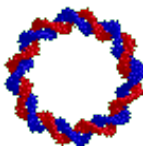
Informationsgehalt von DNA



Welche Themen werden behandelt?

- **Das Genom**

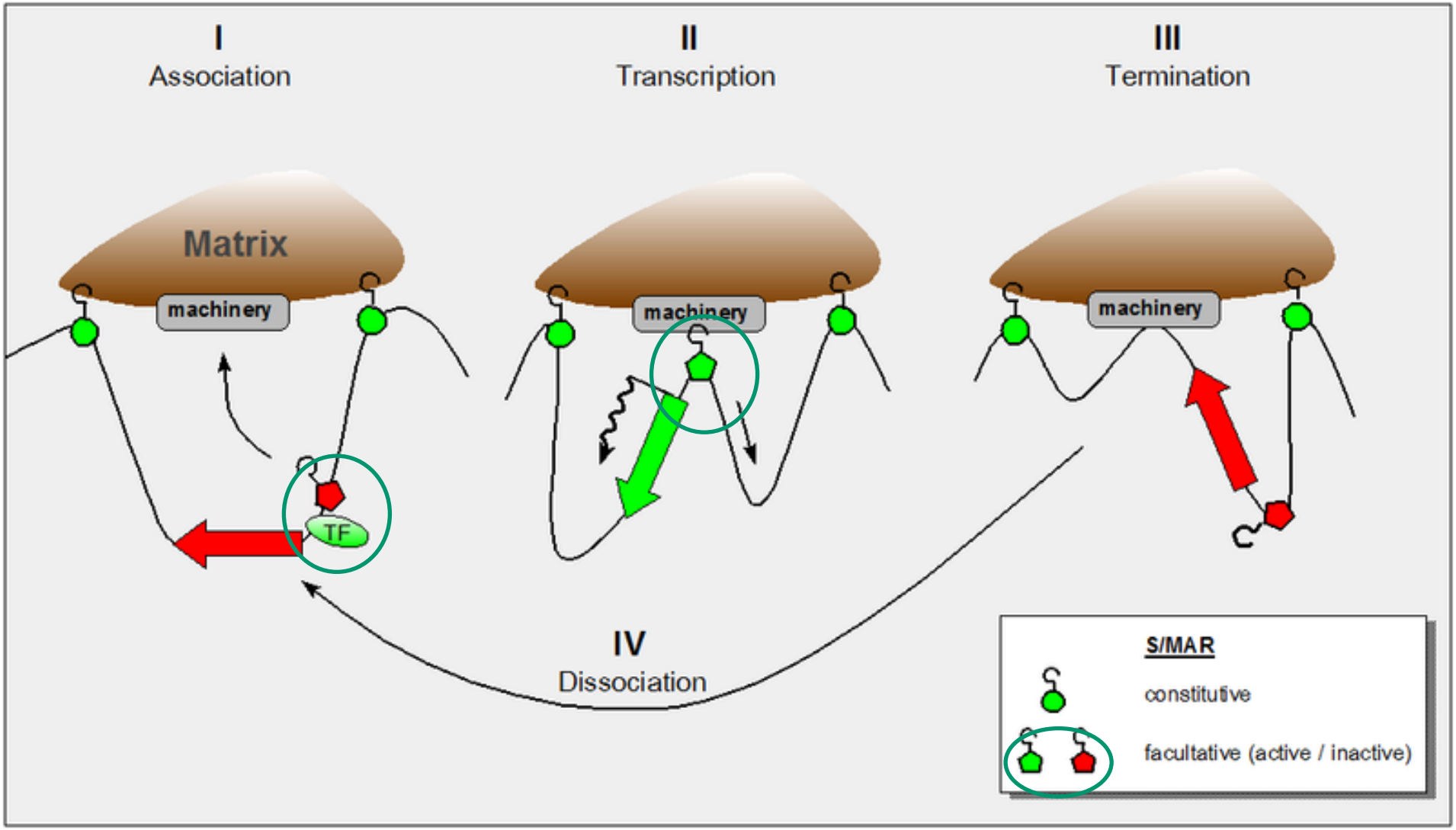
– Chromosomen
  – Organisation
  – Zentromere
  – Telomere – Altern
  – ein aktuelles Thema
  – Revue

Welche Themen werden behandelt?

- **Das Genom**

– Chromosomen
  – Organisation
    – Frage zur Konservierung der MAR
    – Färbung genreicher Regionen

S/MAR attachment regions



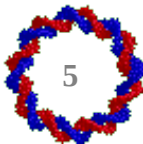
Konservierung von S/MARs

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

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

Galina V. Glazko[1], Eugene V. Koonin[2], Igor B. Rogozin[2] and Svetlana A. Shabalin

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



S/MAR attachment regions

Mensch – Maus: homologe intergenische Abschnitte

HIT „homologous intergenic tracts“ sind durchschnittlich zu 70% konserviert

Fig. 2. Local similarities (HITs) in experimentally identified human matrix–scaffold attachment regions (MARs) and orthologous mouse regions for the CSP-B gene (M62717) flanking sequence. Alignments were constructed using the OWEN program [25].

Glatzko al. (2003) TIGS 19(3): 119 .

S/MAR attachment regions

enthalten verschiedene (unspezifische) Sequenzmotive

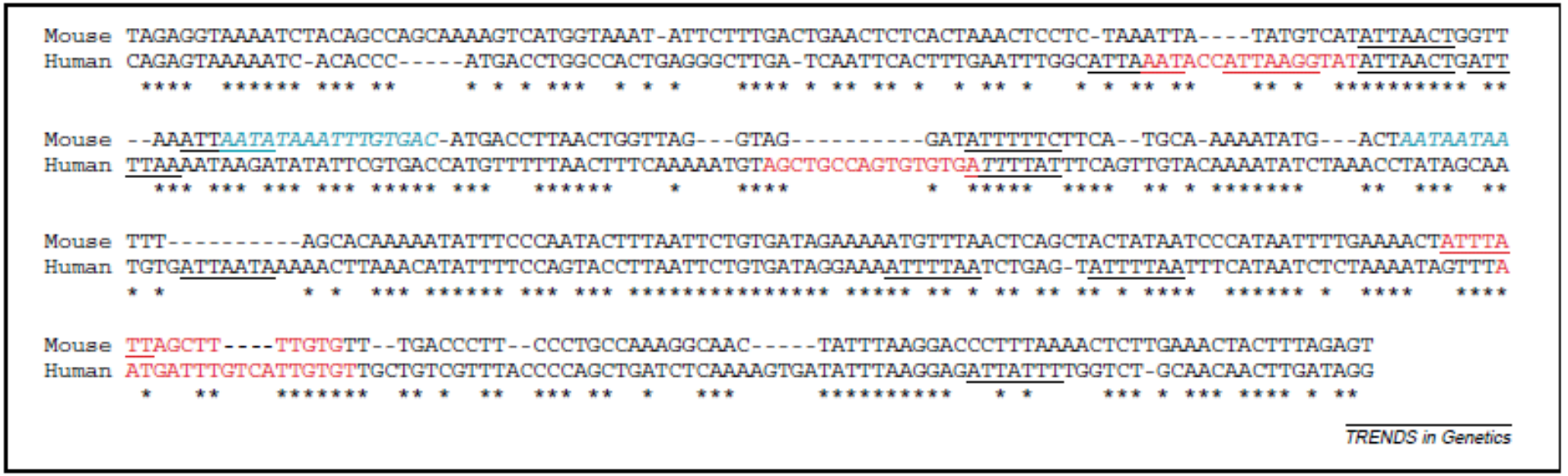


Fig. 1. (a) Alignment of the mouse Igκ matrix attachment region (MAR) and the orthologous region in the human JC κ intron (X67858). Potential replication origins (ORI; Box 1) are underlined; red, topoisomerase II sites; green, MAR recognition signatures (MRSs).

ORI (potentiell) – unterstrichen
Topo II – rot
MAR-Signatur – grün

Glatzko al. (2003) TIGS 19(3): 119 .

S/MAR attachment regions

Box 1. Characteristics of matrix–scaffold attachment regions (MARs)

Sequence motifs (rules) used to classify a DNA sequence as a MAR were deduced by analysis of experimentally characterized MARs. MARs can contain potential replication origins (ORI) and homeotic protein-binding sites, which share the ATTA, ATTTA, ATTTTA motifs; topoisomerase I, II binding and cleavage sites (Topo I, II); AT-rich stretches of 4–20 nucleotides; binding sites for transcription factors; intrinsically curved DNA produced by the $(A)_n$, T_mA_n and some other motifs; kinked DNA generated by the presence of TG, CA, or TA dinucleotides separated from each other by 2–4 or 9–12 nucleotides; polypurine and polypyrimidine stretches, which are known to form triple-helical or H-DNA structures; MAR recognition signatures (MRS), which are two nucleotide motifs located <200 bp apart; sites of stress-induced DNA duplex destabilization [4,5,8,11]. In general, MARs contain ~70% AT. MARs are enriched with all motifs listed above, but each individual MAR does not necessary contain each motif.

Glatzko al. (2003) TIGS 19(3): 119 -.

...

Chromosomen-Zahlen

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

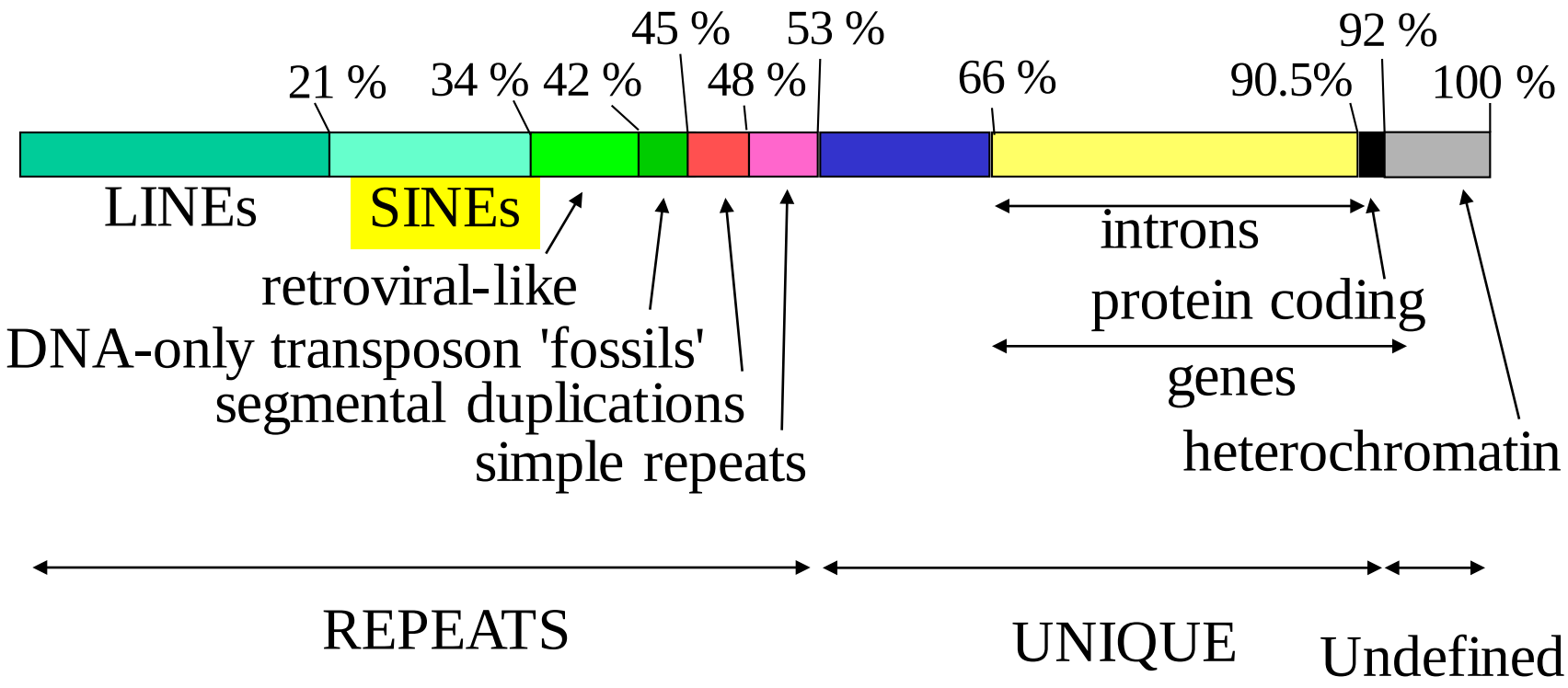
Gen-reiche Chromosomen sind auch reich an Alu-Sequenzen

Repetitive Elemente

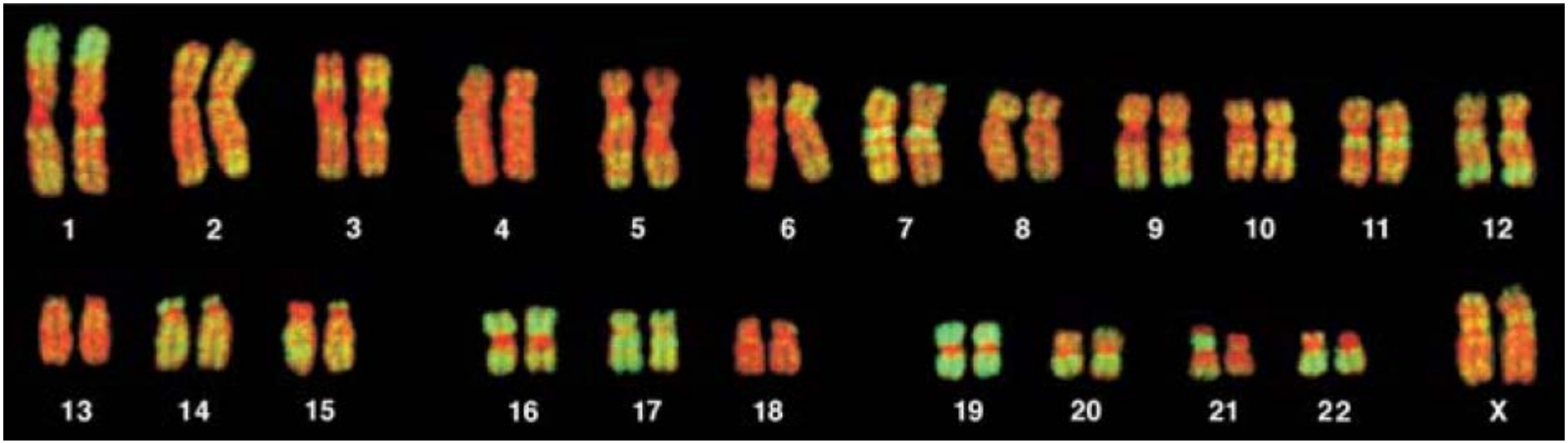
- Alu Elemente:
  - verstreute Repeats (dispersed repeats)
  - charakteristische Schnittstelle AGCT (***Arthrobacter luteus***)
  - G+C reich
  - Auftreten von Alu Elementen deckt sich mit Haushaltsgenen

Gen-reiche Chromosomen sind auch reich an Alu-Sequenzen

„Müll“ im Genom des Menschen



Alu – Sequenzen in humanen Chr



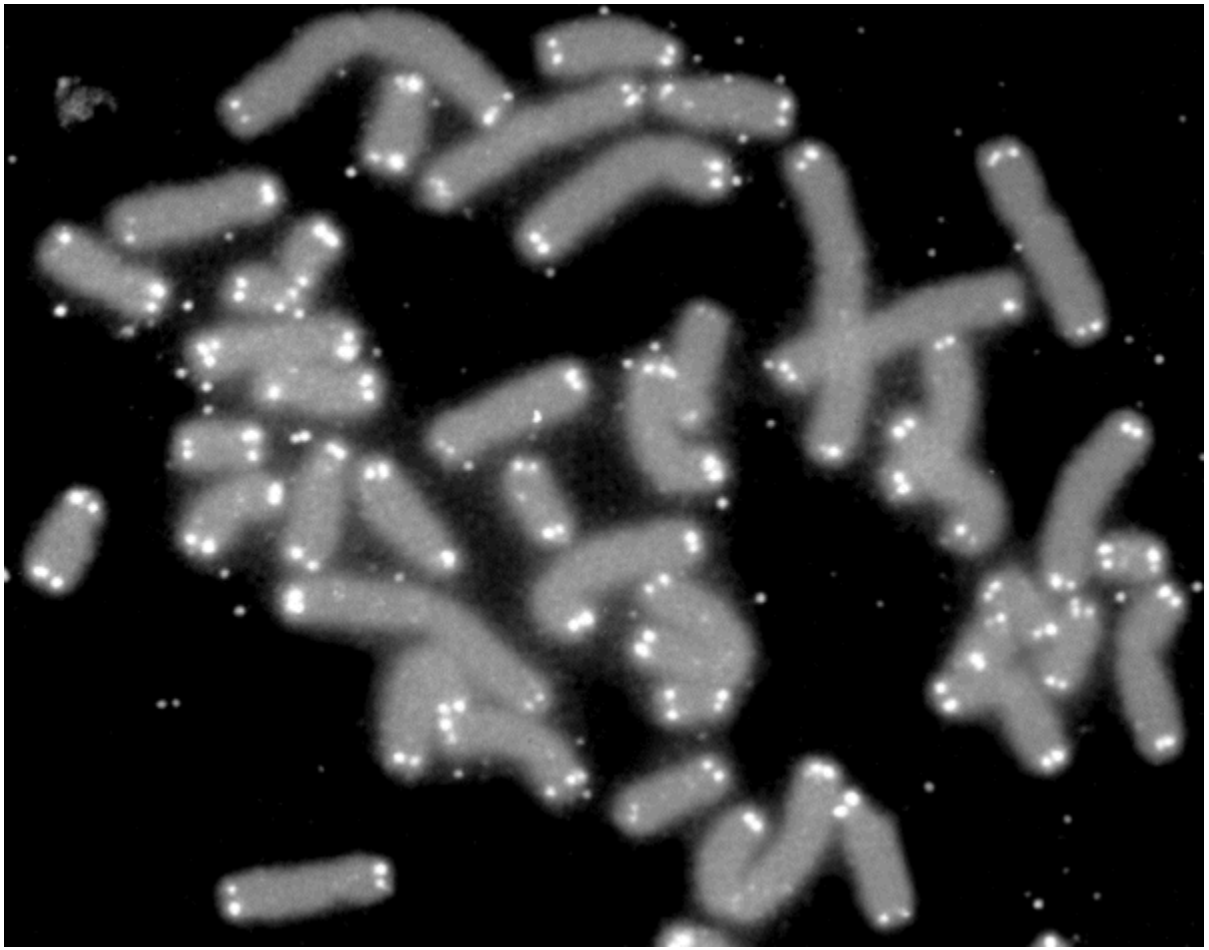
Karyotyp eines humanen Lymphozyten

Probe: Alu-Sequenzen (grün)
DNA: (rot)

Bolzer et al. (2005) Biol 3(5): e157.

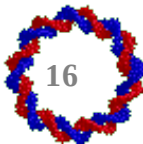
Telomere

Telomerlokalisation



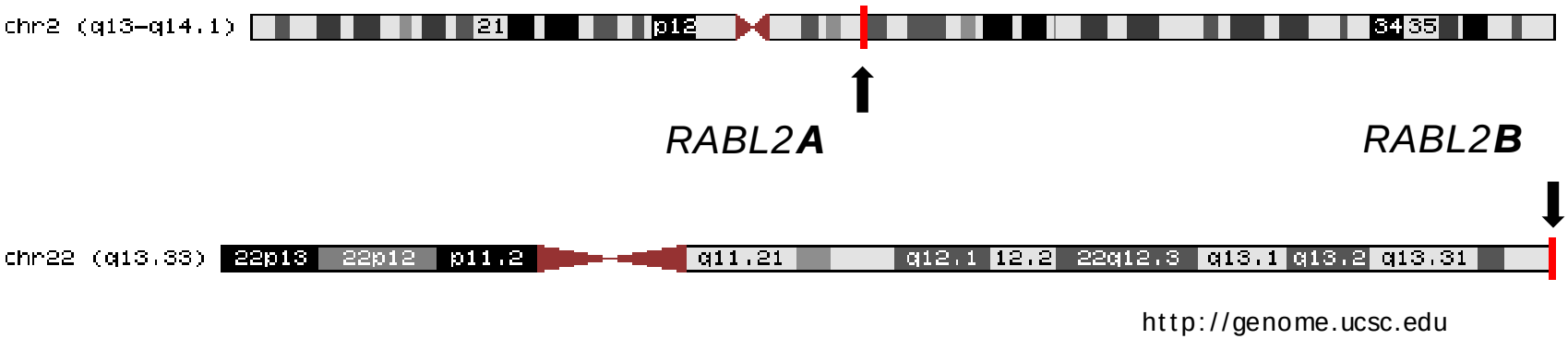
Telomerfunktion

- Mechanismus zur Unterscheidung von Enden von DNA-Strang-Brüchen
- Schutz der Chromosomen-Enden vor Abbau
- Anordnung und Segregation der Chr während der Meiose
- Modifizierung der Genexpression benachbarter Gene **(reversibel)**
- **Gewährleistung der Integrität des Genoms**



Telomerfunktion

- Modifizierung der Genexpression benachbarter Gene **(reversibel)**



- hohe Sequenzähnlichkeit (zwei Aminosaüreaustausche)
- möglicherweise ist die Lage im Chromosom entscheidend

Monaghan and Haussmann (200) TIEE 21(1): 47

Telomerstruktur

Bakterien

- kovalent verbundene Einzelstränge

Eukaryonten

- kurze wiederholte Sequenzeinheiten
- Transposons (*Drosophila*)

Telomermotive

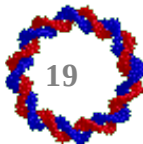
Motive: Mensch T_2AG_3 Hefe $C_{1-3}A/TG_{1-3}$

Telomer

TTAGGGTTAGGGTTAGGG-OH
AATCCC

- kurze, G-reiche, nicht kodierende Sequenzmotive
- doppelsträngig
- am Ende (3‘-OH) Überhang von G (Einzelstrang)
- Länge des Einzelstranges ist variabel in verschiedenen Spezies:

  Mensch: 15,000 Basenpaare
  Maus: 40,000 Basenpaare



Telomermotive

Motive: Mensch T_2AG_3 Hefe $C_{1-3}A/TG_{1-3}$

Telomer

TTAGGGTTAGGGTTAGGG-OH
AATCCC

- bei jeder Zellteilung verkürzt (wenn nicht erhalten)
- Funktionsverlust

  Schwellenwert für Länge unterschritten
  Schädigung der DNA
  Schädigung der assoziierten Proteine
- kaputte Telomere werden als DNA-Doppelstrangbrüche erkannt und Reparaturmaschinerie der Zelle setzt ein: Apoptose (Zelltod) oder replikative Seneszenz (keine Teilung)

Telomermotive

Motive: Mensch T_2AG_3 Hefe $C_{1-3}A/TG_{1-3}$

Telomer

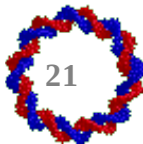
```
TTAGGGTTAGGGTTAGGG-OH
AATCCC
```

- unklar:

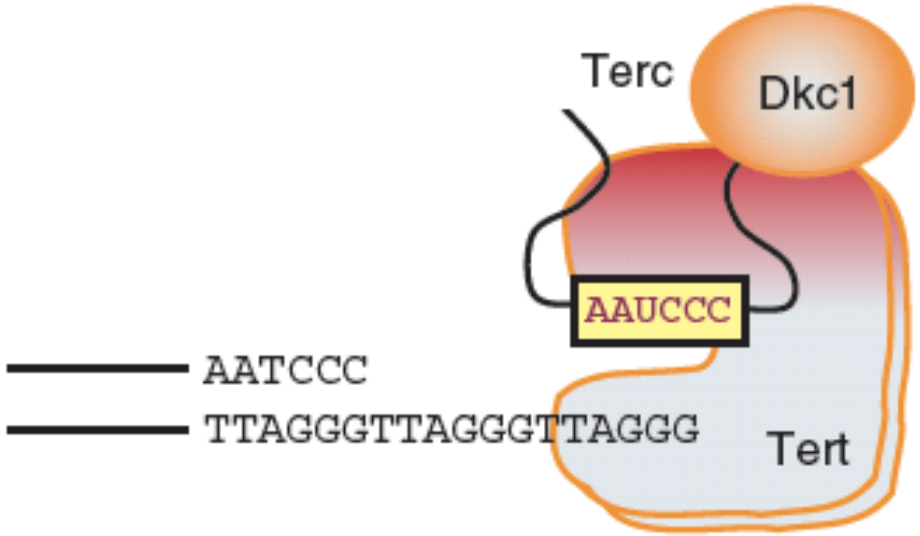
  Sind Telomere an bestimmten Chromosomen wichtiger?

  Was ist der Schwellenwert für die Länge der Telomere -

(offenbar werden Chromosomen-Gruppen erfasst)?



Telomerorganisation im Menschen



Telomerase:

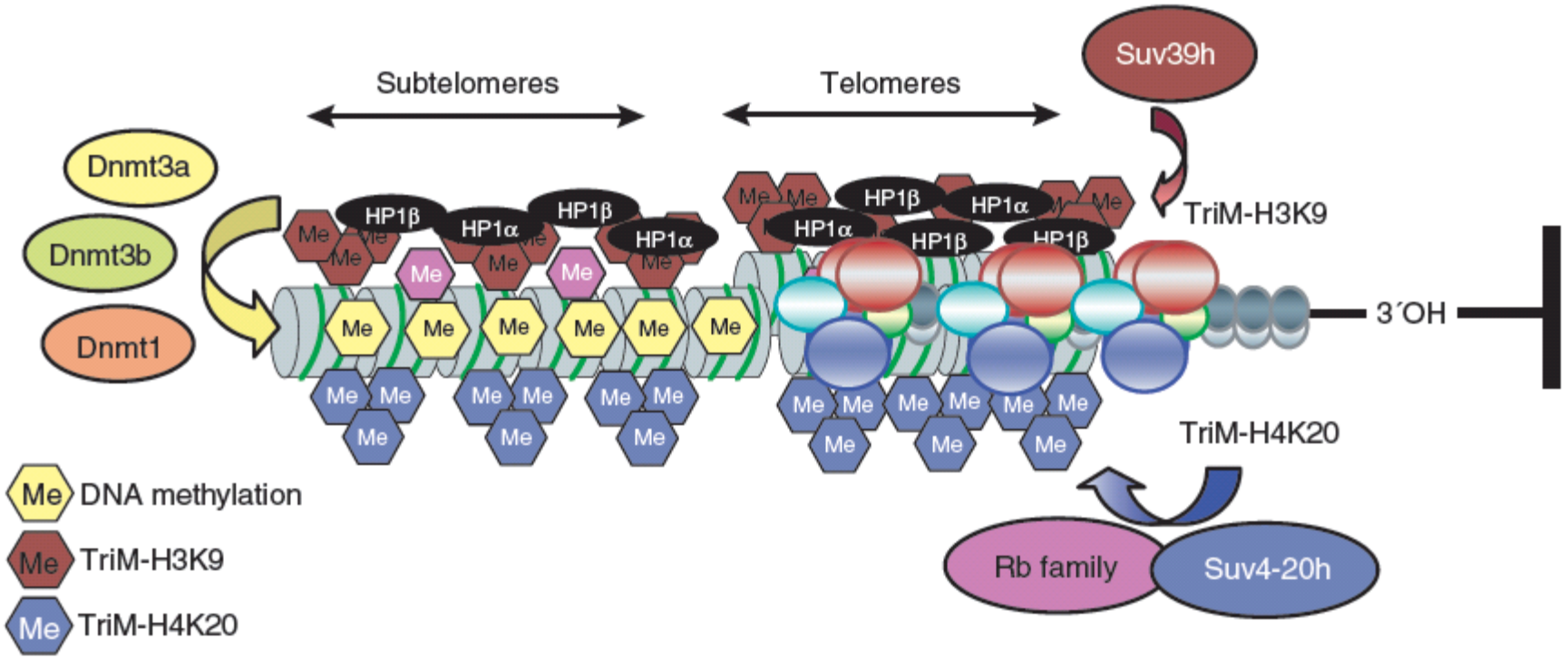
Tert = telomerase assoziierte Reverse Transkriptase

Terc = telomerase assoziierte RNA

Dkc = Dyskerin-Protein zur Stabilisierung des Komplexes

Blasco (2007) Nat Chem Biol 10(3): 640

Telomerorganisation im Menschen

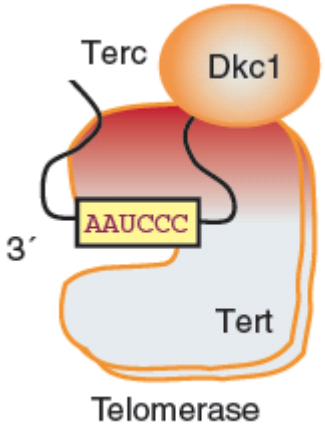


Telomere beinhalten Nucleosomen, die man sonst im Heterochromatin findet.

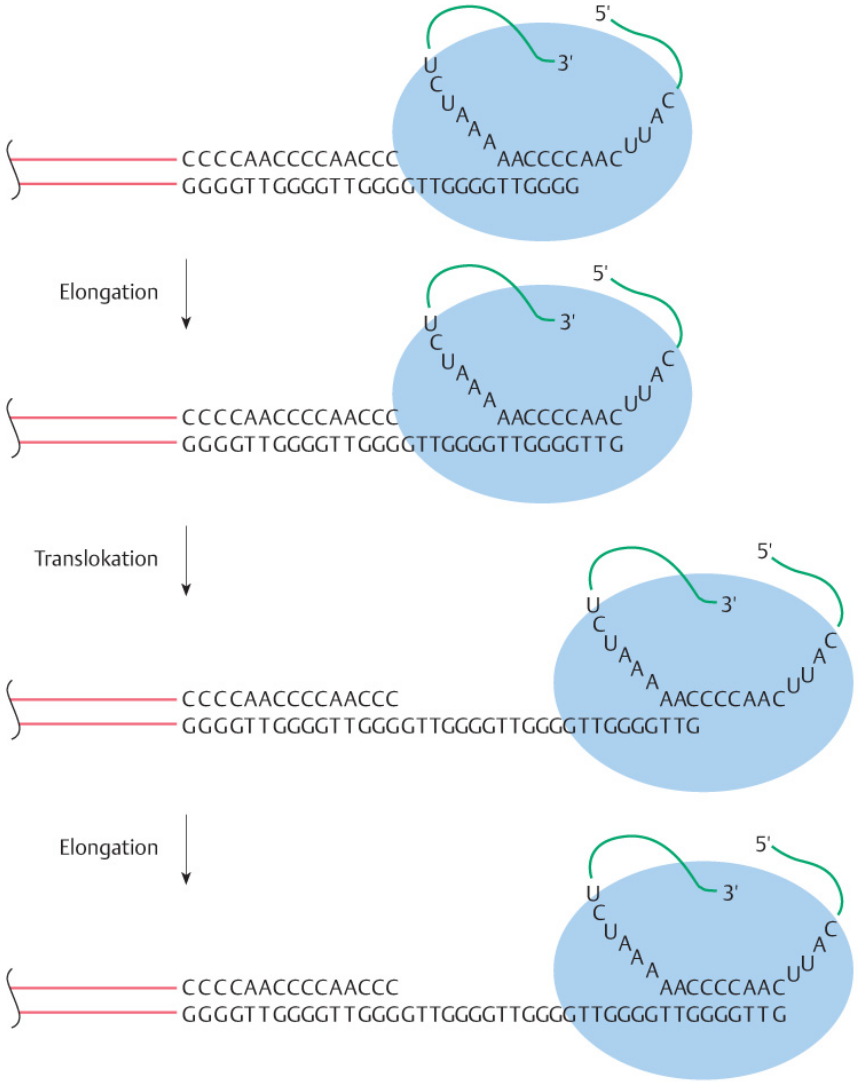
Subtelomere Bereiche sind stark methyliert.

Blasco (2007) Nat Chem Biol 10(3): 640

Telomerorganisation



blau = Tert
grün = Terc

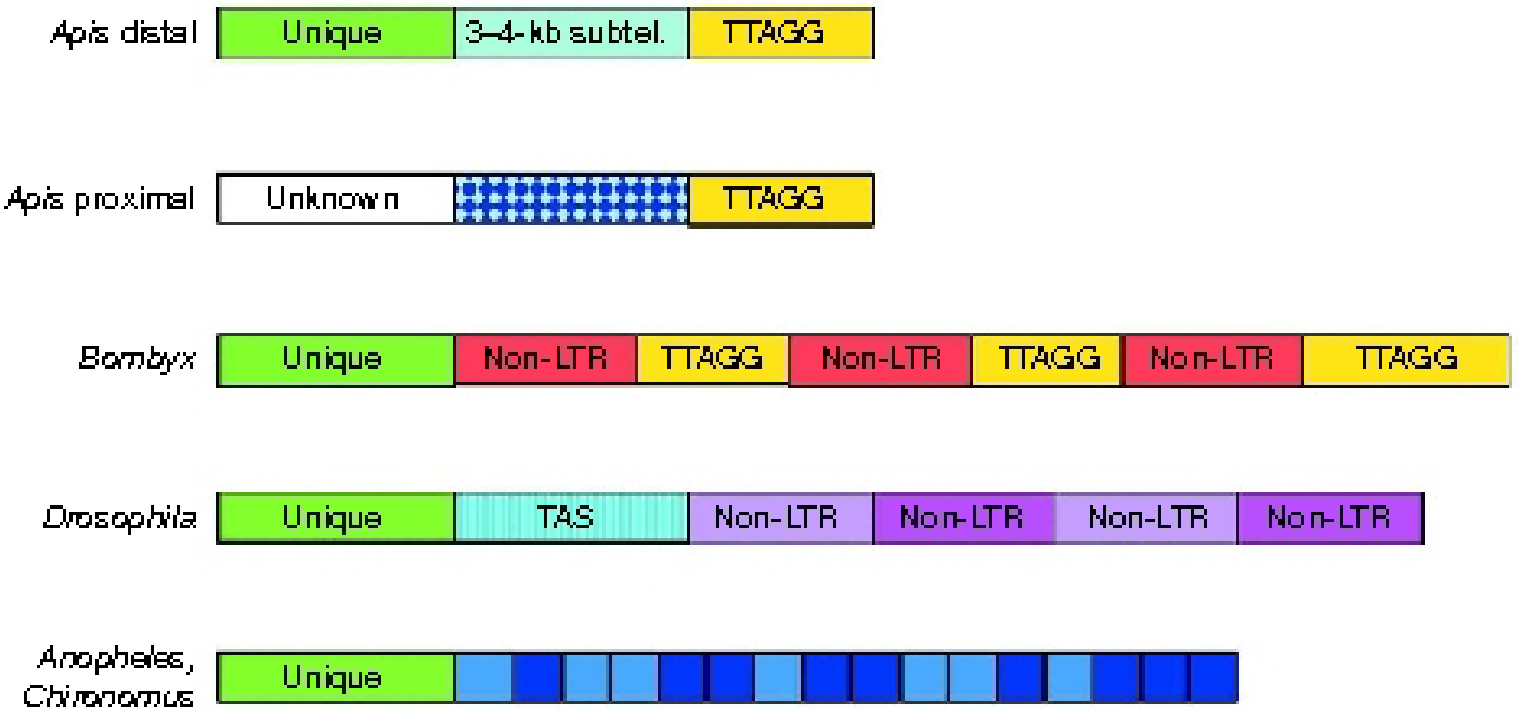


Telomere und Altern

- Telomere verkürzen sich konstant bei jeder Zellteilung
- „mitotische Uhr“ – Aussage über Alter der Zelle
- Länge der Telomere gibt Auskunft zu „Alter“ einer Spezies
- trifft teilweise zu – aber es gilt nicht: kurz = kurz

Telomermotive

Insekten



komplexe Tandem-Wiederholungen

Nature 443, 931-948

END

Aktuelles Thema

Nothobranchius species – natural habitat

… is in seasonal ponds in Africa

N. guentheri

N. rachovii

N. furzeri

Precipitation in natural habitat

...is highly variable

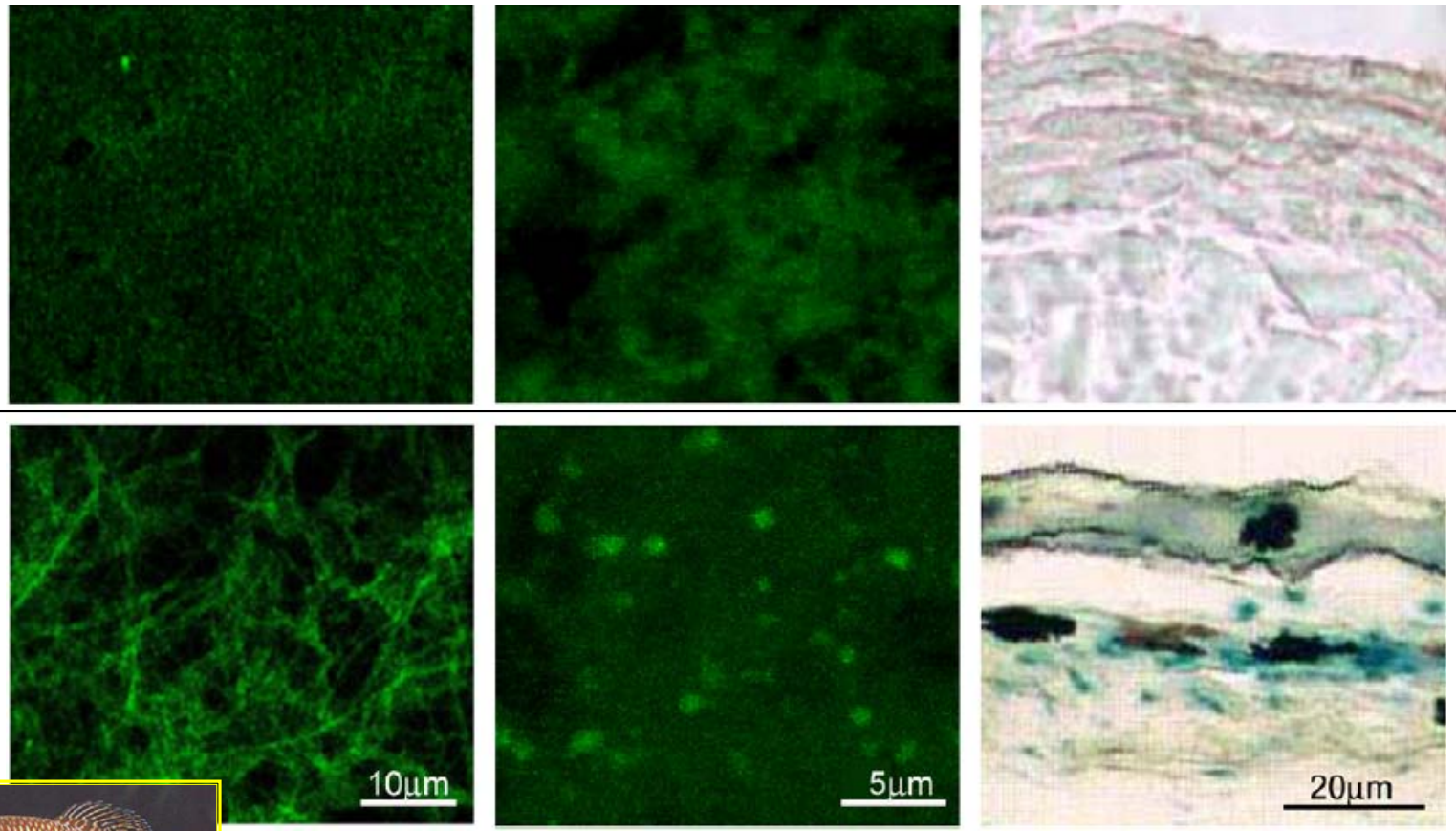
Watters, AKA 42(2): 37 (2009)

Aging related biomarkers

Fluoro-Jade B, Lipofuscin, SA-ß Galactosidase

brain **liver** **skin**

neuronal degeneration | accumulation of aging pigment | cellular senescence



5 weeks

9 weeks

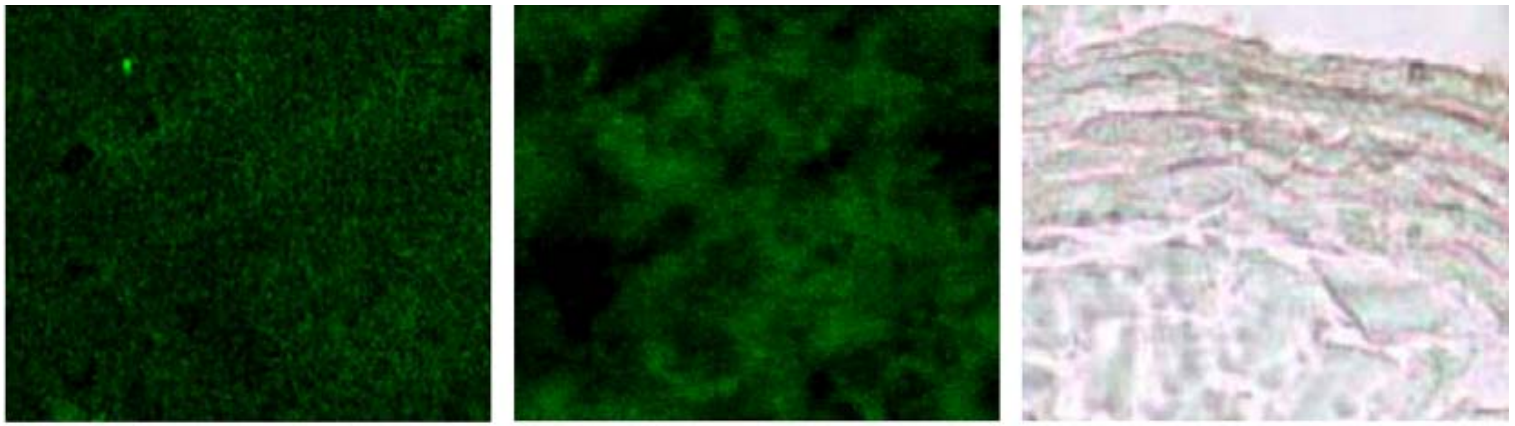
Terzibasi et al., Exp Gerontol 42:81 (2007)

Aging related biomarkers

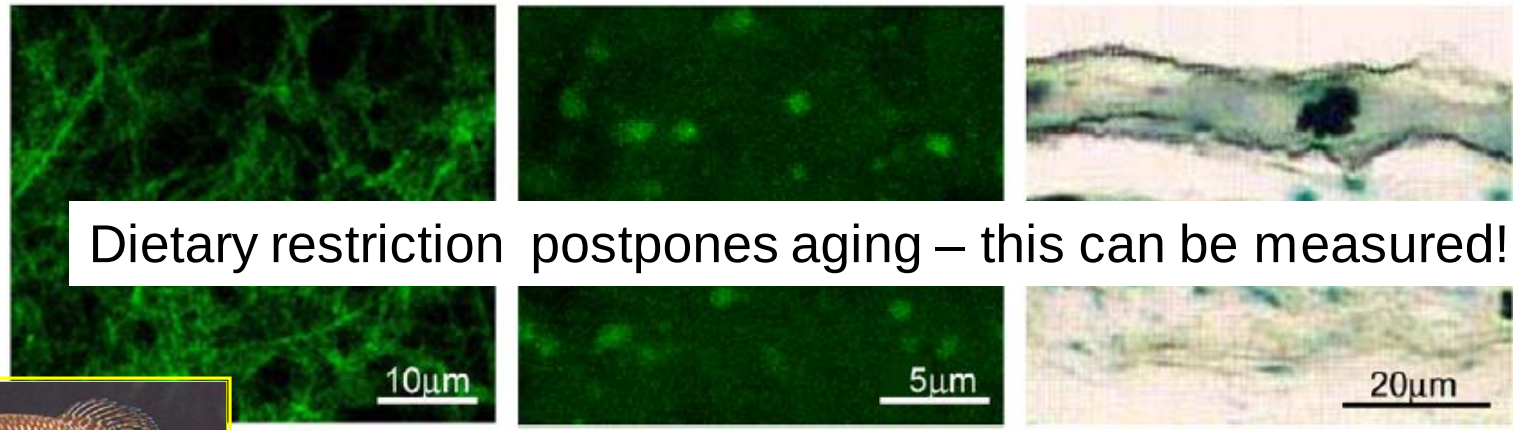
Fluoro-Jade B, Lipofuscin, SA-ß Galactosidase

brain **liver** **skin**

neuronal degeneration accumulation of aging pigment cellular senescence



5 weeks



9 weeks

Dietary restriction postpones aging – this can be measured!

Terzibasi et al., Exp Gerontol 42:81 (2007)

	Life-span increase		Beneficial health effects	
	Dietary restriction	Mutations/ drugs	Dietary restriction	Mutations/ drugs
 Yeast	3-fold	10-fold (with starvation/ DR)	Extended reproductive period	Extended reproductive period, decreased DNA damage/mutations
 Worms	2- to 3-fold	10-fold	Resistance to misexpressed toxic proteins	Extended motility Resistance to mis-expressed toxic proteins and germ-line cancer
 Flies	2-fold	60–70%	None reported	Resistance to bacterial infection, extended ability to fly
 Mice	30–50%	30–50% (~100% in combination with DR)	Protection against cancer, diabetes, atherosclerosis,cardio-myopathy, autoimmune, kidney, and respiratory diseases; reduced neurodegeneration	Reduced tumor incidence; protection against age-dependent cognitive decline, cardio-myopathy, fatty liver and renal lesions. Extended insulin sensitivity
 Monkeys	Trend noted	Not tested	Prevention of obesity; protection against diabetes, cancer, and cardiovascular disease	Not tested
 Humans	Not determined	Not determined (GHR-deficient subjects reach old age)	Prevention of obesity, diabetes, hypertension Reduced risk factors for cancer and cardiovascular disease	Possible reduction in cancer and diabetes

Fig. 1. Experiments on dietary restriction (DR) and genetic or chemical alteration of nutrient-sensing pathways have been performed on a range of model organisms. The results differ widely, and little is known about the long-term effects in humans.

Fontana et al. Science 328: 321(2010)

Life span and food intake

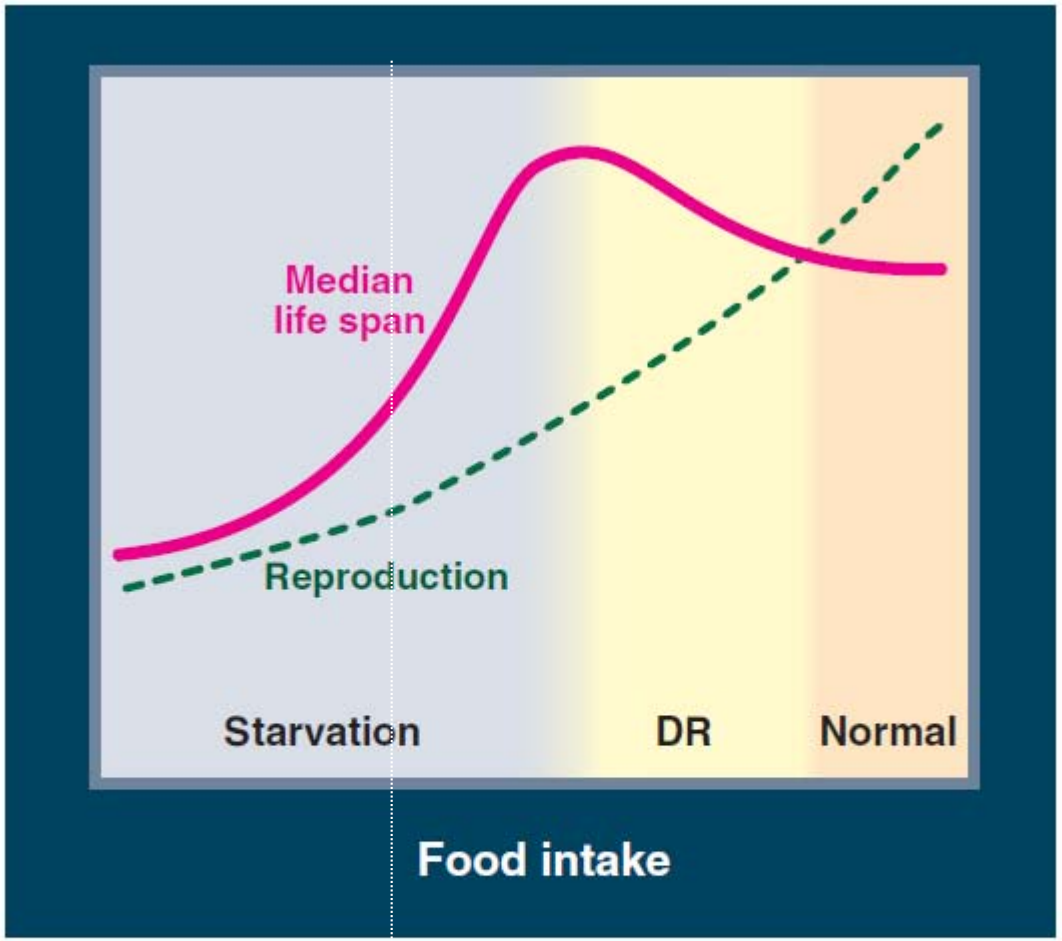


Fig. 2. The median life span and fecundity of higher eukaryotes are negatively affected by a very low food intake. However, life span but not fecundity is optimized by dietary restriction (DR). Unlike what is observed in most higher eukaryotes, starvation extends the yeast chronological life span.

Fontana et al. Science 328: 321(2010)

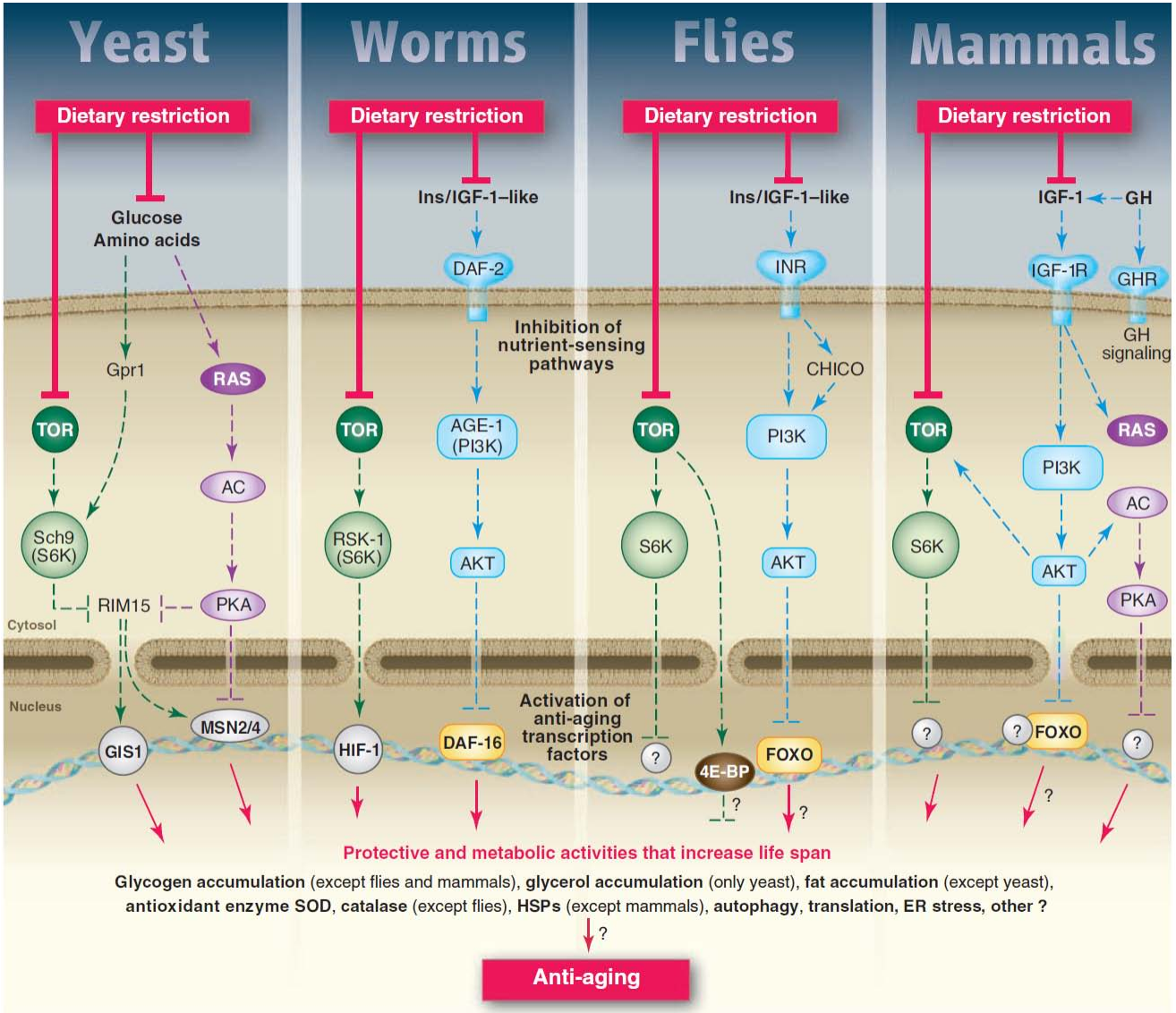
Conserved Nutrient Signalling pathways

Box 1. Nutrient-sensing pathways and aging.

Nutrient-sensing pathways are fundamental to the aging process. Different nutrients can activate different pathways directly or indirectly. For example, in yeast, glucose activates the Ras-AC-PKA pathway, but in mice nutrients increase the level of IGF-1, which, in turn, activates pro-aging pathways in various mammalian cells (Fig. 3). Dietary restriction partially inactivates one or several nutrient-signaling pathways, thereby causing life-span extension in model organisms. In yeast and worms, this effect on longevity requires one or more transcription factors (proteins that regulate the expression of many genes involved, for example, in cellular protection, metabolic pathways, and processing of damaged proteins). In mice, dietary restriction or the inhibition of nutrient signaling can also reduce various age-related diseases, including cancer. These effects on diseases are believed to be a result of delaying the aging process in the various cells associated with the disease. The reason why these pathways are inactivated or partially inactivated by reduced nutrients is apparently simple: During periods of food scarcity, cells and organisms must be able to enter a standby mode in which cell division and reproduction are halted or minimized to allow energy to be available to maintenance systems. The conserved composition and function of anti-aging pathways in the different organisms indicate that most species have developed anti-aging systems to overcome periods of starvation.

Fontana et al. Science 328: 321(2010)

Conserved Nutrient Signalling pathways



Fontana et al. Science 328: 321(2010)

Conserved Nutrient Signalling pathways

Outlook

Extreme dietary restriction can lead to several detrimental health effects such as amenorrhea, infertility, sarcopenia, osteoporosis, and immune deficiencies. Thus, it will be important to examine these negative side-effects in dietary-restricted subjects that are not malnourished. Indeed, experimental studies are required to evaluate the optimal calorie intake and macro- and micro-nutrient composition needed for healthy aging in humans, on the basis of age, sex, genotype, and energy expenditure.

Although adjustment of dietary intake and composition may be realistic and beneficial, the severe dietary restriction that induces major health benefits is not a desirable option for most people.

Fontana et al. Science 328: 321(2010)