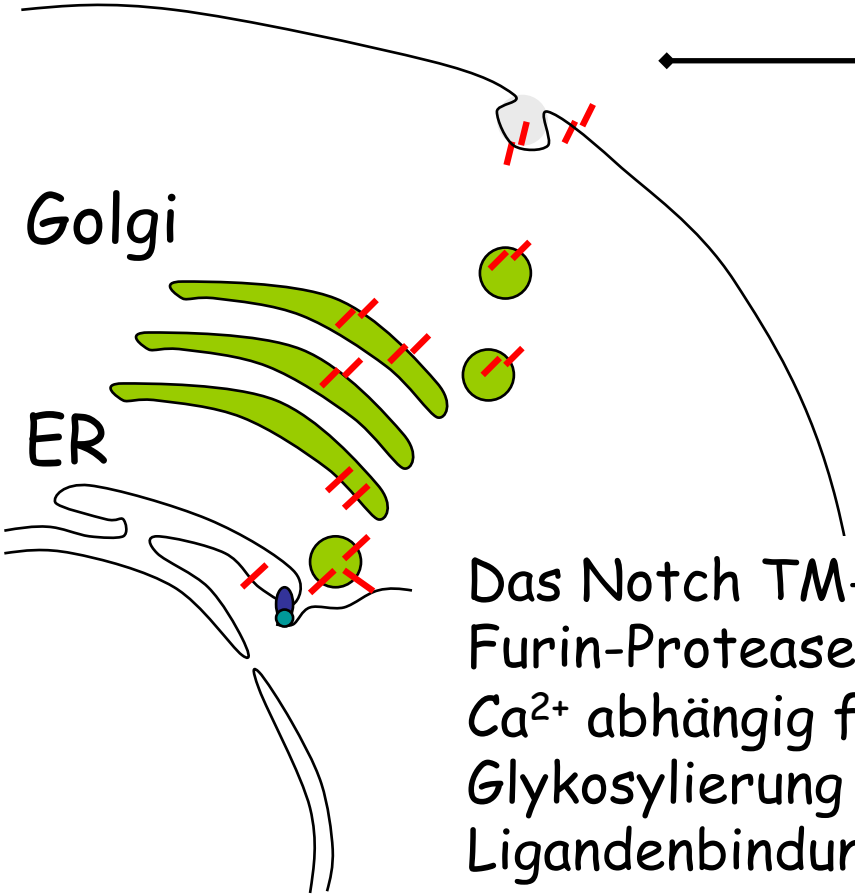


Notch Signaltransduktion



Notch Signaltransduktion

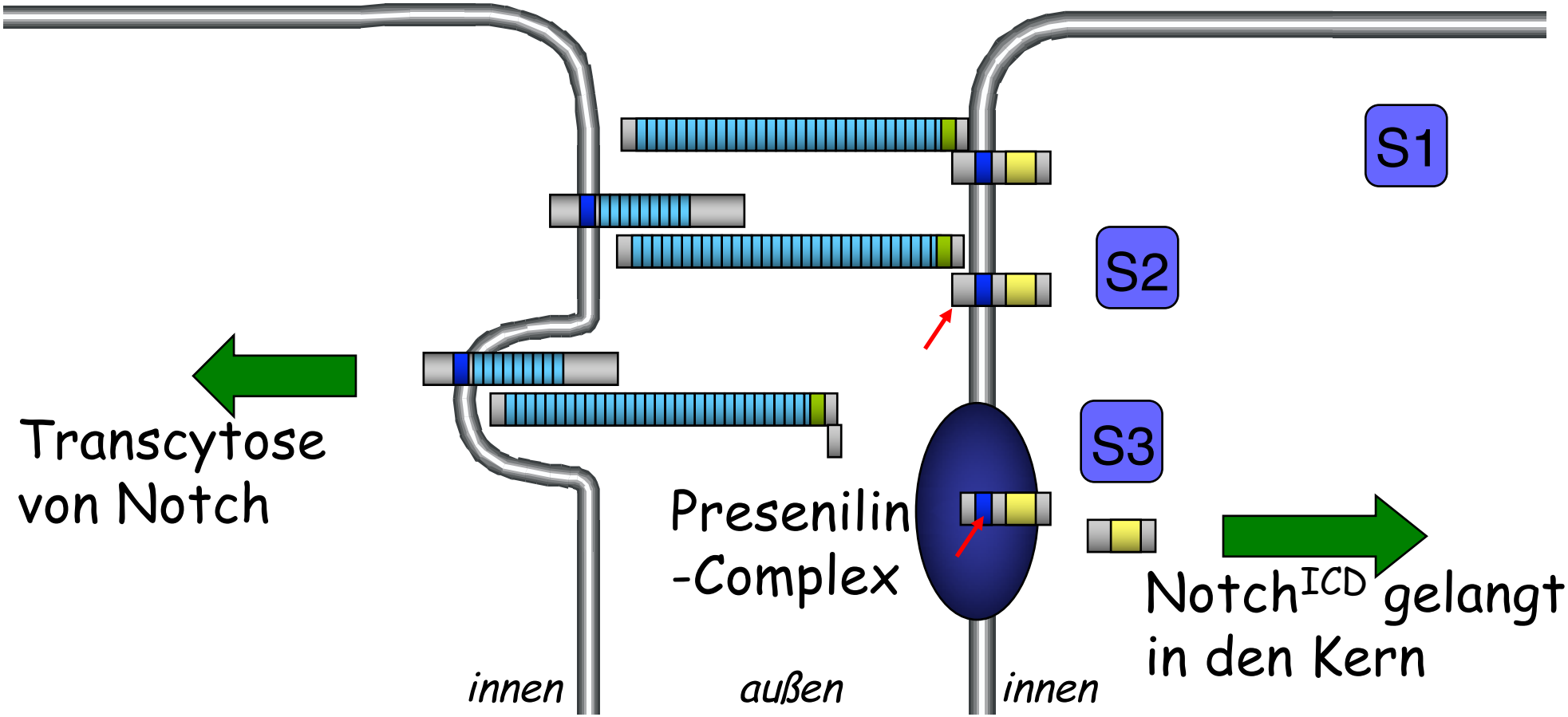




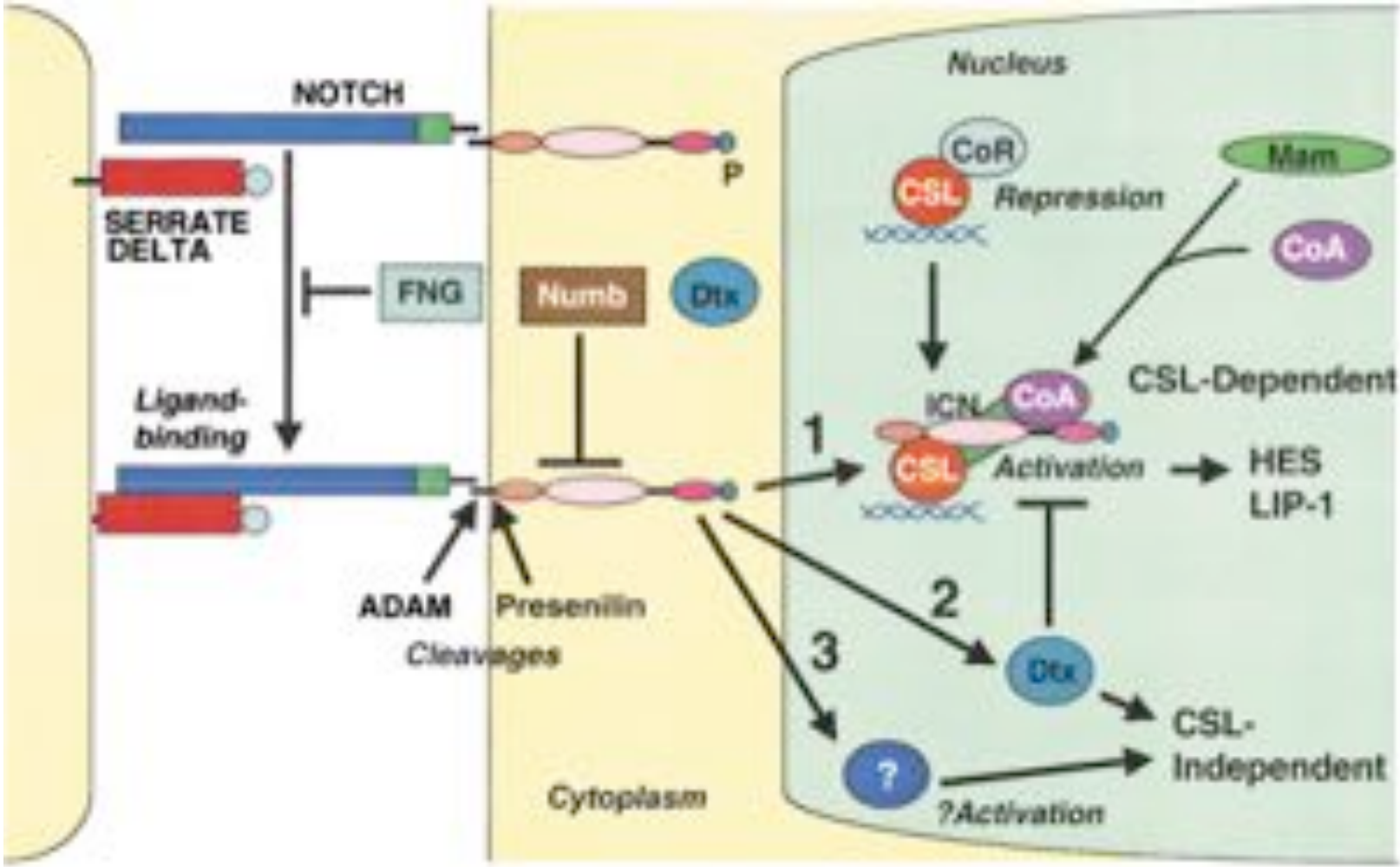
Das Notch TM-Protein wird im Golgi durch eine Furin-Protease prozessiert. Der ECN-Teil wird Ca^{2+} abhängig festgehalten. Zugleich erfolgt eine Glykosylierung durch Fringe, moduliert Ligandenbindung

Der Notch-Signalweg

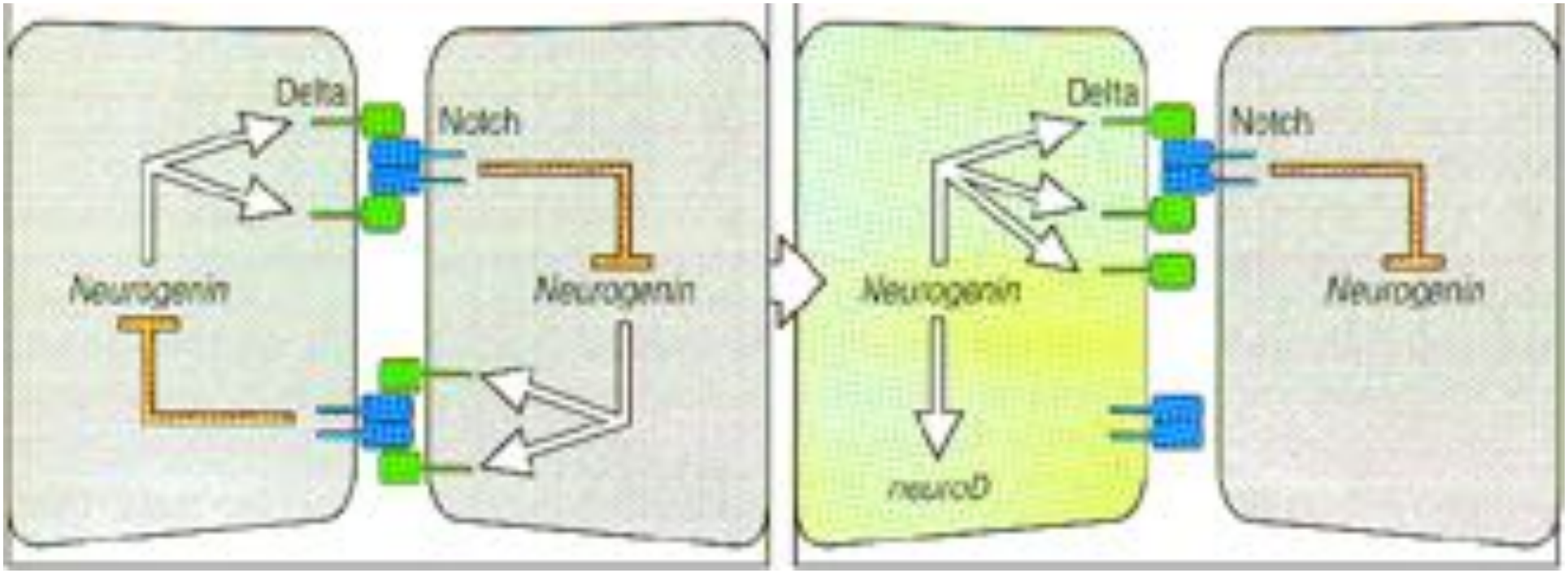
1) Notch wird im Golgi gespalten. S1
2) Notch bindet Delta oder Serrate Liganden (DSL)
   Es kommt zur Spaltung. S2
3) Anschließend wird Notch in der Membran durch
   Presenilin gespalten. Cofactor ist Nicastrin. S3



Notch Signaltransduktion

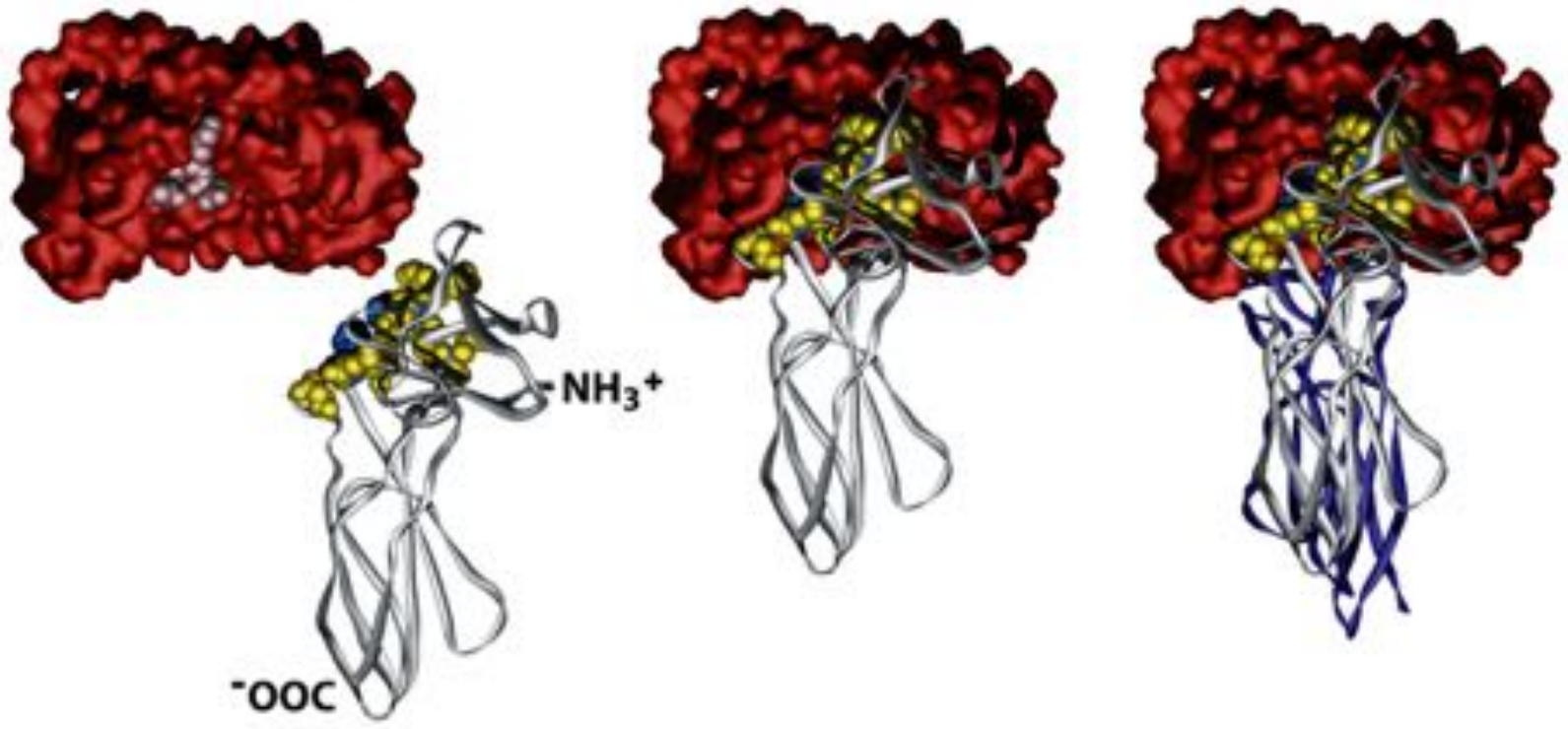


Notch-Delta Interaktion

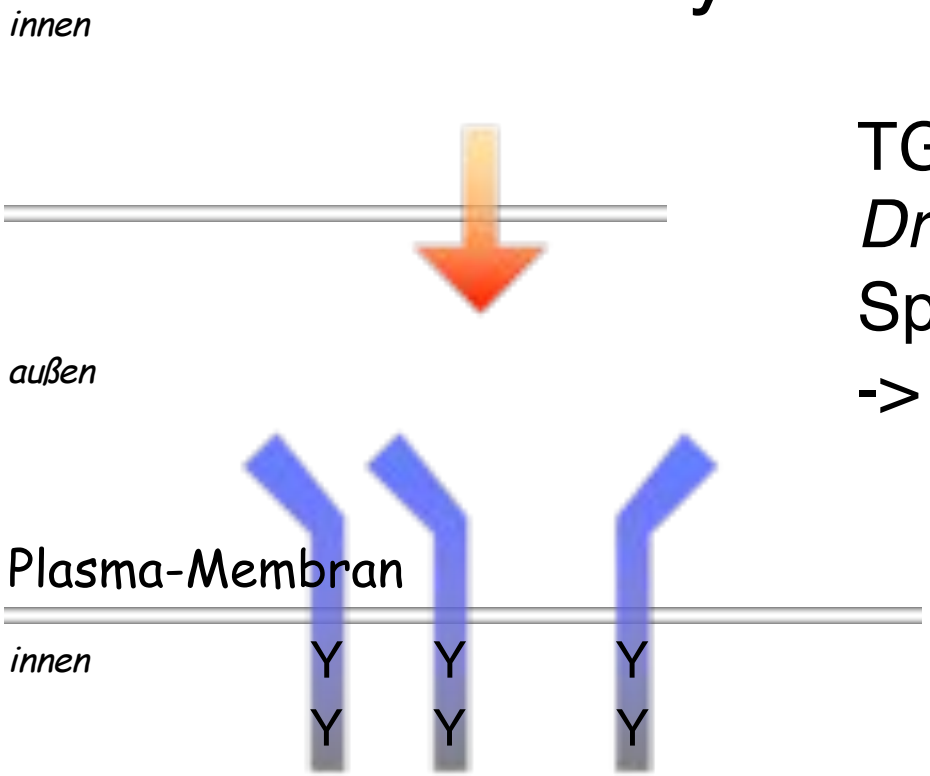


AS-C	Mash/Xash	Delta	Jagged
	Neurogenin	Notch	Notch
E(spl)	Hes		

Rezeptor-Ligand Interaktionen sind extrem spezifisch



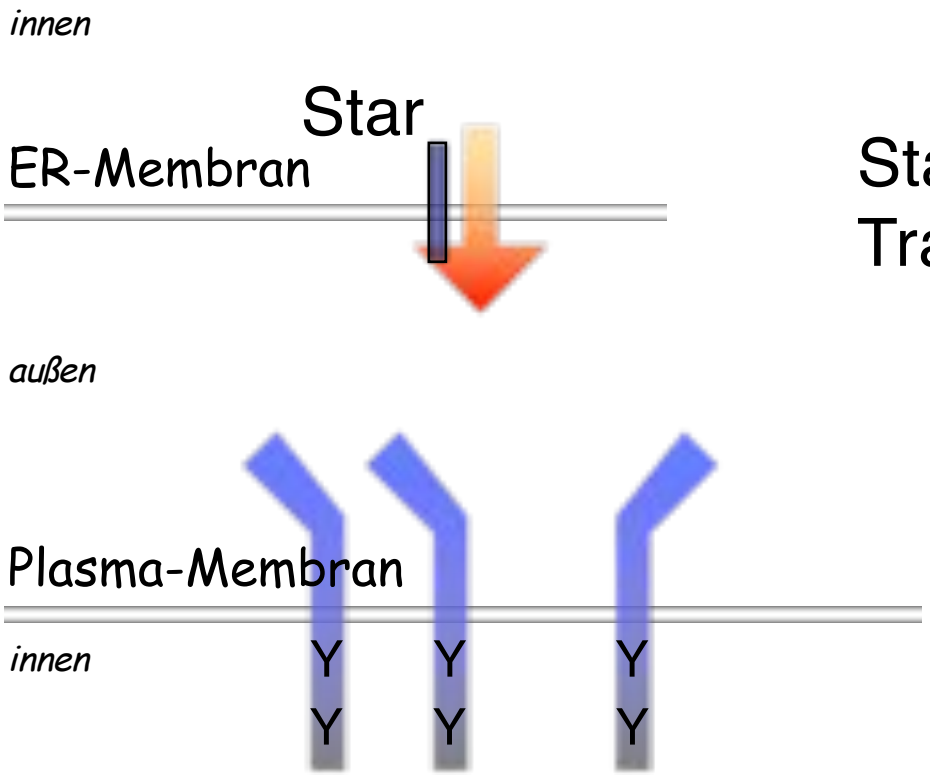
TGFa und verwandte Liganden werden als Membranständige Vorläuferprotein synthetisiert



TGFα -> TACE
Drosophila:
Spitz, Gurken, Keren, Vein
-> Rhomboid und Star

Drosophila EGF-Rezeptor (DER)

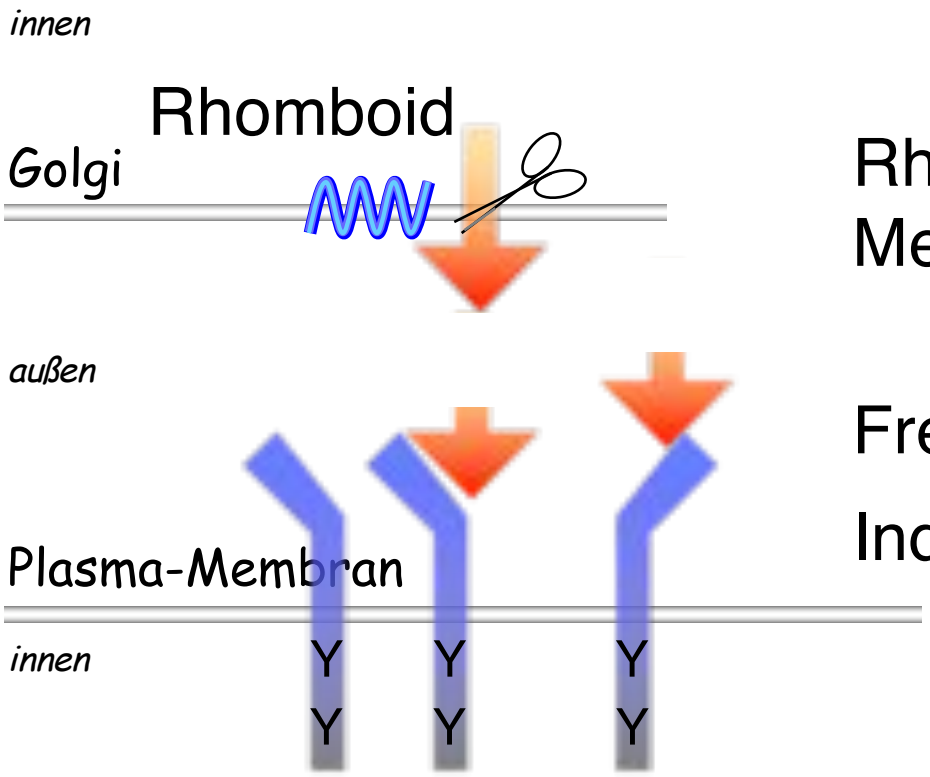
Star reguliert den Transport von Spitz vom ER zum Golgi



Star markiert mSpitz für den Transport zum Golgi

Drosophila EGF-Rezeptor (DER)

Star reguliert Transport von Spitz vom ER zum Golgi



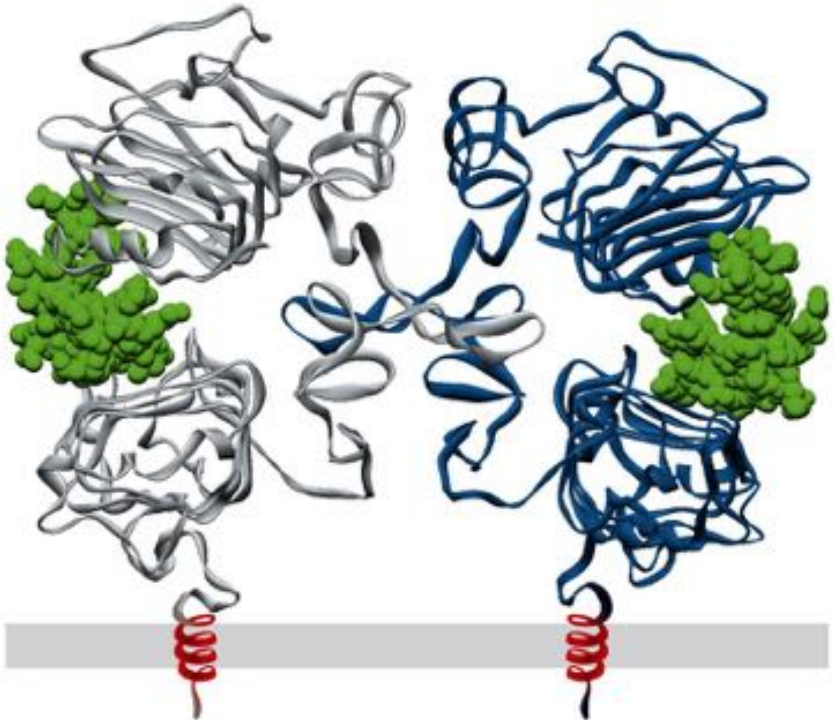
Rhomboid ist eine Intra-Membran Protease

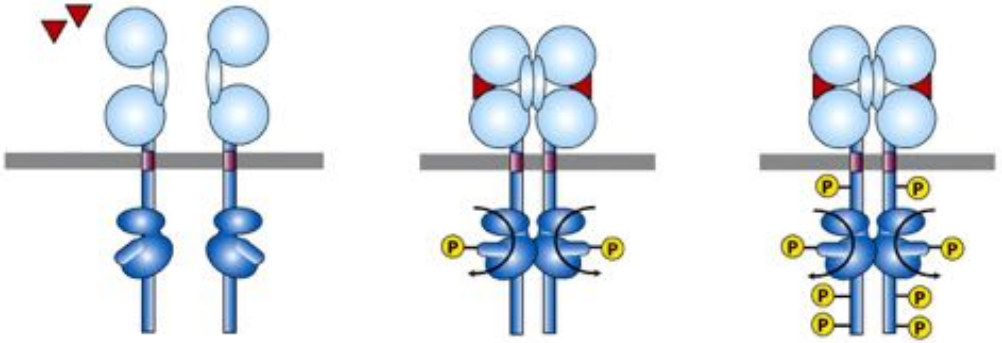
Freies Spitz aktiviert den EGFR

Induziert Dimer-Bildung

Drosophila EGF-Rezeptor (DER)

Bindung von EGF führt zur Rezeptordimerisierung und zur Autophosphorylierung (Tyrosin!)

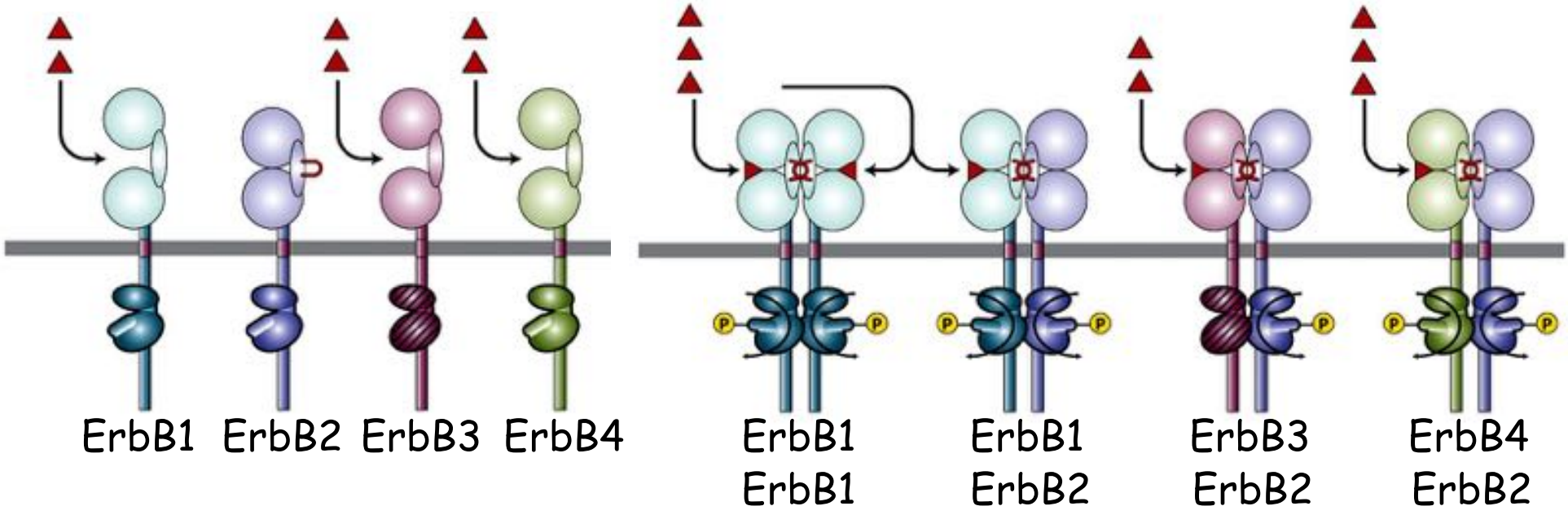




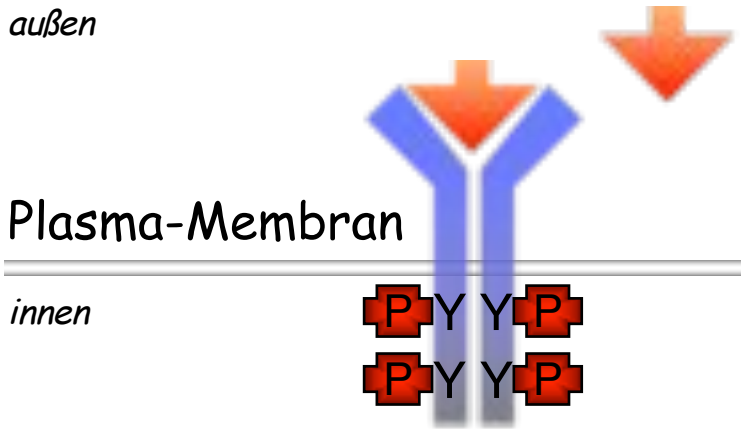
4 verschiedene EGF-Rezeptoren beim Menschen (ErbB1-ErbB4)

EGF
TGFα

Nrg1,2



Aktivierung des EGF-Rezeptors
= Rezeptor-Tyrosin-Kinase (RTK)



Dimer-Bildung

induziert Autophosphorylierung



Src, SH3 SH2 *SH1*

eine cytoplasmatische-Tyrosin-Kinase

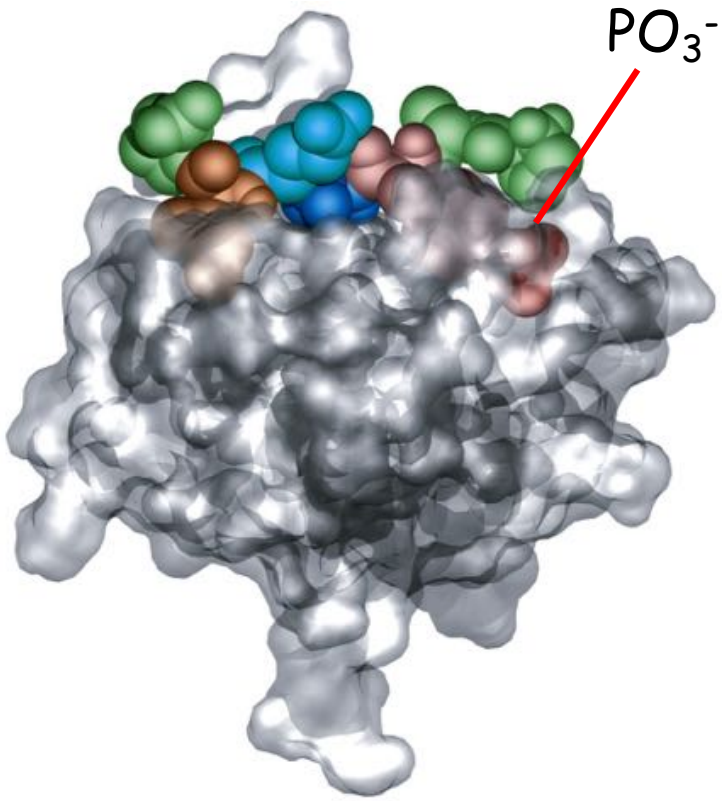


SH2-Domänen binden Phosphotyrosin



SH3-Domänen binden Prolinreiche Sequenzen

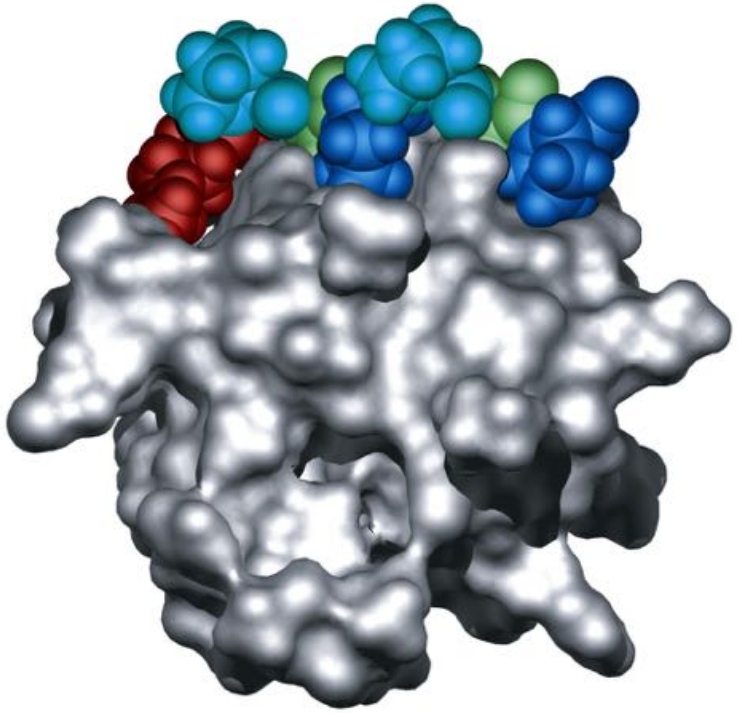
SH2 Domäne



Phospho-Tyrosin Bindung

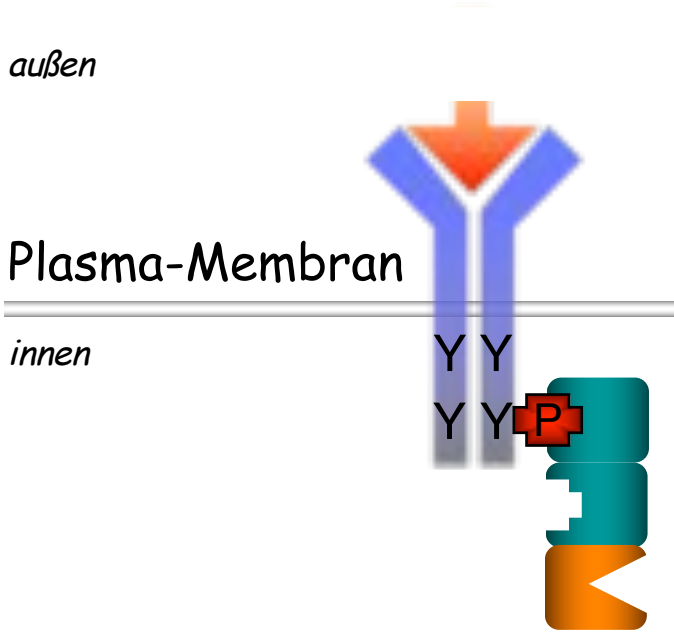
Es gibt auch eine PTB-Domain
(**P**hospho-**T**yrosin **B**inding)

SH3 Domäne



PXXP Interaktion.
Spezifische Protein-Protein Interaktion

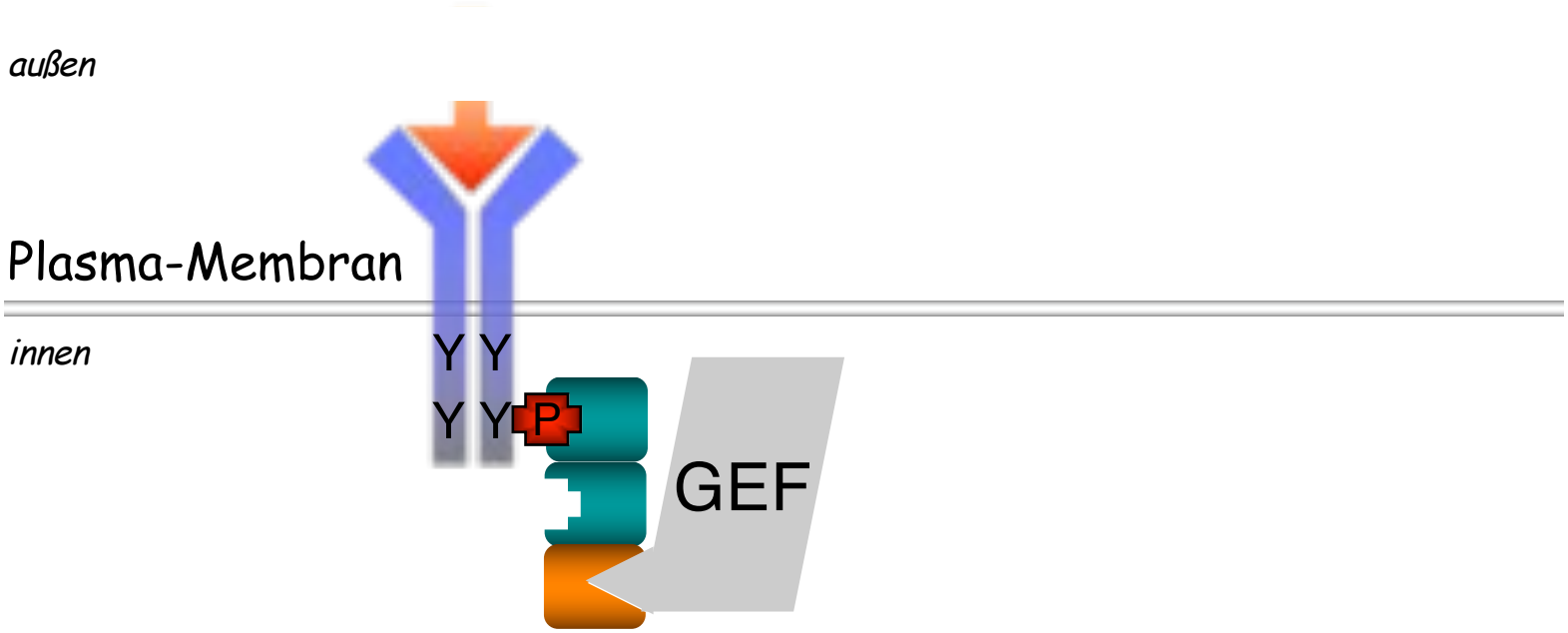
Weiterleitung des Signals



Das (SH2-SH2-SH3) Protein Drk/Grb koppelt aktivierte RTKs an weitere Proteine.

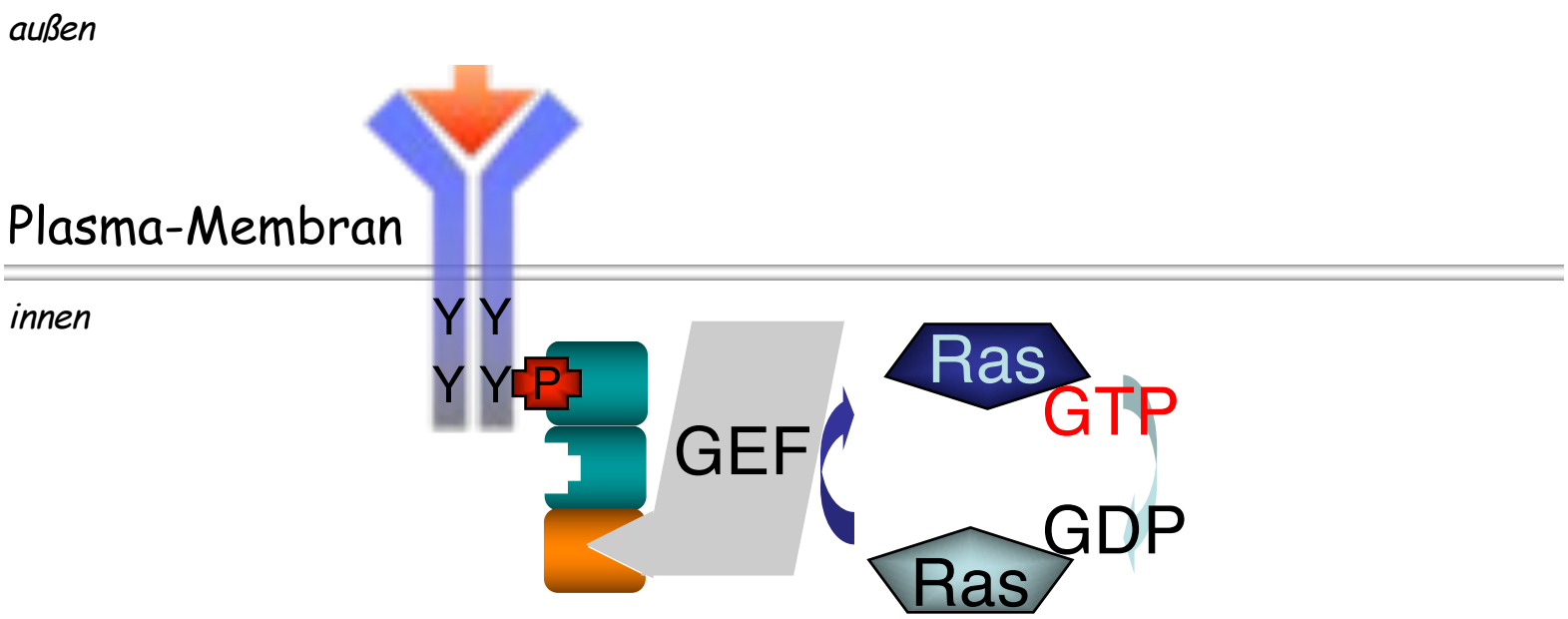
Problem: Spezifität!

Weiterleitung des Signals



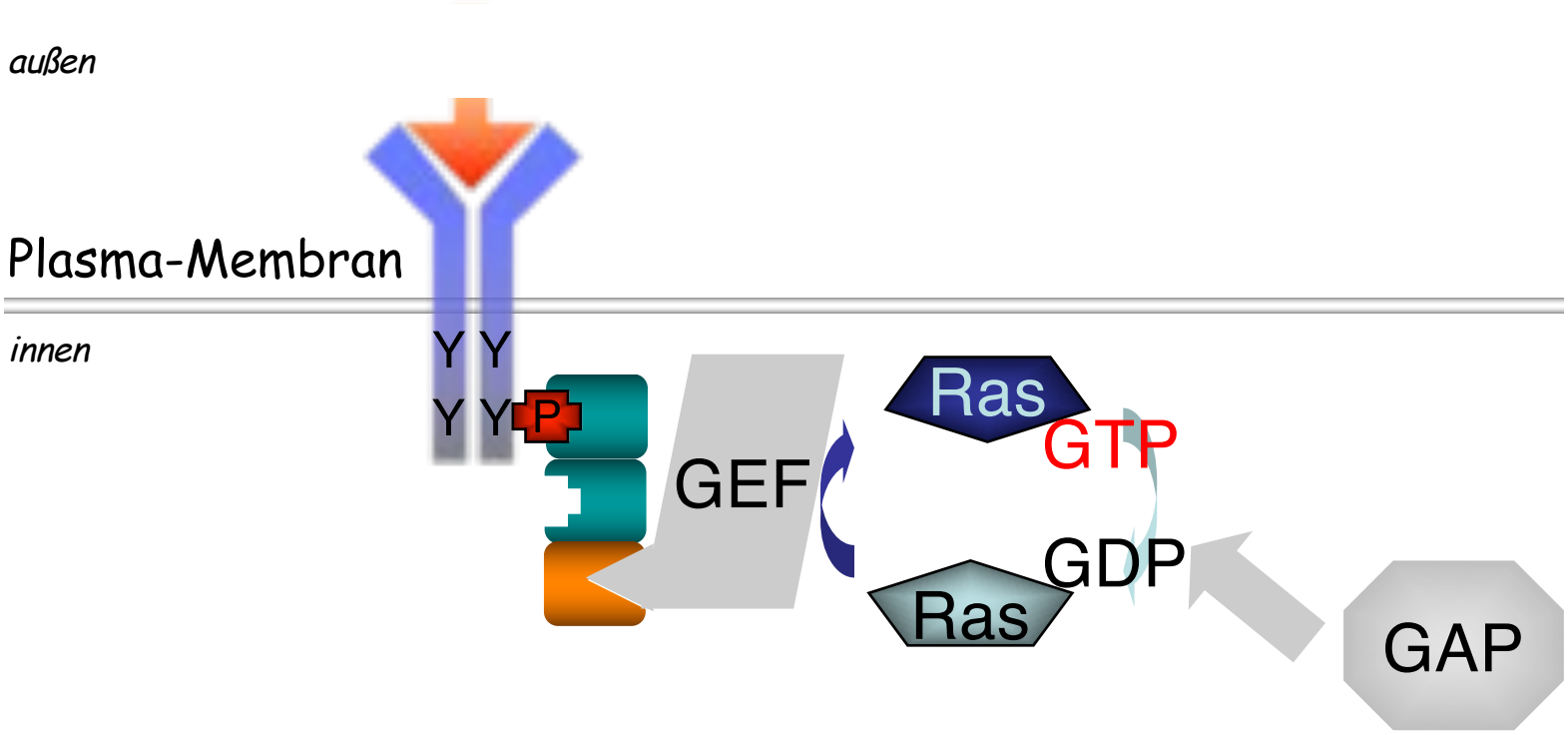
Ein GEF (Guanine exchange factor) aktiviert kleine G-Proteine.
Für EGFR heißt es SOS (Son of Sevenless)

Weiterleitung des Signals



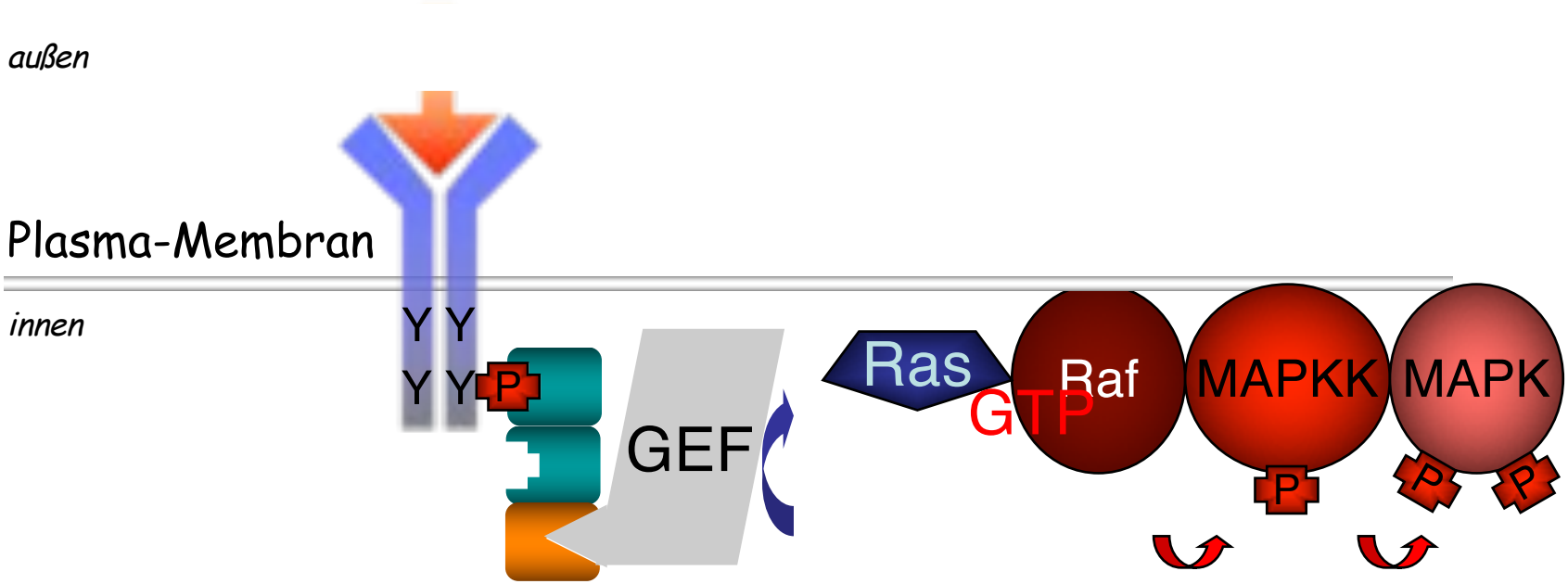
Ein GEF (Guanine exchange factor) aktiviert kleine G-Proteine.
Für EGFR heißt es SOS (Son of Sevenless)

Weiterleitung des Signals



Ein GAP (GTPase activating protein) inaktiviert Ras.

Weiterleitung des Signals



Ras aktiviert die MAP-Kinase Kaskade.
Serin/Threonin (S/T) Kinasen.

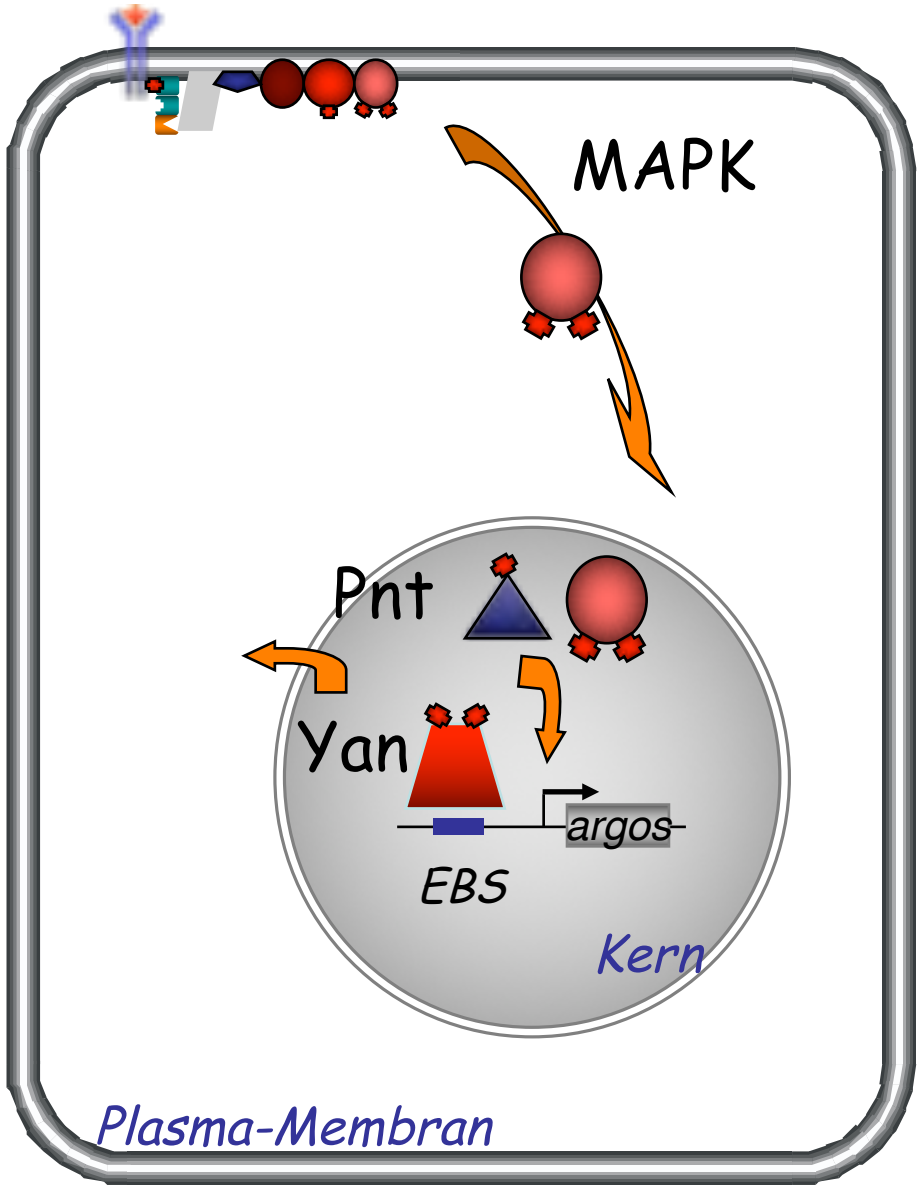
Signalverstärkung

Die MAPK gelangt in den Zellkern und phosphoryliert Transkriptionsfaktoren

Die aktivierte MAPK gelangt in der Kern und phosphoryliert die ETS-Domänen Transkriptionsfaktoren:

Yan = Repressor

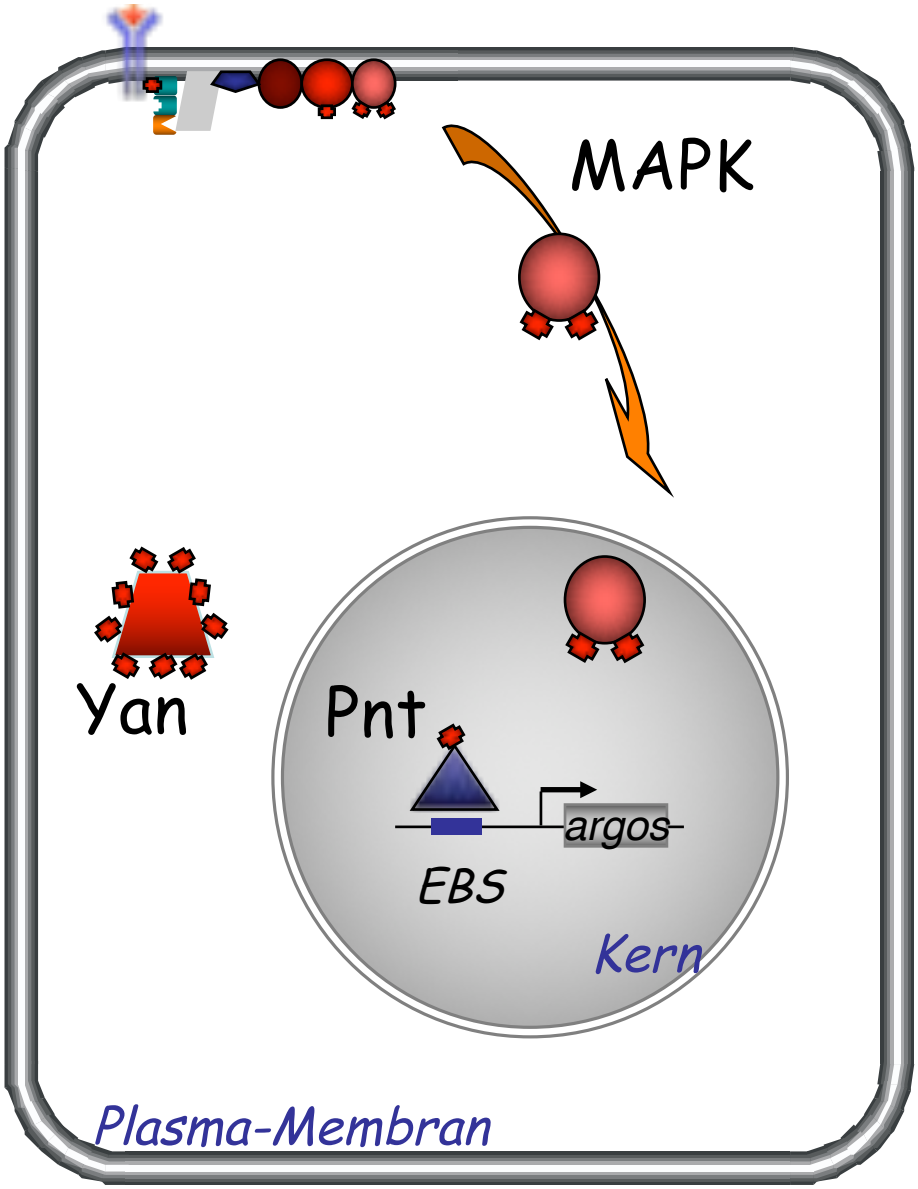
Pnt = Aktivator



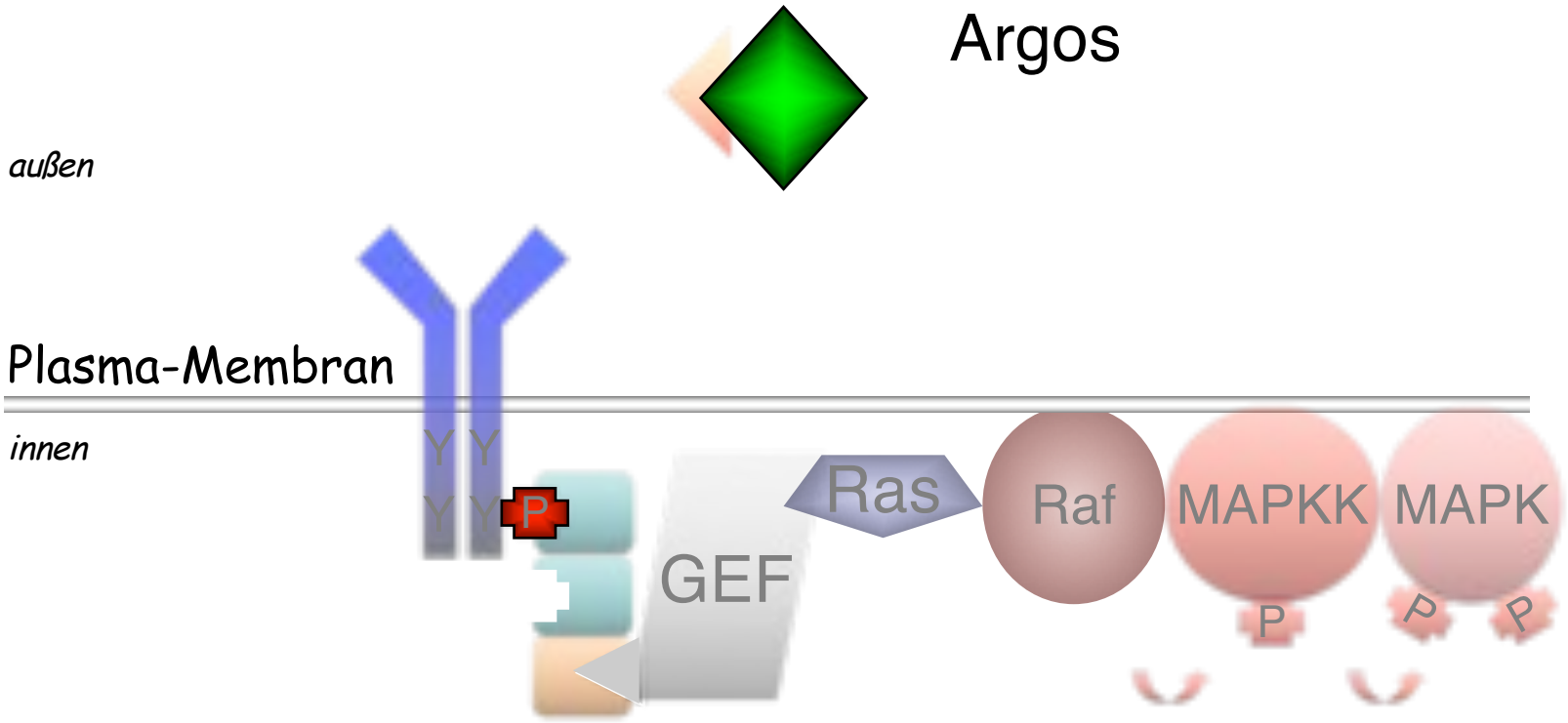
Die MAPK gelangt in den Zellkern und phosphoryliert Transkriptionsfaktoren

Yan wird sehr stark phosphoryliert und verliert die DNA Bindungsfähigkeit, kommt ins Cytoplasma und wird degradiert.

Pnt wird einmal phosphoryliert und hierdurch aktiviert.

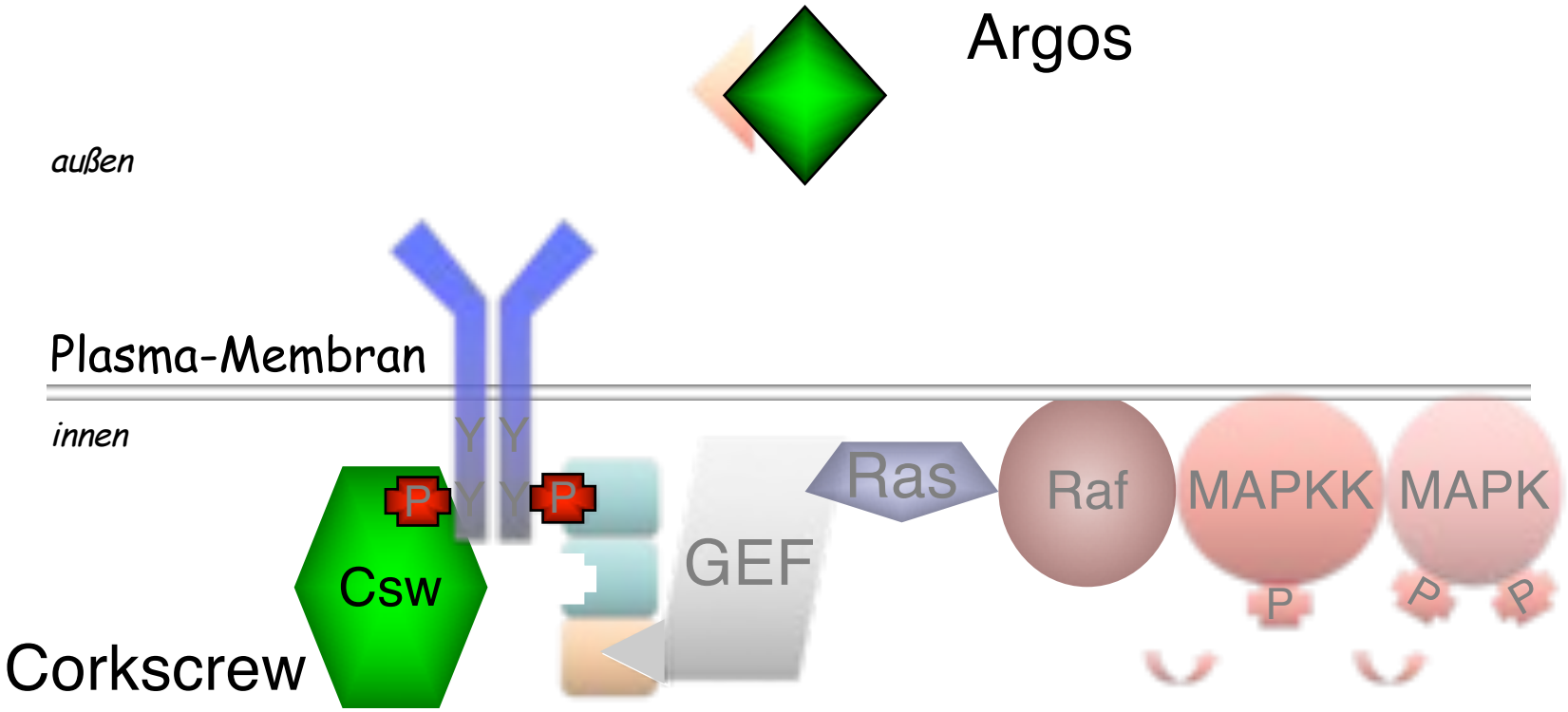


Termination des Signals



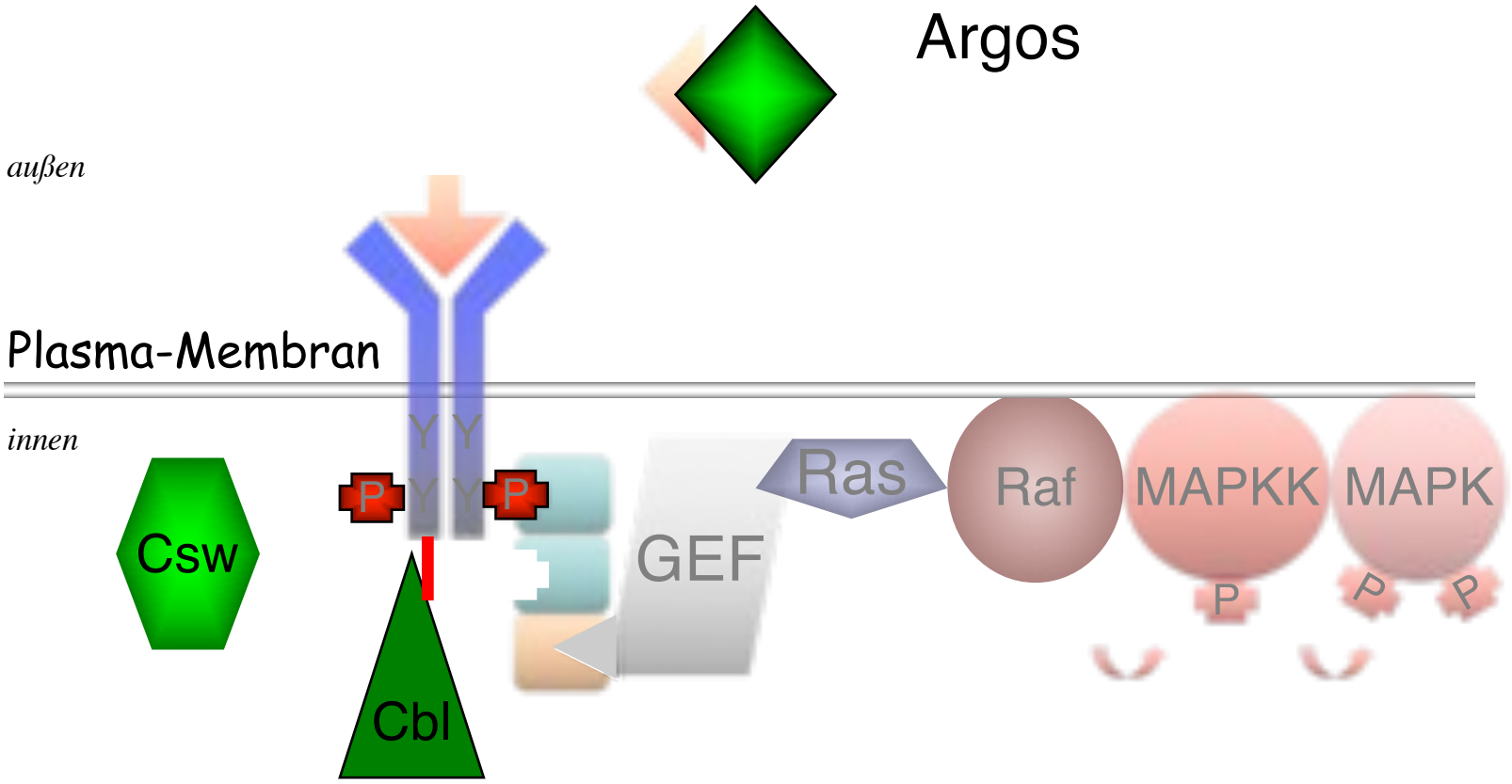
Argos konkuriert mit Spitz um Rezeptoraktivierung
Zielgen von Pnt. Kaskade inaktiviert sich selbst!

Termination des Signals



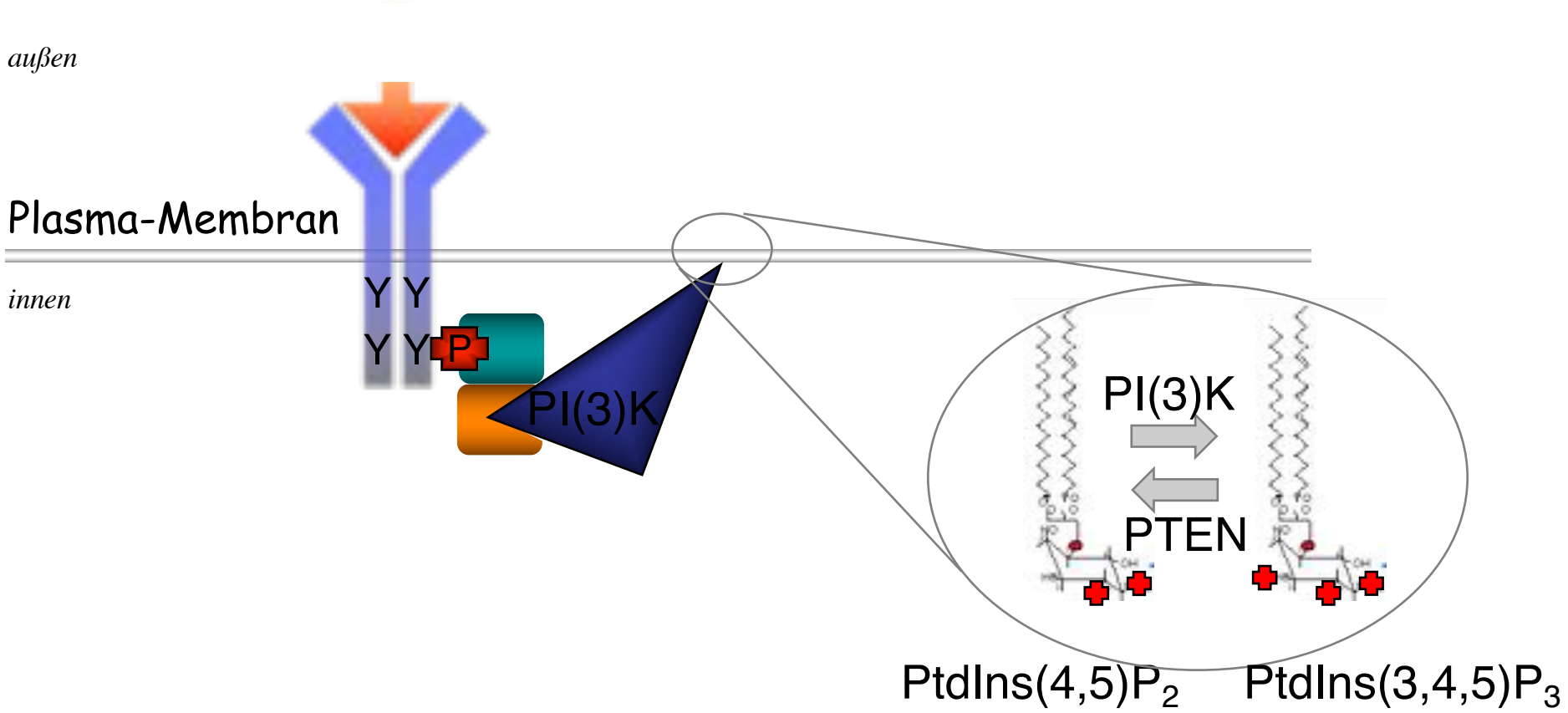
Corkscrew ist eine Phosphatase. Dephosphoryliert RTKs.

Termination des Signals



Cbl ist eine E3 Ubiquitin-Ligase. Mono-Ubiquitinierung von Rezeptoren führt zur Endozytose.

Komplexe Signalketten

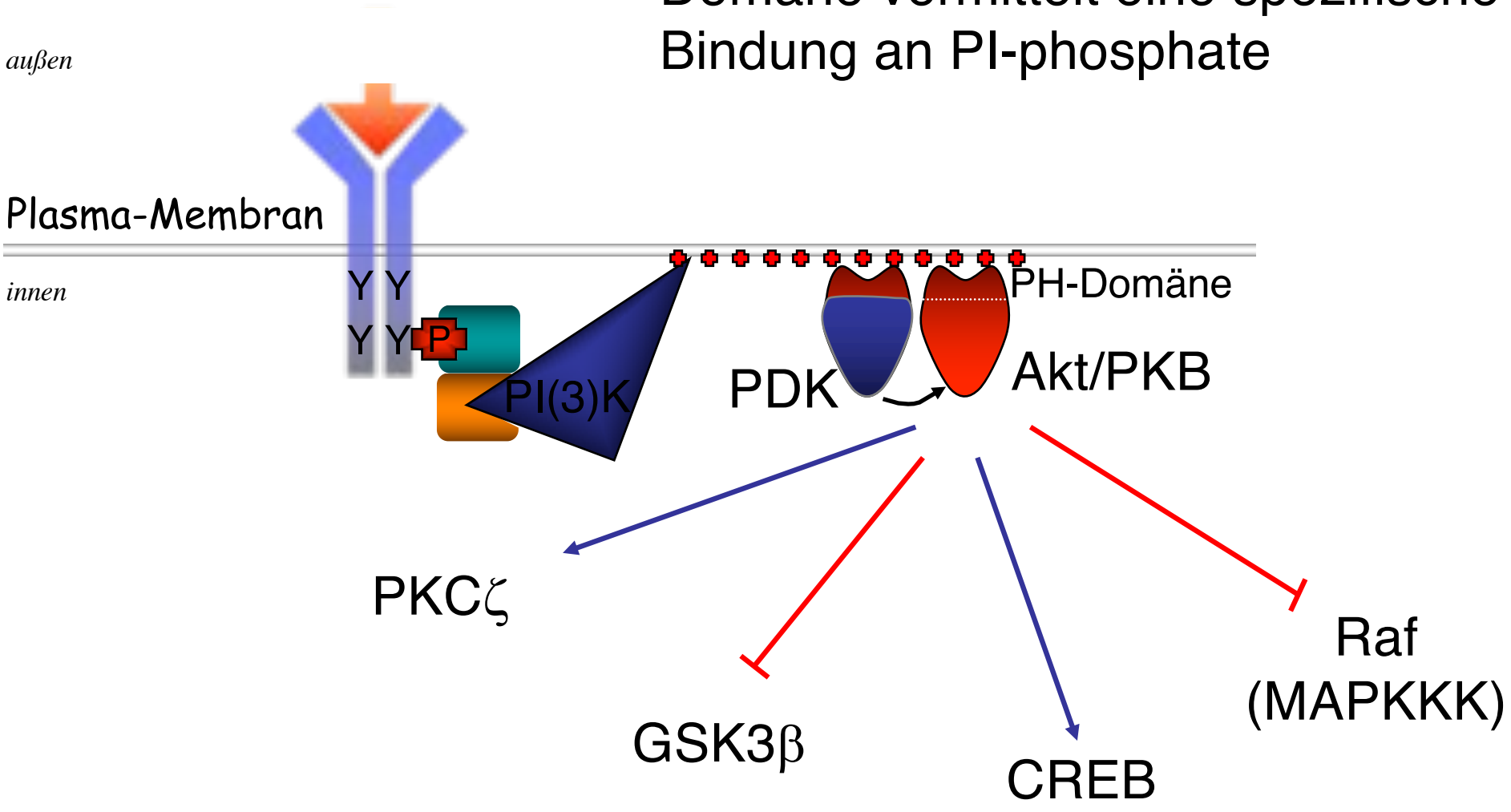


PI(3)K = Phosphatidylinositol-3-Kinase

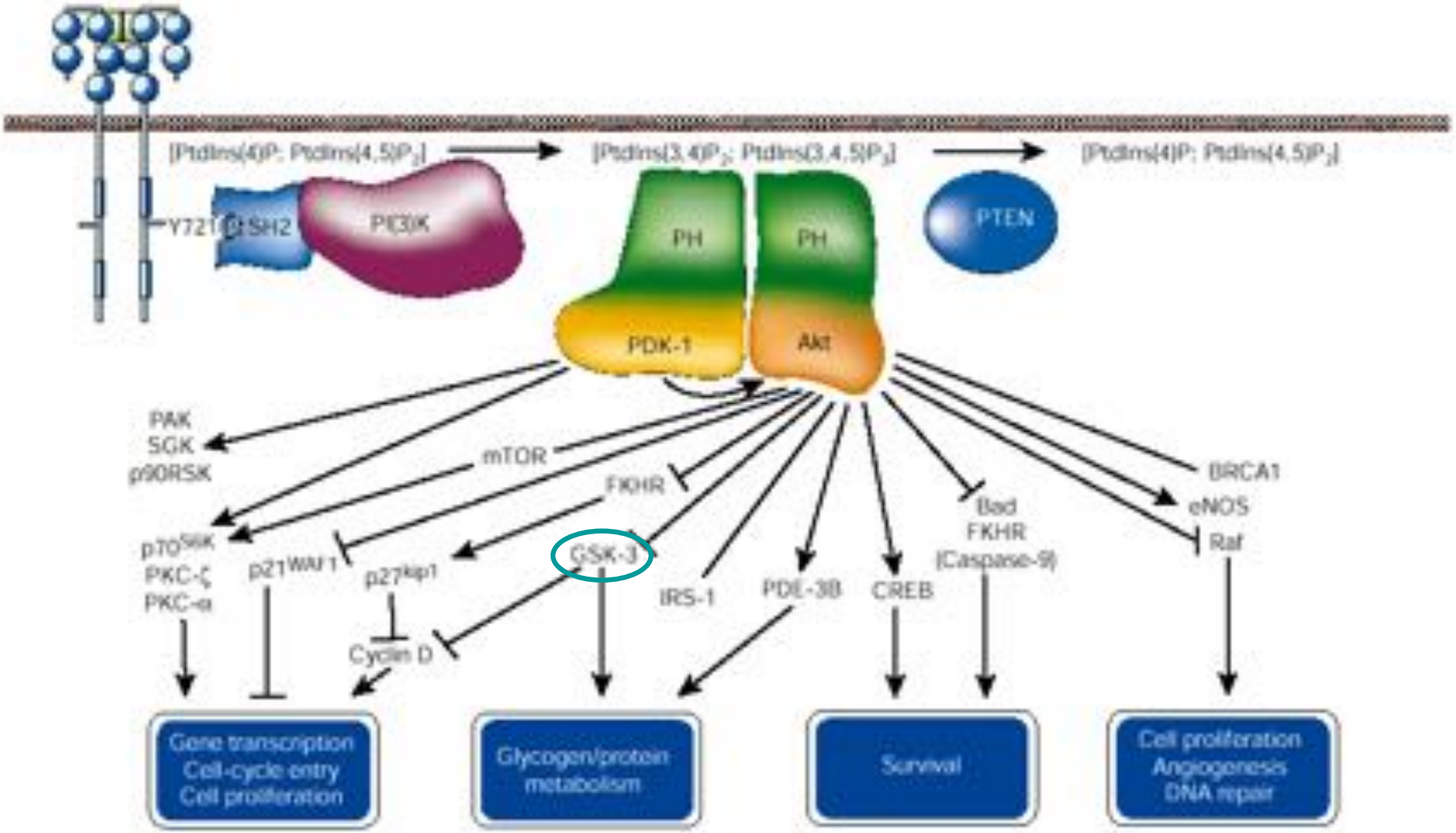
PTEN = Phosphatase and Tensin homolog

Komplexe Signalketten

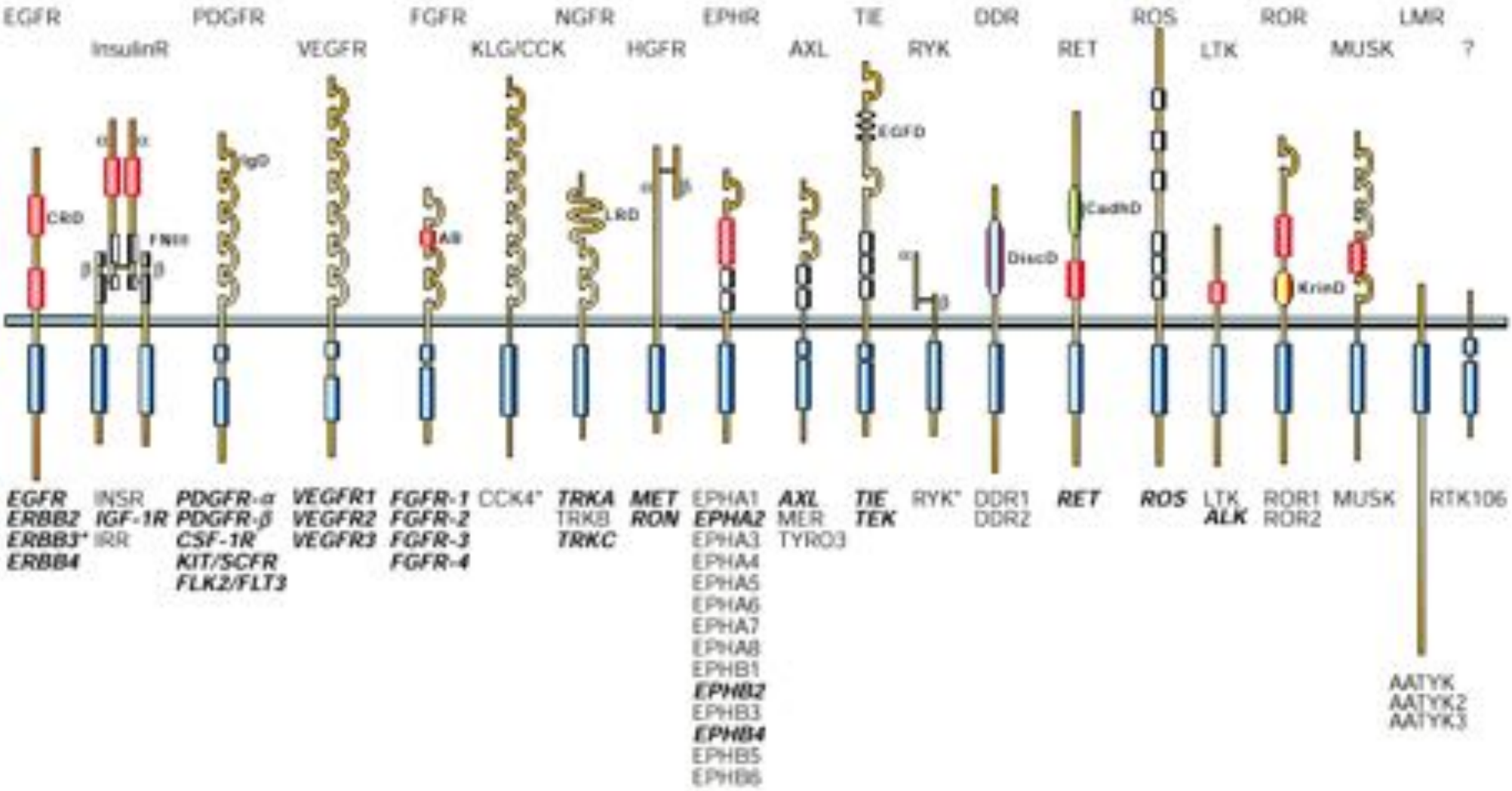
Die Pleckstrin Homologie (PH) - Domäne vermittelt eine spezifische Bindung an PI-phosphate



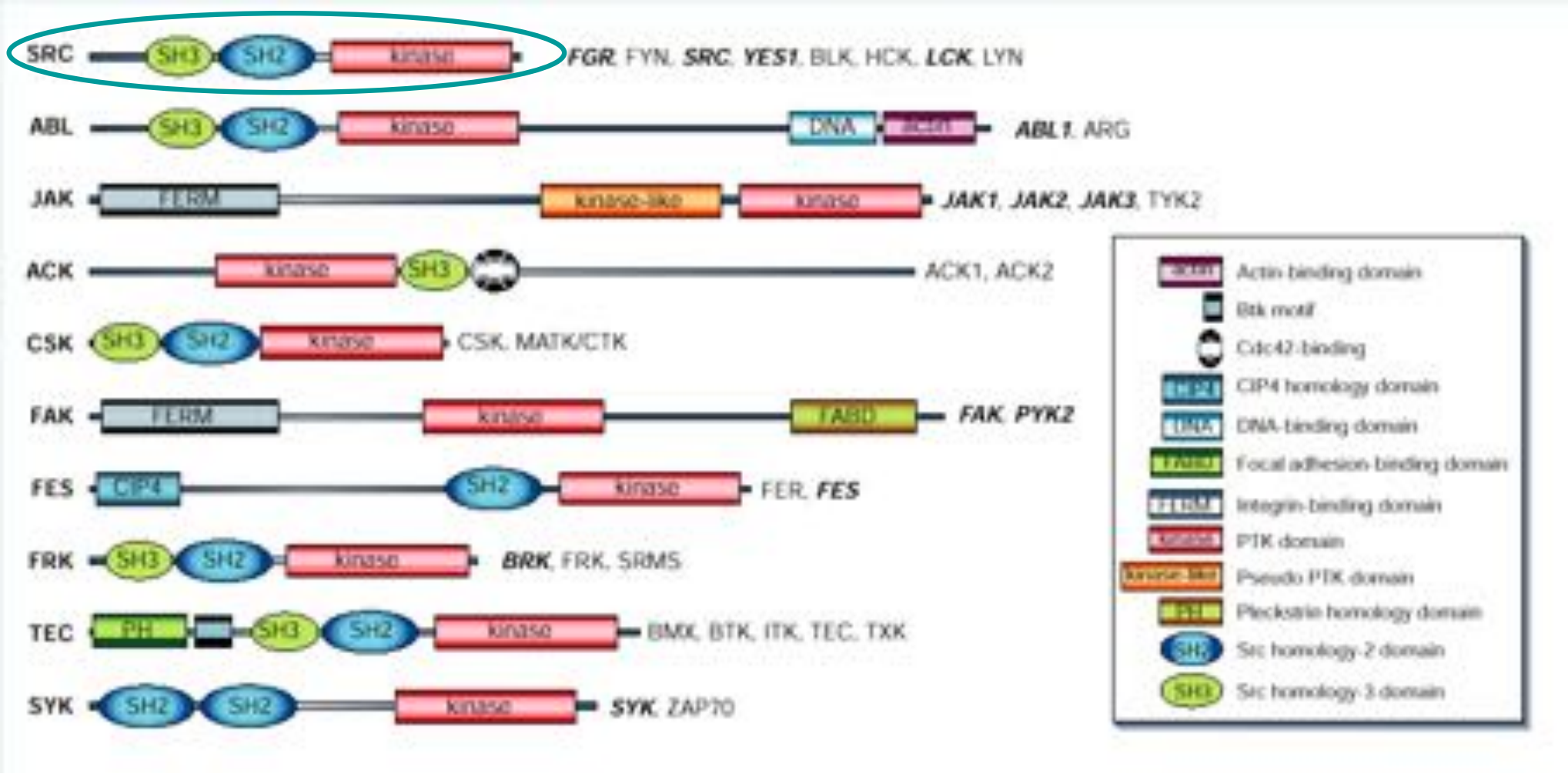
Komplexe Signalwege



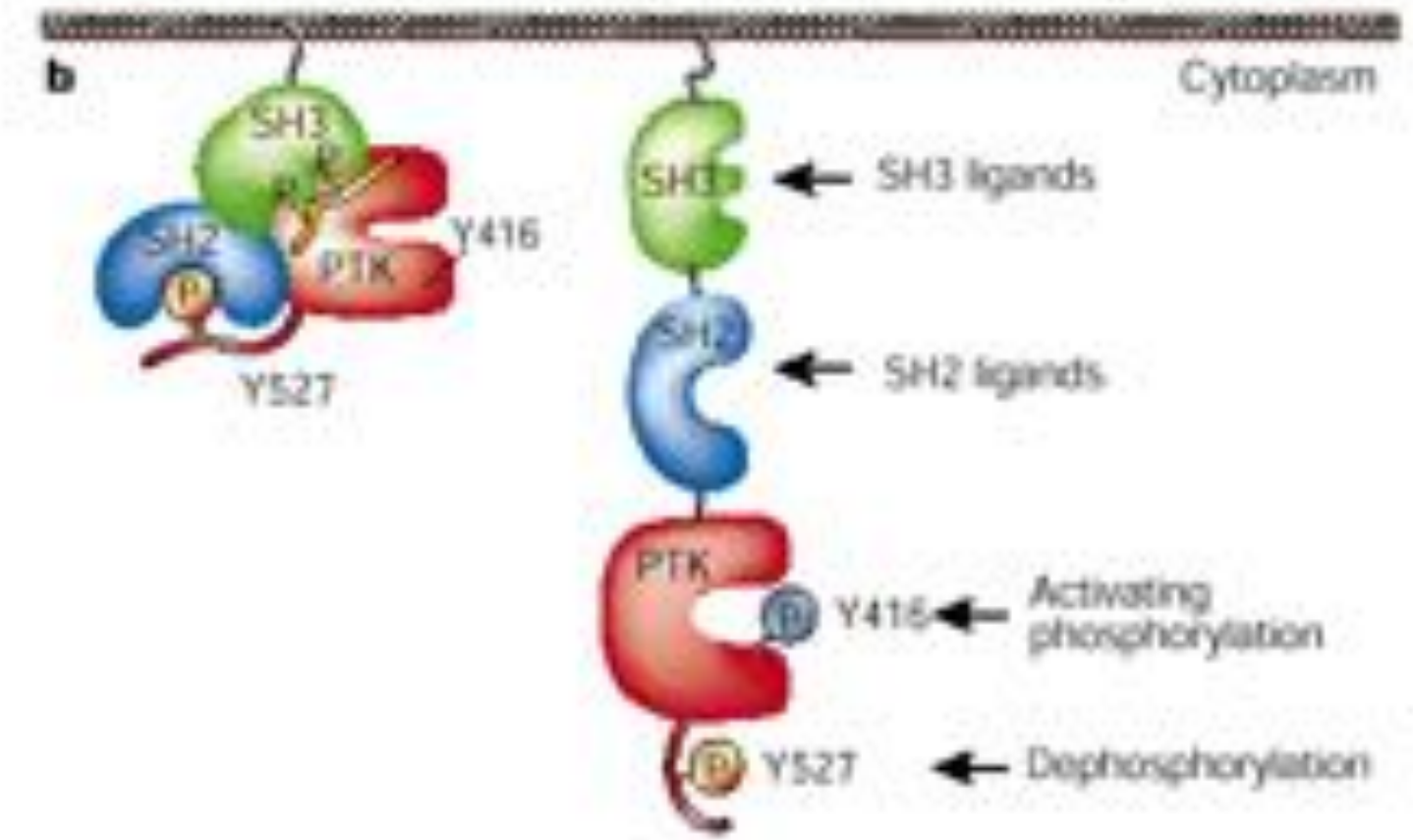
Rezeptor Tyrosin Kinasen (RTKs) des Menschen



Cytoplasmatische Tyrosinkinasen

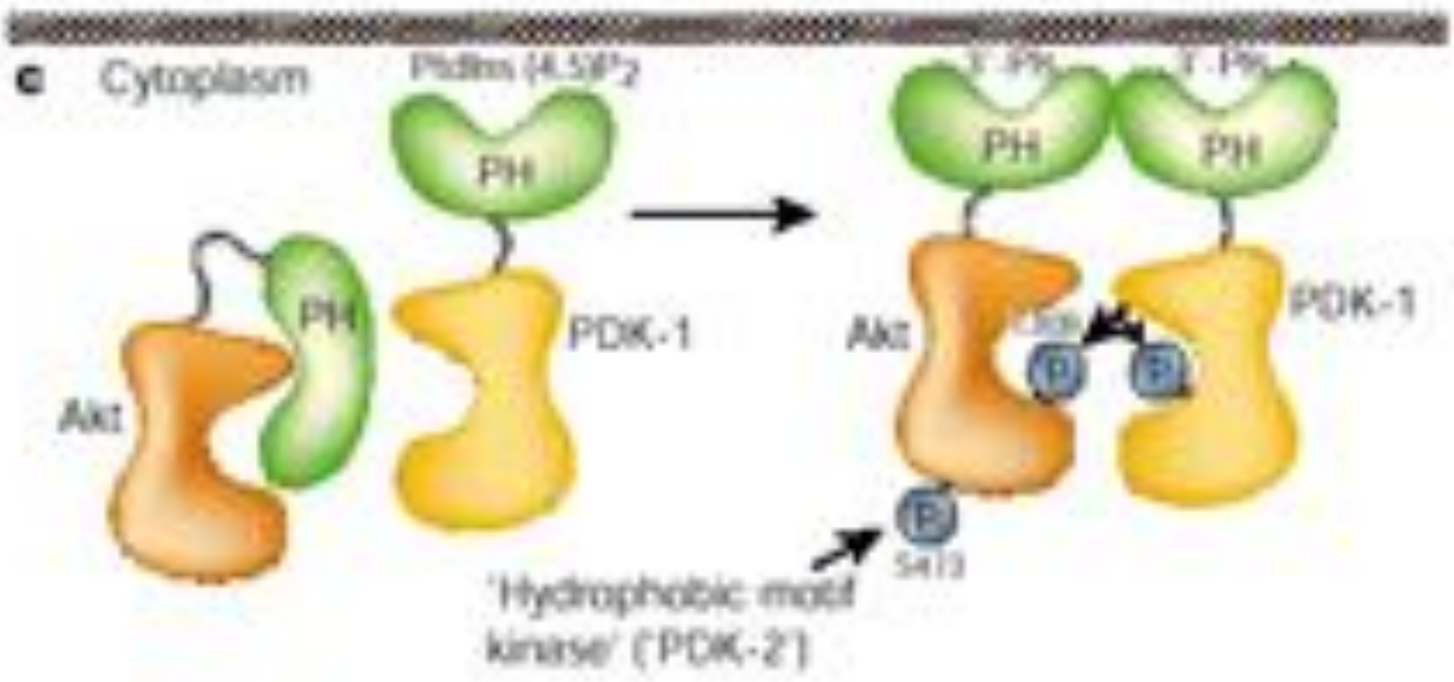


Src - Aktivierung



SH = Src Homology

Akt - Aktivierung



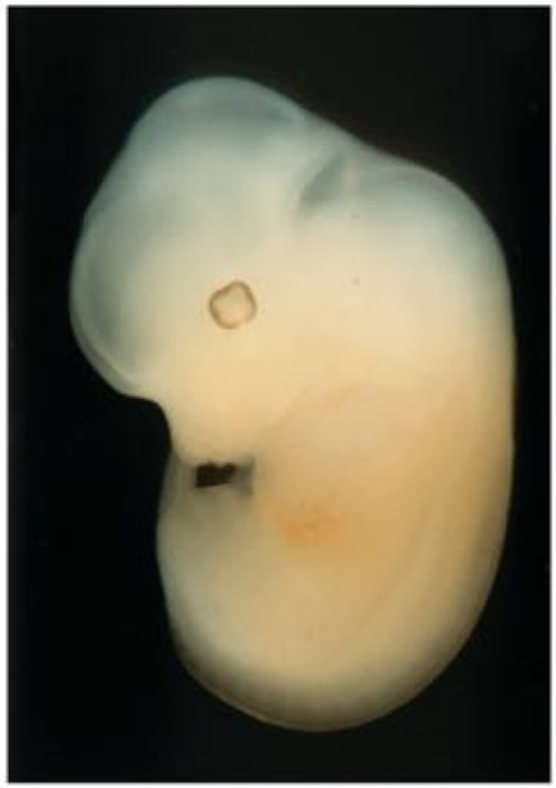
PH = Pleckstrin Homology

JAK/STAT Signalweg

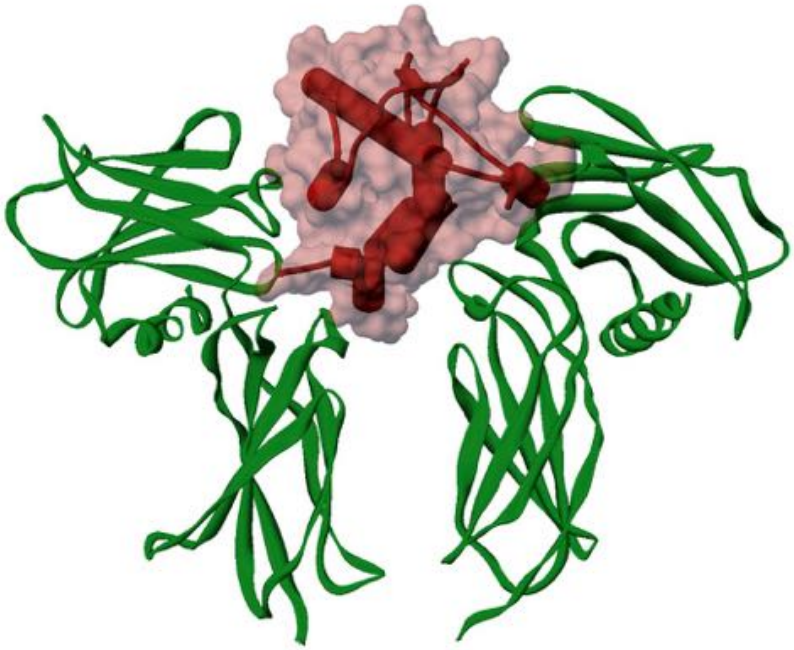
(JAK = Janus Kinasen;
STAT = Signal Transduction and Activation of Transkription)



Wildtyp

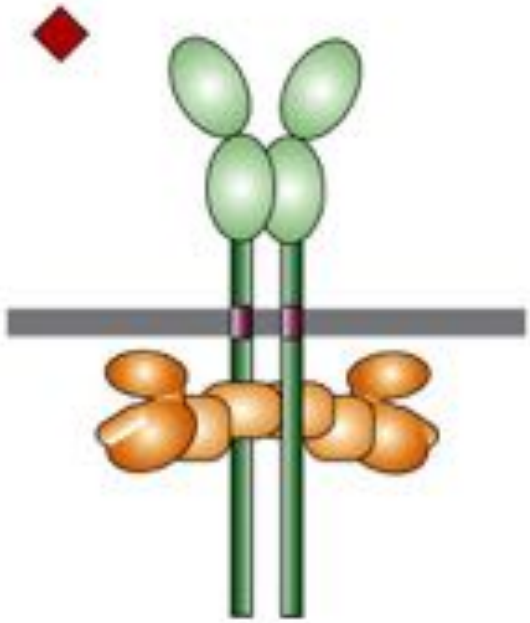


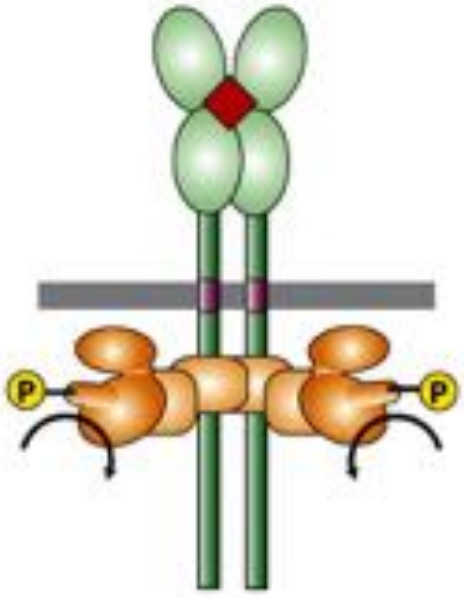
Epo-R $^{-/-}$



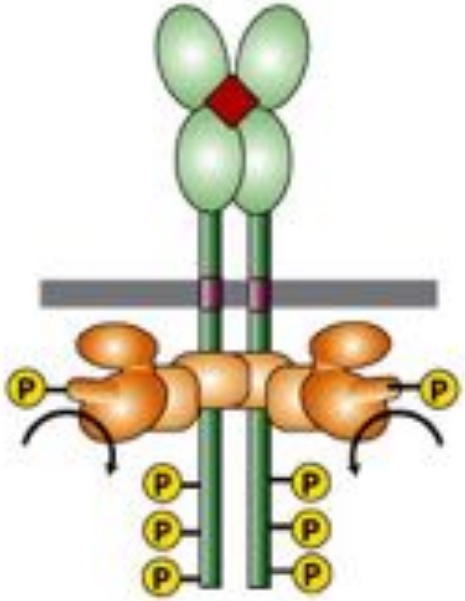
Erythropoietin (Epo), Bindung an den Epo-Rezeptor

Die Aktivierung eines Zytokin-Rezeptors erfolgt über Rezeptorassozierte JAK Kinasen



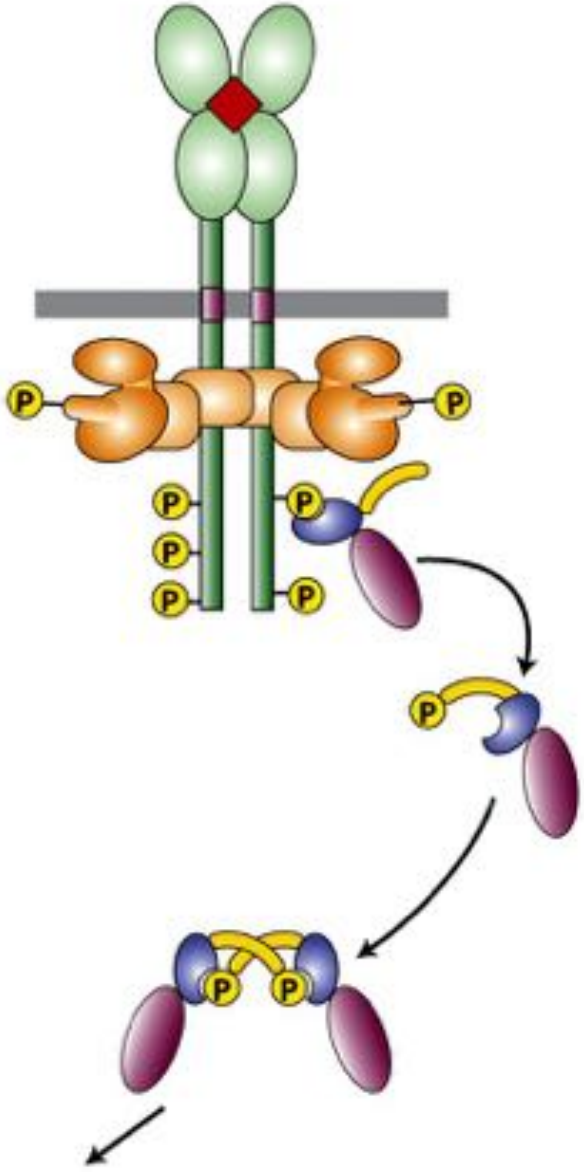


Nach Ligandenbindung JAK phosphorylierung



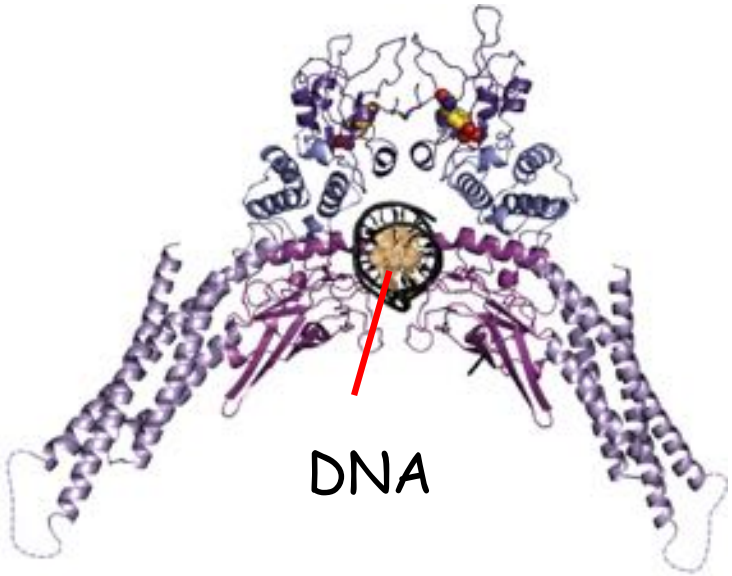
Zusätzliche Phospho-tyrosine sind Docking-Stationen für STAT Transkriptionsfaktoren

JAK/STAT Signalweg

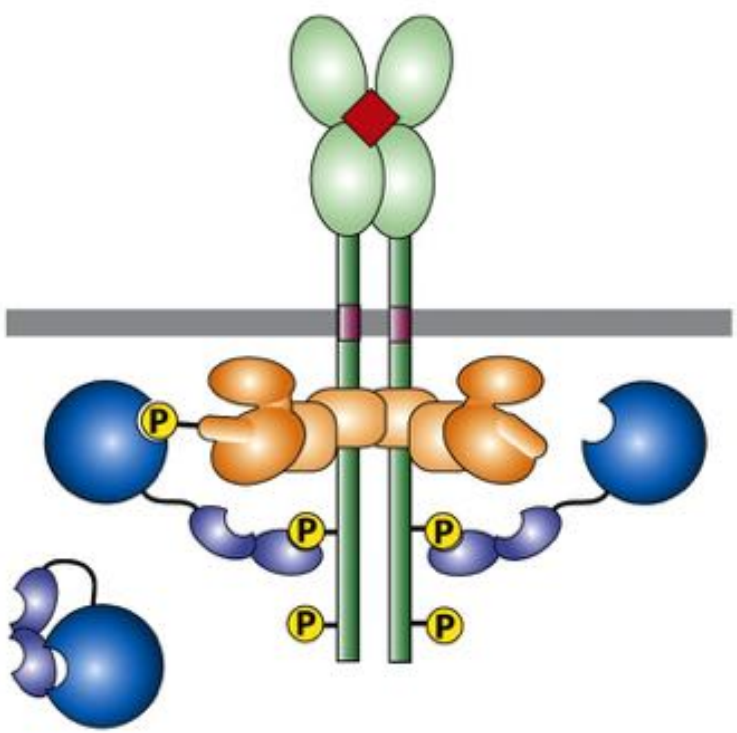


STAT bindet über eine SH2 Domäne, wird von JAK phosphoryliert und ist nach Dimerisierung als TF aktiv.

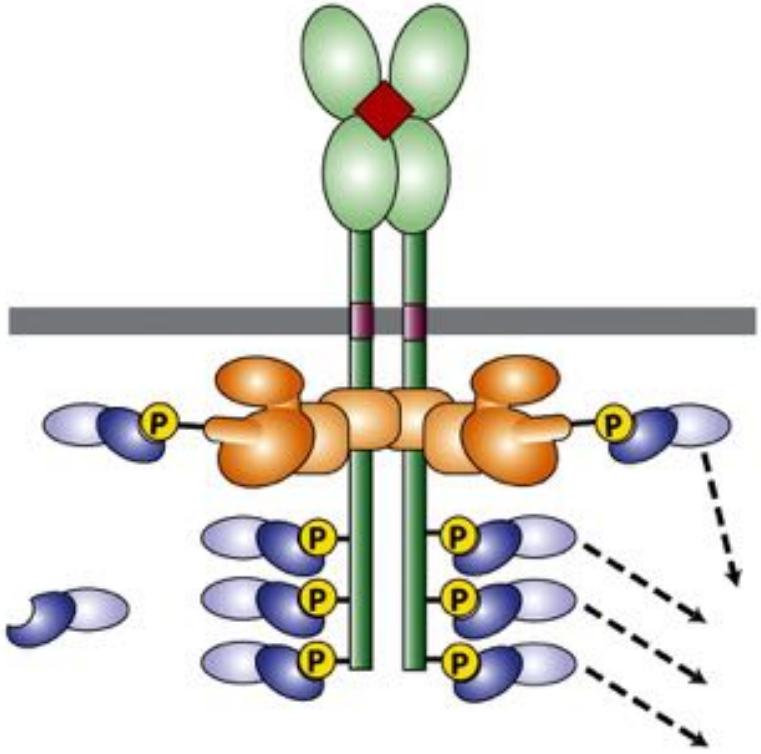
STAT



Inaktivierung des JAK/STAT Signalwegs

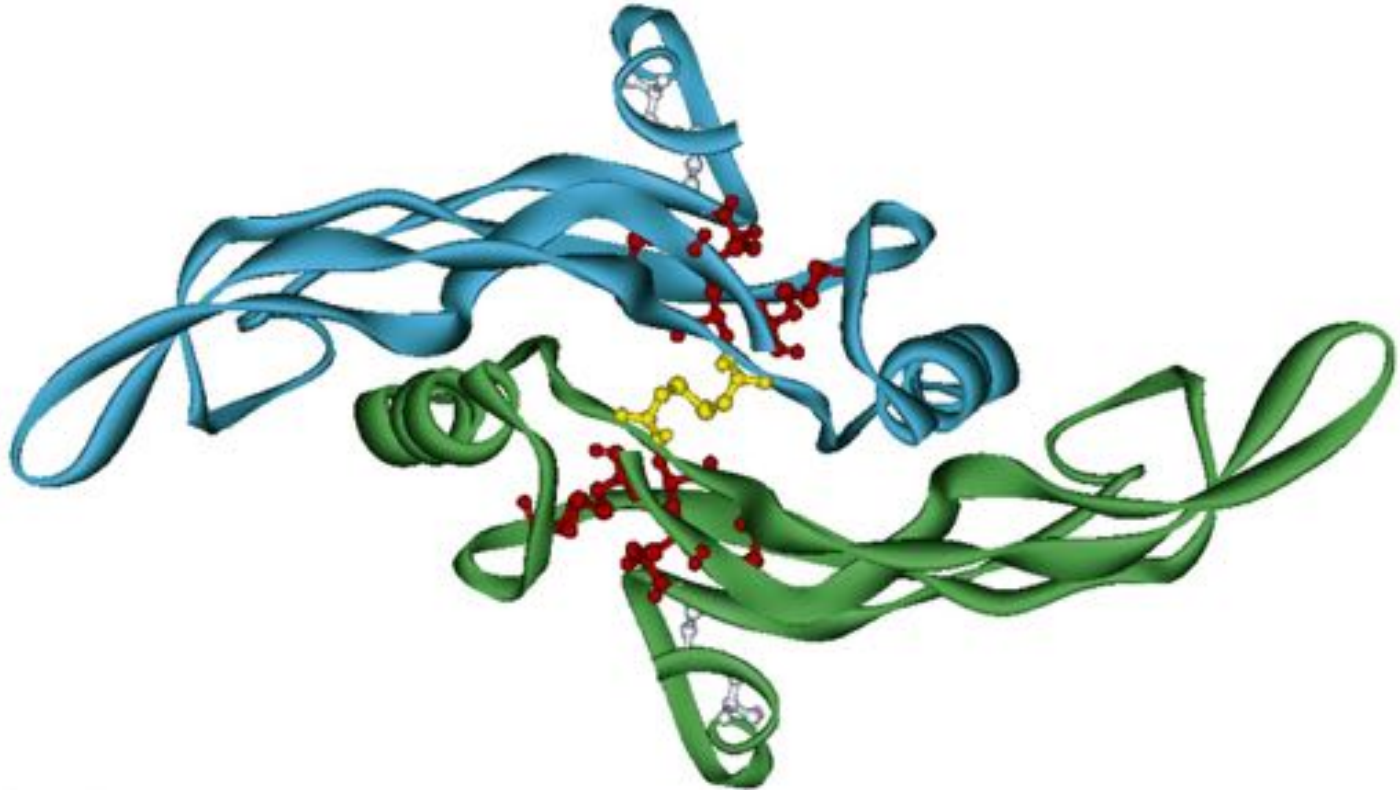


SHP1 Phosphatase

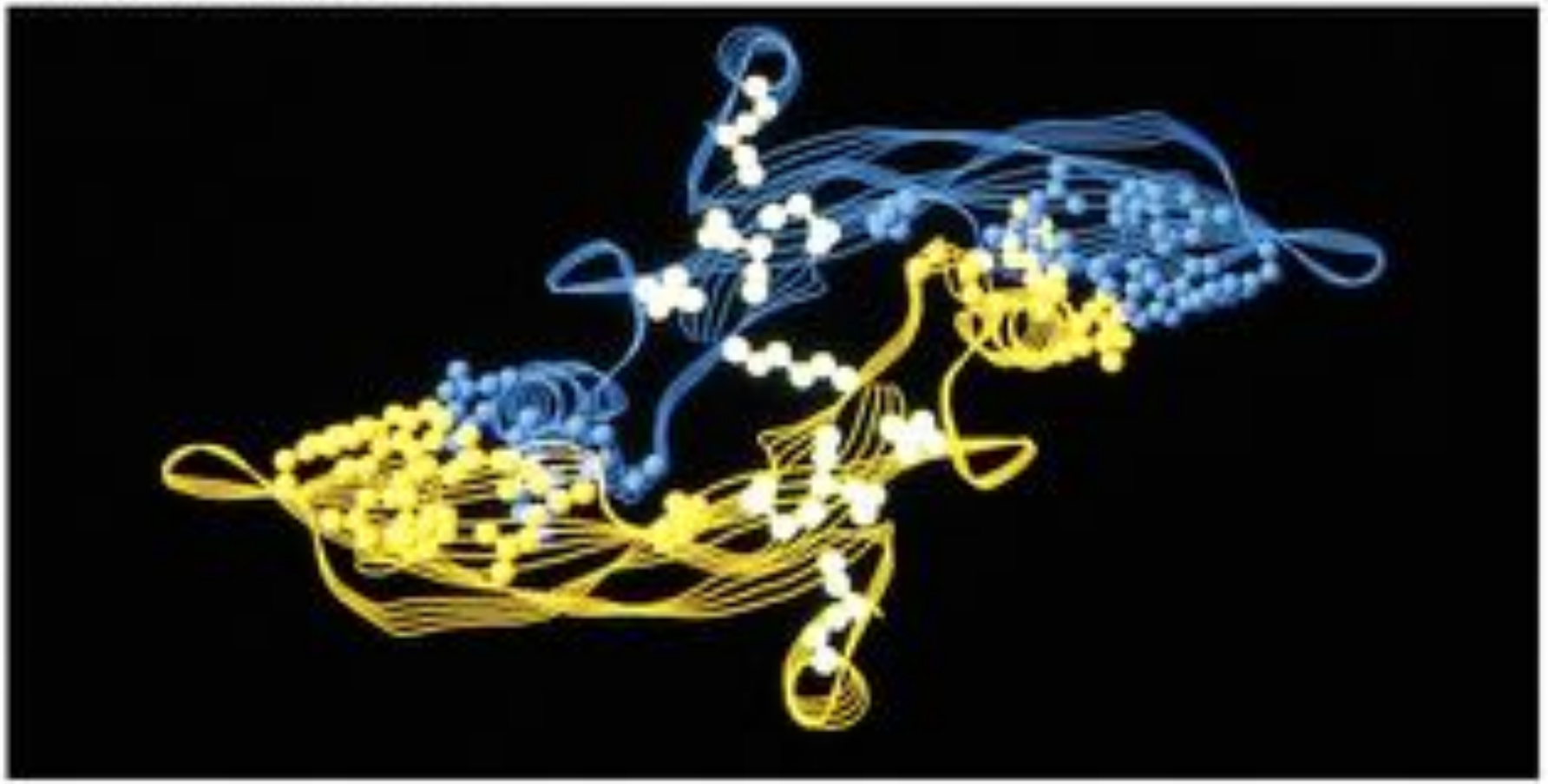


Rekrutierung einer E3
Ubiquitin Ligase

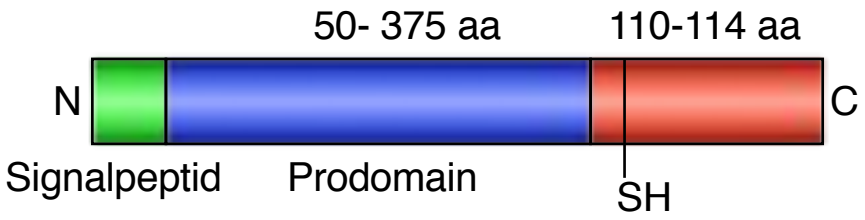
Reifes dimeres TGFβ



Konservierte Raumstruktur des TGFβ



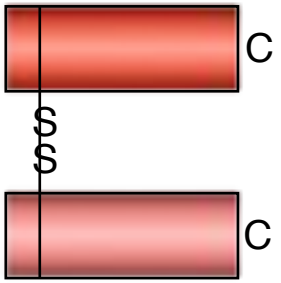
Proteolytische Reifung von TGFβ



Vorläufer



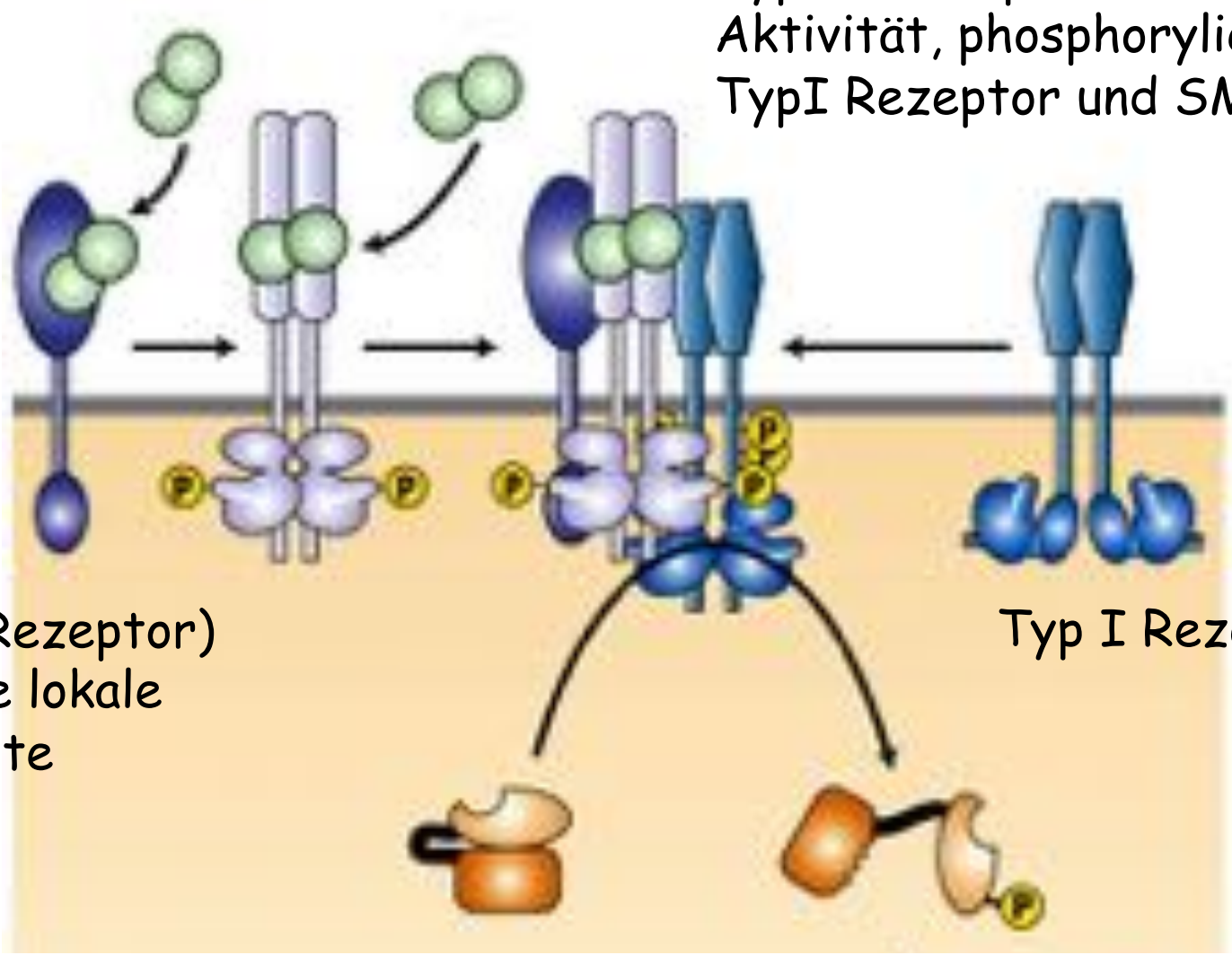
Monomer



Dimer
(Homo- oder
Heterodimer!)

TGFβ (BMP4) Signalweg

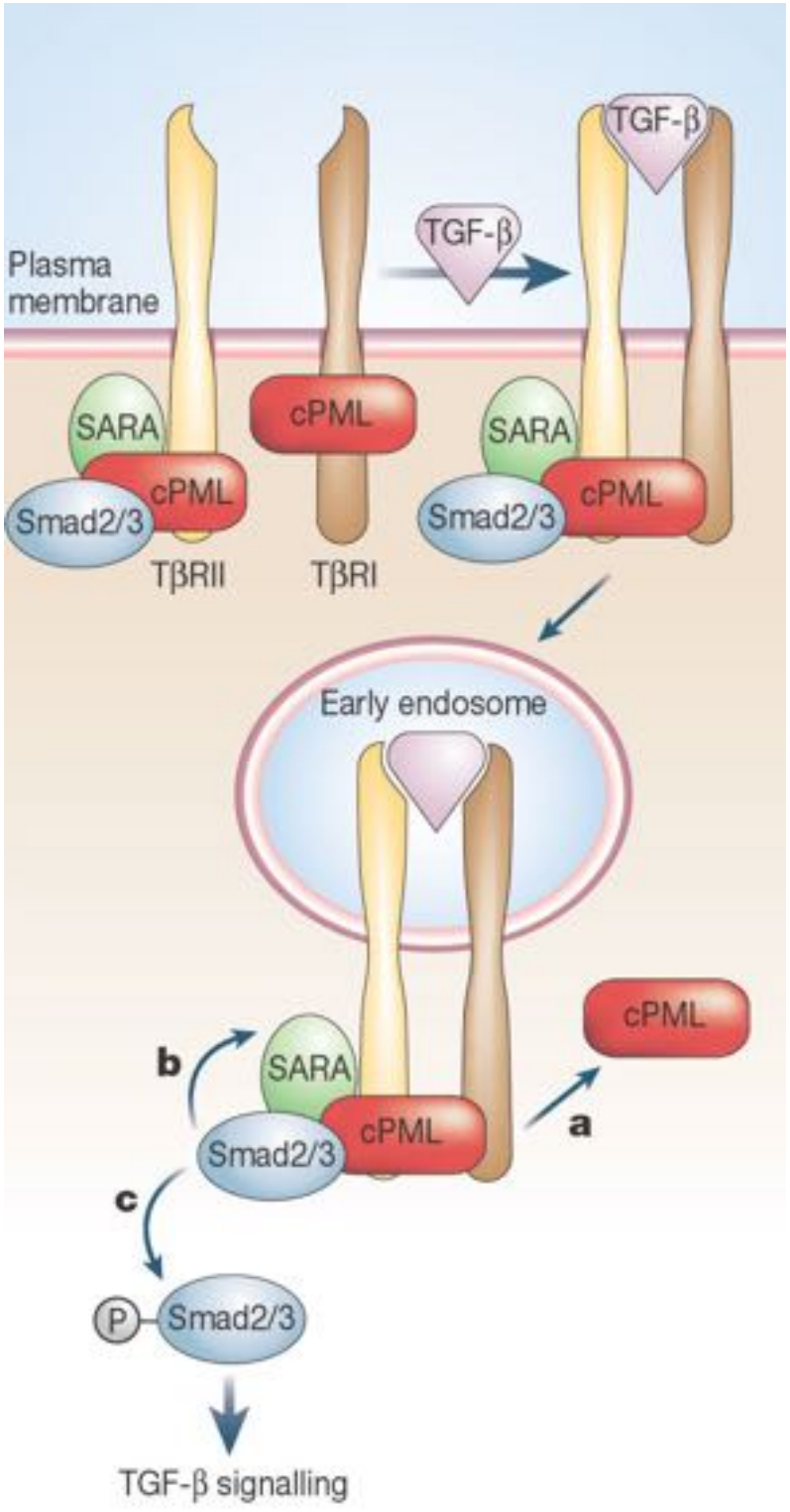
Typ II Rezeptor Kinase Aktivität, phosphoryliert den TypI Rezeptor und SMADs



(Typ III Rezeptor)
Erhöht die lokale Signaldichte

Typ I Rezeptor

Signaltransduktion und Endosomen! SARA



TGFβ (BMP4) Signalweg

Die Signaltransduktion wird zusätzlich über die zelluläre Kompartimentierung reguliert.

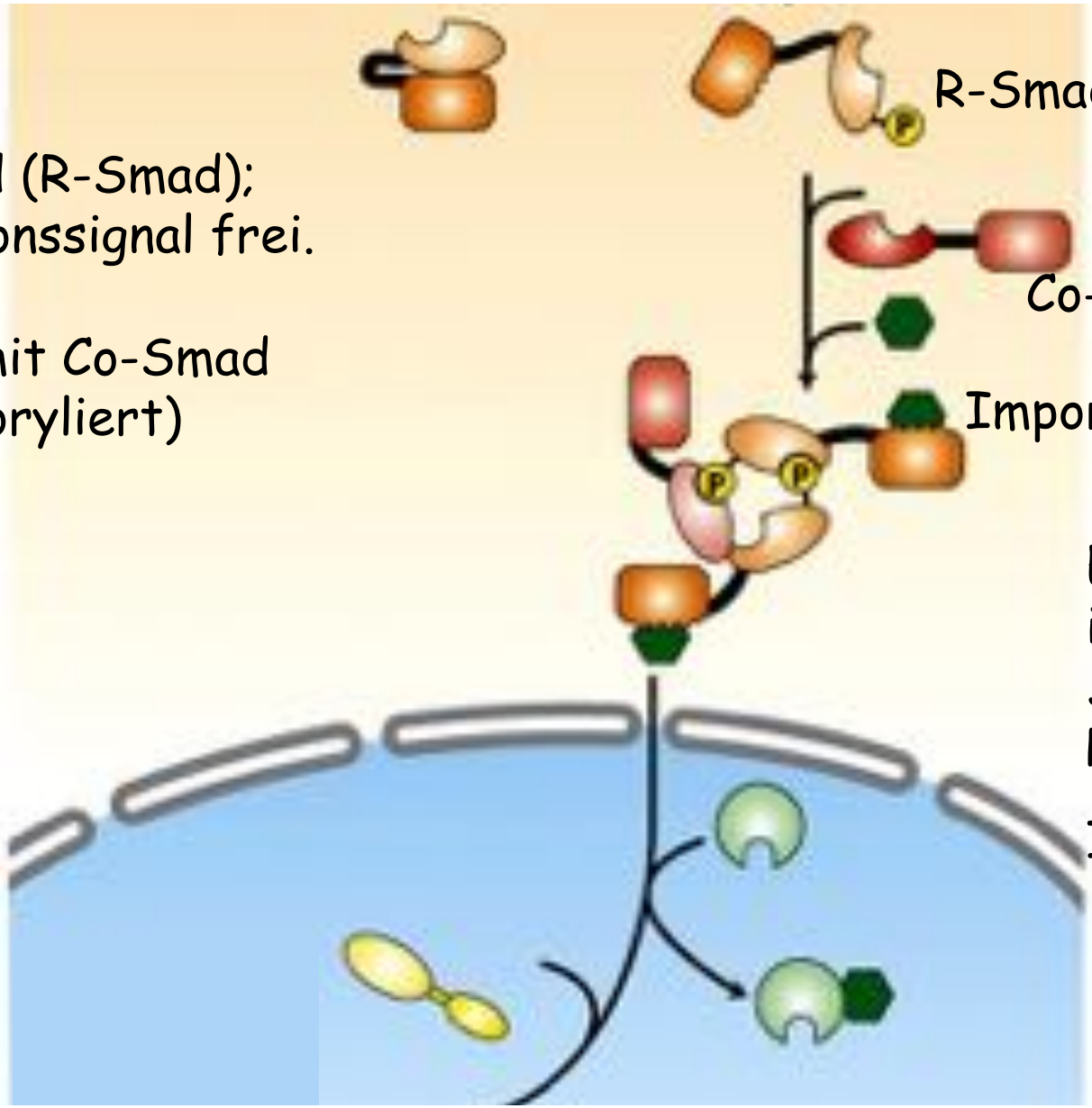
SARA (Smad anchor for receptor activation)

TGFβ (BMP4) Signalweg

Phospho-Smad (R-Smad);
Kernlokalisationssignal frei.

Interaktion mit Co-Smad
(nicht phosphoryliert)

Kerntransport

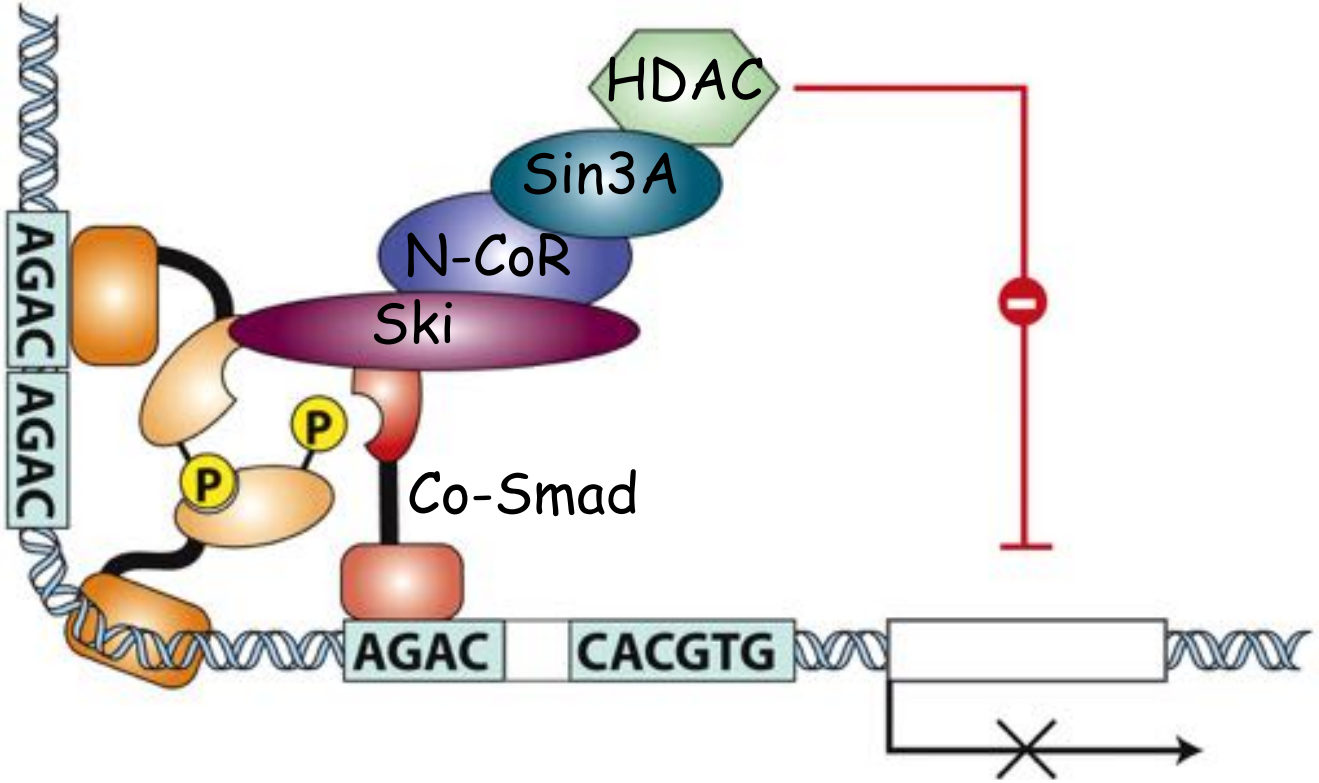


Es gibt auch inhibitorische Smad Proteine:

I-Smad

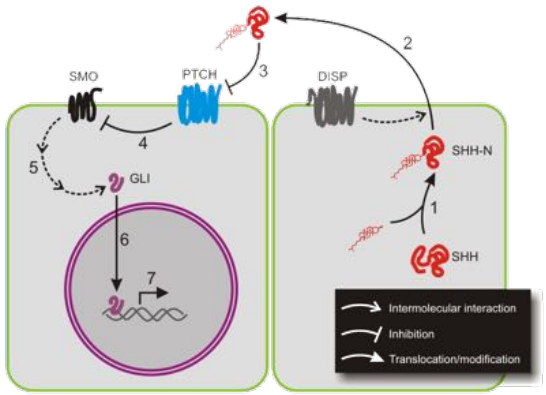
TGFβ (BMP4) Signalweg

Im Kern bindet der Smad-Komplex Sequenzspezifisch und rekrutiert die Histondeacetylase HDAC.



Hedgehog-Signaling

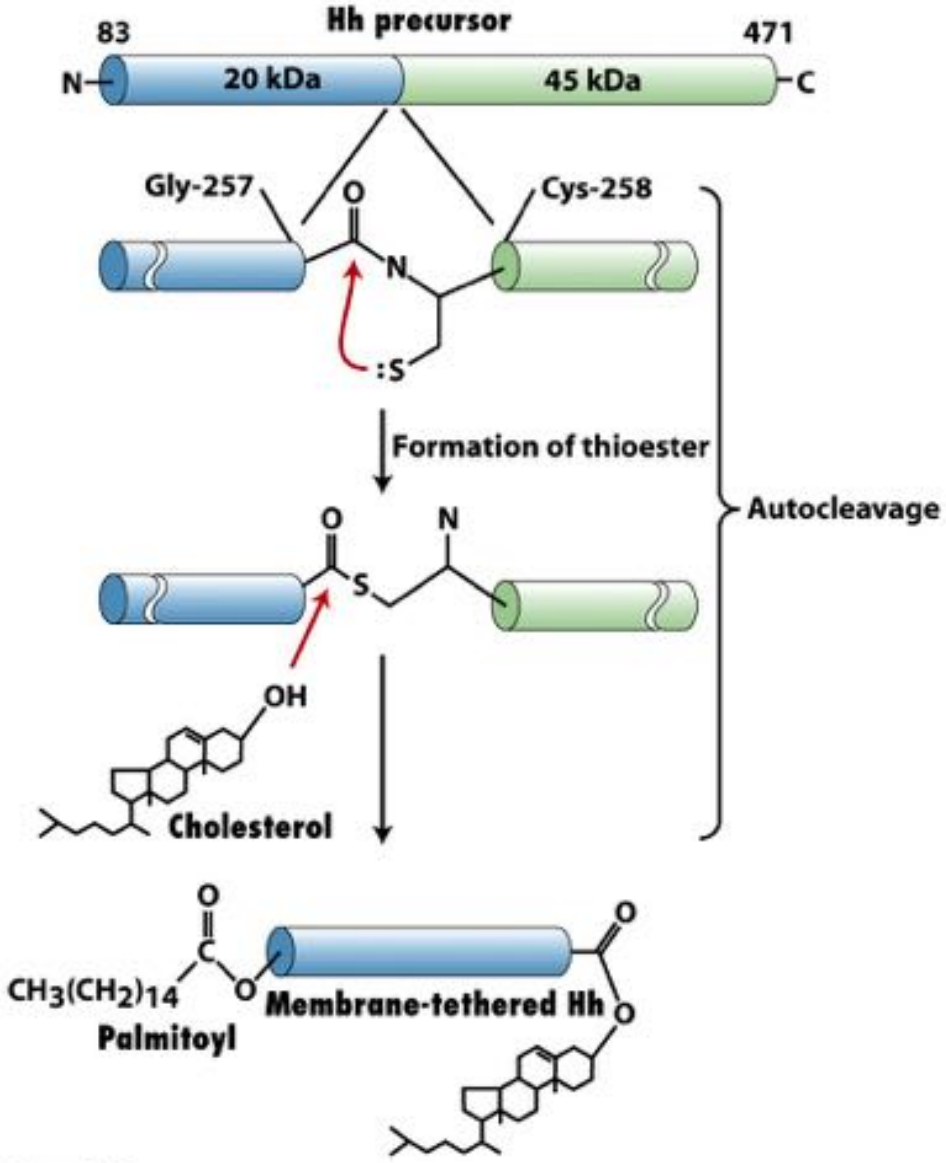




Hedgehog (hh)

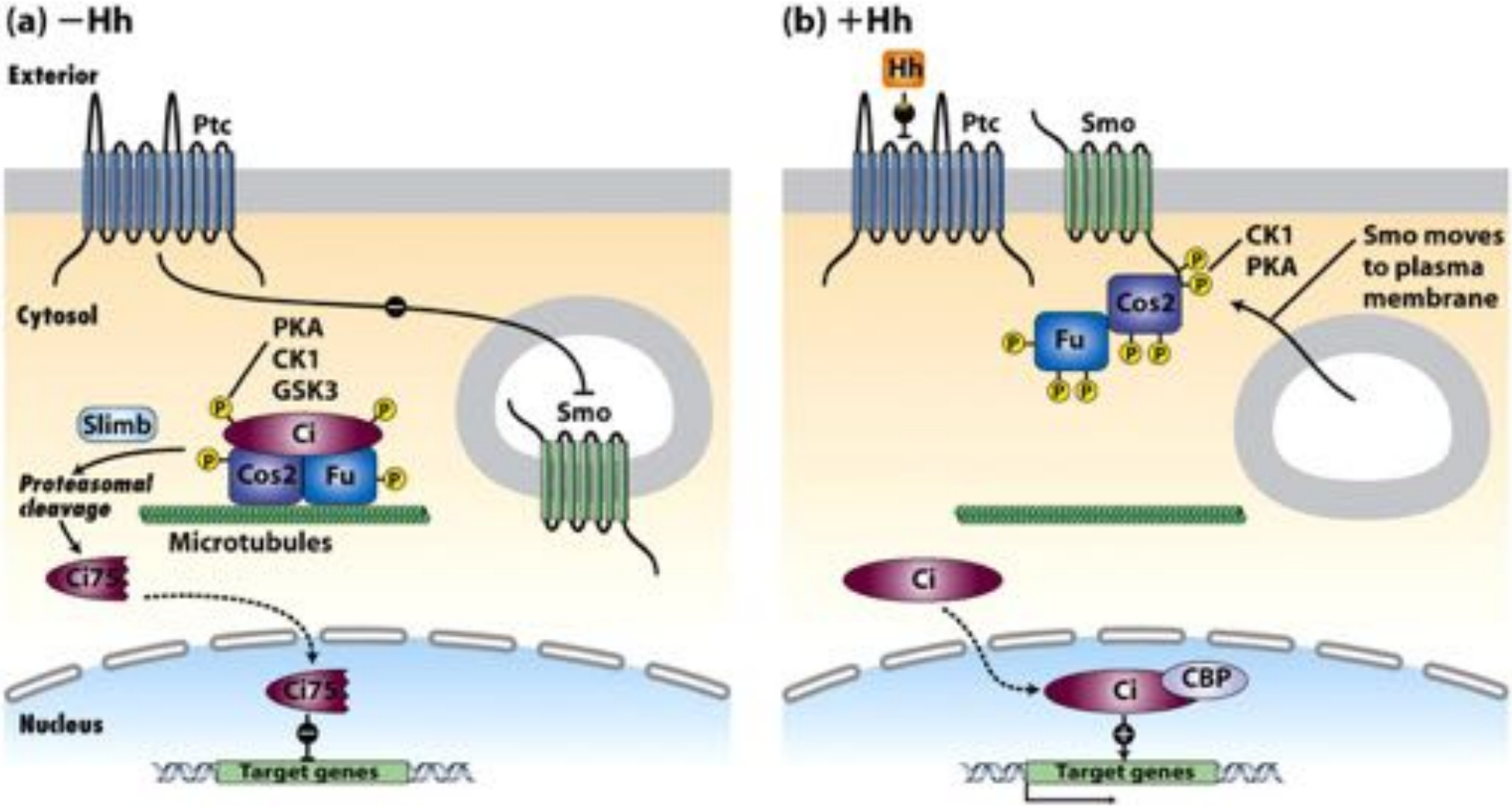
Sonic hedgehog (shh)
Desert hedgehog (dhh)
Indian hedgehog (ihh)

Prozessierung und Lipid-Modifikation von Hedgehog



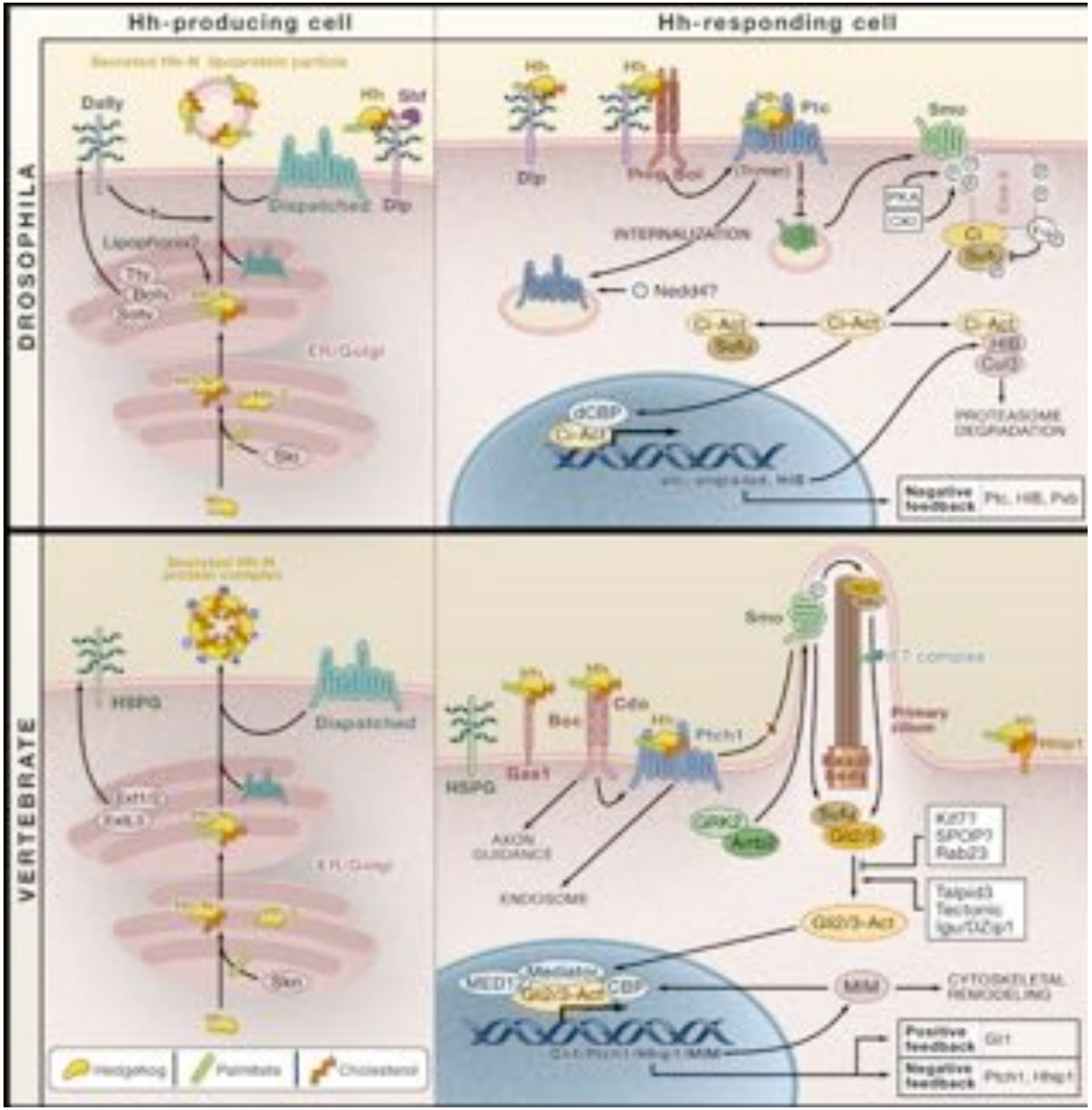
Intein

Hedgehog Signaltransduktion

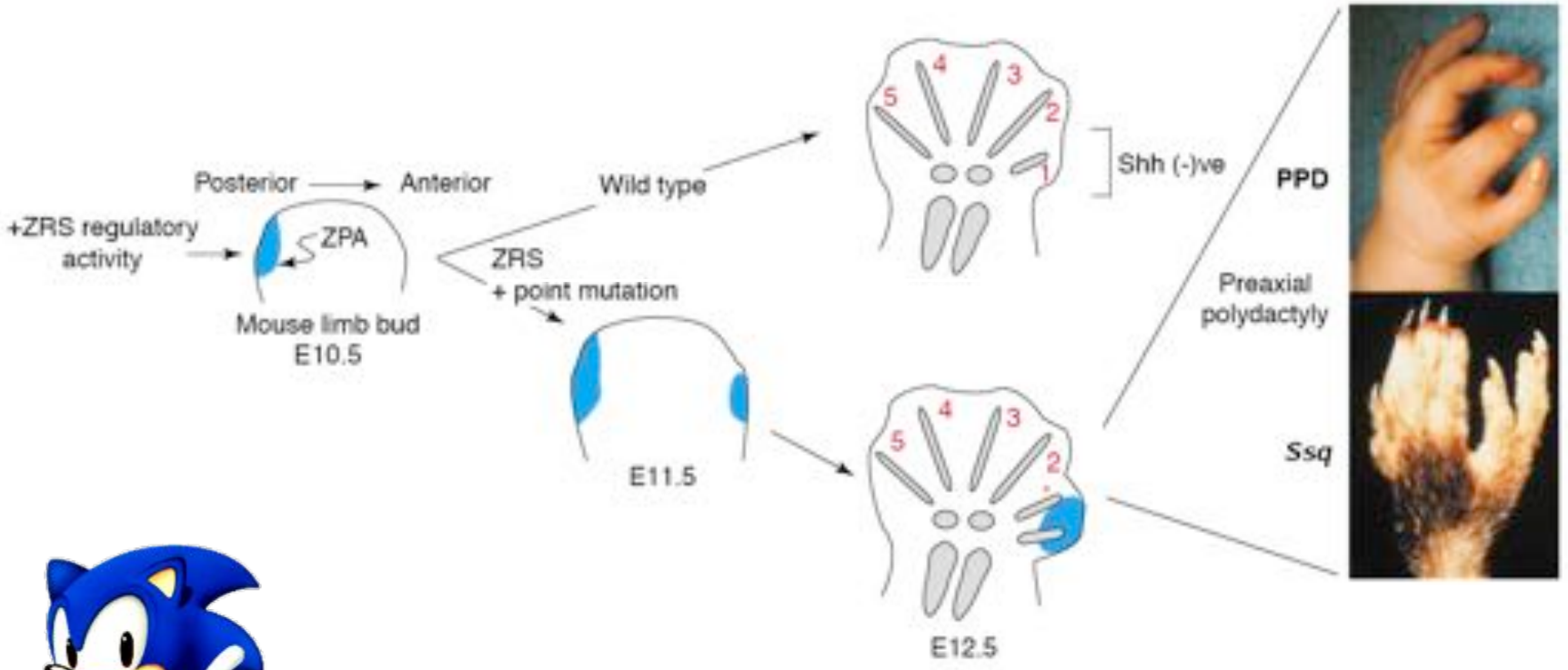


Hh ⊣ ptc ⊣ smo → PKA → Ci

Hedgehog Signaltransduktion II



Defekte in der Shh Signalkaskade, Shh ist ein Morphogen

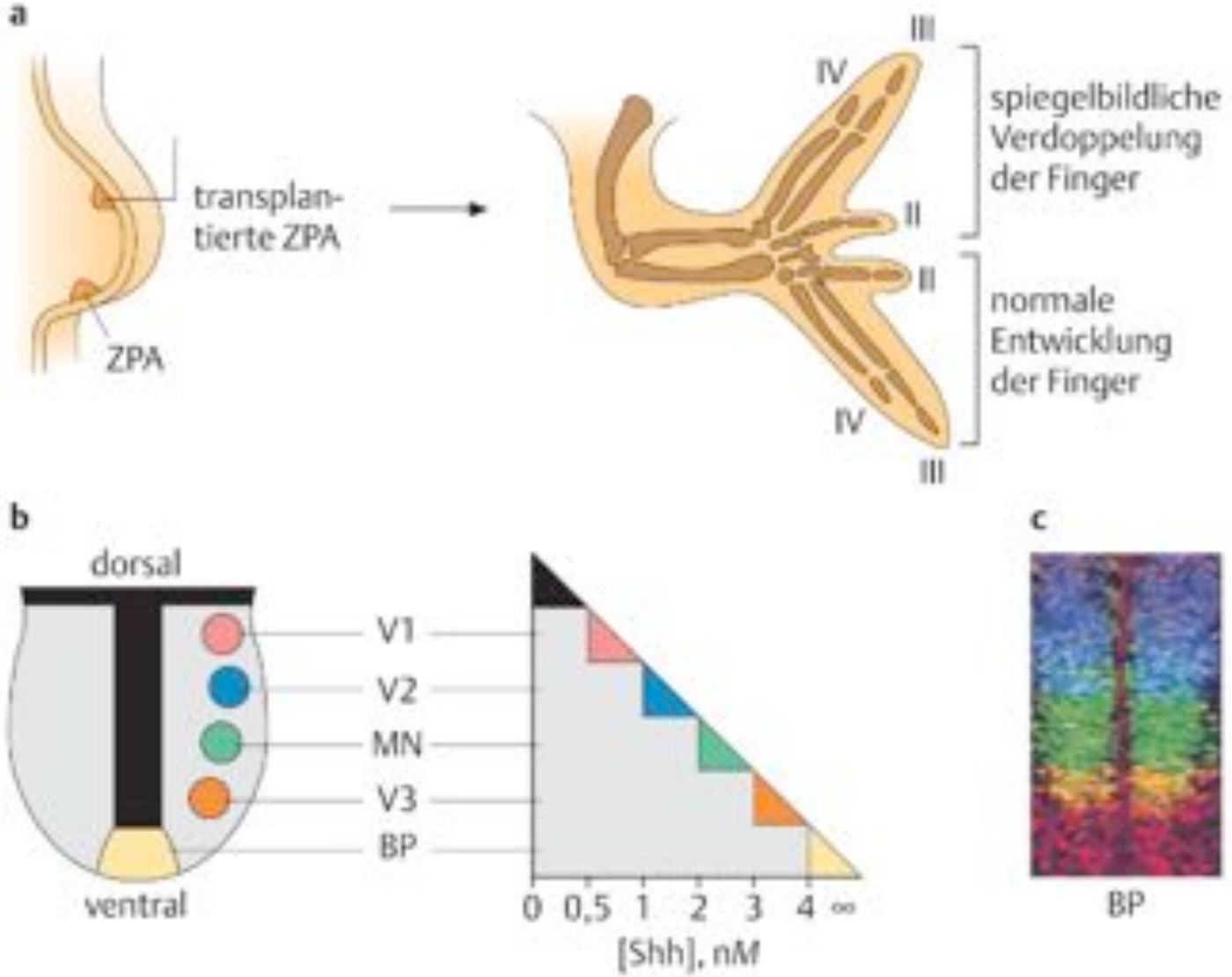




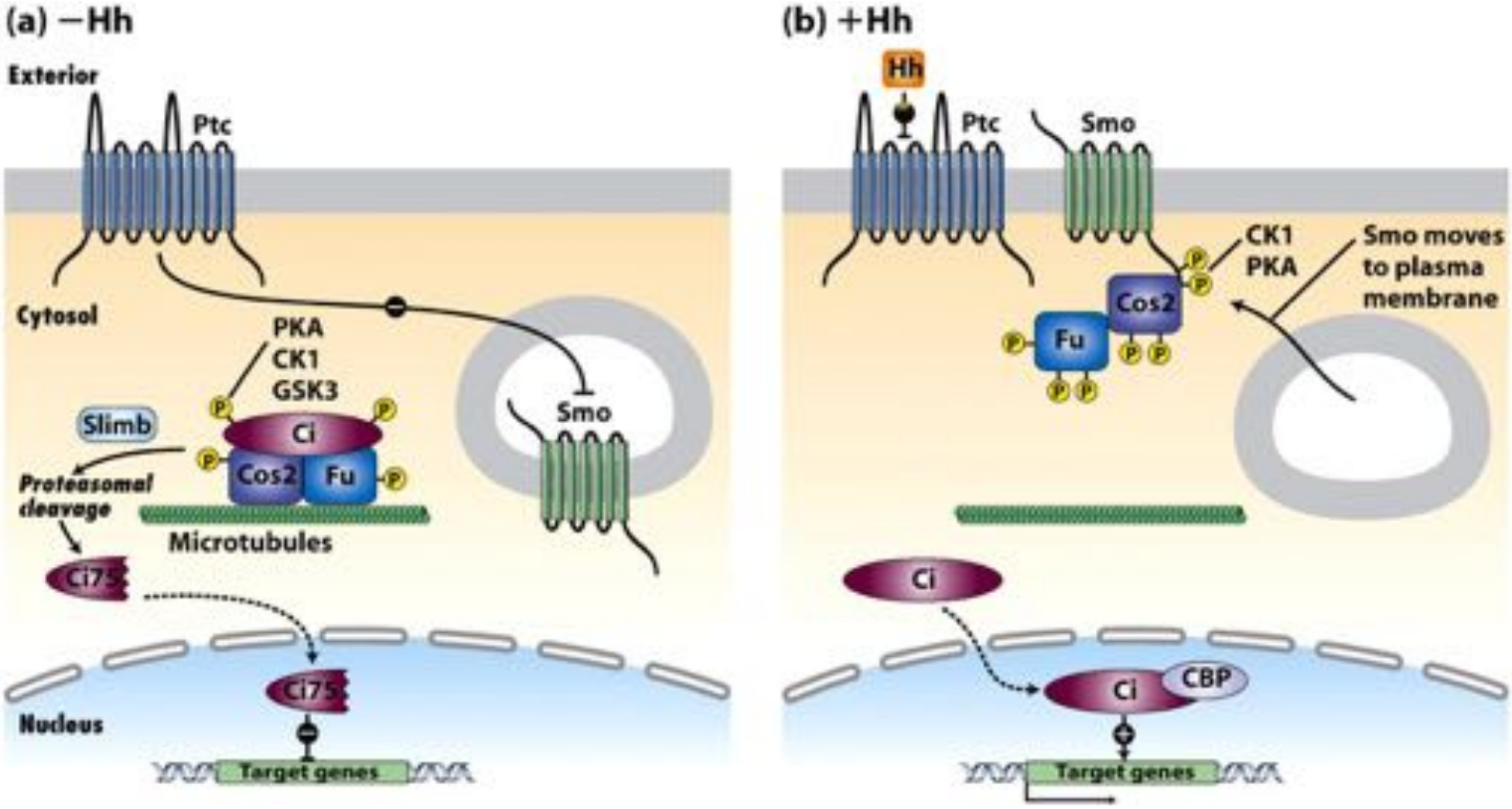
Sonic hedgehog



Morphogen-Gradienten



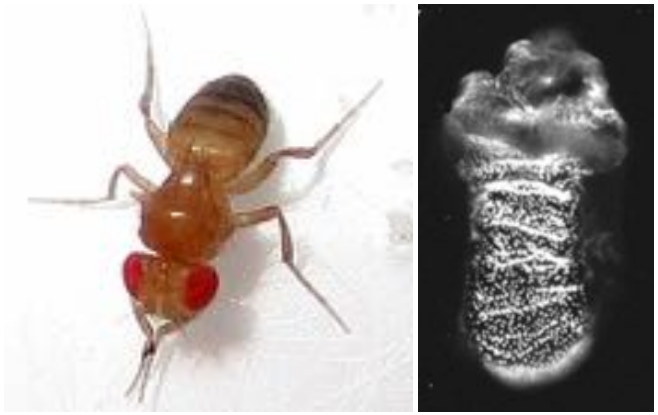
Hedgehog Signaltransduktion



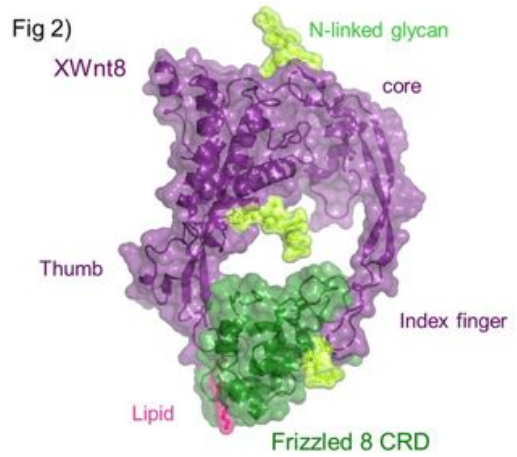
Hh ⊣ ptc ⊣ smo → PKA → Ci

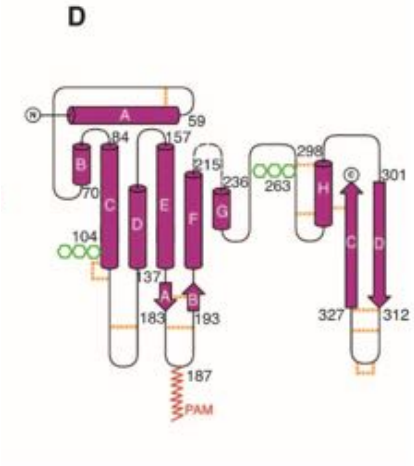
Kanonische wnt Signaltransduktion

Wnt: Synthetischer Name aus wingless und Int.
Konserviertes, sezerniertes Signalmolekül.
22 konservierte Cysteinreste.



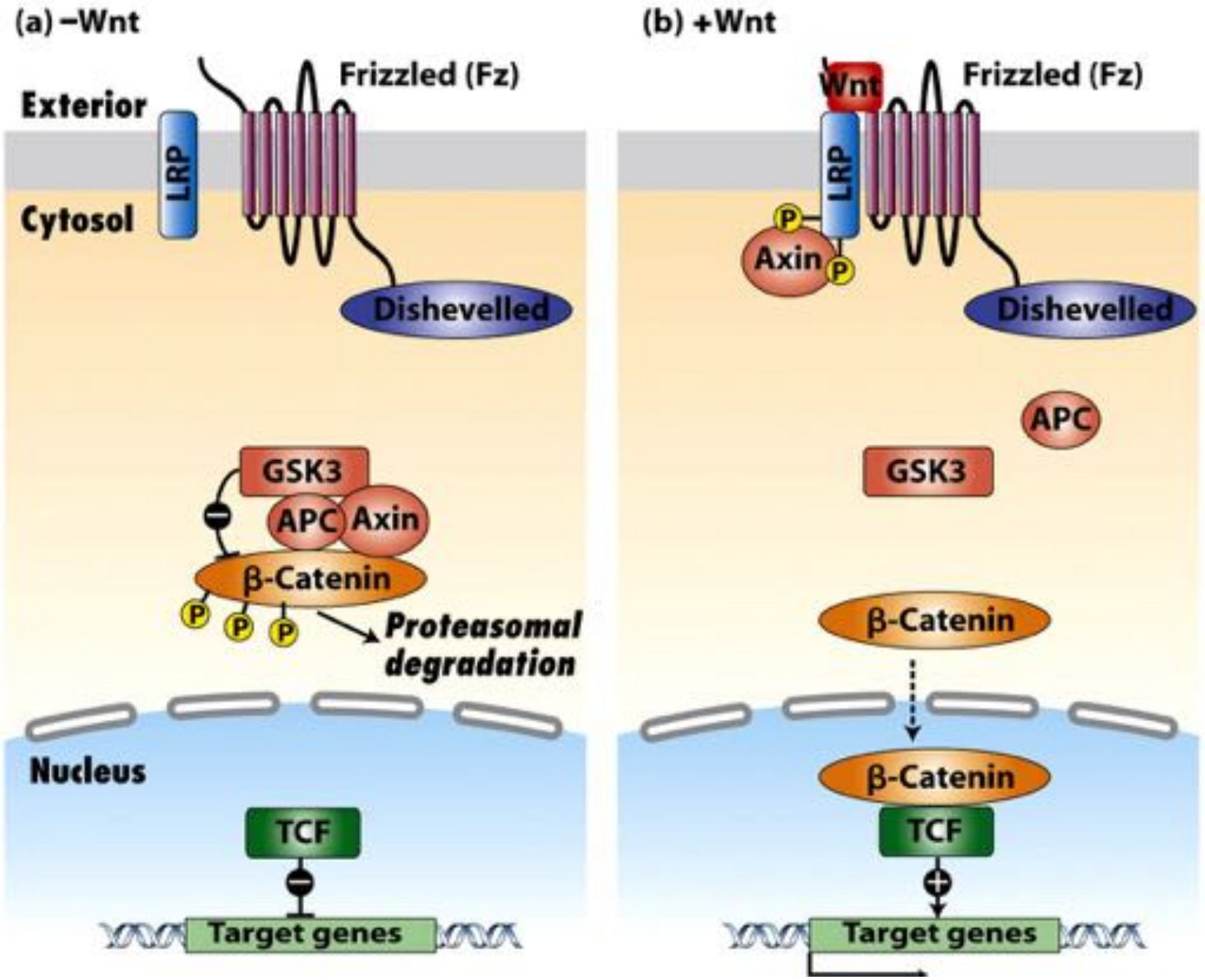
Drei verschiedene wnt Signalwege:
Canonical wnt signaling
PCP signaling (Planar cell polarity)
Ca^{2+} signaling





2012, Janda et al.,

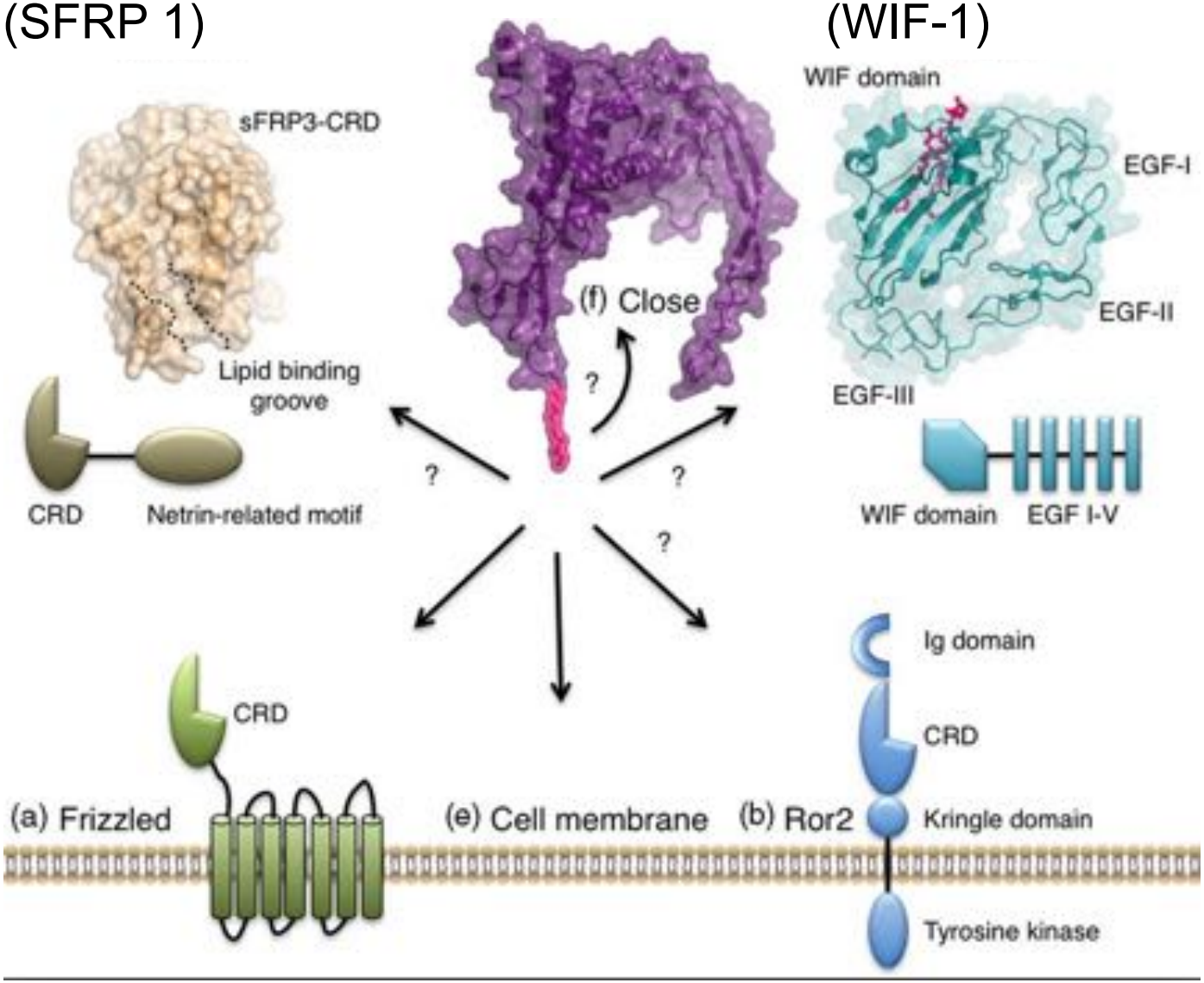
Kanonische wnt Signaltransduktion



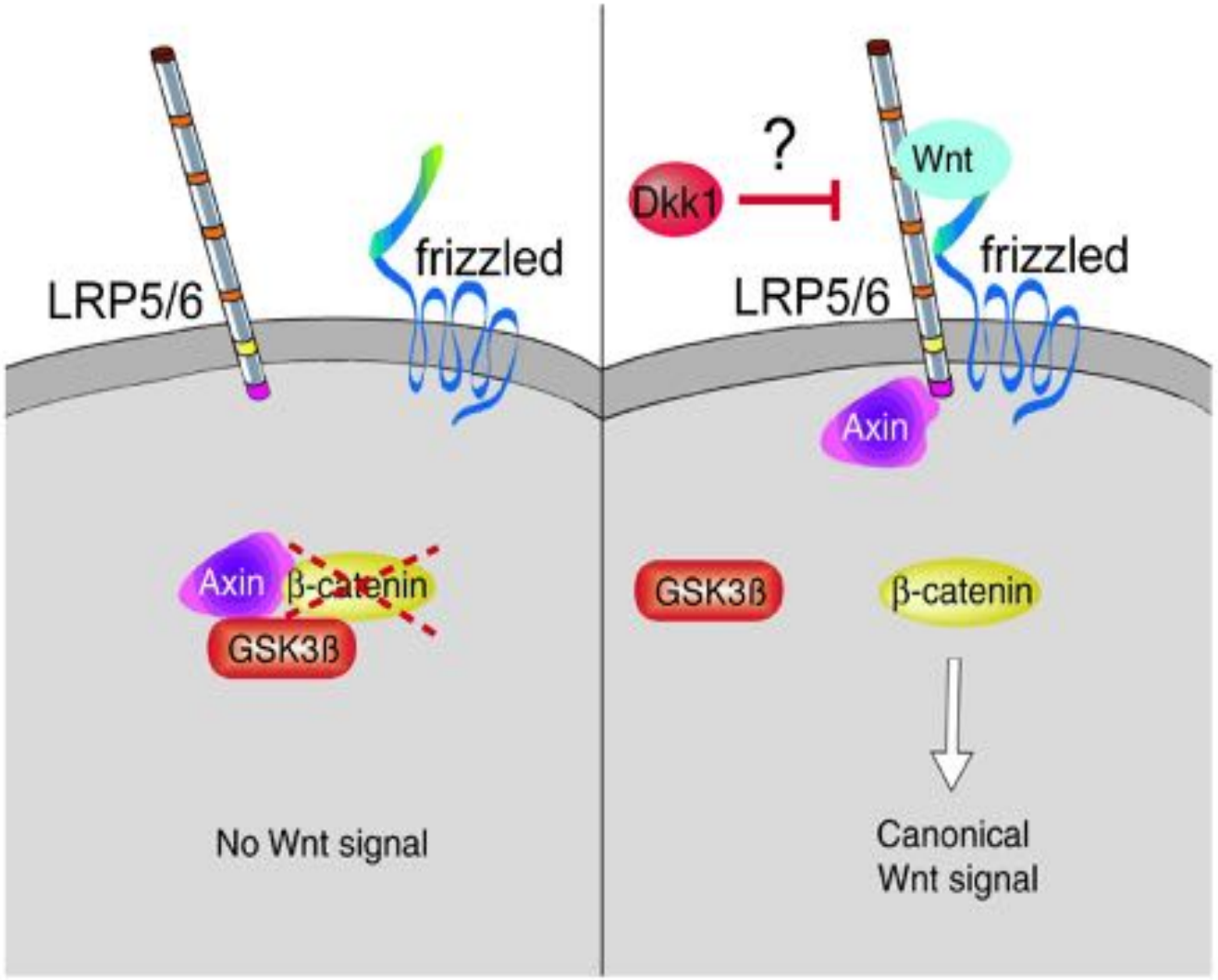
Kanonische wnt Signaltransduktion

Secreted frizzled-related protein 1 (SFRP 1)

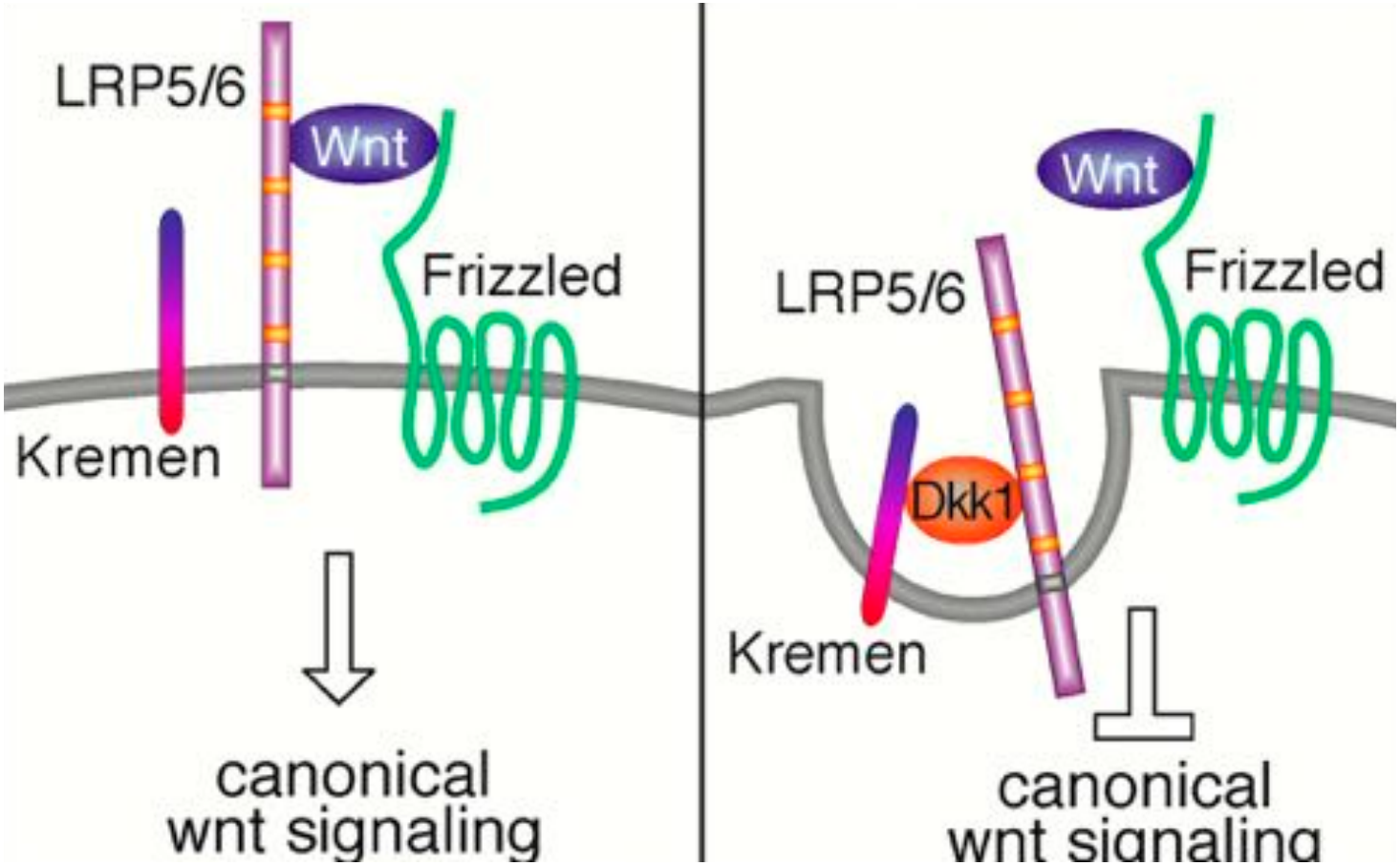
Wnt inhibitory factor-1 (WIF-1)



Der Wingless/Wnt Rezeptor benötigt einen Co-Rezeptor

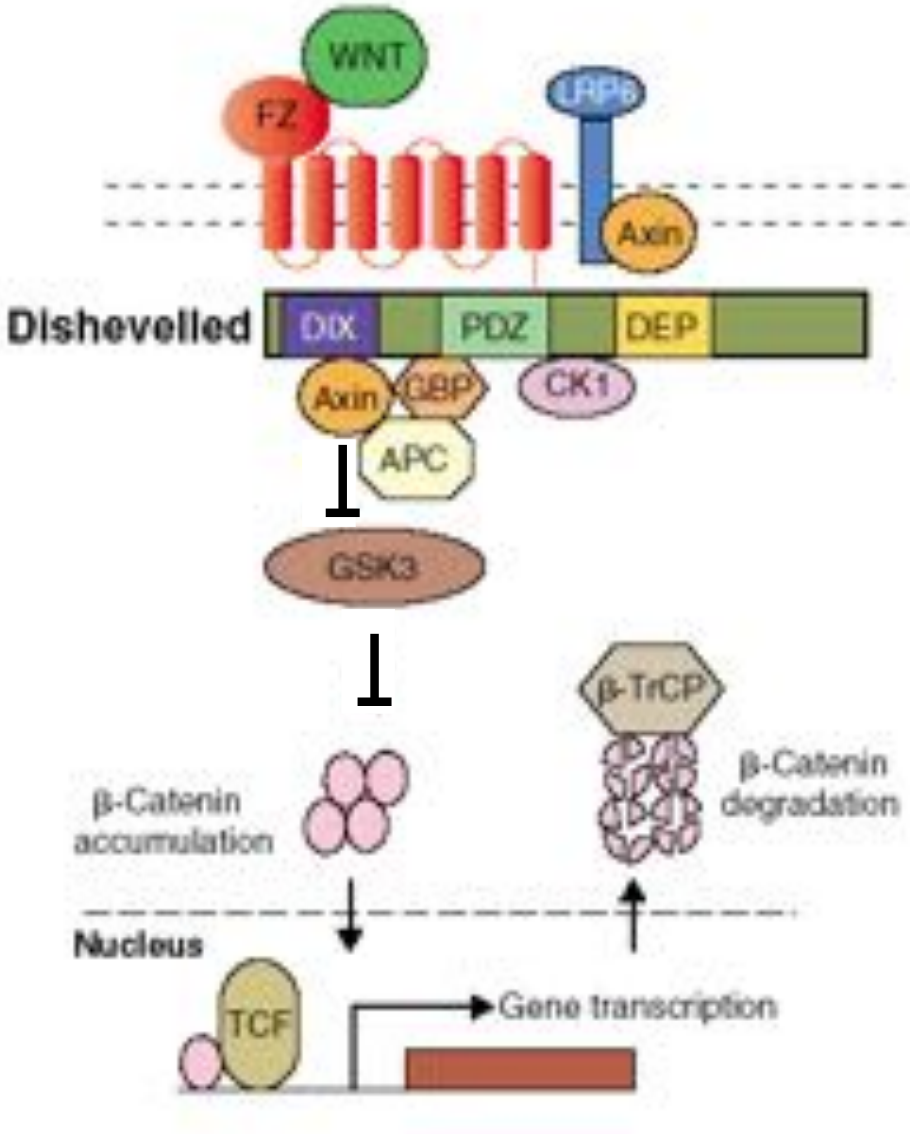


Dickkopf hemmt die Wnt Signaltransduktion über Kremen



Kremen ist Vertebraten-spezifisch!

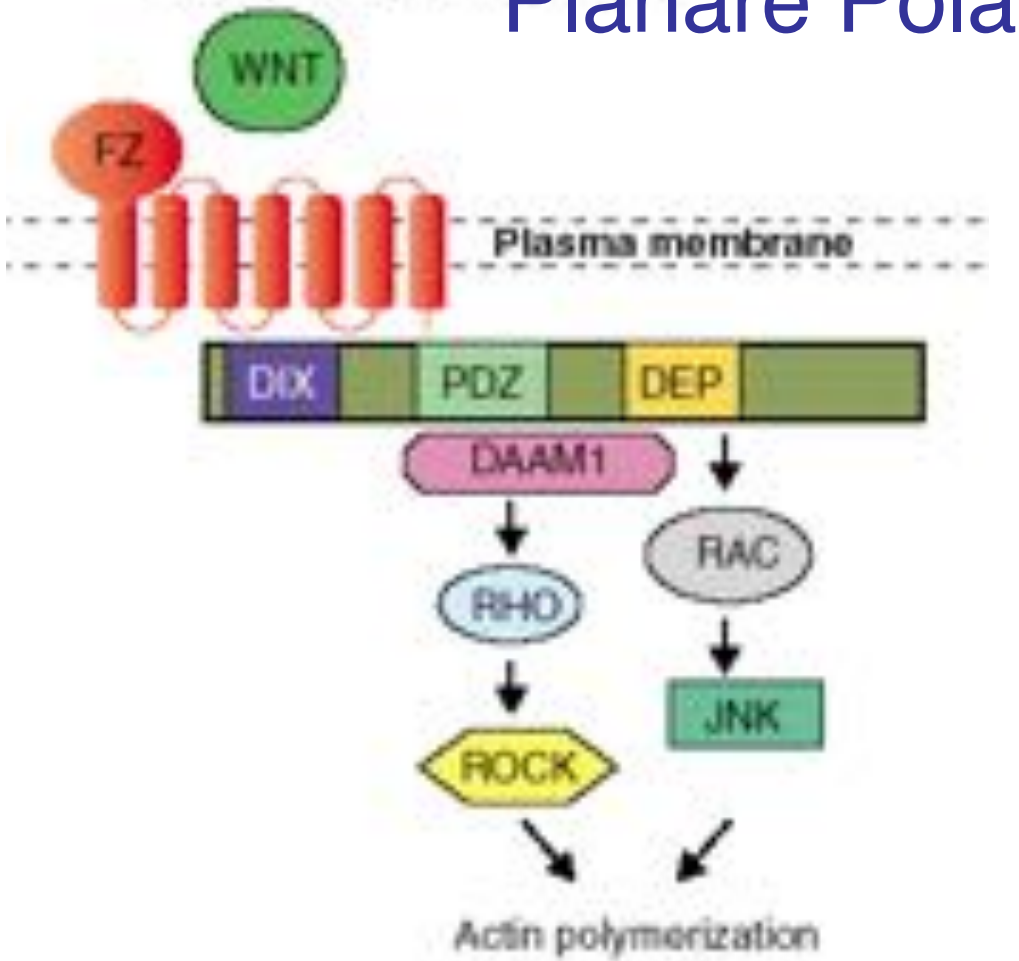
Der “kanonische” wnt Signalweg



DIX:
Dishevelled, Axin
PDZ:
PSD-95, DLG, ZO1
DEP:
Dishevelled, EGL-10, Pleckstrin

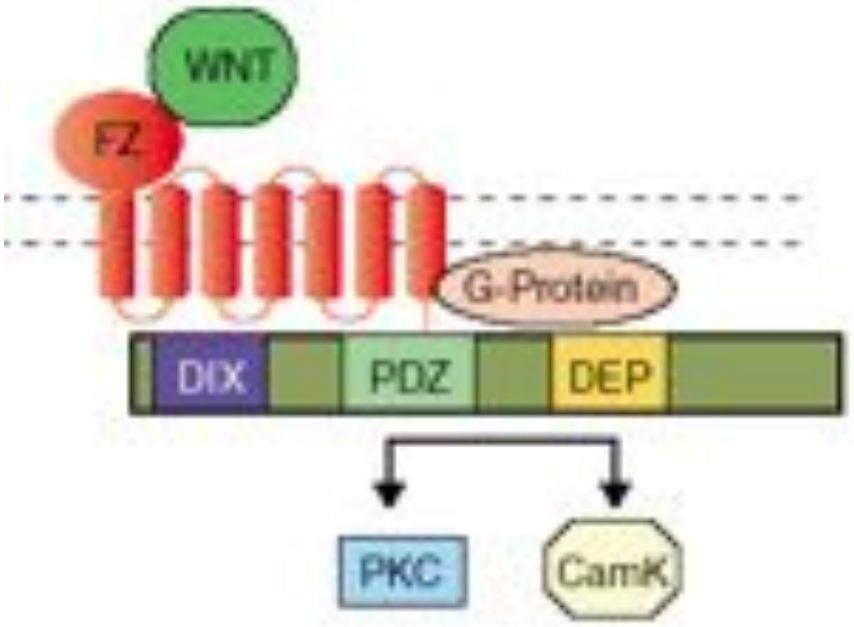
Canonical wnt signaling pathway.

Der “nicht kanonische” wnt Signalweg
Planare Polarität



DIX:
Dishevelled, Axin
PDZ:
PSD-95, DLG, ZO1
DEP:
Dishevelled, EGL-10, Pleckstrin

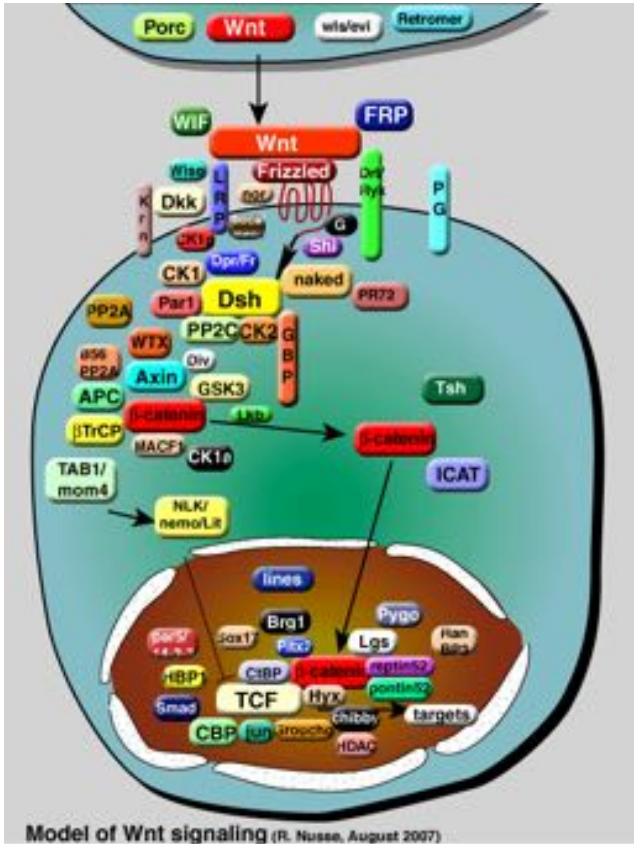
Der Ca^{2+} wnt Signalweg

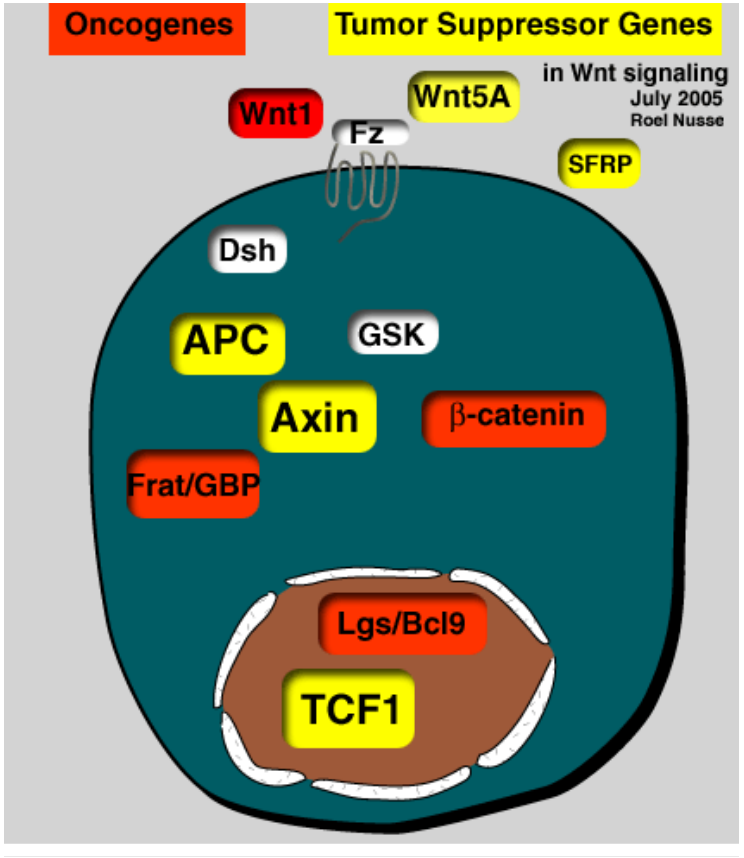


DIX:
Dishevelled, Axin
PDZ:
PSD-95, DLG, ZO1
DEP:
Dishevelled, EGL-10, Pleckstrin

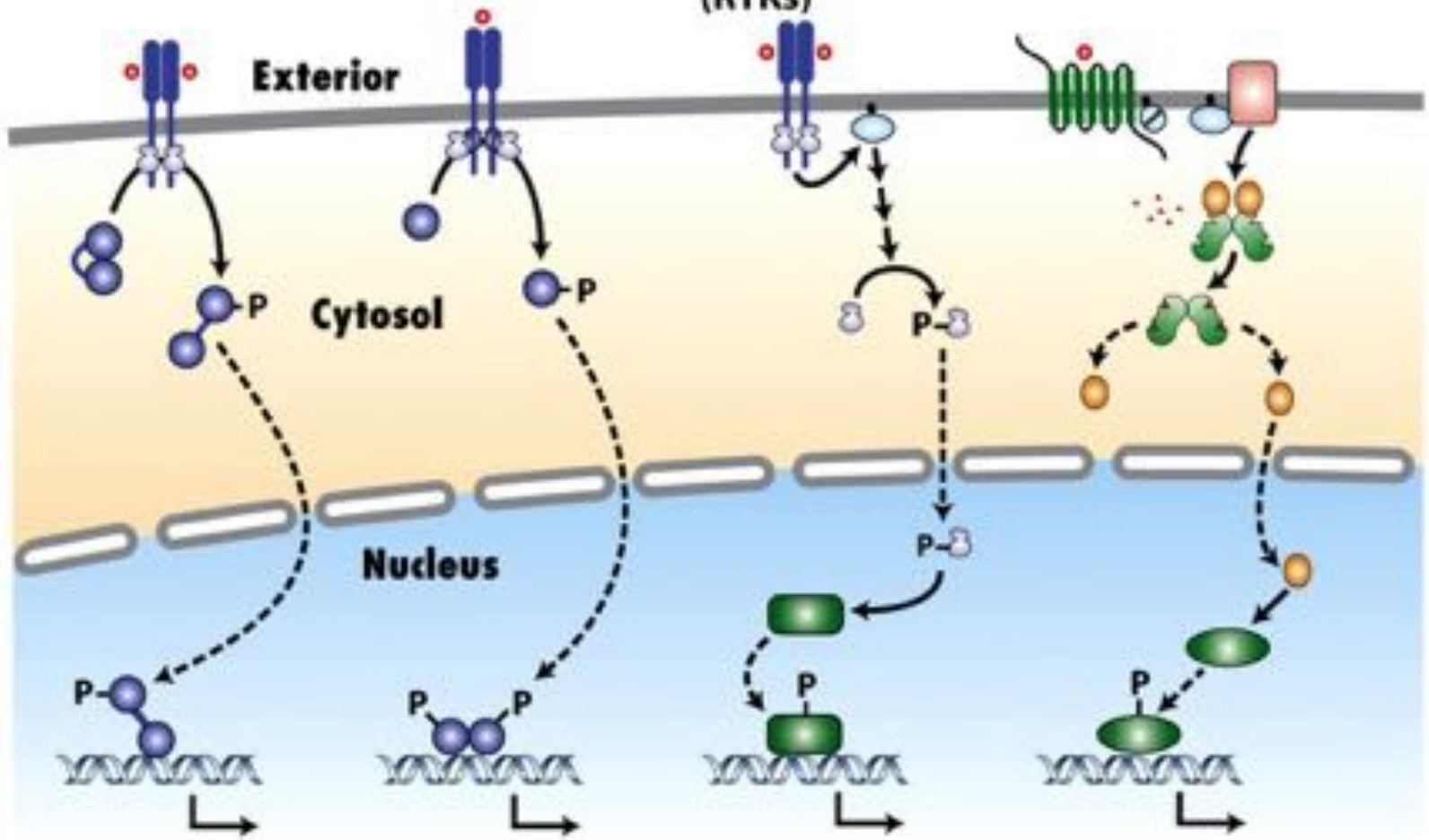
Ca^{2+} pathway.

Wingless Signaltransduktion

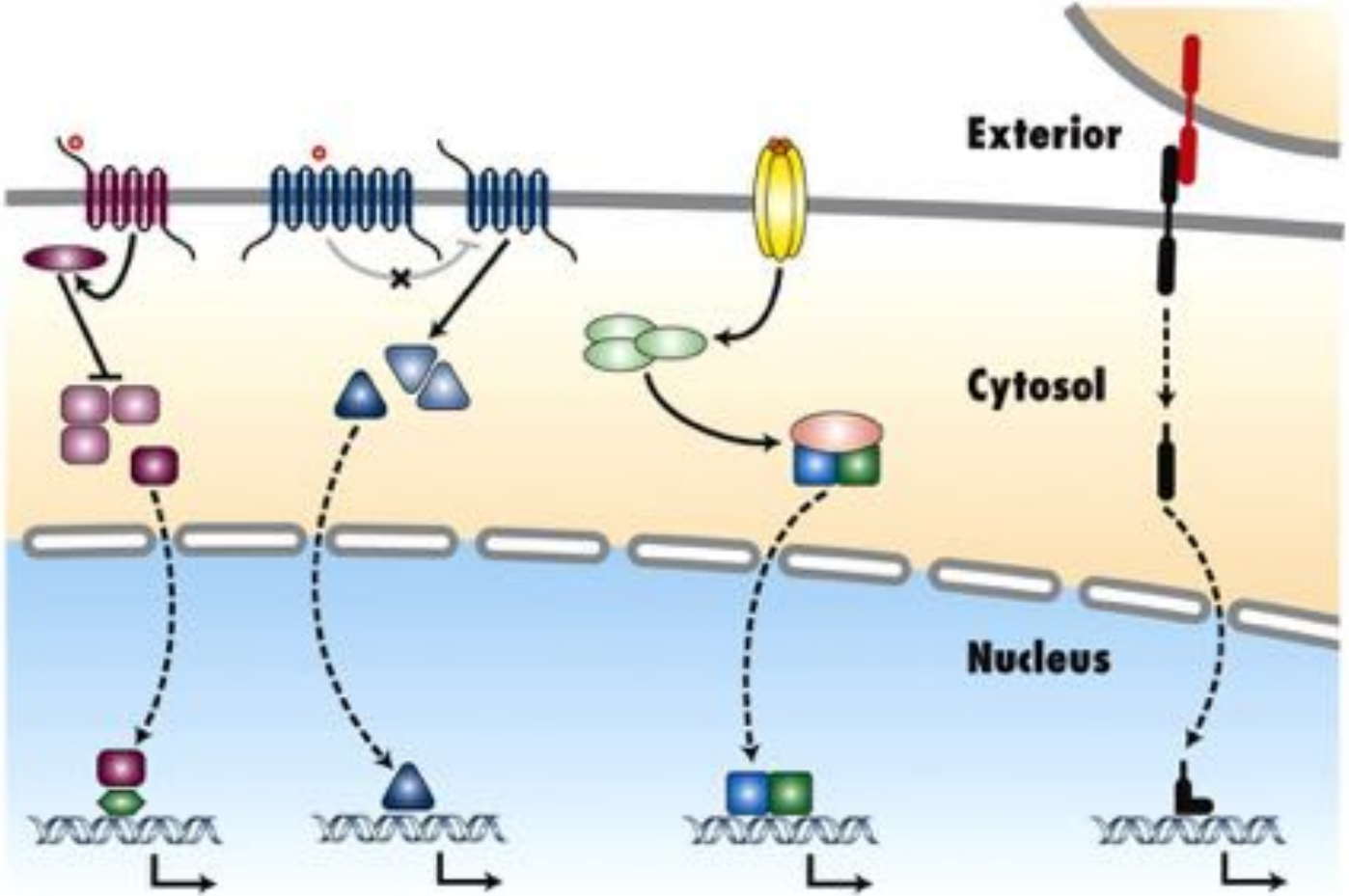




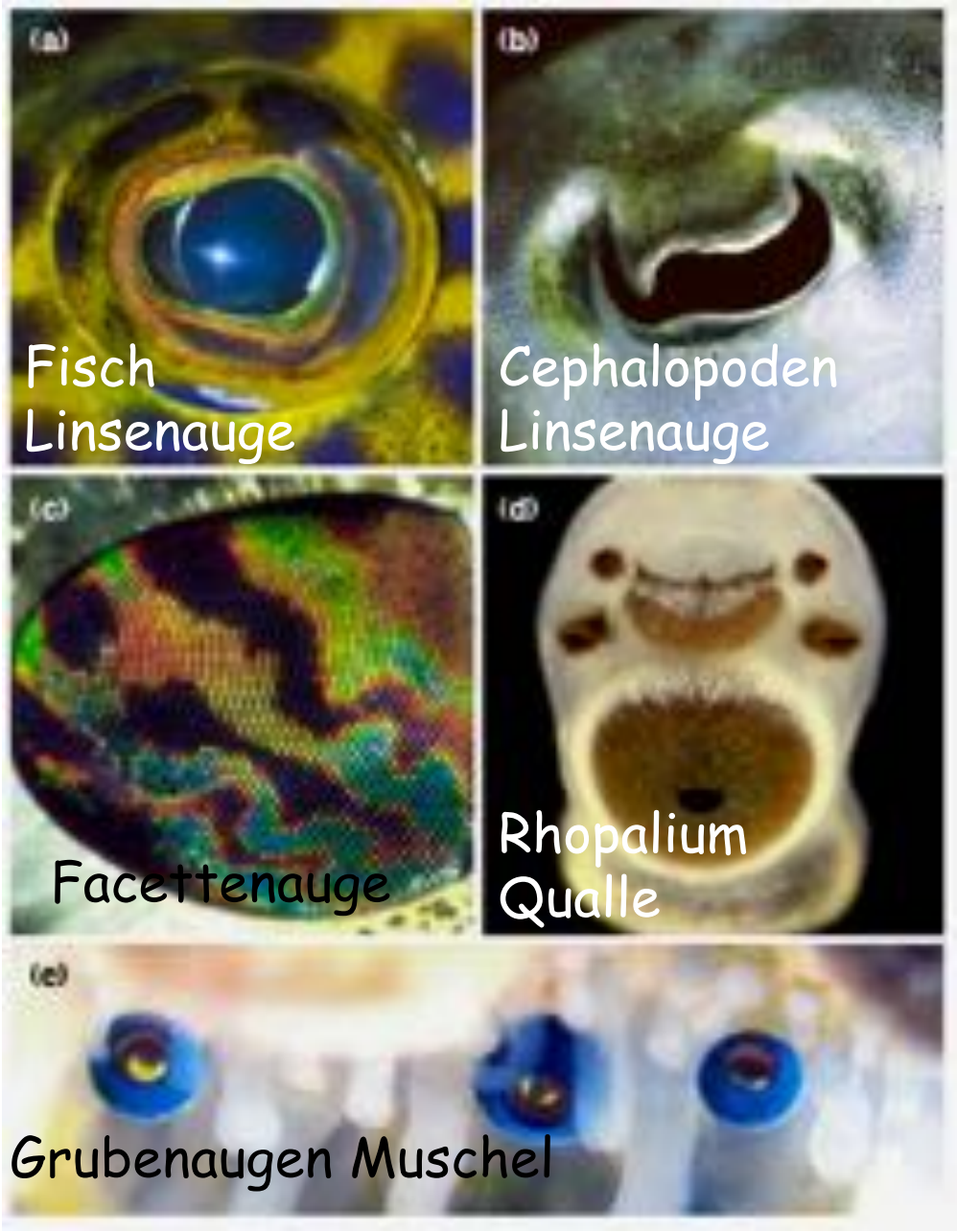
Bedeutung bei Krebs !



Receptor class	(a) TGFβ receptors	(b) Cytokine receptors	(c) Receptor tyrosine kinases (RTKs)	(d) G protein-coupled receptors (GPCRs)
Transcription factor (TF)	Smads	STATs	Various	CREB
Mechanism of TF activation	Phosphorylation in cytosol by activated receptor	Phosphorylation in cytosol by JAK kinase	Phosphorylation by cytosolic kinase in nucleus or cytosol	Phosphorylation in nucleus by protein kinase A

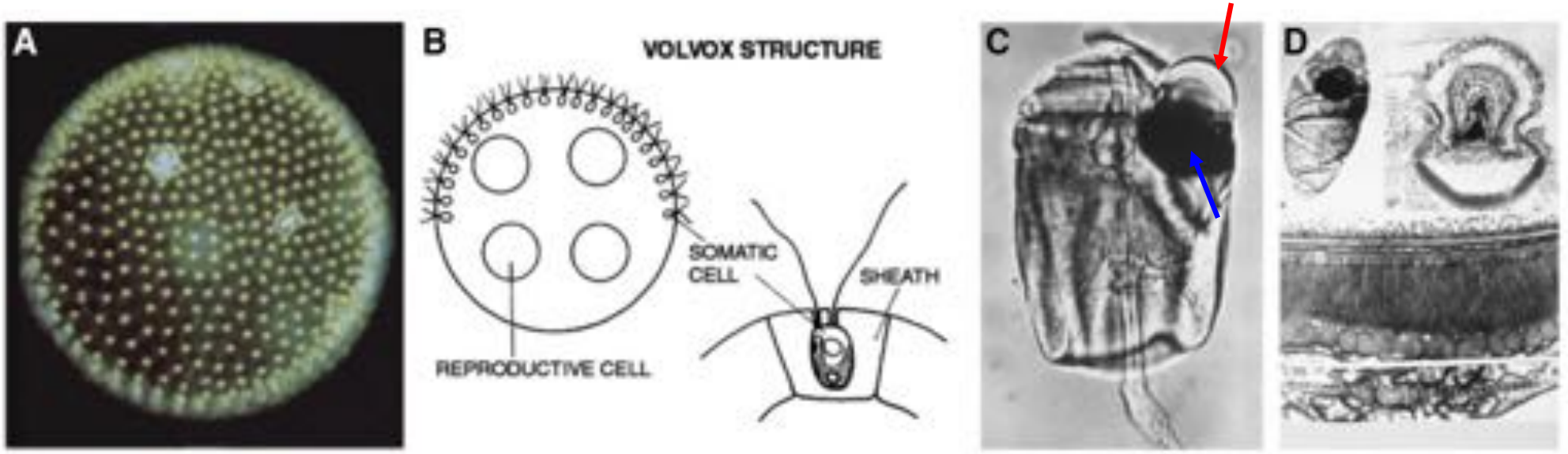
Receptor class	(e) Wnt receptors	(f) Hedgehog receptors	(g) TNFα receptors	(h) Notch (Delta receptors)
				
Transcription factor (TF)	β-Catenin	Ci (activator) Ci fragment (repressor)	NF-κB	Notch cytosolic domain
Mechanism of TF activation	Disassembly of multiprotein complex in cytosol; acts with TCF in nucleus	Disassembly of multiprotein complex in cytosol	Phosphorylation and degradation of inhibitor of NF-κB in cytosol	Proteolytic release of Notch cytosolic domain, which acts with nuclear TFs

Augen werden in allen Tierstämmen gebildet



Nilsson 2004

Augen-Organellen



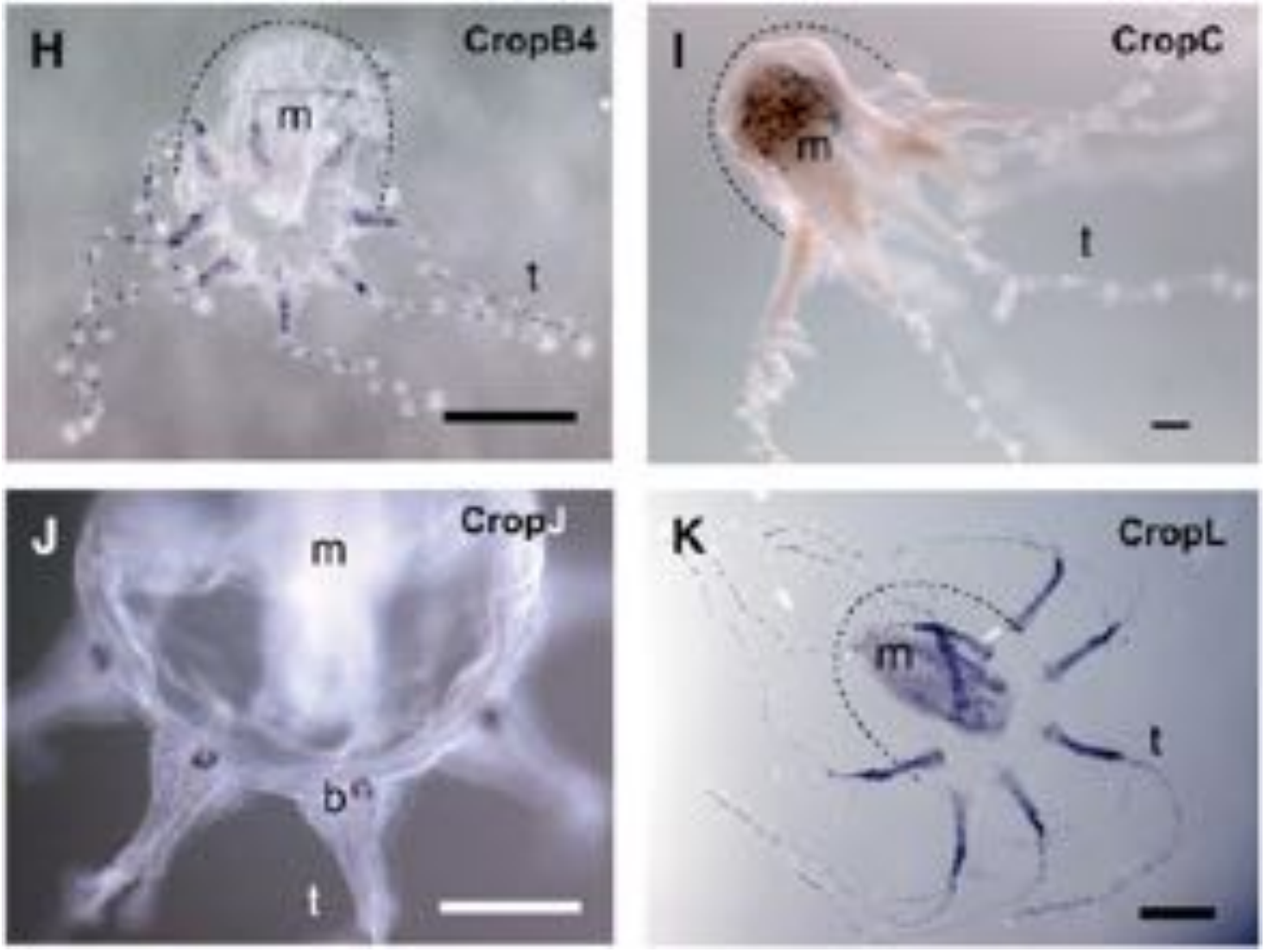
Volvox->
Ocellus im Chloroplast

Dinoflagellaten

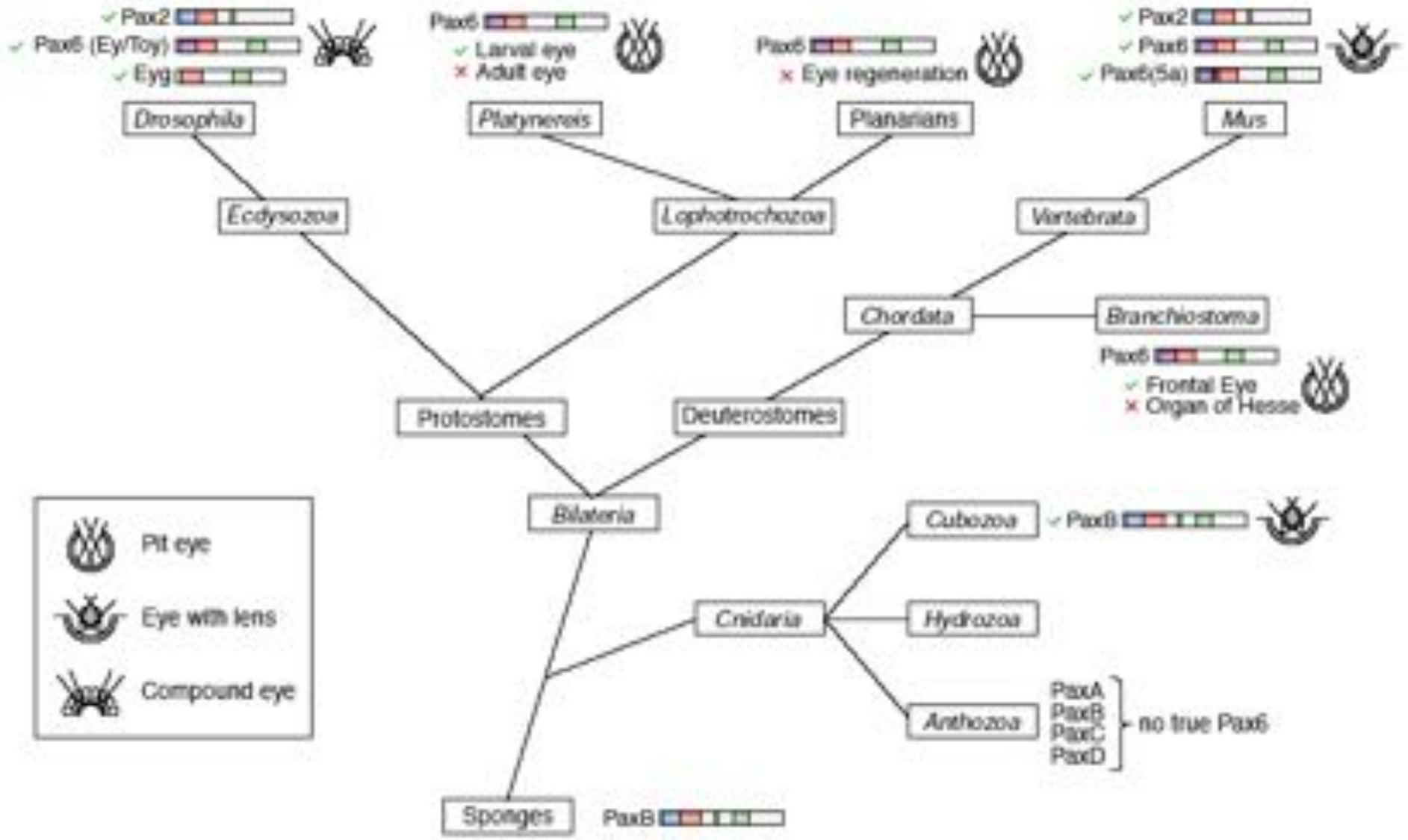
Linse, Pigmentbecher,
Rhabdomere

Rhodopsin als Lichtsensor !

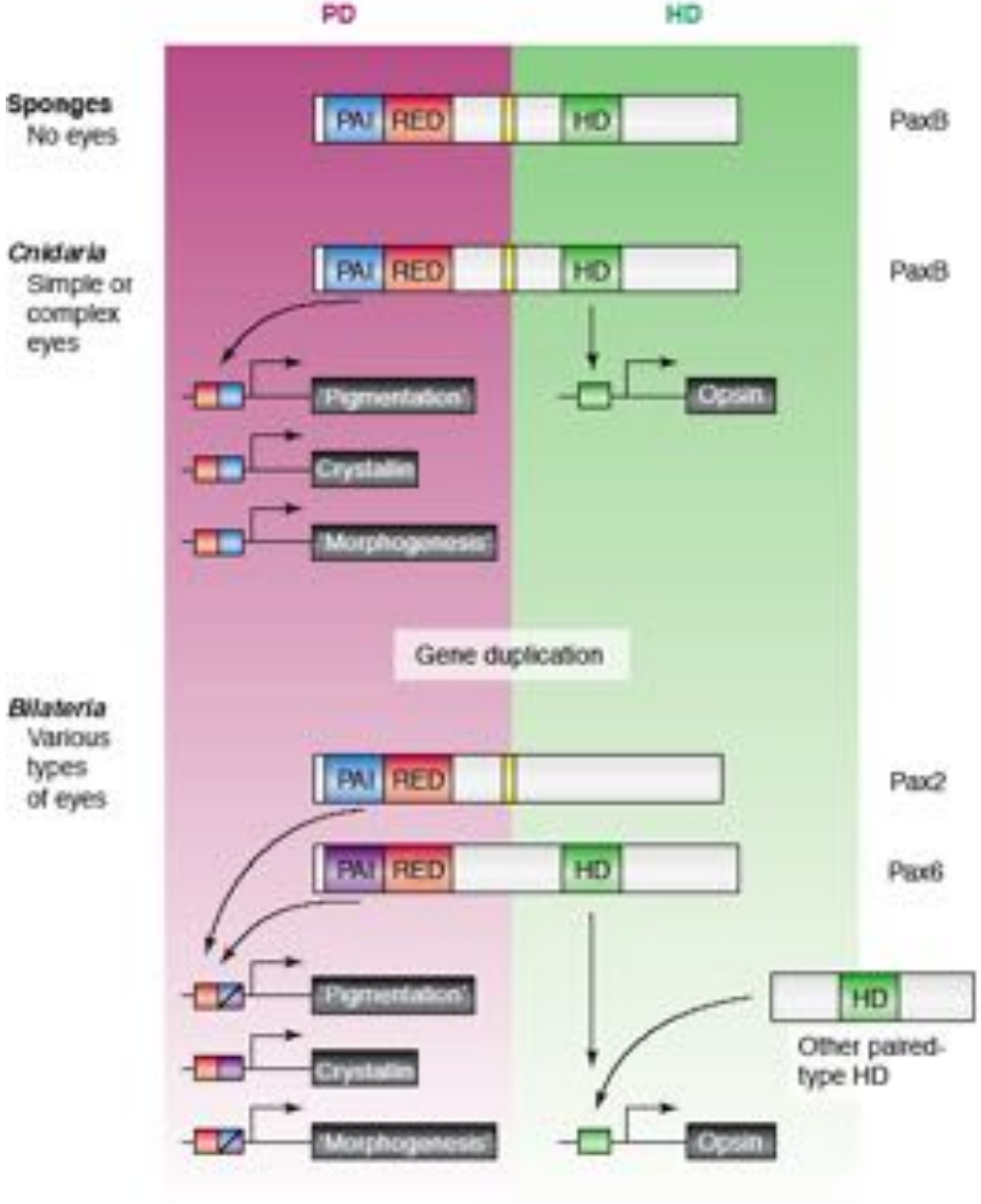
Expression von Quallen Opsin Genen (*in situ*)



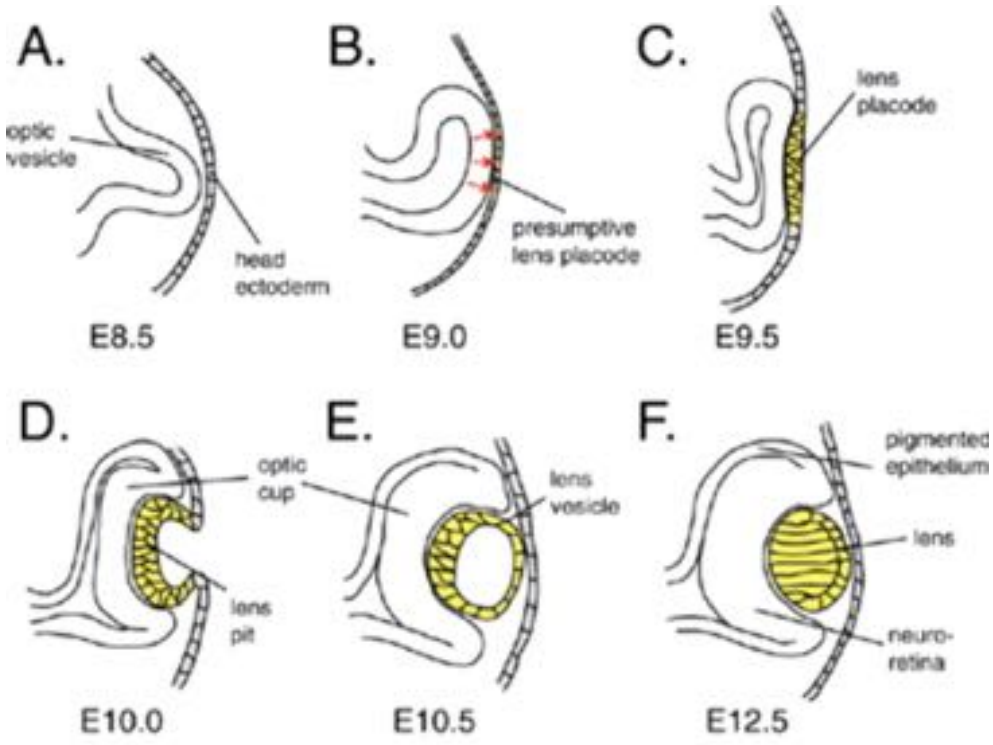
Evolution der Augentypen/Pax-Gene



Evolution des Pax6 Gens



Augenentwicklung bei Vertebraten



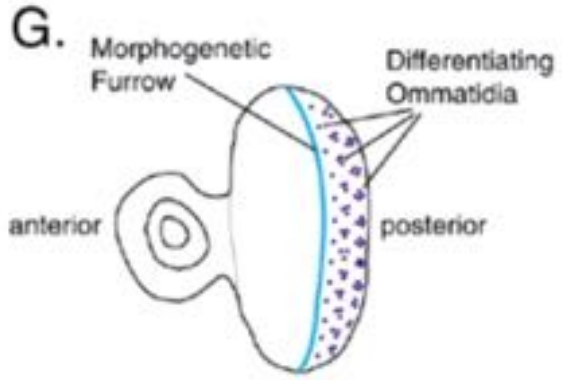








Drosophila:



Linseninduktion

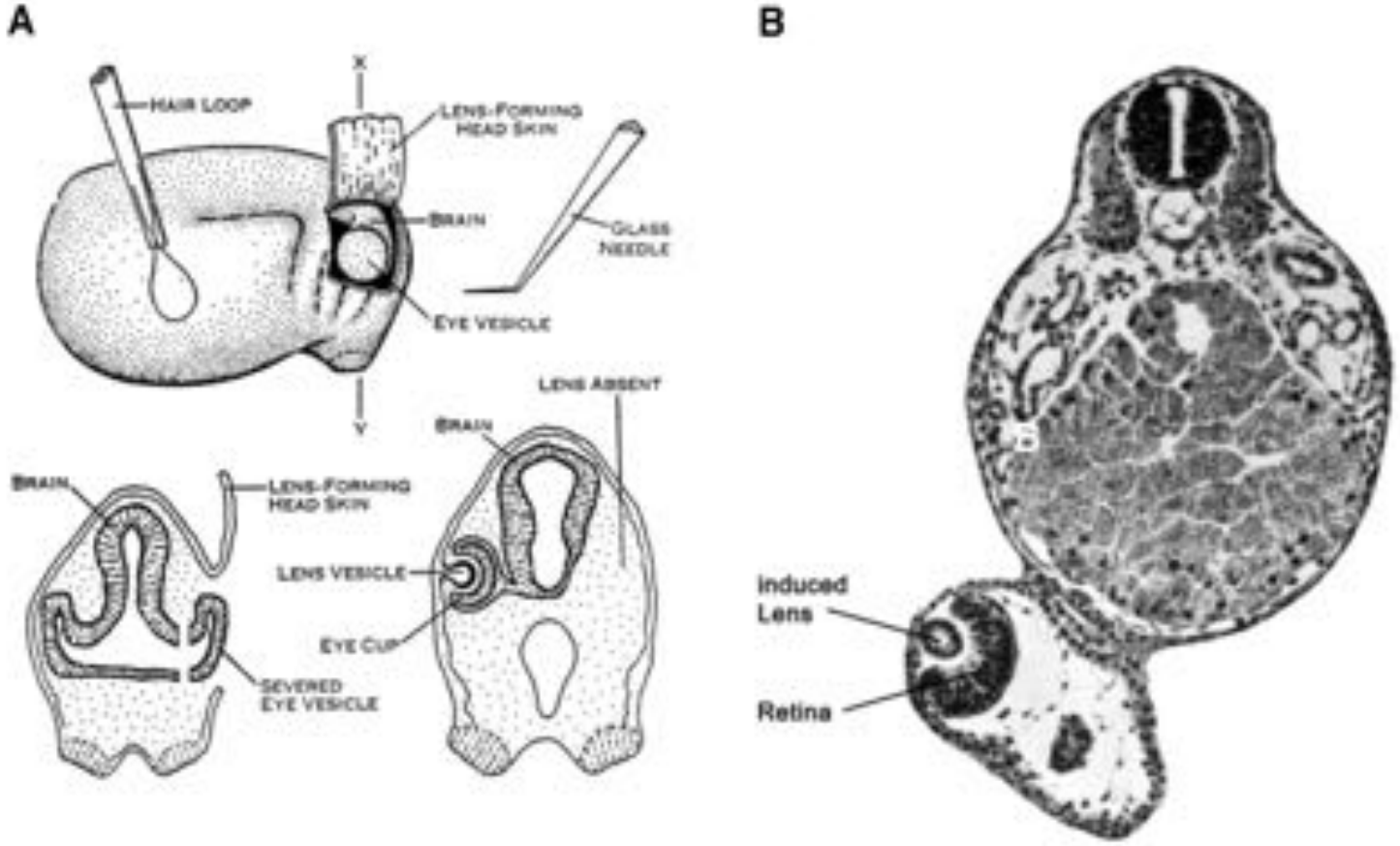
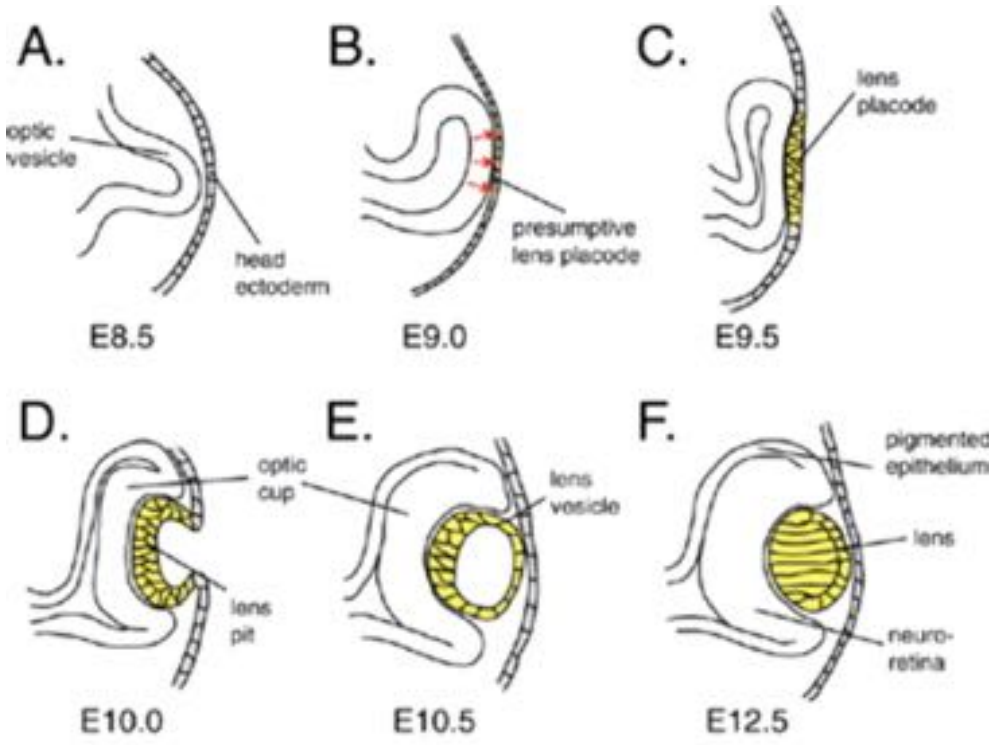
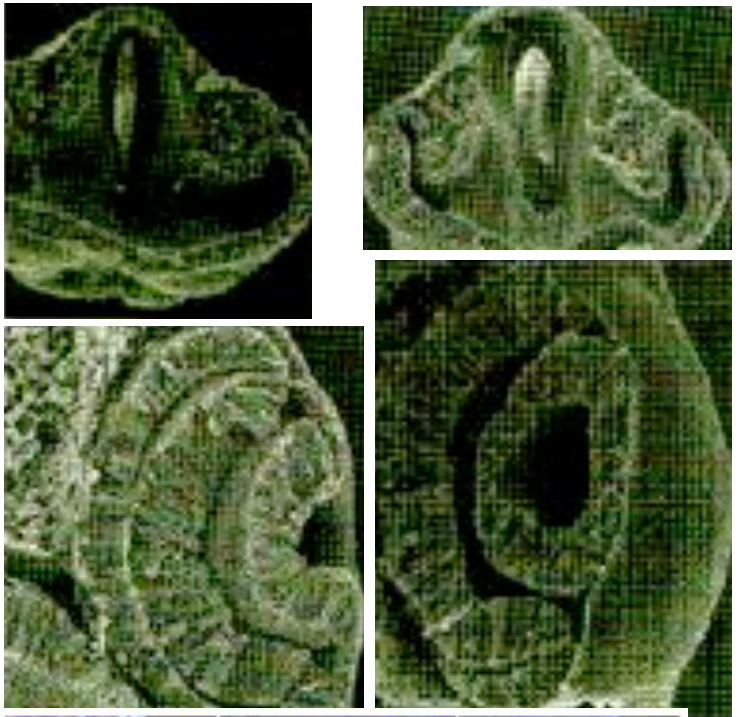
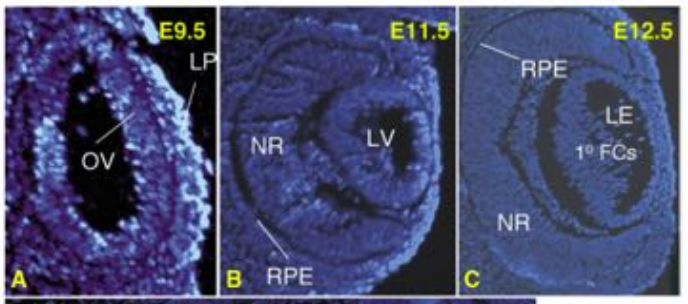


Fig. 1. (A) *Exstirpation of the eye vesicle in an amphibian embryo leading to the absence of both the eye cup and the lens (after Hamburger, 1988).* **(B)** *Section across a* Triturus taeniatus *larva with a transplanted eye cup, which has induced an ectopic lens in the flank of the larva (after Spemann, 1936).*

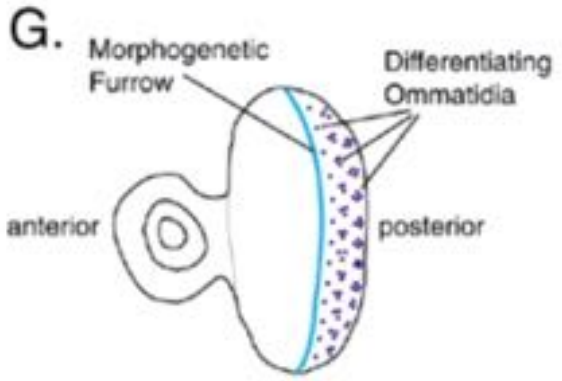
Augenentwicklung bei Vertebraten

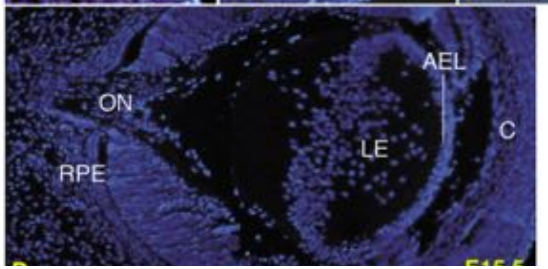




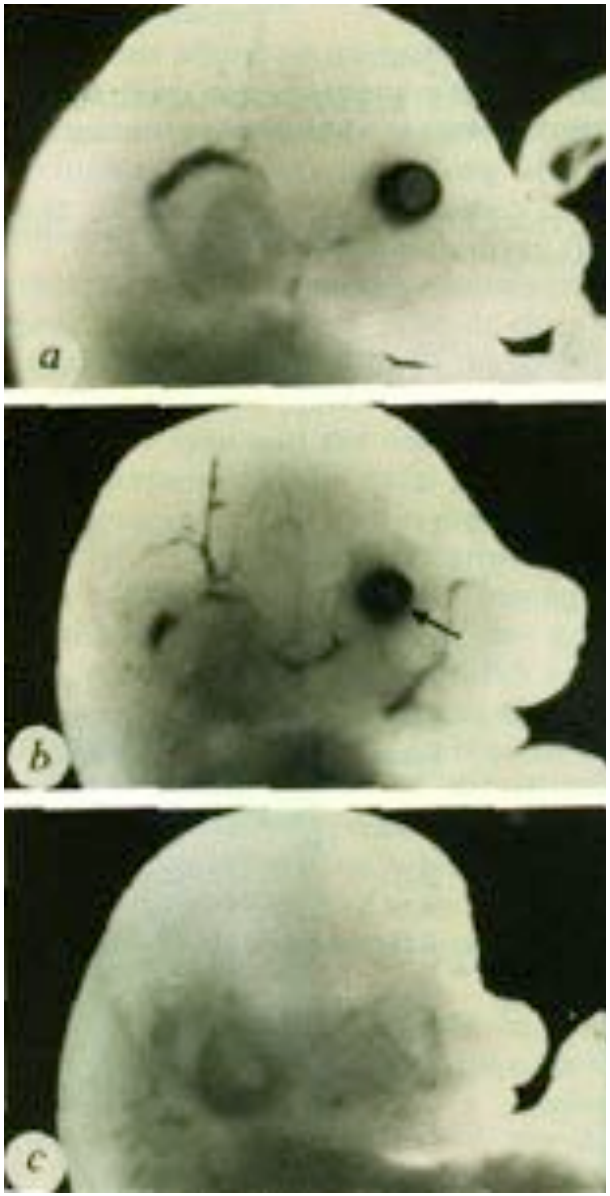


Drosophila:

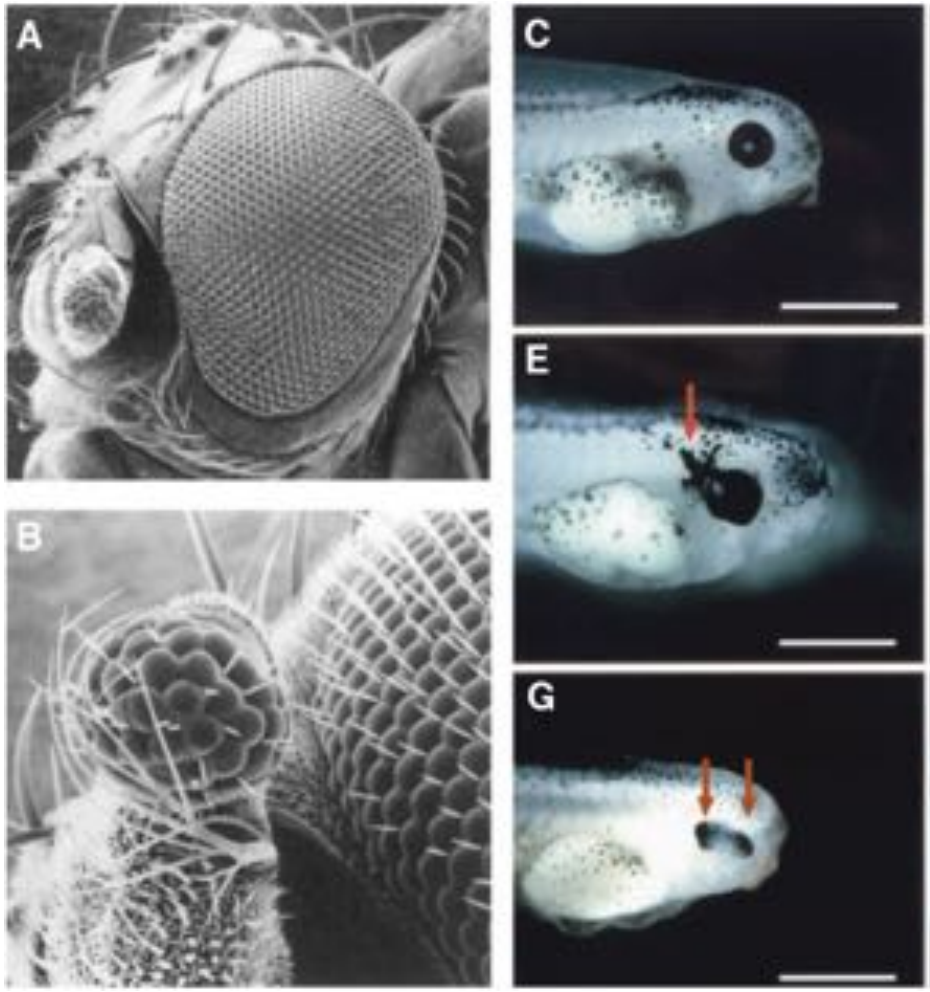




Small eye Mutante der Maus

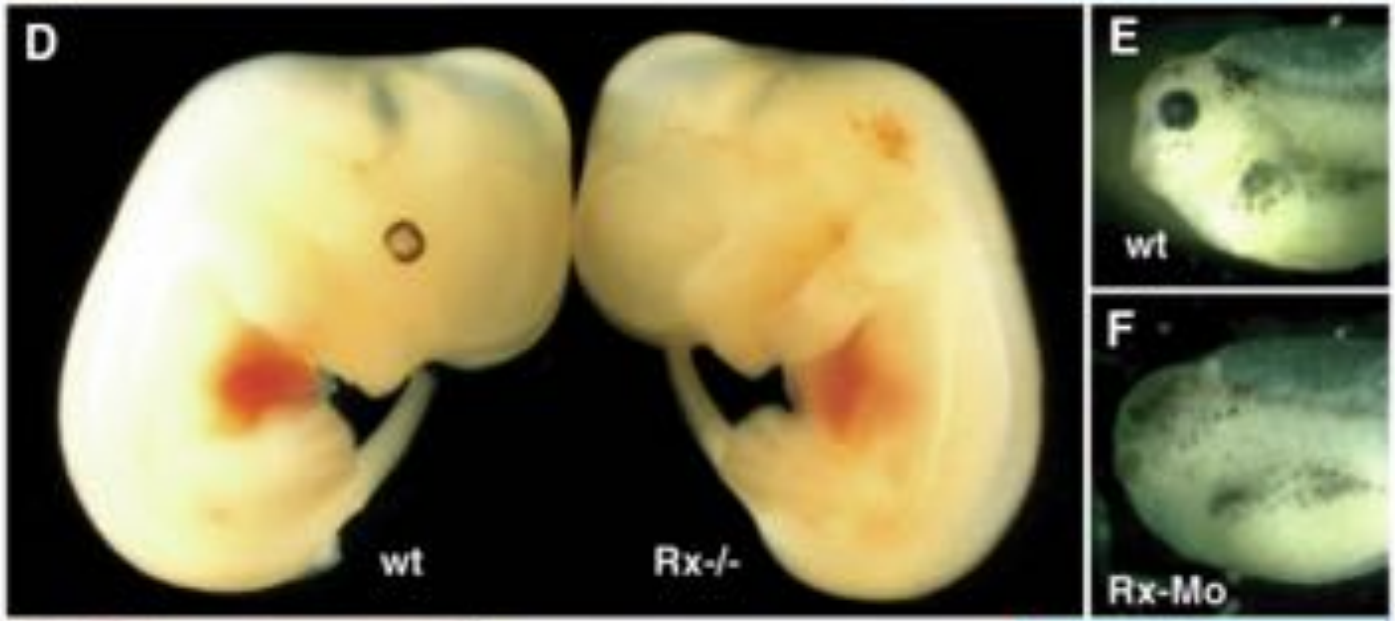


Induktion von Augen nach Pax6 Expression



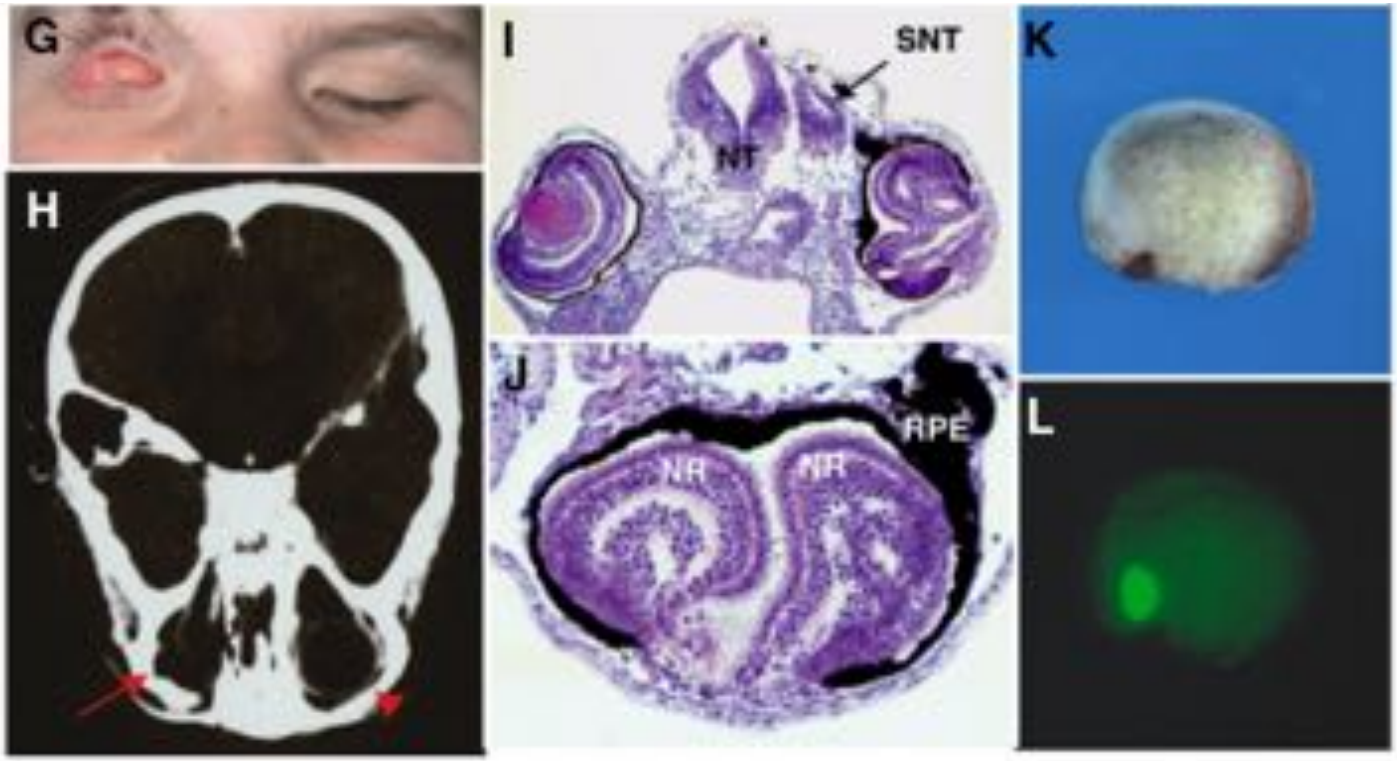
Pax6 ist ein universeller Regulator der Augenentwicklung

Die genetische Kontrolle der Augenentwicklung bei Vertebraten



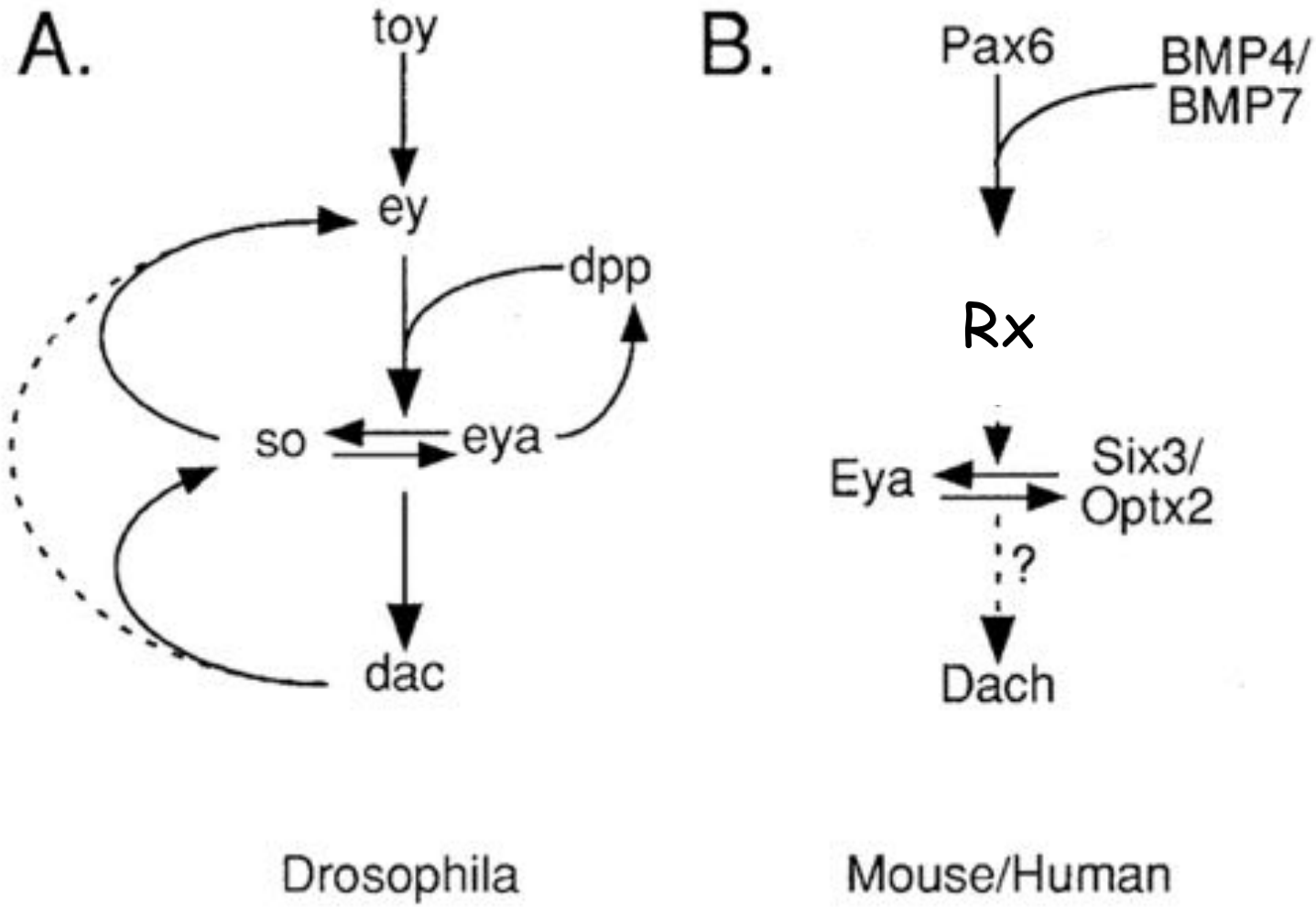
Genetische Kontrolle der Augenentwicklung bei Vertebraten

LOF *Rx* GOF *Rx*



wird durch eine verwandte,
aber nicht identische Genkaskade kontrolliert
eyeless Mutation der Maus (Rx = Retinal Homeobox)

Genetische Kontrolle der Augenentwicklung



Genetische Kontrolle der Augenentwicklung

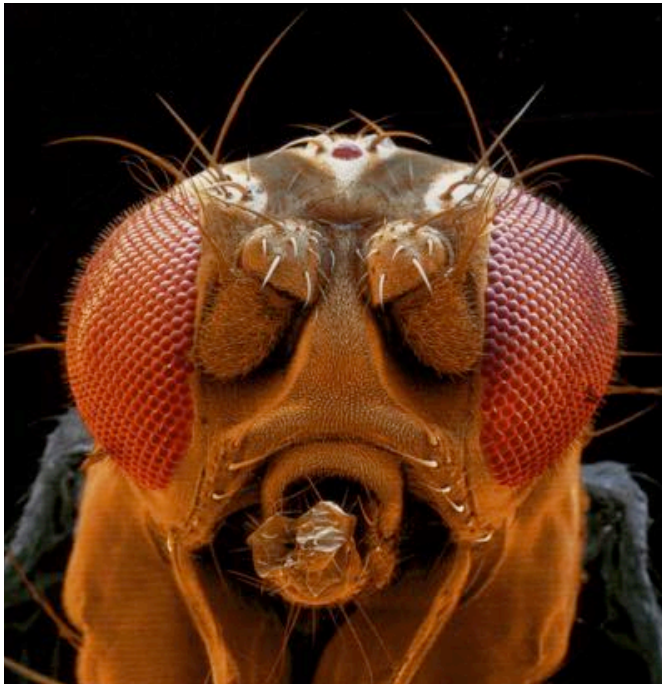
Drosophila	Vertebrate	Eye Phenotype (loss of function)	Refereneces
Ey	*Pax6*	small eyes, anophthalmia, Aniridia	Hill *et al*., 1991; Glaser *et al*., 1994
Eya	*Eya1-3*	*Eya1*: open eyelids *EYA1*: anterior segment anomalies	Xu *et al*., 1997; Azuma *et al*., 2000
So	*Six1/2*	none	Laclef *et al*., 2003
Optix	*Six3/6*	holoprosencephaly, anophthalmia	Gallardo *et al*., 1999; Wallis *et al*., 1999; Pasquier *et al*., 2000; Li *et al*., 2002;
D-six4	*Six4/5*	*Six5*: adult onset cataracts	Sarkar *et al*., 2000; Klesert *et al*., 2000; Winchester *et al*., 1999
Dac	*Dach1*	none	Davis *et al*., 2001
Hh	*Shh*	holoprosencephaly, cyclopia	Chiang *et al*., 1996
Atonal	*Math5* (*Xath5*, *ath5*)	> 80% loss of RGCs	Brown *et al*., 2001; Wang *et al*., 2001
hairy	*Hes1*	premature retinal neurogenesis resulting in a retina with very few of each major type of neuron	Tomita *et al*., 1996

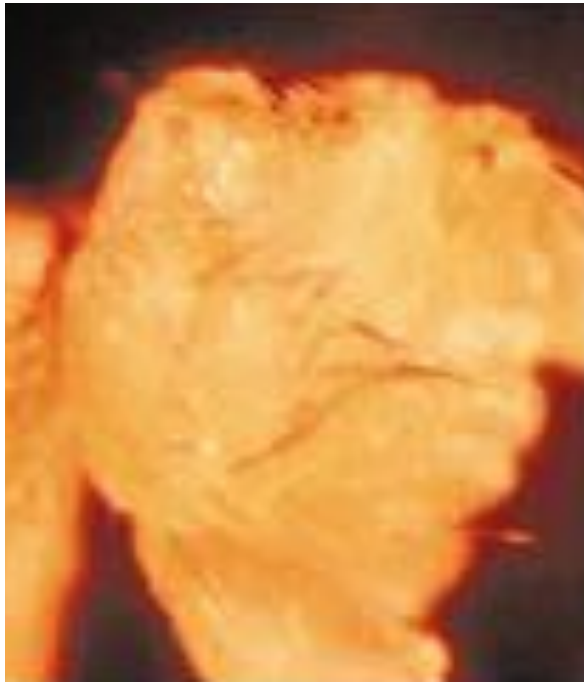
Die Neurogenese von Drosophila und Vertebraten wird von konservierten Signalwegen gesteuert

- Proneurale Gene vermitteln neuronale Kompetenz.
- Die Notch Signalkaskade hilft in der Auswahl der Vorläuferzellen und vermittelt die laterale Inhibtion.
- Die Spezifität einzeler Neuronentypen wird von konservierten Schaltern reguliert (Auge, Motoneurone, D/V Regionalisierung .


SCIENCEphotoLIBRARY

Bei Drosophila hängt die Entwicklung des (Komplex)auges von wenigen Genen ab





eyeless (pax6)

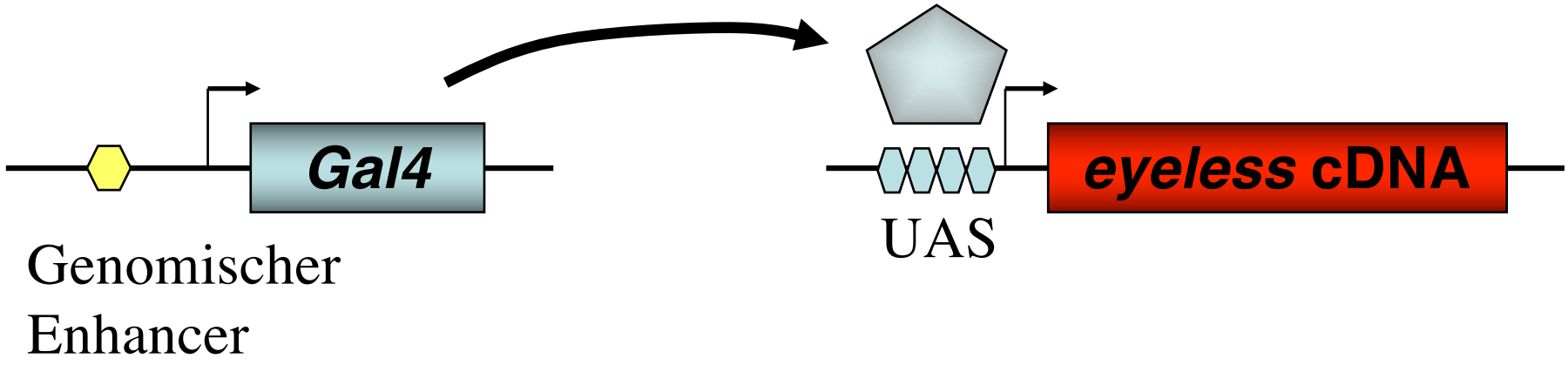
twin of eyeless

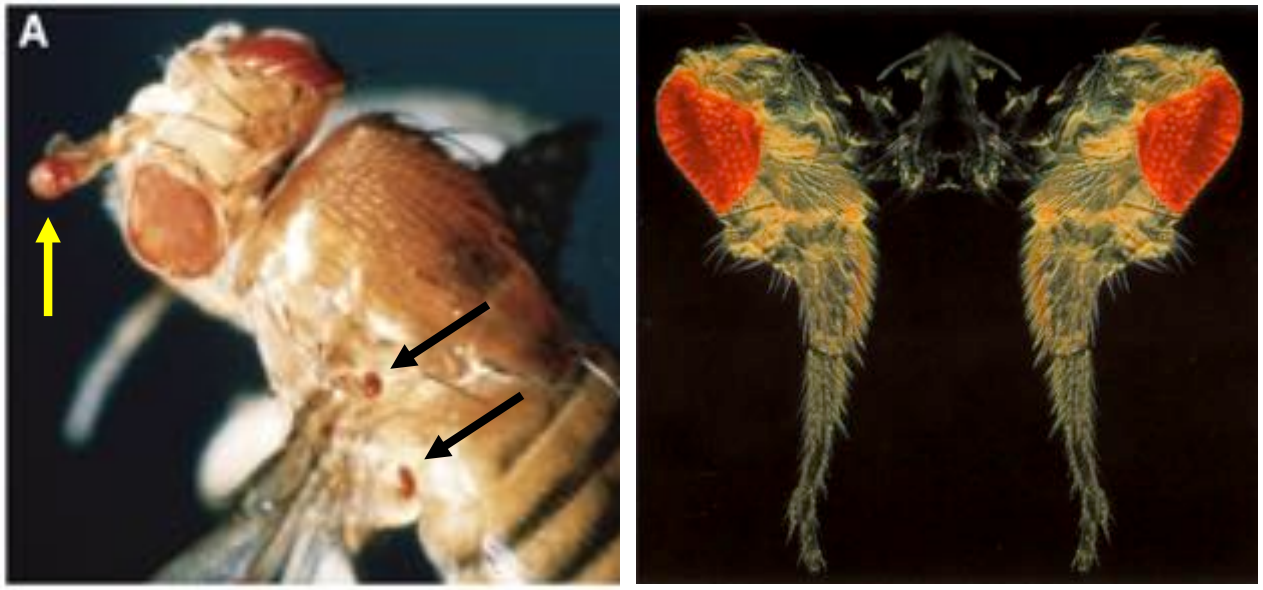
sine oculis

eyes absent

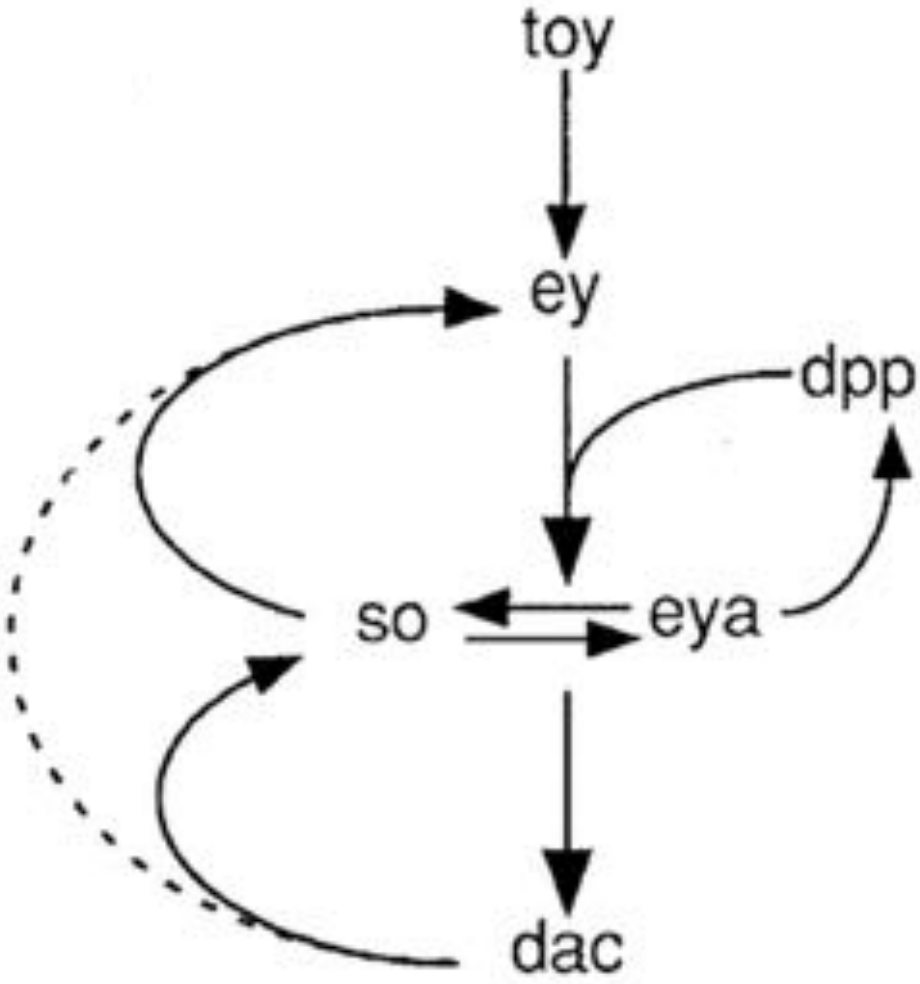
eye gone

Die ektopische Expression von *eyeless* induziert die Augenentwicklung

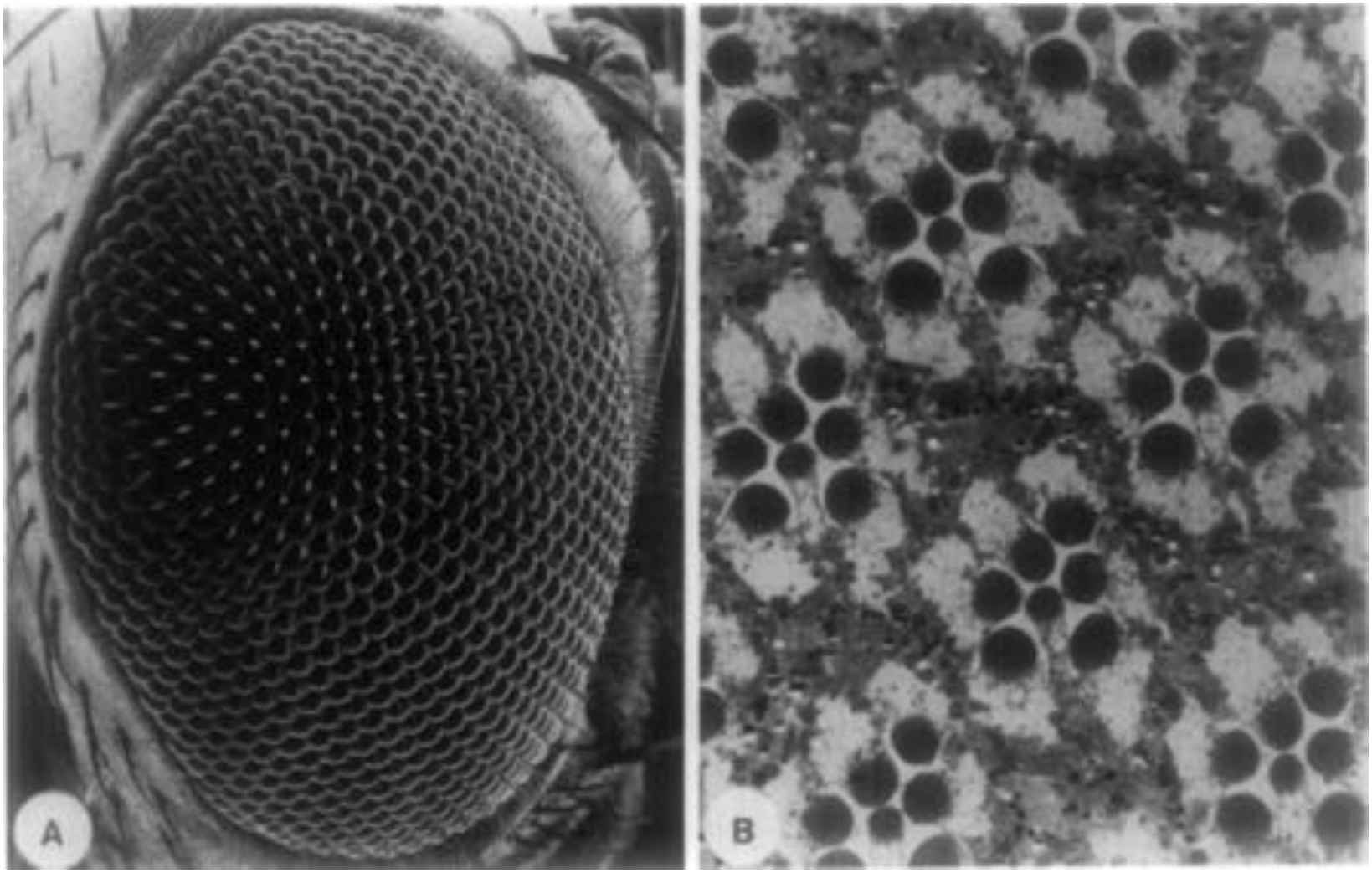




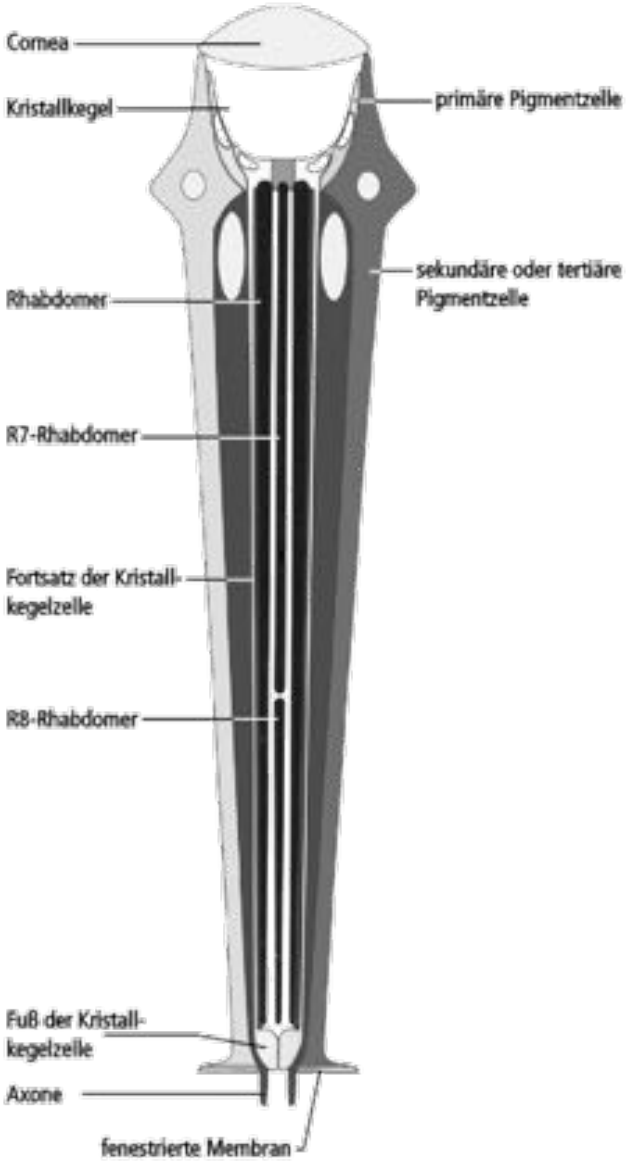
Genetische Kontrolle der Augenentwicklung bei *Drosophila*

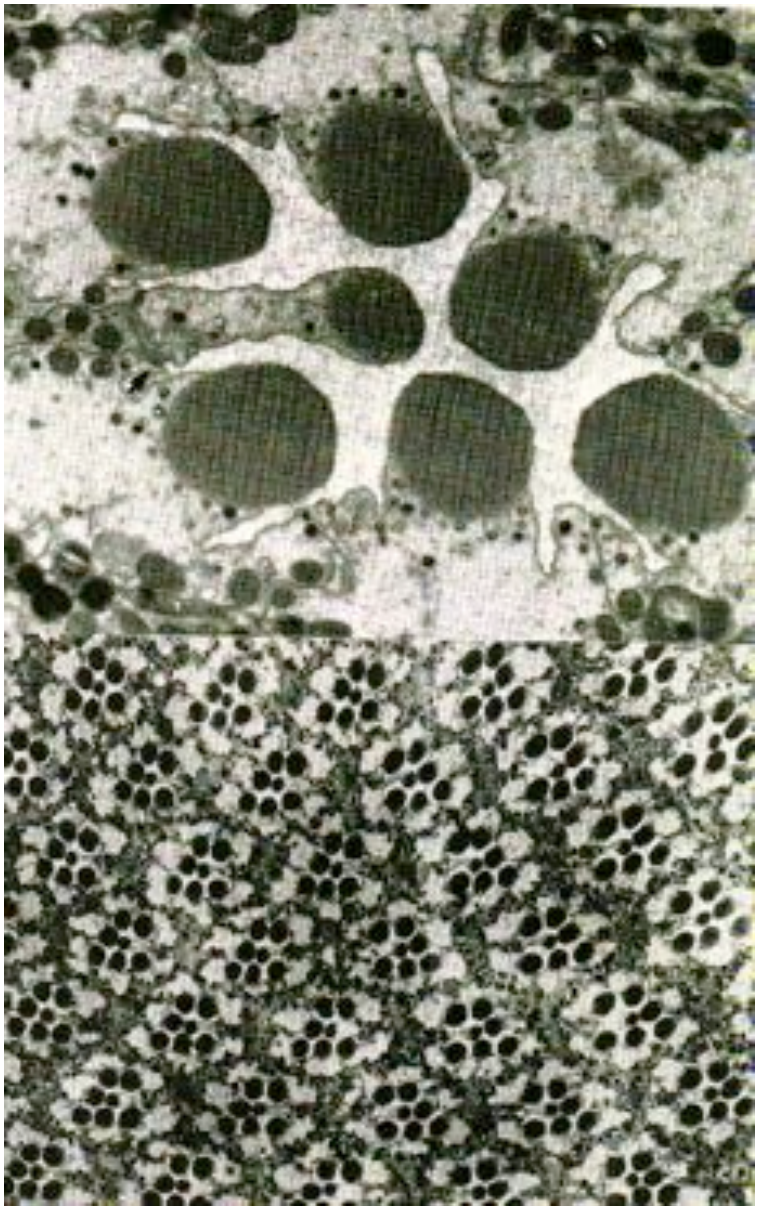


Das *Drosophila* Komplexauge

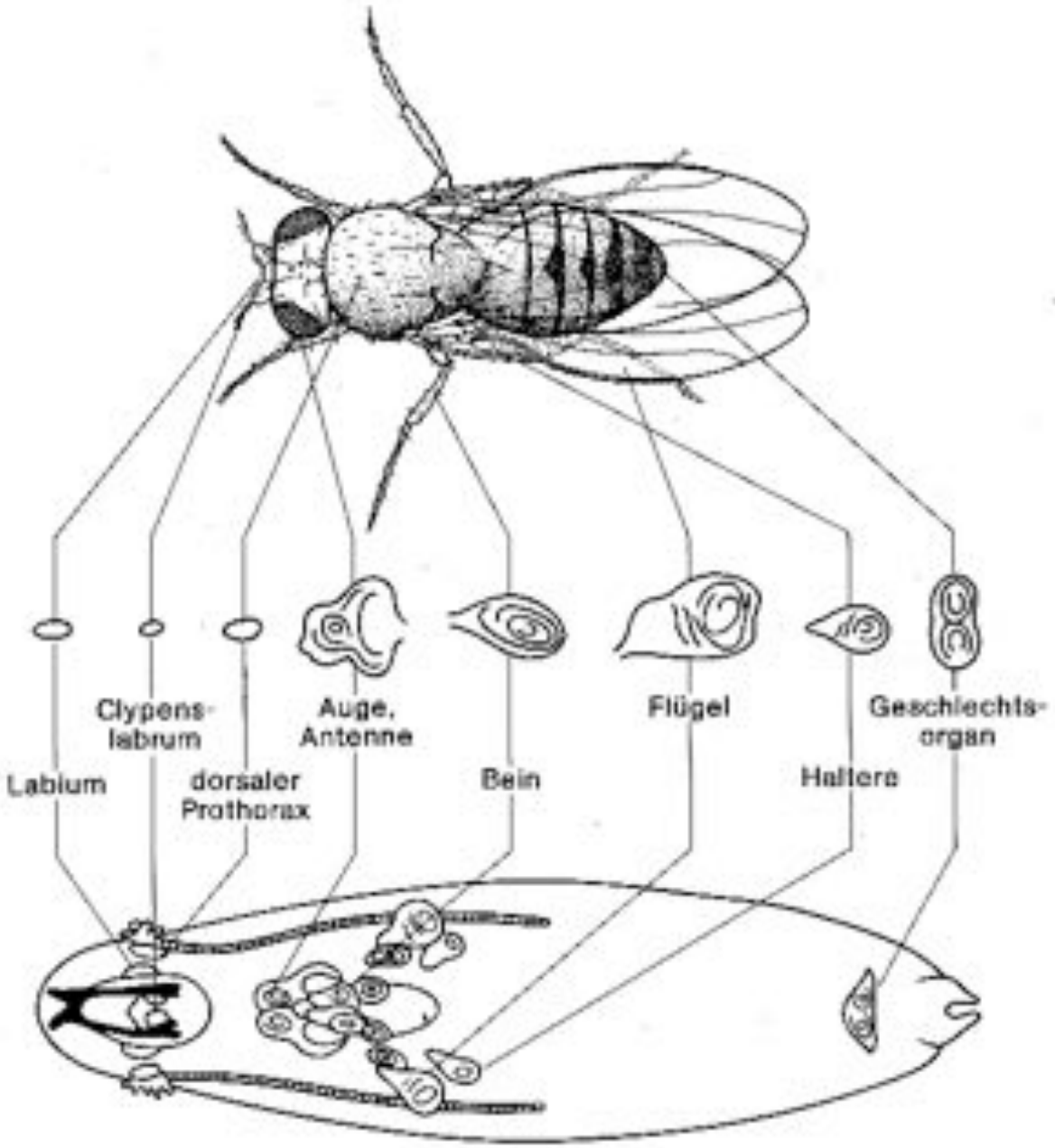


Ommatidienaufbau

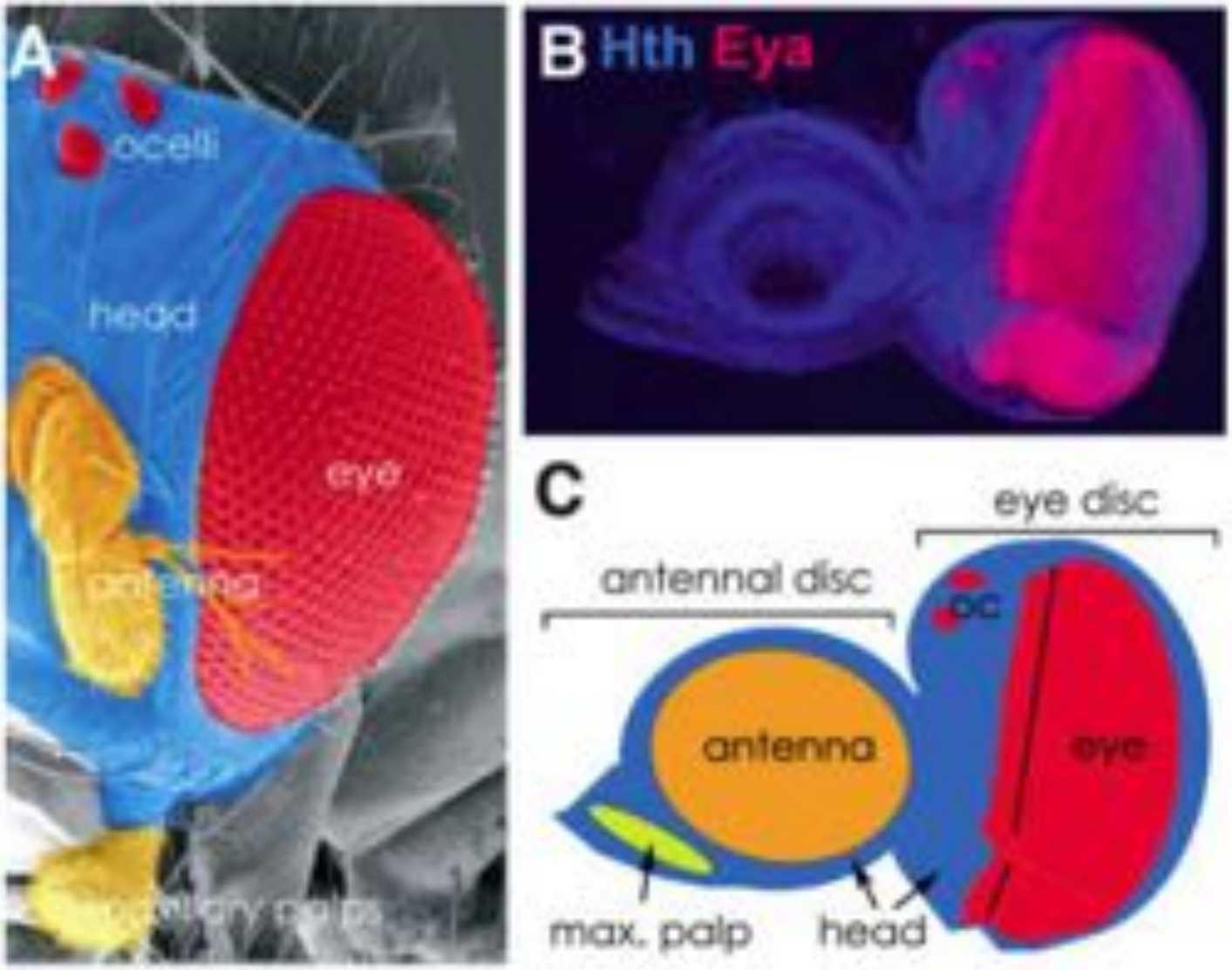




Drosophila Entwicklung

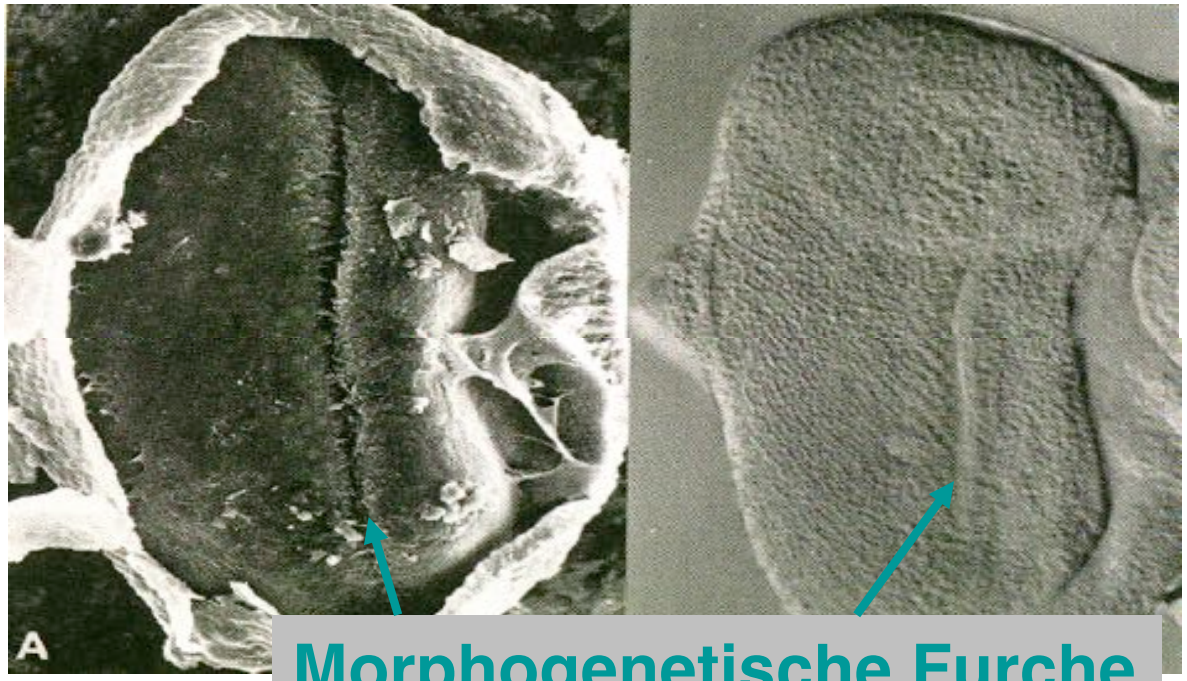


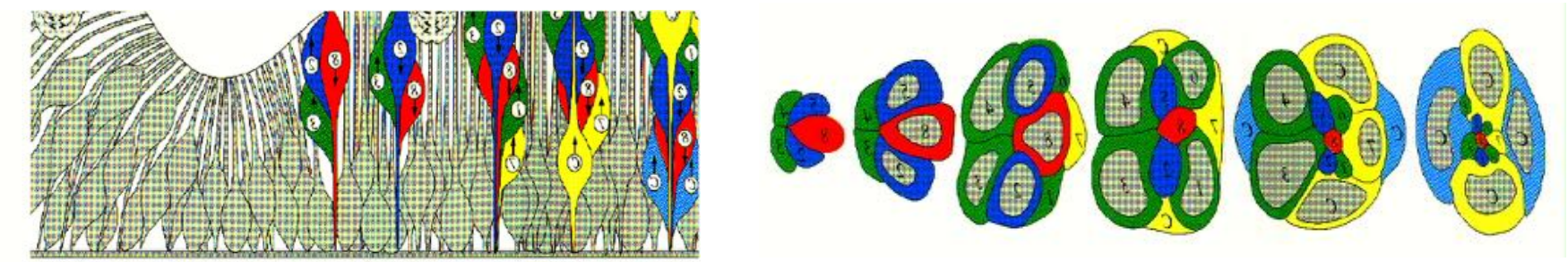
Das Auge und die Kutikula des Kopfes entwickeln sich aus der Augenimaginalscheibe



Dominguez & Casares, 2005

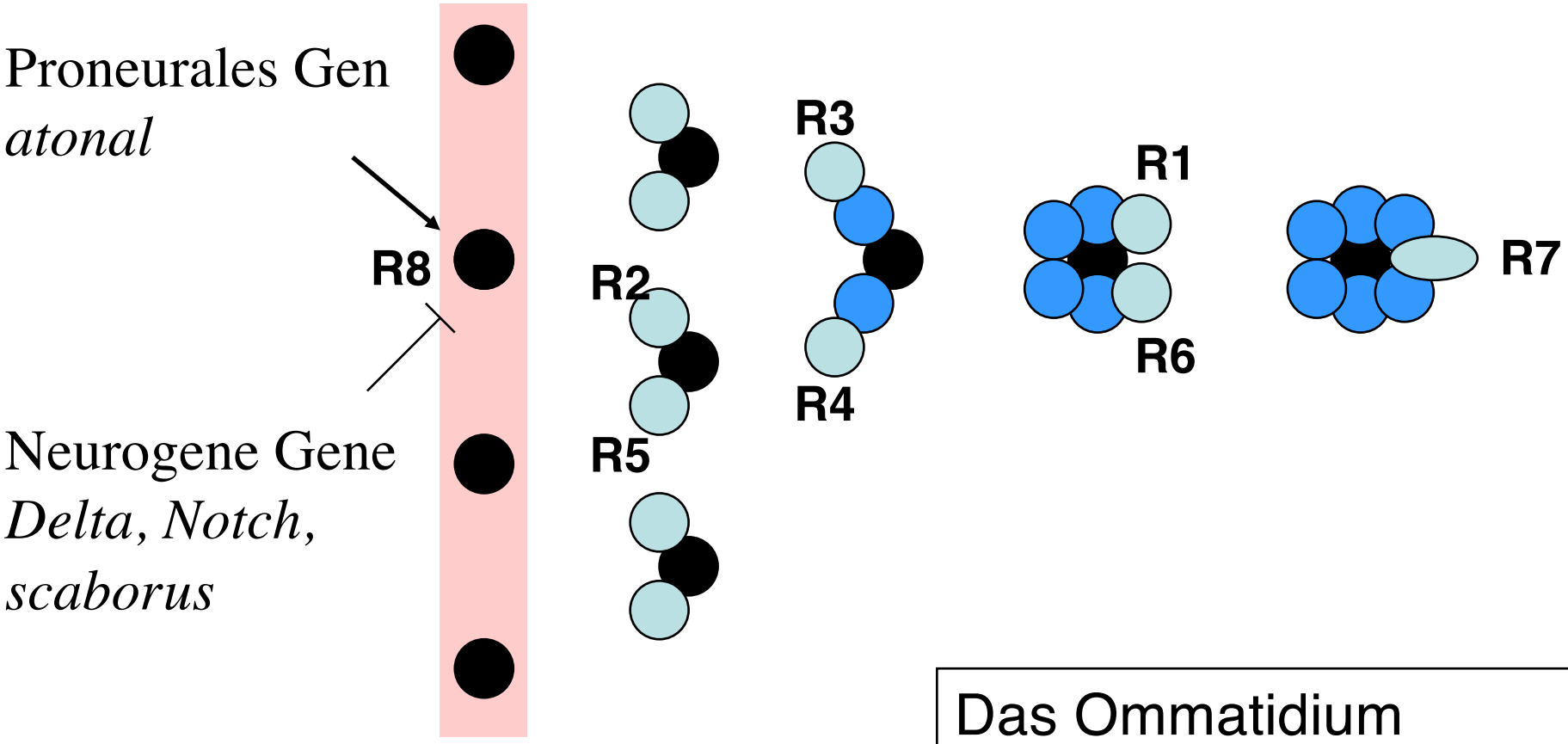
Das *Drosophila* Komplexauge





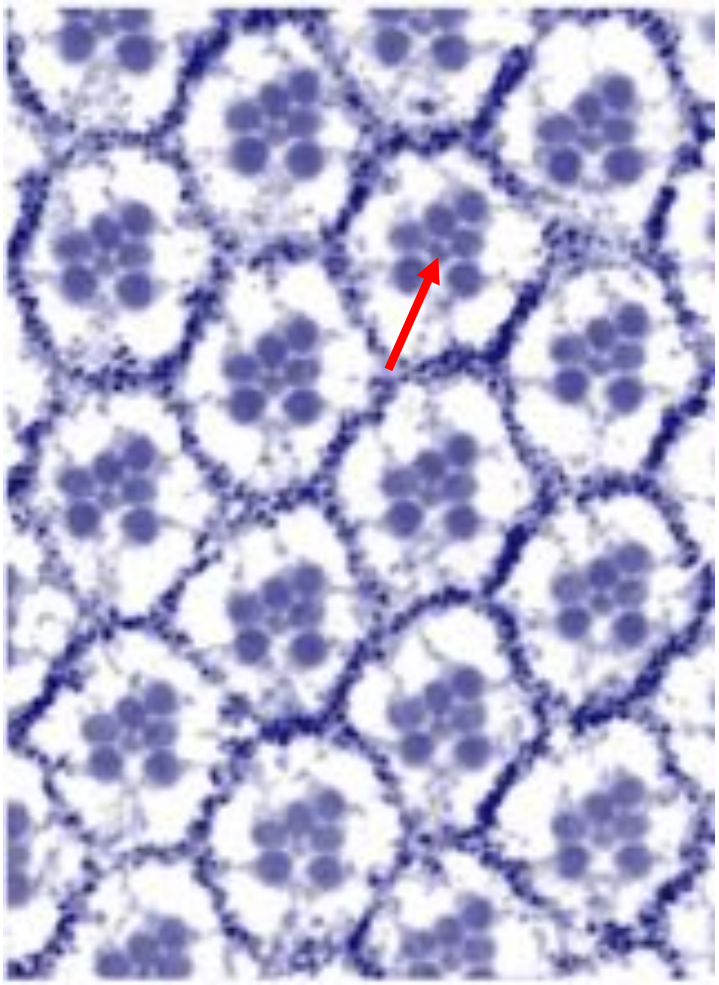
R8 **+R2,5** **+R3,4** **+R1,6** **+R7** +Cone cells

Photorezeptorzellen entwickeln sich sequentiell (R8 zuerst)

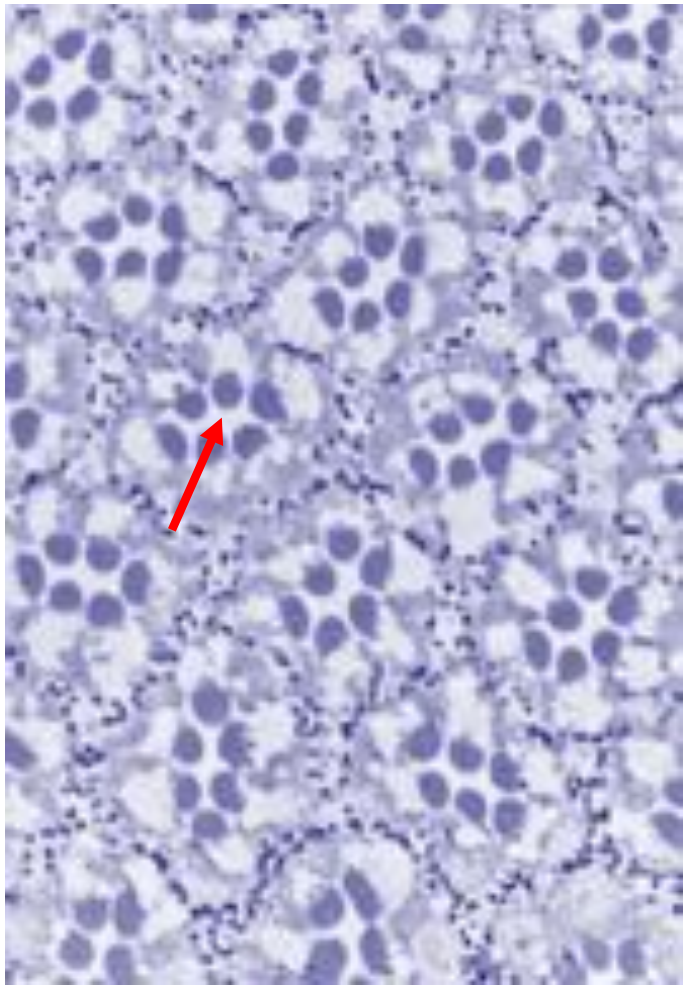


Das Ommatidium entsteht durch induktive Wechselwirkungen!!

Das Gen *sevenless* betrifft die Bildung der R7 Zelle

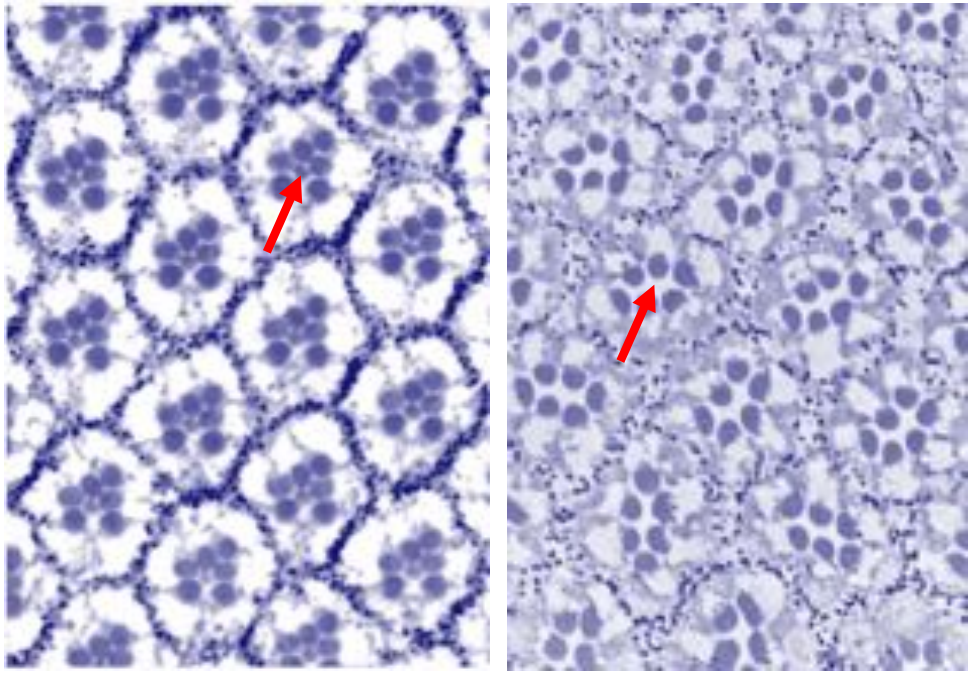


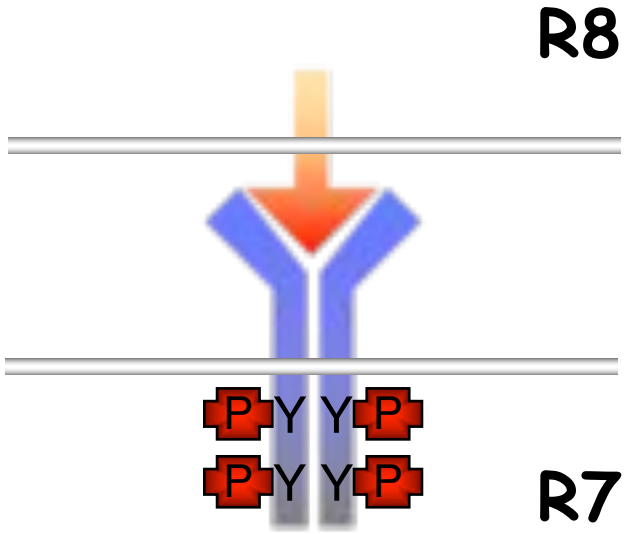
Wildtyp
R7-Zelle



sevenless^{LOF}
Keine R7-Zelle

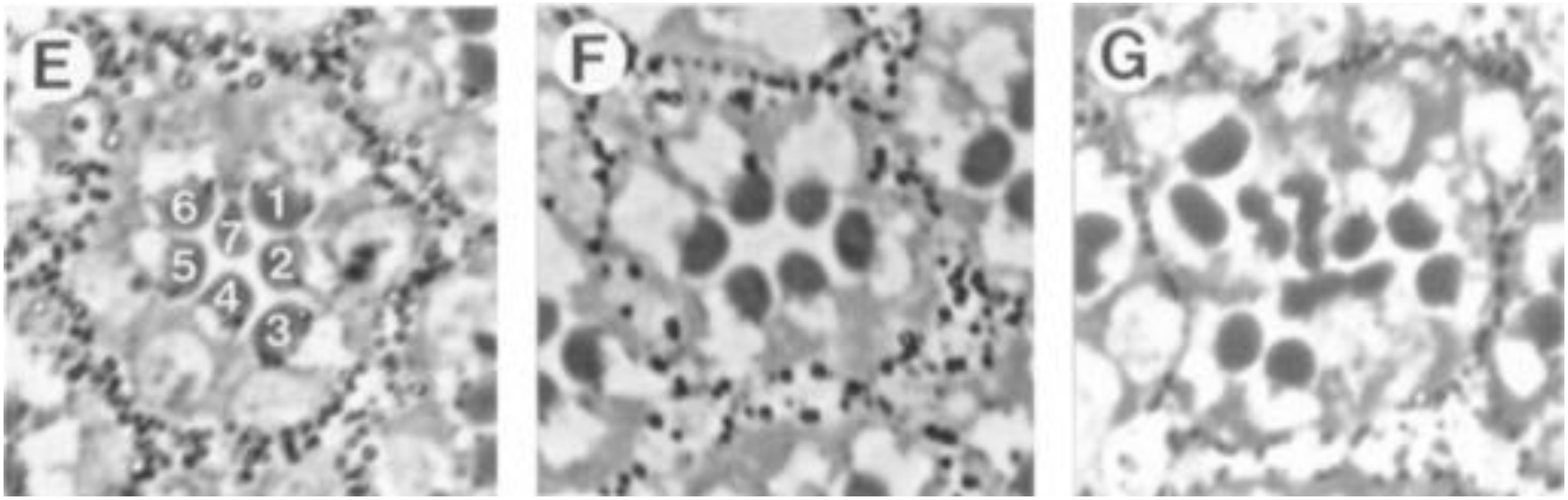
Die Aktivität der Sevenless Rezeptortyrosin Kinase kontrolliert die Bildung der R7 Zelle





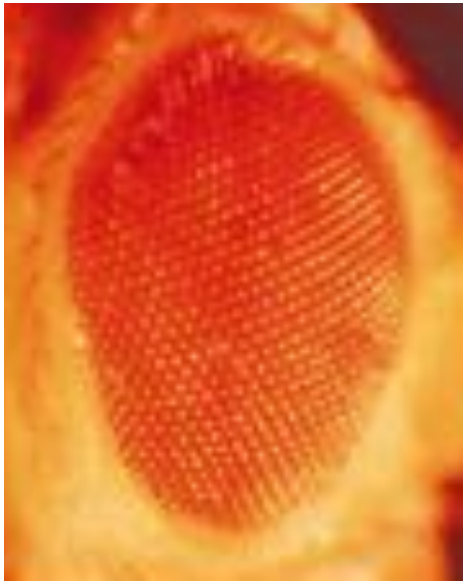
RTK Signaltransduktion

Die Aktivierung von Sevenless führt zur Bildung von zusätzlichen R7-Zellen

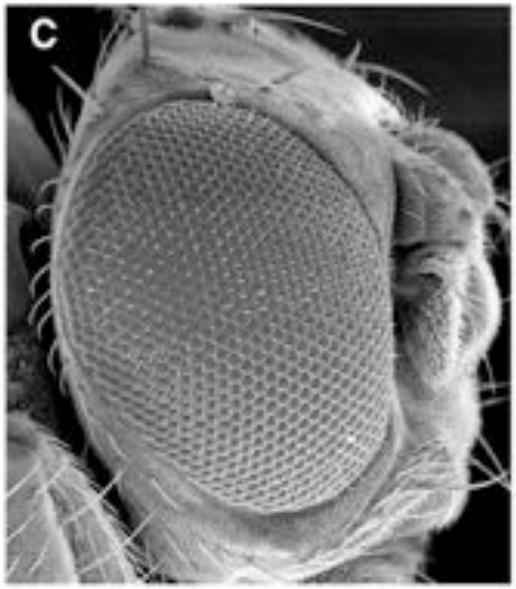


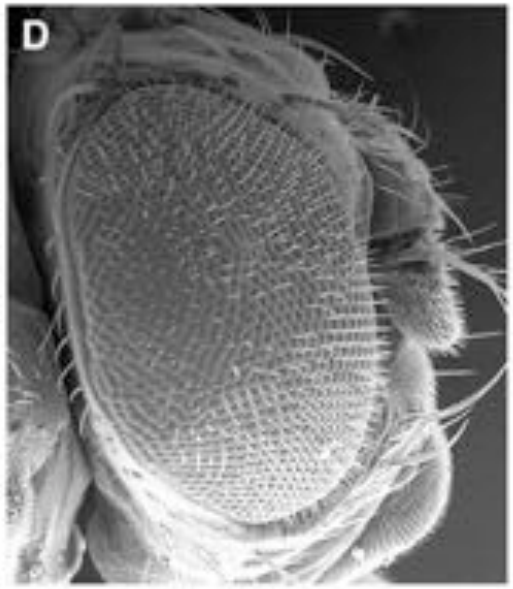
Wildtyp ***sevenless^{LOF}*** ***sevenless^{GOF}***

Die Aktivierung von Sevenless führt zur Bildung von „rauhen“ Augen



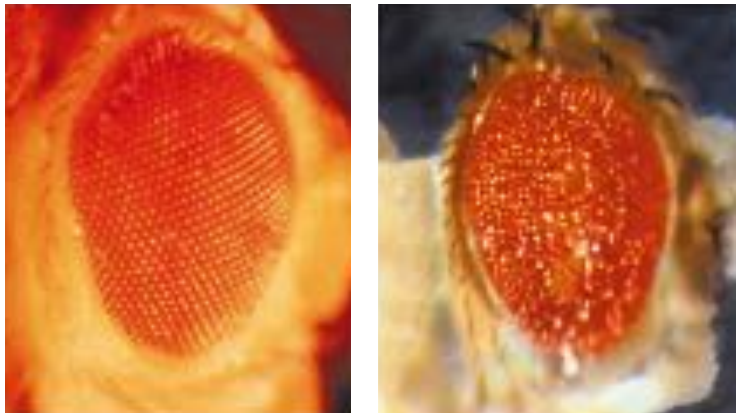




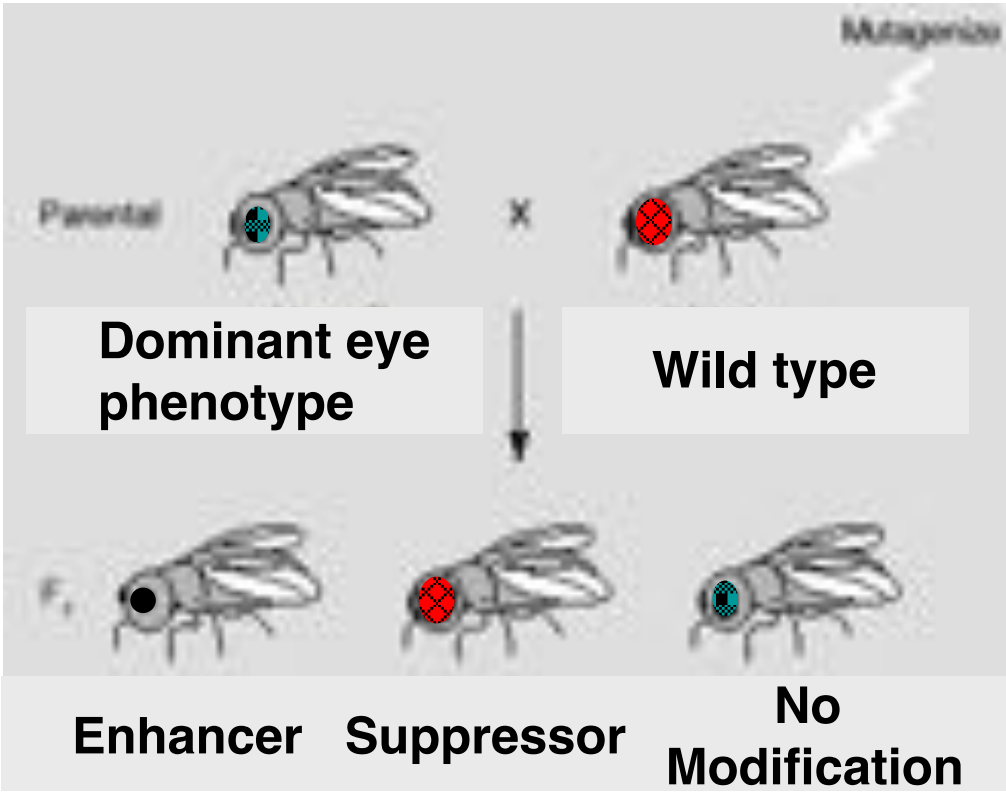


Wildtyp ***sev^{GOF}***

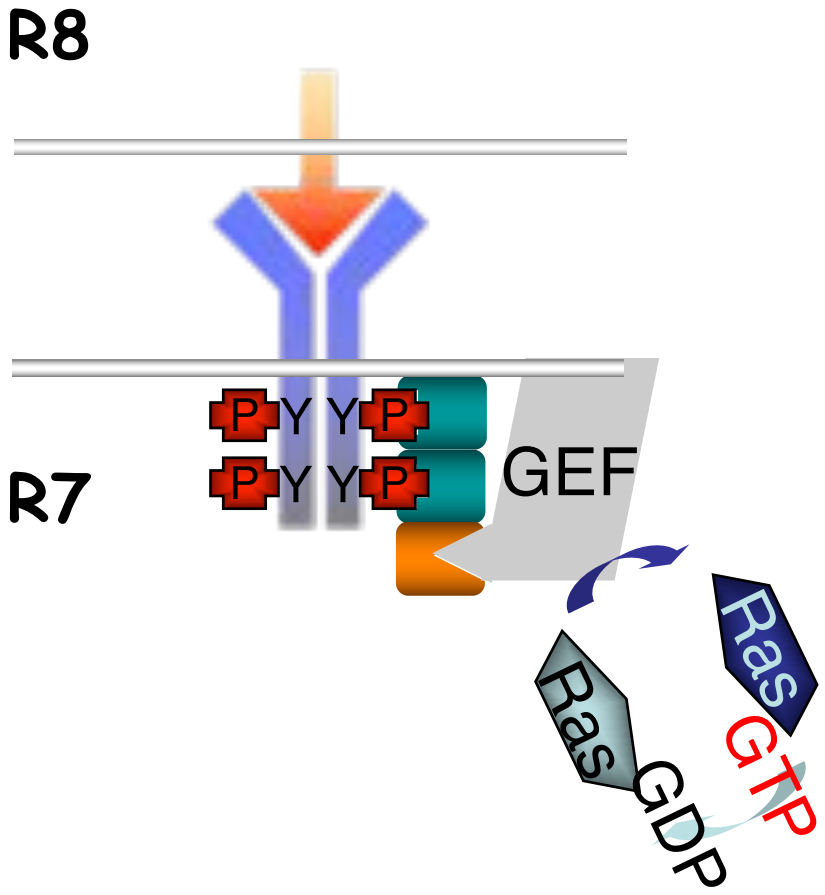
Die Suppression des Sev^{GOF} Phänotyps führte zur Aufdeckung der RTK-Signalkaskade

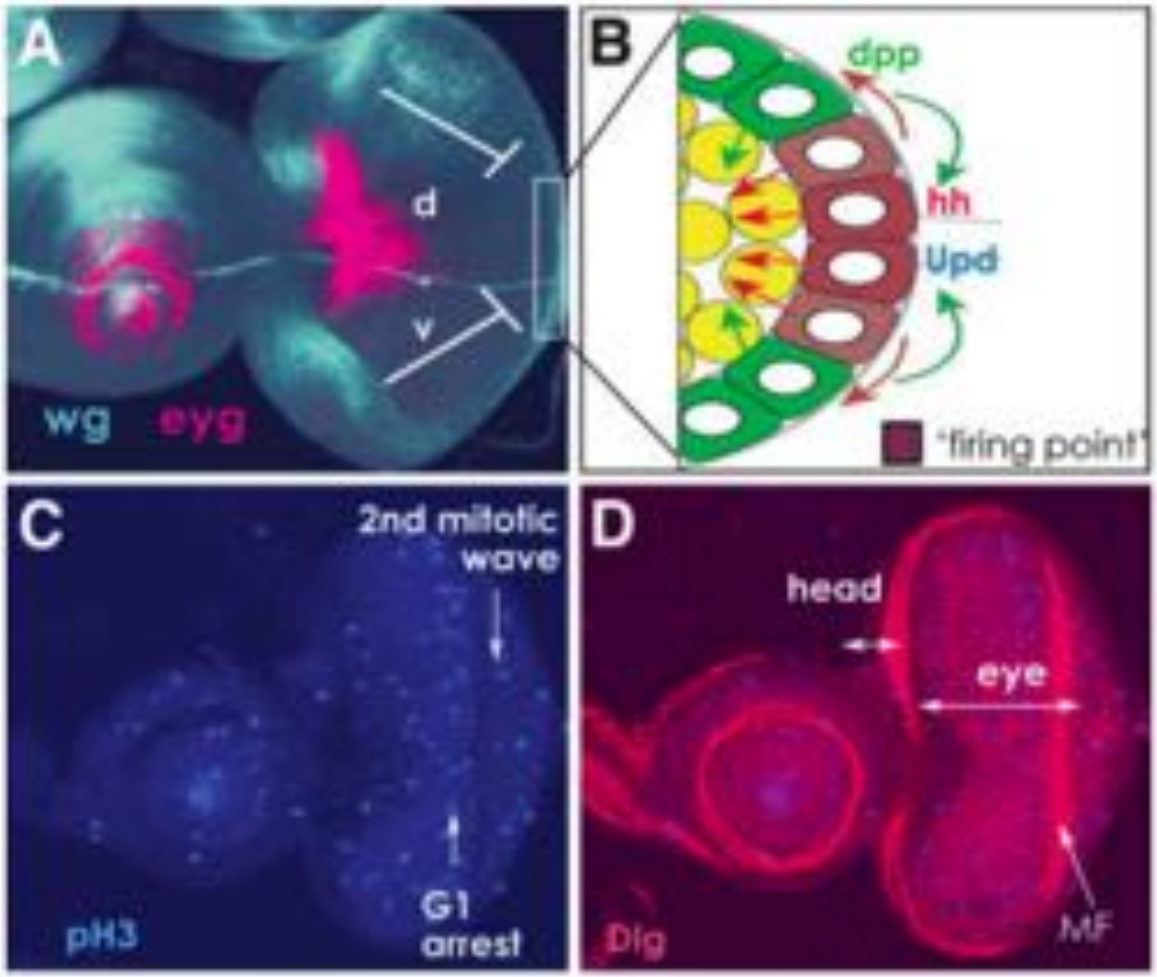


Wildtyp ***sev^{GOF}***

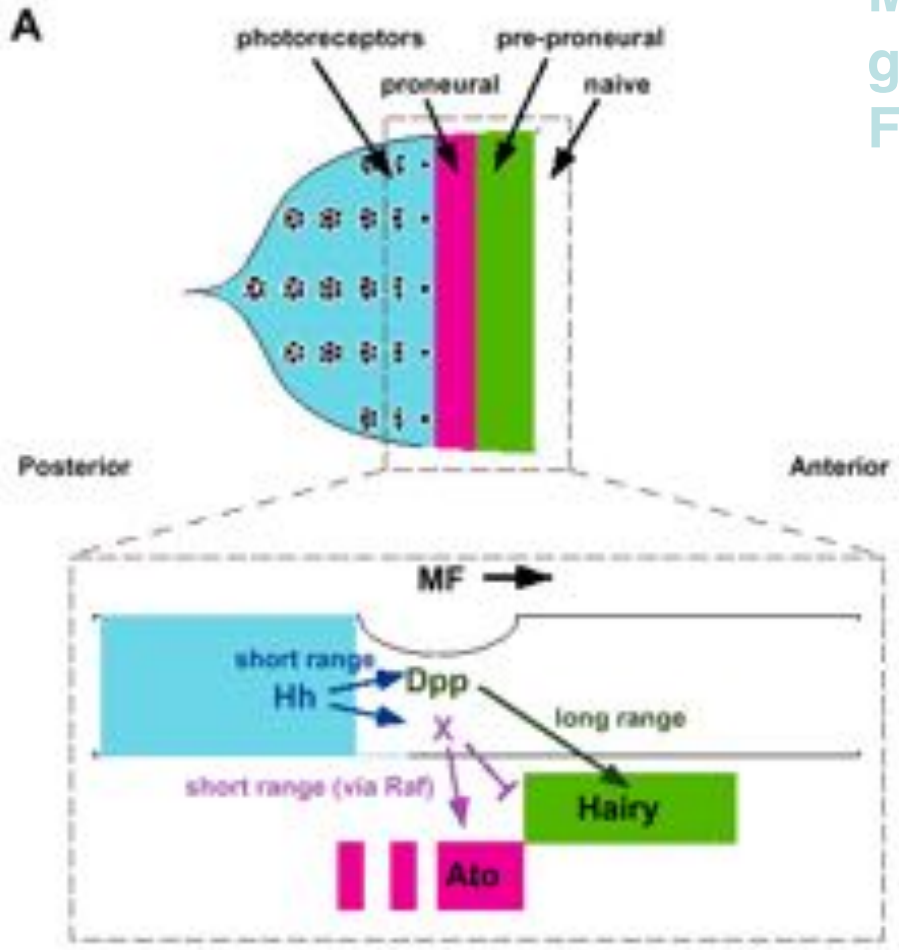


Die Suppression des Sev^{GOF} Phänotyps führte zur Aufdeckung der RTK-Signalkaskade



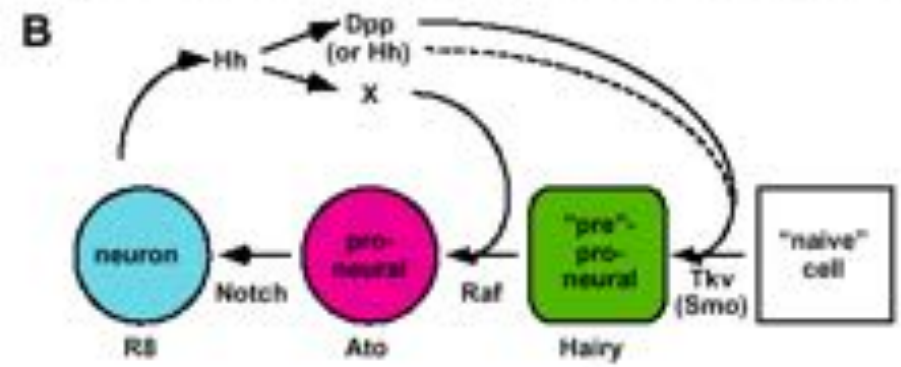

A
d
v
wg
eyg
B
dpp
hh
Upd
"firing point"
C
2nd mitotic wave
G1 arrest
pH3
D
head
eye
MF
Dlg

Die Differenzierungswelle in der Augenscheibe



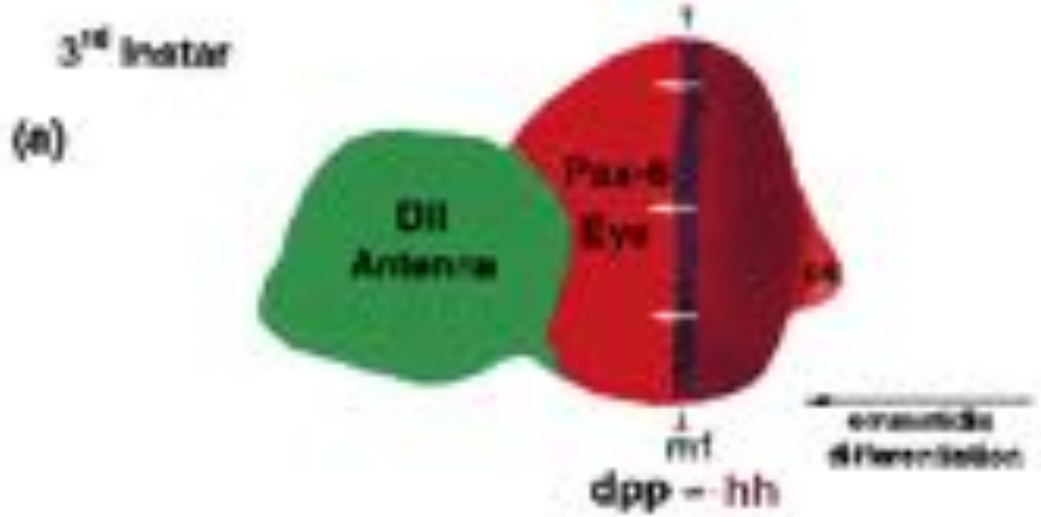
Morpho-genetische Furche

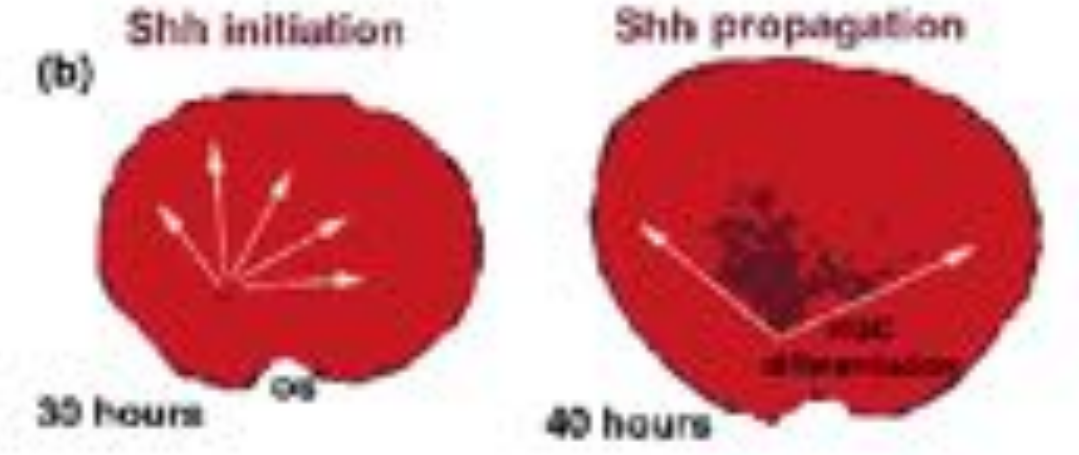




Hedgehog Signalweg

Die Funktion von *hedgehog* scheint konserviert

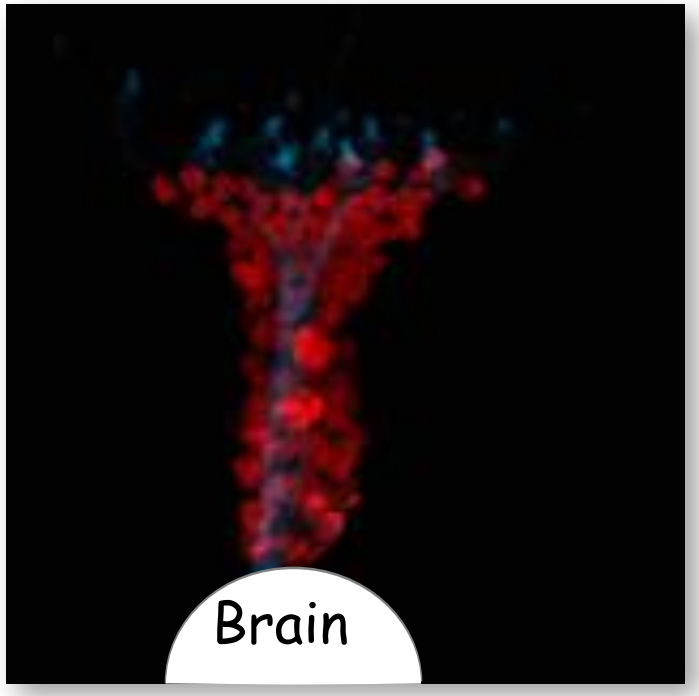


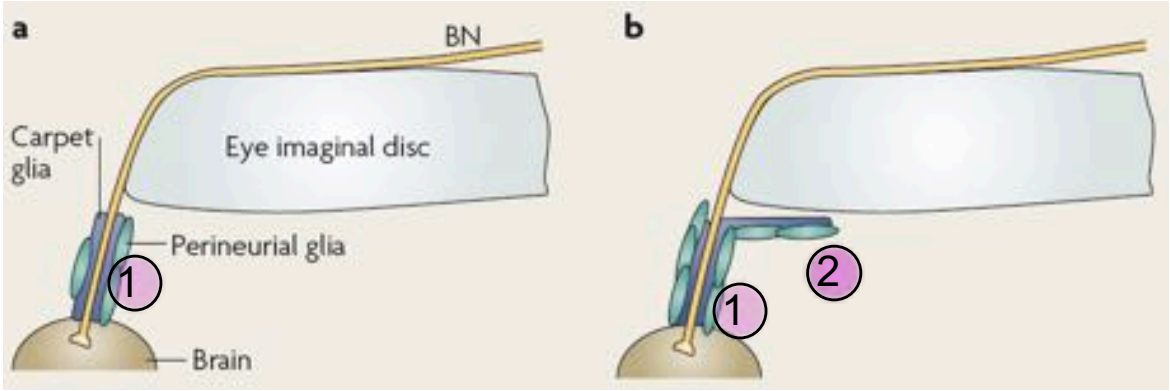


Neurone und Gliazellen in der Augenimaginalscheibe



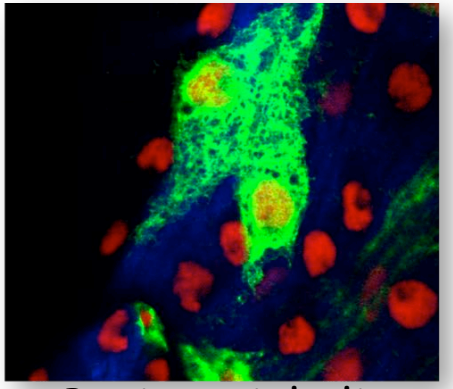
Glial cell migration onto the eye disc





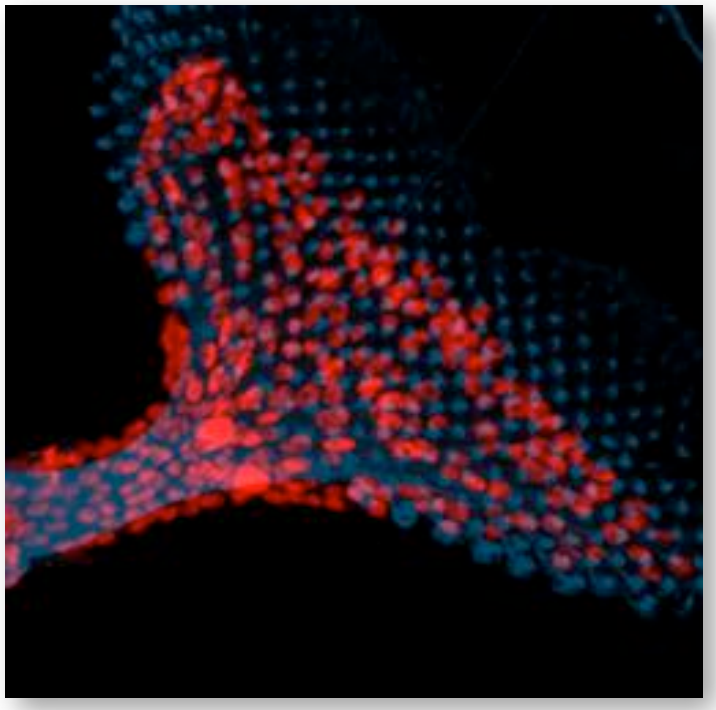
1 Birth

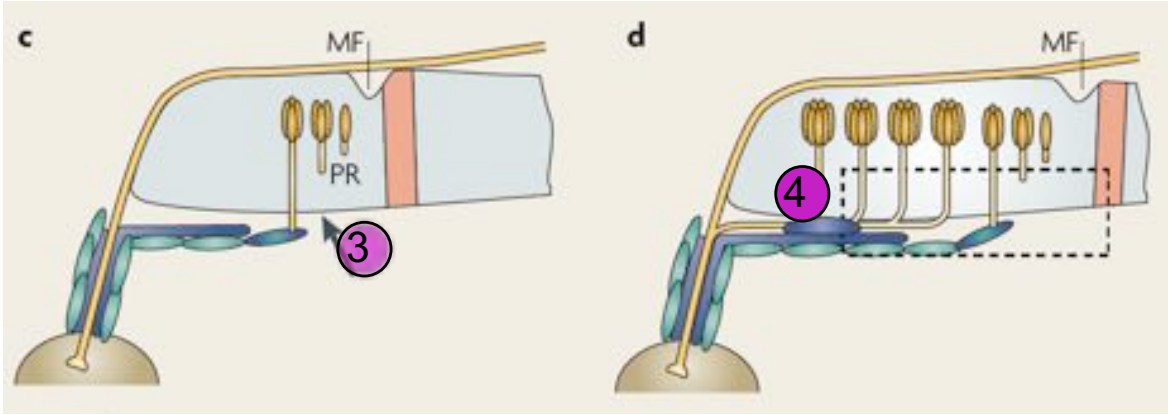
2 Migration



Perineurial glia

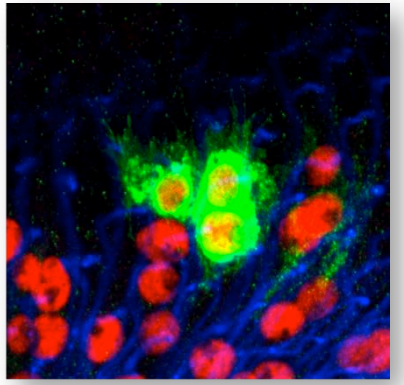
Glial cell migration onto the eye disc

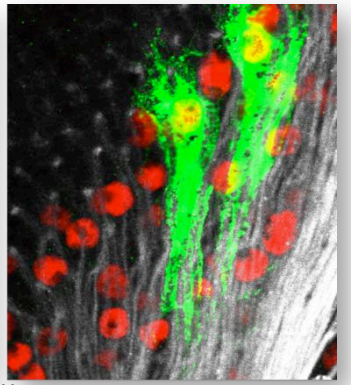




3 First contact with axons, glial cells become competent

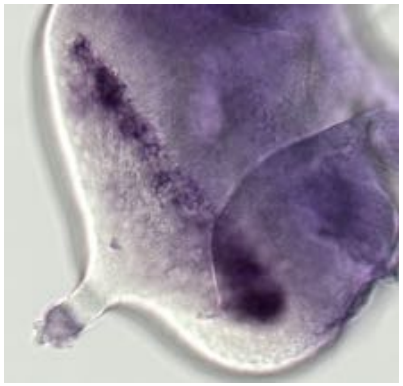
4 Differentiation, wrapping



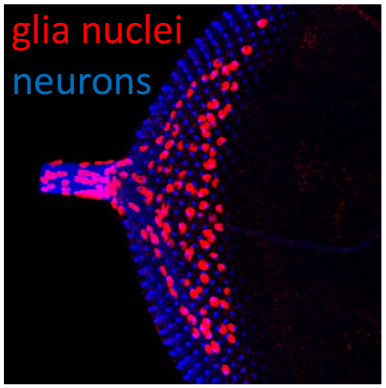


„Competence zone“ Wrapping glia

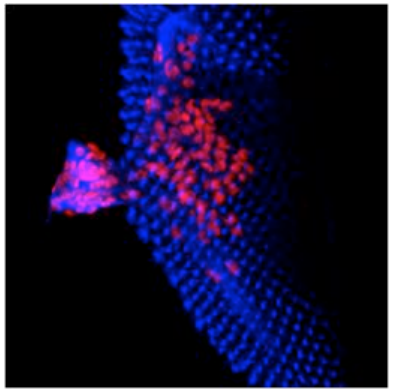
Der FGF-Rezeptor (RTK) kontrolliert die gliale Proliferation und Differenzierung



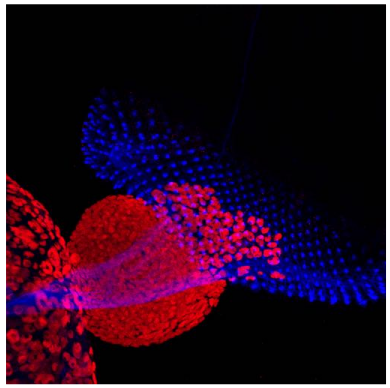
wild type



panglial>>FGFR^{DN}

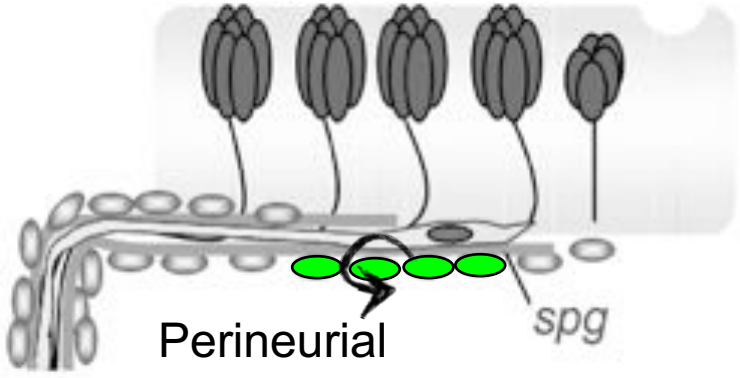


panglial>>FGFR^{act}





FGF-Receptor



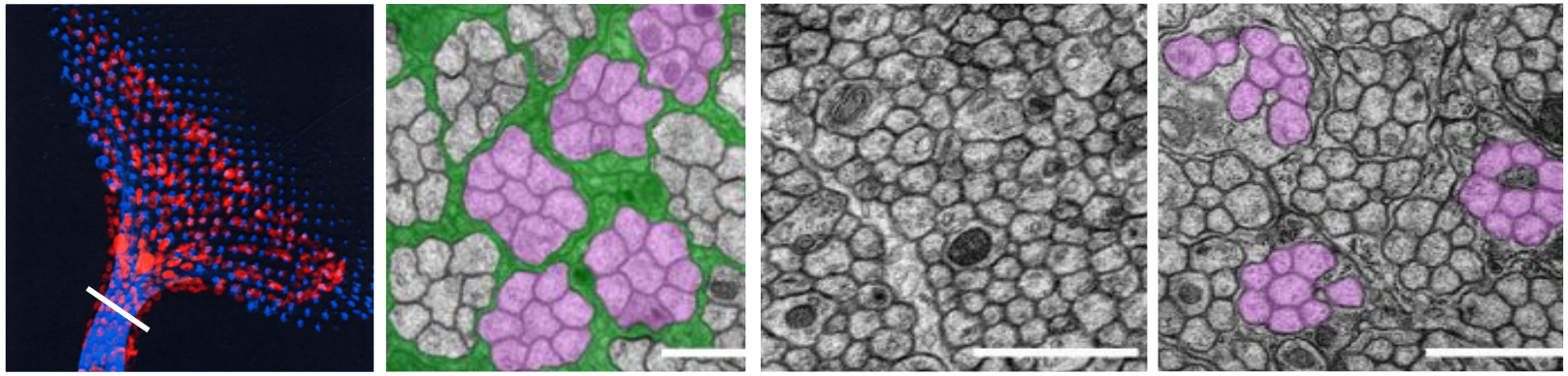
FGF ligand provided by the *spg*

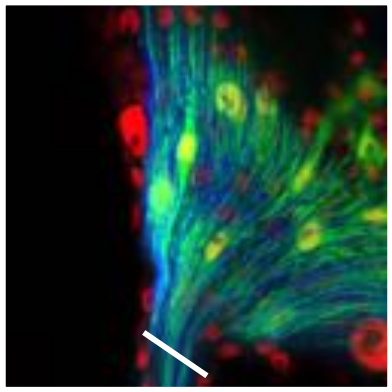
Der FGF-Rezeptor (RTK) kontrolliert die gliale Proliferation und Differenzierung

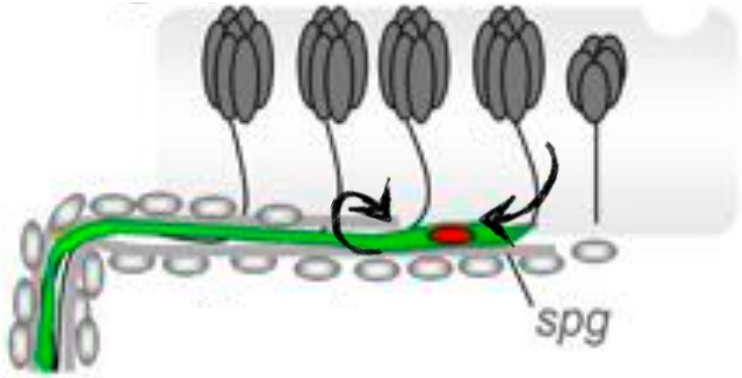
wild type

wrapping glial >>FGFR^{DN}

wrapping glial >>FGFR^{act}

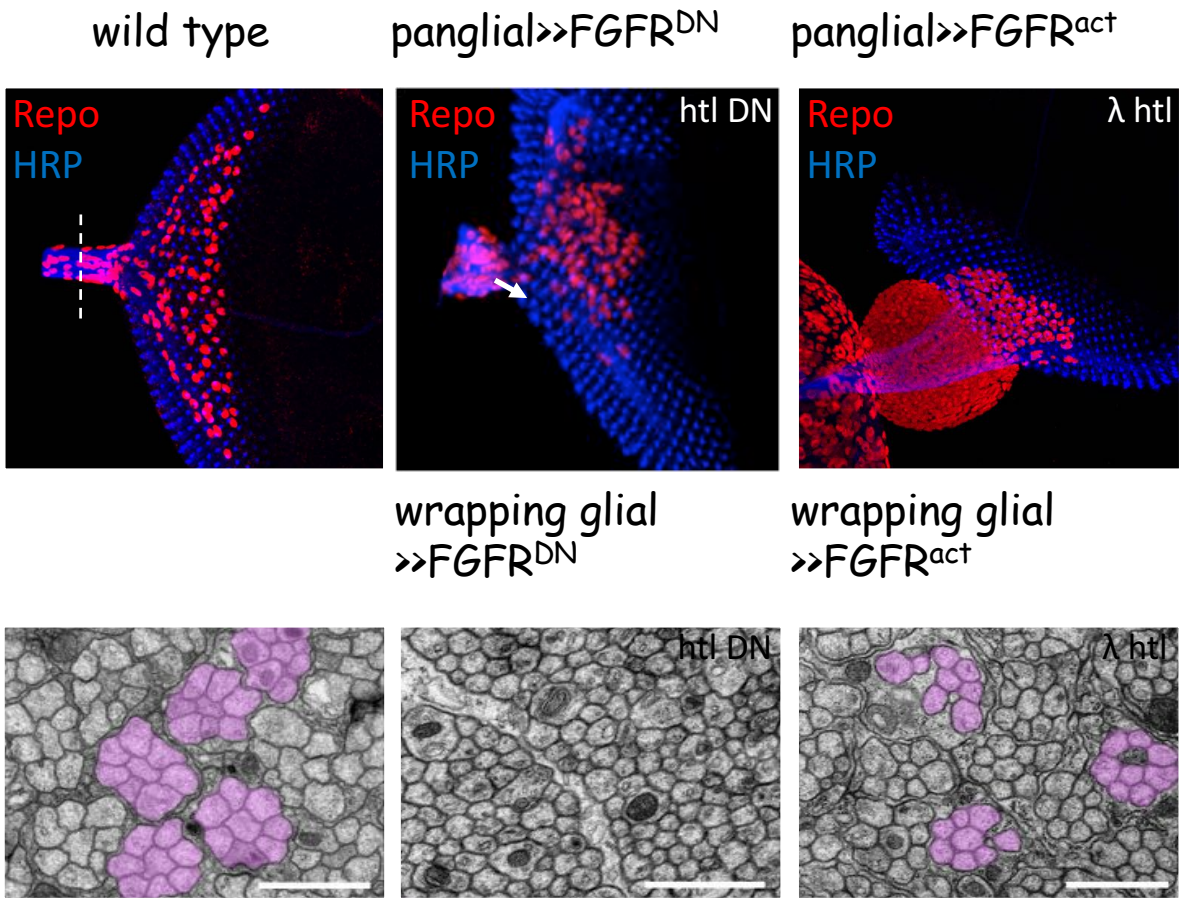


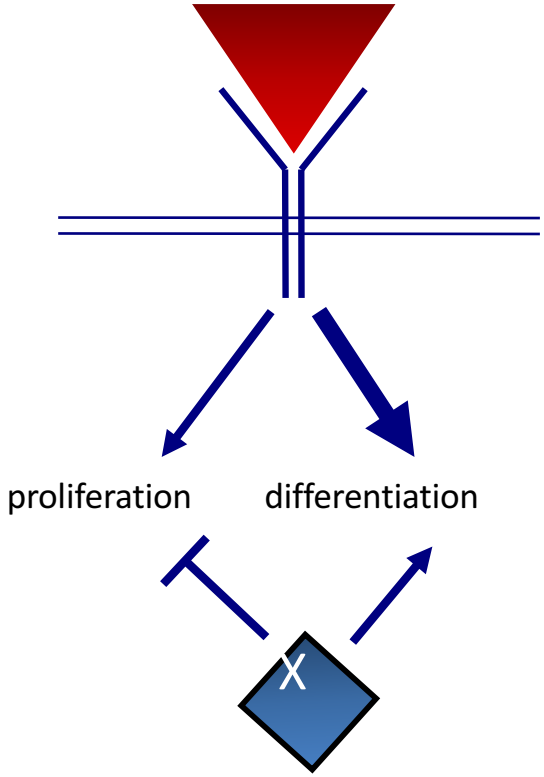




FGF ligand provided by the *spg* and sensory neurons

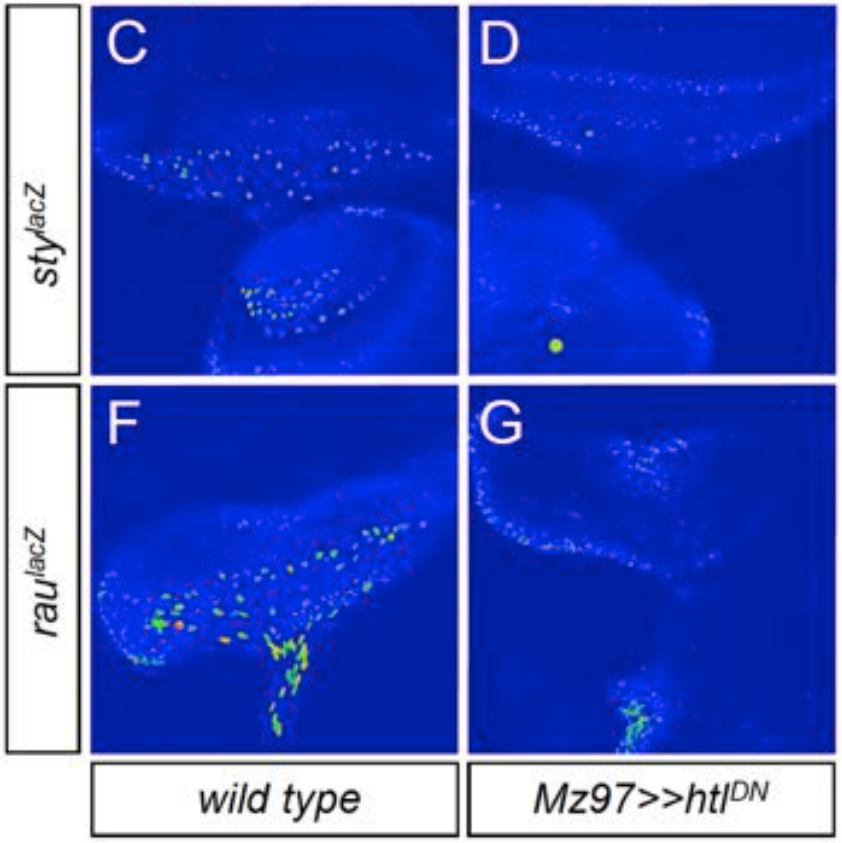
Wie kann ein Rezeptor zwei unterschiedliche Dinge steuern?



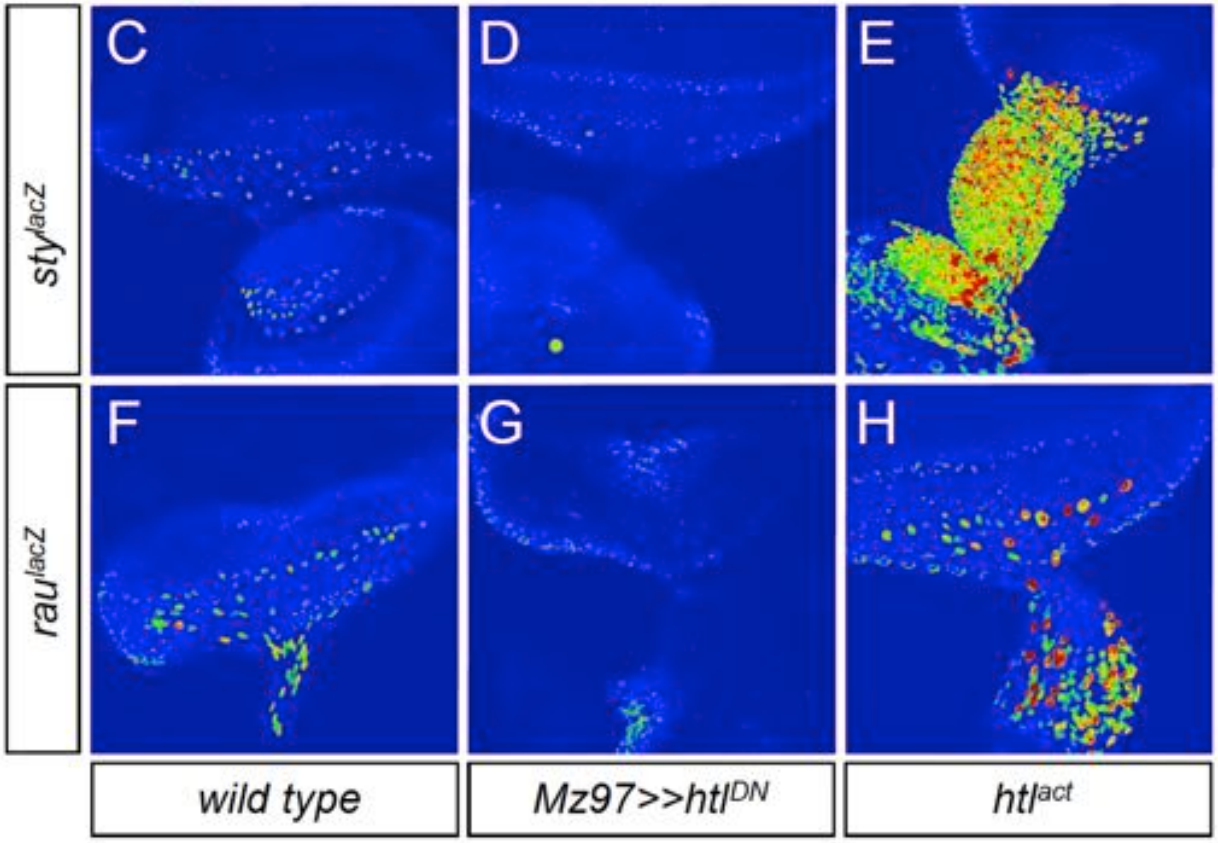


Additional factors in wrapping glia

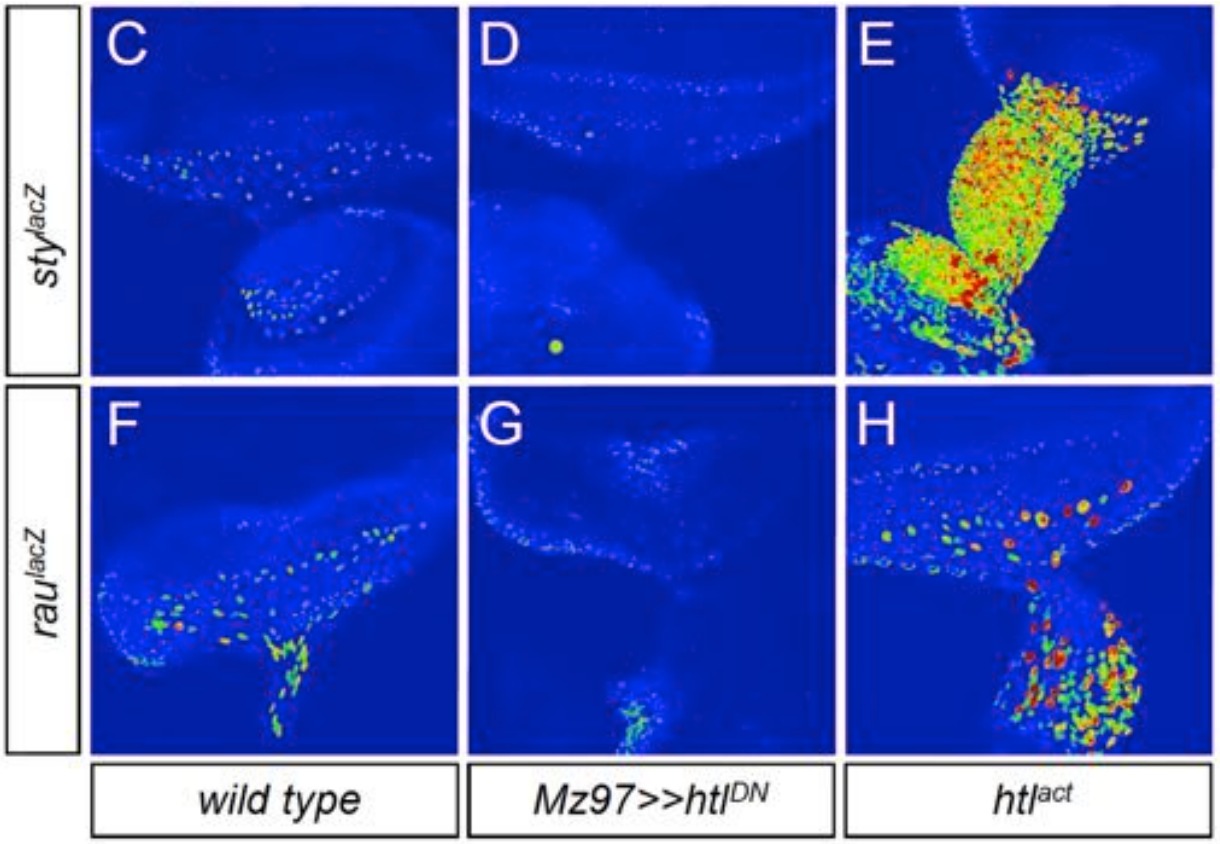
Gene mit spezifischer Expression, die abhängig von der Aktivität des FGF-Rezeptors sind

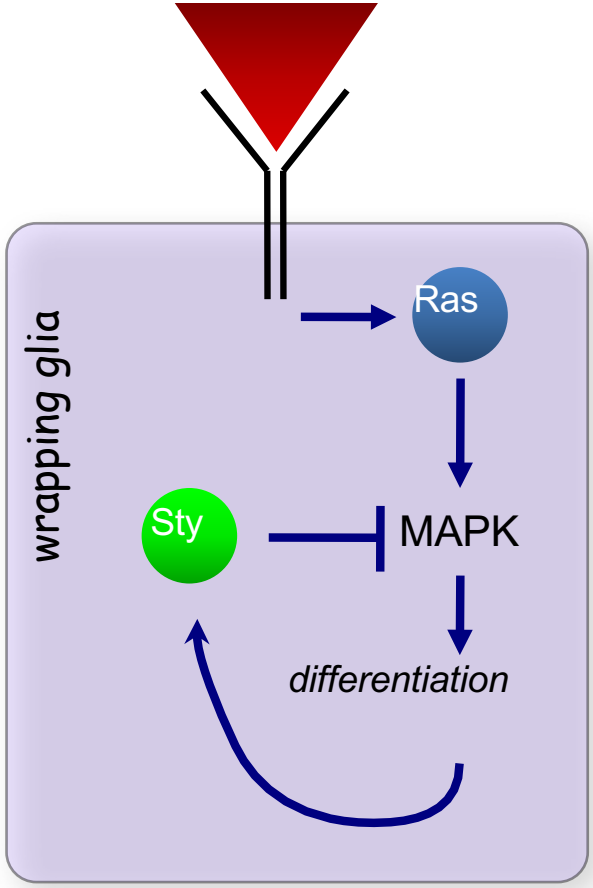


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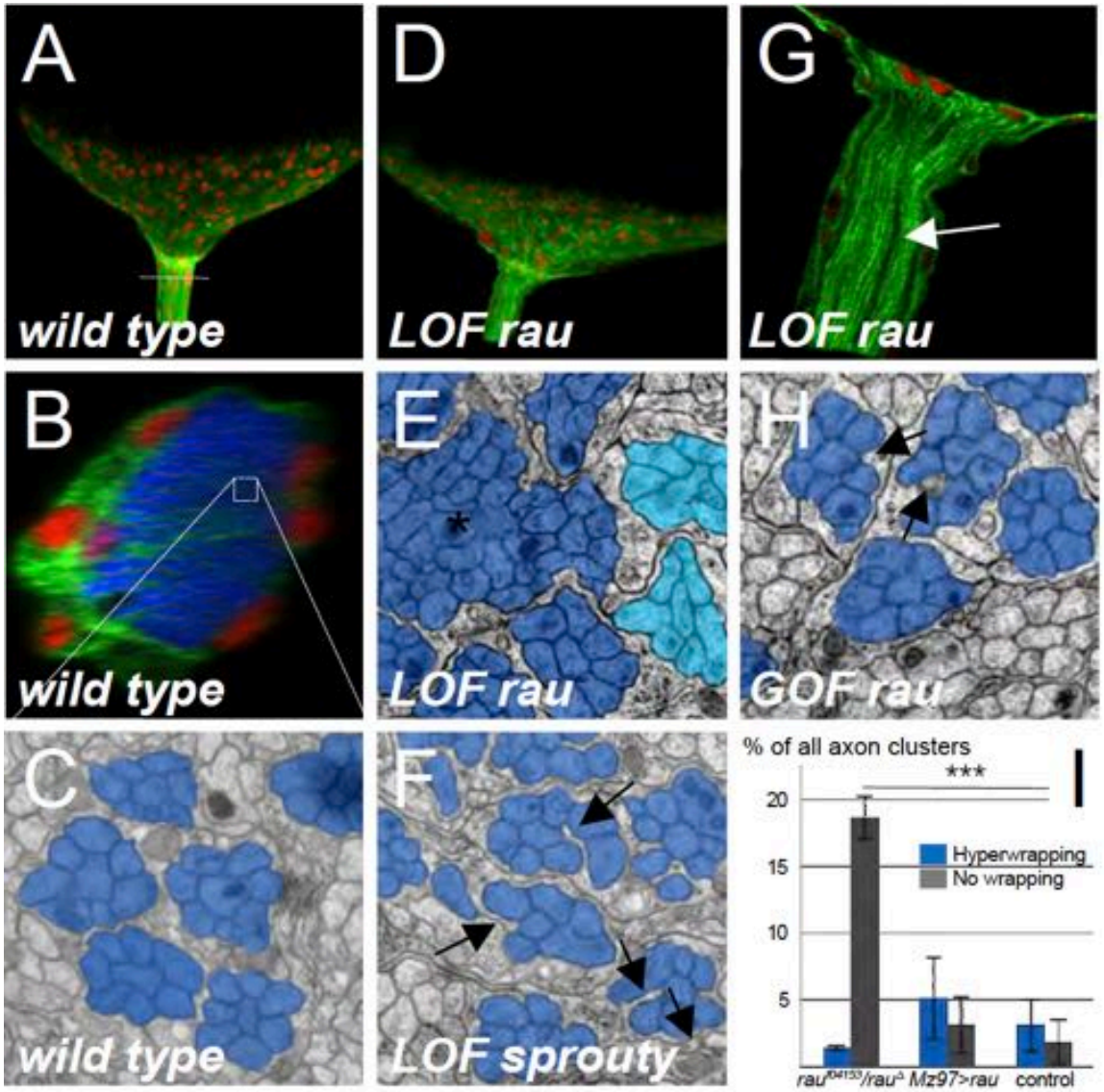


Sprouty ist ein negativer Feedback Regulator des FGF-Rezeptors





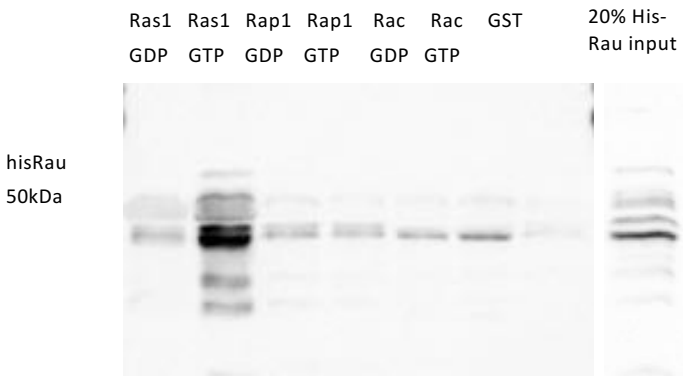
Rau ist ein positiver Feedback Regulator des FGF-Rezeptors



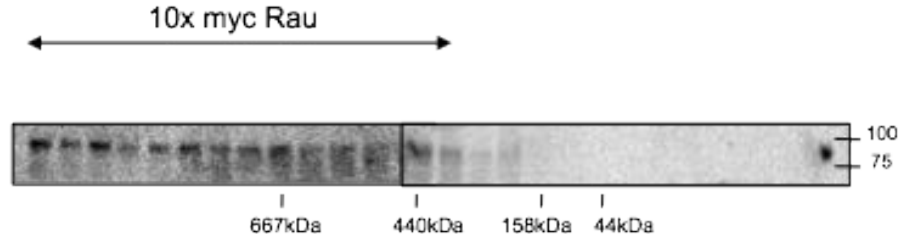
Rau ist ein positiver Feedback Regulator des FGF-Rezeptors



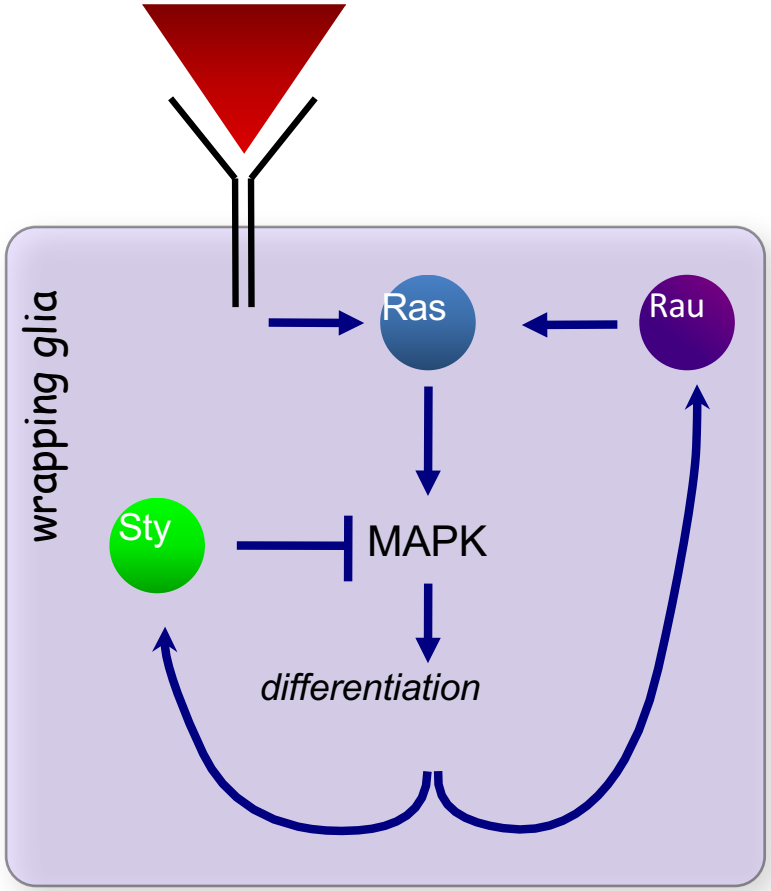
Ras pulldown of his-Rau:



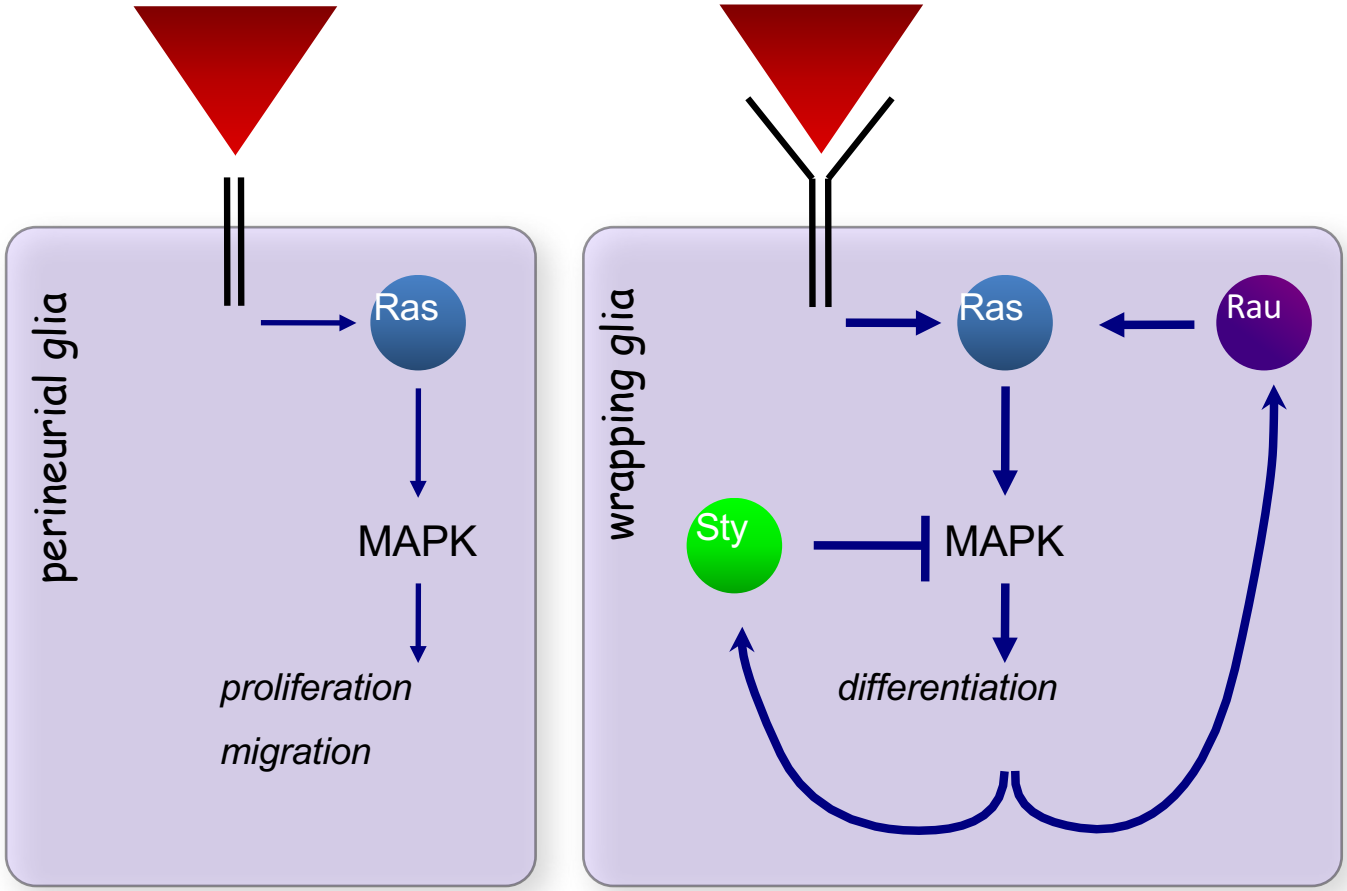
Ras protein input: 30µg



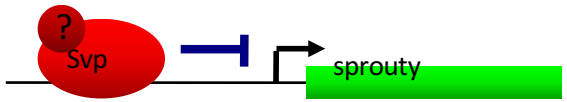
Gel filtration



Rau ist ein positiver Feedback Regulator des FGF-Rezeptors

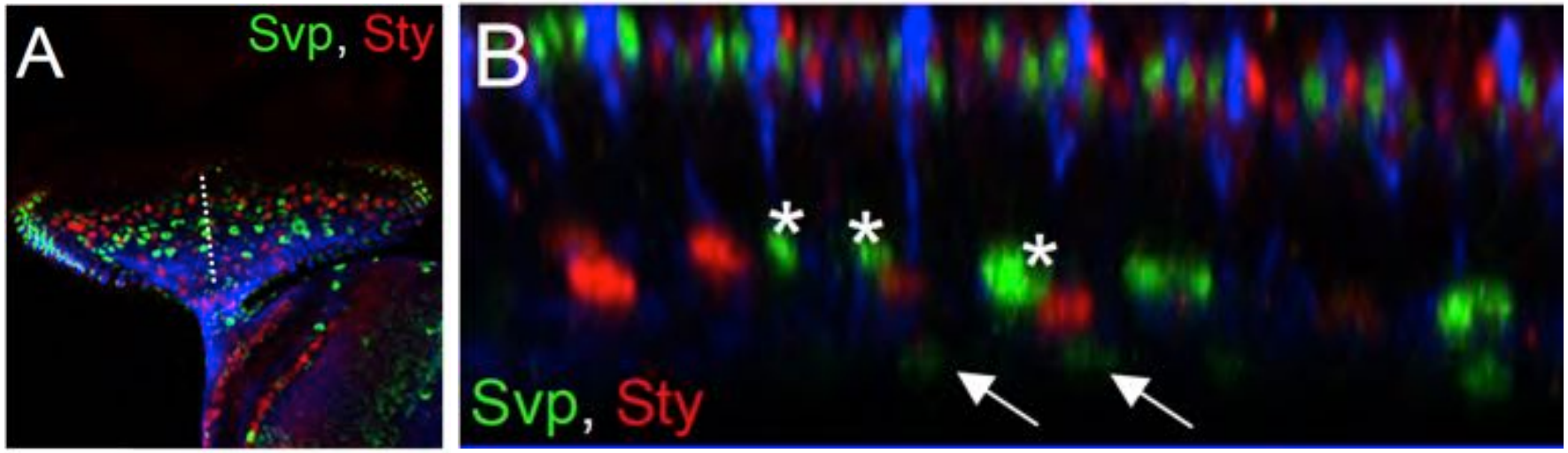


Wechselwirkung von Rau und Sprouty

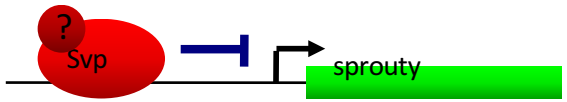


(Iwanami et al, 2005)

During photoreceptor development Sprouty is inhibited by the orphan hormone receptor Seven-up (Svp)

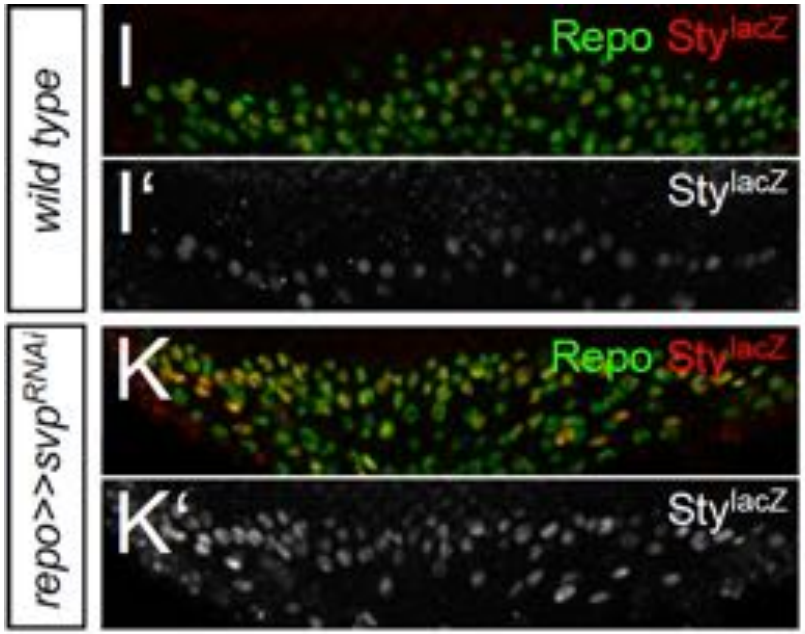


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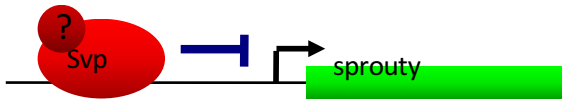


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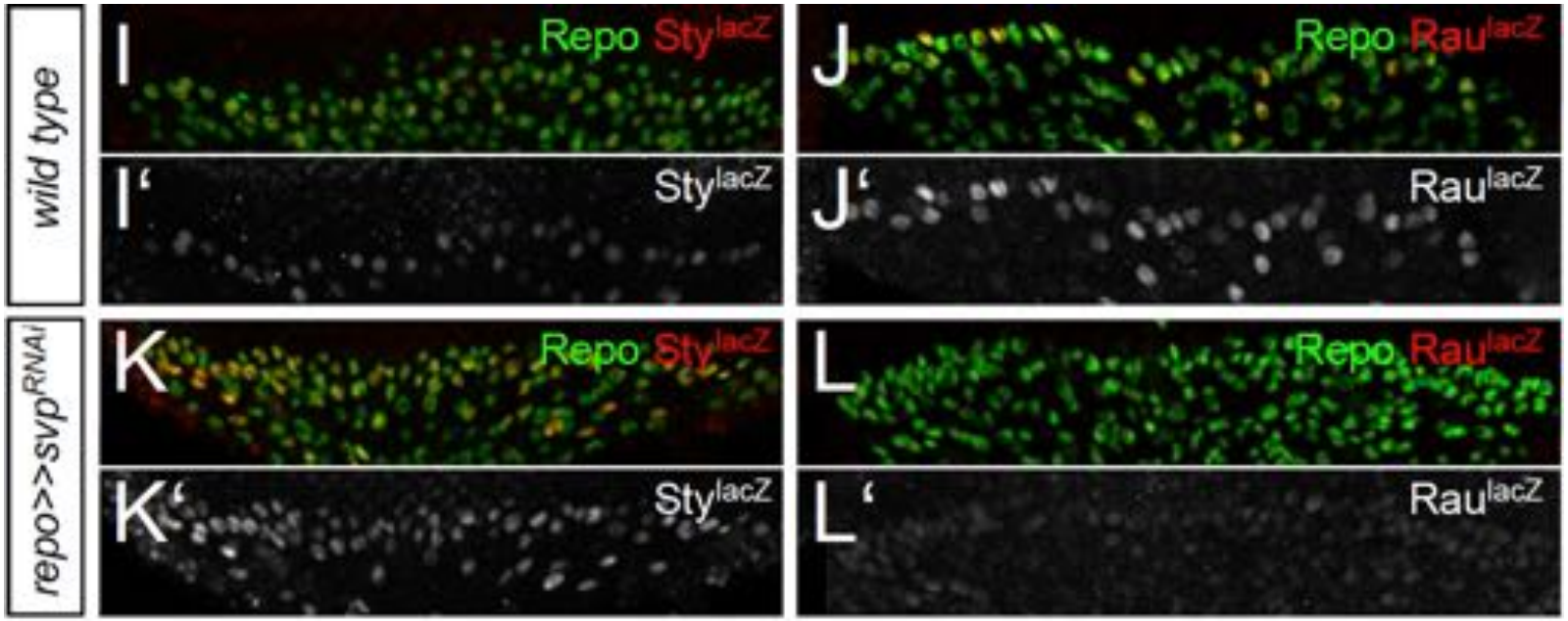


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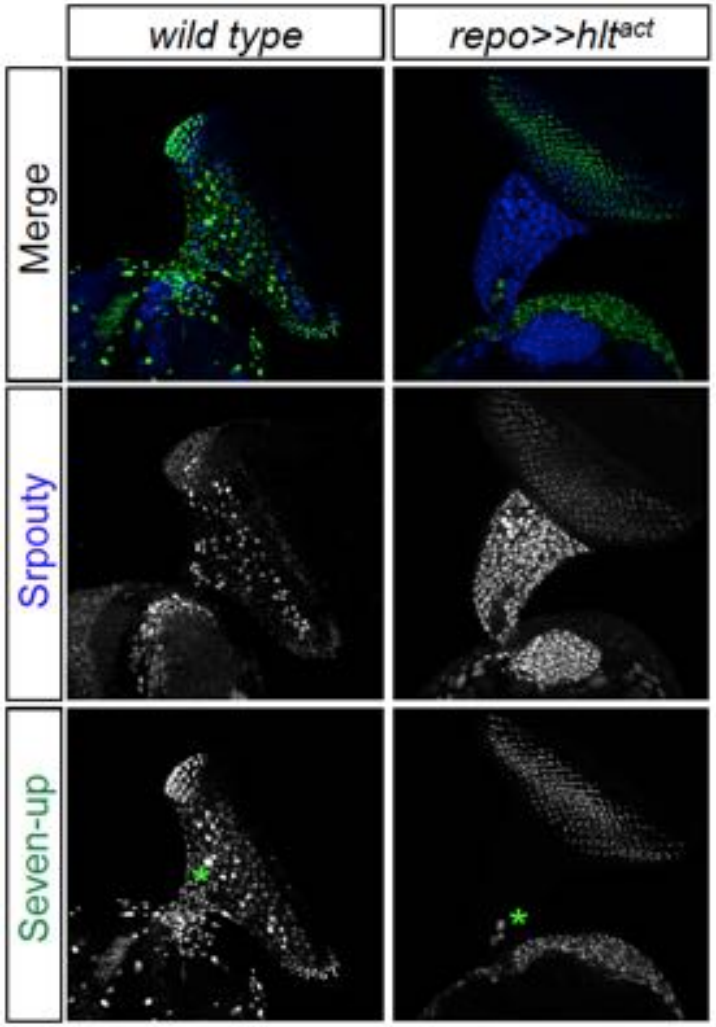


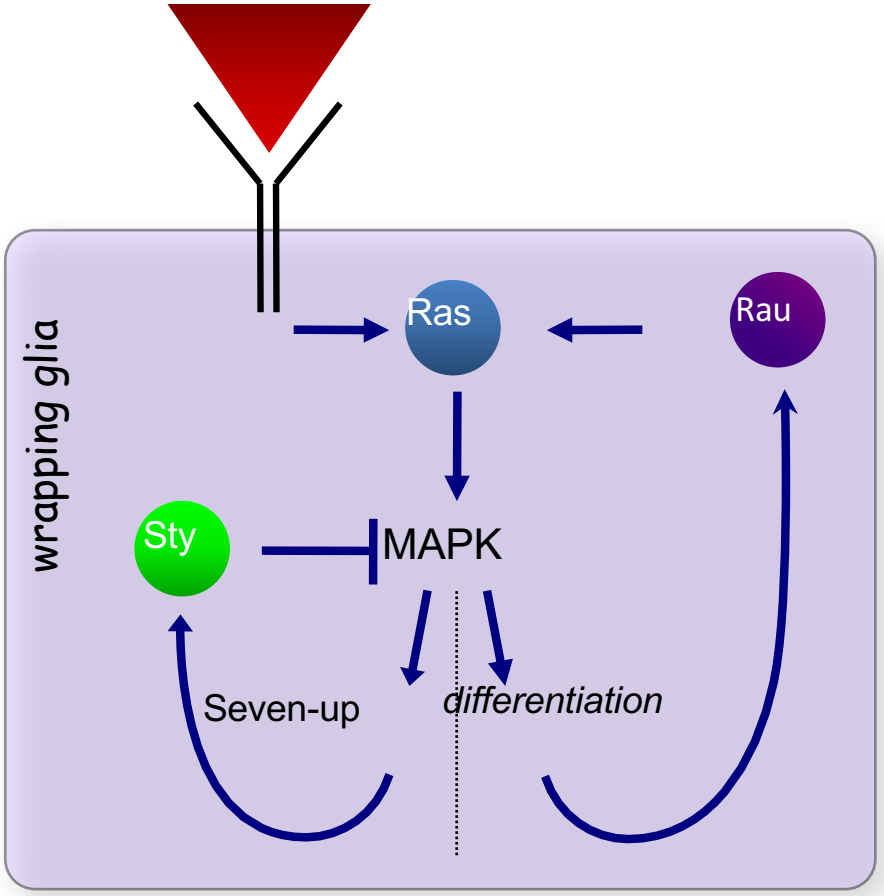
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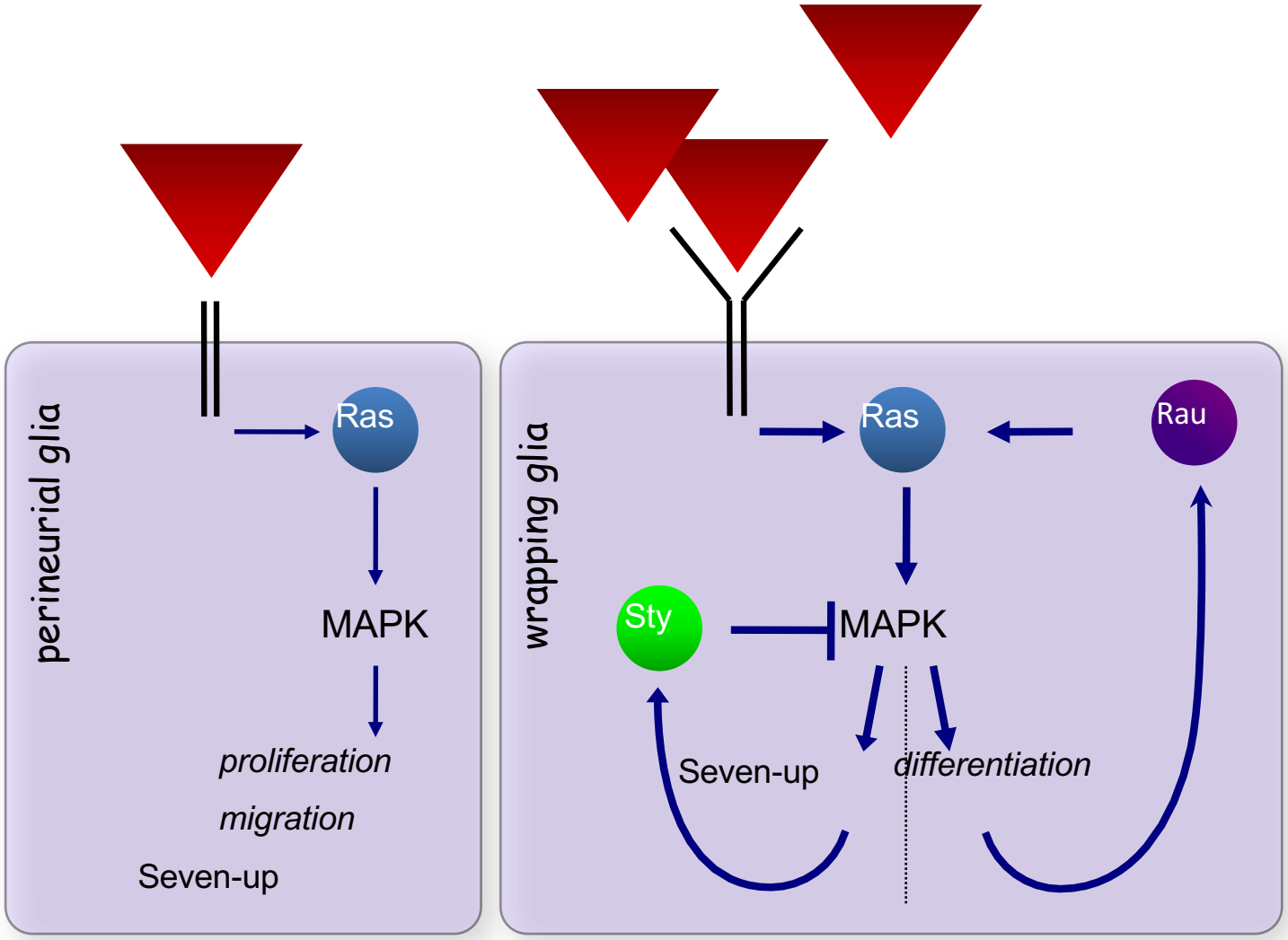


Modell


wild type
repo>>hlt^act
Merge
Srpouty
Seven-up

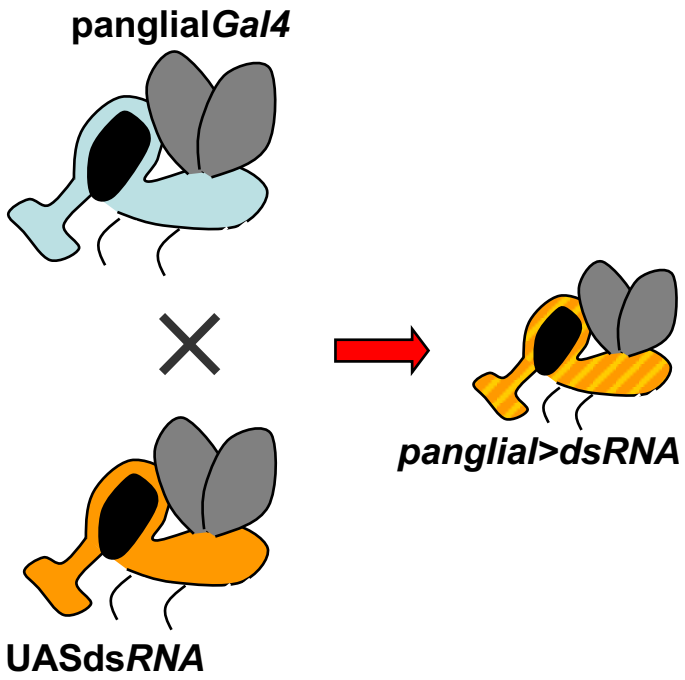

wrapping glia
Ras
Rau
Sty
MAPK
Seven-up
differentiation

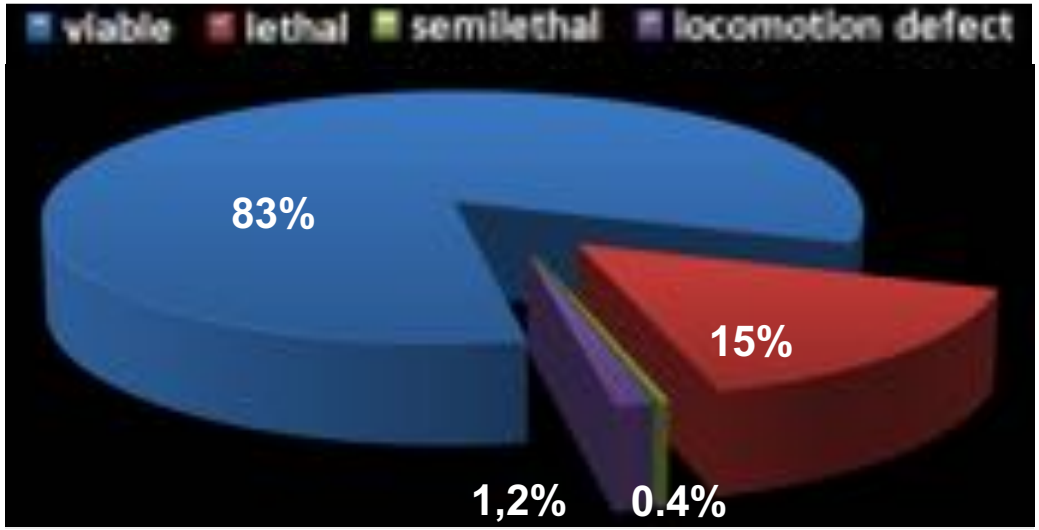
Modell



What determines proliferation and what differentiation?

RNAi Screen um weitere Gene zu finden, die an der glialen Differenzierung beteiligt sind

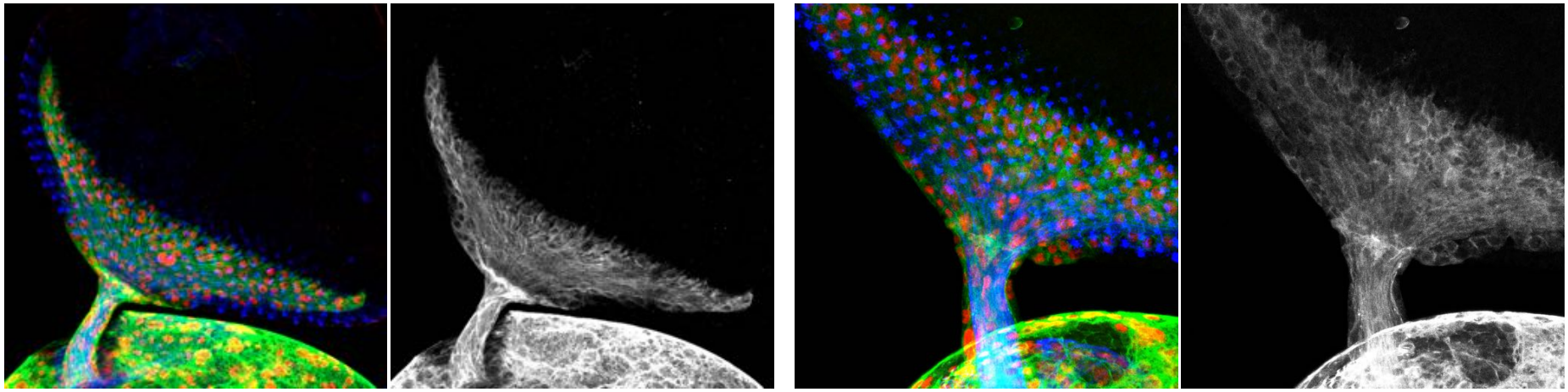


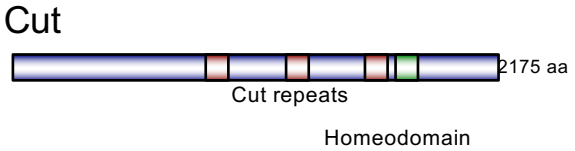


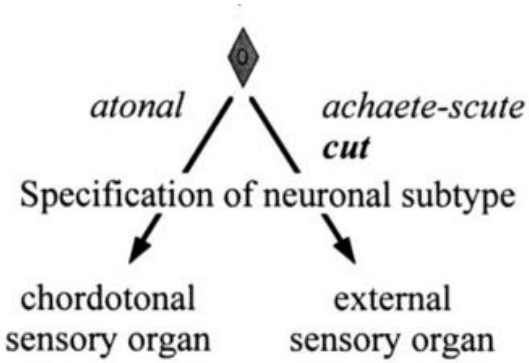
Confocal analysis of the knockdown phenotypes of all genes encoding transcriptional regulators (ca. 900)

Transkriptionsfaktor Cut:

loss of function Analyse (RNAi)

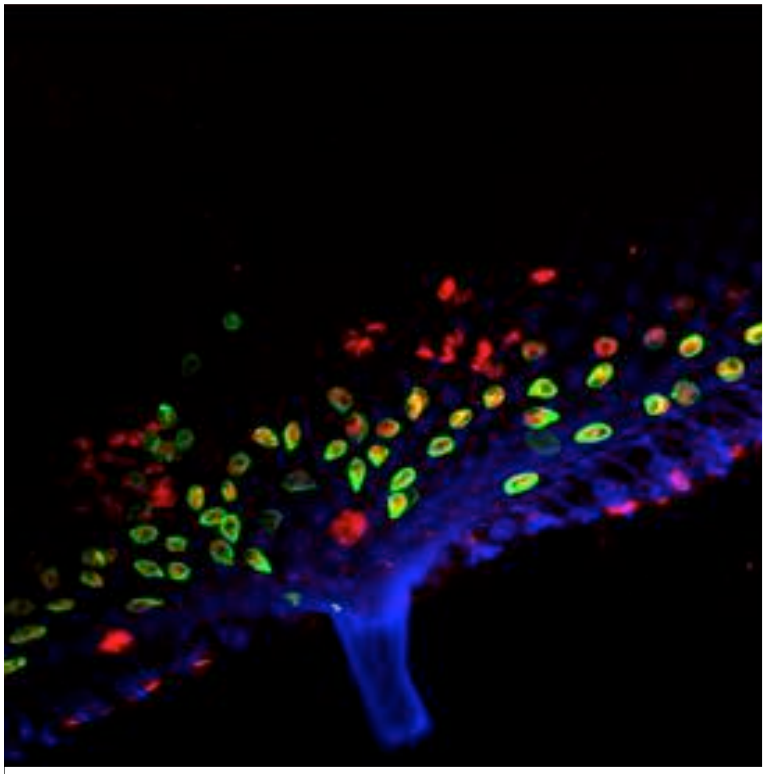


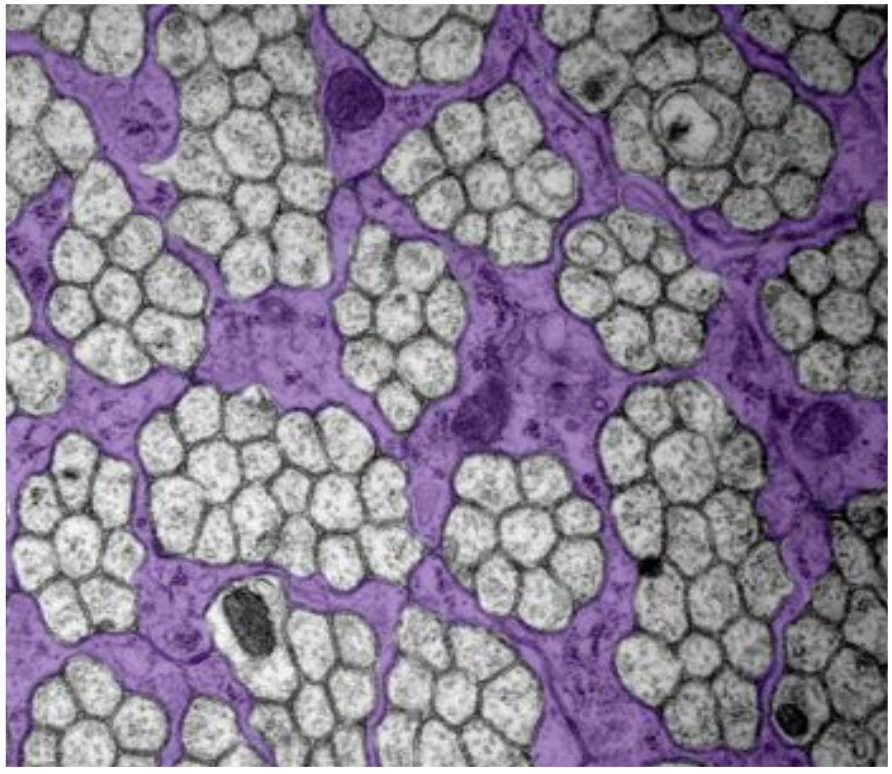




Transkriptionsfaktor Cut:

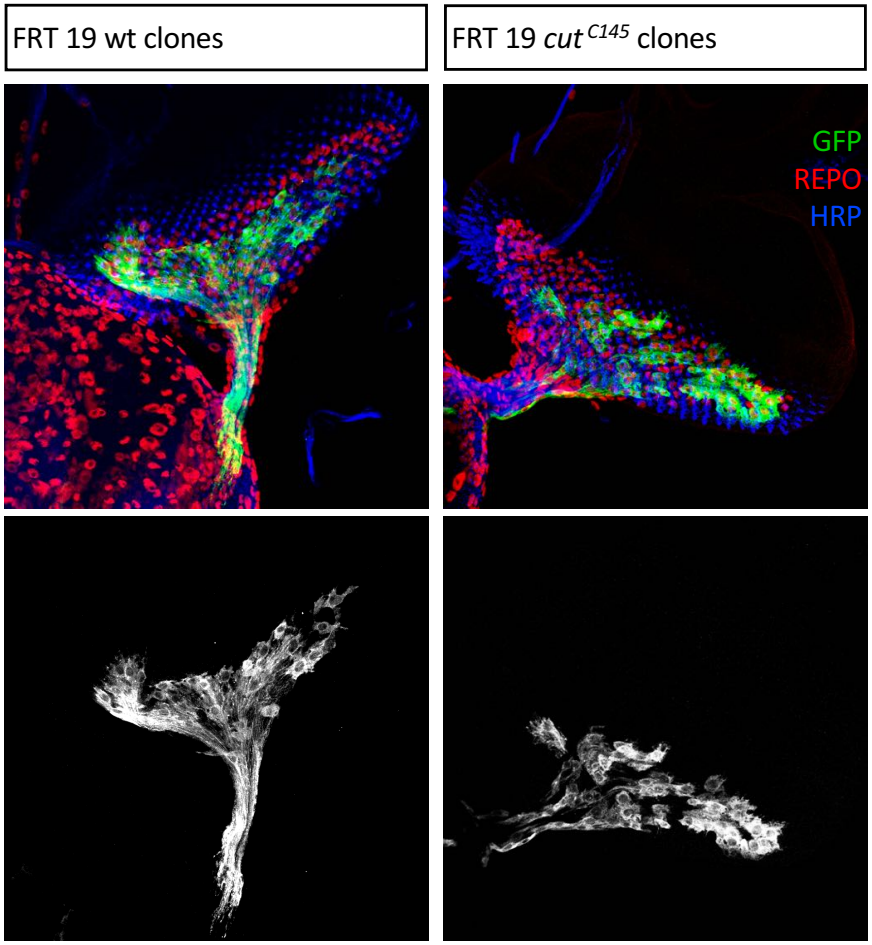
loss of function Analyse (RNAi)

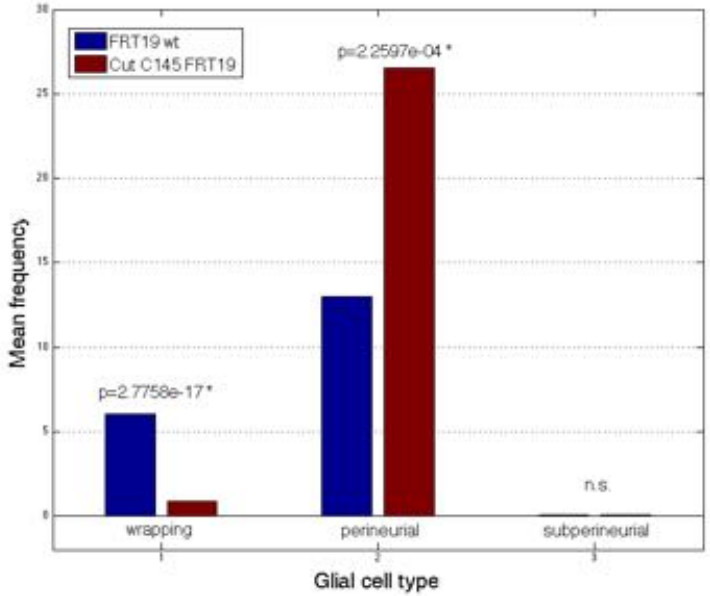




Transkriptionsfaktor Cut:

loss of function Analyse (MARCM)

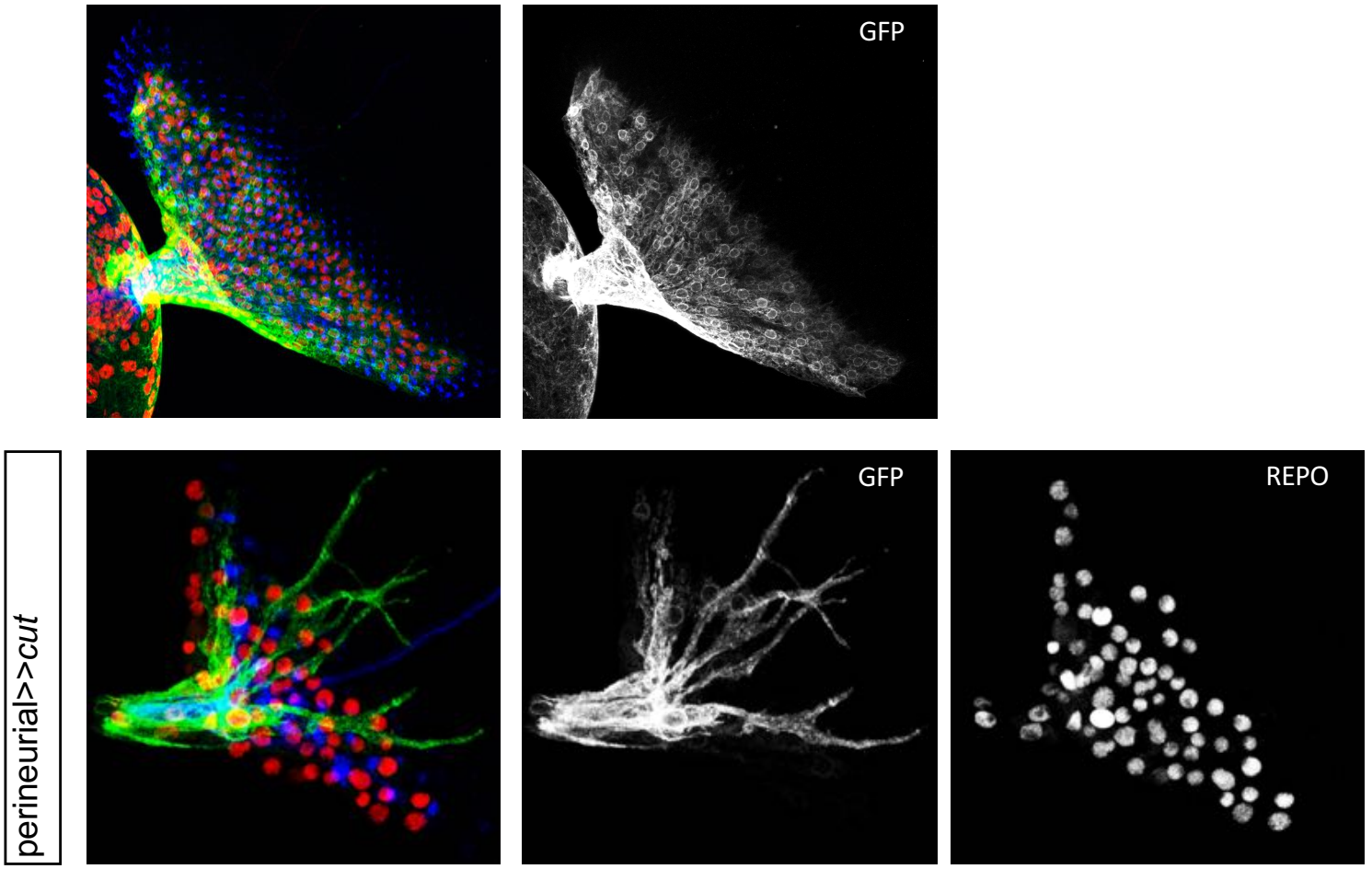




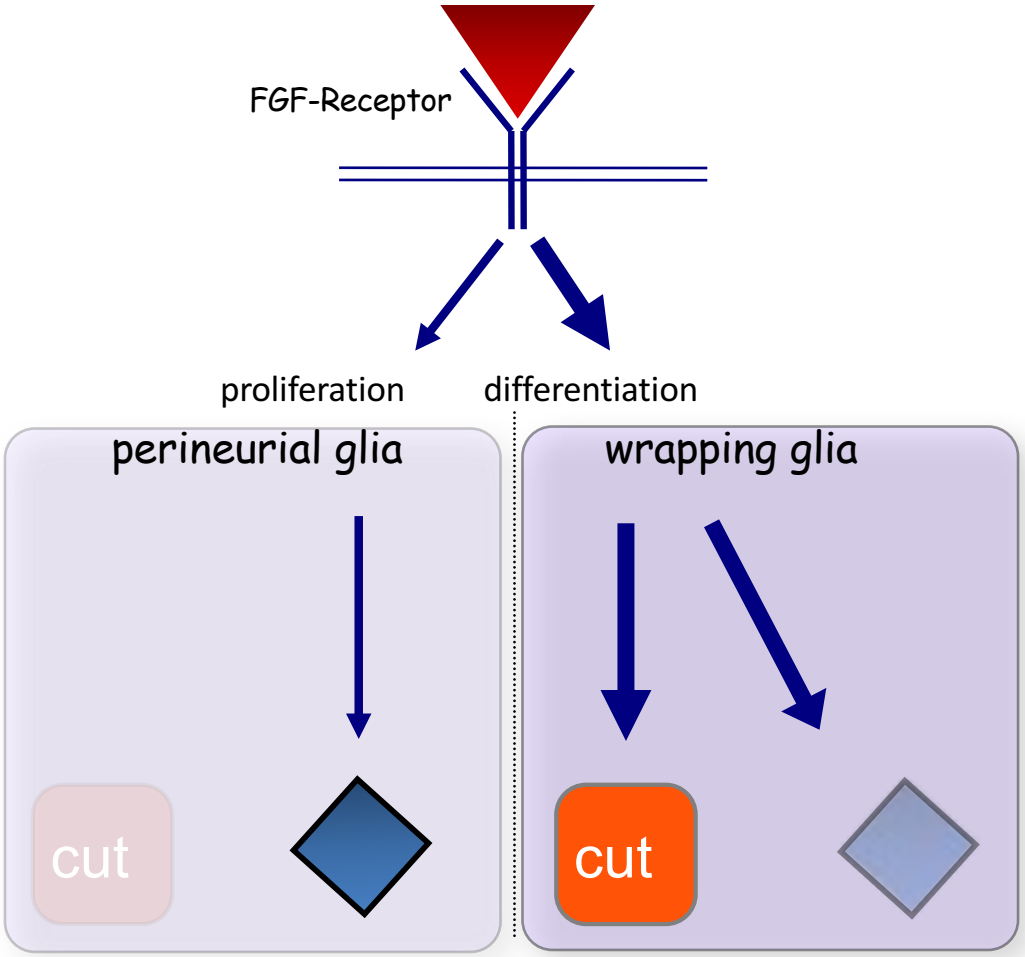
cut is required for wrapping glial cell fate

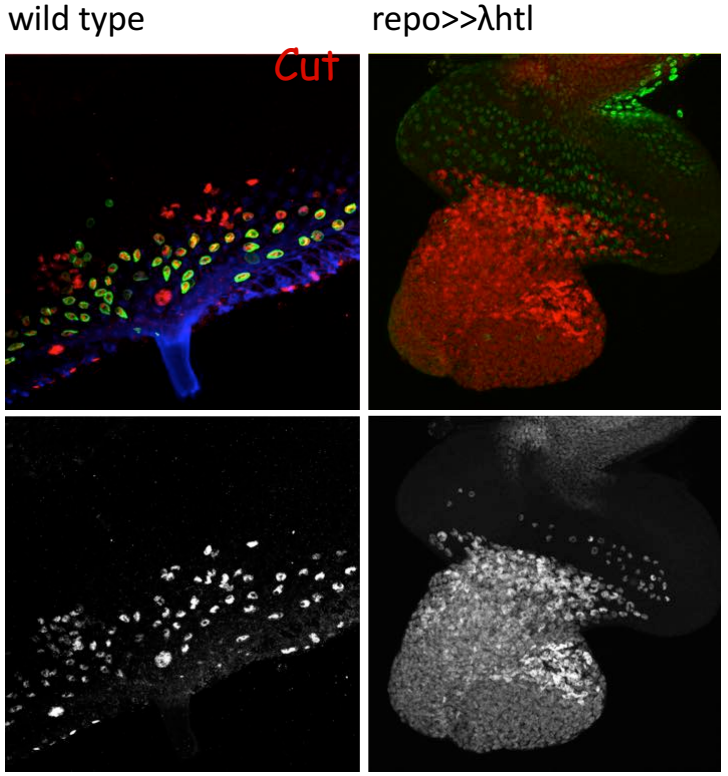
Transkriptionsfaktor Cut:

gain of function Analyse (RNAi)

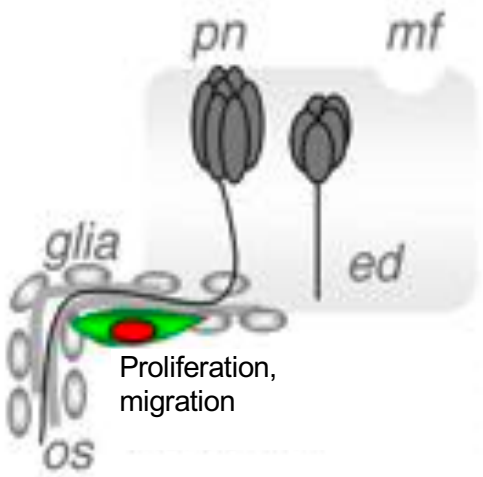


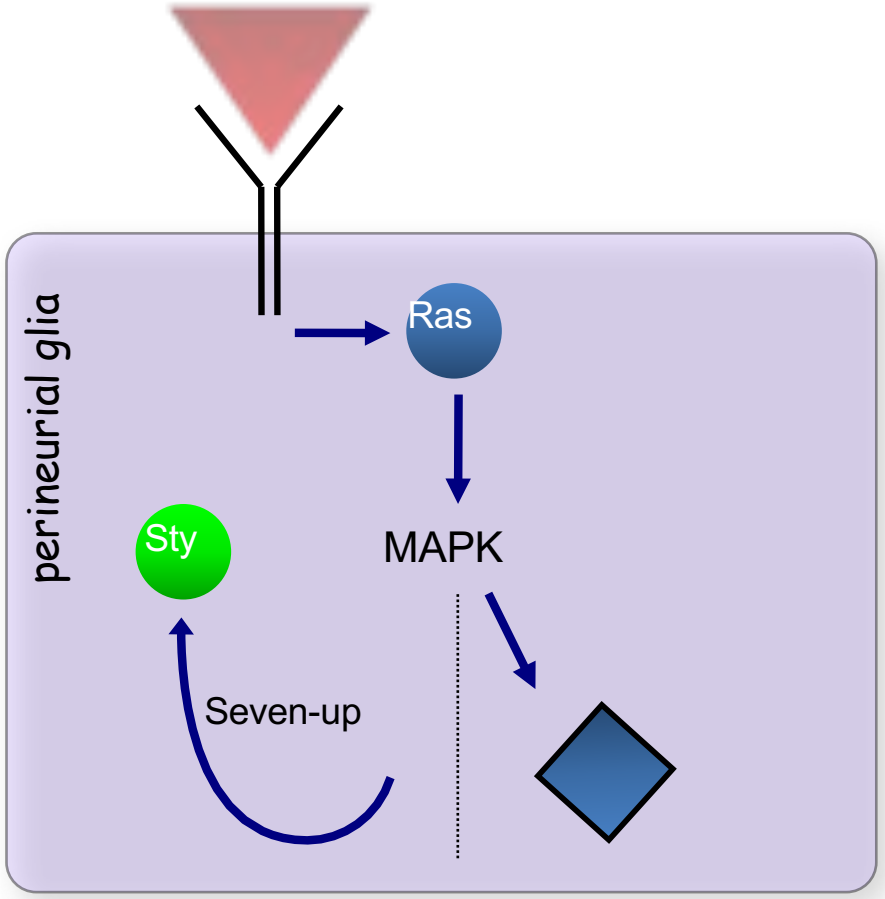
Interaktion FGF-Rezeptor und Cut





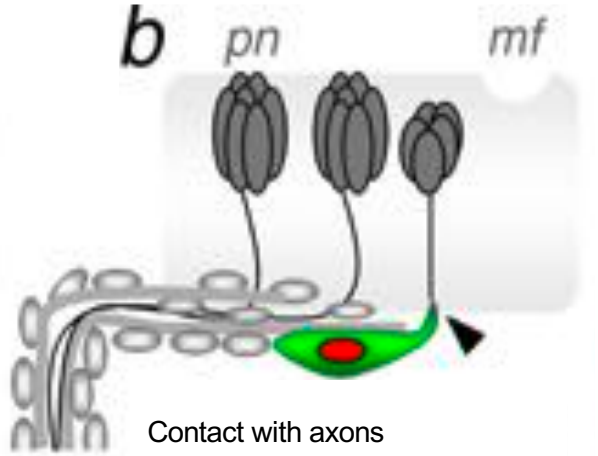
Modell

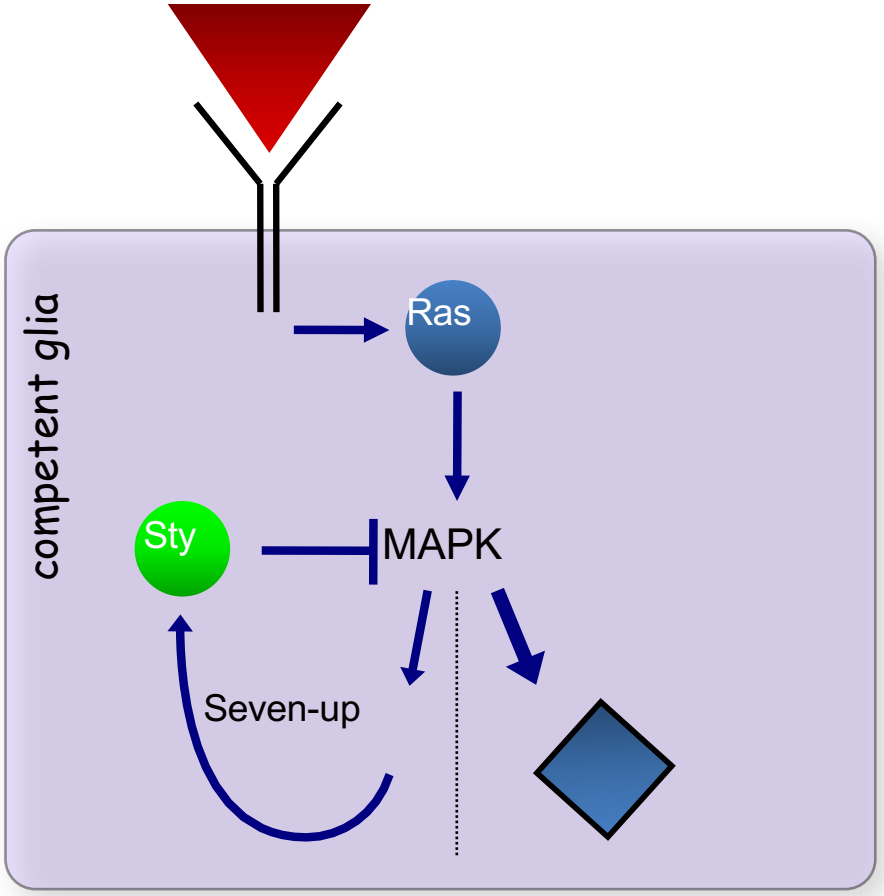




Low levels of FGF-R activity promote proliferation and migration

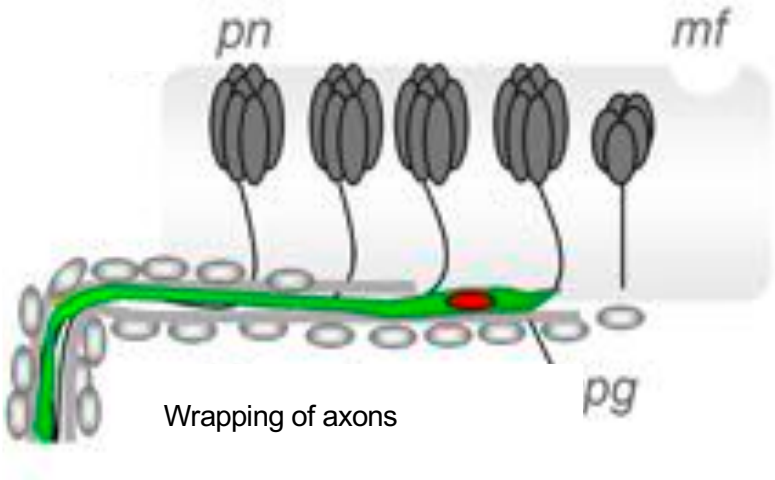
Modell

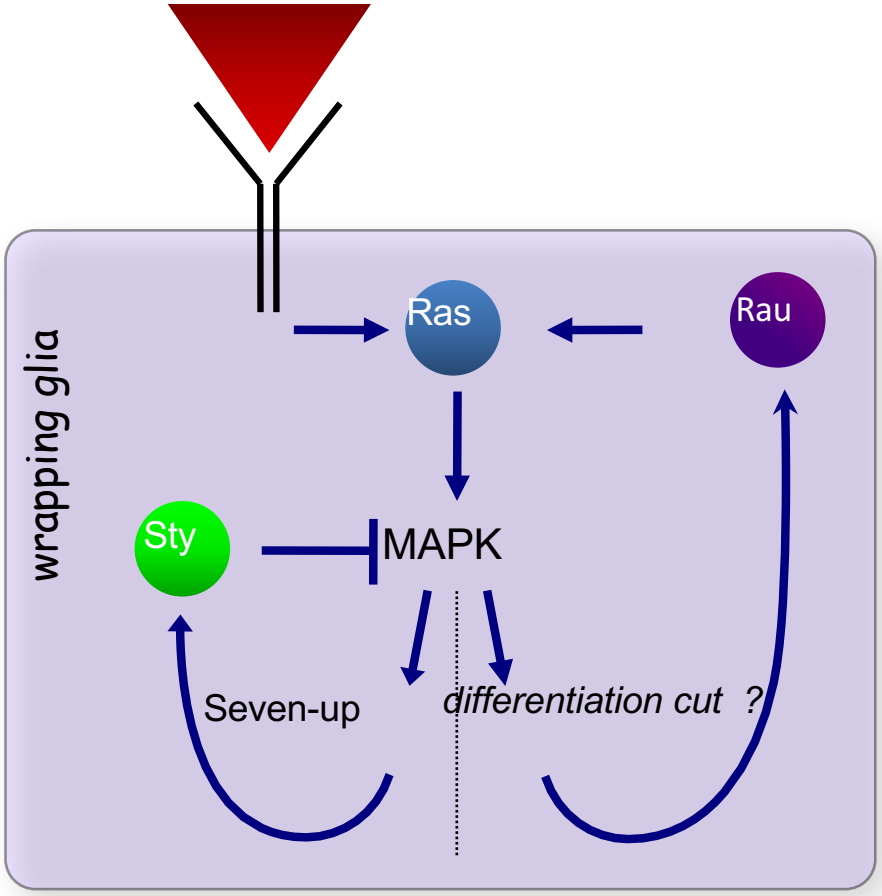




Highs levels of FGF-R activity during first axon contact suppress and sustain FGF-R signaling via Srpouty and Rau and induce Cut expression, triggering differentiation

Modell





Moderate levels of FGF-R activity are maintained during differentiation