WS 2018/19 Modul 8 „Allgemeine und Molekulare Genetik“

JOHANNES GUTENBERG UNIVERSITÄT MAINZ

Chromosomen und Chromatin bei Eukaryoten

Kap. 9, 10, 11, 29

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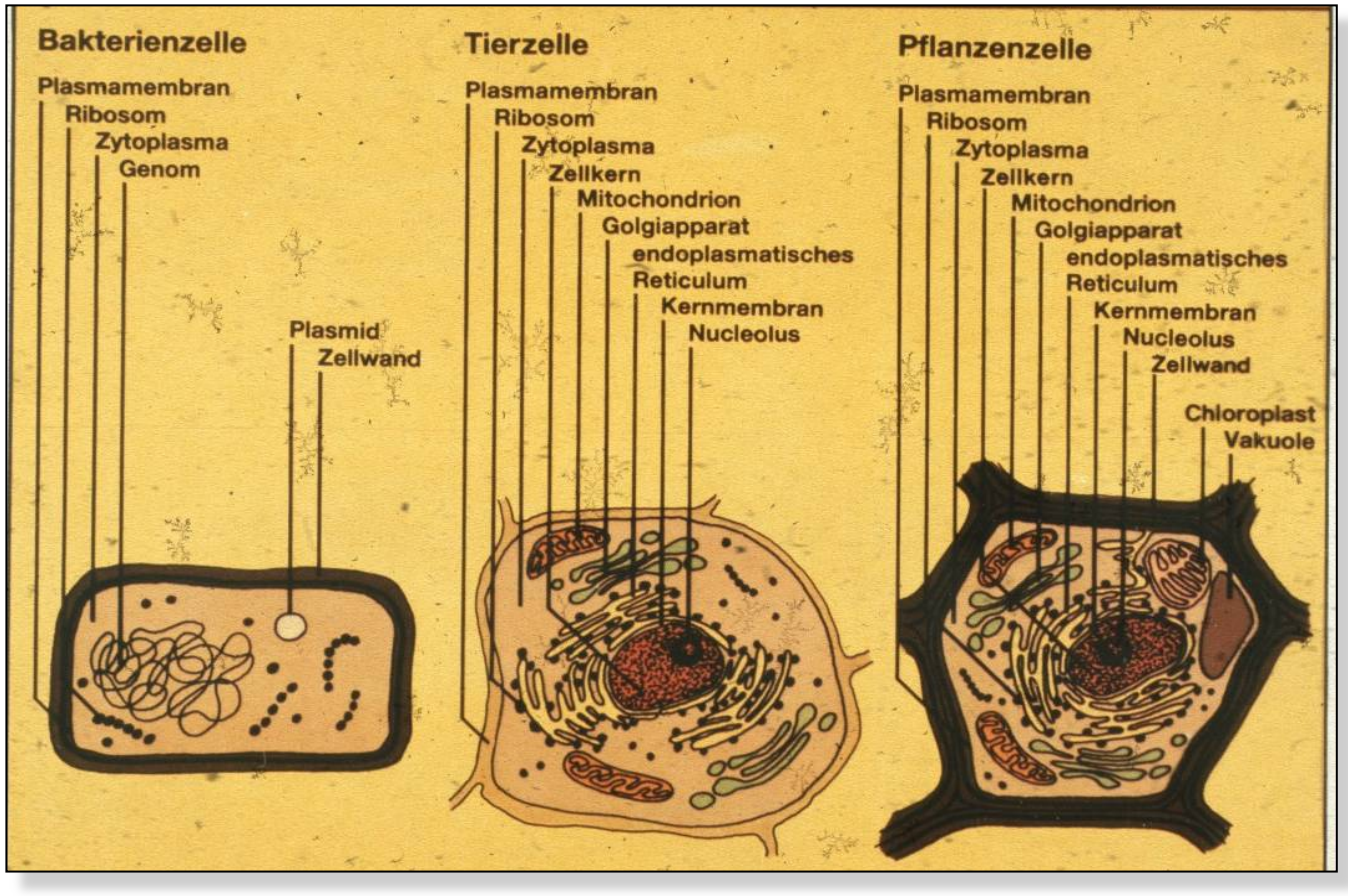
Chromatin

Walter Flemming
1879



= das Material, aus dem Chromosomen bestehen

- Chromatin im engeren Sinne findet sich im **Zellkern** eukaryotischer Zellen
- auch die DNA in Bakterien, Mitochondrien und Chloroplasten ist aber mit Proteinen besetzt („Nukleoid“)

Wilhelm von Waldeyer-Hartz
1888

Ein Chromosom...

• ist ein Komplex aus **DNA, Proteinen** und **RNA**.

• besteht bei Eukaryoten aus einem oder mehreren linearen DNA-Fäden (**1 Chromatide = 1 Doppelhelix**).

• garantiert die korrekte **Verteilung der Erbinformation** bei der Mitose und Meiose.

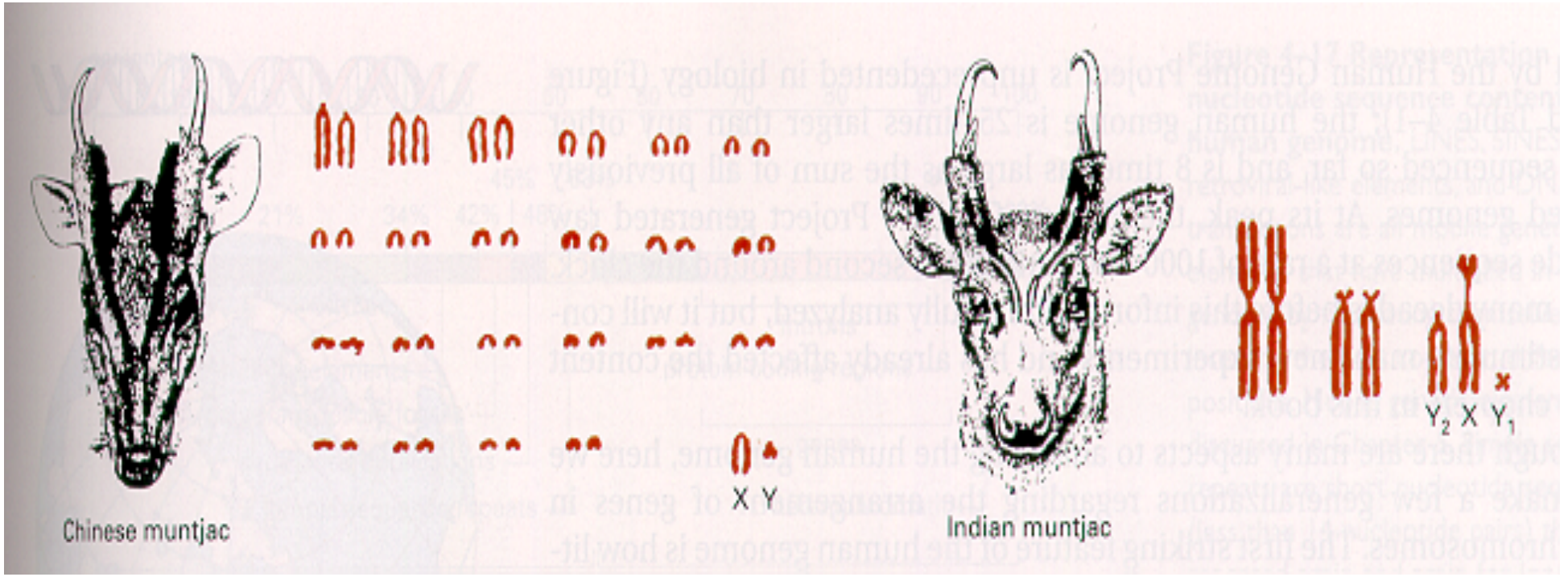
Genomgrößen & Chromosomenzahl

Organism	Genome size (Mb)[a]	Chromosome number[a]
Yeast (*Saccharomyces cerevisiae*)	14	16
Slime mold (*Dictyostelium*)	70	7
Arabidopsis thaliana	70	5
Corn	5,000	10
Onion	15,000	8
Lily	50,000	12
Nematode (*Caenorhabditis elegans*)	100	6
Fruit fly (*Drosophila*)	165	4
Toad (*Xenopus laevis*)	3,000	18
Lungfish	50,000	17
Chicken	1,200	39
Mouse	3,000	20
Cow	3,000	30
Dog	3,000	39
Human	3,000	23

[a] Both genome size and chromosome number are for haploid cells.
Mb = millions of base pairs.

1 Mb
=
10^6 Bp

Genomgrößen & Chromosomenzahl



M.W. Strickberger (2000) Evolution. Sudbury, MA

- keine Korrelation!
- Chromosomen können während der Evolution fusionieren oder Teile sich separieren!

Die Anzahl der Chromosomen...

- ist in den verschiedenen Zellen eines Organismus **konstant** (Ausnahme: B-Chromosomen).

- ist **artspezifisch** und korreliert nicht mit der Genomgröße.

- beim Menschen:
  2n = 46 mit 44 Autosomen und 2 Gonosomen, X bzw. Y

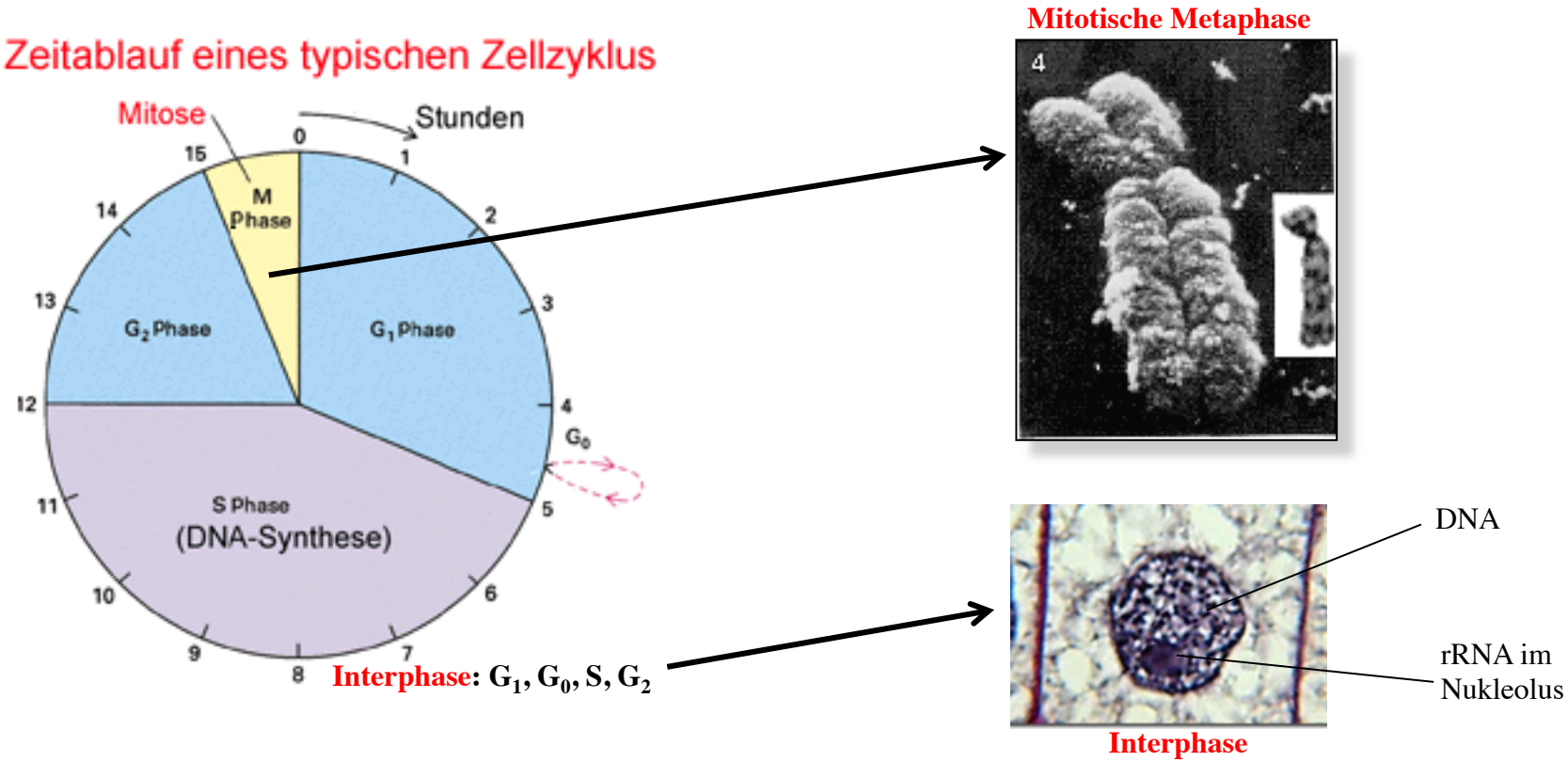
THE CHROMOSOME NUMBER OF MAN

By *JOE HIN TJIO* and *ALBERT LEVAN*

ESTACION EXPERIMENTAL DE AULA DEI, ZARAGOZA, SPAIN, AND CANCER CHROMOSOME LABORATORY, INSTITUTE OF GENETICS, LUND, SWEDEN

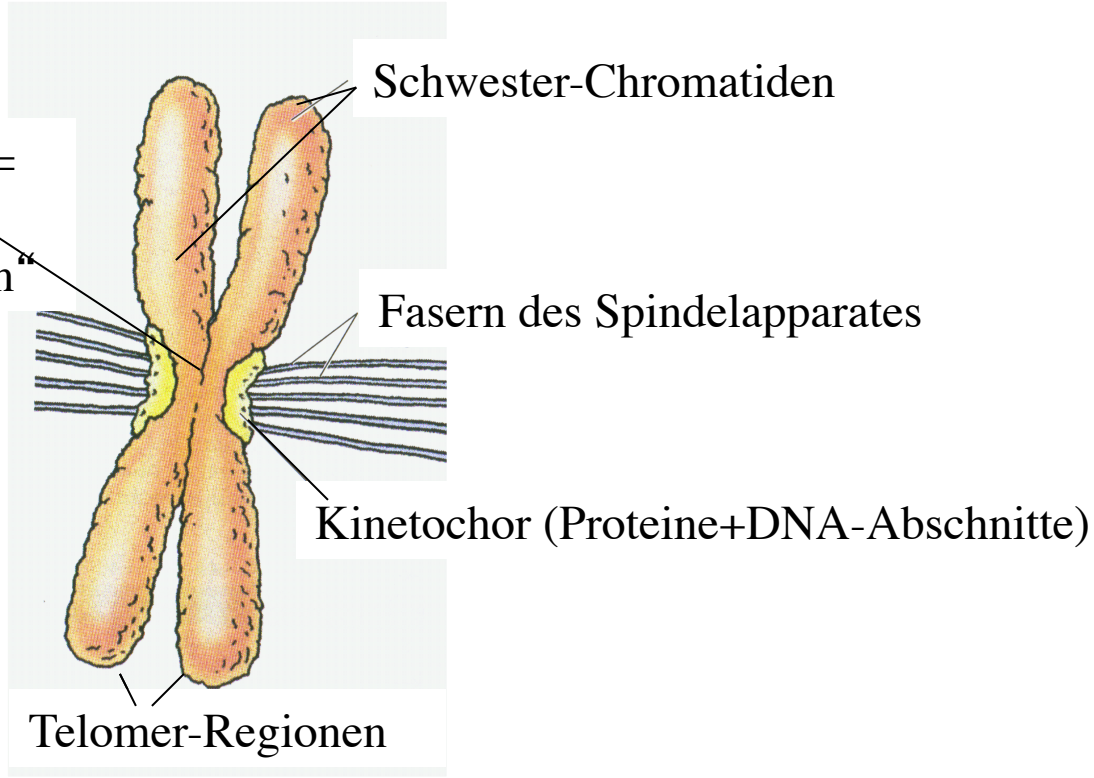
Hereditas 1956 !!

Chromosomen sind dynamische Strukturen



...und durchlaufen während des Zellzyklus (**Interphase** => **Mitose**) Dekondensation und Kondensation.

Merkmale von Metaphase-Chromosomen




A 35-year romance with a seafaring microbe p. 1006
Social ties and policy reforms in China's S&T system pp. 1019 & 1022
Designing zeolites to react pp. 1028 & 1032
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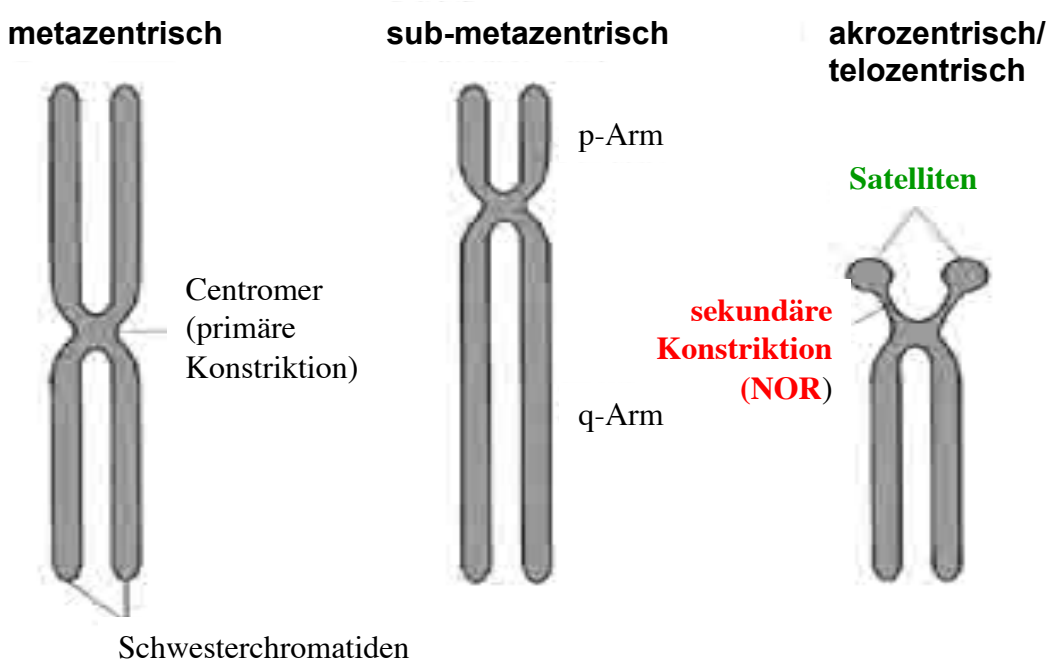
Remodeling the yeast genome piece by piece p. 1038

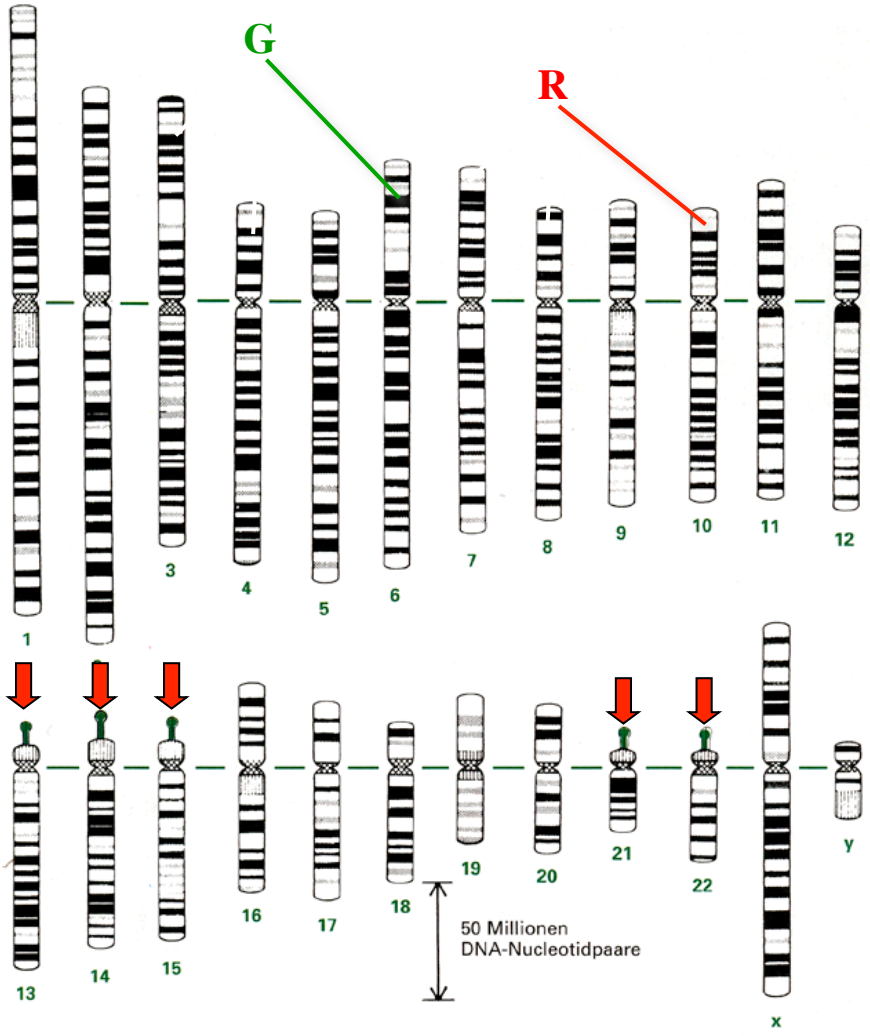
Die Lage des Centromers variiert



• In der **sekundären Konstriktion** der akrozentrischen Chromosomen 13, 14, 15, 21 und 22 liegen die **Gene für die rRNA**, die bei Transkription in der Interphase den **Nukleolus** bilden (NOR = nucleolus organizing region).

• Die als „**Satelliten**“ bezeichneten Endstücke der Chromosomen haben NICHTS mit der repetitiven Satelliten-DNA der Centromere (s.u.) zu tun.

Identifikation der Chromosomen über G- und R-Banden (Giemsa-Färbung)

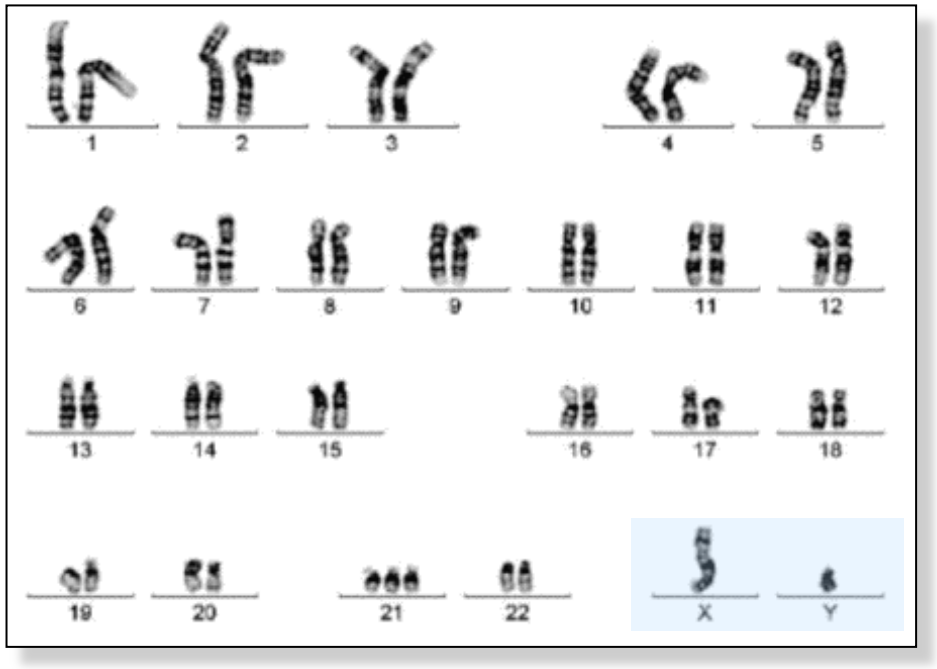


- **R-Banden** (revers: nicht gefärbt nach Trypsin-Behandlung): überdurchschnittlich viele Gene, GC-reich, frühe Replikation, beim Menschen reich an SINE/Alu-Transposons

- **G-Banden** (Giemsa-gefärbt nach Trypsin-Behandlung): wenige Gene, AT-reich, späte Replikation, beim Menschen reich an LINE-Transposons

Chromosomale Aberrationen im humanen Karyotyp

Karyogramm



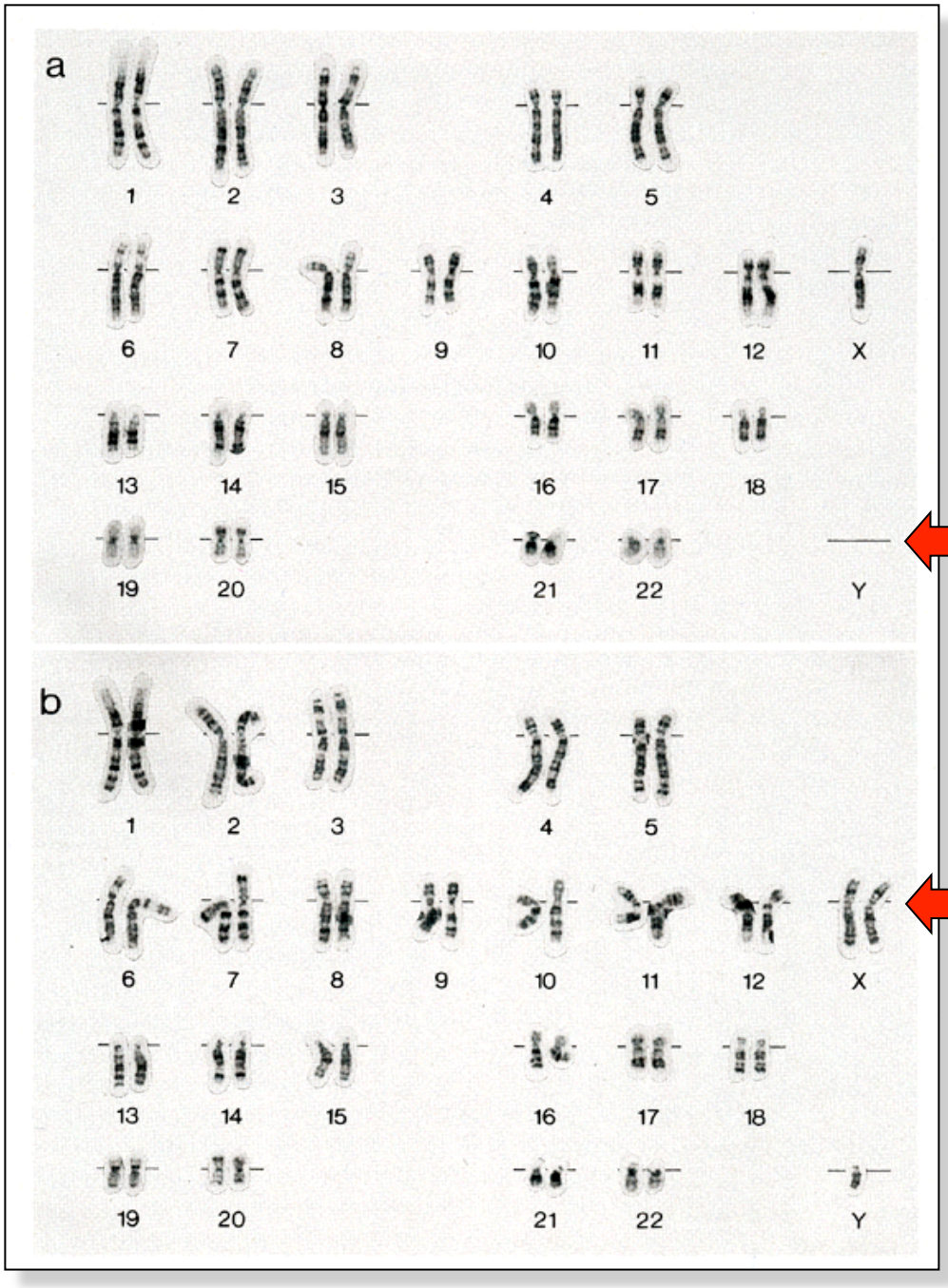
Autosomen

Gonosomen

Down-Syndrom: 47, XY, +21

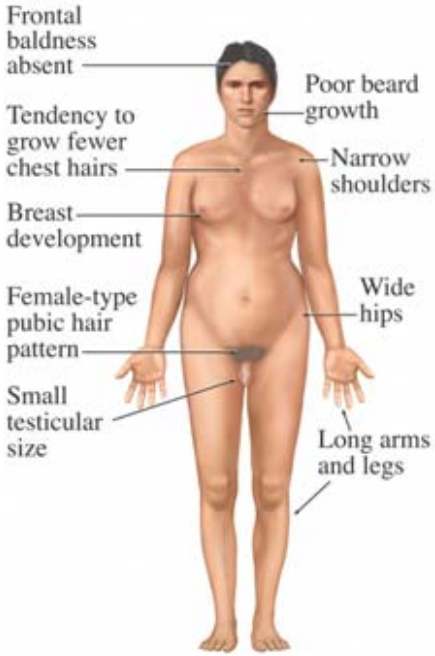
Karyotyp

Gonosomale Aberrationen

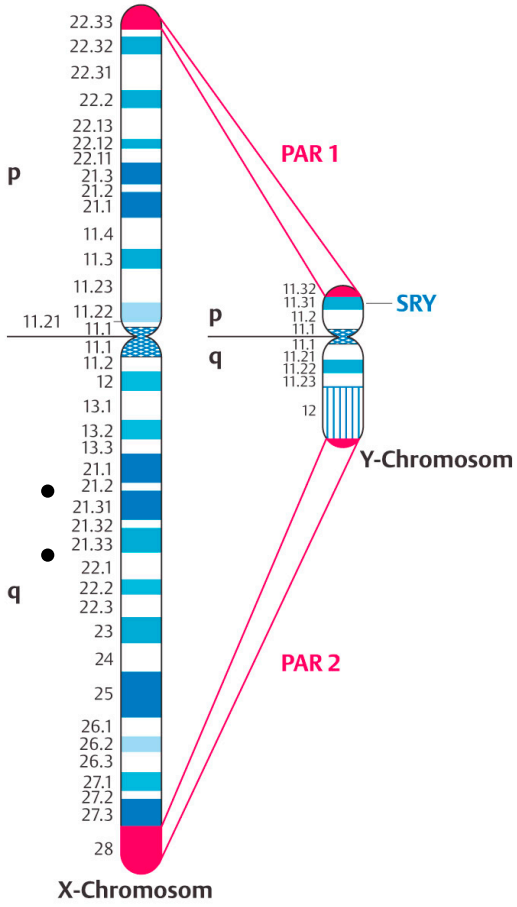


Turner-Syndrom
45, X0

Klinefelter-Syndrom
47, XXY

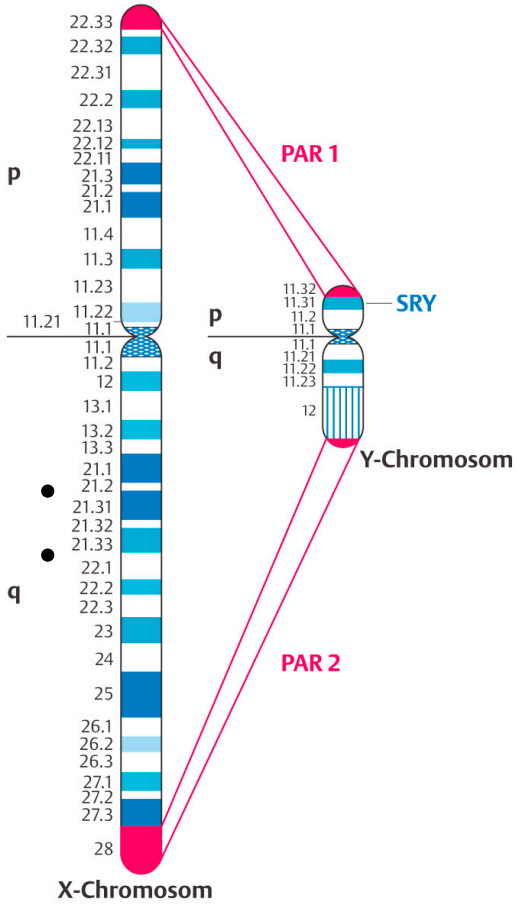


Geschlechtschromosomen bei Säugern: X und Y



- Männchen **heterogametisch** (XY)
- Weibchen **homogametisch** (XX)
- X-Chromosom 165 Mb, ca. 1100 Gene
- Y-Chromosom 60 Mb, nur 60 Gene

Geschlechtsbestimmung: *Ein* Gen macht den Mann!



SRY-Gen:

- **s**ex determining **r**egion of **Y**
- **dominanter Geschlechtsbestimmer, bewirkt männlichen Phänotyp**
- **„Transkriptionsfaktor“**

Nov. 2013

Sciencexpress Resea

Two Y Genes Can Replace the Entire Y Chromosome for Assisted Reproduction in the Mouse

Yasuhiro Yamauchi, Jonathan M. Riel, Zoia Stoytcheva, Monika A. Ward*

Institute for Biogenesis Research, John A. Burns School of Medicine, University of Hawaii, 1960 East-West Road, Honolulu, HI, 96822, USA.

*Corresponding author. E-mail: mward@hawaii.edu

The Y chromosome is thought to be important for male reproduction. We have previously shown that, with the use of assisted reproduction, live offspring can be obtained from mice lacking the entire Y chromosome long arm. Here, we demonstrate that live mouse progeny can also be generated by using germ cells from males with the Y chromosome contribution limited to only two genes, the testis determinant factor *Sry* and the spermatogonial proliferation factor *Eif2s3y*. *Sry* is believed to function primarily in sex determination during fetal life. *Eif2s3y* may be the only Y chromosome gene required to drive mouse spermatogenesis, allowing formation of haploid germ cells that are functional in assisted reproduction. Our findings are relevant, but not directly translatable, to human male

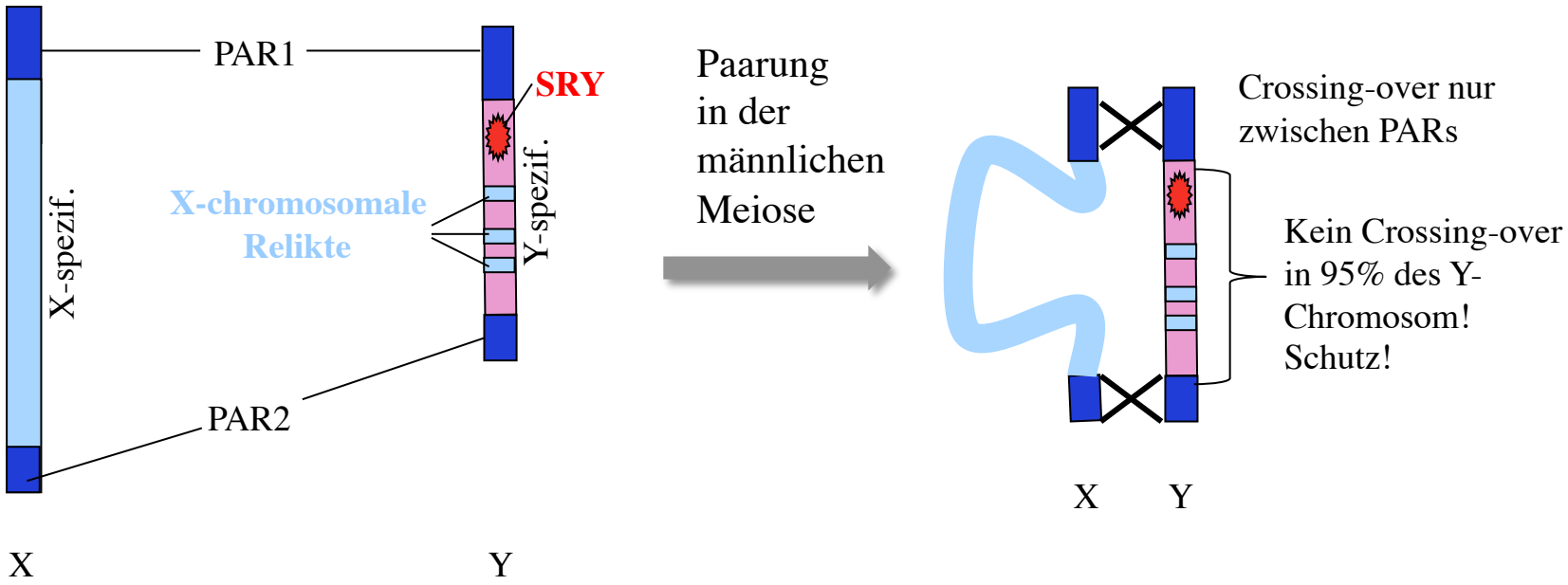
...zweites essenzielles Gen sorgt also für Fertilität!

Dann sprach Gott, der Herr: Es ist nicht gut, dass der Mensch allein bleibt. Er formte aus dem Ackerboden alle Tiere. Gott ließ den Menschen in einen tiefen Schlaf fallen, nahm eine seiner Rippen und baute aus ihr eine Frau. Und der Mensch sprach: Das endlich ist Bein von meinem Bein und Fleisch von meinem Fleisch. Frau soll sie heißen.

Genesis 2.4

Säuger-Geschlechtschromosomen

- **Das Y ist ein degeneriertes X-Chromosom!**

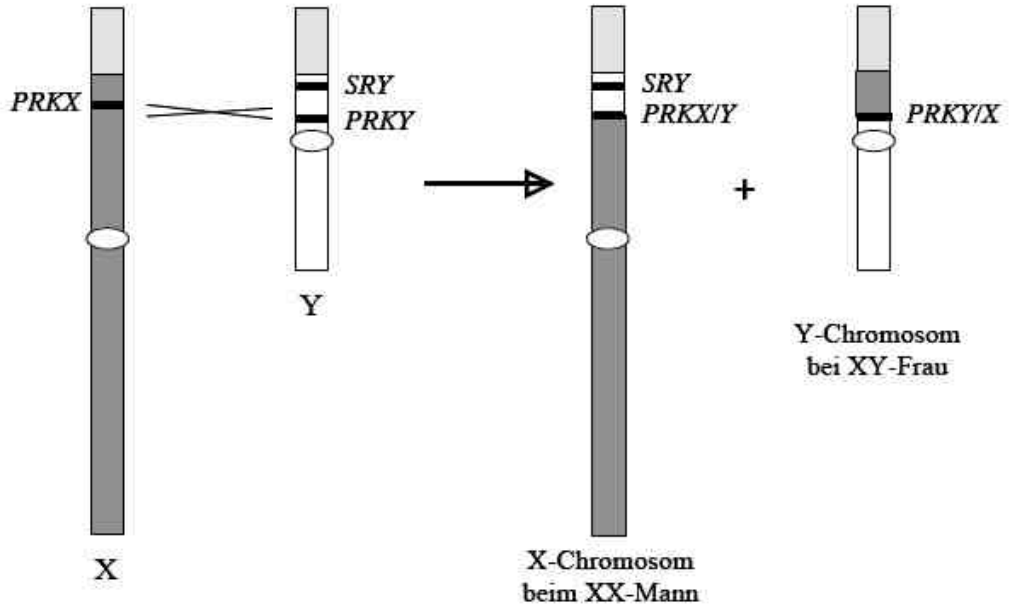


- Paarung der **p**seudo-**a**utosomalen **R**egionen (**PAR**) von X und Y während der männlichen Meiose => Crossing-over findet statt!

Vorsicht! SRY liegt nur 5 kb von PAR1-Grenze entfernt...

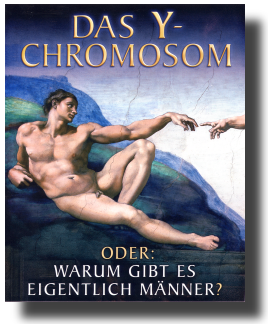
- **Unfall in 1 von 20 000 männlichen Meiosen:**

Crossing-over außerhalb der PAR1 zwischen den homologen Genen PRKX und PRKY führt zu **Translokation** von SRY.



XX-Männer !!

XY-Frauen !!



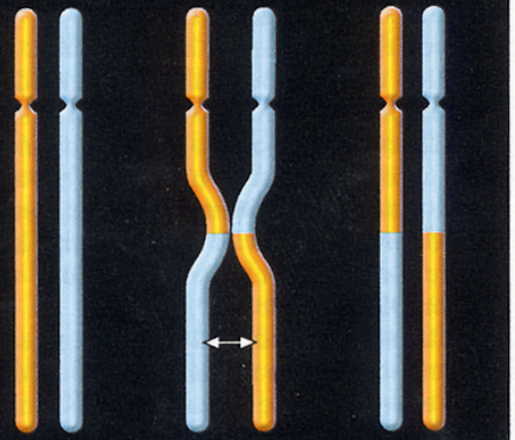
„Selbst-Heilung“ des Y

Aus: Der Spiegel, Okt. 2003

Wie das Y-Chromosom seine Wunden heilt

Im Zellkern jeder Menschenzelle befinden sich 22 Chromosomenpaare sowie das Geschlechtschromosomenpaar XX oder XY. Im Verlaufe der Reifung von Eizelle und Spermium tauschen die Chromosomenpaare untereinander genetisches Material aus. Diese Rekombination erhöht die genetische Vielfalt und kann zugleich Fehler im Erbgut entfernen. Das geschrumpfte Y-Chromosom jedoch kann nur bedingt rekombinieren und muss sich auf anderem Wege behelfen.

zwei X-Chromosomen (Frau)

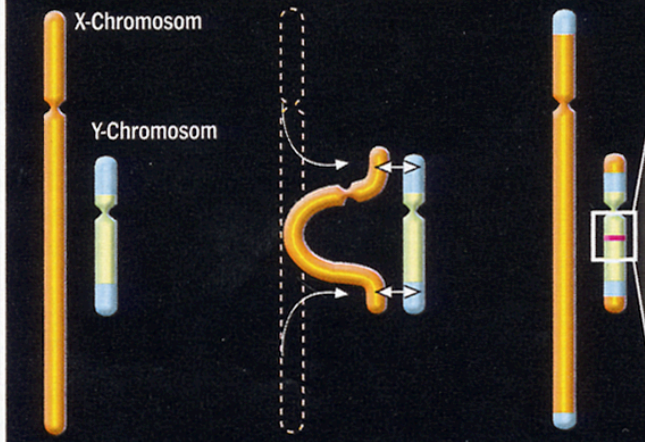


Die Chromosomen lagern sich aneinander.

Sie tauschen Teile ihrer DNS aus (Rekombination).

Defekte Bereiche konnten mit gesunden ausgetauscht werden.

X- und Y-Chromosom (Mann)



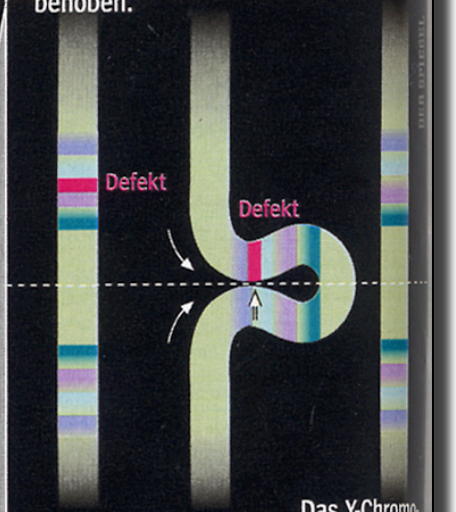
Das Y-Chromosom ist dreimal kleiner als das X-Chromosom.

Der DNS-Austausch ist nur an den Enden der Chromosomen möglich.

Keine Rekombination im mittleren Bereich des Y-Chromosoms.

Palindrome zur Reparatur des Y-Chromosoms

Viele DNS-Bausteine auf den Y-Chromosomen sind wie Spiegelbilder (Palindrome) angeordnet. Fehler werden durch Ausgleich behoben.



Das Y-Chromosom hat sich selbst geheilt.

Wird das Y-Chromosom verschwinden?

ARTICLE

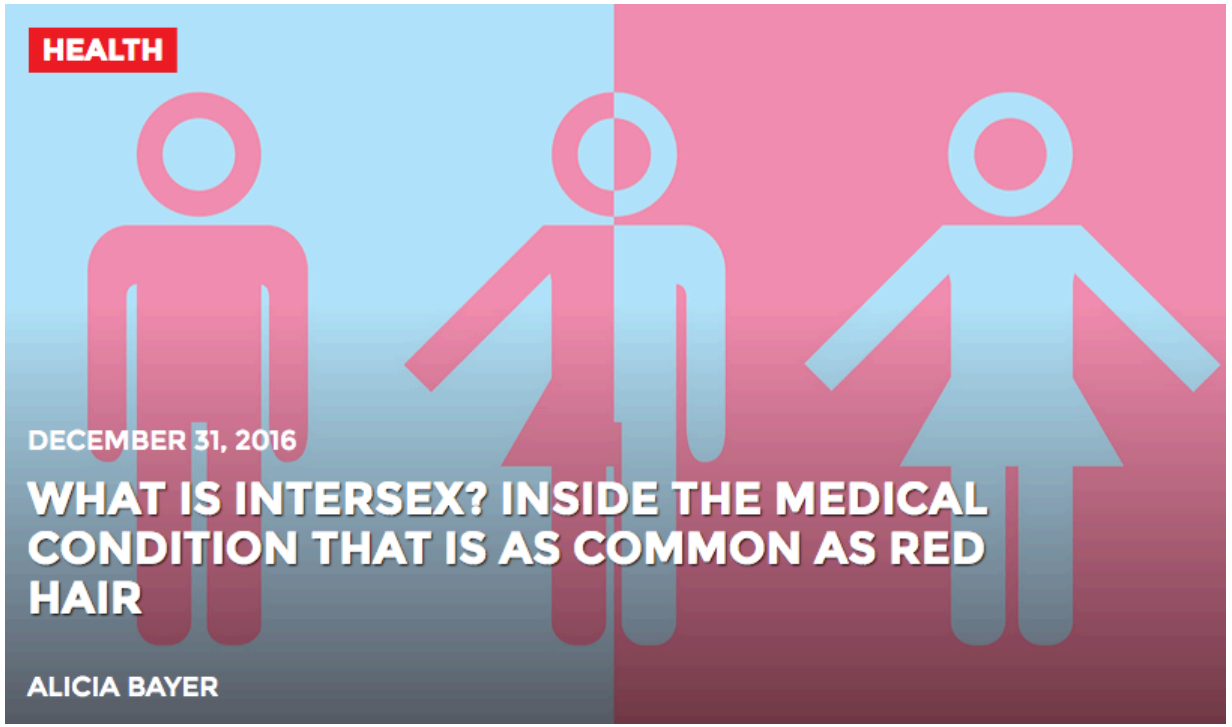
doi:10.1038/nature13206

Mammalian Y chromosomes retain widely expressed dosage-sensitive regulators

Daniel W. Bellott[1], Jennifer F. Hughes[1], Helen Skaletsky[1], Laura G. Brown[1], Tatyana Pyntikova[1], Ting-Jan Cho[1], Natalia Koutseva[1], Sara Zaghlul[1], Tina Graves[2], Susie Rock[2], Colin Kremitzki[2], Robert S. Fulton[2], Shannon Dugan[3], Yan Ding[3], Donna Morton[3], Ziad Khan[3], Lora Lewis[3], Christian Buhay[3], Qiaoyan Wang[3], Jennifer Watt[3], Michael Holder[3], Sandy Lee[3], Lynne Nazareth[3], Jessica Alföldi[1], Steve Rozen[1], Donna M. Muzny[3], Wesley C. Warren[2], Richard A. Gibbs[3], Richard K. Wilson[2] & David C. Page[1]

The human X and Y chromosomes evolved from an ordinary pair of autosomes, but millions of years ago genetic decay ravaged the Y chromosome, and only three per cent of its ancestral genes survived. We reconstructed the evolution of the Y chromosome across eight mammals to identify biases in gene content and the selective pressures that preserved the surviving ancestral genes. Our findings indicate that survival was nonrandom, and in two cases, convergent across placental and marsupial mammals. We conclude that the gene content of the Y chromosome became specialized through selection to maintain the ancestral dosage of homologous X–Y gene pairs that function as broadly expressed regulators of transcription, translation and protein stability. We propose that beyond its roles in testis determination and spermatogenesis, the Y chromosome is essential for male viability, and has unappreciated roles in Turner's syndrome and in phenotypic differences between the sexes in health and disease.

1-2 / 100 Geburten!



Das Säuger-X-Chromosom

Nature 17. März 2005

The X factor

The sequence of the 'feminine' X chromosome is a prime hunting ground for geneticists interested in the evolution of the cognitive and cultural sophistication that defines the human species. Erika Check reports.

P. GINTER

"If higher cognitive abilities were a critical step in our evolution, it makes sense that you'd find those functions on the X chromosome."

— Hunt Willard

Opinion TRENDS in Genetics Vol.17 No.12 De

A high density of X-linked genes for general cognitive ability: a run-away process shaping human evolution?

Ulrich Zechner, Monika Wilda, Hildegard Kehrer-Sawatzki, Walther Vogel, Rainald Fundele and Horst Hameister

The incidence of mental disability is 30% higher in males than in females. We have examined entries in the OMIM database that are associated with mental disability and for several other common defects. Our findings indicate that compared with the autosomes, the X chromosome contains a significantly higher number of genes that, when mutated, cause mental impairment. We propose that these genes are involved in the development of cognitive abilities and thus exert a large X-chromosome effect on general intelligence in humans. We discuss these conclusions with regard to the conservation of the vertebrate X-chromosomal linkage group and to human evolution.

Gershoni and Pietrokovski *BMC Biology* (2017) 15:7
DOI 10.1186/s12915-017-0352-z

BMC Biology

RESEARCH ARTICLE Open Access

The landscape of sex-differential transcriptome and its consequent selection in human adults

Moran Gershoni* and Shmuel Pietrokovski

Abstract

Background: The prevalence of several human morbid phenotypes is sometimes much higher than intuitively expected. This can directly arise from the presence of two sexes, male and female, in one species. Men and women have almost identical genomes but are distinctly dimorphic, with dissimilar disease susceptibilities. Sexually dimorphic traits mainly result from differential expression of genes present in both sexes. Such genes can be subject to different, and even opposing, selection constraints in the two sexes. This can impact human evolution by differential selection on mutations with dissimilar effects on the two sexes.

Results: We comprehensively mapped human sex-differential genetic architecture across 53 tissues. Analyzing available RNA-sequencing data from 544 adults revealed thousands of genes differentially expressed in the reproductive tracts and tissues common to both sexes. Sex-differential genes are related to various biological systems, and suggest new insights into the pathophysiology of diverse human diseases. We also identified a significant association between sex-specific gene transcription and reduced selection efficiency and accumulation of deleterious mutations, which might affect the prevalence of different traits and diseases. Interestingly, many of the sex-specific genes that also undergo reduced selection efficiency are essential for successful reproduction in men or women. This seeming paradox might partially explain the high incidence of human infertility.

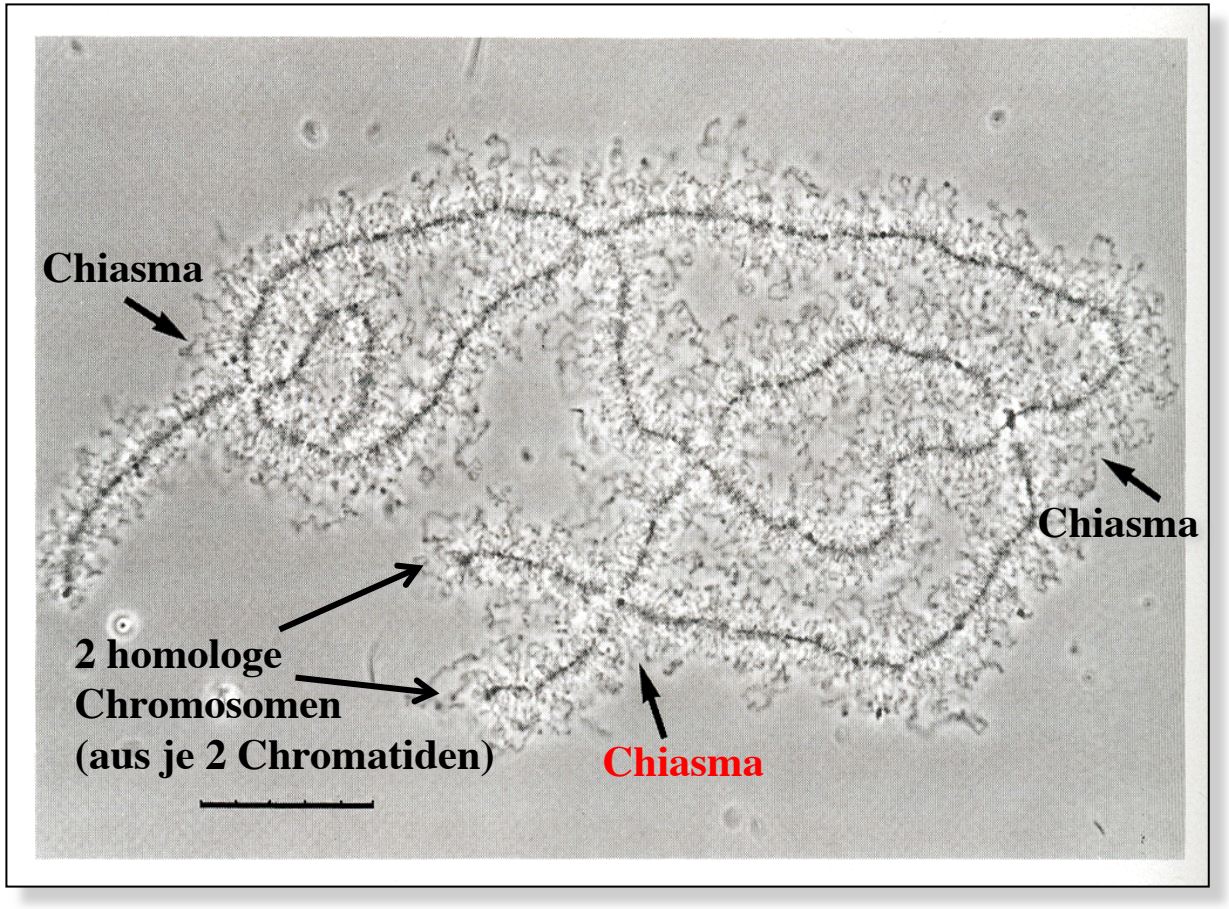
Conclusions: This work provides a comprehensive overview of the sex-differential transcriptome and its importance to human evolution and human physiology in health and in disease.

Keywords: Sex-differential expression, Sex-differential selection, Sexual dimorphism

Ca. 6000 Gene geschlechtsspezifisch reguliert!!

„Spezialchromosomen /1“

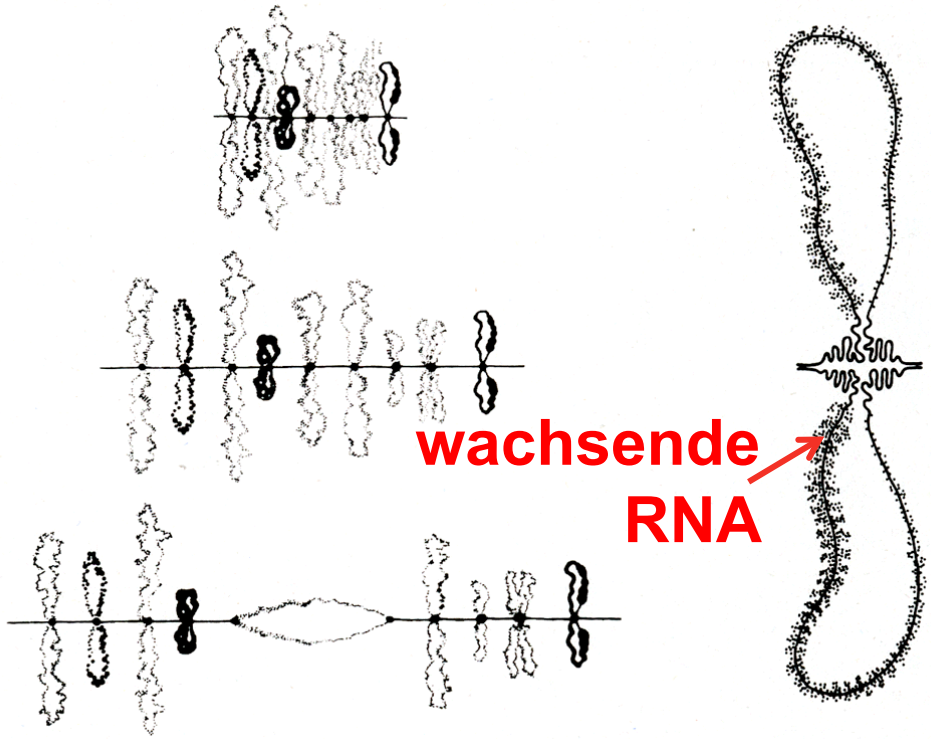
Lampenbürstenchromosomen



- in Prophase I der weiblichen und männlichen **Meiose**

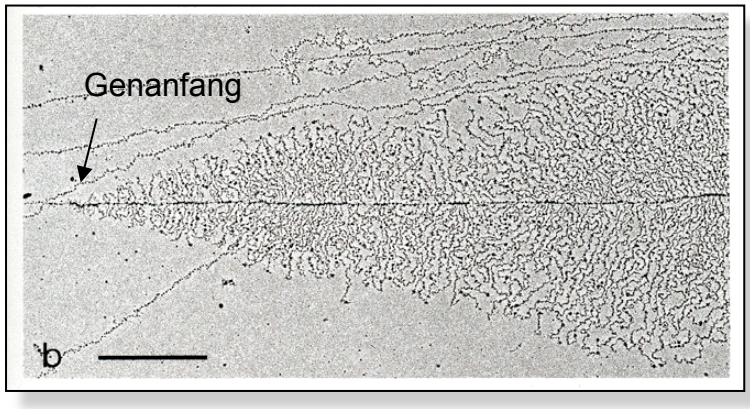
- besonders schön in Oocyten von Amphibien

Lampenbürstenchromosomen



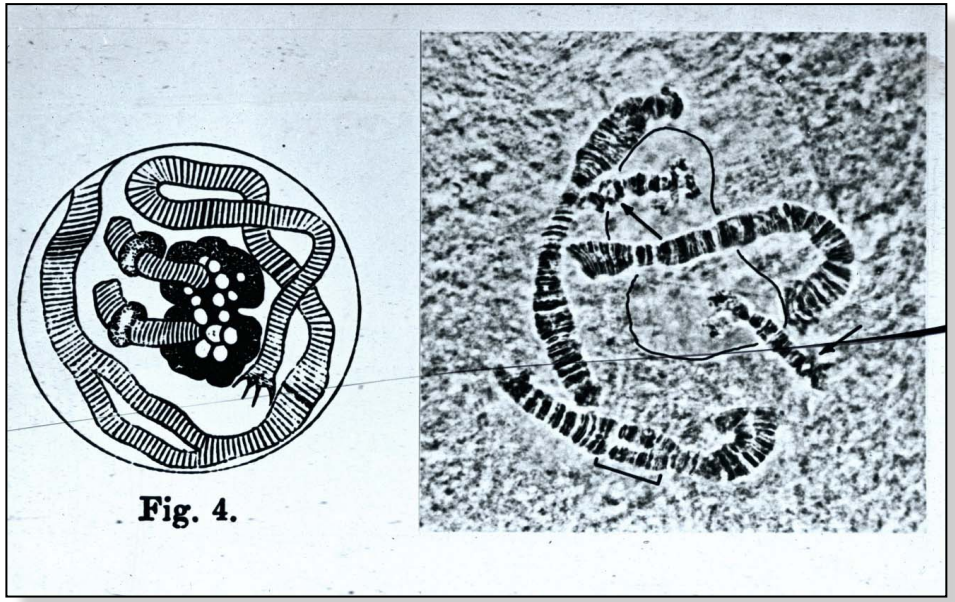
- Jedes Chromosom besteht erwartungsgemäß aus zwei Chromatiden.

- An den Schleifen findet starke Transkription statt (Funktion +/- unklar).



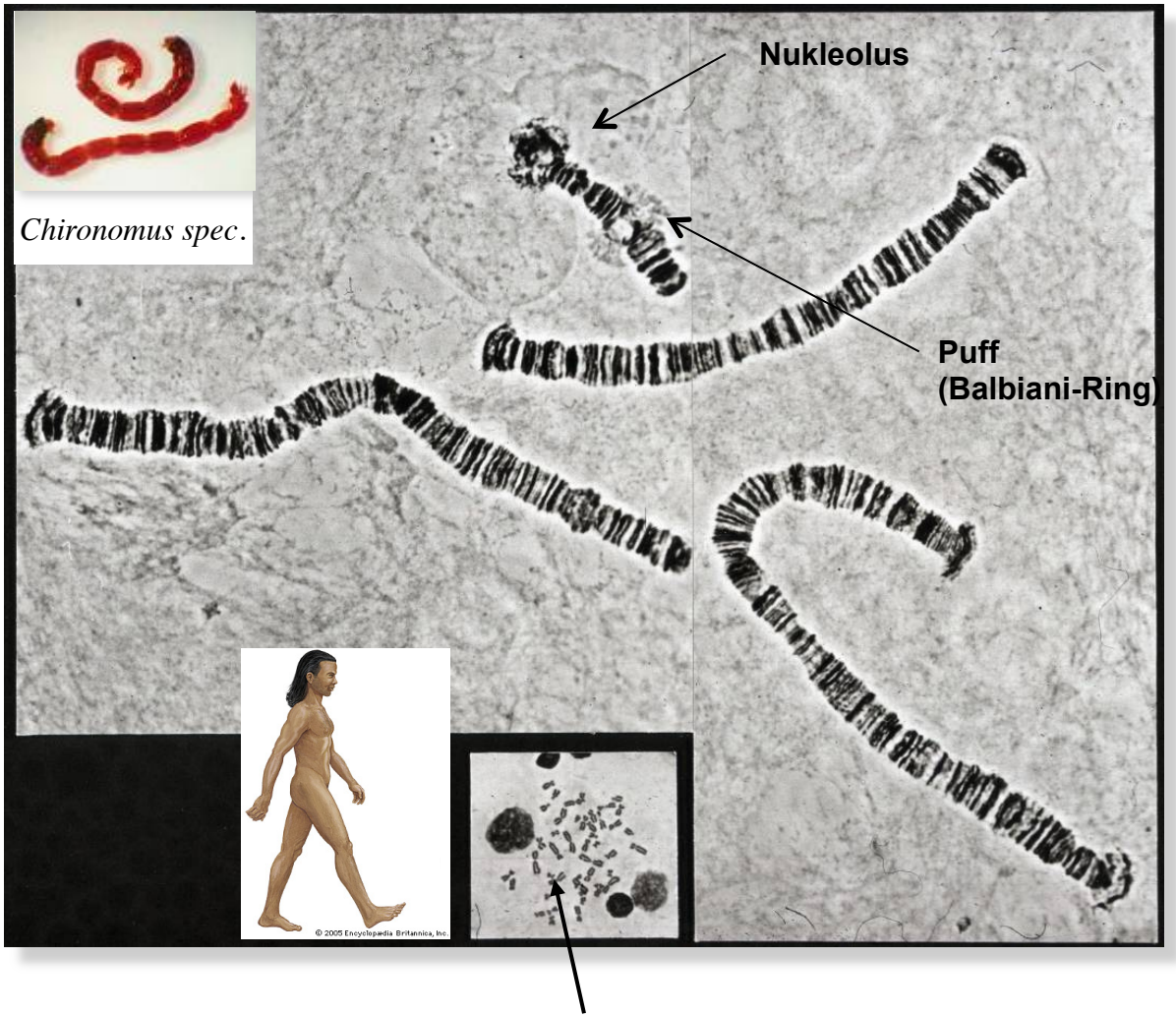
„Spezialchromosomen /2“

Polytäne Riesenchromosomen



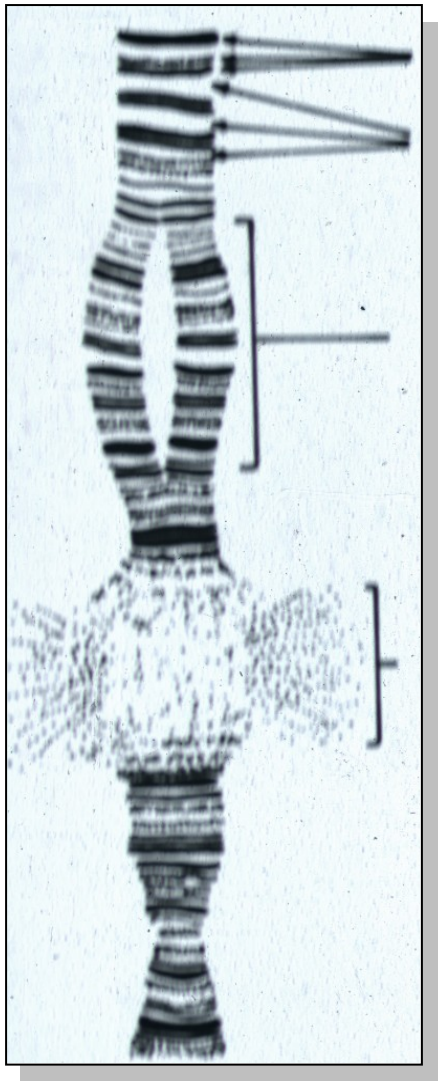
- entdeckt durch Balbiani, 1881
- in **Speicheldrüsenzellen von Dipteren**, aber auch in Ciliaten, Pflanzen, Collembolen etc.
- **Interphase**-Chromosomen
- charakteristisches Banden-Interbanden-Muster sowie „**Puffs**“

Riesenchromosomen sind XXX*L*...



Menschliche Metaphase-Chromosomen bei gleicher Vergrößerung!

Struktur von Polytänchromosomen



Banden

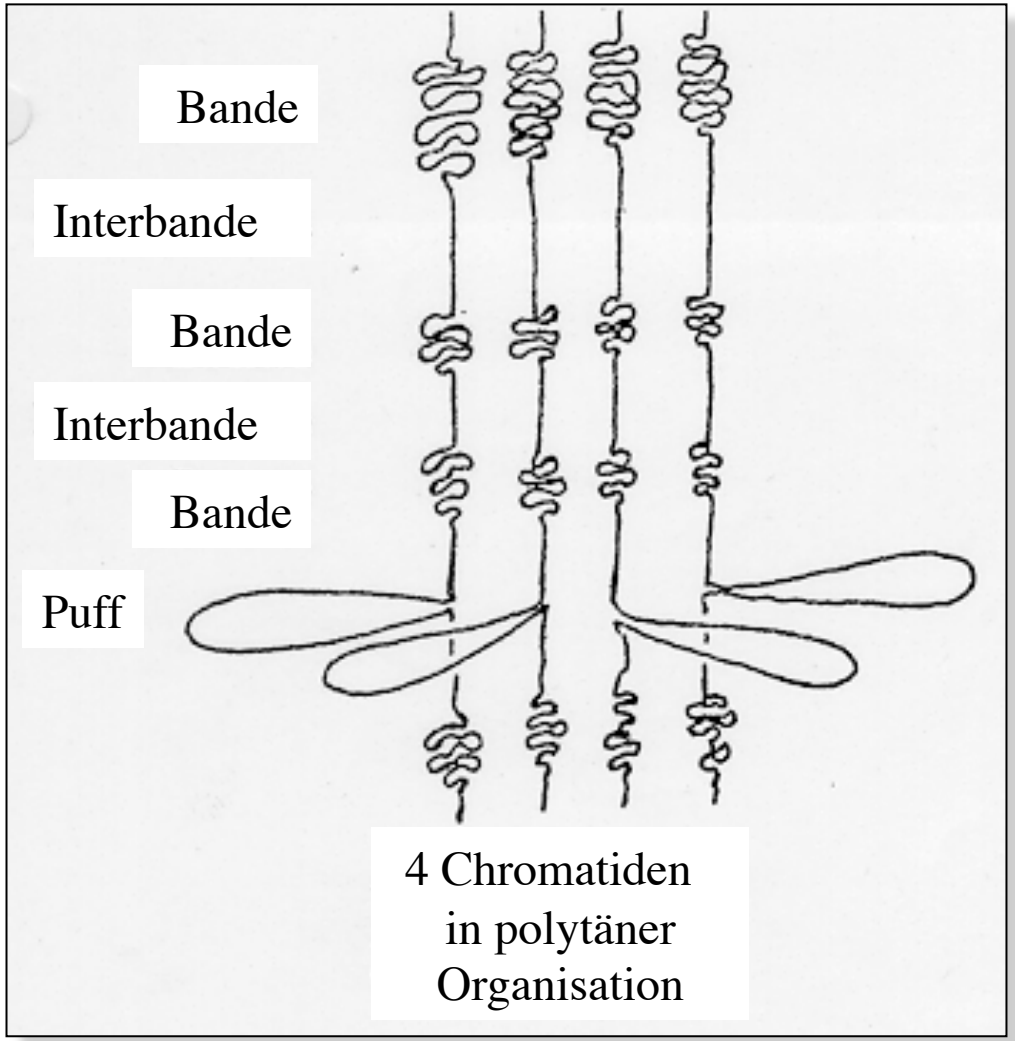
Interbanden

Chromosomenkörper aus den gepaarten 2 Homologen (=**somatische Paarung**; nur bei Dipteren)!
Jedes Chromosom besteht zudem aus **mehreren Tausend Chromatiden* !!**

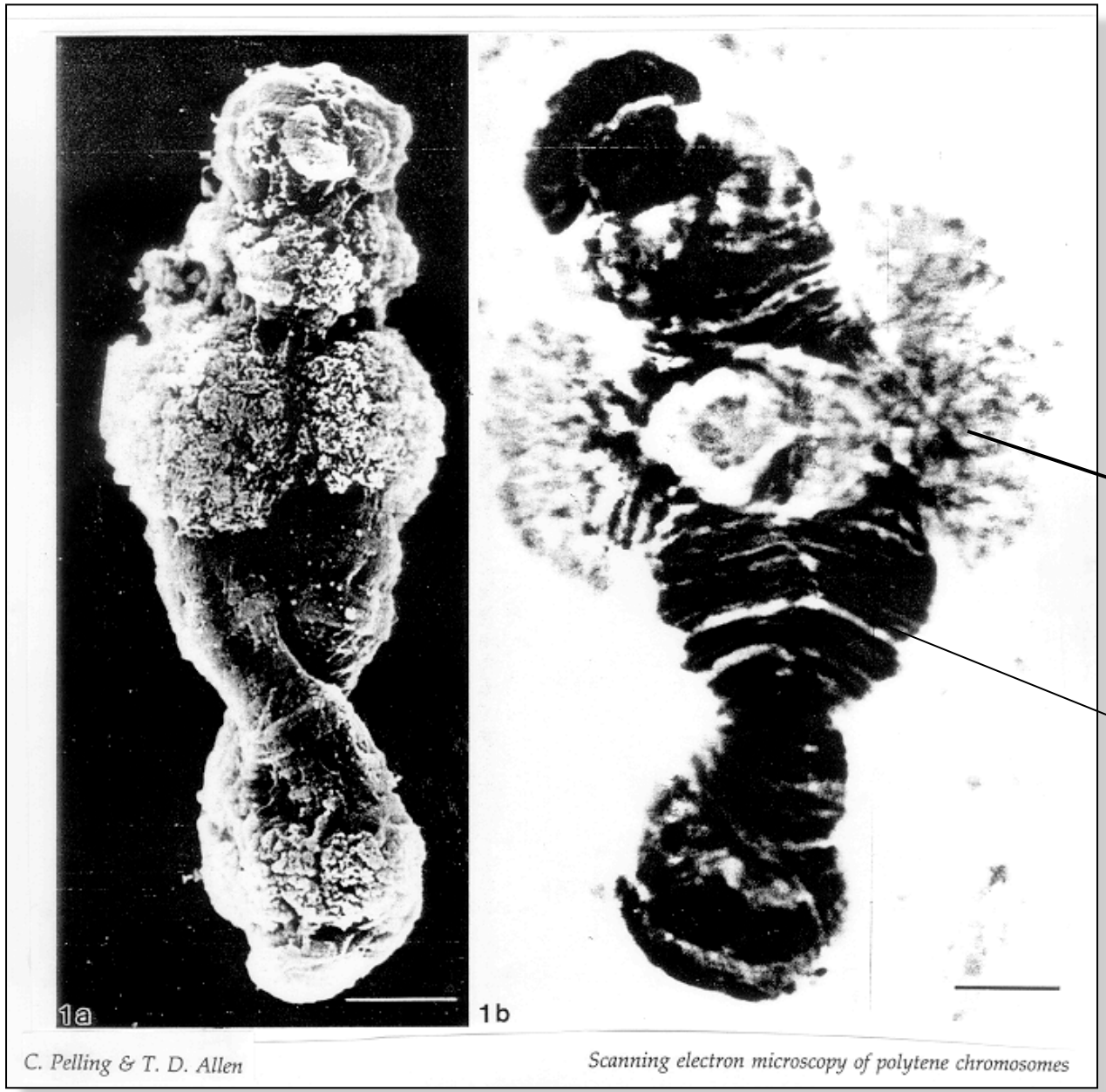
Puff bzw. Balbiani-Ring
(**besonders starke Transkription**)

- **Die vielen Chromatiden sind durch sukzessive Replikationsereignisse ohne nachfolgende Zellteilung entstanden („Endomitose“).**

Struktur von Polytänchromosomen



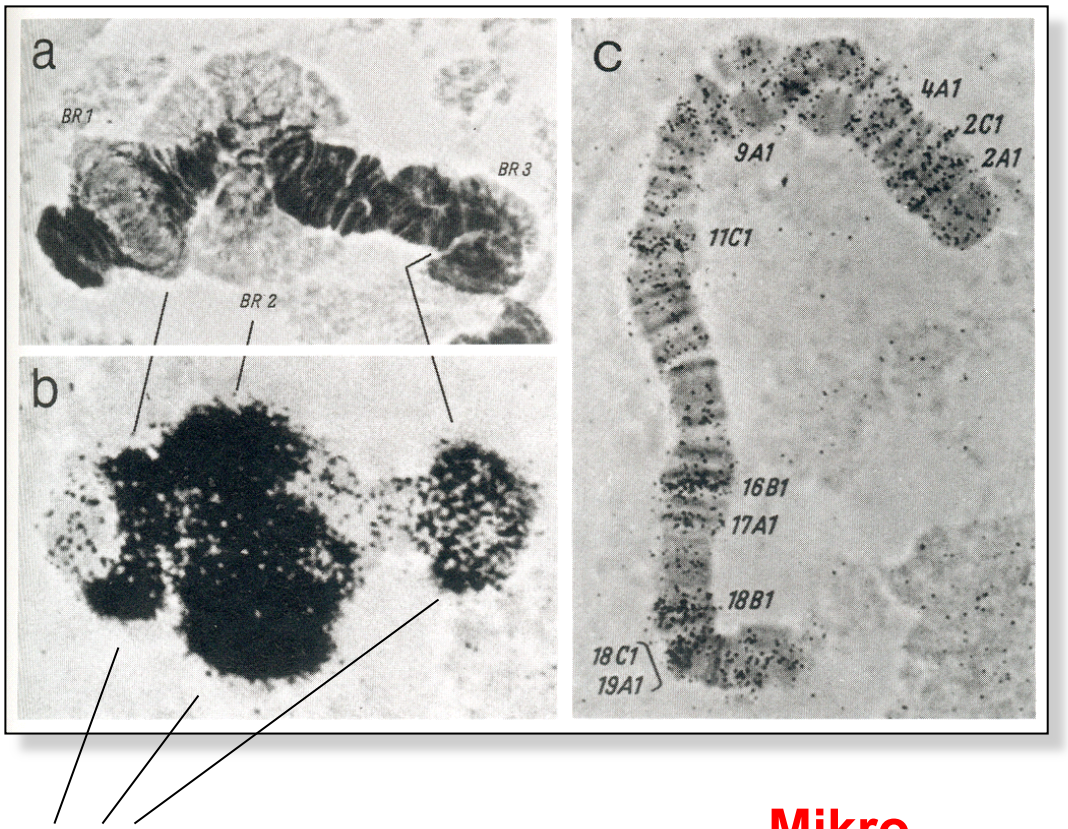
Polytän-chromosomen im Raster-EM



Riesen-Puffs („Balbiani-Ringe“)

Homologen-Paarung!

Polytänchromosomen zeigen Genaktivität...

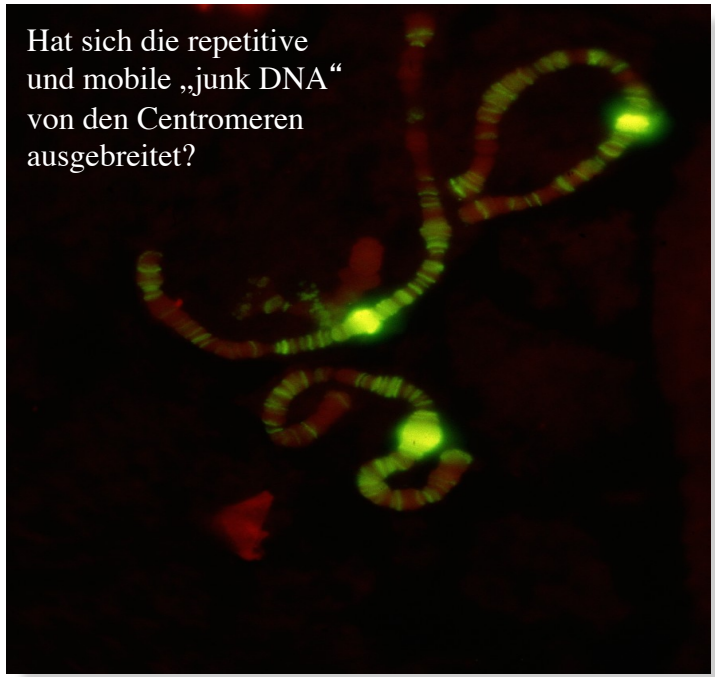


Nachweis von RNA durch Einbau von ^{3}H-Uridin → **Mikro-Autoradio-graphie**

- **Puffs** sind sehr aktive Genorte mit stark auf-gelockerter Chromatin-Struktur.

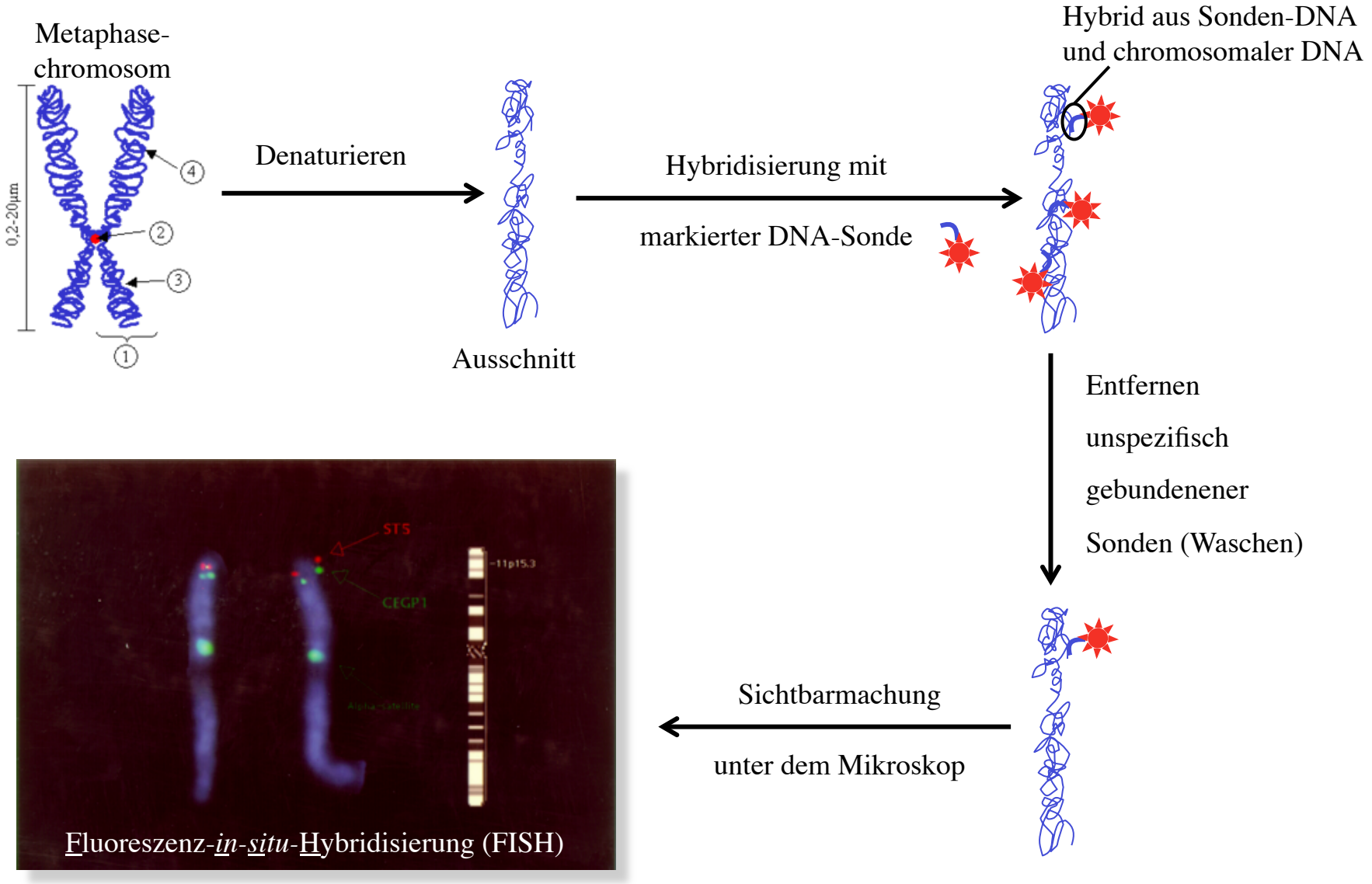
- **„Balbiani-Ringe“** sind große Puffs mit Genen, die das Spinnsekret der Insektenlarven kodieren.

Gen-Kartierung an Polytän-chromosomen

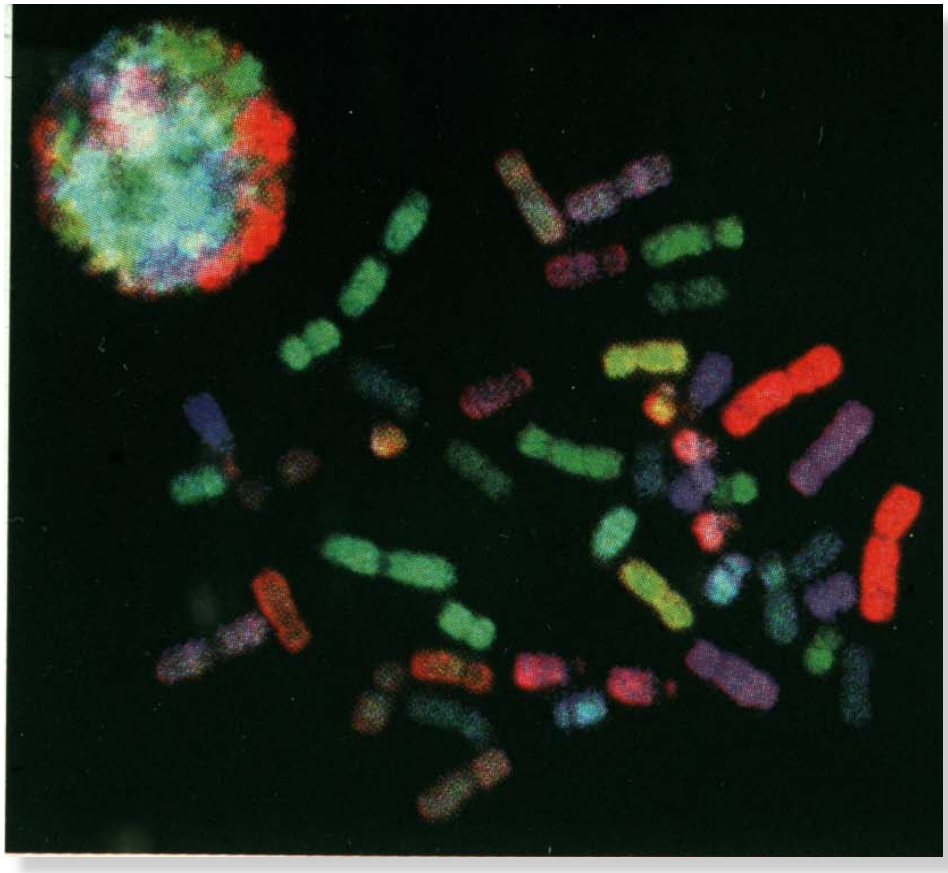


Lokalisation von ‚single copy‘-Genen und repetitiver DNA durch Fluoreszenz-*In-situ*-Hybridisierung (FISH)

Gen-Kartierung an Metaphase-Chromosomen



Chromosomen-“Painting“

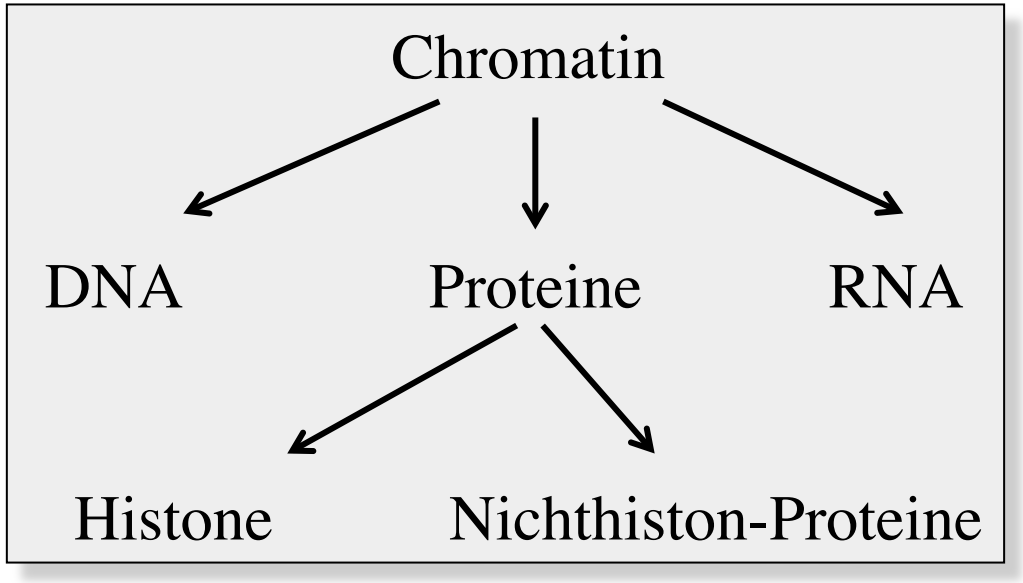


- *In-situ*-Hybridisierung mit unterschiedlich markierter DNA **ganzer Chromosomen** als Sonden

- Diagnose von Chromosomen-veränderungen

- Evolution der Chromosomenstruktur

Molekularbiologie des CHROMATINS



Funktionen des Chromatins...

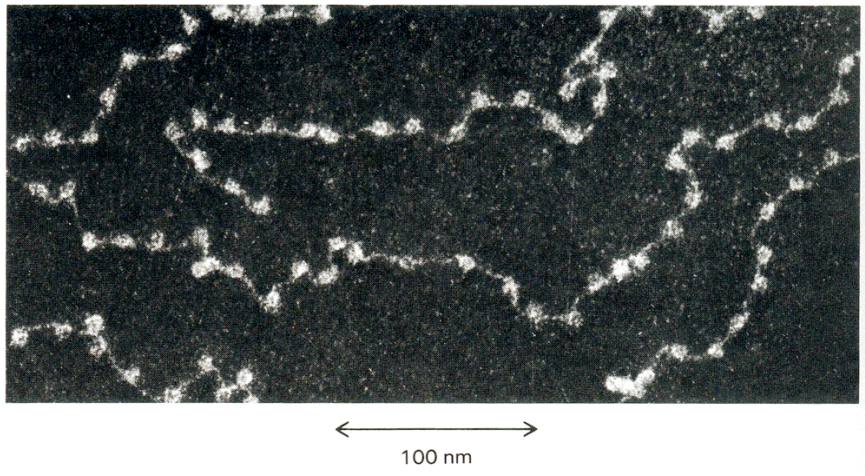
...**Verpackung** der DNA

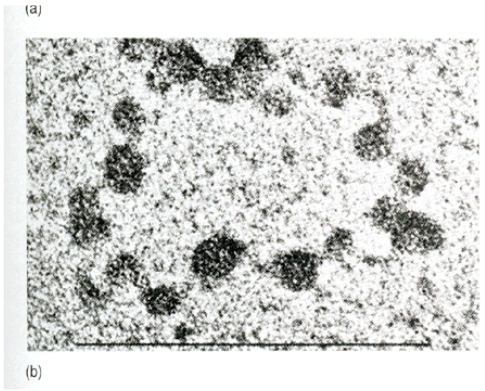
> Schutz vor Nukleasen
> Transport
> Chromosomenpaarung

...**Kondensation/Dekondensation**

> Steuerung der Genaktivität

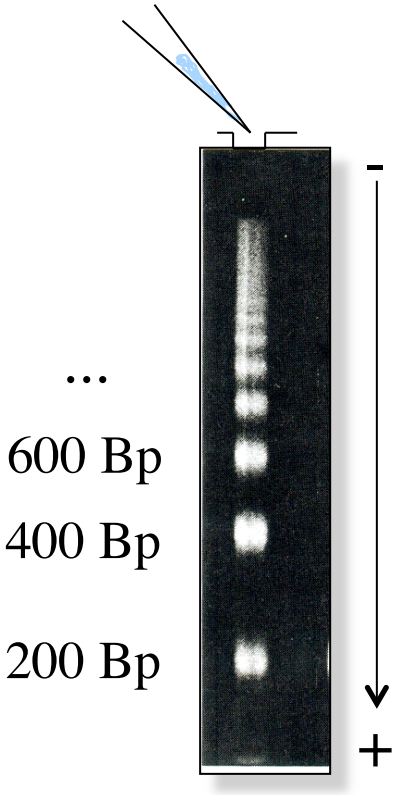
Die Basis-Einheit des Chromatins sind die Nukleosomen





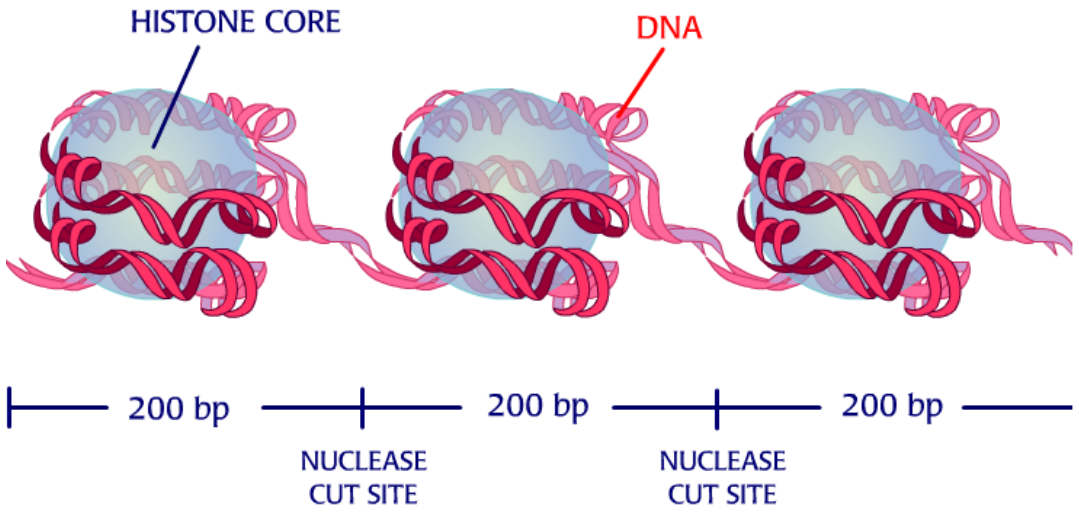
elektronenmikroskopische Aufnahmen von Nukleosomen

Entdeckung der Nukleosomen



gelelektrophoretische Auftrennung von Nuklease-behandeltem Chromatin

Interpretation:



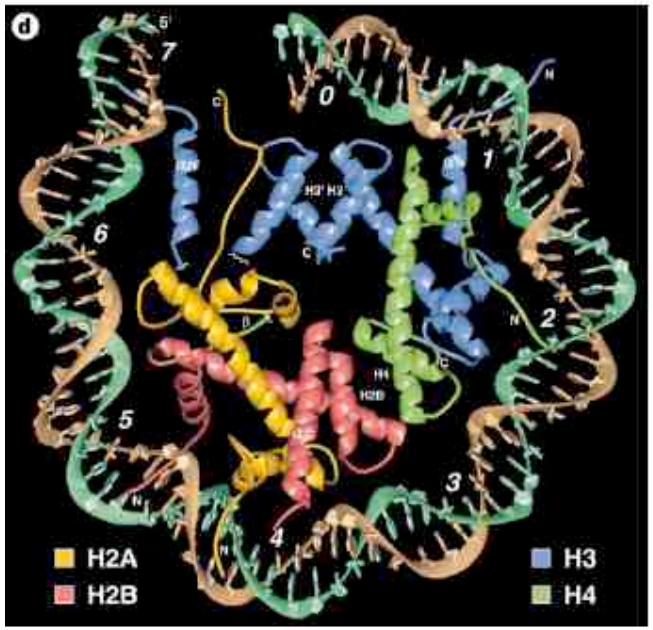
http://www.dnaftb.org/dnaftb/29/concept/index.html

Crystal structure of the nucleosome core particle at 2.8 Å resolution

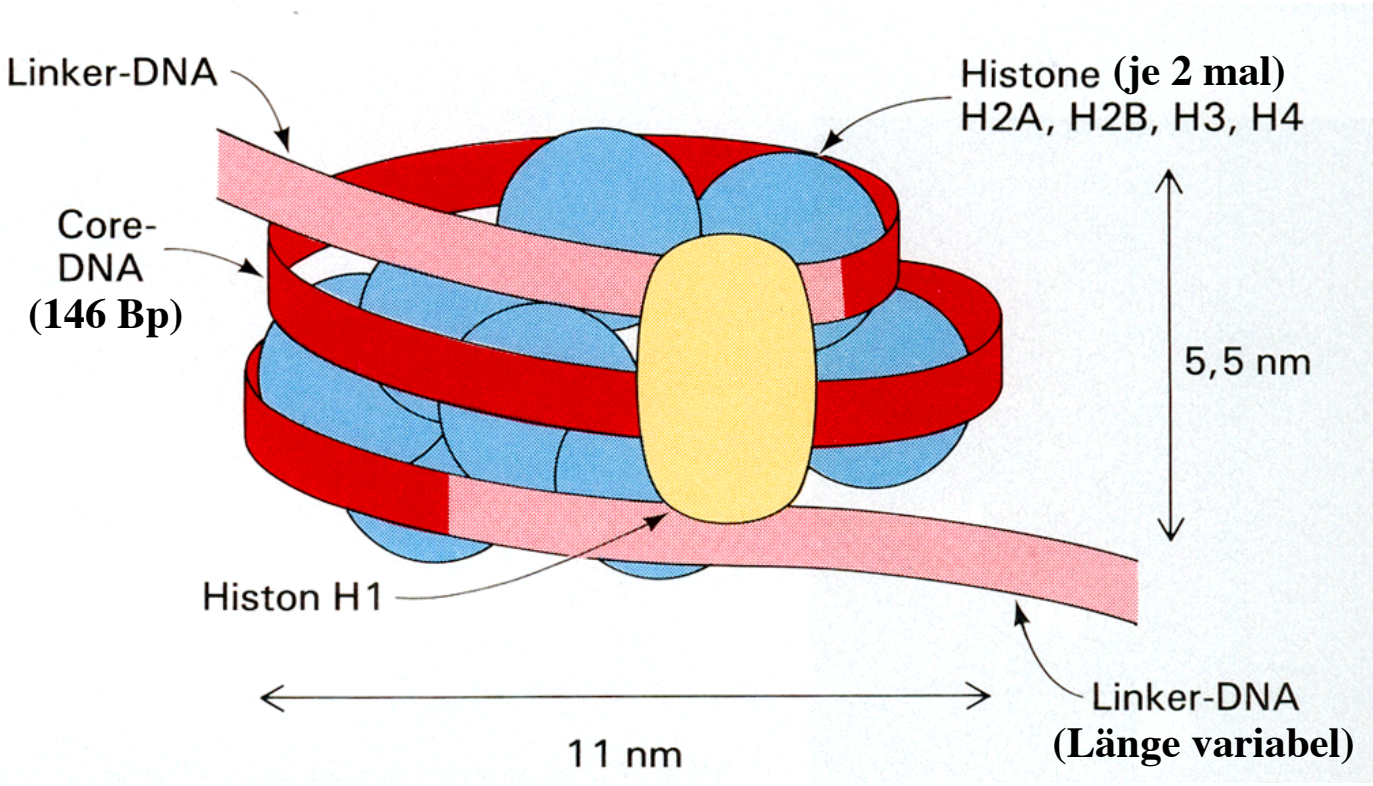
Karolin Luger, Armin W. Mäder, Robin K. Richmond, David F. Sargent & Timothy J. Richmond

Institut für Molekularbiologie und Biophysik ETHZ, ETH-Hönggerberg, CH-8093 Zürich, Switzerland

The X-ray crystal structure of the nucleosome core particle of chromatin shows in atomic detail how the histone protein octamer is assembled and how 146 base pairs of DNA are organized into a superhelix around it. Both histone/histone and histone/DNA interactions depend on the histone fold domains and additional, well ordered structure elements extending from this motif. Histone amino-terminal tails pass over and between the gyres of the DNA superhelix to contact neighbouring particles. The lack of uniformity between multiple histone/DNA-binding sites causes the DNA to deviate from ideal superhelix geometry.



Arithmetik des Nukleosoms



1 Nukleosom = Histon-Oktamer + Histon H1 + ca. 200 Bp DNA

Nukleosomale DNA ist um den Faktor 6 kondensiert.

Die Histone...

Table 4.2 The Major Histone Proteins

positive Ladung!!!

Histone[a]	Molecular weight	Number of amino acids	Percentage Lys + Arg
H1	22,500	244	30.8
H2A	13,960	129	20.2
H2B	13,774	125	22.4
H3	15,273	135	22.9
H4	11,236	102	24.5

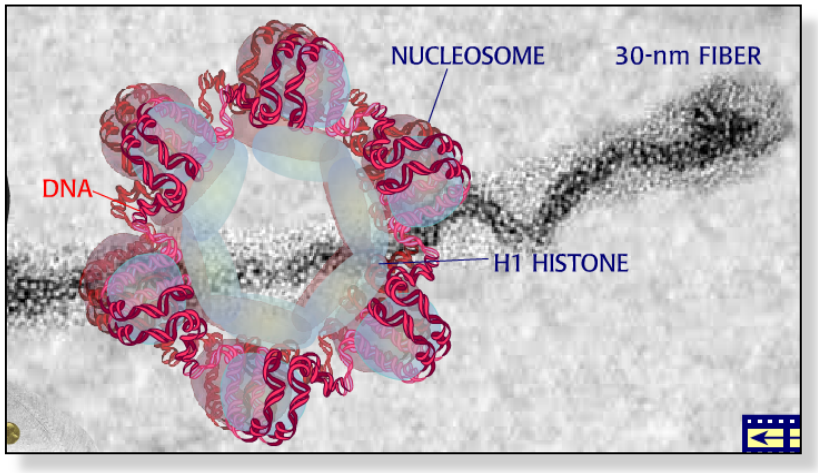
[a] Data are for rabbit (H1) and bovine histones.

- interagieren mit der neg. geladenen DNA über positiv die geladenen Aminosäuren Lys und Arg
- sind äußerst konservative Proteine während der Evolution.

Die Histone

- H2A, H2B, H3, H4 bilden den **Histone-Core**.

- **H1** „versiegelt“ die DNA bei Ein- und Austritt aus dem core-Nukleosom und dient dem Aufbau einer übergeordneten Chromatinstruktur (Solenoid).



http://www.dnaftb.org/dnaftb/29/concept/index.html

Organisationsstufen des Chromatins

Kondensation

-

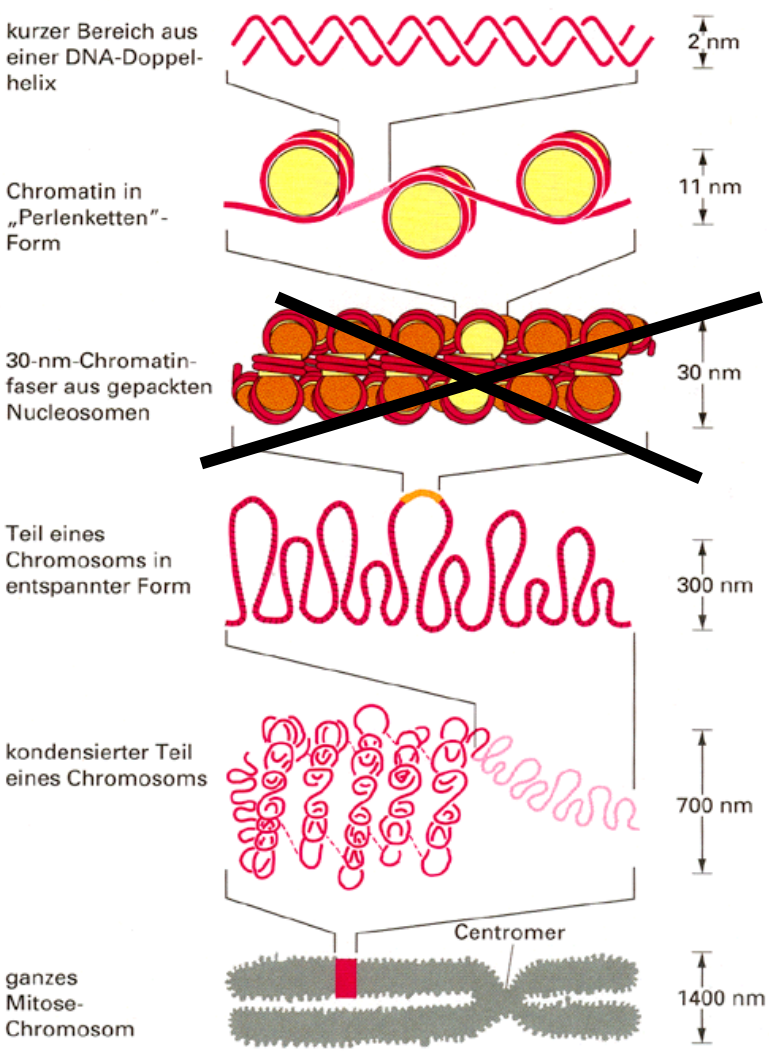
aktiv trans-kribierte Gene — **6**

+ H1, Gene weniger aktiv — **40**

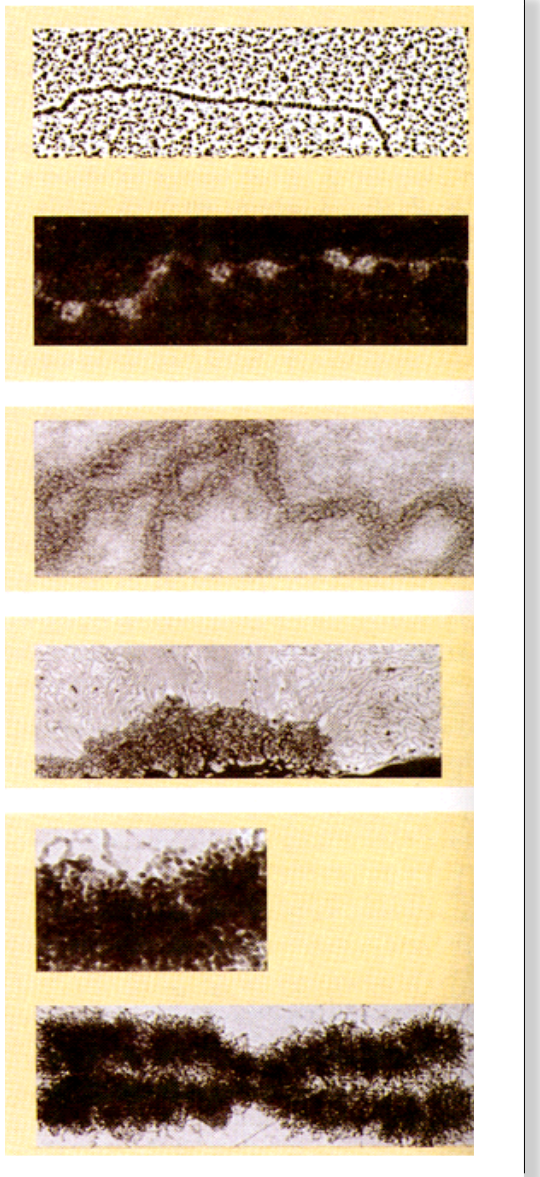
+ scaffold-Proteine, Interphase — **1700**

+ weitere scaffold-Proteine, Metaphase — **12000**

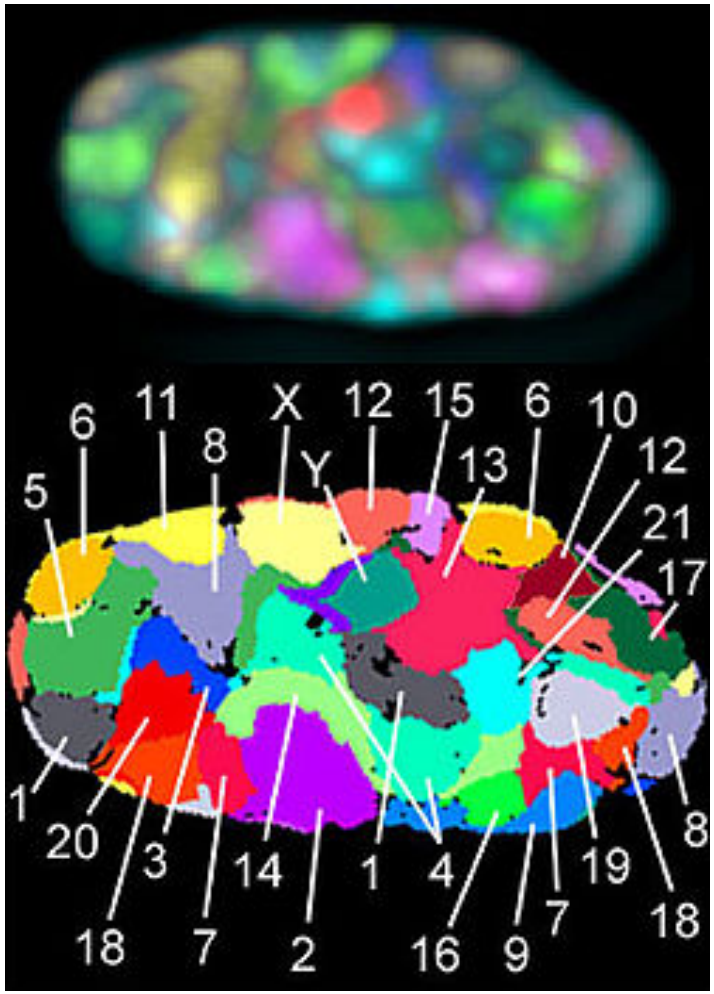
50000



GESAMTERGEBNIS: JEDES DNA-MOLEKÜL WURDE ALS MITOSECHROMOSOM GEPACKT, DAS 50 000MAL KÜRZER ALS IN AUSGEDEHNTER FORM IST



Chromosomen-Territorien im Interphase-Zellkern



BIOLOGIE IN UNSERER ZEIT

VBiO Verband Biologie, Biowissenschaften & Biomedizin in Deutschland

DOI:10.1002/biuz.201610601

Teil 1: Chromosomenterritorien und Chromatindomänen

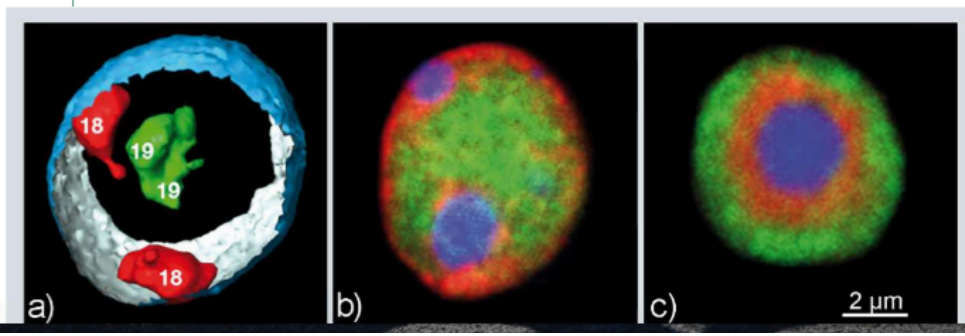
Der Zellkern – eine Stadt in der Zelle

THOMAS CREMER | MARION CREMER | CHRISTOPH CREMER

Zellkerne sind die Informations- und Steuerzentrale der Zellen aller eukaryotischen Lebewesen. Neue Erkenntnisse zeigen, dass sie also viel mehr sind als „chemische Reagenzgläser", in denen komplizierte biochemische Prozesse an Chromatinfäden in wässriger Lösung ablaufen. In ihrer Architektur sind sie mit einer Stadt in der Zelle vergleichbar, deren Funktionen von der räumlichen Anordnung der darin enthaltenen DNA, RNA und Proteine abhängen.

Die Zellkerne unserer Körperzellen sind Winzlinge mit einem Durchmesser von etwa 10 µm (1 µm = 1/1000 mm) und vielfältigen Gestalten. In Bindegewebszellen haben sie die Form flacher Ellipsoide, Lymphozytenkerne sind kugelförmig und Granulozytenkerne vielfältig eingebuchtet. Diese Winzlinge haben es in sich. Zu ihren Funktionen gehört die präzise Regulation der Transkription von vielen tausend Genen, die Verdoppelung der Chromosomen und der darin enthaltenen DNA vor jeder Zellteilung sowie ständige Reparaturprozesse, die zur Erhaltung der Integrität des

ABB. 1 | CHROMOSOMENTERRITORIEN UND VERÄNDERUNGEN DER ZELLKERNARCHITEKTUR



The secret life of histones

Cell, Vol. 114, 673–688, September 19, 2003, Copyright ©2003 by Cell Press

Involvement of Histone H1.2 in Apoptosis Induced by DNA Double-Strand Breaks

- Eines von mehreren Histon H1-Genen des Menschen wird bei DNA-Doppelstrangbrüchen aktiviert. Das exprimierte Protein (H1.2) wandert zu den Mitochondrien und leitet dort den programmierten Zelltod („**Apoptose**“) ein!

The dark side of histones…

Nat Med. 2009 Nov;15(11):1318-21. Epub 2009 Oct 25.

Extracellular histones are major mediators of death in sepsis.

Xu J, Zhang X, Pelayo R, Monestier M, Ammollo CT, Semeraro F, Taylor FB, Esmon NL, Lupu F, Esmon CT.

Oklahoma Medical Research Foundation, Oklahoma City, OK, USA.

Comment in:

Nat Med. 2009 Nov;15(11):1245-6.

Hyperinflammatory responses can lead to a variety of diseases, including sepsis. We now report that extracellular histones released in response to inflammatory challenge contribute to endothelial dysfunction, organ failure and death during sepsis. They can be targeted pharmacologically by antibody to histone or by activated protein C (APC). Antibody to histone reduced the mortality of mice in lipopolysaccharide (LPS), tumor necrosis factor (TNF) or cecal ligation and puncture models of sepsis. Extracellular histones are cytotoxic toward endothelium in vitro and are lethal in mice. In vivo, histone administration resulted in neutrophil margination, vacuolated endothelium, intra-alveolar hemorrhage and macro- and microvascular thrombosis. We detected histone in the circulation of baboons challenged with Escherichia coli, and the increase in histone levels was accompanied by the onset of renal dysfunction. APC cleaves histones and reduces their cytotoxicity. Co-infusion of APC with E. coli in baboons or histones in mice prevented lethality. Blockade of protein C activation exacerbated sublethal LPS challenge into lethality, which was reversed by treatment with antibody to histone. We conclude that extracellular histones are potential molecular targets for therapeutics for sepsis and other inflammatory diseases.

Nicht-Histon-chromosomale Proteine (NHCPs)

z. B.

- **Histon-<u>modifizierende</u> Proteine:**
  Histon-Acetylasen, HATs
  Histon-Deacetylasen, HDACs

- **alle weiteren Transkriptions- und Replikationsproteine**

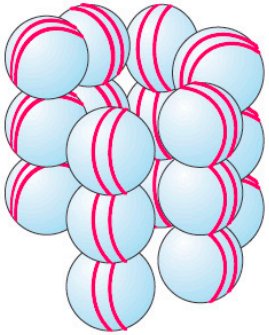
Veränderungen des Chromatins durch Histon-Modifikationen

aktiv

„offenes“ Chromatin

Histon-Methylierung,
Histon-Deacetylierung,
Einlagerung von Histonen und Heterochromatin-Proteinen,
DNA-Methylierung

Histon-Demethylierung,
Histon-Acetylierung,
Verlust von Histonen und Heterochromatin-Proteinen

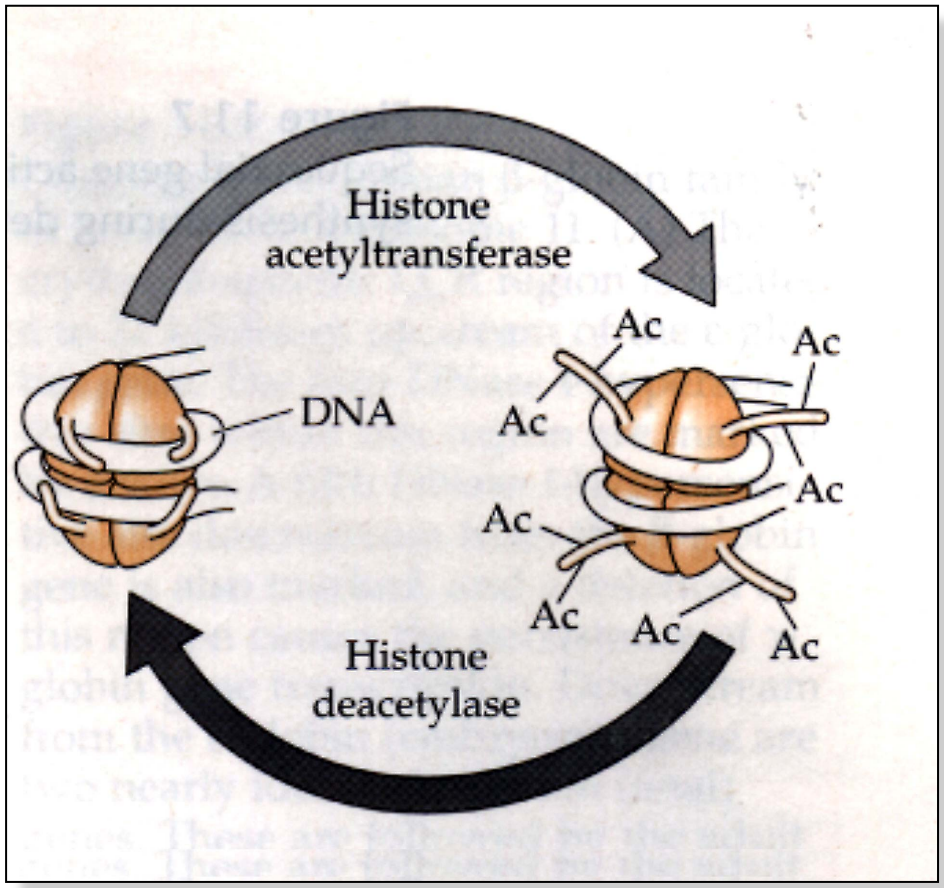


kondensiertes Chromatin

inaktiv

…entdecke die Möglichkeiten!

Veränderungen des Chromatins durch Histon-Acetylierung



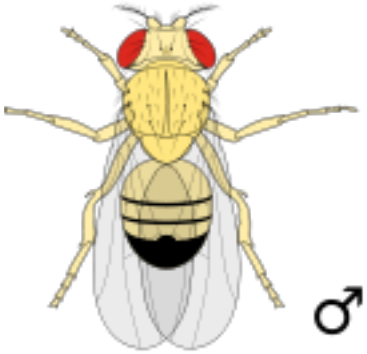
Acetylgruppe (-) wird an N-terminale **Lysin-Reste (+)** angeheftet und neutralisiert so die Interaktion mit der **DNA (-)**.

Der Vorgang ist reversibel.

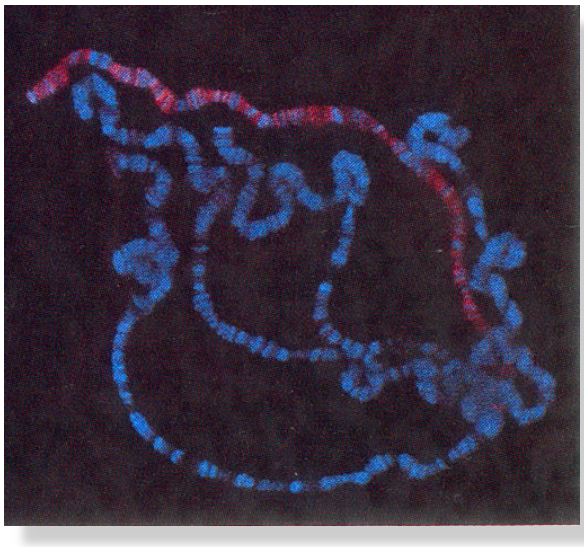
Animation: http://www.chemistry.mtu.edu/~mthomps/res/research.html

Beispiel:

Hyper-Acetylierung von Histon H4 steigert die Genexpression



Drosophila-Männchen
X-Chromosom



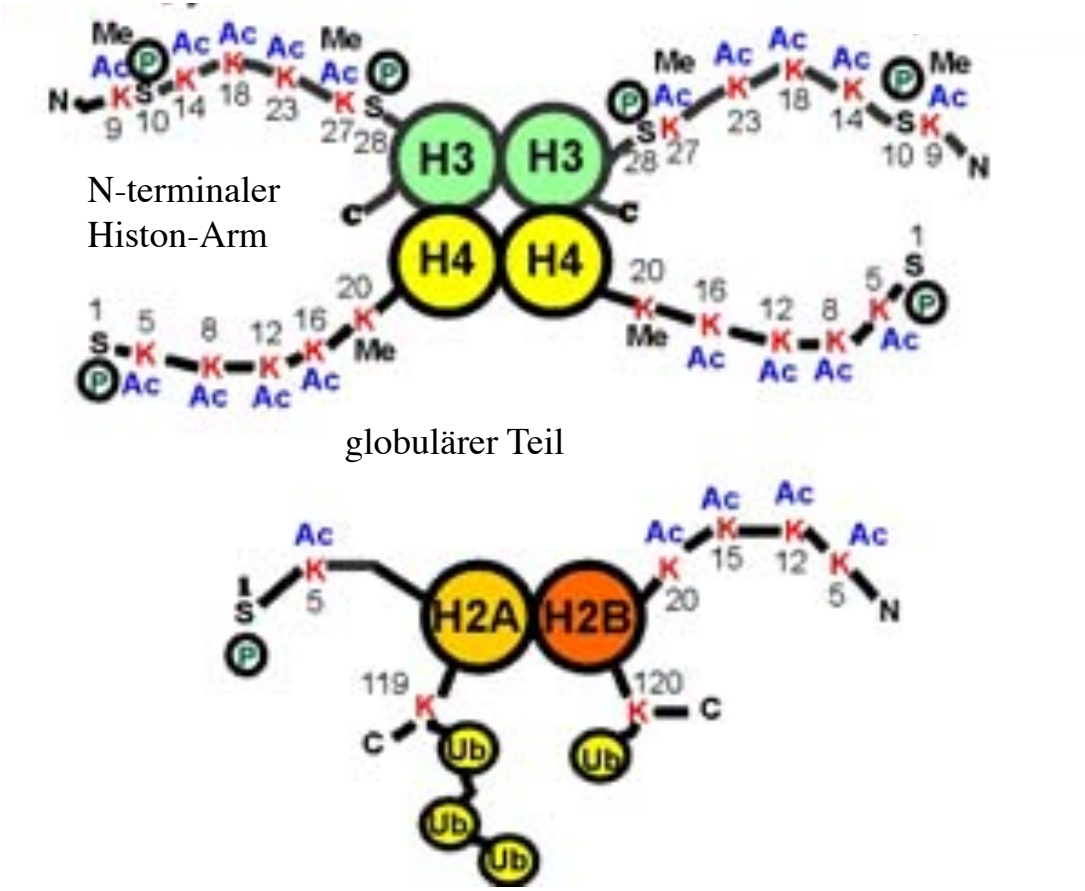
Nachweis von Histon H4 Lys^{Ac} durch spezifischen Antikörper (**rot**)

- Hyper-Acetylierung **verdoppelt (!)** Transkriptionsaktivität des einzigen X-Chromosoms in Männchen.
- **Dosiskompensation zwischen den Geschlechtern, bei Fliegen* durch Hochregulation der X-Gene in Männchen!**

Säuger machen das anders...

Strahl and Allis (2000) Nature 403, 41-45

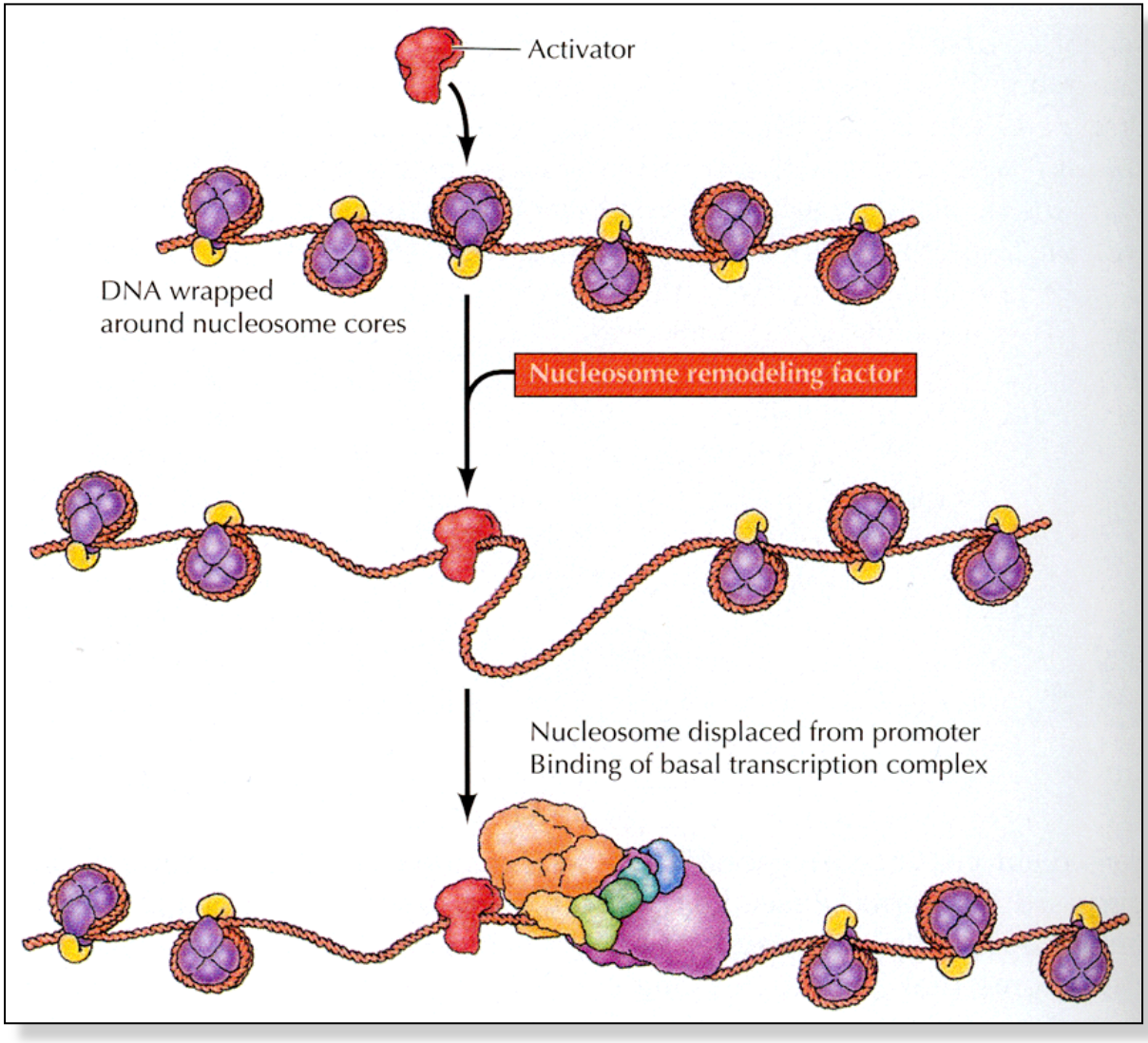
Fine-tuning des Chromatins an den Histonen



- Mehr als **50 Enzyme** können das Chromatin an unterschiedlichen Stellen modifizieren!

- **2^{50} Kombinationen** für 40 Mio. Nukleosomen: ein *„histone code“*?

Nukleosomenstruktur bei der Transkription



- Promotor-Bereiche aktiver Gene sind oft frei von Nukleosomen.

- **Histone in aktiven Genen werden modifiziert.** Nukleosomenstruktur wird +/- aufgelöst.

- Die Histone verlassen jedoch wohl nicht komplett die DNA.

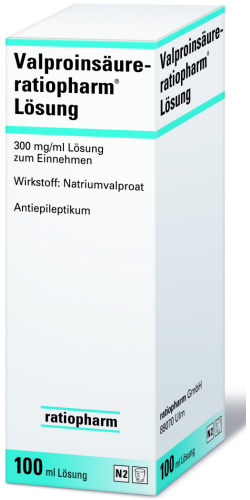
• Chromatin ist dynamisch und steuert dadurch die Aktivität der Gene !

• Bindung der Histone an die DNA kann durch Enzyme modifiziert, d.h. reversibel gelockert bzw. gefestigt werden.

• In „offenem“ (dekondensierten) Chromatin können Gene leichter transkribiert werden.

• Wir können die Chromatinstruktur therapeutisch beeinflussen...

Histon-Deacetylasen-Inhibitoren in der Medizin

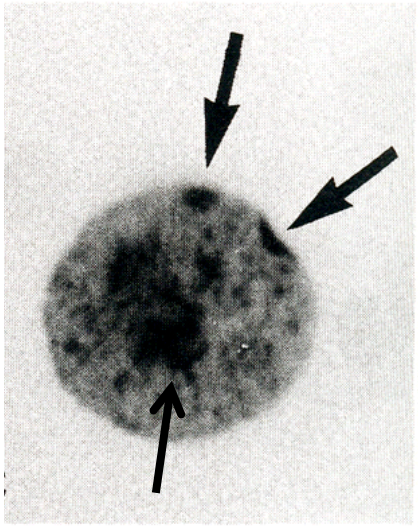


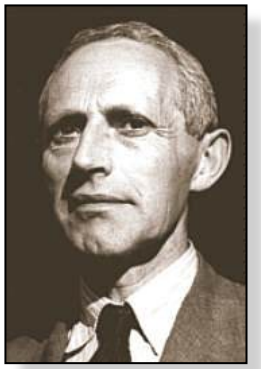
- HATs > stimulieren Genaktivität
- HDACs > reduzieren Genaktivität

<u>Problem: inaktivierte Tumorsuppressorgene!</u>

- HDAC-Inhibitoren (HDI) können Anti-Tumorgene **reaktivieren**!
- offenbar relativ wenige Nebenwirkungen auf andere Gene
- breite Verwendung: **Krebs, Epilepsie**, **Alzheimer**...

Euchromatin und Heterochromatin





E. Heitz, 1928

Interphase-Zellkern:

- *de*kondensiertes **Eu**chromatin
- kondensiertes **Hetero**chromatin (Pfeile)

Euchromatin und Heterochromatin

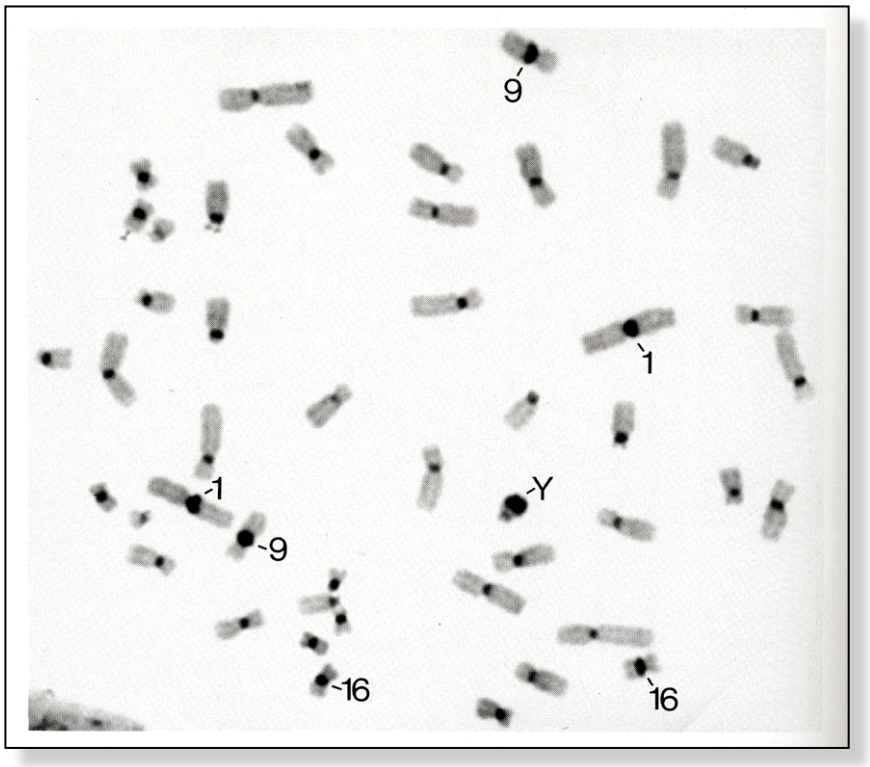
• **Konstitutives Heterochromatin** ist in allen Zellen und allen Phasen des Zellzyklus heterochromatisch.

• **Fakultatives Heterochromatin** ist lediglich in „manchen“ Zellen heterochromatisch.

• **Euchromatin** ist in der Interphase dekondensiert und aktiv.

Konstitutives Heterochromatin

Menschliche Metaphase-Chromosomen nach **„C-Banding“**

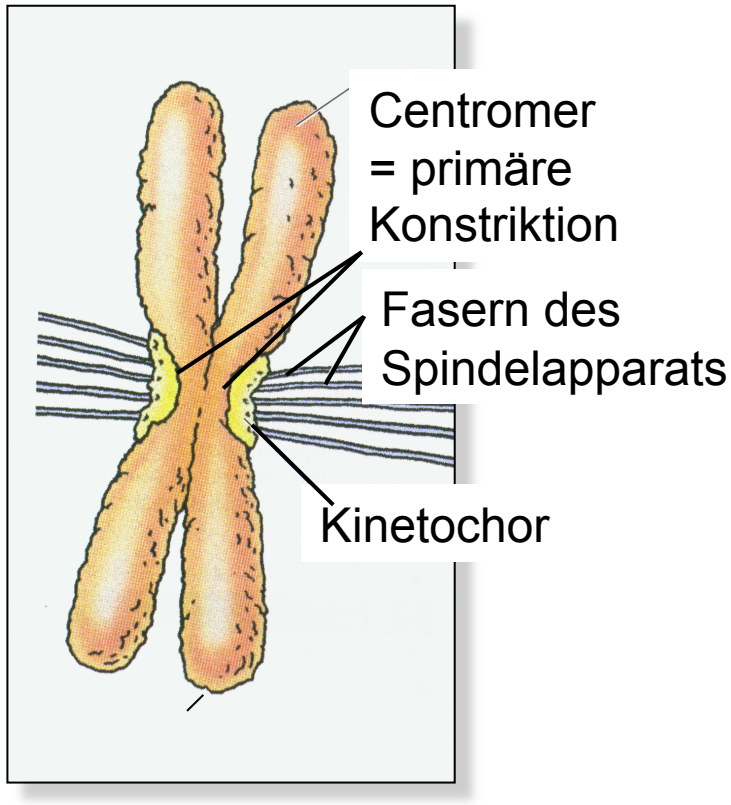


Konstitutiv hetero-

chromatische **Centromere**

werden spezifisch gefärbt

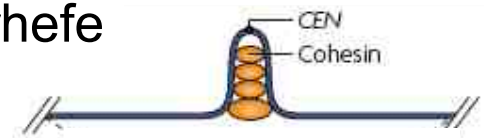
Das Centromer...



- ist konstitutiv heterochromatisch.
- organisiert Anheftung des Kinetochors.
- hält Schwesterchromatiden bis in späte Metaphase zusammen.
- **ist unerlässlich für richtige Chromosomenverteilung.**

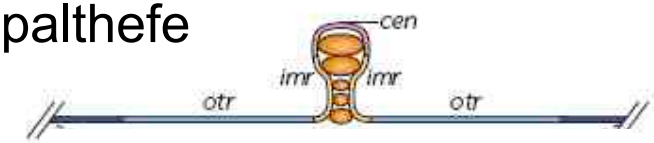
Centromere von Eukaryoten

Bäckerhefe



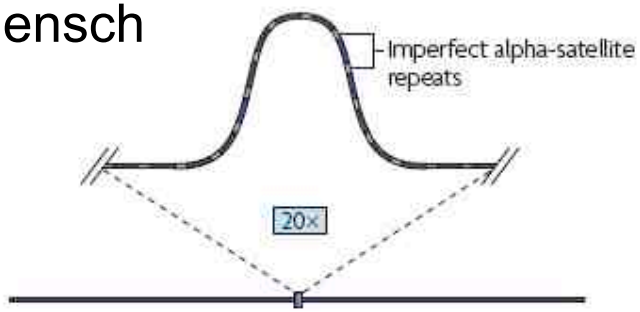
- nur 120 Bp lang!
- spezifische Sequenz

Spalthefe



- schon 70 kbp
- repetitive DNA

Mensch



- > 130 Mbp
- tandem-repetitive DNA („alpha-Satelliten-DNA“)

Struktur der Centromere in höheren Eukaryoten

- Tandem-repetitive DNA (syn. „Satelliten-DNA“)

- oft chromosomen- und artspezifische repetitive DNA
- weitgehend genleer
- verpackt mit speziellen Chromatinproteinen
  (z. B. CENP A, Histon H3-ähnlich)

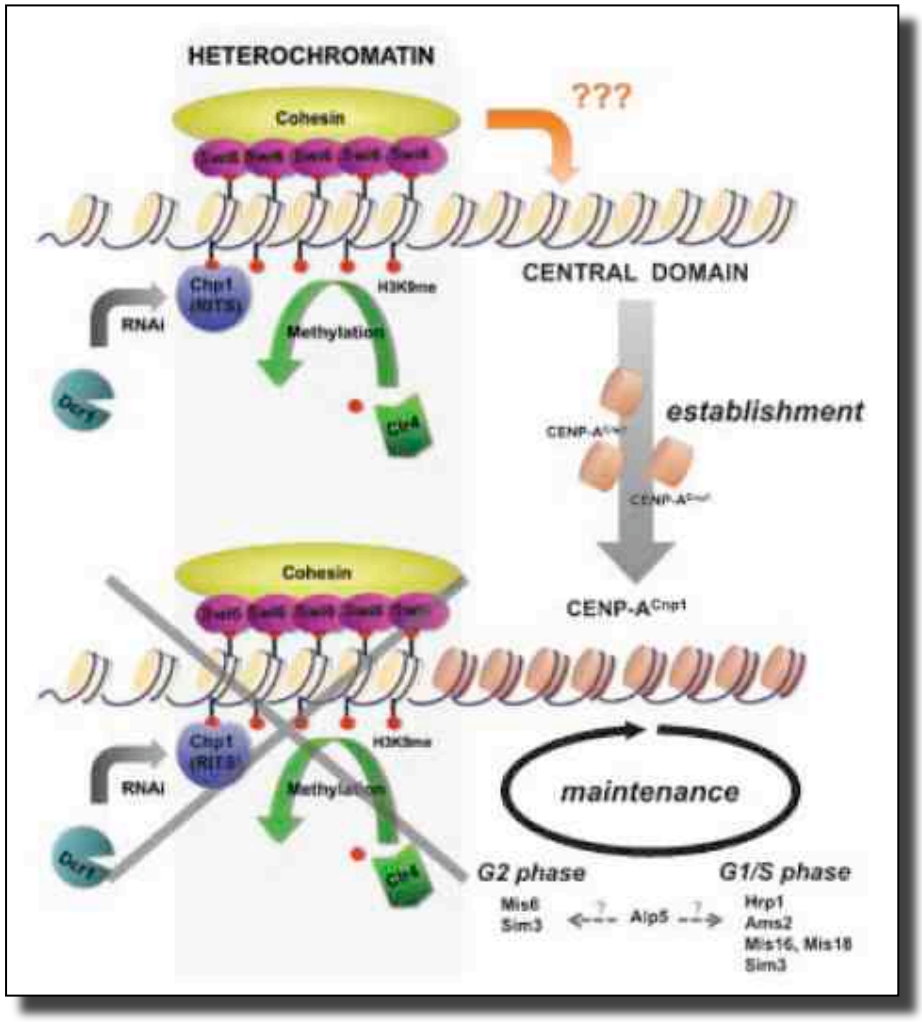
Centromere in höheren Eukaryoten

Centromere sind essentielle Strukturen in Chromosomen. Ohne sie keine Verteilung der Chromatiden...

Ein Centromer wird nicht primär durch seine DNA-Sequenz festgelegt, sondern durch seine spezielle Form von Chromatin.

Diese Chromatinstruktur wird von Zelle zu Zelle vererbt!

Zweigeteilte Centromer-Struktur in höheren Eukaryoten?

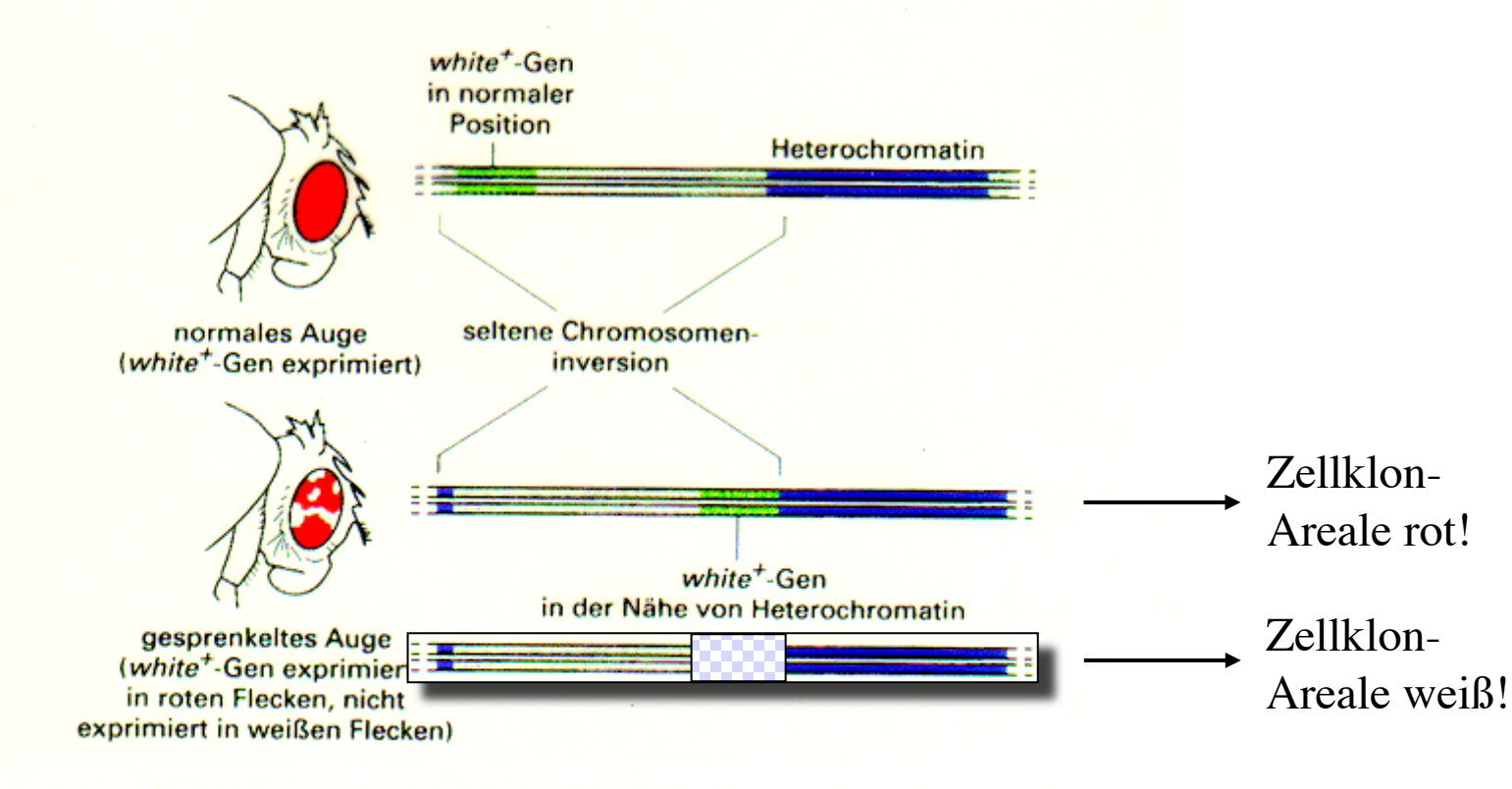


Heterochromatin (mit HP1/pink, siRNA-Komplexen/blau und DNA-5mCyt/rot) dirigiert Einbau von CENP-A (als Ersatz von Histon H3) in seiner Nachbarschaft. Das CENP-A-Chromatin organisiert dann das Kinetochor...

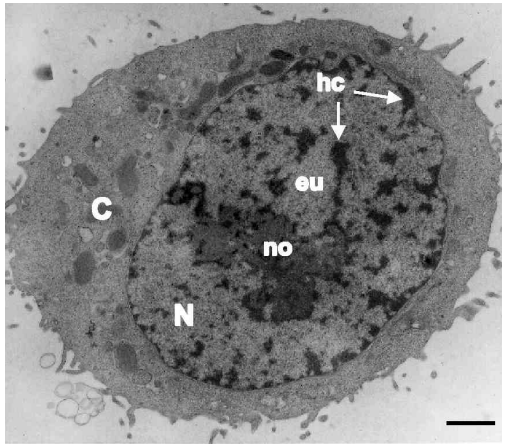
Figure 2. A model for CENP-A^{Cnp1} establishment and maintenance. The model is based on the model described in Folco et al.[1] A schematic centromere with heterochromatin is shown. At the top, heterochromatin components (Clr4^{KMT1}, H3K9me2, Swi6^{HP1}, RNAi) are required to establish CENP-A^{Cnp1} de novo on the central domain of the mini-chromosomes introduced by transformation. This process may also involve cohesins. However, the question remains exactly how the nearby heterochromatin directs the establishment of CENP-A^{Cnp1} chromatin. The bottom panel illustrates that, for maintenance of CENP-A^{Cnp1}, heterochromatin and the RNAi mechanism are not needed. Instead several other factors that deposit CENP-A^{Cnp1} at different stage of the cell cycle are involved. There are two main pathways for deposition. The G_2 pathway involves the loading factor Mis6[11] Sim4[12] and the chaperone Sim3.[22] The G_1/S pathway requires the remodeller Hrp1,[23] chaperones Mis16 and Mis18[24] the GATA-like transcription factor Ams2.[25] Histone acetylation by the lysine acetyltransferase enzyme Alp5[26] and histone deacetylation by a yet unidentified HDAC enzyme are also important for deposition of CENP-A^{Cnp1} in either G_2 or G_1/S or in both pathways.

Konstitutives Heterochromatin kann benachbarte Gene inaktivieren!

Position effect variegation (PEV)
am Beispiel der Augenfarbe von Drosophila melanogaster



Konstitutives Heterochromatin...



- ist stark **kondensiert** (dunkel).
- ist transkriptionell **wenig aktiv**.
- enthält **wenig Gene**.
- besteht aus **repetitiver DNA**.
- ist häufig im Bereich der **Centromere** lokalisiert.
- ist **spät-replizierend** in der S-Phase.
- kann benachbarte Gene inaktivieren (**Positionseffekt**).

Fakultatives Heterochromatin:
Das X-Chromosom der Säuger

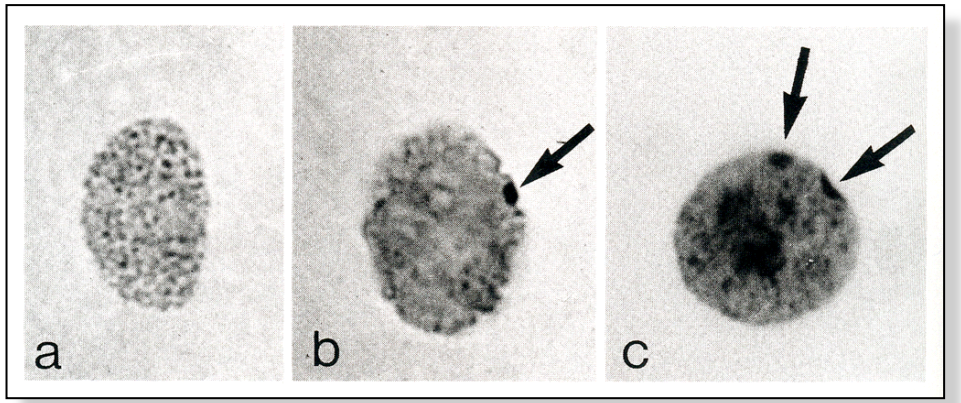
Mary F. Lyon, 1961:

Zum Zwecke der **‚Dosiskompensation‘** ist eines der beiden X-Chromosomen im weiblichen Geschlecht bei Säugern inaktiv.*

Das inaktive, heterochromatische X wird als Barr-Körperchen bezeichnet (Barr and Bertram 1949).

* *Remember Drosophila!* Die Fliege hatte das Problem anders gelöst.

Geschlechtsbestimmung über Mundschleimhaut-Abstrich

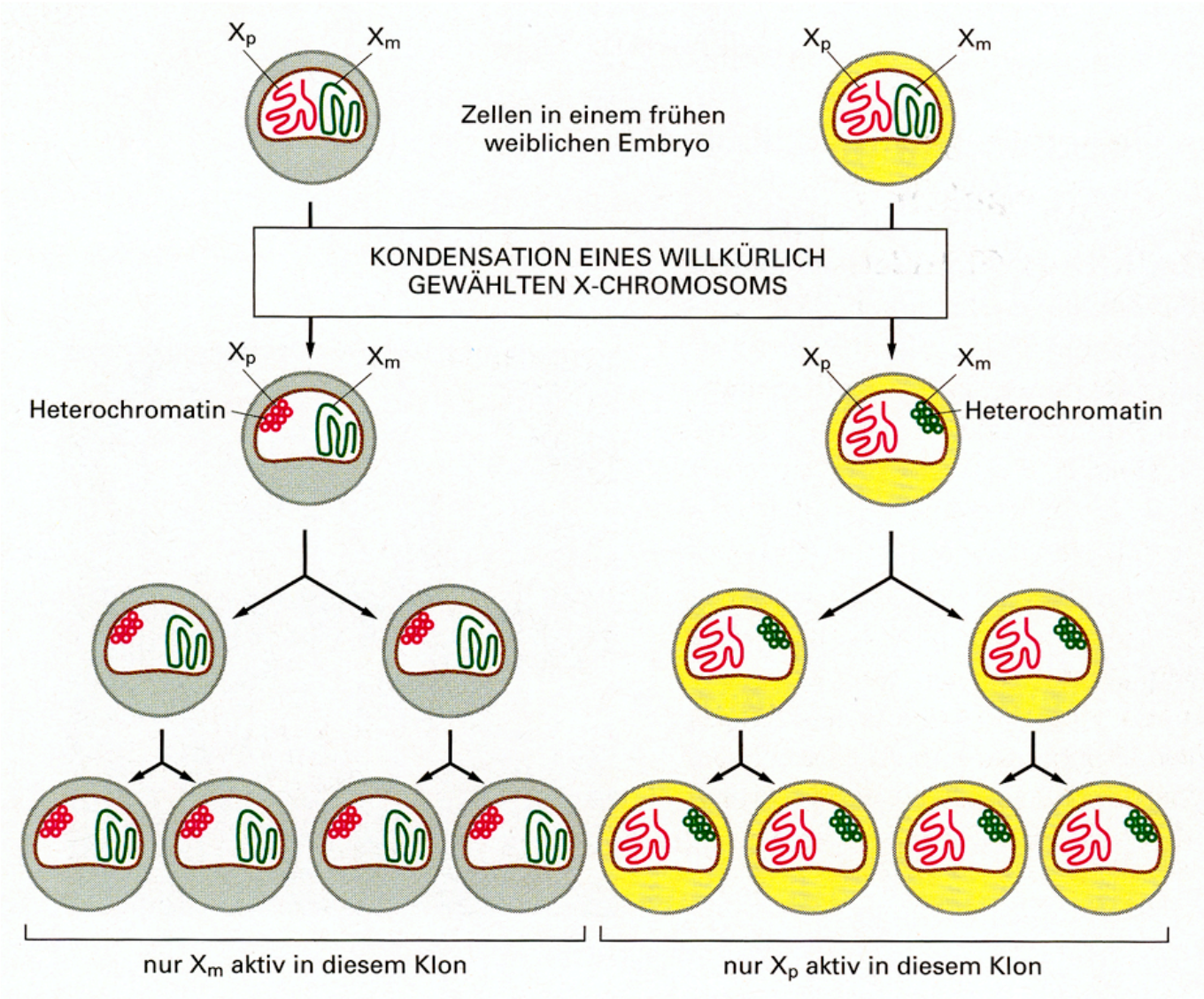


XY ~~XX~~ ~~XXX~~

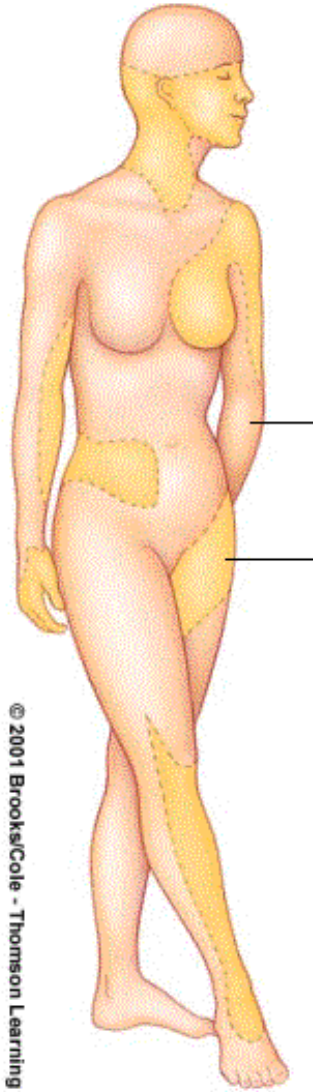
Barr-Körperchen in menschlichen Interphase-Zellkernen

In weiblichen Säugern sind alle X-Chromosomen bis auf eines heterochromatisch (= inaktiv) !

X-Inaktivierung in *Weibchen*



...und ihre Konsequenz!



z.B. Anhidrotische ektodermale Dysplasie

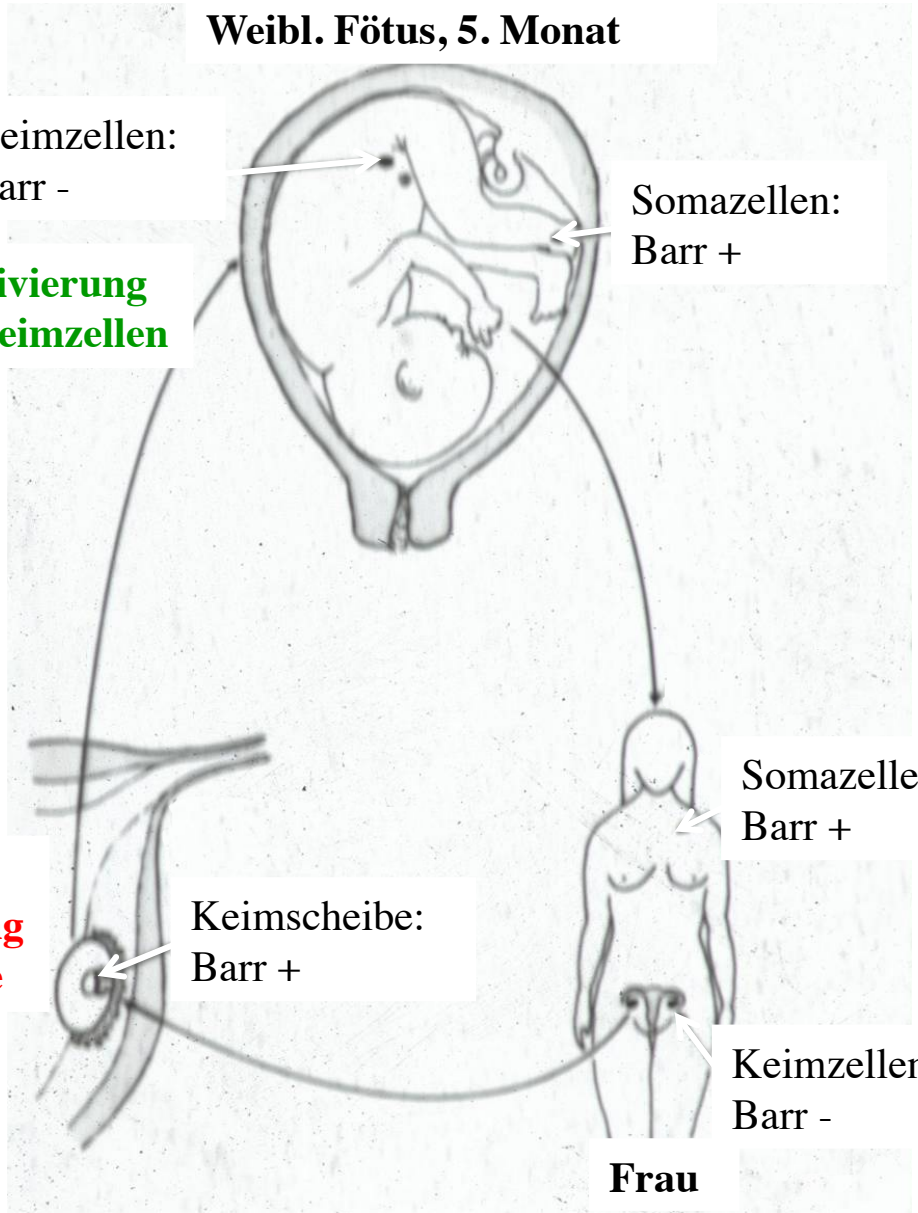
Normale Hautareale:
In diesen Zellen ist das X mit mutiertem Gen inaktiviert

Hautareale ohne Schweissdrüsen:
In diesen Zellen ist das X mit normalem Gen inaktiviert

Bei **Heterozygotie** an einem X-chromosomalen Genlokus sind weibliche Säuger ein **„genetisches Mosaik“.**

(stark vereinfachter)

Zyklus der X-Heterochromatisierung



https://www.nature.com/scitable/topicpage/x-chromosome-x-inactivation-323

Updates
- New post in Green Science: I leave and heave a sigh and say goodbye
- New post in Saltwater Science: Thanks for the good times!
- New topic in Women in Science: Where to find Laura Hoopes' Women in Science future thoughts?
- New post in Saltwater Science: Microplastic in the ocean
- New topic in Women in Science: LAB GIRL, exciting new woman-in-science biography

Topic Rooms
Within this Subject (43)
- Chromosome Analysis (10)
- Chromosome Number (4)
- Chromosome Structure (6)

ADVANCED

CHROMOSOMES AND CYTOGENETICS | Lead Editor: Clare O'Connor

X Chromosome: X Inactivation

By: Janice Y. Ahn (*Harvard Medical School*) & J. T. Lee, Ph.D. (*Department of Genetics, Harvard Medical School*)

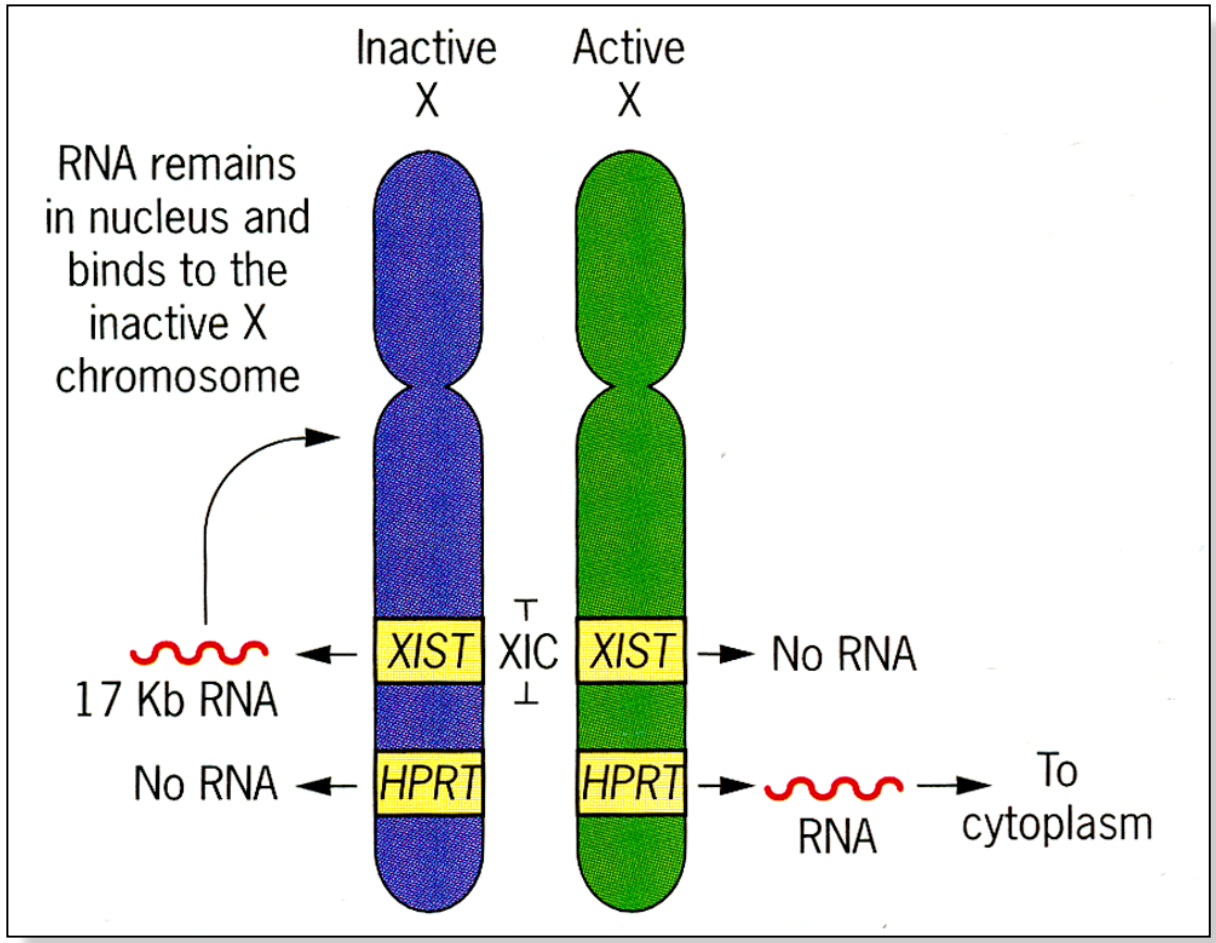
Citation: Ahn, J. & Lee, J. (2008) X chromosome: X inactivation. *Nature Education* 1(1):24

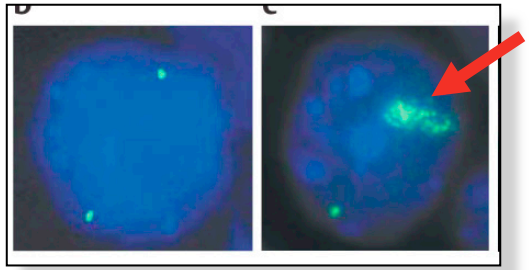
Females (XX) carry twice as many X-linked genes on their sex chromosomes as males (XY). How do cells control gene expression to manage this potentially lethal dosage problem?

Aa Aa Aa

Unlike the gene-poor Y chromosome, the X chromosome contains over 1,000 genes that are essential for proper development and cell viability. However, females carry two copies of the X chromosome, resulting in a potentially toxic double dose of X-linked genes. To correct this imbalance, mammalian females have evolved a unique mechanism of dosage compensation distinct from that used by organisms such as flies and worms. In particular, by way of the process called X-chromosome inactivation (XCI), female mammals transcriptionally silence one of their two Xs in a complex and highly coordinated manner (Lyon, 1961). The inactivated X chromosome then condenses into a compact structure called a Barr body, and it is stably maintained in a silent state (Boumil & Lee, 20

Molekularer Mechanismus der X-Heterochromatisierung





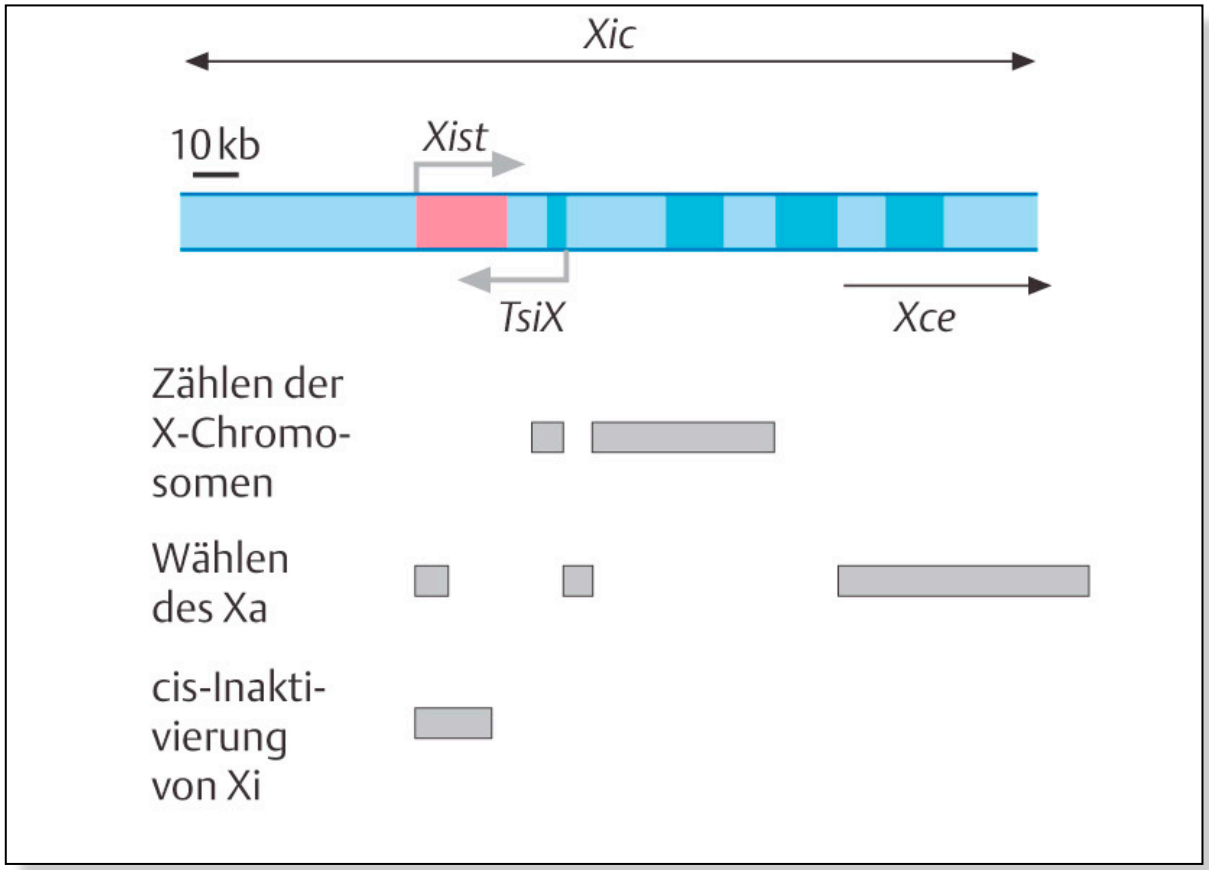
XIST-RNA.
vor Inaktiv. bei Inaktiv.

XIC = X-inactivation center

XIST = X inactive specific transcript

HPRT = Hypoxanthin-Phosphoribosyltransferase (Beispiel-Gen)

Molekularer Mechanismus der X-Heterochromatisierung



?

Molekularer Mechanismus der X-Heterochromatisierung

• Inaktivierung der X-Gene wird begleitet von komplizierten Modifikationen der Histone und einer Methylierung der Cytosine (5mC) in Genschaltern.

„**X inactivation** is associated with chromosome-wide establishment of inactive chromatin. Although this is classically regarded as facultative heterochromatin that is uniform in nature, the exact distribution of associated epigenetic marks is not well defined. Here we have analysed histone modifications in human somatic cells within two selected regions of the X chromosome. Intergenic, coding and promoter regions are segregated into differentially marked chromatin. **H3K27me3** is most prominent in intergenic and silenced coding regions, but is associated with some active coding regions as well. Histone **H3/H4 acetylation** and **H3K4me3** are locally enriched at promoter regions but do not necessarily mark continuing transcription. Remarkably, **H3K9me3** is predominant in coding regions of active genes, a phenomenon that is not restricted to the X chromosome. **These results argue against the exclusiveness of individual marks to heterochromatin or euchromatin, but rather suggest that composite patterns of interdependent or mutually exclusive modifications together signal the gene expression status.**“ EMBO Rep. 28.04.2006

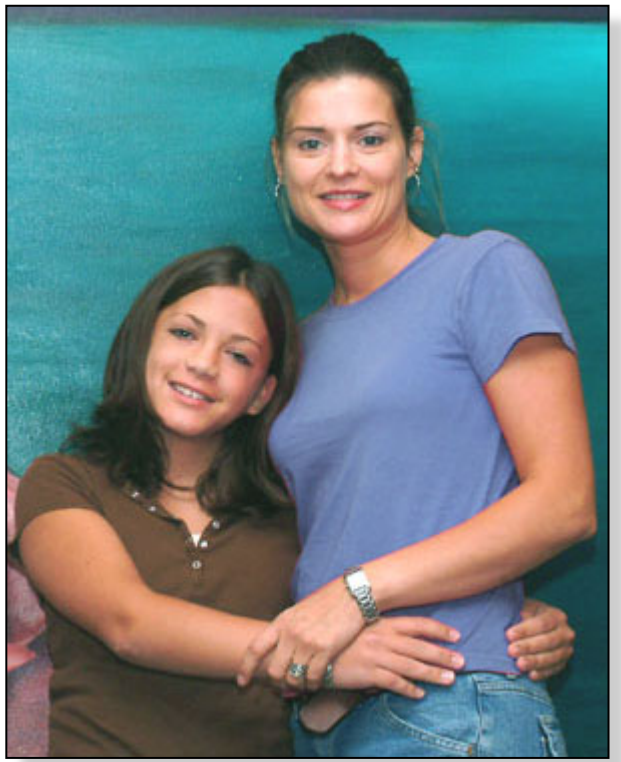
Der Chromatin-Status reguliert die Gene eines ganzen Chromosoms!

Q: Warum sind XO-Frauen phänotypisch erkrankt?

1:4000 Embryos

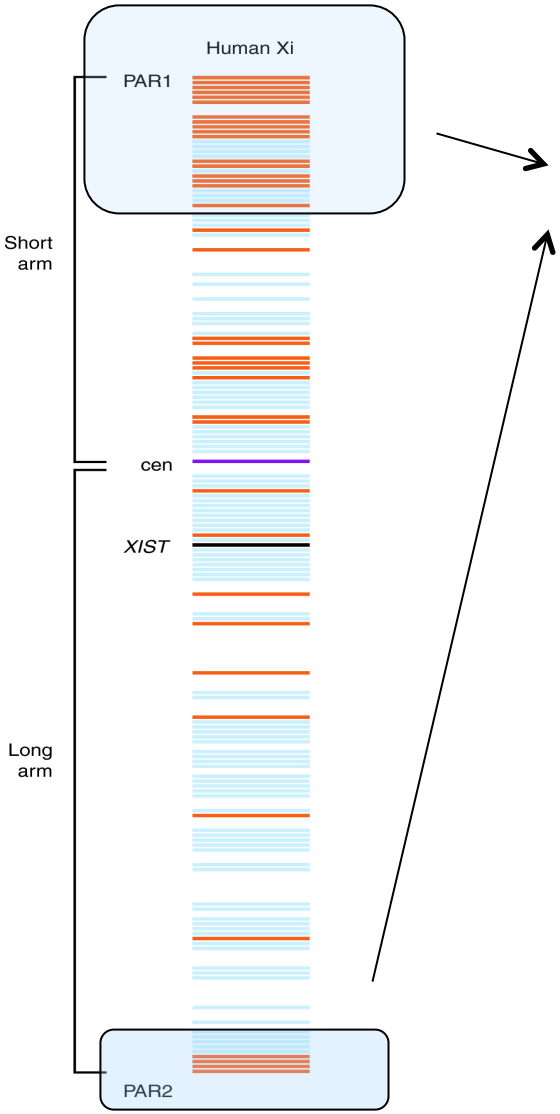
84% letal während Schwangerschaft

- Organ-Fehlbildungen
- Infertilität
- Nackenfalte
- Klein
- Intelligenz und Lebenserwartung oft normal



Turner-Syndrom

A: Manche X-Gene enkommen der Inaktivierung!

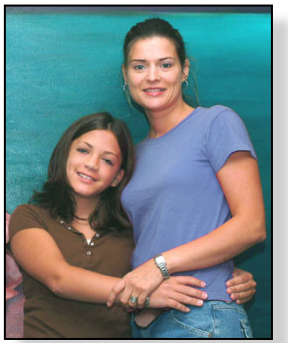


Gene in den PAR-Regionen des X sind ja auch im Männchen doppelt vorhanden!

Sie dürfen also im Weibchen nicht inaktiviert werden!

SHOX-Gen (short stature Homeobox) liegt in PAR1.

Defizienz in XO erklärt Turner-Phänotyp teilweise.

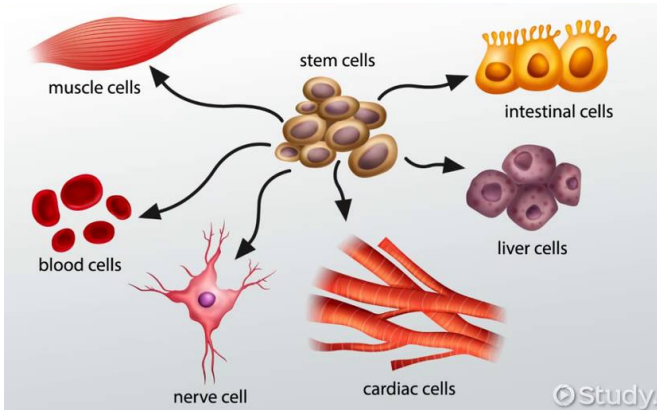


Q:

- Woher weiß eigentlich ein inaktives X, dass es in Tochterzellen inaktiv bleiben soll?

- Woher wusste das Zentromer, dass es in Tochterzellen Zentromer (und damit inaktives Heterochromatin) bleiben soll?

- Woher weiß überhaupt eine Zelle, was sie „werden soll“? Und wie kann sie ihre Identität aufrecht erhalten? (zelluläres Gedächtnis)



Die DNA-Sequenz ist ja überall gleich...

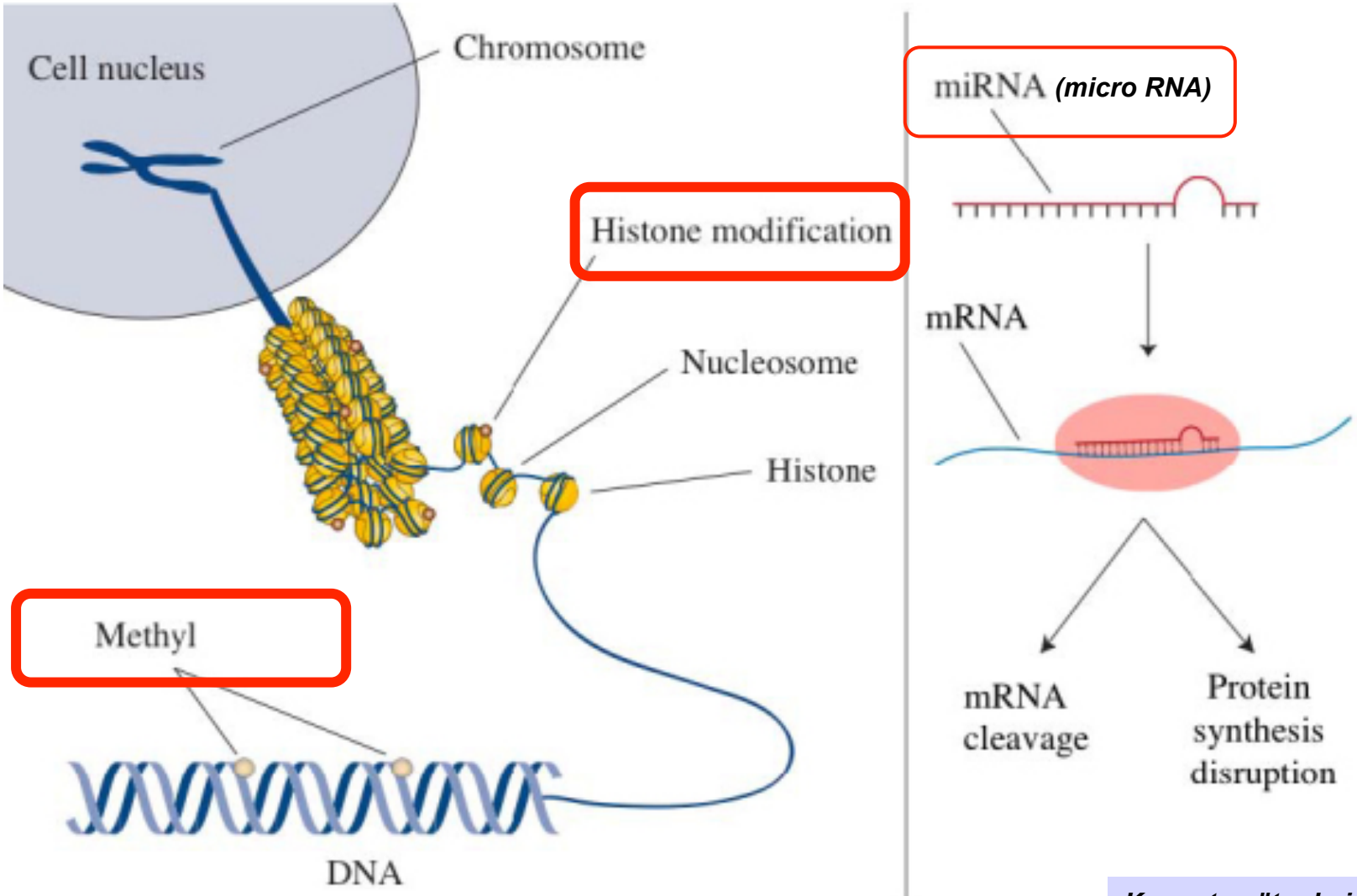
Epigenetik

(„daneben“)

Vererbung wird nicht nur durch den genetischen Code der DNA festgelegt, sondern auch durch

- Modifikationen der DNA-Basen
  (Cytosin-Methylierung)
- Veränderungen des Chromatins
  (Histon-Modifikation, spezielle Histon-Varianten u. andere Heterochromatin-Proteine, RNA-Komplexe)

Epigenetische Prozesse



Kommt später beim Thema „Genregulation“

Epigenetische Prozesse...

...regeln die Genaktivität, die auf diese Weise vererbt werden kann.

Beispiele:

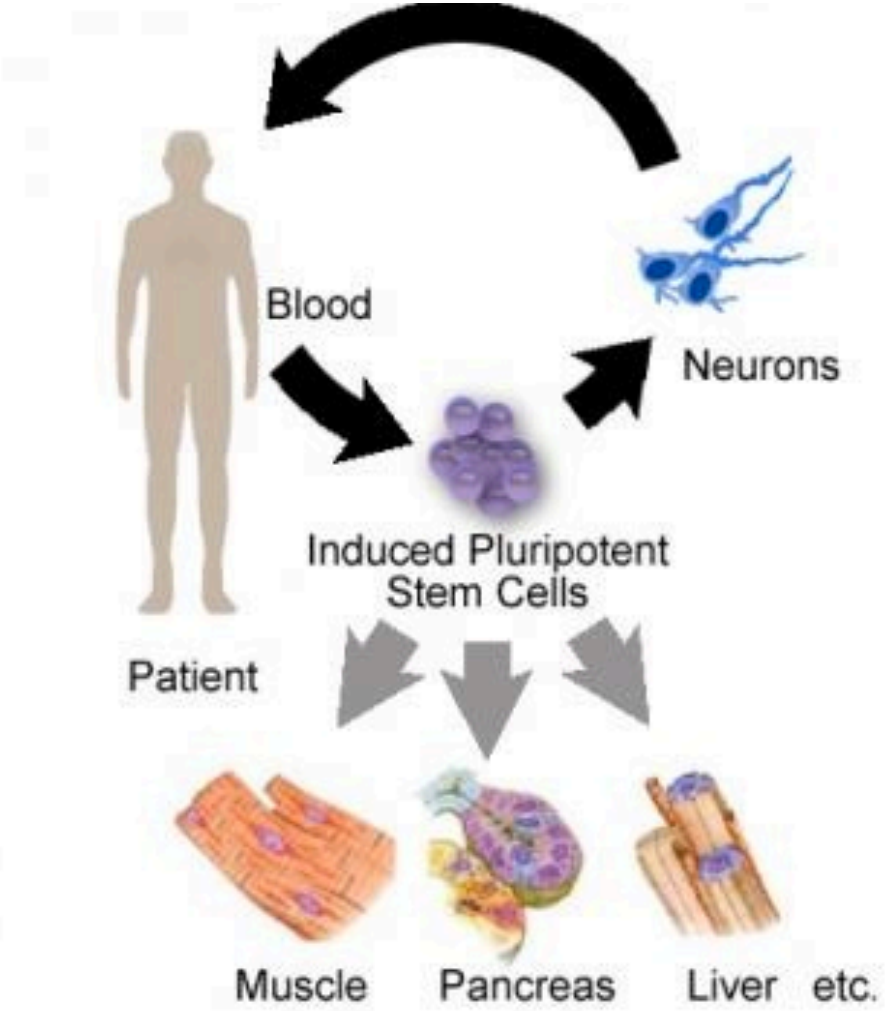
- Festlegung und Vererbung der **Centromer-Struktur**
- **X-Chromosom-Status** bei Gendosis-Kompensation
- **Imprinting**: unterschiedliche (!!) Aktivität väterlicher und mütterlicher Gene (n = ca. 80) während der Entwicklung
- **zelluläres Gedächtnis & Zelldifferenzierung**

Stammzellen: „Reset“ des Epigenoms

Beseitigung epigenetischer Markierungen

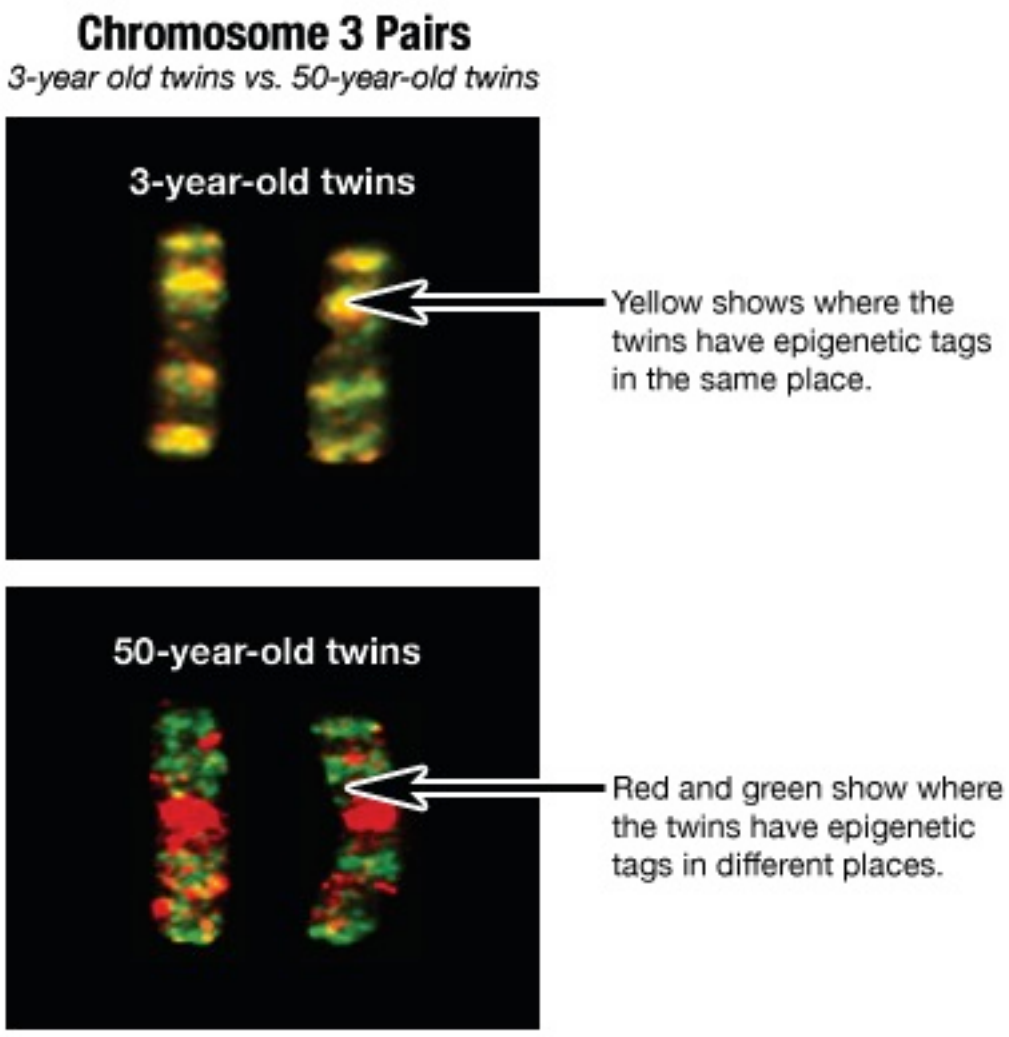
- Induzierte pluripotente Stammzellen (iPSC)

Yamanaka, Gurdon 2012



Nature or Nurture?

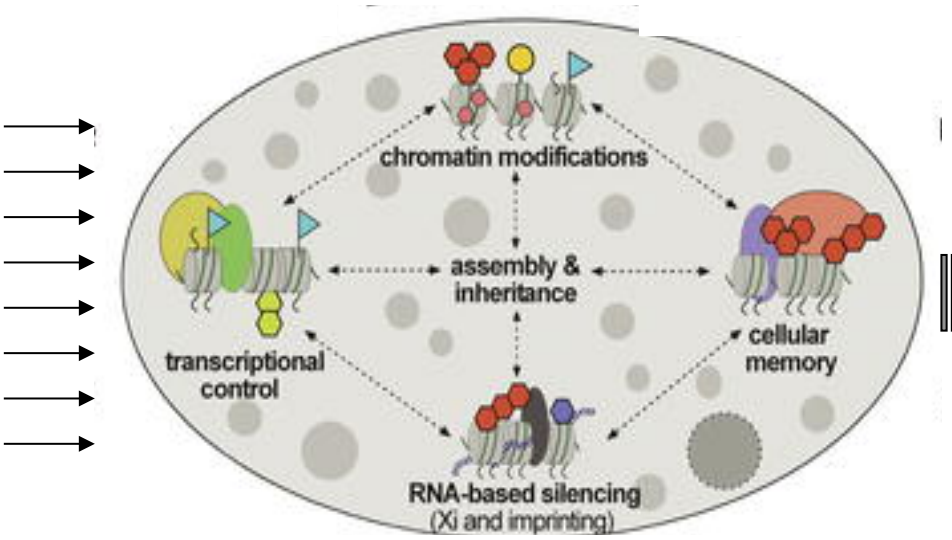
DNA sequence or chromatin?



http://learn.genetics.utah.edu/content/epigenetics/

Nature *AND* Nurture!

Umwelt



Ontogenese
Erkrankung

Umwelt nimmt über die Epigenetik erheblichen Einfluss direkt auf das Erbmaterial!

Bsp: Fellfärbung (Agouti-Gen)

Nature Reviews Genetics 8. April 2007

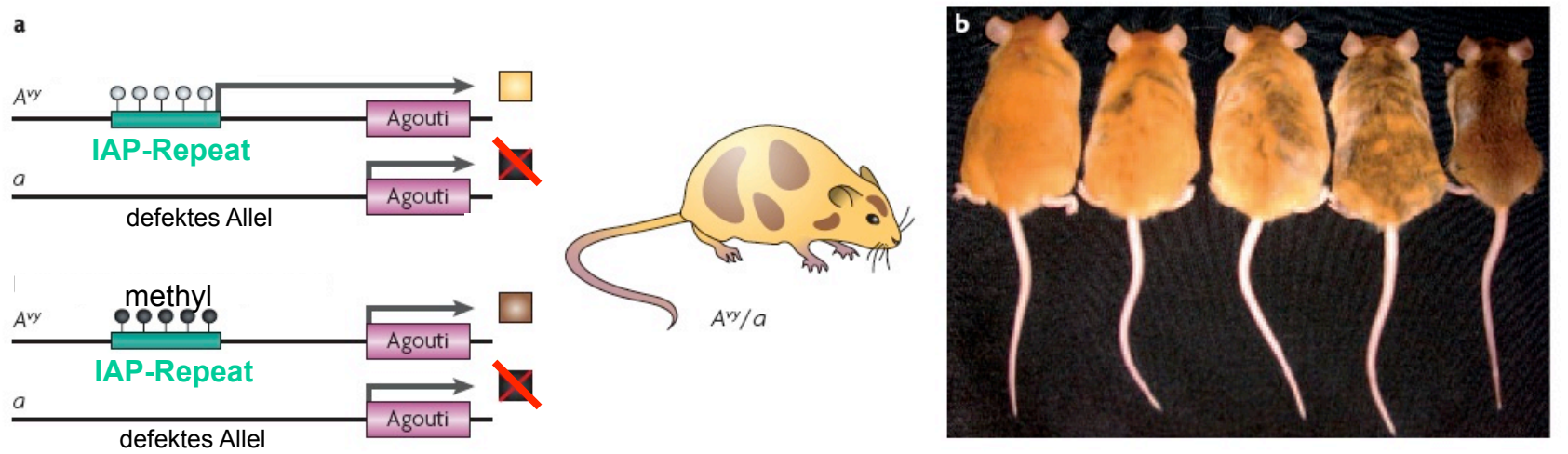


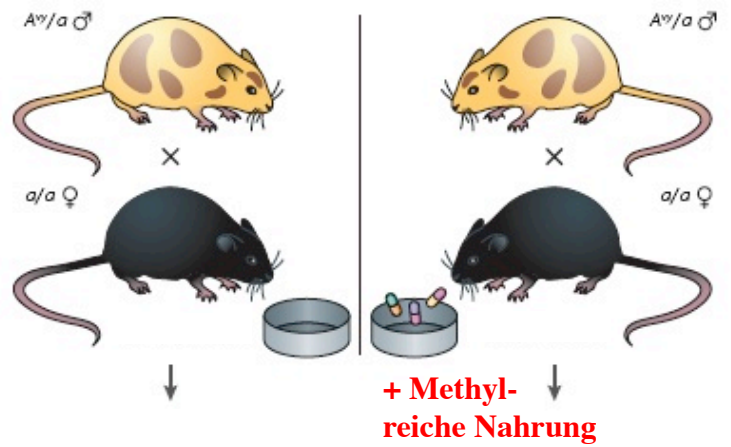
Figure 1 | **Epigenetic regulation of metastable epialleles. a** | Epigenetic regulation of the agouti gene in A^{vy}/a mice. White-filled circles indicate unmethylated CpG sites and black-filled circles indicate methylated CpG sites. Phaeomelanin (the product of the agouti gene) is not produced from the *a* allele because the agouti gene is mutated (shown as a box marked with a red cross). Two potential epigenetic states of the A^{vy} allele can occur within cells of A^{vy}/a mice. The IAP (intracisternal A particle) that lies upstream of the agouti gene can remain unmethylated, allowing ectopic expression of the gene from the IAP and resulting in a yellow coat colour (top). Alternatively, the IAP can be methylated, so that the gene is expressed under its normal developmental controls, leading to a brown coat colour (bottom). If the IAP methylation event occurs later in development and does not affect all embryonic cells, the offspring will have a mottled appearance (illustrated on the right). **b** | Genetically identical week 15 A^{vy}/a mouse littermates are shown, representing five coat-colour phenotypes. Mice that are predominately yellow are also clearly more obese than the brown mice. Part **b** reproduced with permission from REF. 20 © (2006) National Institute

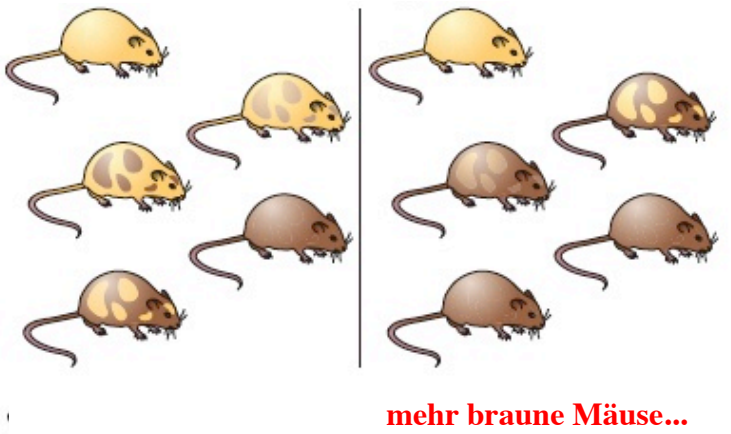
Das IAP-Repeat ist ein Transposon.

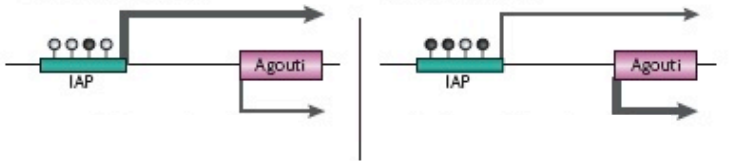
Oben: keine Methylierung > Agouti-Gen vom Promotor der IAP-Sequenz „ferngesteuert“ > gelbes Fell

Unten: IAP methyliert > Agouti unter eigener Kontrolle > braunes Fell

Umwelt > DNA > Phänotyp







Gelbe Mäuse haben auch mehr Krebs und Diabetes!

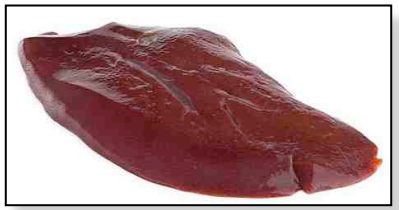
Ernährung beeinflusst über Epigenetik das Auftreten von Erkankungen!

https://de.wikipedia.org/wiki/Hongerwinter

Epigenetik:
„Du bist, was deine Mutter isst“

- Methylgruppen-reiche Ernährung vorteilhaft
  (Methionin, Betain, Cholin oder -als Vorstufe - Folsäure)

- Vitamin B12 und Zink als Co-Faktoren



Leslie *BMC Medicine* 2013, **11**:27
http://www.biomedcentral.com/1741-7015/11/27

BMC Medicine

COMMENTARY Open Access

Multigenerational epigenetic effects of nicotine on lung function

Frances M Leslie

Abstract

A recent preclinical study has shown that not only maternal smoking but also grandmaternal smoking is associated with elevated pediatric asthma risk. Using a well-established rat model of *in utero* nicotine exposure, Rehan *et al.* have now demonstrated multigenerational effects of nicotine that could explain this 'grandmother effect'. F1 offspring of nicotine-treated pregnant rats exhibited asthma-like changes to lung function and associated epigenetic changes to DNA and histones in both lungs and gonads. These alterations were blocked by co-administration of the peroxisome proliferator-activated receptor-γ agonist, rosiglitazone, implicating downregulation of this receptor in the nicotine effects. F2 offspring of F1 mated animals exhibited similar changes in lung function to that of their parents, even though they had never been exposed to nicotine. Thus epigenetic mechanisms appear to underlie the multigenerational transmission of a nicotine-induced asthma-like phenotype. These findings emphasize the need for more effective smoking cessation strategies during pregnancy, and cast further doubt on the safety of using nicotine replacement therapy to reduce tobacco use in pregnant women.

Please see related article: http://www.biomedcentral.com/1741-7015/10/129

Background

The negative health effects of tobacco use in adult smokers are well established [1]. On average, smoking leads to more than 400,000 premature deaths in the United States each year, with an overall decrease in life expectancy of 14 years. The major adverse health consequences of smoking include cancer, cardiovascular disease and respiratory disorders. Since many women continue to smoke during pregnancy, the negative impact of tobacco can begin before birth [2]. Maternal smoking is now the single most important preventable risk factor for Sudden Infant Death Syndrome, which results from developmental delays in the neural control of cardiopulmonary function [1,2]. Children of smokers are also more prone to respiratory diseases, such as asthma. One surprising finding is that a grandmother's tobacco use is associated with increased risk of early childhood asthma, even if the mother did not smoke while pregnant [3]. Rehan *et al.* [4] have recently used a well-established rat model of *in utero* nicotine exposure to determine the possible mechanisms underlying this clinical observation (Figure 1). They found that maternal nicotine exposure exerted adverse effects on lung development, not only for the immediate offspring but also for the next generation. They also identified epigenetic mechanisms involved in this multigenerational transmission. This paper will review these groundbreaking findings and discuss their potential clinical implications.

Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse

Patrick O McGowan[1,2], Aya Sasaki[1,2], Ana C D'Alessio[3], Sergiy Dymov[3], Benoit Labonté[1,4], Moshe Szyf[2,3], Gustavo Turecki[1,4] & Michael J Meaney[1,2,5]

Maternal care influences hypothalamic-pituitary-adrenal (HPA) function in the rat through epigenetic programming of glucocorticoid receptor expression. In humans, childhood abuse alters HPA stress responses and increases the risk of suicide. We examined epigenetic differences in a neuron-specific glucocorticoid receptor (NR3C1) promoter between postmortem hippocampus obtained from suicide victims with a history of childhood abuse and those from either suicide victims with no childhood abuse or controls. We found decreased levels of glucocorticoid receptor mRNA, as well as mRNA transcripts bearing the glucocorticoid receptor 1_F splice variant and increased cytosine methylation of an NR3C1 promoter. Patch-methylated NR3C1 promoter constructs that mimicked the methylation state in samples from abused suicide victims showed decreased NGFI-A transcription factor binding and NGFI-A–inducible gene transcription. These findings translate previous results from rat to humans and suggest a common effect of parental care on the epigenetic regulation of hippocampal glucocorticoid receptor expression.

Nature Neuroscience 9. Jan 2009

https://www.nature.com/polopoly_fs/1.14816!/menu/main/topColumns/topLeftColumn/pdf/507022a.pdf

THE *SINS* OF THE FATHER

The roots of inheritance may extend beyond the genome, but the mechanisms remain a puzzle.

BY VIRGINIA HUGHES

When Brian Dias became a father last October, he was, like any new parent, mindful of the enormous responsibility that lay before him. From that moment on, every choice he made could affect this newborn son's physical and psychological development. But, unlike most new parents, Dias was also aware of the influence of his past experiences — not to mention those of his parents, his grandparents and beyond.

Where one's ancestors lived, or how much they valued education, can clearly have effects that pass down through the generations. But what about the legacy of their health: whether they smoked, endured famine or fought in a war?

As a postdoc in Kerry Ressler's laboratory at Emory University in Atlanta, Georgia, Dias had spent much of the two years before his son's birth studying these kinds of questions in mice. Specifically, he looked at how fear associated with a particular smell affects the animal's and leaves an imprint on the brains of their descendants.

Dias had been exposing male mice to acetophenone — a chemical with a sweet, almond-like smell — and then giving them a mild foot shock. After being exposed to this treatment five times a day for three days, the mice became reliably fearful, freezing in the presence of acetophenone even when they received no shock.

Ten days later, Dias allowed the mice to mate with unexposed females. When their young grew up, many of the animals were more sensitive to acetophenone than to other odours, and more likely to be startled by an unexpected noise during exposure to the smell. Their offspring — the 'grandchildren' of the mice trained to fear the smell — were also jumpier in the presence of acetophenone. What's more, all three generations had larger-than-normal 'M71 glomeruli', structures where acetophenone-sensitive neurons in the nose connect with neurons in the olfactory bulb. In the January issue of *Nature Neuroscience*[1], Dias and Ressler suggested that this hereditary transmission of environmental information was the result of epigenetics — chemical changes to the genome that affect how DNA is packaged and expressed without altering its sequence.

Biologists first observed this 'transgenerational epigenetic inheritance' in plants. Tomatoes, for example, pass along chemical markings that control an important ripening

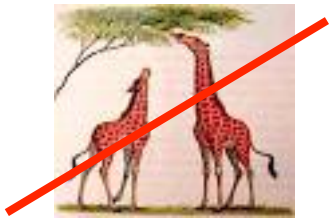
EpiGenetik:
Lamarck revisited!

• Umwelteinflüsse können über Generationen hinweg die Vererbung von Merkmalen verändern!

• Bedeutung für die Makroevolution noch unklar.

• *trans-generational effects* häufiger bei Pflanzen (wegen fehlender Keimbahn)

• Es entstehen jedoch wohl so keine neuen Arten, wie Lamarck es postuliert hat !!



Handel and Ramagopalan *BMC Medical Genetics* 2010, **11**:73
http://www.biomedcentral.com/1471-2350/11/73

BMC Medical Genetics

DEBATE Open Access

Is Lamarckian evolution relevant to medicine?

Adam E Handel[1,2] and Sreeram V Ramagopalan*[1,2]

Abstract

Background: 200 years have now passed since Darwin was born and scientists around the world are celebrating this important anniversary of the birth of an evolutionary visionary. However, the theories of his colleague Lamarck are treated with considerably less acclaim. These theories centre on the tendency for complexity to increase in organisms over time and the direct transmission of phenotypic traits from parents to offspring.

Discussion: Lamarckian concepts, long thought of no relevance to modern evolutionary theory, are enjoying a quiet resurgence with the increasing complexity of epigenetic theories of inheritance. There is evidence that epigenetic alterations, including DNA methylation and histone modifications, are transmitted transgenerationally, thus providing a potential mechanism for environmental influences to be passed from parents to offspring: Lamarckian evolution. Furthermore, evidence is accumulating that epigenetics plays an important role in many common medical conditions.

Summary: Epigenetics allows the peaceful co-existence of Darwinian and Lamarckian evolution. Further efforts should be exerted on studying the mechanisms by which this occurs so that public health measures can be undertaken to reverse or prevent epigenetic changes important in disease susceptibility. Perhaps in 2059 we will be celebrating the anniversary of both Darwin *and* Lamarck.