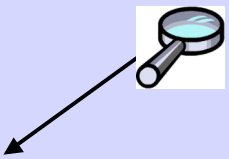
Evolution in Action:
Mutationen schaffen Variabilität

1. Genom-Mutationen

2. Chromosomen-Mutationen

   Deletion, Duplikation, Inversion, Translokation



3. "intragenische" Mutationen

   Punktmutation/Substitution, Deletion, Duplikation, Inversion, Transposition

Thomas Hankeln SS 2010

JOHANNES GUTENBERG UNIVERSITÄT MAINZ

„Intragenische" Mutationen

Nukleotidaustausche und auch kleinere Indels sind diejenigen Unterschiede, die am häufigsten zur Bestimmung der molekularen Evolution von Genen verwendet werden.

...aber auch größere strukturelle Rearrangements in Genomen („rare genomic changes") haben einen wichtigen phylogenetischen Informations-gehalt.

„Intragenische" Mutationen

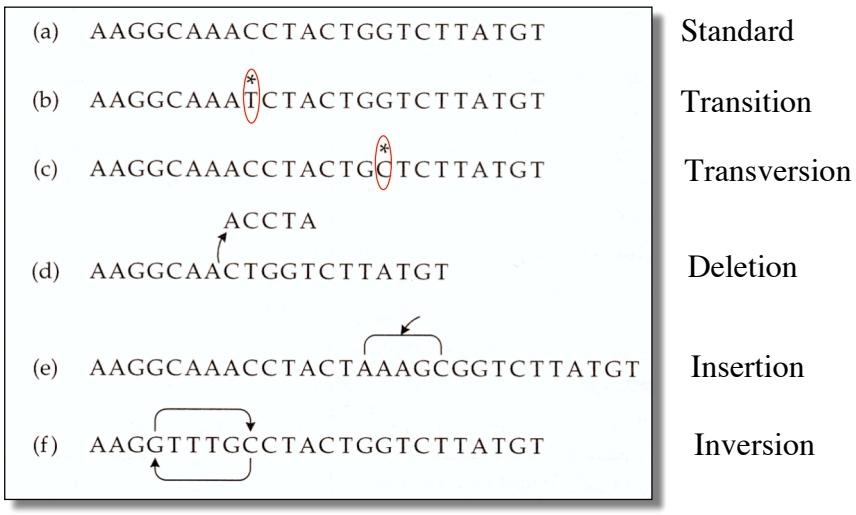
Basensubstitutionen

- Transition = Pur>Pur oder Pyr>Pyr
- Transversion = Pur<>Pyr

Insertionen/Deletionen (Indels)

- führen in Genen zu Leseraster-Verschiebungen

„Intragenische" Mutationen



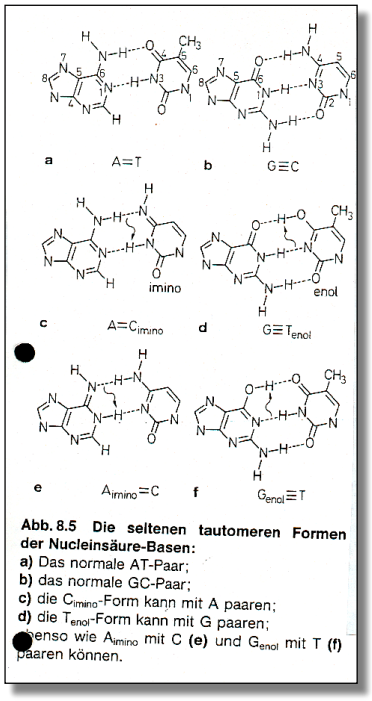


Abb. 8.5 Die seltenen tautomeren Formen der Nucleinsäure-Basen:
a) Das normale AT-Paar;
b) das normale GC-Paar;
c) die C_{imino}-Form kann mit A paaren;
d) die T_{enol}-Form kann mit G paaren;
e) ebenso wie A_{imino} mit C **(e)** und G_{enol} mit T **(f)** paaren können.

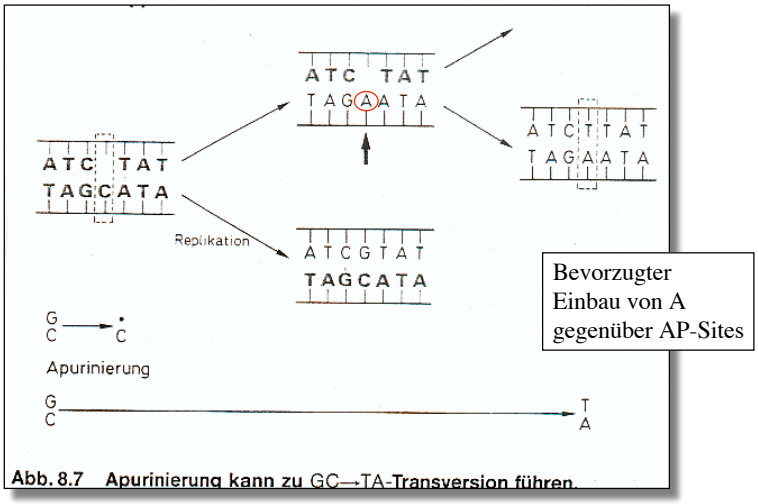
Spontan-Substitutionen durch Basen-Tautomerie

Häufigkeit 10^{-4}

> Transitionen

Spontane Transversionen durch Basenverlust

AP-Stellen: Purine sind besonders säureempfindlich



Bevorzugter Einbau von A gegenüber AP-Sites

Abb. 8.7 Apurinierung kann zu GC→TA-Transversion führen.

Spontane Desaminierungen an Basen

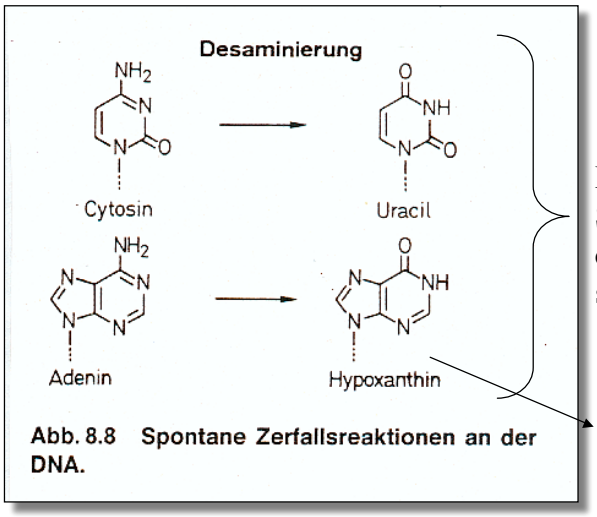


Abb. 8.8 Spontane Zerfallsreaktionen an der DNA.

Meist von Reparatur-Systemen als fremd erkannt;
sonst Transitionen

paart wie G

5-Methyl-Cytosin : ein Mutations-HotSpot

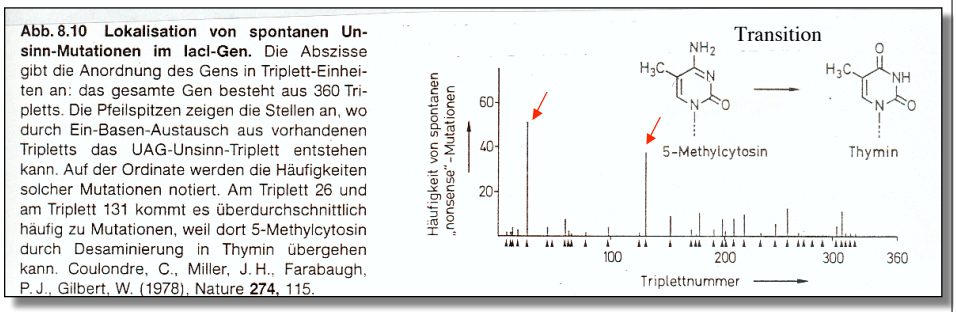
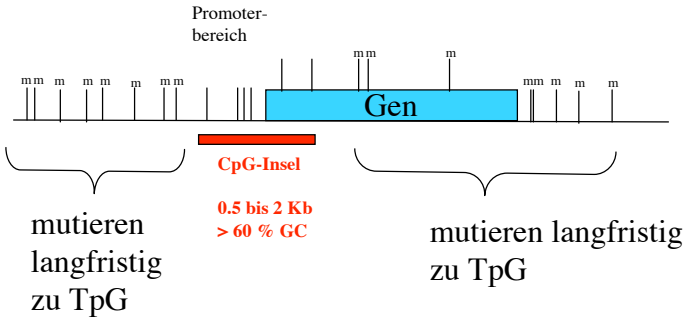


Abb. 8.10 Lokalisation von spontanen Unsinn-Mutationen im lacI-Gen. Die Abszisse gibt die Anordnung des Gens in Triplett-Einheiten an: das gesamte Gen besteht aus 360 Tripletts. Die Pfeilspitzen zeigen die Stellen an, wo durch Ein-Basen-Austausch aus vorhandenen Tripletts das UAG-Unsinn-Triplett entstehen kann. Auf der Ordinate werden die Häufigkeiten solcher Mutationen notiert. Am Triplett 26 und am Triplett 131 kommt es überdurchschnittlich häufig zu Mutationen, weil dort 5-Methylcytosin durch Desaminierung in Thymin übergehen kann. Coulondre, C., Miller, J. H., Farabaugh, P. J., Gilbert, W. (1978), Nature **274**, 115.

- in Säugern: während der Evolution „Zerfall" von $C^{m}pG$-Dinukleotiden zu TpG

CpG-Inseln im Säugergenom

- in Säugern: 60-90 % aller Cytosine in CpGs methyliert
- Promoter-Bereiche aktiver Gene sind untermethyliert
- während der Evolution „Zerfall“ von C^{m}pG zu TpG (Soll-Häufigkeit bei 41% GC ist 4%, tatsächlich nur 0.8 %)



Mutationsspektrum und -Häufigkeit in menschlichen Zellen

aus: TIG 14(3), 1998

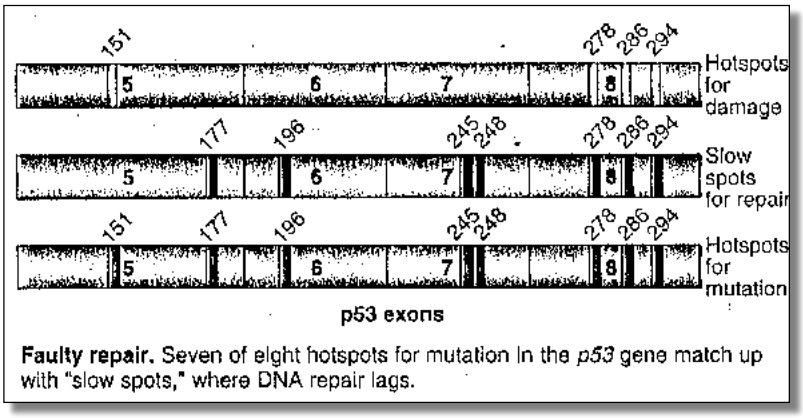
TABLE 1. Endogenous DNA lesions in human cells

Lesion	Mode of formation	Number of residues generated daily per human genome	Genome steady state level in normal, repair-proficient cells
Uracil	Cytosine deamination	400	~1
Thymine (opposite guanine)	5-Methylcytosine deamination	30	10–20
Hypoxanthine	Adenine deamination	10	~1
8-Oxoguanine	Guanine oxidation	~1000	~1
faPy	Guanine oxidation	~200	~5
Thymine glycol and similar oxidized pyrimidines	Pyrimidine oxidation	~500	~5
Etheno C	Lipid peroxidation of cytosine	~200	~5
Etheno A	Lipid peroxidation of adenine	~200	~5
3-Methyladenine	SAM methylation of adenine	600	~5
7-Methylguanine	SAM methylation of guanine	4000	3000
O^6-Methylguanine	Genomic alkylation by endogenous nitrosamines	~200	~1
Abasic site	Hydrolytic depurination	9000	~5

This table was prepared and presented in a talk by Tomas Lindahl (ICRF, UK) one of the organizers of the meeting, who kindly provided it for this report. It is not intended to be an exhaustive list of all the types of damage discussed. The values for hydrolytic and alkylation damage are based on the measured rates of generation and of repair, while the values for oxidative damage are based on measured rates of repair and on approximate rates of generation of lesions estimated from data with microbial mutants.

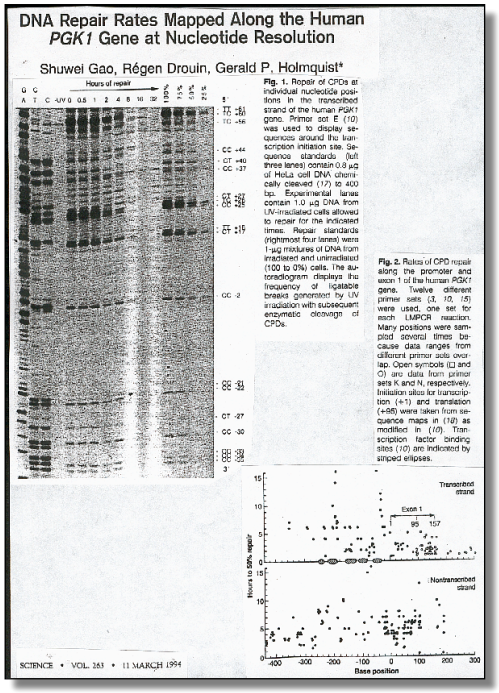
Fehlerhafte Reparatur als zusätzliche Ursache von Mutations-Hotspots

Bsp. Tumorsuppressorgen p53



Faulty repair. Seven of eight hotspots for mutation in the *p53* gene match up with "slow spots," where DNA repair lags.

Science 263 (1994) pp1436


DNA Repair Rates Mapped Along the Human *PGK1* Gene at Nucleotide Resolution

Shuwei Gao, Régen Drouin, Gerald P. Holmquist*

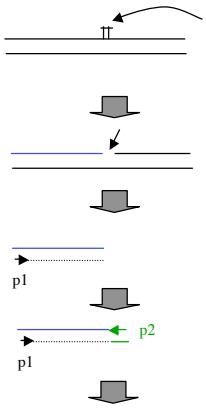
SCIENCE • VOL. 263 • 11 MARCH 1994

Kartierung der Reparatur-Effizienz

- präferentielle Reparatur des transkribierten Stranges
- langsame Reparatur im Promoter an TF-Bindestellen
- Reparaturrate variiert bis Faktor 15 innerhalb eines Gens

Kartierung von Reparaturprozessen

1. Zellen mutagenisieren (hier: UV)
2. zeitabhängig Proben entnehmen:



+ T4 Endonuklease V
+ E. coli Photolyase
>attackieren Cyclobutandimere

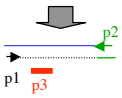
„nick“

Denaturieren

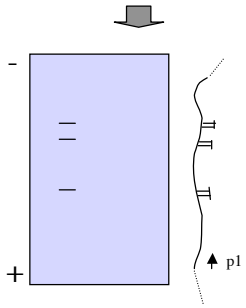
Auffüllen des 2. Stranges von p1 aus

Ligation eines ds Linkers

Ligation-mediated PCR (p1+p2)



Denaturieren,
Laden auf denat. Gel,
Blotten,
Hybridisierung mit Sonde (p3)



Mutationen sind das Resultat von...

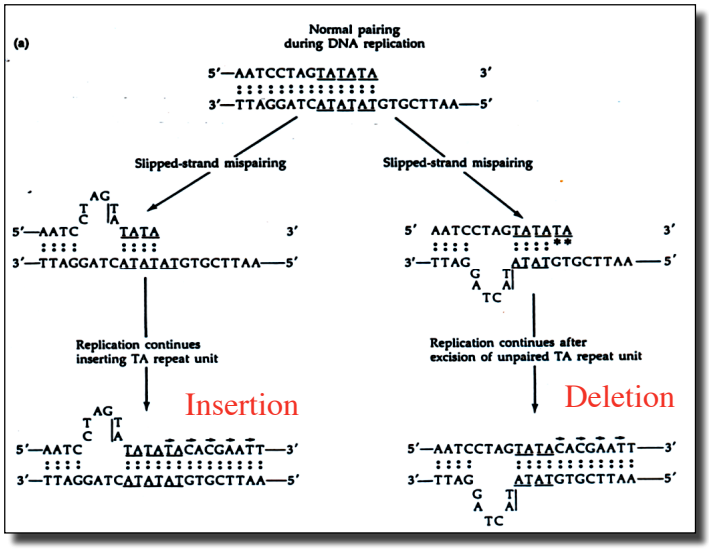
...Konzentration endogener und exogener Mutagene

... Genauigkeit der DNA-Polymerasen

... proof-reading-Aktivität der Polymerasen

... prä-und post-replikativen Reparaturmechanismen

„Slippage"-Fehler bei der Replikation



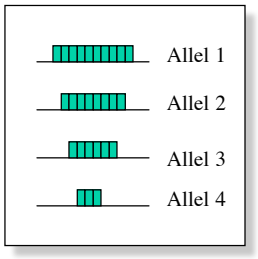
Streisinger 1966

Entstehung und Längeninstabilität von „Mikrosatelliten"-DNA

- Mikrosatelliten-DNA, simple tandem repeats (STR), simple sequences
- DNA mit sich wiederholenden Sequenz-Motiven von 2 bis etwa 10 Nukleotiden

  z.B. $(CA)_{25}$; $(CAA)_{13}$; $(GAAA)_{17}$
- ca. 100 000 CA-Dinucleotid-Mikrosatelliten in einem typischen Säugergenom
- Bei der DNA-Replikation verändert sich durch „slippage" häufig die Länge von STRs
- Mikrosatellitensequenzen sind hochpolymorph; viele verschiedene Allele in der Population

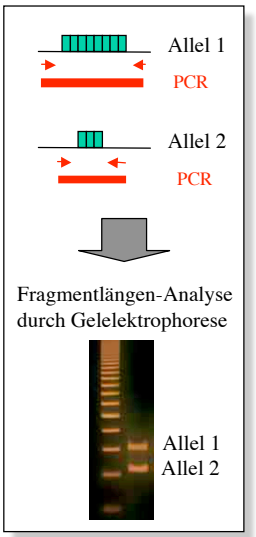
Längeninstabilität von (Mikro)Satelliten-DNA



‚Instabilität' = Längenänderung
= Zugewinn oder Verlust von Einheiten

„VNTR"	variable number of tandem repeats
„HVR"	hypervariable regions

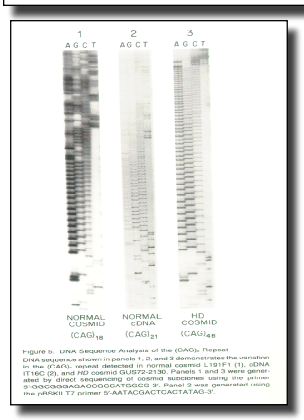
Anwendung: DNA-Fingerprinting



- stabile Vererbung nach Mendel ermöglicht Abstammungsnachweis
- hohe Zahl an unterschiedlichen Allelen in der Population ermöglicht Identifizierung von Individuen

Cell, Vol. 72, 971–983, March 26, 1993, Copyright © 1993 by Cell Press

A Novel Gene Containing a Trinucleotide Repeat That Is Expanded and Unstable on Huntington's Disease Chromosomes



(CAG)n

- HD -Allel > 36 x CAG
- Normal meist < 30 x CAG

…polymorph, aber stabil vererbt von Generation zu Generation

Trinukleotid-Erkrankungen

Protein: loss-of-function

Protein: gain-of-function

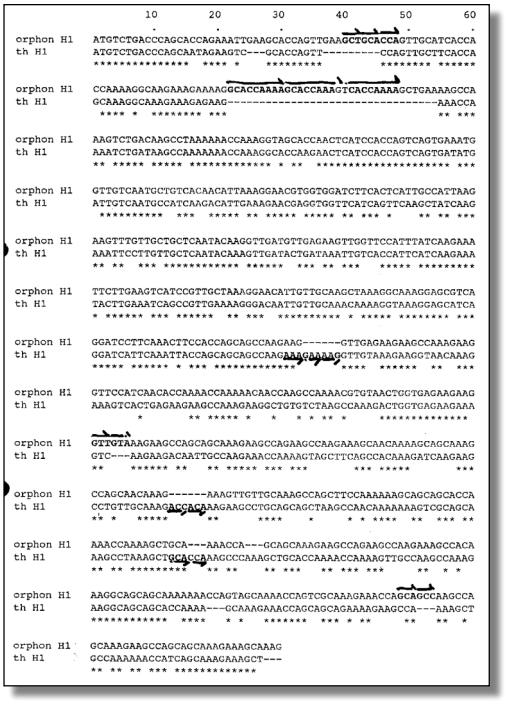
RNA: gain-of-function

188 T. Hankeln et al.

Table 1. Summary of triplet repeat diseases in humans

Repeat sequence	Condition	Gene	Repeat localisation	Repeat number	
				Normal	Disease
CGG	FRAXA	FMR-1	5′-Untranslated	6–52	200–1000
CGG	FRAXE	?		6–25	200–1000
CGG	FRA11B (Jacobsen syndrome)	CBL2	?	~11	400–800
CAG	SBMA (Kennedy disease)	Androgen receptor	ORF	12-33	<100
CAG	Huntington	Huntington	ORF	9–30	<150
CAG	SCA 1	Ataxin	ORF	9–39	<100
CAG	DRPLA/HRS	Atropin	ORF	9–23	<100
CAG	Machado-Joseph	MJD 1	ORF	16–36	<100
CTG	Myotonic dystrophy	DM kinase (DM-1)	3′-Untranslated	5–40	200–4000

ORF, open reading frame.

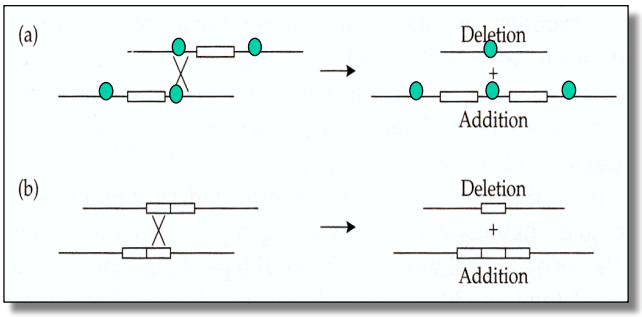


„slippage" während der Evolution von Genen

Entstehung von Histon H1- Gen-Varianten in Zuckmücken

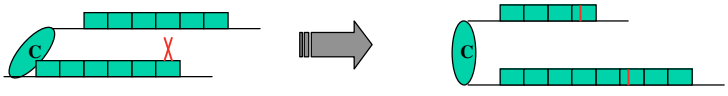
Verschiedene Spielarten der Rekombination führen zu großräumigen Rearrangements im Genom

Duplikationen und Deletionen durch „inäquales Crossingover“



- zwischen Schwesterchromatiden (!!) in der Mitose
- oder zwischen Nicht-Schwesterchromatiden in der Meiose

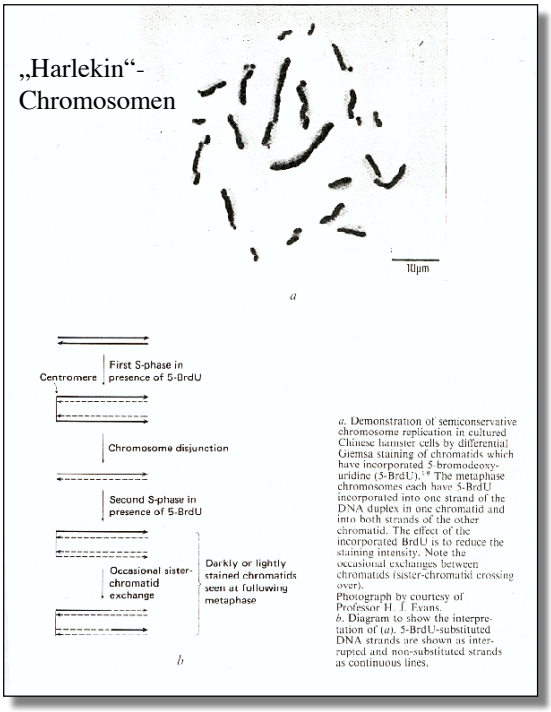
Duplikationen und Deletionen durch „inäquales Crossingover“



SCE Sister chromatid exchange

Speziell in Tandem-Clustern führt SCE zu „Hypervariabilität“

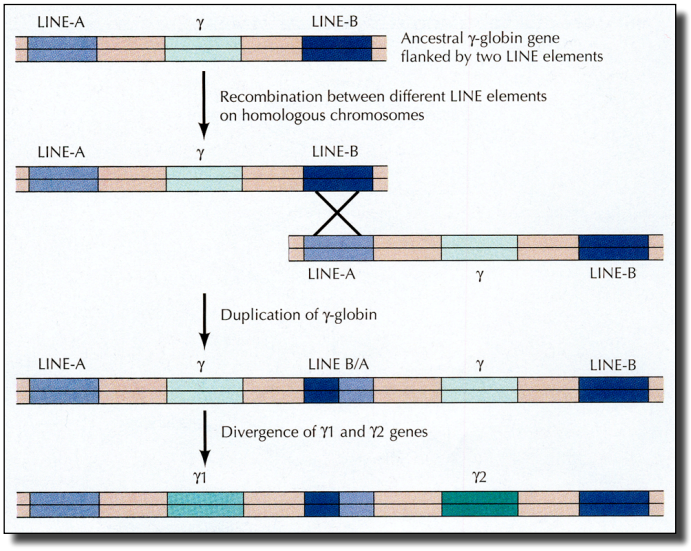
„Harlekin“-
Chromosomen



a, Demonstration of semiconservative chromosome replication in cultured Chinese hamster cells by differential Giemsa staining of chromatids which have incorporated 5-bromodeoxyuridine (5-BrdU).[1] The metaphase chromosomes each have 5-BrdU incorporated into one strand of the DNA duplex in one chromatid and into both strands of the other chromatid. The effect of the incorporated BrdU is to reduce the staining intensity. Note the occasional exchanges between chromatids (sister-chromatid crossing over).
Photograph by courtesy of Professor H. J. Evans.
b, Diagram to show the interpretation of (*a*). 5-BrdU-substituted DNA strands are shown as interrupted and non-substituted strands as continuous lines.

Das Taylor-Experiment zur semikonservativen DNA-Replikation beweist häufige **Schwesterstrang-Crossingover**

Entstehung von Globingen-Duplikaten durch inäquales Crossingover



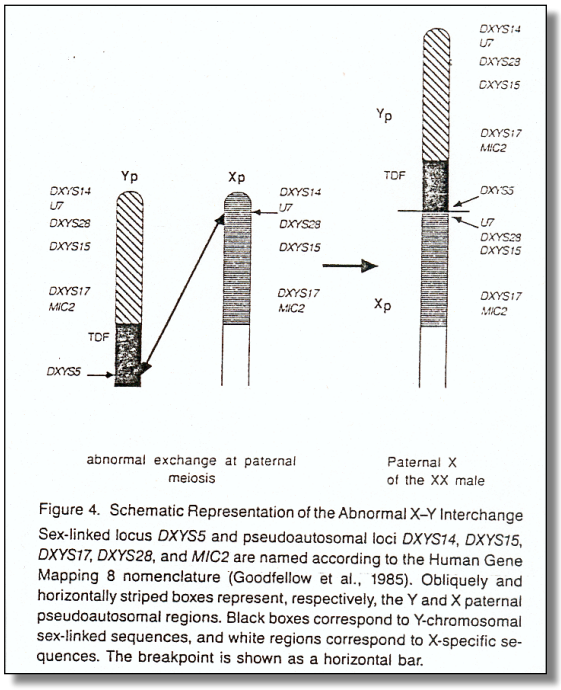


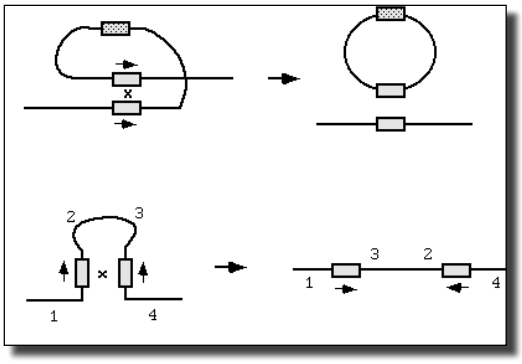
Figure 4. Schematic Representation of the Abnormal X–Y Interchange

Sex-linked locus *DXYS5* and pseudoautosomal loci *DXYS14*, *DXYS15*, *DXYS17*, *DXYS28*, and *MIC2* are named according to the Human Gene Mapping 8 nomenclature (Goodfellow et al., 1985). Obliquely and horizontally striped boxes represent, respectively, the Y and X paternal pseudoautosomal regions. Black boxes correspond to Y-chromosomal sex-linked sequences, and white regions correspond to X-specific sequences. The breakpoint is shown as a horizontal bar.

Inäquales CO zwischen homologen Sequenzen auf unterschiedlichen Chromosomen kann Translokationen erzeugen

>XX-Männer!!

Weitere Mechanismen der Genom-Instabilität durch Rekombination



Deletion durch Intra-Chromatid-Rekombination zwischen ‚direct repeats'

Inversion durch Intra-Chromatid-Rekombination zwischen ‚inverted repeats'

Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A

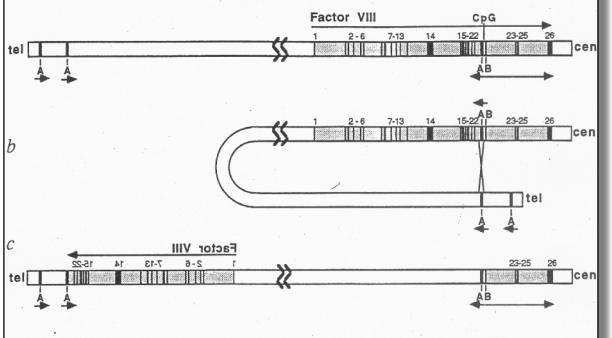


Fig. 1 Diagram of the factor VIII gene and illustration of the inversion model. *a*, Region of Xq28 that includes the factor VIII gene, oriented with the telomere at the left, is depicted. Three copies of the A gene are indicated, two lying upstream of factor VIII and one inside intron 22. The location of the B transcript is also shown. The arrows indicate the direction of transcription of the factor VIII and internal A and B genes. The direction of the upstream A genes is hypothesized to be as shown. *b*, Proposed homologous recombination between the intron-22 copy of gene A and one of the two upstream copies. A crossover between these two identical regions, oriented as shown, would result in an inversion of sequence between the two recombined A genes (*c*). A recombination could involve either of the upstream A genes, but only one is presented. The crossover could occur anywhere in the region of homology which includes the A genes.

Inversion

- 47% aller Hämophilie A-Mutationen
- Gen ist auf X lokalisiert
- praktisch alle Mutationen entstehen in Männern!!

➢führt Fehlen eines 2. X-Chromosoms in der männl. Meiose zu einer Fehl-faltung des X und damit zur häufigen Inversion?

Genom-Rearrangements als Ursache genetischer Erkrankungen

TABLE 1. Physical features of regions associated with genomic disorders

Trait	Rearrangement type	Distance between repeats (kb)	Repeat length (bp)
Color blindness	DEL	0	39 000
α-Thalassemia	DEL	3.7 or 4.2	4000
Growth hormone deficiency	DEL	6.7	2200
Debrisoquine sensitivity	DEL	9.3	2800
Hunter mucopolysaccharidosis	INV	20	3000
Glucocorticoid-remediable aldosteronism	DUP	45	10 000
Hemophilia A	INV	500	9500
CMT1A/HNPP	DUP/DEL	1500	24 011
X-linked ichthyosis	DEL	1900	20 000
Williams syndrome	DEL	~2000	>30 000
Smith–Magenis syndrome/dup(17)(p11.2)	DEL/DUP	~5000	>200 000

Abbreviations: DEL, deletion; DUP, duplication; INV, inversion.

TIG 14(10) 1998, 417ff

Mechanisms of tandem duplication in the Duchenne muscular dystrophy gene include both homologous and nonhomologous intrachromosomal recombination

Xiuyuan Hu, Peter N.Ray and Ronald G.Worton

Genetics Department, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, and Department of Molecular and Medical Genetics, University of Toronto, Toronto, Canada

Communicated by W.Gehring

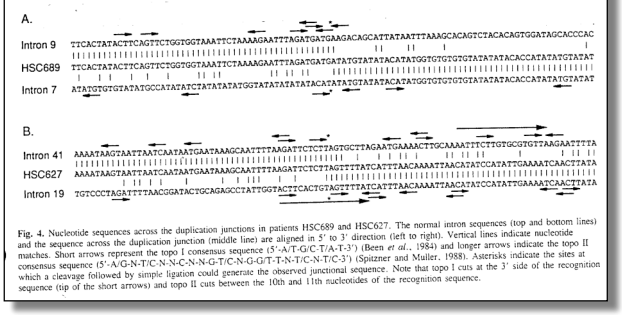


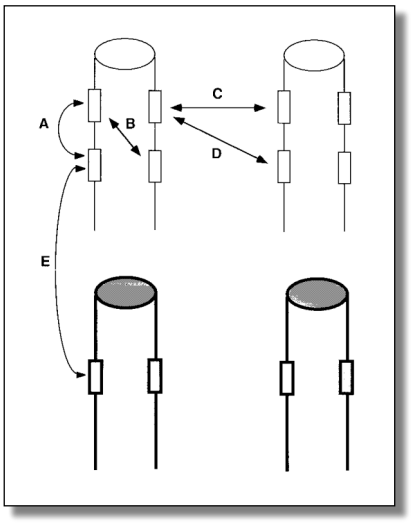
Fig. 4. Nucleotide sequences across the duplication junctions in patients HSC689 and HSC627. The normal intron sequences (top and bottom lines) and the sequence across the duplication junction (middle line) are aligned in 5' to 3' direction (left to right). Vertical lines indicate nucleotide matches. Short arrows represent the topo I consensus sequence (5'-A/T-G/C-T/A-T-3') (Been *et al.*, 1984) and longer arrows indicate the topo II consensus sequence (5'-A/G-N-T/C-N-N-C-N-N-G-T/C-N-G-G/T-T-N-T/C-N-T/C-3') (Spitzner and Muller, 1988). Asterisks indicate the sites at which a cleavage followed by simple ligation could generate the observed junctional sequence. Note that topo I cuts at the 3' side of the recognition sequence (tip of the short arrows) and topo II cuts between the 10th and 11th nucleotides of the recognition sequence.

‚Illegitimes' Crossingover

= non-homologous end joining (NHEJ)

Im Extremfall müssen rekombinierende Orte nicht einmal deutliche Sequenz-übereinstimmungen haben

Genom-Instabilität per Rekombination: …entdecke die Möglichkeiten!



A. Intra-Chromatid-Rekomb.

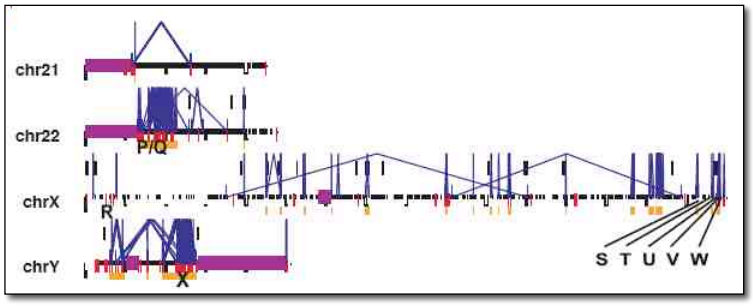
B. inäqualer SCE

C. klassisches CO (Meiose)

D. inäquales CO zwischen Nicht-Schwesterchromatiden

E. „Ektopisches" CO zwischen nicht-homologe Chromosomen

Segmentale Duplikationen (SD) im Humangenom



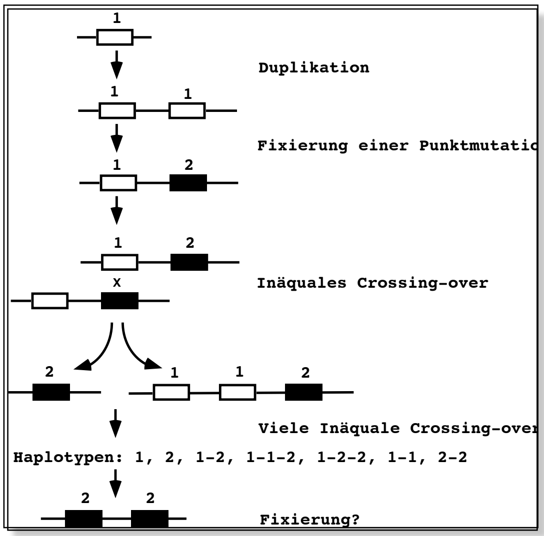
- low-copy repeats; 1-400 kb; dispergiert; > 90 % Nt-Identität
- 5% des Gesamtgenoms
- Nicht-allele homologe Rekombination (NAHR)
- Ursache für >25 Erkrankungen
- Basis für viele individuelle Unterschiede

Bailey et al. 2002, Science

Ein weiterer Effekt von wiederholter Rekombination:

Konzertierte Sequenzevolution

„konzertierte Evolution“ bei Genduplikaten



mehrfaches inäquales Crossingover kann zu einer ‚Sequenzhomogenisierung‘ führen

(„crossover fixation“)

Entstehung und 'Homogenisierung' von Tandem-Repetitionen durch inäquales CO

RANDOM STARTING SEQUENCE

3033123233002101222000202320020003210101320302000331012321031122330203112020233301301321020100103200
0222320012210010111132312310323323023131312311103221330112133121120011210321320013211333002220200011
13212010231303231233221103130031022233123030033321311011311130031112331002003103020120310101300202223
3312223011201000232130033231133211211320223003322023010331002013310111221322111110003031221310222332
2332333223200001032212230322330133022022302133303100313001033032221231130231313231220122032012121213

FINAL SEQUENCE AFTER 200 CYCLES

2033310322103221032210322103221032210322103221032210322003221032210322103221032200322103221032210322
1032210322103221032200322103221032210322103221032210322103221032210322103221032210322103221032210322
1032210322003221032210322003221032210322103221032210322103221032210322103221032210322103221032200322
1032210322103221032210322003221032210322103220032210322103221032210322103221032200322103221032210322
1032210322103221032210322103221032210322103221032220030121213

Fig. 2. Random starting sequence and final sequence after 200 cycles in one of the simulations summarized in Table 1 (fourth row). Each of the digits from 0 to 3 stands for a different one of the four possible base pairs. There is a small amount of anomalous sequence at each end of the array. This is because anomalous terminal sequences can only be lost by crossover at chance regions of homology within the terminal sequence; as the terminal sequences become small, such a crossover becomes highly improbable and the anomalous sequence persists.

Gerald P. Smith
1973, 1976

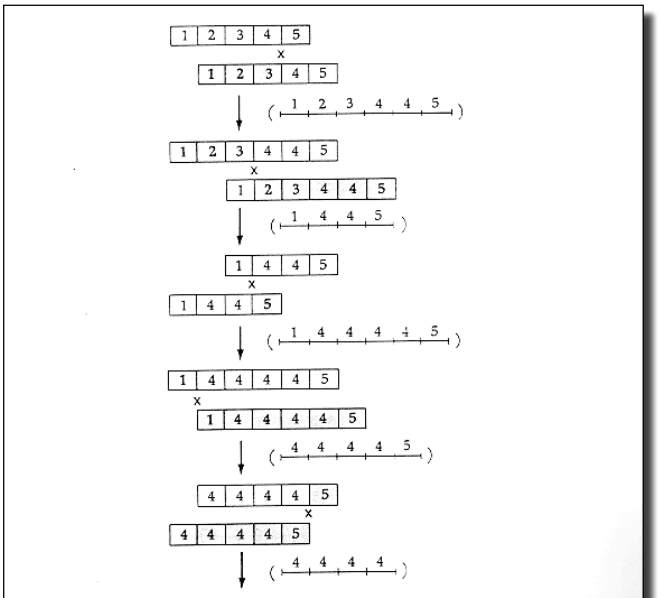
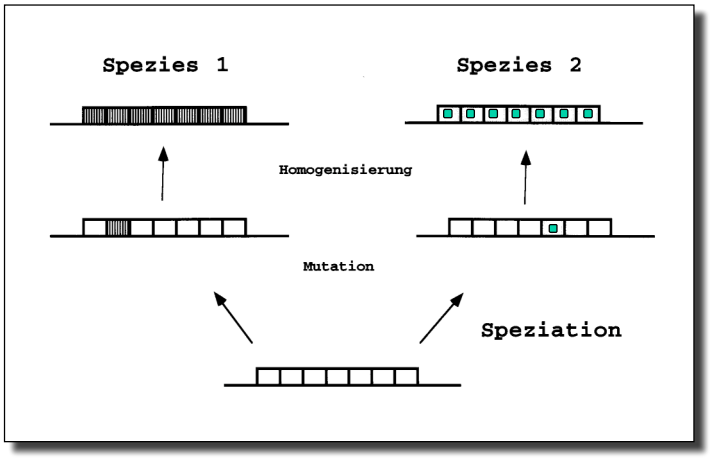


Figure 19. Concerted evolution by unequal crossing-over. Repeated cycles of unequal crossover events cause the duplicated genes on each chromosome to become progressively more homogenized. The process also affects the number of repeated sequences on each chromosome. From Ohta (1980).

Konzertierte Evolution durch „cross-over fixation" in tandem-repetitiver Satelliten-DNA

Spezies- und Chromosomen-Spezifität von Satelliten-DNA-Sequenzen



DNA-Abschnitte können sogar Sequenzinfo austauschen:

Genkonversion / Genkorrektur

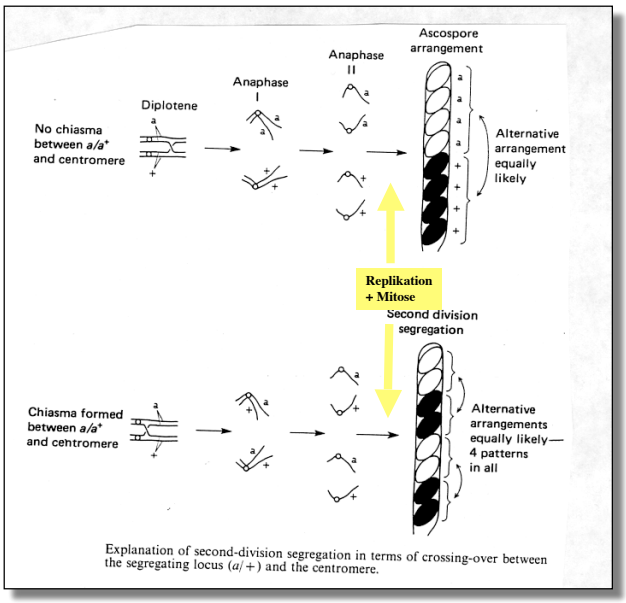
Genkonversion

- **nicht-reziproke Übertragung genetischer Information** (Donor > Rezipient)

- zwischen Allelen oder zwischen nicht-allelen Genduplikaten an verschiedenen Orten im Genom

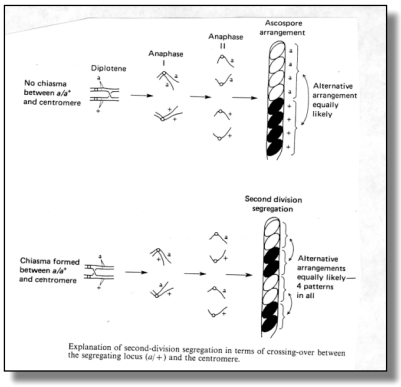
- **2 mögliche Mechanismen**: (1) Heteroduplex-Bildung bei klass. Rekombination
  (2) über cDNA-Intermediate

Meiose bei Ascomyzeten

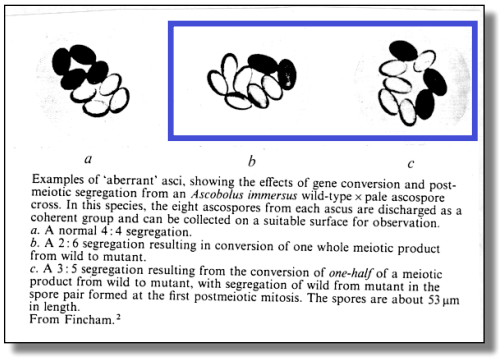


Meioseprodukte (Ascosporen) können mikroskopisch ausgewertet und genetisch interpretiert werden

Aberrante (nicht-mendelsche) Segregation bei Ascosporen: „Genkonversion"



„Mendel"



„aberrant" > „ Allele konvertiert"

Genkonversion und Rekombination

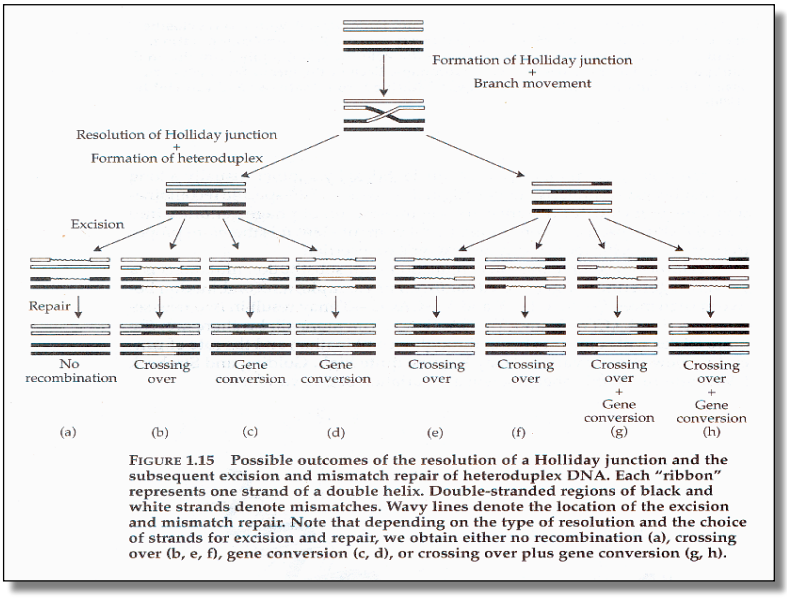
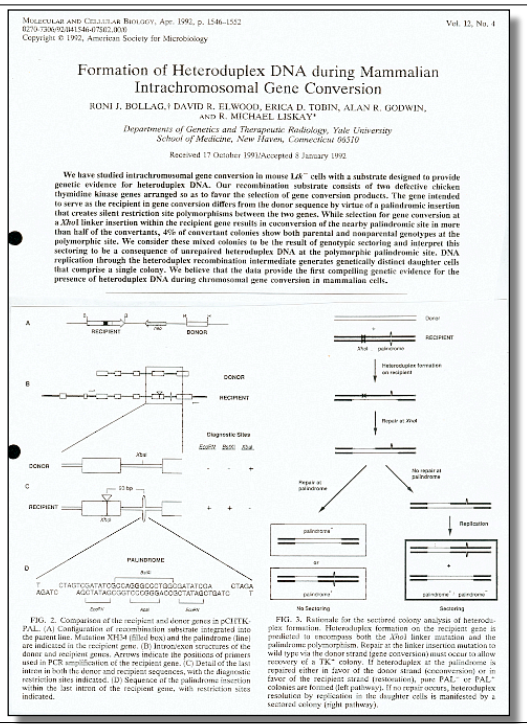


FIGURE 1.15 **Possible outcomes of the resolution of a Holliday junction and the subsequent excision and mismatch repair of heteroduplex DNA. Each "ribbon" represents one strand of a double helix. Double-stranded regions of black and white strands denote mismatches. Wavy lines denote the location of the excision and mismatch repair. Note that depending on the type of resolution and the choice of strands for excision and repair, we obtain either no recombination (a), crossing over (b, e, f), gene conversion (c, d), or crossing over plus gene conversion (g, h).**



Formation of Heteroduplex DNA during Mammalian Intrachromosomal Gene Conversion

RONI J. BOLLAG,† DAVID R. ELWOOD, ERICA D. TOBIN, ALAN R. GODWIN, AND R. MICHAEL LISKAY*

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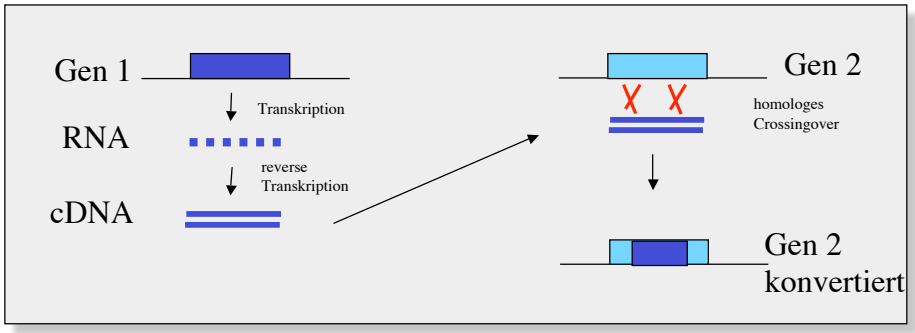
Received 17 October 1991/Accepted 8 January 1992

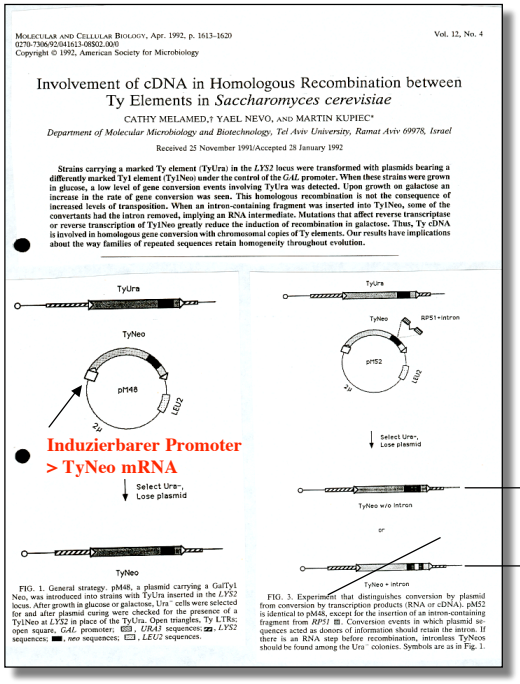
We have studied intrachromosomal gene conversion in mouse Ltk⁻ cells with a substrate designed to provide genetic evidence for heteroduplex DNA. Our recombination substrate consists of two defective chicken thymidine kinase genes arranged so as to favor the selection of gene conversion products. The gene intended to serve as the recipient in gene conversion differs from the donor sequence by virtue of a palindromic insertion that creates silent restriction site polymorphisms between the two genes. While selection for gene conversion at a XhoI linker insertion within the recipient gene results in coconversion of the nearby palindromic site in more than half of the convertants, 4% of convertant colonies show both parental and nonparental genotypes at the polymorphic site. We consider these mixed colonies to be the result of genotypic sectoring and interpret this sectoring to be a consequence of unrepaired heteroduplex DNA at the polymorphic palindromic site. DNA replication through the heteroduplex recombination intermediate generates genetically distinct daughter cells that comprise a single colony. We believe that the data provide the first compelling genetic evidence for the presence of heteroduplex DNA during chromosomal gene conversion in mammalian cells.

Beweis für die Ausbildung von Heteroduplizes bei der Genkonversion

Genkonversion via cDNA



Beweis für Genkonversion via cDNA



MOLECULAR AND CELLULAR BIOLOGY, Apr. 1992, p. 1613–1620 Vol. 12, No. 4
0270-7306/92/041613-08$02.00/0
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Involvement of cDNA in Homologous Recombination between Ty Elements in *Saccharomyces cerevisiae*

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Received 25 November 1991/Accepted 28 January 1992

Strains carrying a marked Ty element (TyUra) in the *LYS2* locus were transformed with plasmids bearing a differently marked Ty1 element (Ty1Neo) under the control of the *GAL* promoter. When these strains were grown in glucose, a low level of gene conversion events involving TyUra was detected. Upon growth on galactose an increase in the rate of gene conversion was seen. This homologous recombination is not the consequence of increased levels of transposition. When an intron-containing fragment was inserted into Ty1Neo, some of the convertants had the intron removed, implying an RNA intermediate. Mutations that affect reverse transcriptase or reverse transcription of Ty1Neo greatly reduce the induction of recombination in galactose. Thus, Ty cDNA is involved in homologous gene conversion with chromosomal copies of Ty elements. Our results have implications about the way families of repeated sequences retain homogeneity throughout evolution.

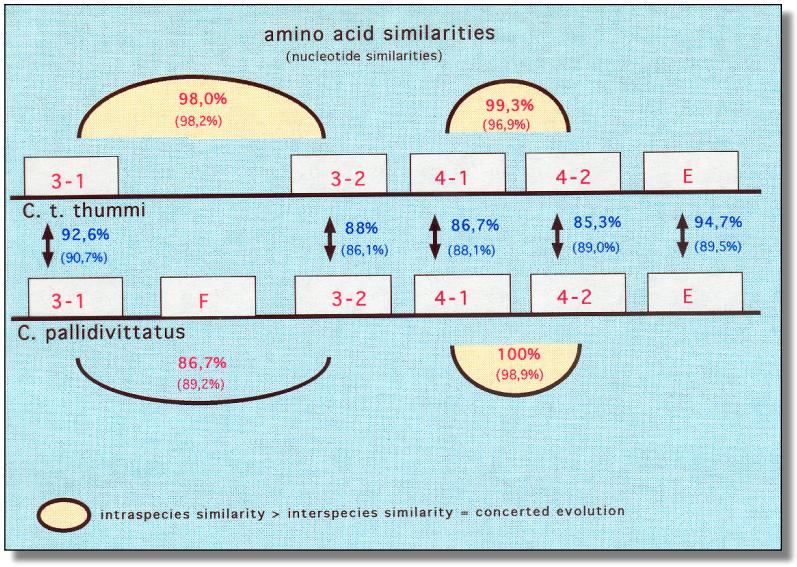
TyUra
TyNeo
pM48
2μ
LEU2
Induzierbarer Promoter > TyNeo mRNA
Select Ura−, Lose plasmid
TyNeo
FIG. 1. General strategy. pM48, a plasmid carrying a GalTy1 Neo, was introduced into strains with TyUra inserted in the *LYS2* locus. After growth in glucose or galactose, Ura$^+$ cells were selected for and after plasmid curing were checked for the presence of a Ty1Neo at *LYS2* in place of the TyUra. Open triangles, Ty LTRs; open square, *GAL* promoter; ▨, *URA3* sequences; ▨, *LYS2* sequences; ■, *neo* sequences; ▭, *LEU2* sequences.

TyUra
TyNeo
RPS1+intron
pM52
2μ
LEU2
Select Ura−, Lose plasmid
TyNeo w/o intron
or
TyNeo + intron
FIG. 3. Experiment that distinguishes conversion by plasmid from conversion by transcription products (RNA or cDNA). pM52 is identical to pM48, except for the insertion of an intron-containing fragment from *RPS1* ▨. Conversion events in which plasmid sequences acted as donors of information should retain the intron. If there is an RNA step before recombination, intronless TyNeos should be found among the Ura$^+$ colonies. Symbols are as in Fig. 1.

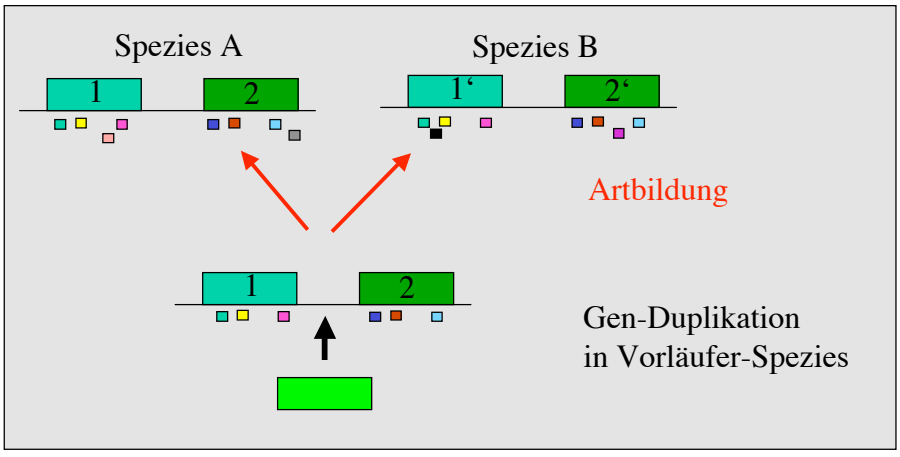
Intron weg, daher Konversion über cDNA

Keine Konversion über intron-haltige Plasmidsequenzen

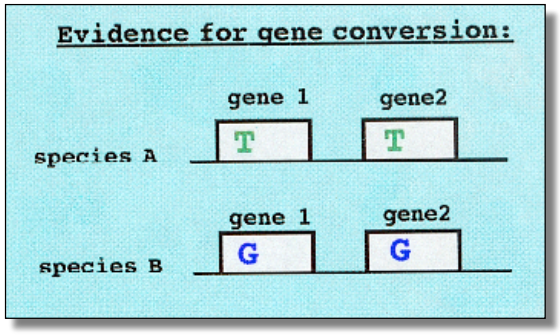
Genkonversion führt zur „konzertierten" Evolution von Genfamilien



Was wäre ohne Genkonversion zu erwarten?

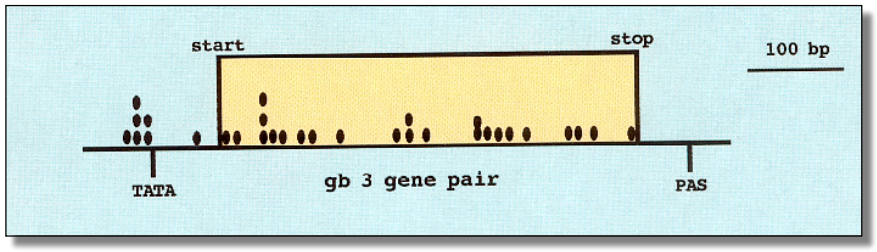


Wie erkennt man konvertierte Basenpositionen?



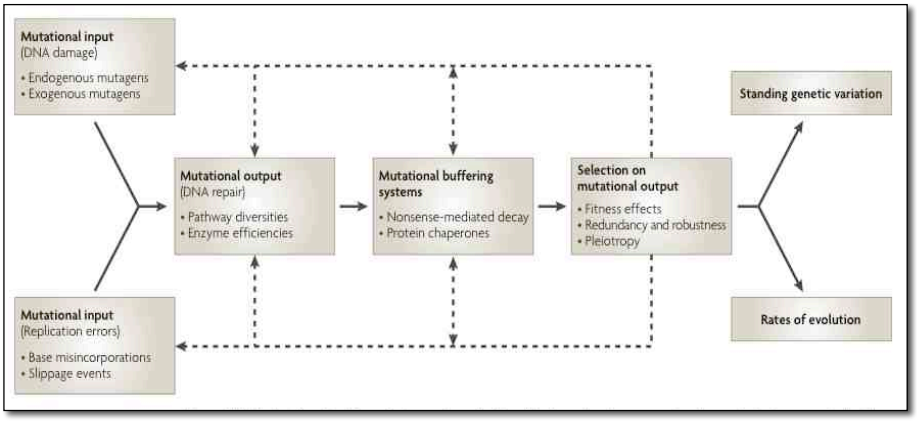
Innerartliche Übereinstimmung besser als zwischenartliche Übereinstimmung!!

Beispiel: Konvertierte Basenpositionen in Hämoglobingenen



Frage: Sind diese Konversionsereignisse via cDNA-Intermediat erfolgt?

Wie häufig sind Mutationen?



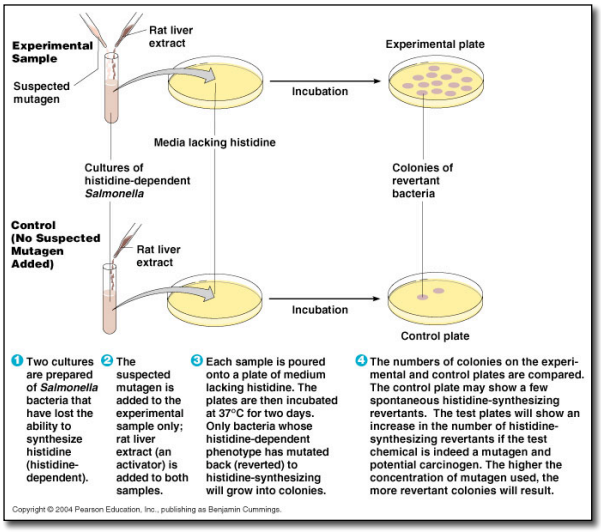
Nat Rev Genet (2007) 8:619

Wie häufig sind Mutationen?

- direkte Messung in höheren Organismen schwierig
- indirekte Abschätzung der Mutationsrate durch Analyse von funktionslosen Pseudogenen:

  ca. 10^{-9} Nukleotidsubstitutionen /Sequenzposition
- Substitutionen 10 x häufiger als Indels
- Mutationsrate in „hypervariablen“ Mikrosatelliten : z.B. 10^{-3}
- Mutationsrate in Säuger-mt-DNA 10fach höher als in Kerngenen
- Mutationsraten in Viren bis 10^{-2}!

Bestimmung der Mutationsrate (Ames-Test)



Rates of DNA Sequence Evolution in Experimental Populations of *Escherichia coli* During 20,000 Generations

Richard E. Lenski,[1] Cynthia L. Winkworth,[2] Margaret A. Riley[2] **JME (2003) 56:498**

- Mutationsratenbestimmung ohne zweifelhafte Zeitangaben!

- Teil-Sequenzierung (je 500 Bp) von 36 Genen in klonalen 12 E. coli-Populationen nach 20 000 Generationen (6 Gen/Tag!)

- 4 Populationen haben Defekte in DNA-Reparatursystemen entwickelt
  ➢„Mutator-Stämme“

- Hochrechnung aufs ganze Genom:
  > ca. 250 synonyme Substitutionen in Mutator-Stämmen
  > nur ca. 3 synonyme Substitutionen in „normalen“ Stämmen

  (Mutationsrate: 1,44 x 10^{-10} pro Bp pro Generation)

PNAS (2002) 99: 13950

Natural radioactivity and human mitochondrial DNA mutations

Lucy Forster*†‡, Peter Forster†§, Sabine Lutz-Bonengel¶, Horst Willkomm‖, and Bernd Brinkmann*

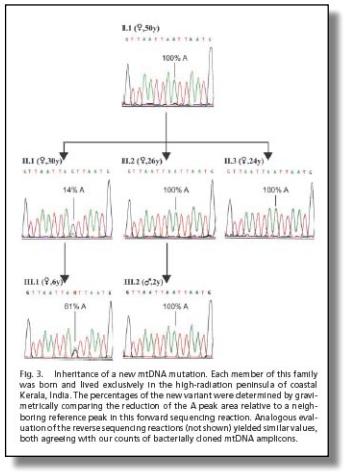


Fig. 3. Inheritance of a new mtDNA mutation. Each member of this family was born and lived exclusively in the high-radiation peninsula of coastal Kerala, India. The percentages of the new variant were determined by gravimetrically comparing the reduction of the A peak area relative to a neighboring reference peak in this forward sequencing reaction. Analogous evaluation of the reverse sequencing reactions (not shown) yielded similar values, both agreeing with our counts of bacterially cloned mtDNA amplicons.

• natürliche Radioaktivität in Kerala, Indien, 10x höher als Durchschnitt (ca 12 000 uSv/Jahr)
• aber keine Besonderheiten im Auftreten von genetischen Erkrankungen etc.

➢22 Mutationen in exponierter Gruppe vs. 1 Mutation in Kontroll-Gruppe

➢Mutationen an gleichen Stellen, die auch während der Evolution die häufigsten Veränderungen zeigen

ARTICLES

Human minisatellite mutation rate after the Chernobyl accident

Nature (1996) 380: 683

Yuri E. Dubrova*†, Valeri N. Nesterov‡, Nicolay G. Krouchinsky‡, Vladislav A. Ostapenko‡, Rita Neumann†, David L. Neil† & Alec J. Jeffreys†

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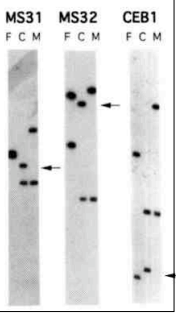
‡ Research Institute for Radiation Medicine, Mogilev Branch, Mogilev, 212004, Belarus

Germline mutation at human minisatellite loci has been studied among children born in heavily polluted areas of the Mogilev district of Belarus after the Chernobyl accident and in a control population. The frequency of mutation was found to be twice as high in the exposed families as in the control group. Mutation rate in the Mogilev families was correlated with the level of caesium-137 surface contamination, consistent with radiation induction of germline mutation.

THE accident on 26 April 1986 at reactor 4 of the Chernobyl nuclear power station resulted in the largest reported accidental release of radioactive material[1]. Many regions within the European part of the former Soviet Union were heavily contaminated by radioactive fallout. In the first three months after the accident, acute irradiation of humans occurred through external and internal exposure to iodine-131 with a half-life of 8 days[2]. Following ^{131}I decay, exposure to more stable isotopes, mainly ^{137}Cs, became the main source of radiation risk for people in contaminated regions.

DNA fingerprints were produced from all families by using multilocus minisatellite probe 33.15 (ref. 8) and two hypervariable single-locus minisatellite probes MS1 and MS31 (loci *D1S7*, *D7S21*)[9]. In addition, most families were DNA profiled with the minisatellite probes MS32 and CEB1 (loci *D1S8*, *D2S90*)[9,14]. These probes, chosen for their relatively high mutation rates[9,10,14], provided sufficient information to verify the parentage of all children analysed, even in the presence of mutation[10]. Mutants were identified as novel DNA fragments present in the



Mutationsrate

Curr. Biol. 15. Sept 2009

- Rate von Basensubstitutionen auf menschlichem Y-Chromosom in „Echtzeit“ bestimmt

- Familienstammbaum mit 13 Generationen!!
- Illumina-Sequenzierung fluoreszenzsortierter Y-DNA von zwei Männern (maximal zeitlich getrennt)

- **3×10^{-8} Mutationen / Nt / Generation**

Haben Basenaustausche eine bestimmte „Richtung“?

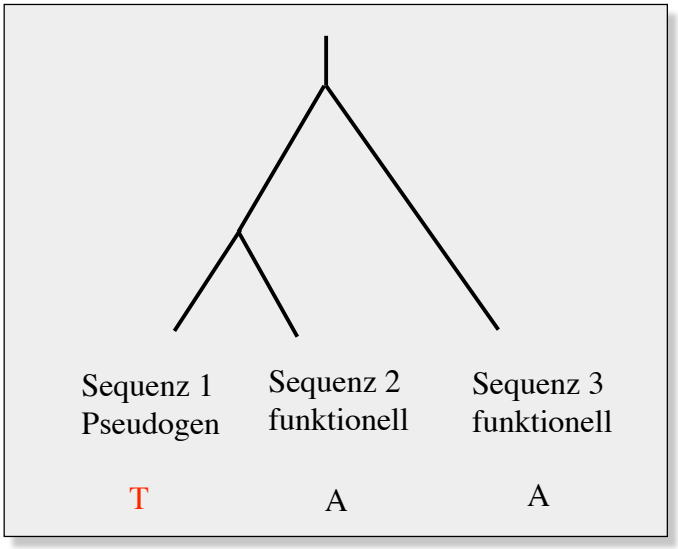


TABLE 1.5 Pattern of nucleotide substitution in pseudogenes[a]

From	To A	To T	To C	To G	Row totals
A	—	4.7±1.3	5.0±0.7	9.4±1.3	19.1
	—	(5.3±1.4)	(5.6±0.8)	(10.3±1.4)	(21.2)
T	4.4±1.1	—	8.2±1.3	3.3±1.2	15.9
	(4.8±1.1)	—	(9.2±1.3)	(3.6±1.3)	(17.6)
C	6.5±1.1	21.0±2.1	—	4.2±0.5	31.7
	(7.1±1.3)	(18.2±2.3)	—	(4.2±0.6)	(29.5)
G	20.7±2.2	7.2±1.1	5.3±1.0	—	33.2
	(18.6±1.9)	(7.7±1.3)	(5.5±1.3)	—	(31.8)
Column totals	31.6	32.9	18.5	16.9	
	(30.5)	(31.2)	(20.3)	(18.1)	

From Gojobori et al. (1982b) and Li et al. (1984).

[a]Table entries are the inferred percentages (f_{ij}) of base changes from i to j based on 13 mammalian pseudogene sequences. Values in parentheses were obtained by excluding all CG dinucleotides from comparison.

- Transitionen (59,3%) häufiger als erwartet (33%)
- G und C mutieren am häufigsten
- 64,5 aller Mutationen resultieren in A od. T (erwartet: 50%)

Mutationen sind zeitlich zufällig

Luria, Delbrück 1943

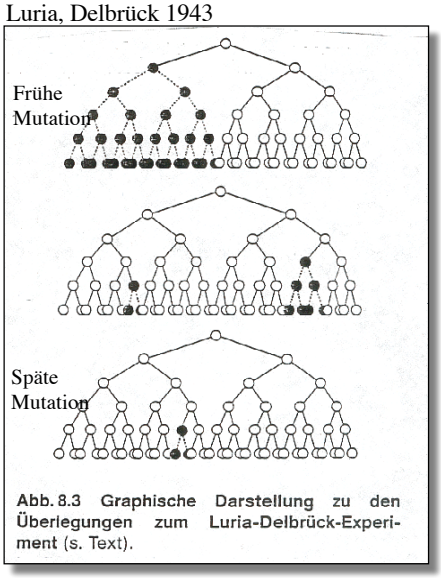


Abb. 8.3 Graphische Darstellung zu den Überlegungen zum Luria-Delbrück-Experiment (s. Text).

Eine Kultur Ab-sensitiver Bakterien aufgeteilt; zu verschiedenen Zeitpunkten auf Ab-haltigen Platten auf resistente Kolonien selektiert:

Viele Kulturen	wenige Kulturen	Sehr wenige Kulturen

„Fluktuation“ widerspricht der Erwartung einer Anpassungsreaktion, bei der (bei etwa gleicher Zahl ausgeplatteter Bakterien) ungefähr ähnliche Anzahlen Resistenter pro Platte zu erwarten wären

SPAM:
selection-promoted adaptive mutation

„It may seem a deplorable imperfection of nature that mutability is not restricted to changes that enhance the adeptness of their carriers.“ - Theodosius Dobzhansky 1970

- **Bakterien (und Eukaryoten?) besitzen Mechanismen zur Erhöhung der Mutationsrate unter Stressbedingungen!!**
- Punktmutationen, Frameshifts, Genamplifikation
- nicht zielgerichtet in dem unter Selektionsdruck stehenden Gen, daher kein Lamarck-Phänomen

Nature Genetics Reviews 2 (2001) S. 504ff

Wo in der Keimbahn von Tieren enstehen die meisten Mutationen?

Was sind die Mechanismen?

Haldane 1947:

„If mutation is due to faulty copying of genes at nuclear division, we might expect it to be commoner in males than in females“

Gibt es tatsächlich mehr Mutationen in der männlichen Keimbahn?

Wenn ja, wäre damit die Fehlerhaftigkeit der Replikation der vorherrschende Mutationsmechanismus!!!

Zellteilungen in der Keimbahn

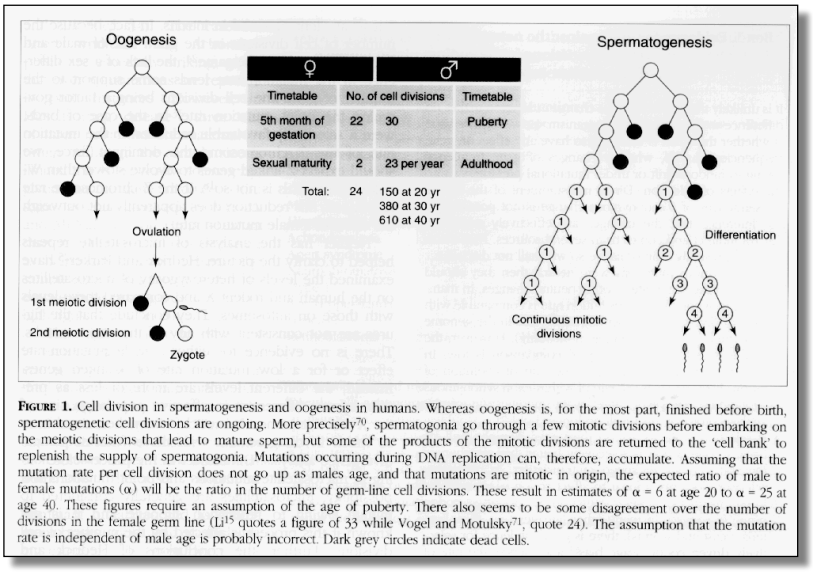


FIGURE 1. Cell division in spermatogenesis and oogenesis in humans. Whereas oogenesis is, for the most part, finished before birth, spermatogenetic cell divisions are ongoing. More precisely[70], spermatogonia go through a few mitotic divisions before embarking on the meiotic divisions that lead to mature sperm, but some of the products of the mitotic divisions are returned to the 'cell bank' to replenish the supply of spermatogonia. Mutations occurring during DNA replication can, therefore, accumulate. Assuming that the mutation rate per cell division does not go up as males age, and that mutations are mitotic in origin, the expected ratio of male to female mutations (α) will be the ratio in the number of germ-line cell divisions. These result in estimates of $\alpha = 6$ at age 20 to $\alpha = 25$ at age 40. These figures require an assumption of the age of puberty. There also seems to be some disagreement over the number of divisions in the female germ line (Li[15] quotes a figure of 33 while Vogel and Motulsky[71], quote 24). The assumption that the mutation rate is independent of male age is probably incorrect. Dark grey circles indicate dead cells.

Zellteilungen in der Säuger-Keimbahn

Teilungen Oogenese/Spermatogenese			
Mensch	ca. 30 /	ca. 200	(6x)
Maus	27 /	57	(2x)
Ratte	29 /	58	

Mutationsrate in der männlichen vs. der weiblichen Keimbahn

M/F ratio $\alpha = u_m / u_f$ u ist Mutationsrate

- autosom. Sequenz stammt von M u. W mit gleicher Wahrscheinl.keit
  Autosomale Mutationsrate A = $(u_m + u_f) / 2$
- X-chromos. Sequenz ist 2/3 der Zeit in W, 1/3 der Zeit in M:
  X-chrom. Mutationsrate X = $(u_m + 2u_f) / 3$
- Y-chromos. Mutationsrate $Y = u_m$

Es ergibt sich $\mathbf{Y / X = 3\alpha / (2 + \alpha)}$

(d. h., durch Vergleich Y- und X-chromosomaler, homologer Sequenzen kann man den Wert für α bestimmen)

In der Tat...

Male-driven evolution of DNA sequences

Lawrence C. Shimmin, Benny Hung-Junn Chang & Wen-Hsiung Li*

Center for Demographic and Population Genetics, University of Texas, PO Box 20334, Houston, Texas 77225, USA

TABLE 1 Number of nucleotide substitutions per 100 sites between intron sequences

Sequences	X intron				Y intron		
	Human	Orang	Baboon	Sq. monkey	Human	Orang	Baboon
Orang X	2.1±0.6						
Baboon X	3.0±0.7	3.5±0.8					
Sq. monkey X	7.6±1.2	7.3±1.1	7.7±1.2				
Human Y	30.6±2.8	31.1±2.8	31.1±2.8	32.2±2.9			
Orang Y	31.6±2.9	32.6±2.9	32.1±2.9	33.3±3.0	4.0±0.8		
Baboon Y	30.2±2.8	31.2±2.9	30.7±2.8	31.6±2.9	6.3±1.1	5.9±1.0	
Sq. monkey Y	32.4±2.9	34.0±3.0	33.2±2.9	32.8±2.9	18.4±2.0	17.8±1.9	17.9±2.0

The mean and standard error are estimated according to ref. 12, a method that takes into account unequal frequencies of the four nucleotides. Gaps are not included in the comparison.

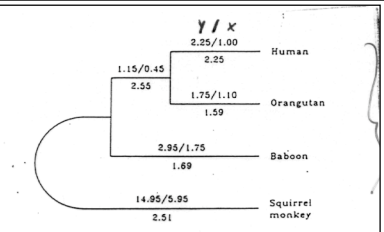


FIG. 2 The numbers above each branch are branch lengths estimated from the Y-linked and the X-linked intron sequences (Table 1), respectively, and the number below each branch is the ratio of the preceding two numbers. The least-squares method was used for the estimation.

Vergleich ZFX / ZFY

Y / X im Menschen ca. 2,25

Dies ergibt $\alpha = 6$!!!

TABLE 1. Estimates of the ratio (α) of the number of point mutations of paternal origin to those of maternal origin leading to dominant autosomal disorders of humans

Disease	Gene	Mutation type	α	No. of mutations	Refs
Multiple endocrine neoplasia type 2B (MEN 2B)[a]	*RET*	Point	∞	25	34
MEN 2A	*RET*	Point	∞	10	33
Hirschsprung disease	*RET*	Point	0	3	35
Achondroplasia	*FGFR3*	Point[b]	∞	53	31, 32
Apert syndrome	*FGFR2*	Point	∞	57	30
Neurofibromatosis type 1	*NF1*	Not large deletions[c]	4.5[d]	11	55
von Hippel–Lindau disease	*VHL*	Point	1.3	7	36
Retinoblastoma	*RB1*	Not large deletions[c]	8.5	38	72

[a]Maternally derived mutations have now been described[73].
[b]Nearly all achondroplasia mutations are point mutations involving a G→A transition at the same residue[74].
[c]Probably point mutations and/or small deletions.
[d]Other reports show a higher male bias [α = ∞, N = 10 (Ref. 37); α = 6, N = 14 (Ref. 75)] but the sort of mutations are unknown although they are probably either point mutations or small deletions.
[e]Probably point mutations and/or small deletions but more likely to be predominantly the latter (e.g. see Ref. 76).

Viele genetische Erkrankungen haben ihren Ursprung in der männlichen Keimbahn!

Mutationen und der genetische Code

- der genetische Code ist „degeneriert“, aber präzise

TABLE 1.2 The universal genetic code

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

Synonyme Codons

Abweichungen vom „universellen“ genetischen Code

Table 1.4 Some other genetic codes that differ from the standard code.

	Codons						
Organelle/Organisms	UGA	AUA	AAA	AGR	CUN	CGG	UAR
Standard genetic code	Ter	Ile	Lys	Arg	Leu	Arg	Ter
Mitochondrial code							
Vertebrate	Trp	Met	•	Ter	•	•	•
Ascidian	Trp	Met	•	Gly	•	•	•
Echinoderm	Trp	•	Asn	Ser	•	•	•
Drosophila	Trp	Met	•	Ser	•	•	•
Yeast	Trp	Met	•	•	Thr	•	•
Protozoan	Trp	•	•	•	•	•	•
Mold	Trp	•	•	•	•	•	•
Coelenterate	Trp	•	•	•	•	•	•
Nuclear code							
Tetrahymena	•	•	•	•	•	•	Gln
Mycoplasma	Trp	•	•	•	•	•	•
Euplotid	Cys	•	•	•	•	•	•

Note: • Indicates identity with the standard code. R = A or G and N = T, C, A, or G.

Abweichungen vom „universellen" genetischen Code

• alternative Initiations-Codons:

- GUG>UUG> AUU in Eubakterien
- AUA>GUG>UUG>AUC>AAG in Hefe

• „unassigned" codons, z.B. „CCG" in Mycoplasma capriolium
> Translation hält an, aber Protein bleibt am Ribosom

Ein nicht-universeller Code entsteht...

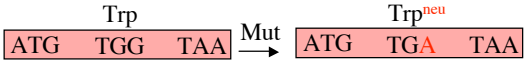
Mycoplasma capriolum benutzt UGG und UGA für Trp

• 75% AT, d.h. Mutationsdruck GC>AT

alle TGA-Stopcodons mutieren zu
TAA > TGA wird nicht-benutztes Codon

tRNA-Trp^{CCA}

...paart mit Trp-Codon UGG
wegen G-U-Wobble-Paarung



einige TGG-Trp-Codons in Genen
mutieren zu TGA
> TGA wird Trp-Codon

Mutation mag in der Evolution des Codes einen größeren Effekt haben als die Selektion für Veränderung!

Die Selektion gegen Veränderung dagegen ist stark und hat zu einer sehr weitgehenden Konservierung des universellen Codes geführt.

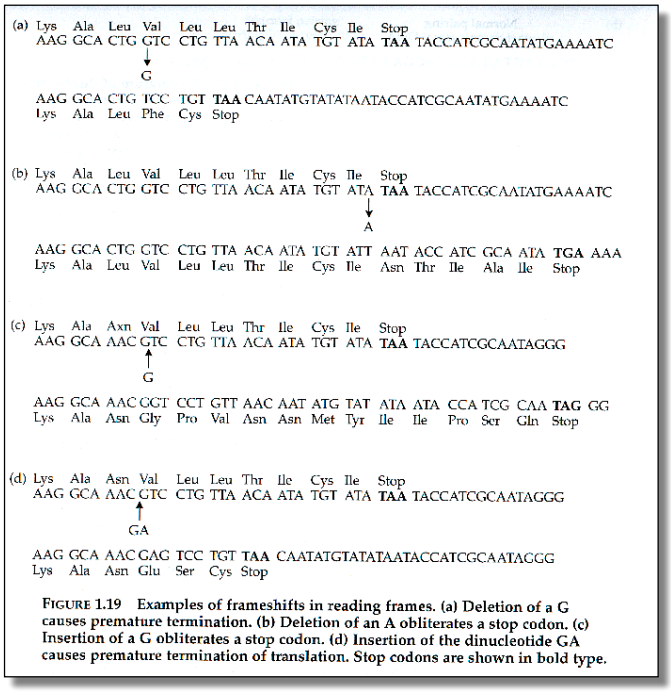


FIGURE 1.19 Examples of frameshifts in reading frames. (a) Deletion of a G causes premature termination. (b) Deletion of an A obliterates a stop codon. (c) Insertion of a G obliterates a stop codon. (d) Insertion of the dinucleotide GA causes premature termination of translation. Stop codons are shown in bold type.

Nt-Indels können das Leseraster verändern

Evolution von Indels in proteinkodierenden Genen

- 4x mehr Aminosäure-Deletionen als Insertionen in Genen unter Selektion beobachtet
- ausgeglichenes Ins:Del-Verhältnis in Pseudogenen und nicht-kod. Bereichen

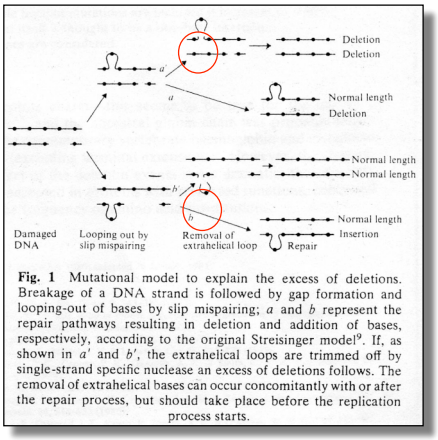


Fig. 1 Mutational model to explain the excess of deletions. Breakage of a DNA strand is followed by gap formation and looping-out of bases by slip mispairing; *a* and *b* represent the repair pathways resulting in deletion and addition of bases, respectively, according to the original Streisinger model[9]. If, as shown in *a'* and *b'*, the extrahelical loops are trimmed off by single-strand specific nuclease an excess of deletions follows. The removal of extrahelical bases can occur concomitantly with or after the repair process, but should take place before the replication process starts.

<u>Modell:</u>

Transiente extrahelikale Einzelstränge werden präferentiell durch Nukleasen vor einem Replikationsschritt abgebaut

Basensubstitutionen in Genen

- synonym / „silent“
- nicht-synonym / „replacement“

> missense
> nonsense

TABLE 1.5 Relative frequencies of different types of mutational substitutions in a random protein-coding sequence

Substitution	Number	Percent
Total in all codons	549	100
Synonymous	134	25
Nonsynonymous	415	75
Missense	392	71
Nonsense	23	4
Total in first codons	183	100
Synonymous	8	4
Nonsynonymous	175	96
Missense	166	91
Nonsense	9	5
Total in second codons	183	100
Synonymous	0	0
Nonsynonymous	183	100
Missense	176	96
Nonsense	7	4
Total in third codons	183	100
Synonymous	126	69
Nonsynonymous	57	31
Missense	50	27
Nonsense	7	4

64 Codons
x 9 Mutationen
= 549 mögliche
Substitutionen

Synonyme Mutationen sind nicht unbedingt „silent"!

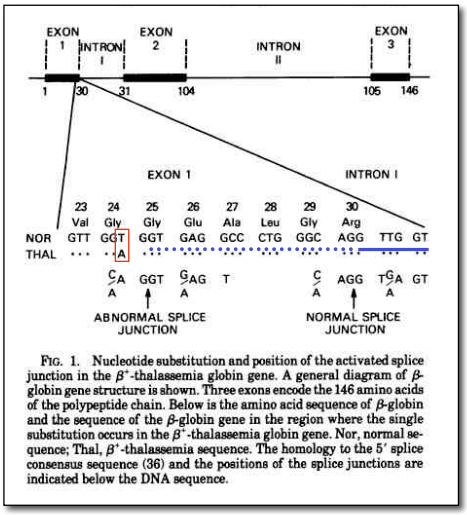


FIG. 1. Nucleotide substitution and position of the activated splice junction in the β^+-thalassemia globin gene. A general diagram of β-globin gene structure is shown. Three exons encode the 146 amino acids of the polypeptide chain. Below is the amino acid sequence of β-globin and the sequence of the β-globin gene in the region where the single substitution occurs in the β^+-thalassemia globin gene. Nor, normal sequence; Thal, β^+-thalassemia sequence. The homology to the 5′ splice consensus sequence (36) and the positions of the splice junctions are indicated below the DNA sequence.

- „stille Mutationen" in Exons und auch Intronmutationen können das Spleißen verändern!

Nature Genetics Reviews
3 (2002), S. 285ff