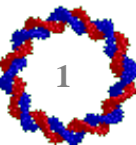
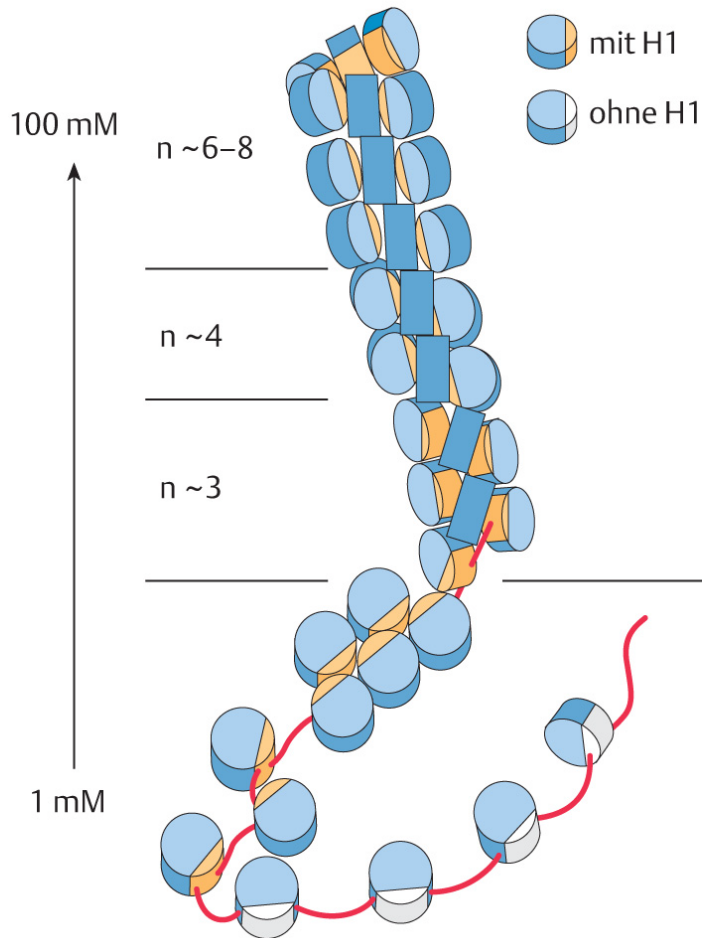


Welche Themen werden behandelt?

- **Das Genom**
- Chromosomen
 - Organisation
 - Zentromere



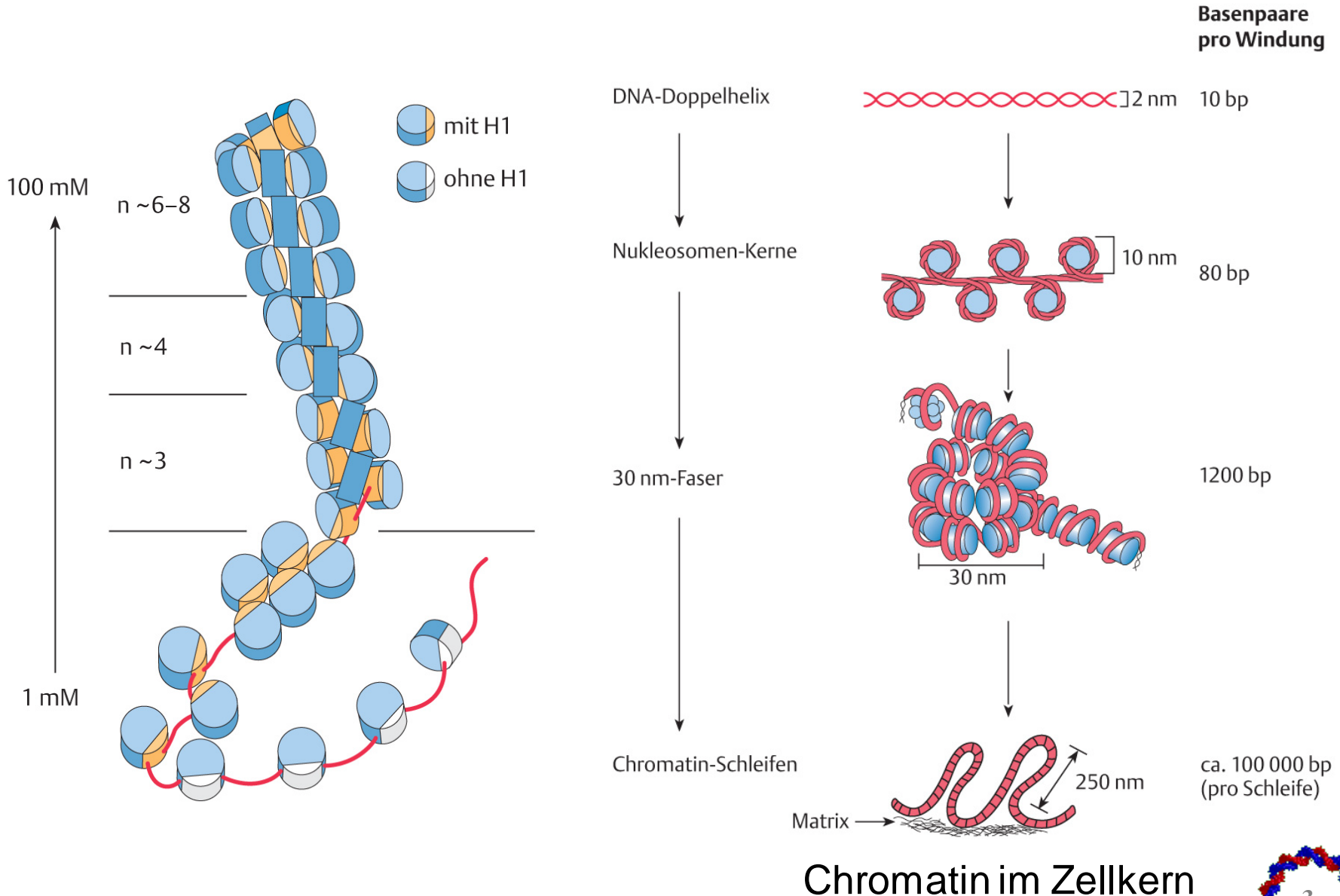
Chromosomenorganisation



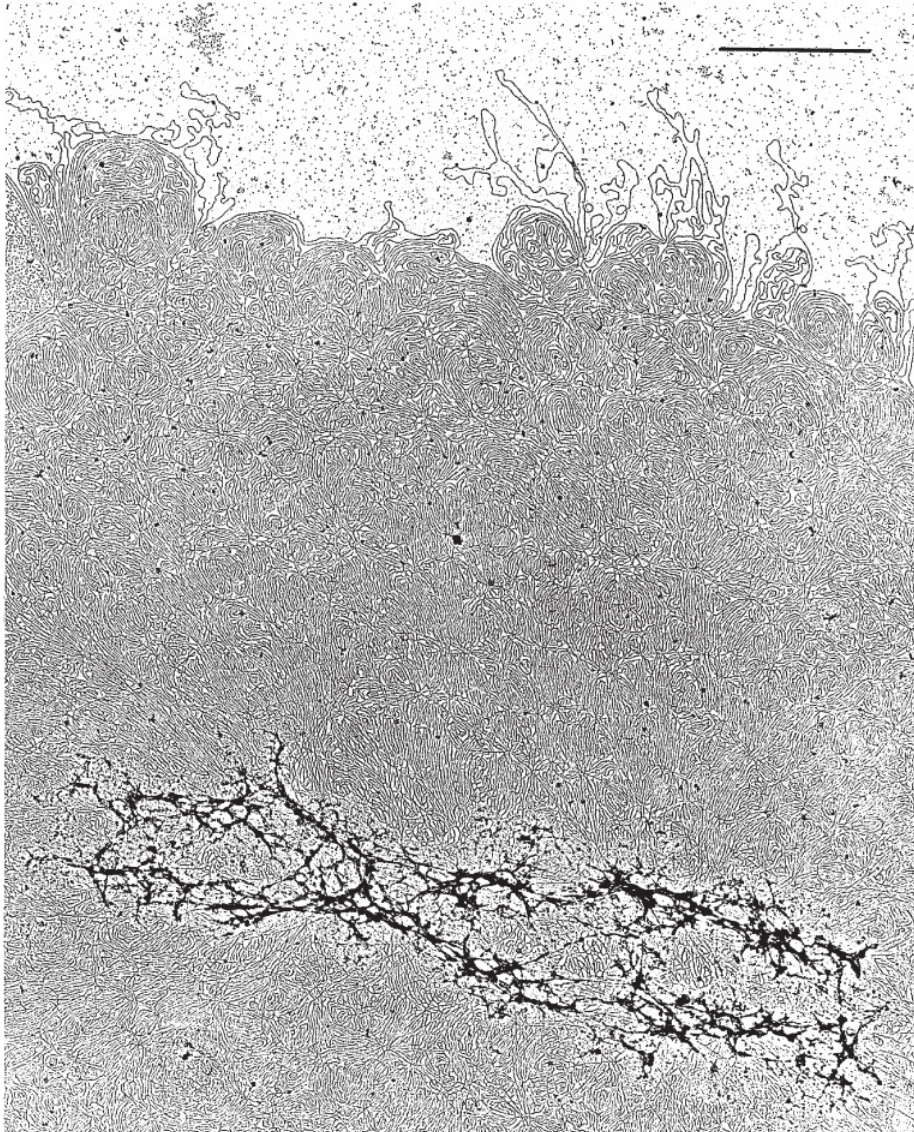
Nucleosomen im Chromatin



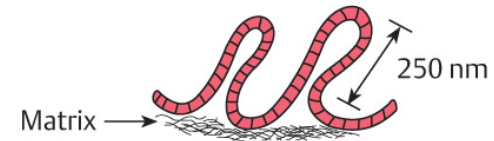
Chromosomenorganisation



Chromosengerüst



Proteinfreie DNA am Chromosomengrundgerüst



ca. 100 000 bp
(pro Schleife)

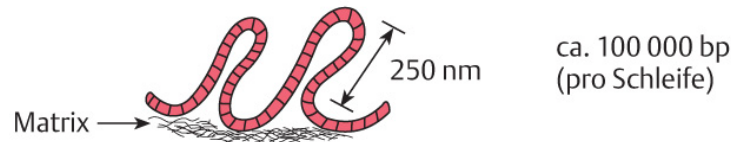
Proteingerüst
=
scaffold

scaffold Bestandteile:

- DNA-Topoisomerase
- SMC-Proteine
(*structural maintenance of chromosomes*)



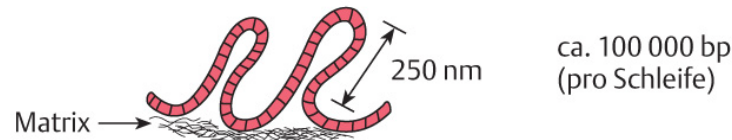
S/MAR attachment regions



S/MAR = *scaffold/ matrix attachment regions*

- nicht zufällig auftretend: benachbart zu transkribierten Regionen oder in 5' Introns
- Bindung erfolgt an AT-reiche DNA-Abschnitte, aber nicht primär sequenzbestimmt
- Chromatin-Schleifen von ca. 50,000 – 100,000 nt = Chromatin- Domänen
- Assoziationsstellen für Proteinkomplexe bei
 - Transkription
 - DNA Replikation
 - Rekombination
 - Chromsomenkondensation

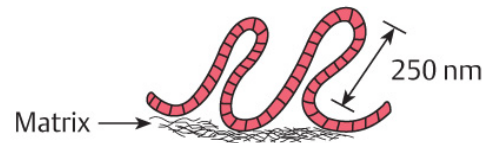
S/MAR attachment regions



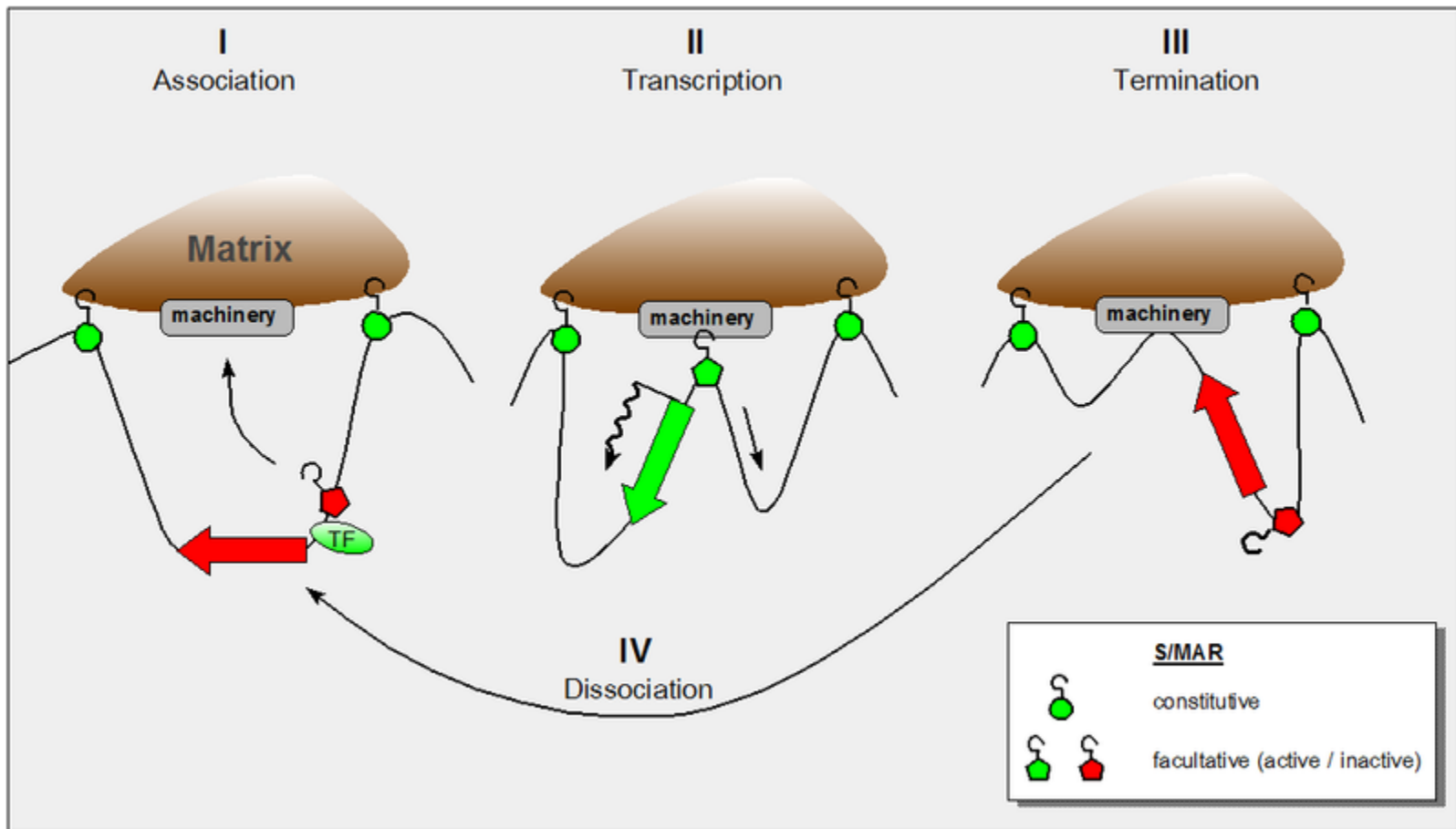
- Abgrenzung von Transkriptionseinheiten - Chromatindomänen
- Plattform für die Bindung von Proteinkomplexen innerhalb von Domänen (Duplexdestabilisierungspotential – Erkennungsstelle für Enzyme)
- konstitutive / fakultative *attachment regions*
 - Unterscheidung bezüglich der Länge der *attachment regions*
 - konstitutiv: SAR (in allen Zelltypen, immer)
 - fakultativ: MAR (nicht in allen Zelltypen, temporär)



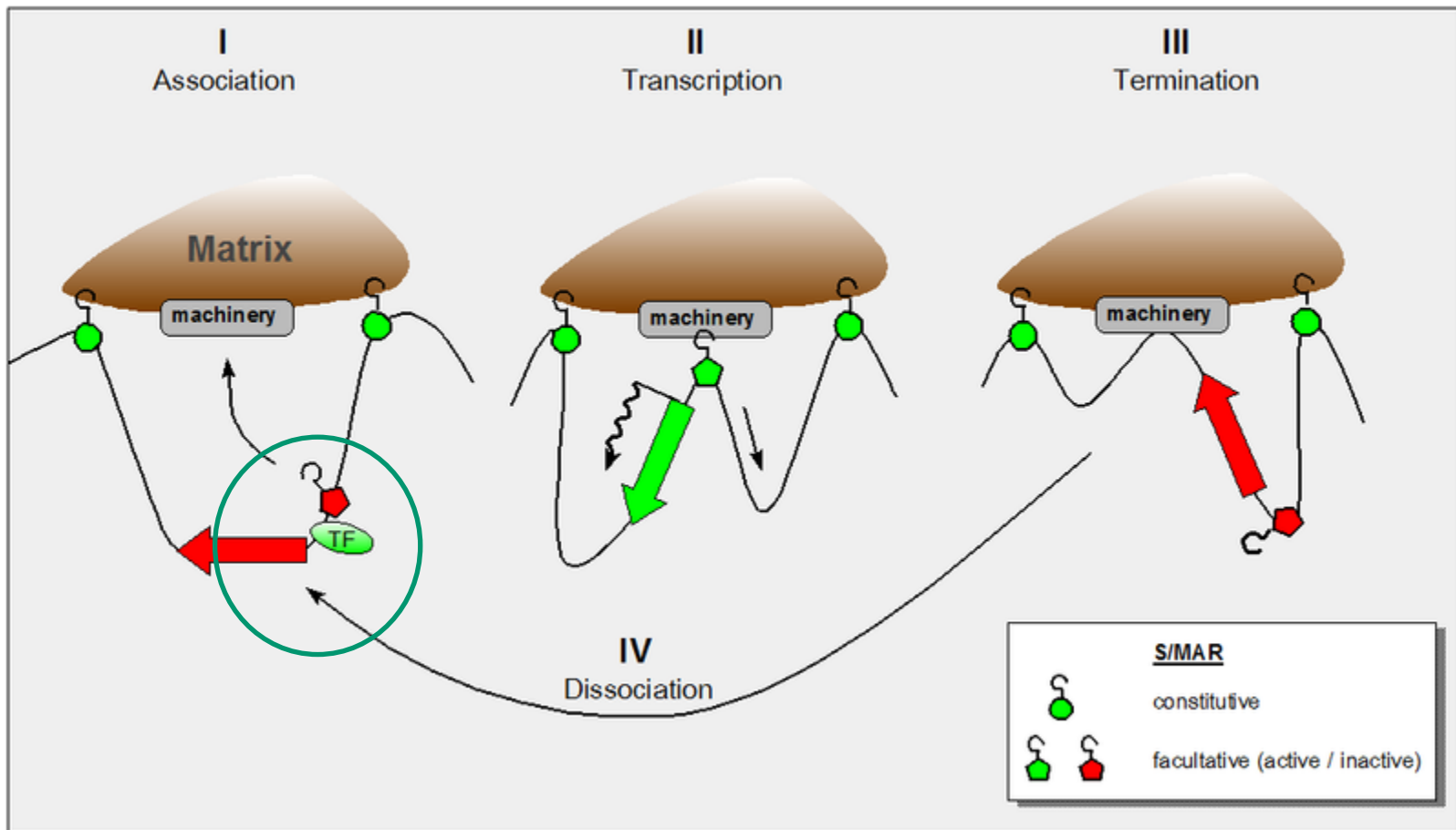
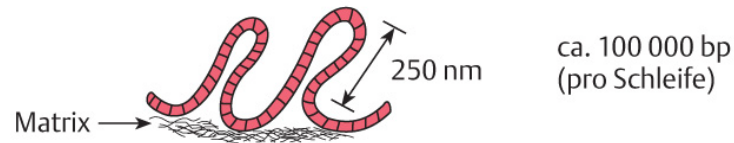
S/MAR attachment regions



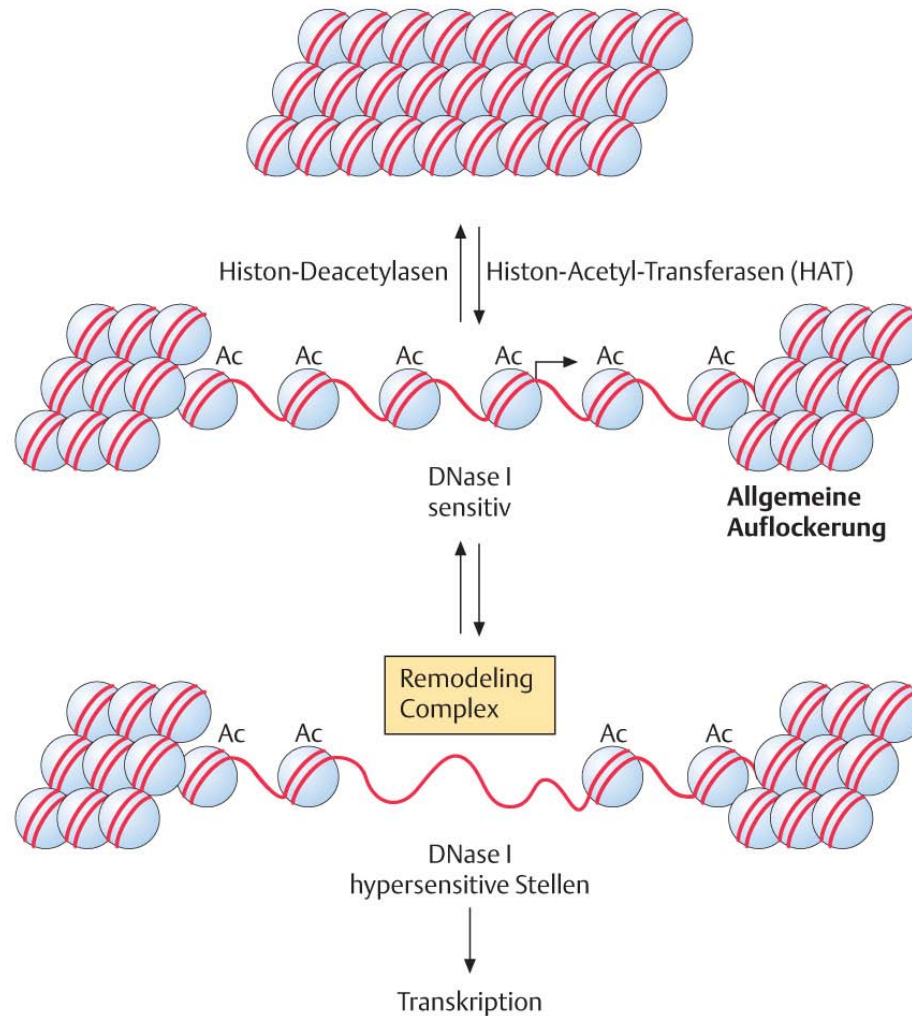
ca. 100 000 bp
(pro Schleife)



S/MAR attachment regions



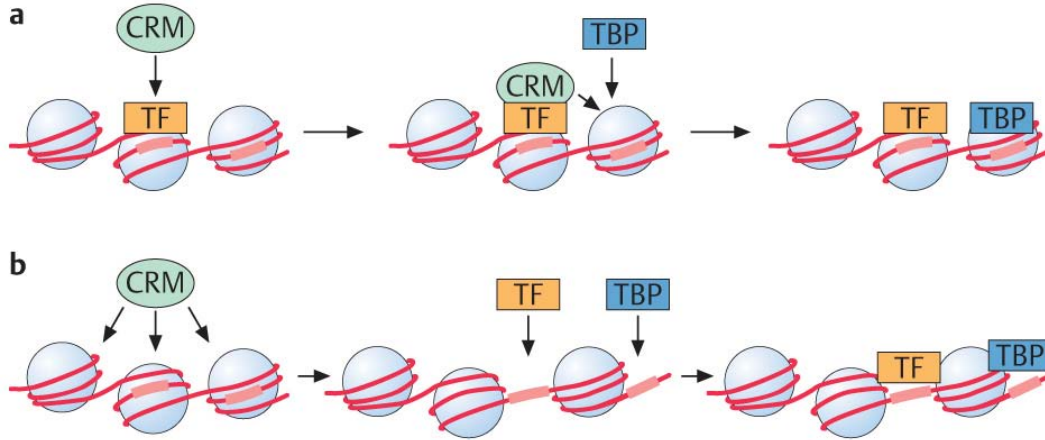
Dynamische Chromatinstruktur



Lage oder Struktur von Nucleosomen wird geändert

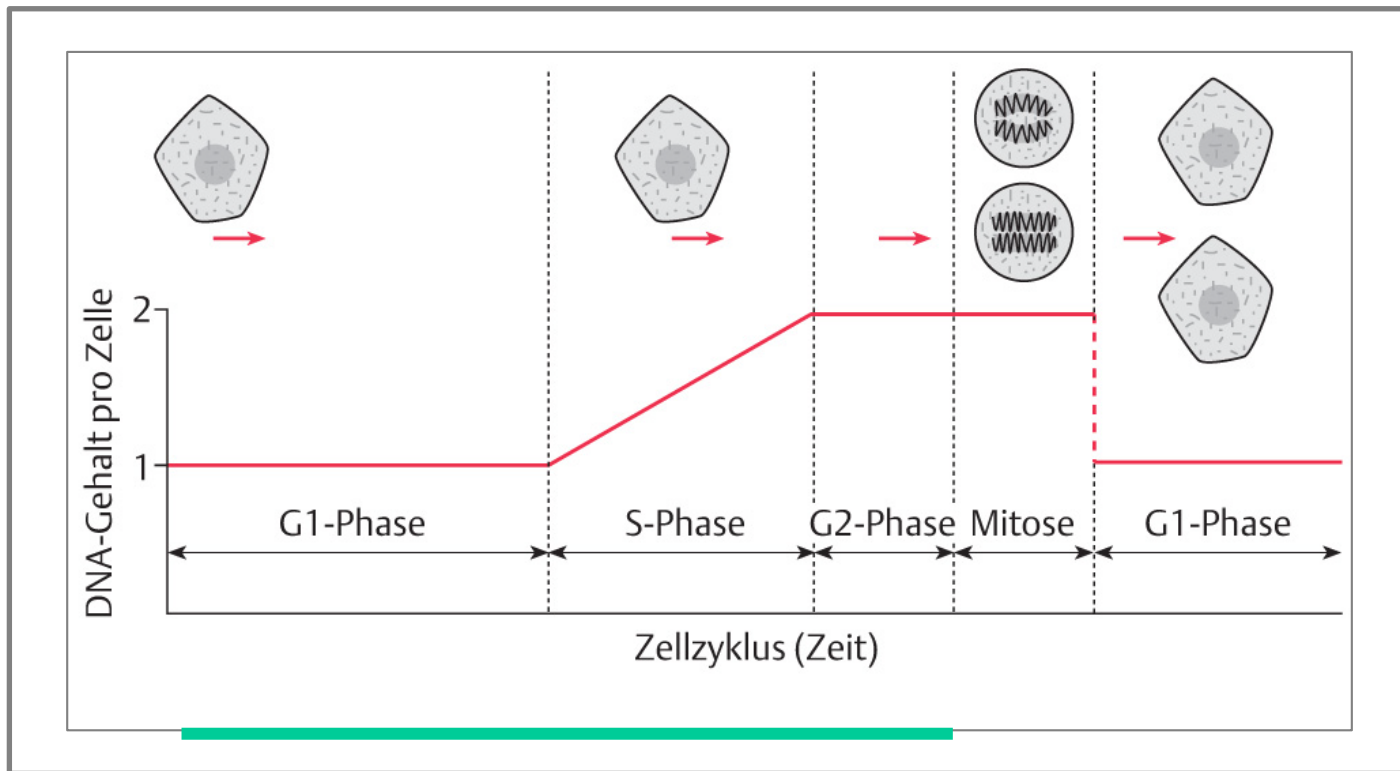
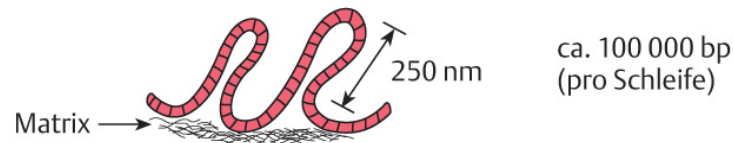


Chromatin Remodeling Complex



- a) Struktur der Nucleosomen wird verändert
- b) Nucleosomen werden verdrängt

Replikation an der Kernmatrix

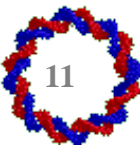


2 - 20 h

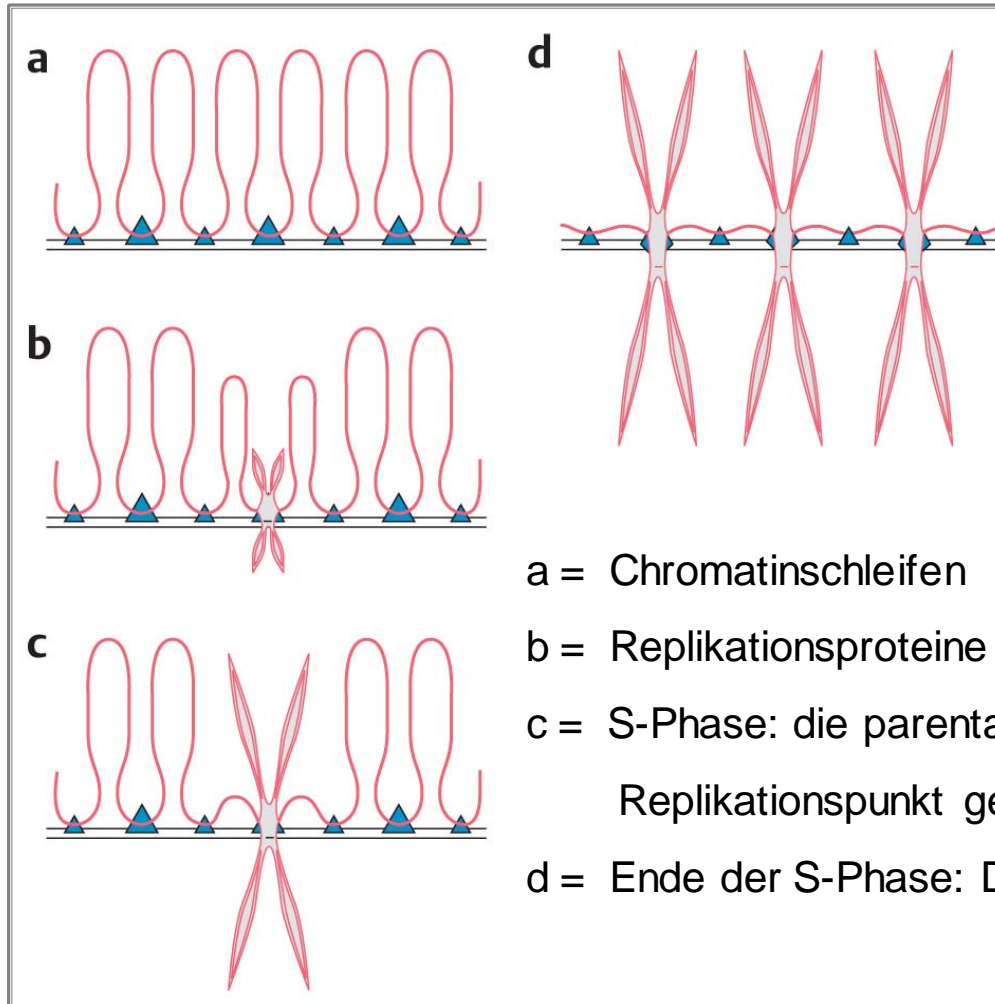
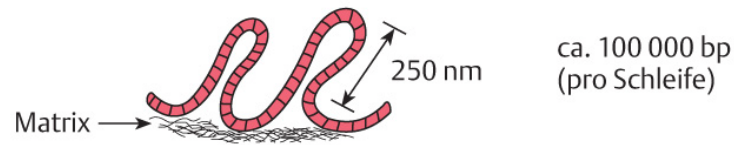
6 - 10 h

2 - 4 h

3 - 4 h



Replikation an der Kernmatrix



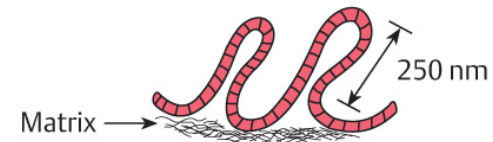
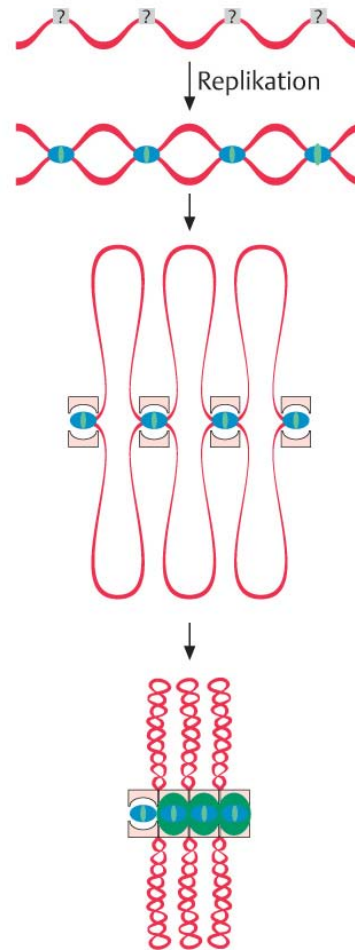
a = Chromatinschleifen

b = Replikationsproteine am Replikationsstart




c = S-Phase: die parentale DNA wird durch den Replikationspunkt gezogen

d = Ende der S-Phase: DNA ist repliziert

Replikation an der Kernmatrix



ca. 100 000 bp
(pro Schleife)

-  Topoisomerase II
-  Condensin
-  Cohesin

Konservierung von S/MARs

Trends in Genetics Volume 19, Issue 3, March 2003, Pages 119-124

A significant fraction of conserved noncoding DNA in human and mouse consists of predicted matrix attachment regions

Galina V. Glazko¹, Eugene V. Koonin², Igor B. Rogozin² and Svetlana A. Shabalina

Noncoding DNA in the human–mouse orthologous intergenic regions contains ‘islands’ of conserved sequences, the functions of which remain largely unknown. We hypothesized that some of these regions might be matrix–scaffold attachment regions, MARs (or S/MARs). MARs comprise one of the few classes of eukaryotic noncoding DNA with an experimentally characterized function, being involved in the attachment of chromatin to the nuclear matrix, chromatin remodeling and transcription regulation. **To test our hypothesis, we analyzed the co-occurrence of predicted MARs with highly conserved noncoding DNA regions in human–mouse genomic alignments. We found that 11% of the conserved noncoding DNA consists of predicted MARs.** Conversely, more than half of the predicted MARs co-occur with one or more independently identified conserved sequence blocks. An excess of conserved predicted MARs is seen in intergenic regions preceding 5 ends of genes, suggesting that these MARs are primarily involved in transcriptional control.

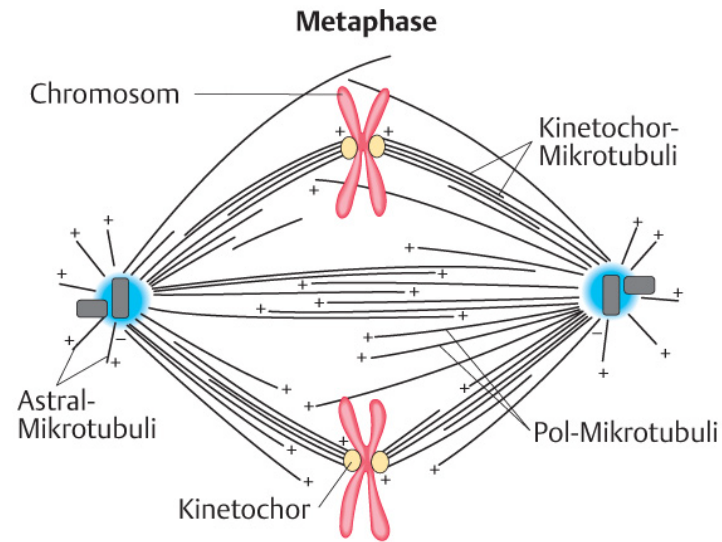
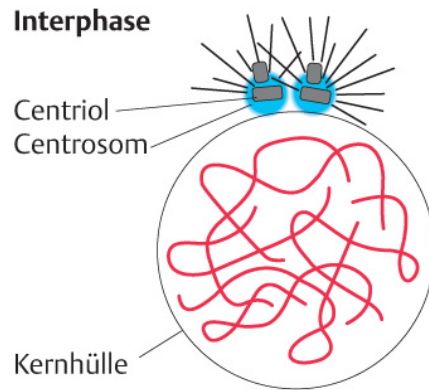


Chromosomenorganisation

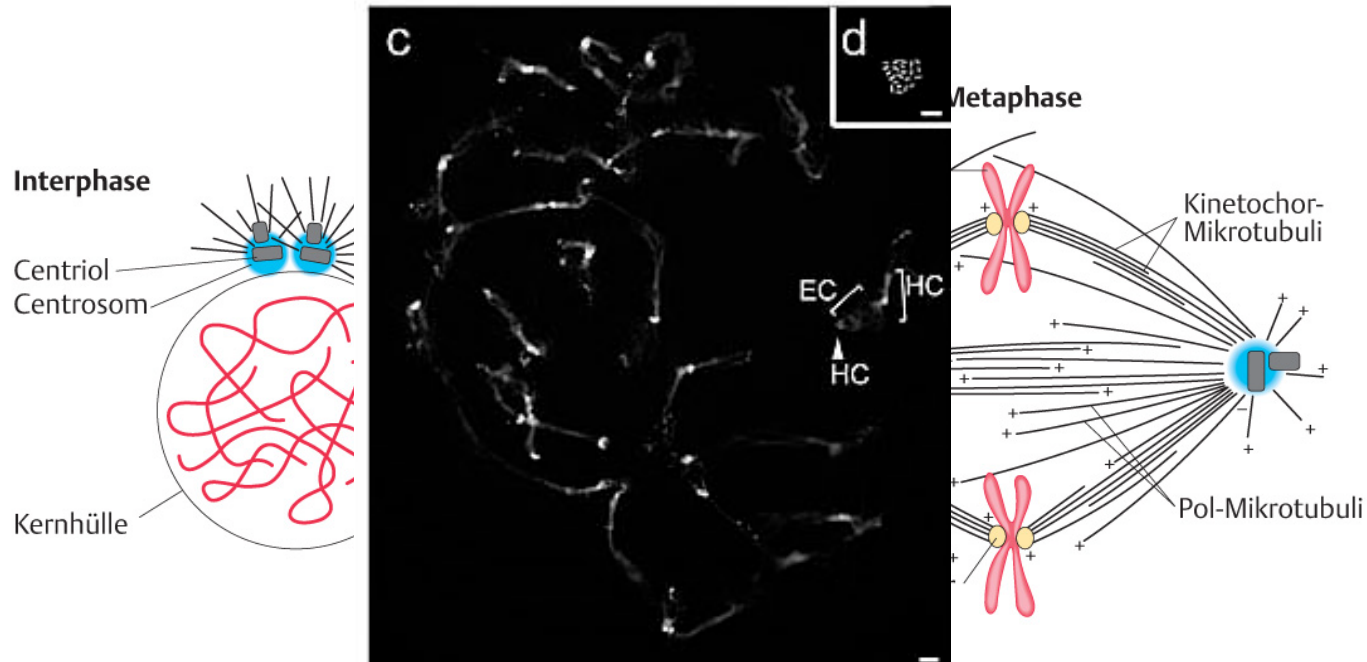
- Chromosomen verändern ihre Struktur während des Zellzyklus
- Spiralisierungsgrad der DNA ist unterschiedlich
- Chromosomen in der Interphase sind entspiralisiert
- Chromosomen in der Metaphase zeigen am dichtesten gepackte DNA
- Bänderung in der Metaphase



Mitose: Prophase vs Metaphase

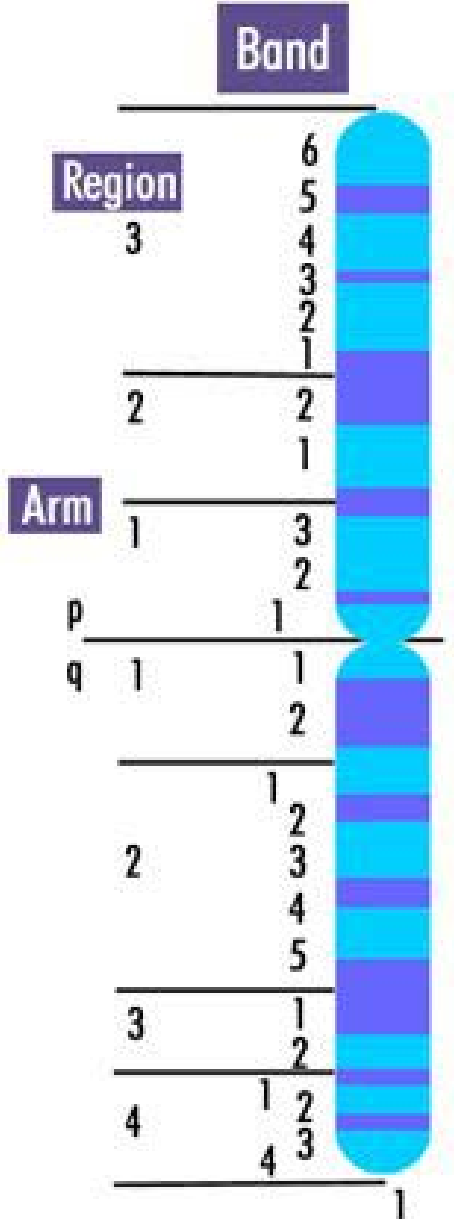


Mitose: Prophase vs Metaphase



Phaseolus coccineus cv. Preisgewinner

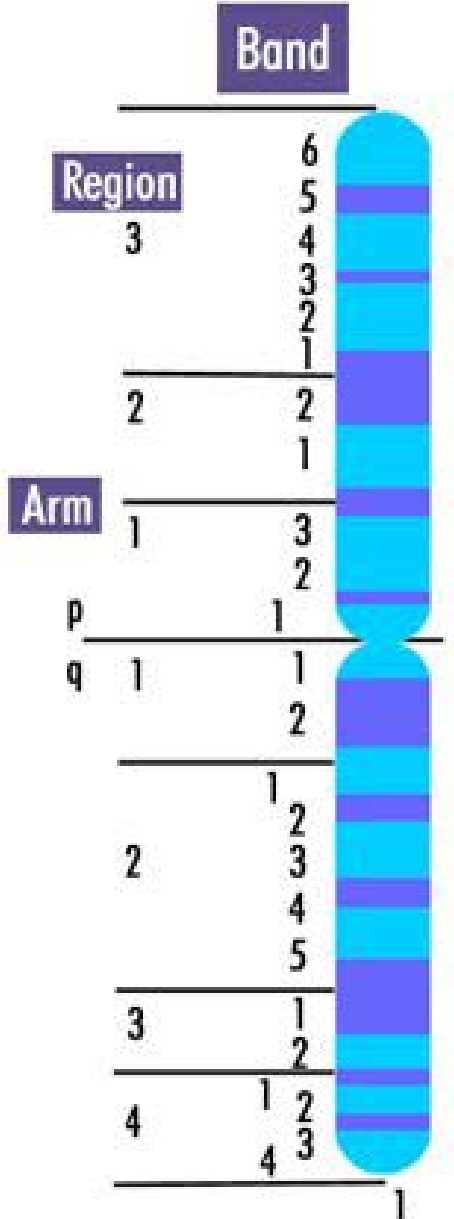
Chromosomenbänderung



Zählung:

- p-Arm
- q-Arm
- vom Centromer nach außen
- bis 1q21.34

Chromosomenbänderung

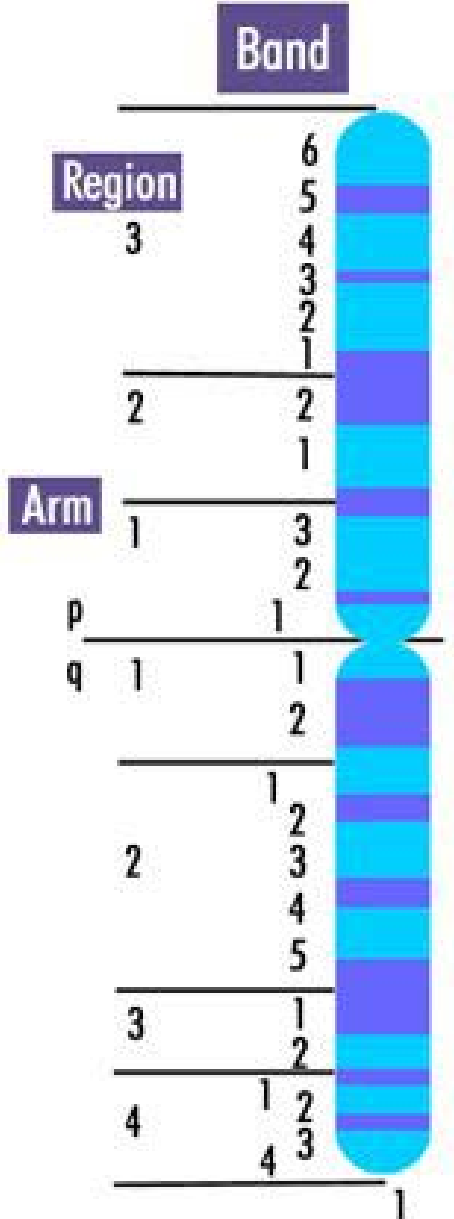


Zählung:

- p-Arm
- q-Arm
- vom Centromer nach außen
- bis 1q21.34

- Zahl abhängig von Kondensierung der Chr
- Metaphase: ca. 400 Bande
- Prophase: ca. 850 Bande

Chromosomenbänderung



Zählung:

- p-Arm
- q-Arm
- vom Centromer nach außen
- bis 1q21.34

- Zahl abhängig von Kondensierung der Chr
- Metaphase: ca. 400 Bande
- Prophase: ca. 850 Bande

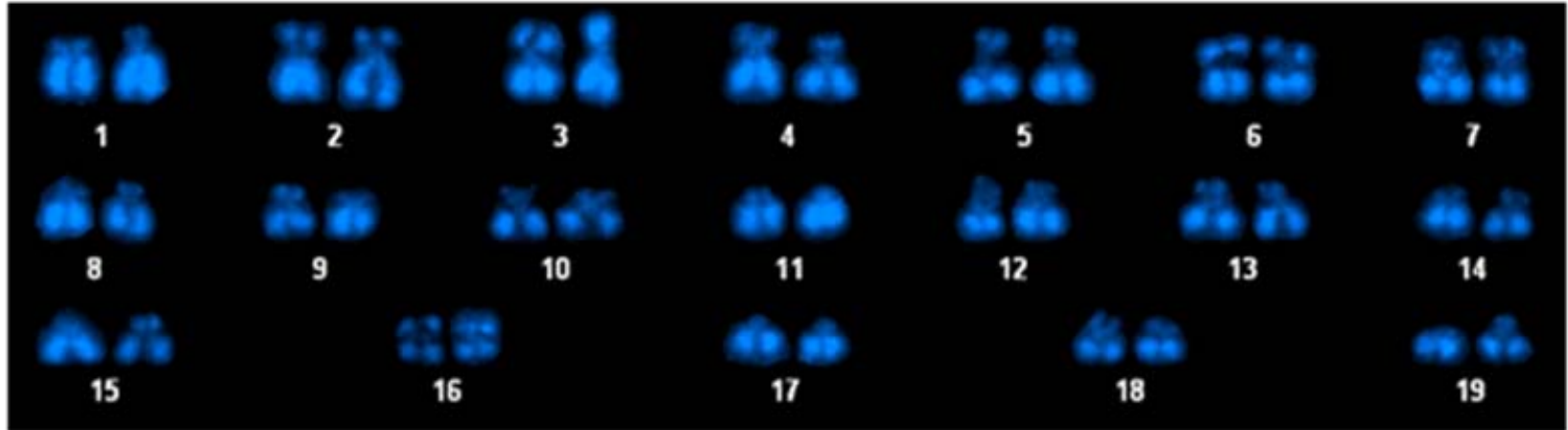
- genreiche und genarme Regionen werden unterschieden:

G-Banden: G+C-arm, spät dupliziert

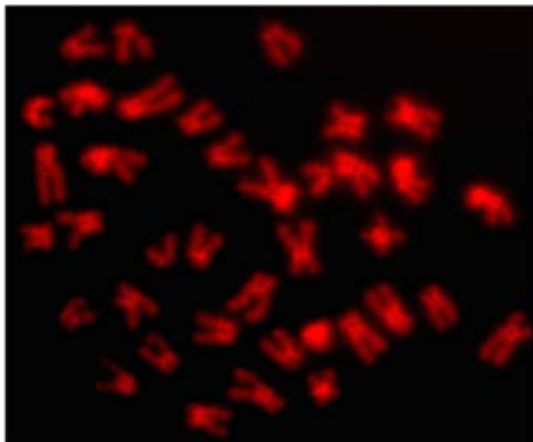
R-Banden: G+C-reich, früh dupliziert

DNA - Färbungen

Karyotyp des Fisches *Nothobranchius*



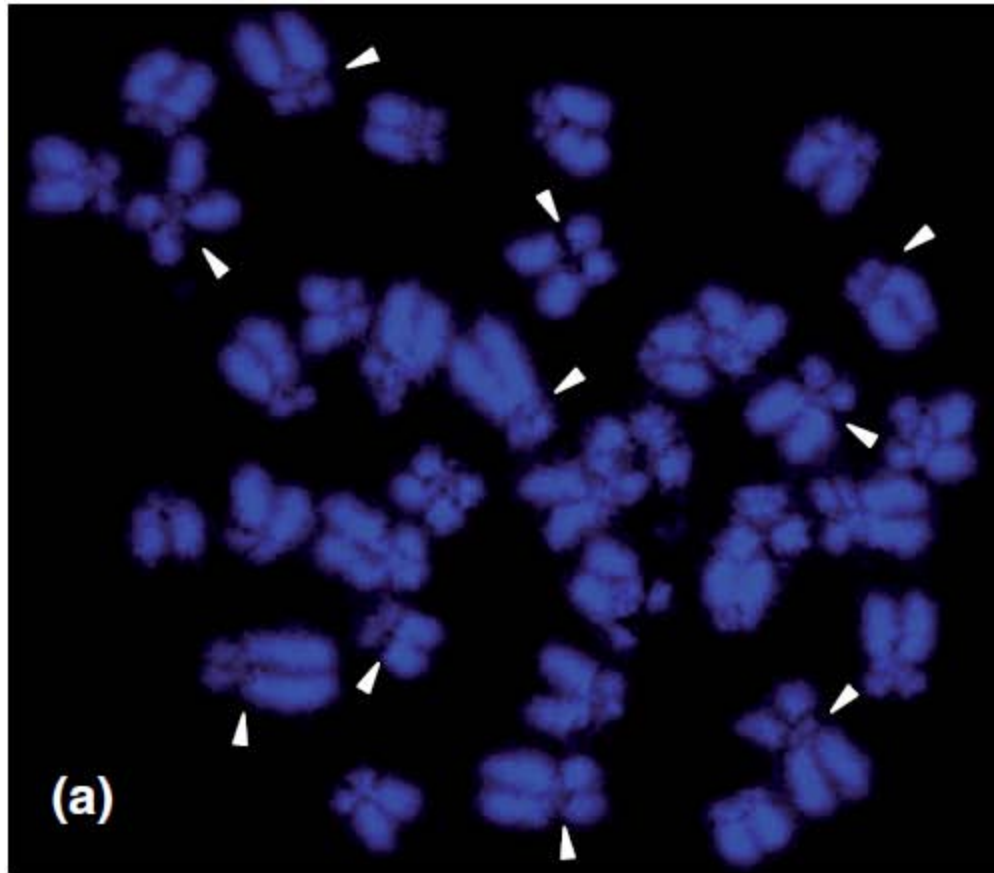
DAPI (4'6'-diamidino-2-phenylindol) - Färbung zeigt weniger stark gefärbte Heterochromatinbereiche



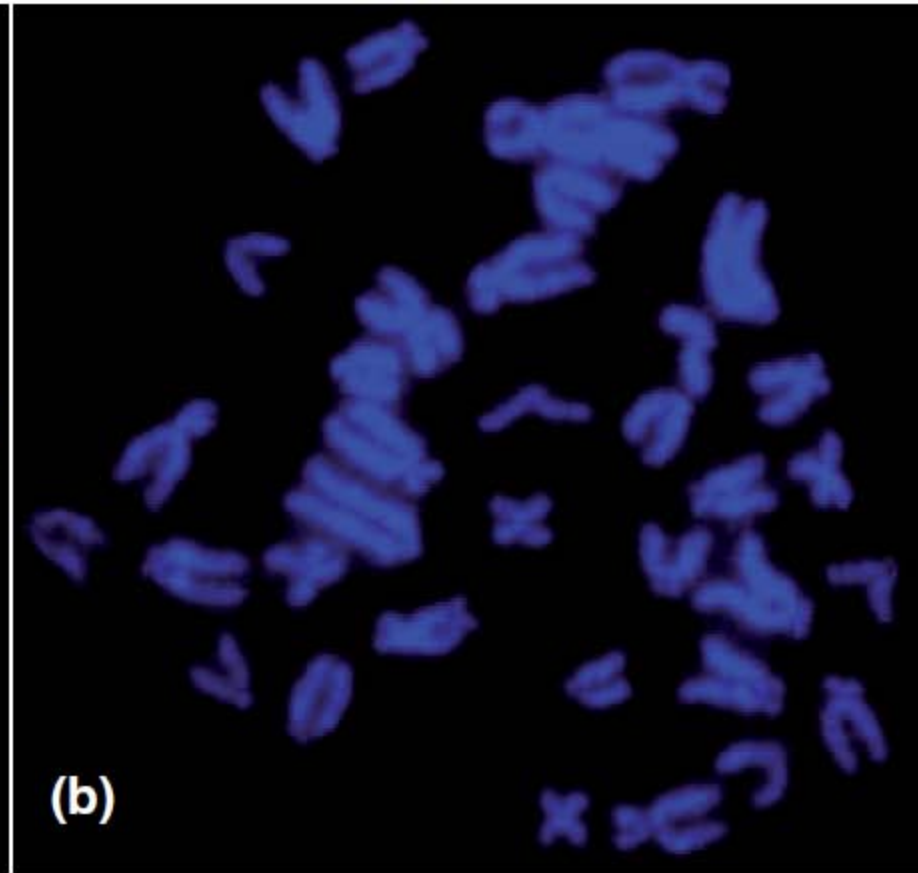
Distamycin A/mithramycin
Färbung zeigt
G+C reiche Strukturen

Bandenfärbungen

Karyotyp zweier *Nothobranchius* Spezies



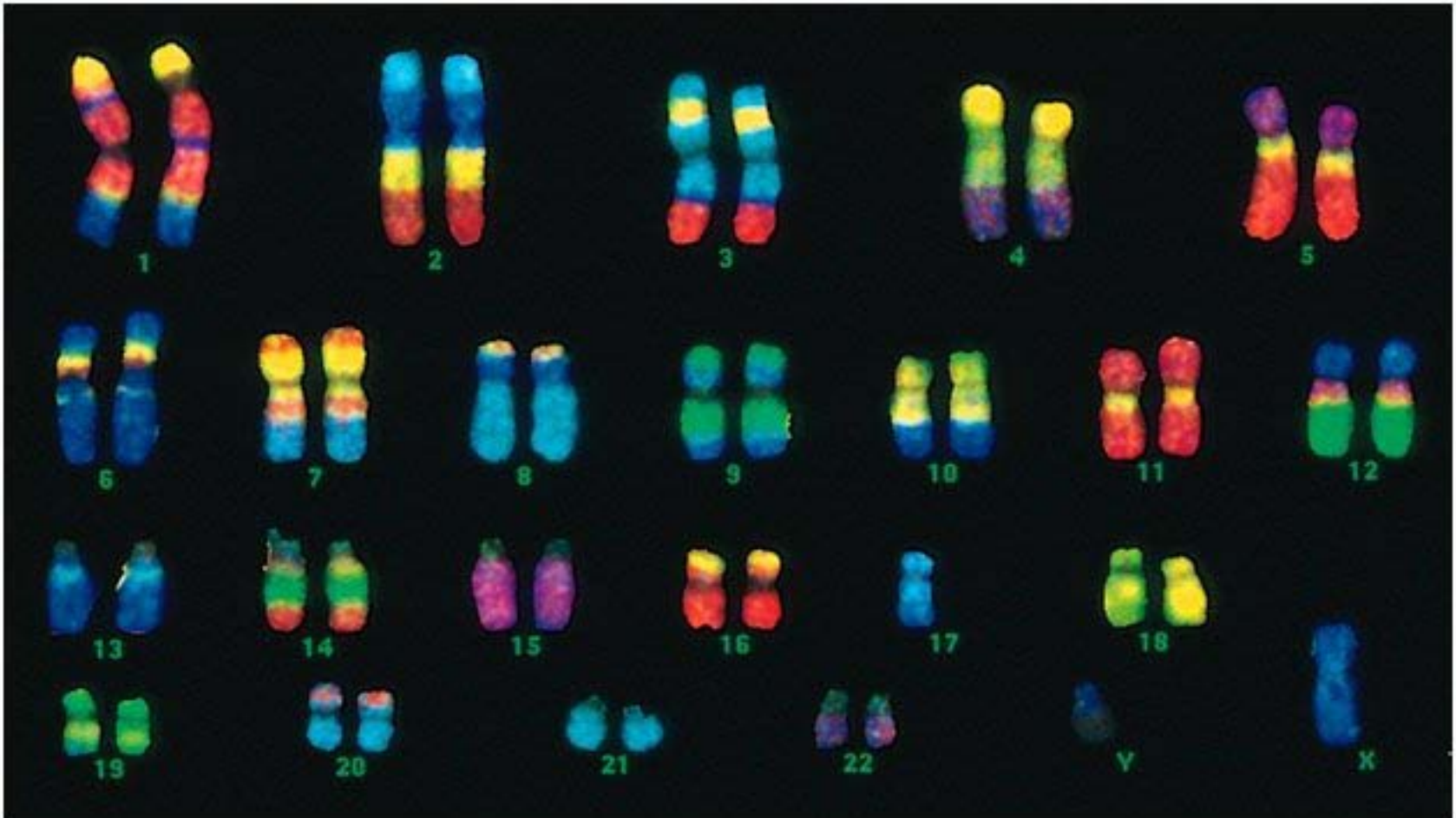
N.furzeri



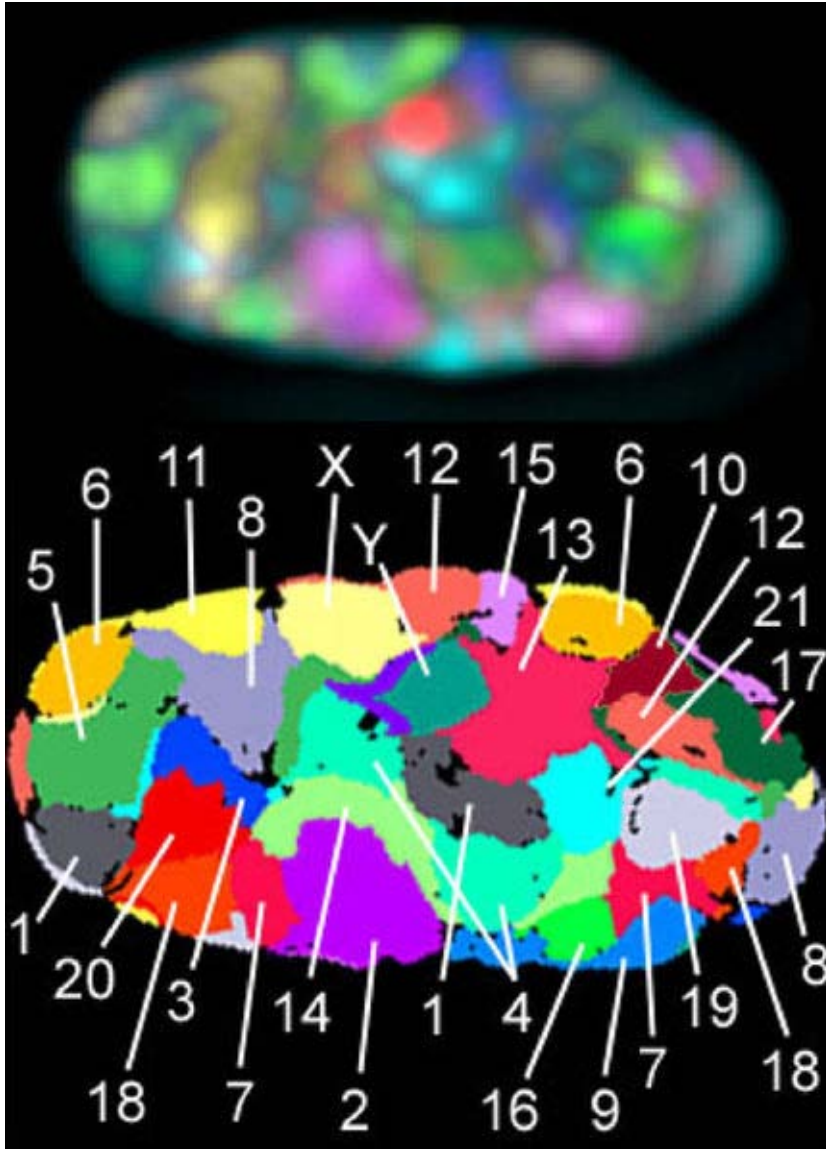
N.orthonotus

Chromosome painting

Der Karyotyp des Menschen



Chromosomenterritorien



Zellkern eines menschlichen Fibroblasten, in dem alle 24 verschiedenen Chromosomen (1- - 22, X und Y) mittels FISH mit einer unterschiedlichen Kombination von insgesamt 7 Fluorochromen angefärbt wurden.

Falschfarben-Darstellung aller Chromosomenterritorien, die in dieser Fokusebene sichtbar sind, nach Computer-Klassifikation.

Chromosomenterritorien

“Chromosome positioning patterns are statistical representations of chromosome positions but do not provide information about the precise coordinates of a given chromosome in a given nucleus. It is important to realize that, although significant non-random chromosome positions can be described, they contain a significant degree of uncertainty and merely indicate a preferred, probabilistic position of a given chromosome in the cell nucleus.”



Chromosomen-Zahlen

Chromosom	Daten
1	247 Mb
21	47 Mb
19	64 Mb – 3000 Gene
18	76 Mb – 600 Gene
Y	58 Mb – 200 Gene

Chromosomen

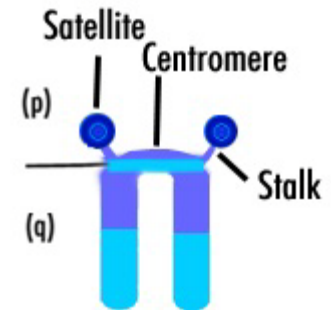
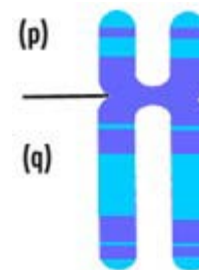
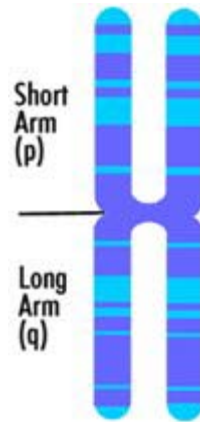
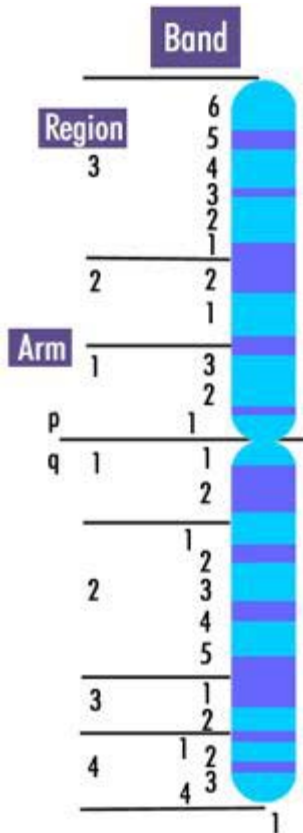
- Zahl ist charakteristisch für jeden Organismus
- Zahl sagt nichts aus über Größe oder Organisationshöhe des Organismus

Spezies	Zahl der Chromosomen (2n)
Taufliege	8
Hund	78
Erbse	14
Mensch	46
Kartoffel	48
Rind	60



Zentromere

Zentromerlokalisierung

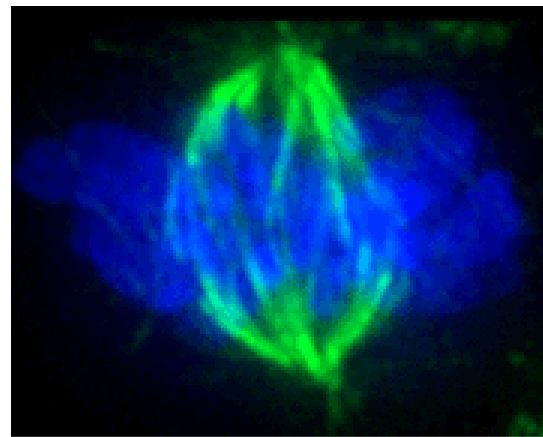


metazentrisch submetazentrisch acrozentrisch

Zentromerfunktion

Bindestelle für die Kinetochorkomplexproteine
benutzt in der Mitose und der Meiose,
um die Chromatiden mittels des
Spindelapparates auseinanderzuziehen

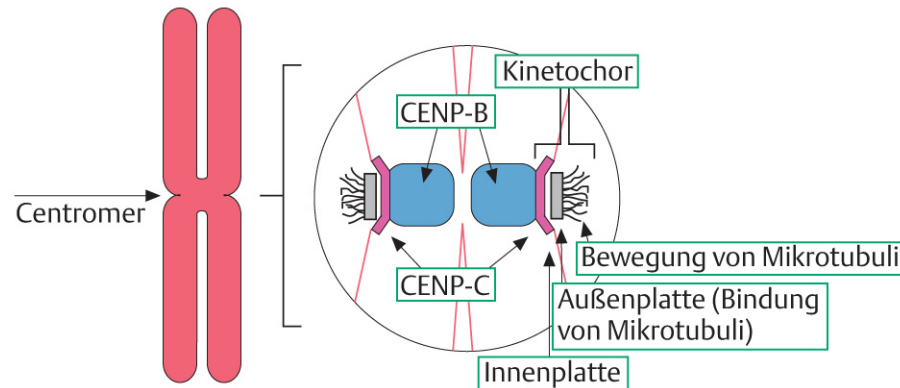
Chromosomen = blau
Spindelapparat = grün



Zentromerorganisation

einfache Motive

- etwas über 100 Basen wie z.B. in Bäckerhefe (AT-reich)
- an diese binden Proteine: CENP zum Aufbau des Kinetochors



Zentromerorganisation

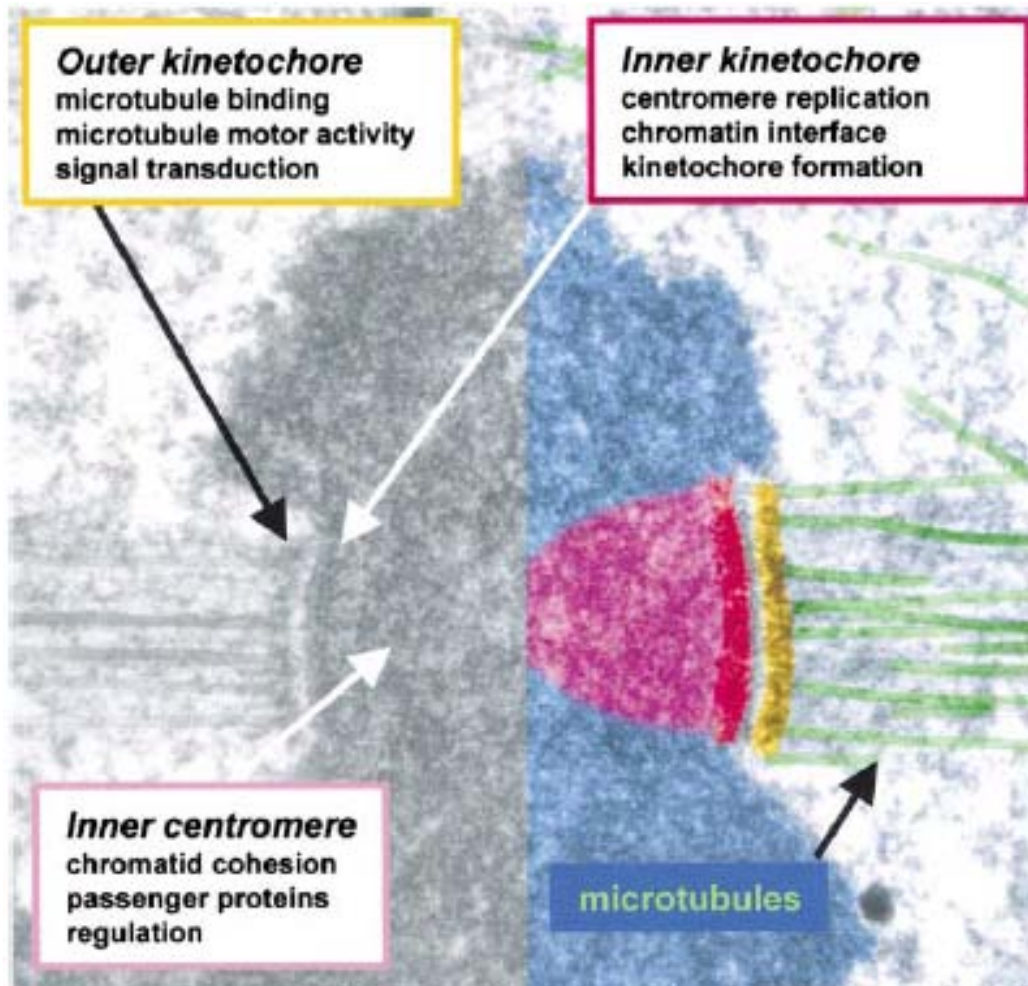
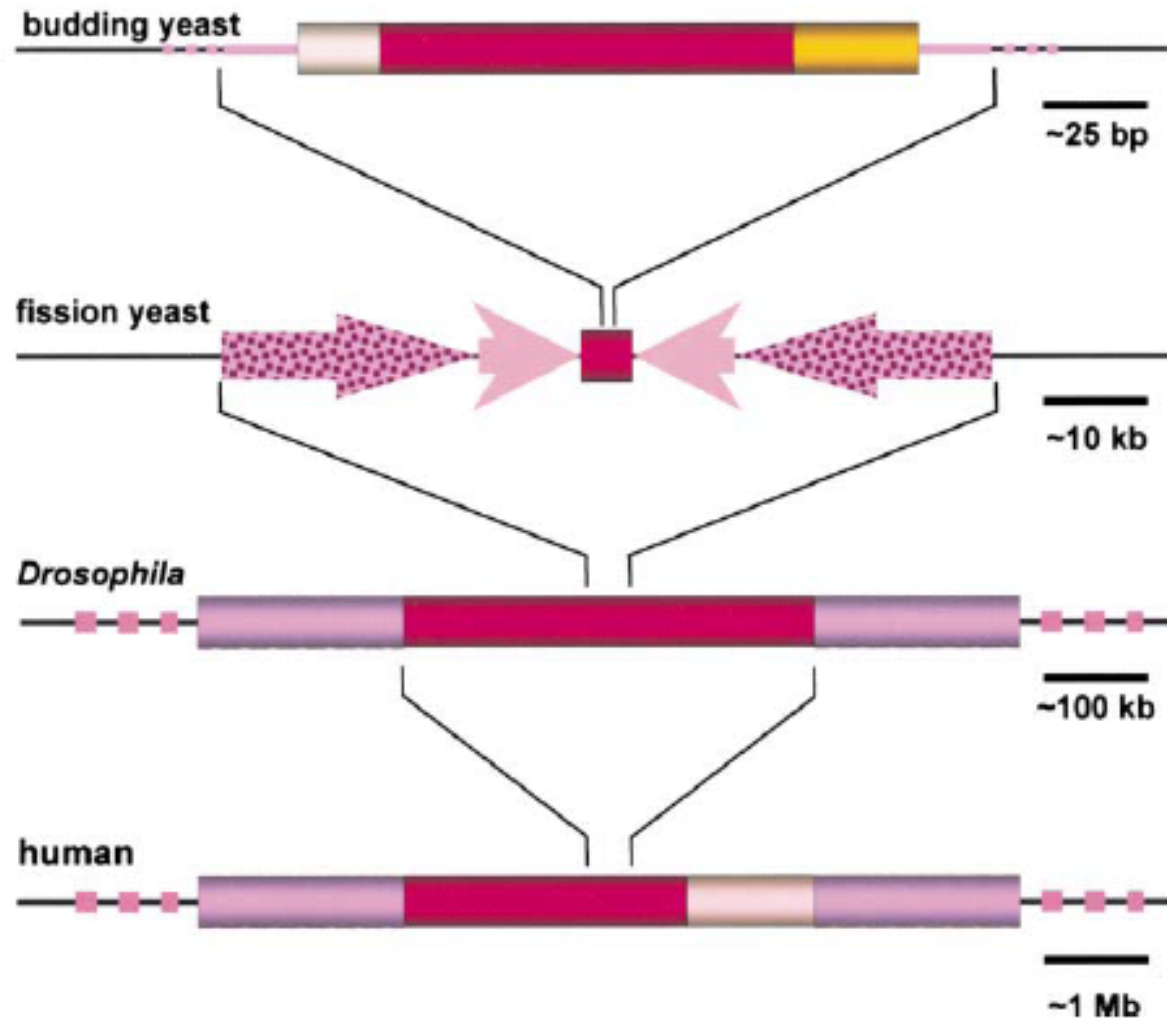


Figure 1. Organization of Centromeres

(A) Overall organization of the centromere. A mitotic chromosome has been sectioned along the plane of the spindle axis, revealing the symmetric bipolar organization of a chromosome fully engaged on the spindle. (Right) Key elements have been pseudo colored. (Violet) The inner centromere, a heterochromatin domain that is a focus for cohesins and regulatory proteins such as Aurora B and Kin I. (Red) The inner kinetochore, a region of distinctive chromatin composition attached to the primary constriction. (Yellow) The outer kinetochore, the site of microtubule binding, is comprised of a diverse group of microtubule motor proteins, regulatory kinases, microtubule binding proteins, and mitotic checkpoint proteins.

Cleveland et al. (2003) Cell (112): 407-21

Zentromerorganisation

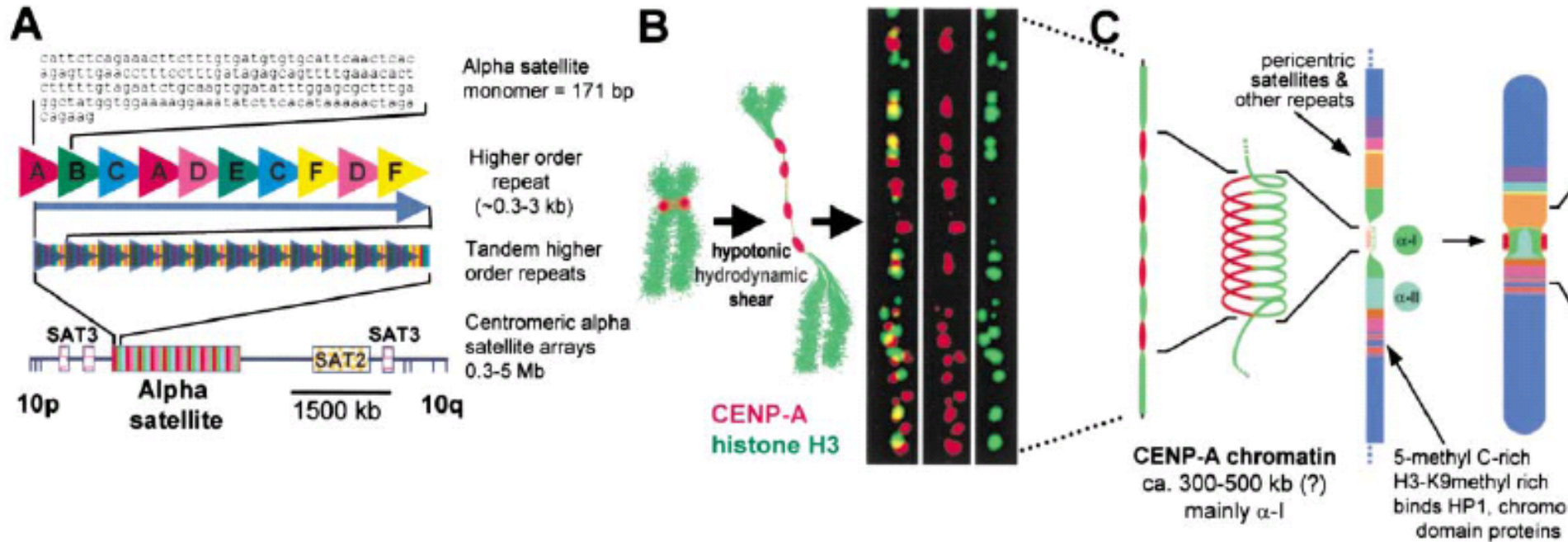


(B) Schematic illustration of centromere loci. Organization of centromeric DNA sequences from the four example organisms. (Top) Budding yeast with a 125 bp centromere comprised of three sequence domains (pink, red, yellow). Fission yeast centromeres show an organized structure, with a nonconserved central core (red), flanking inner repeats (pink arrows) at which the CENP-A-containing nucleosomes assemble, and conserved outer repeats (stippled purple). The *Drosophila* centromere spans ~400 kb (red) embedded in constitutive heterochromatin (purple). (Bottom) Human centromeres have sizes approaching 10 Mb and are comprised of α -I satellite DNA (red) and a more divergent, less regular α -II satellite (pink), flanked by heterochromatin (purple).

Cleveland et al. (2003) Cell (112): 407-21



Zentromerorganisation im Menschen



- A - Hierarchische Organisation von Alpha-Satelliten-DNA
- B - Diskontinuierliche Anordnung von Zentromerproteinen
- C - Faltung von Zentromerchromatin in mitotischen Chromosomen

Zentromermotive

einfache Motive von etwas über 100 Basen wie z.B. in Bäckerhefe

in den meisten Organismen besteht das Zentromer aus:

- Satellitensequenzen
- Transposablen Elementen

Repetitivität ist Voraussetzung für die Funktion
Regionen ohne Funktionsverlust austauschbar
Größe bis zu einigen Megabasen

Holozentrische Chromosomen

z.B. in *Caenorhabditis elegans* gibt es keine spezifische Zentromerposition.

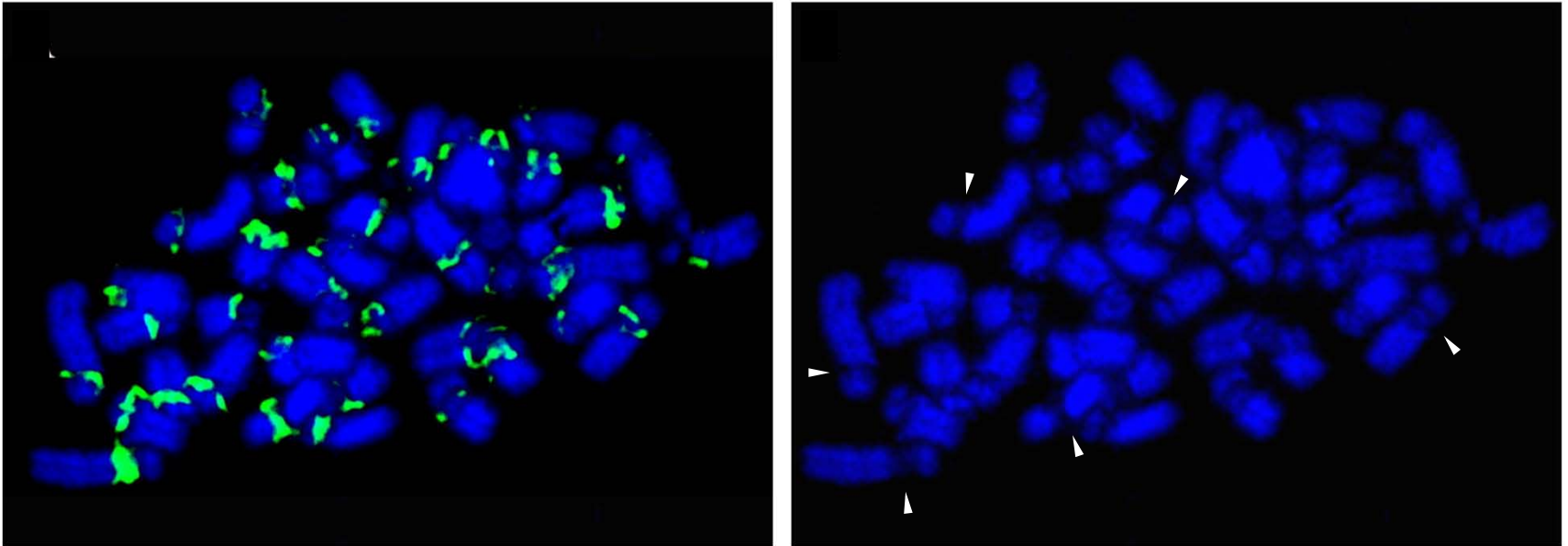


N. furzeri Zentromere

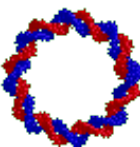


Genome

G+C rich – 77 bp minisatellite

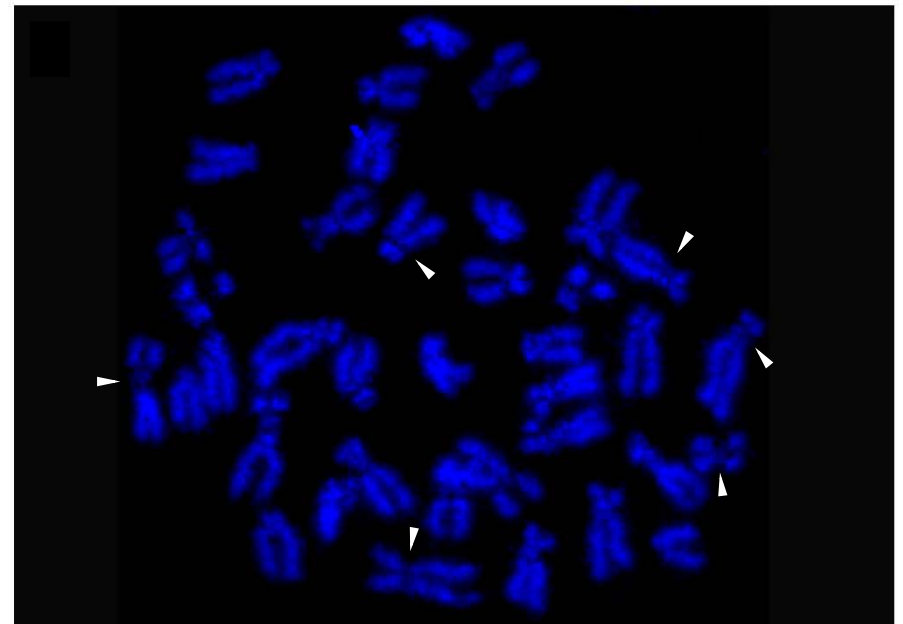
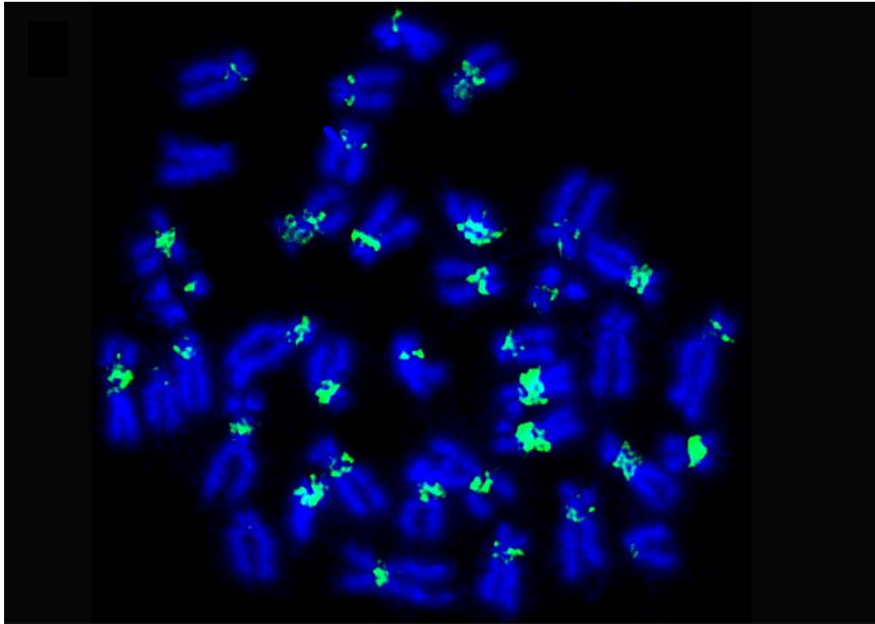


G+C rich tandem repeats map to centromeric regions

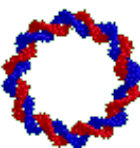


Genome

G+C rich – 77 bp minisatellite

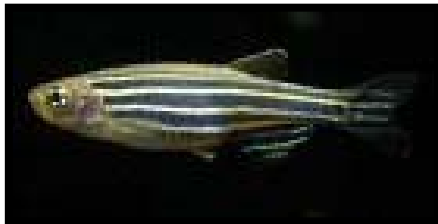
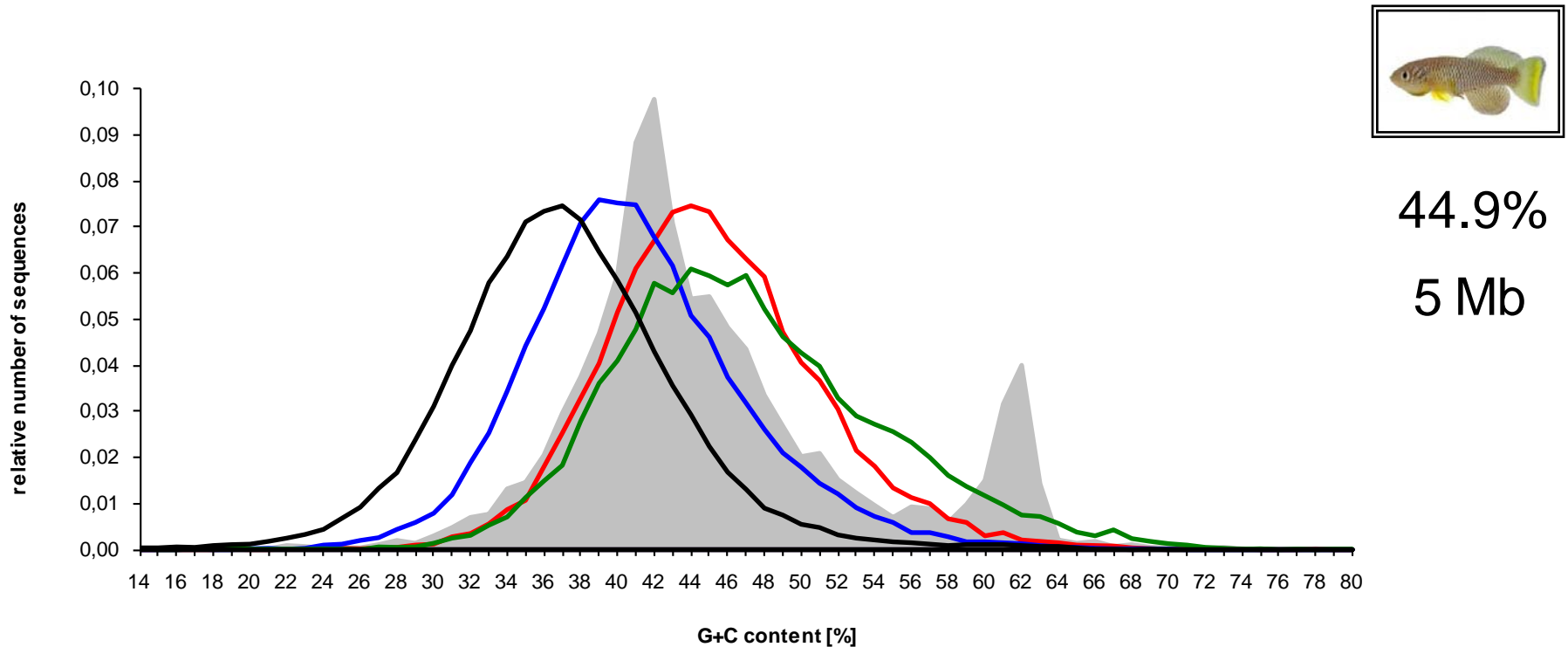


G+C poor tandem repeats also map to centromeric regions



Genomic landscape of *N.furzeri*

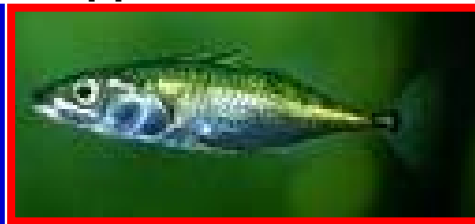
G+C



36.6%



40.5%



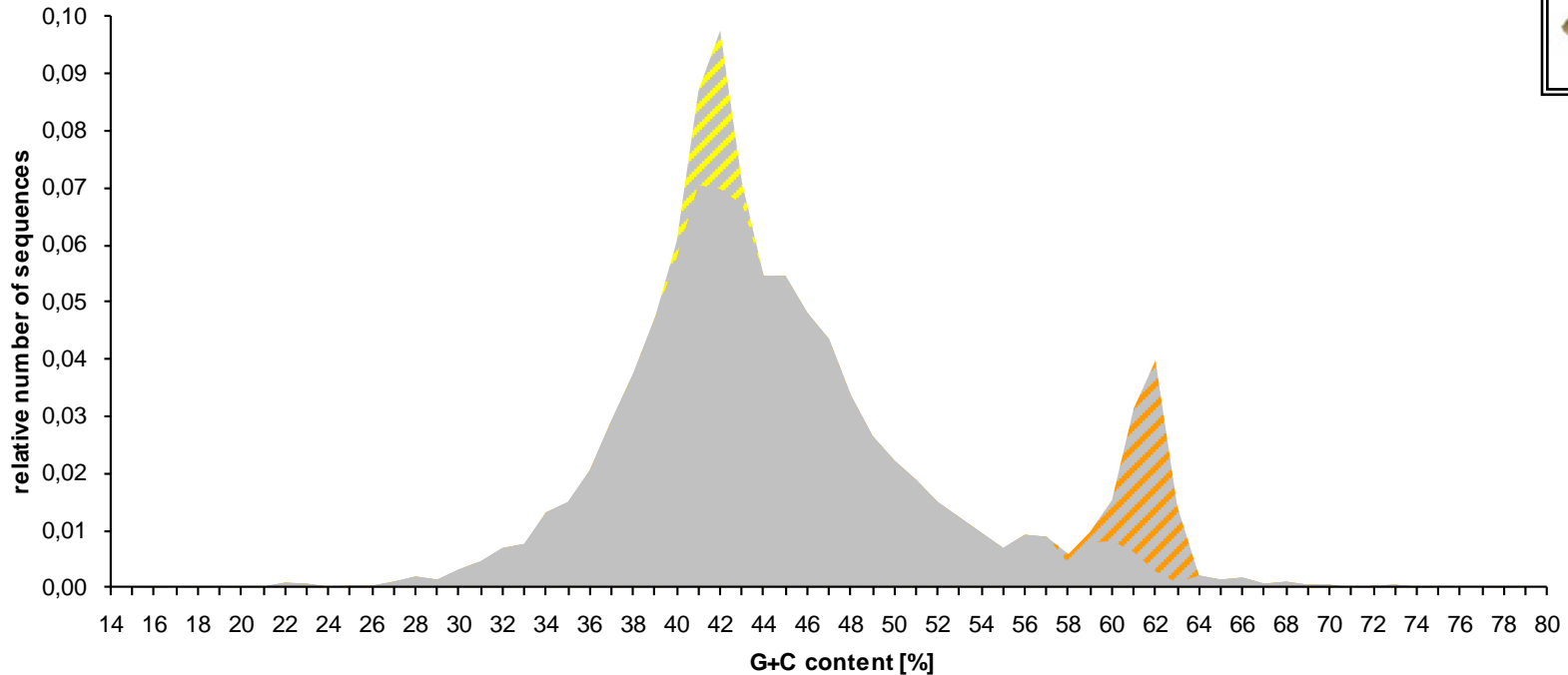
44.6%



46.4%

Genomic landscape of *N.furzeri*

G+C



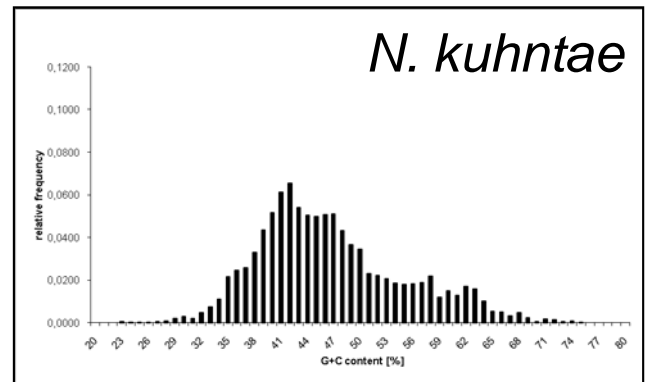
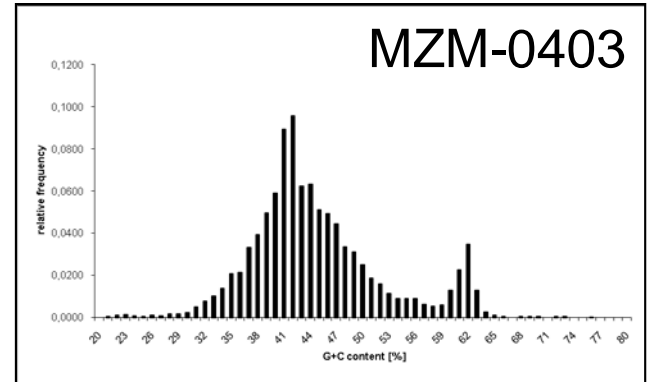
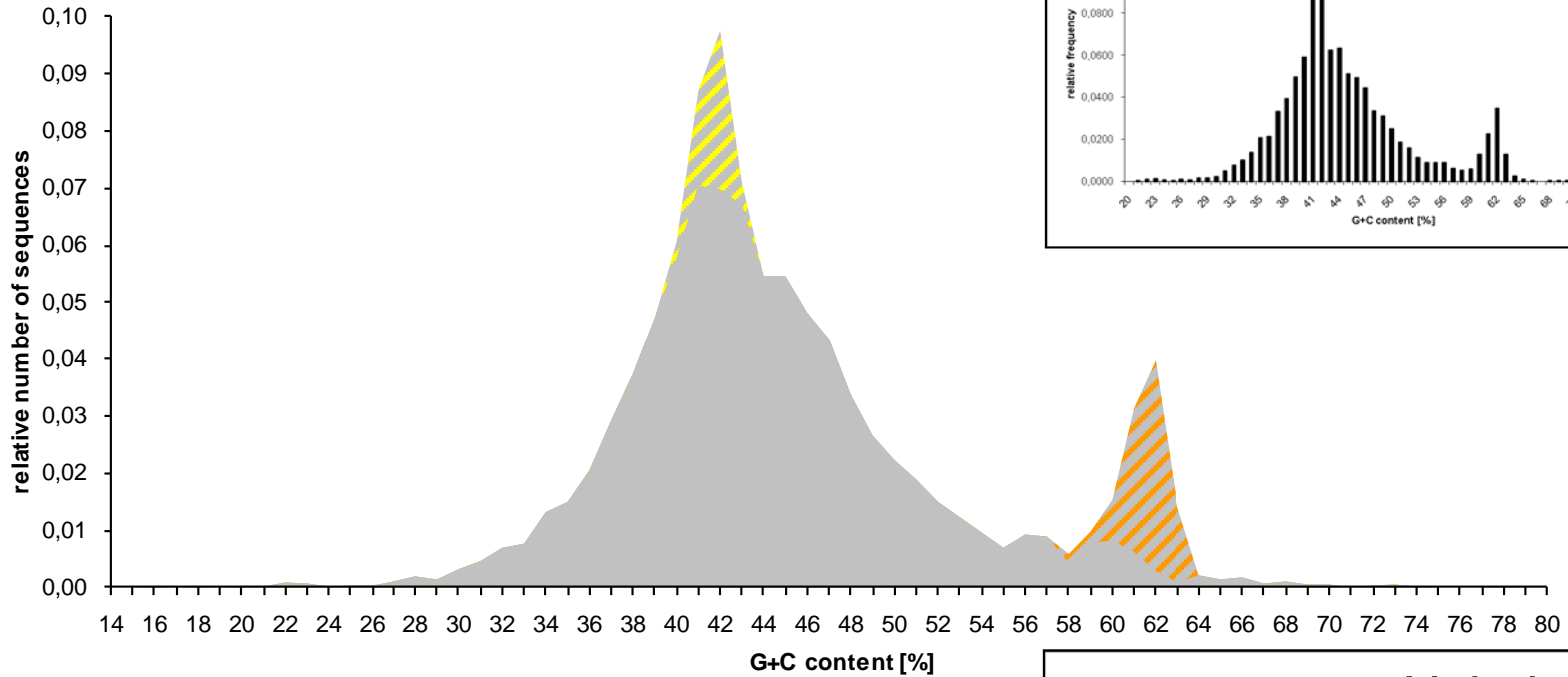
44.9%

5 Mb

G+C poor (yellow) and G+C rich (orange) tandem repeats

Genomic landscape of *N.furzeri*

G+C



END

