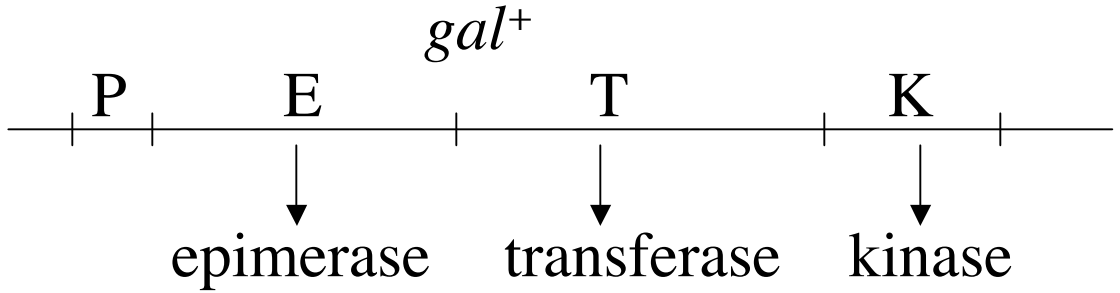
FOGA-III: HOW DOES GENETIC CHANGE HAPPEN? - NATURAL GENETIC ENGINEERING OF GENOME STRUCTURE

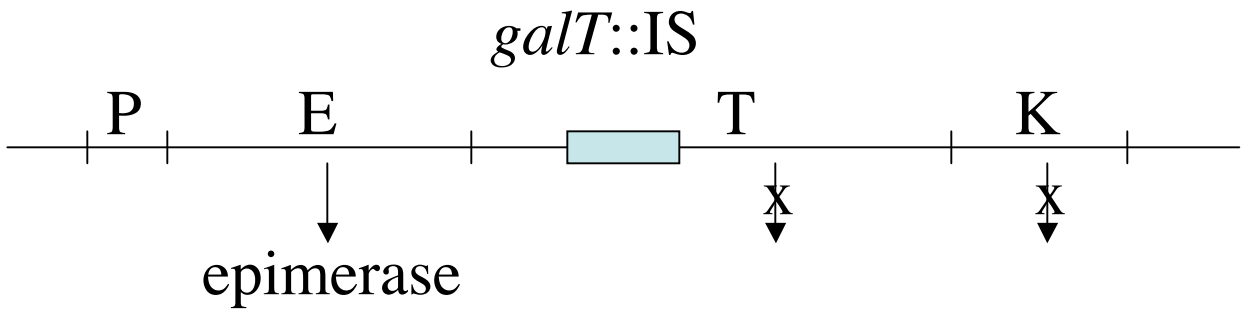
- Cells have a large toolbox of biochemical systems that carry out genome restructuring at all levels of complexity
- Sequenced genomes display structures and relationships that reveal the evolutionary importance of natural genetic engineering functions
- Natural genetic engineering functions are subject to cellular regulation and control

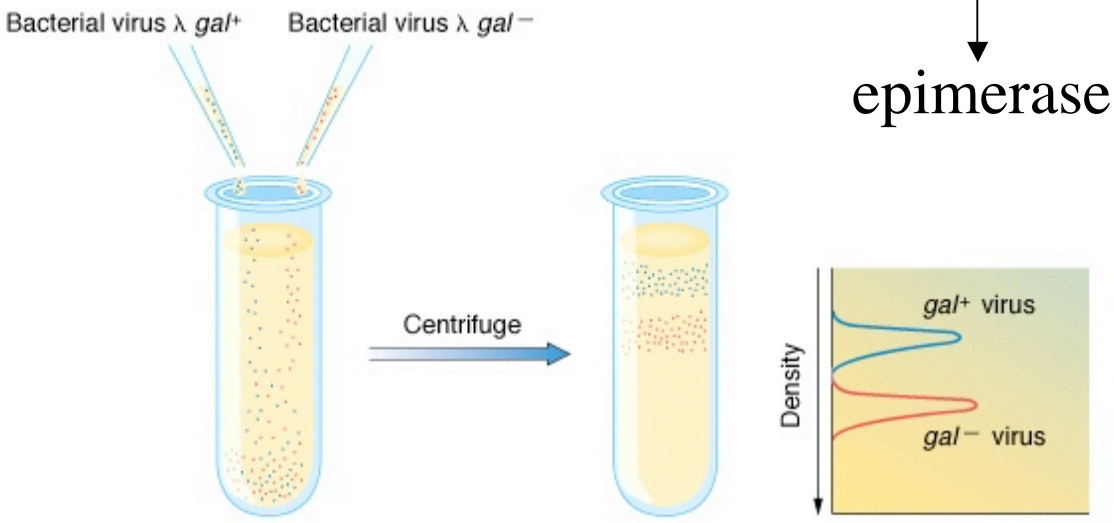
Outline

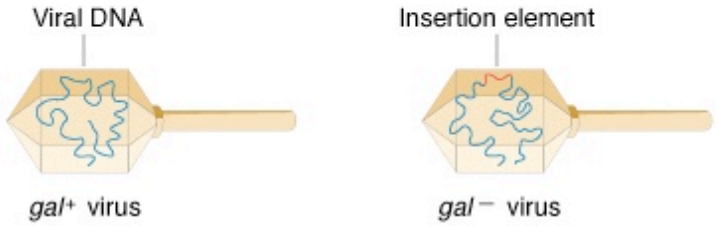
- Personal history with natural genetic engineering
- The mammalian immune system
- Natural genetic engineering in evolution
- Non-random features of natural genetic engineering
- Advantages of evolution by natural genetic engineering

Mobile DNA - IS elements



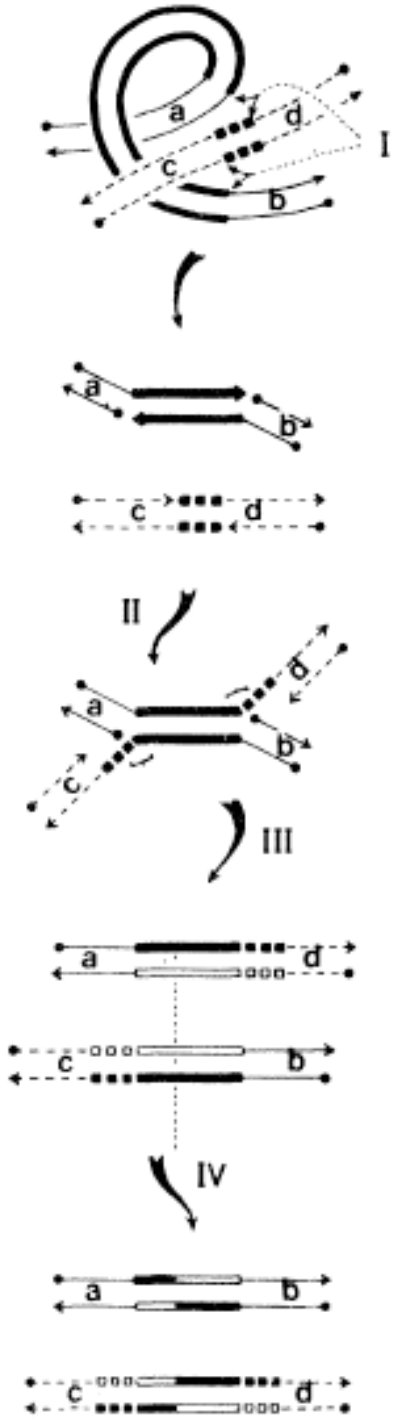


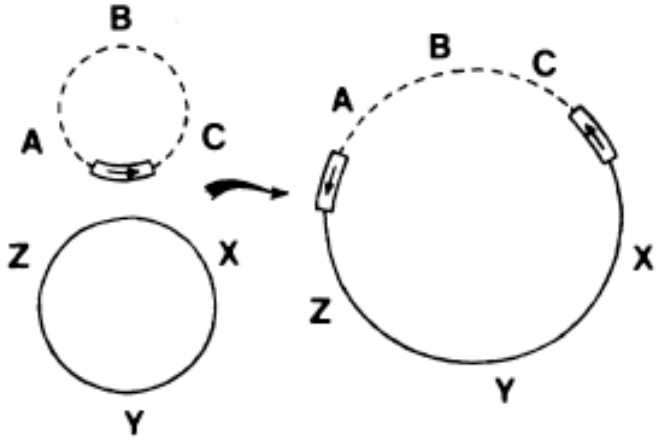


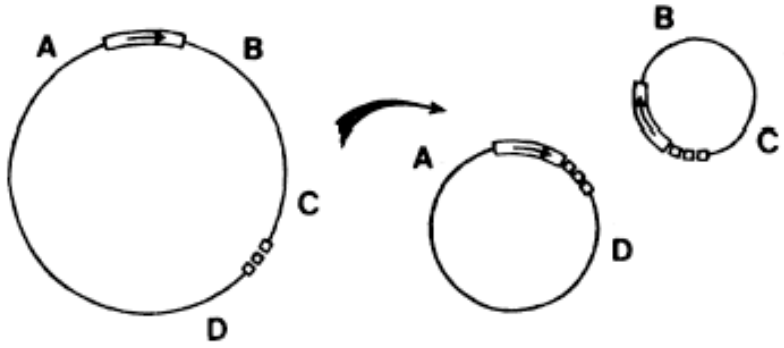


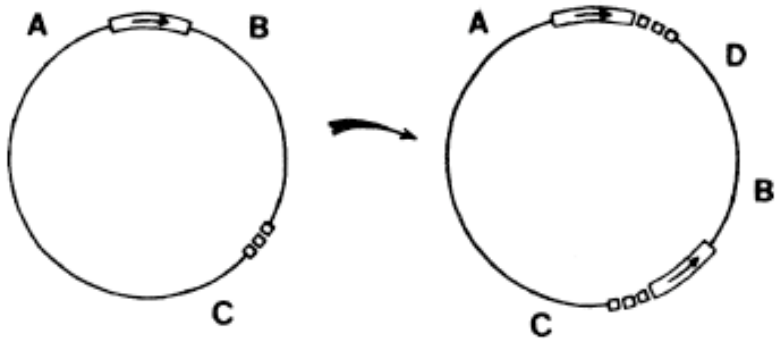
Shapiro JA. Mutations caused by the insertion of genetic material into the galactose operon of Escherichia coli. J Mol Biol. 1969 Feb 28;40(1):93-105.

Replicative transposition and DNA rearrangements



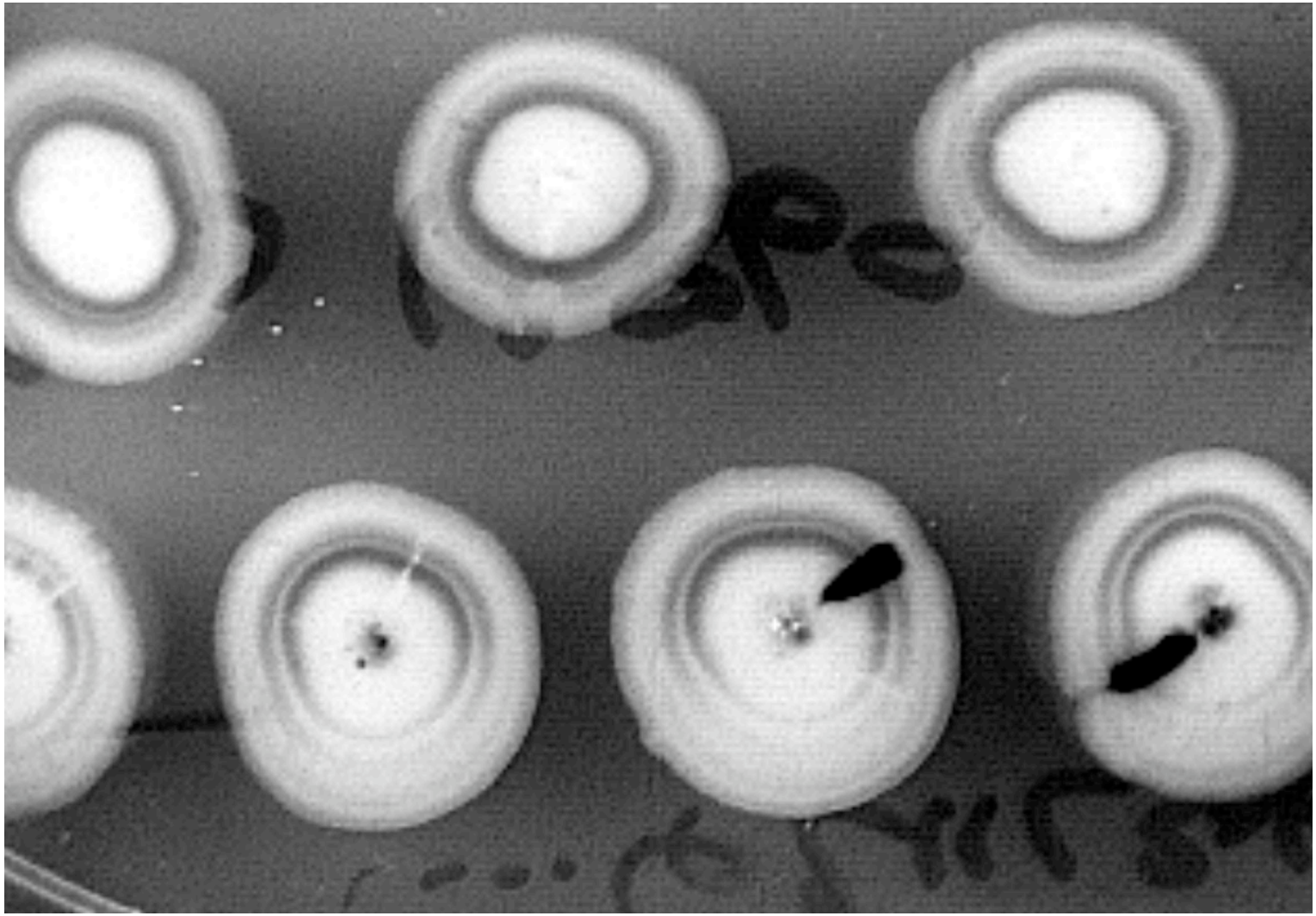






Shapiro, J. 1979. "A molecular model for the transposition and replication of bacteriophage Mu and other transposable elements." Proc. Nat. Acad. Sci. U.S.A. 76, 1933-1937.

Differential Replicative Transposition of Mu*dlac* in *E. coli* Colonies - Starvation Triggered



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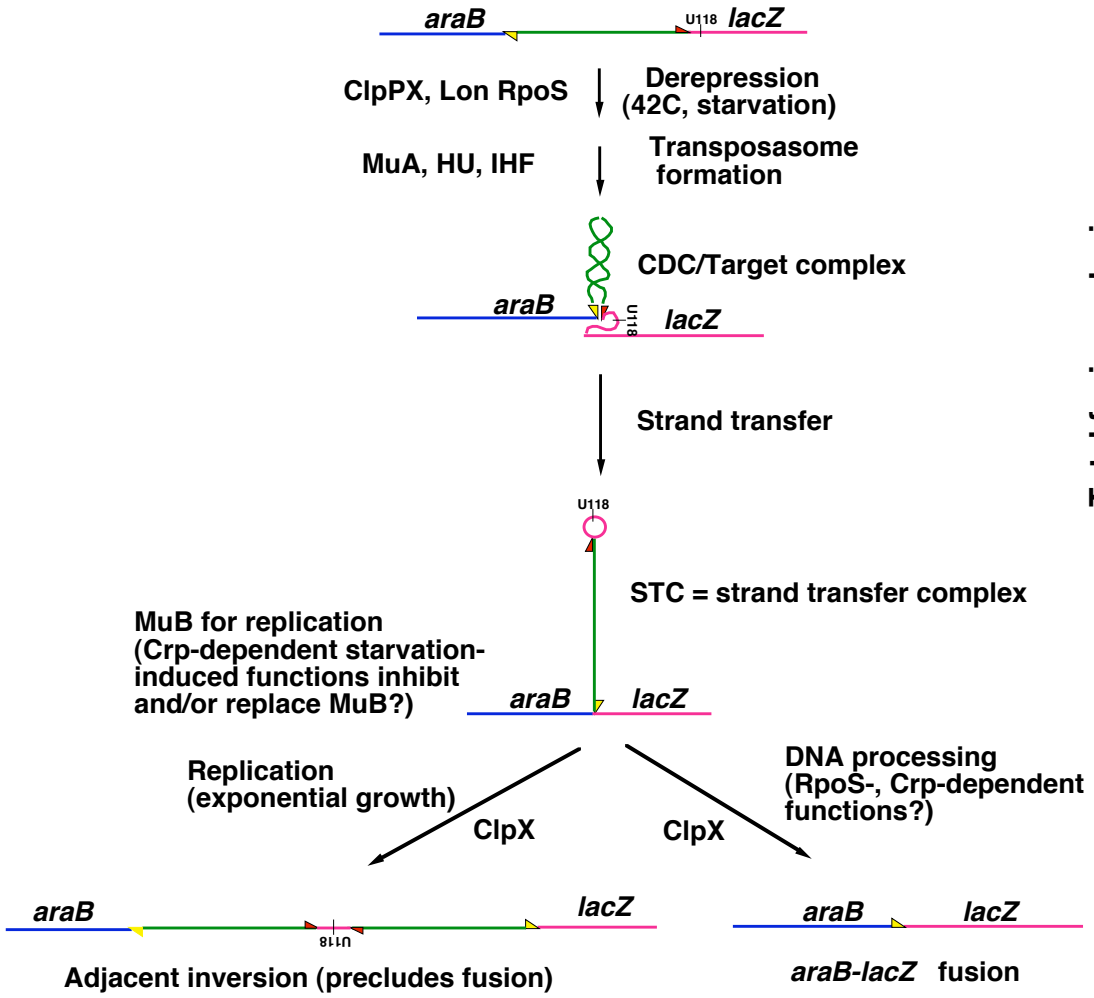
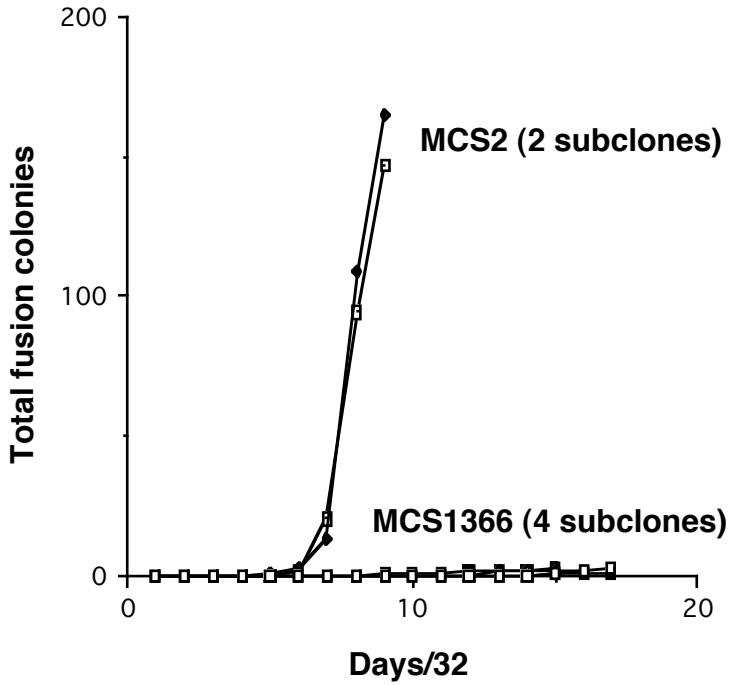
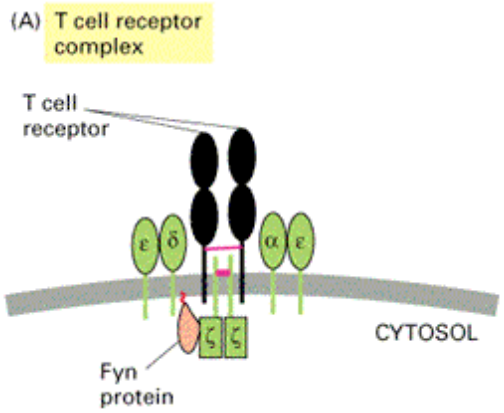
Stress-induced *ara-lac* fusions and adaptive mutation

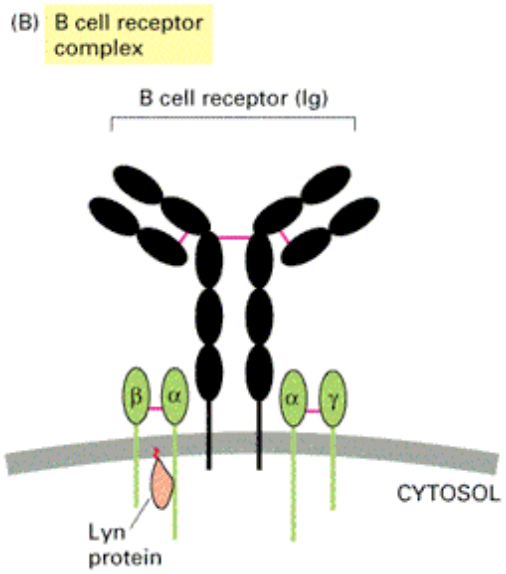


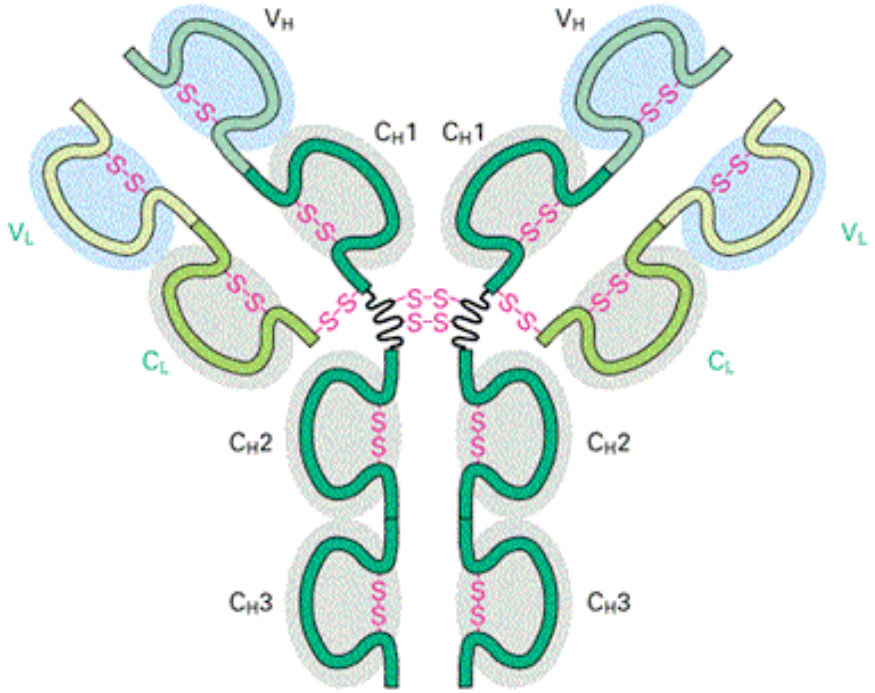


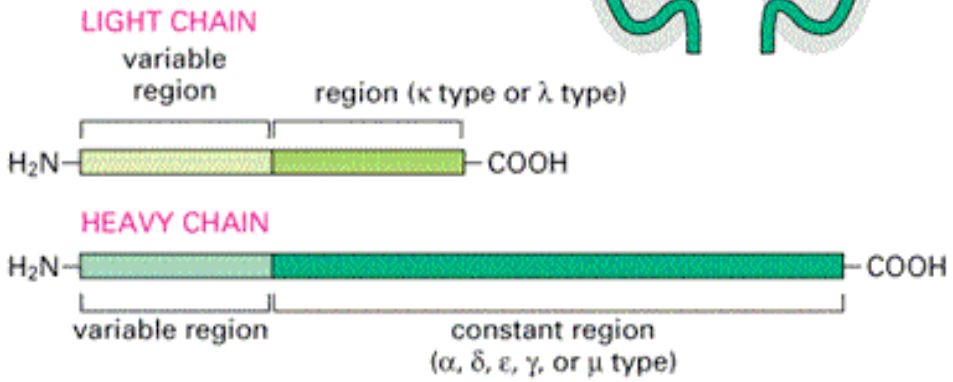
Shapiro, J.A. 1997b. Genome organization, natural genetic engineering, and adaptive mutation. *Trends in Genetics* **13**, 98-104

Immune Systems Receptors: How to generate virtually infinite diversity with finite coding capacity

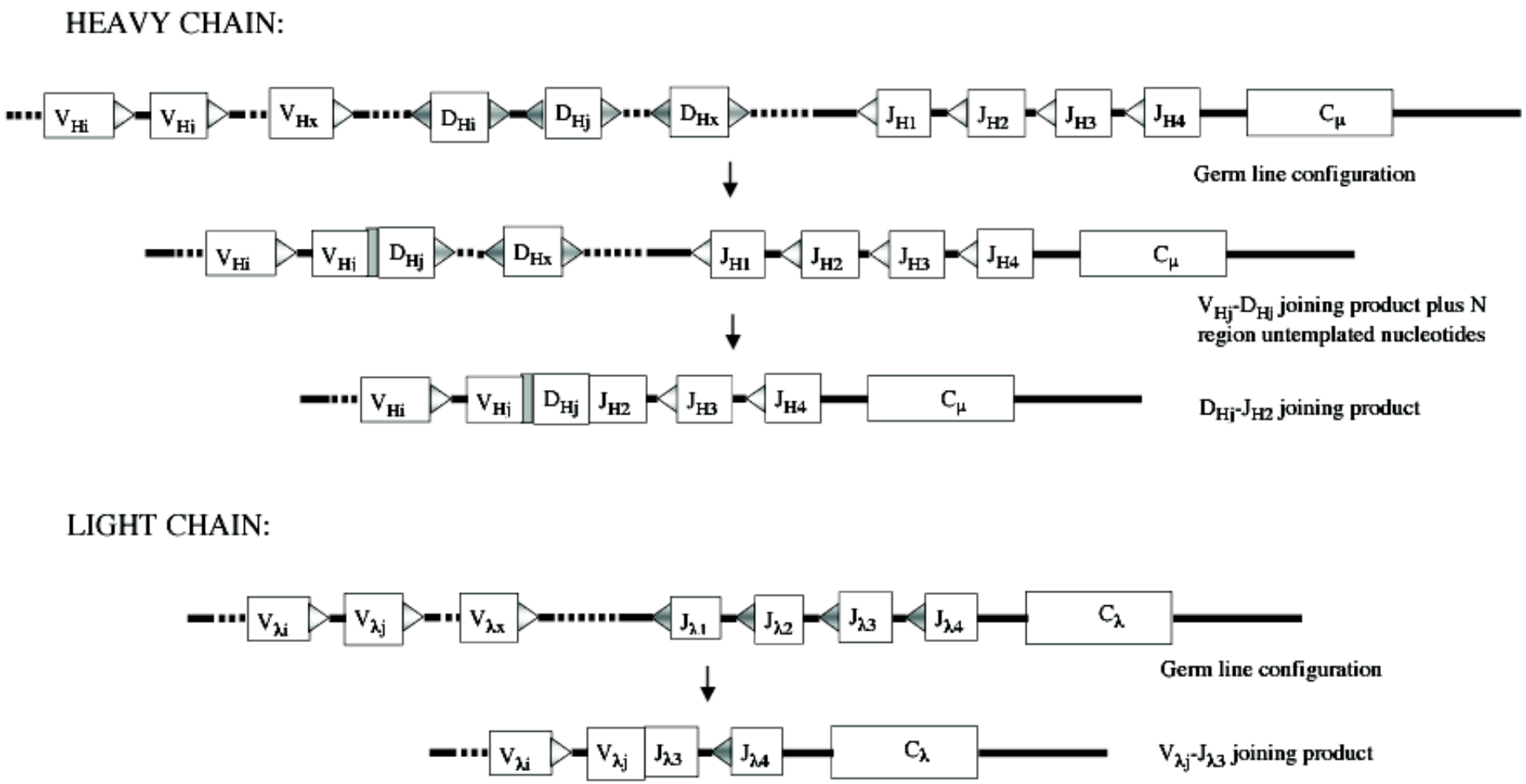




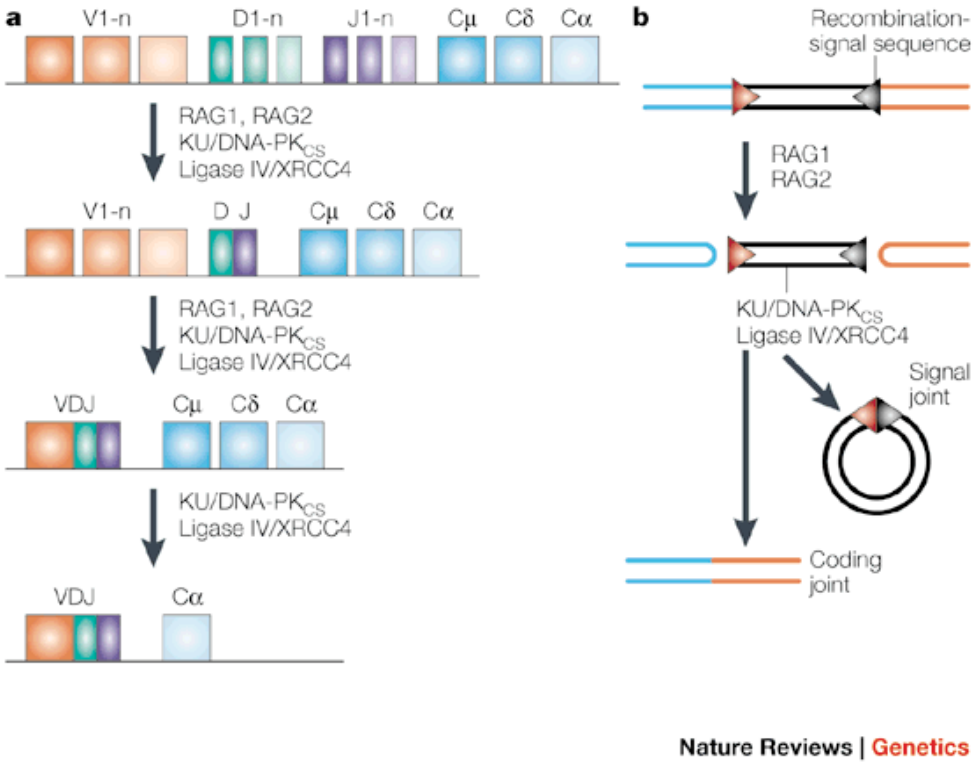


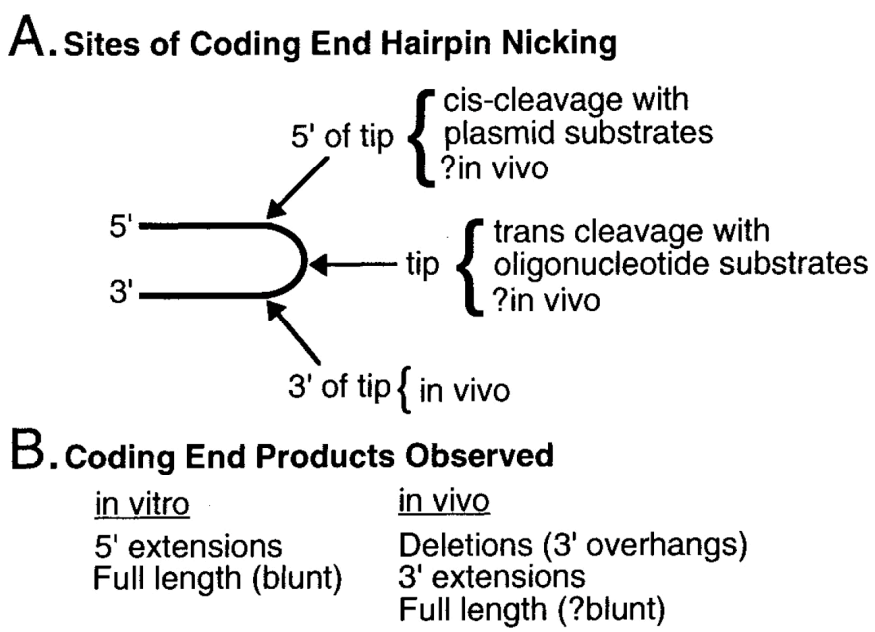


Combinatorial Diversity: assembling immunoglobulin coding sequences from cassettes



Junctional Flexibility: Augmenting Diversity





D. C. van Gent, J. H. Hoeijmakers, R. Kanaar, Chromosomal Stability And The Dna Double-Stranded Break Connection Nature Rev. Genet. 2, 196 (2001)

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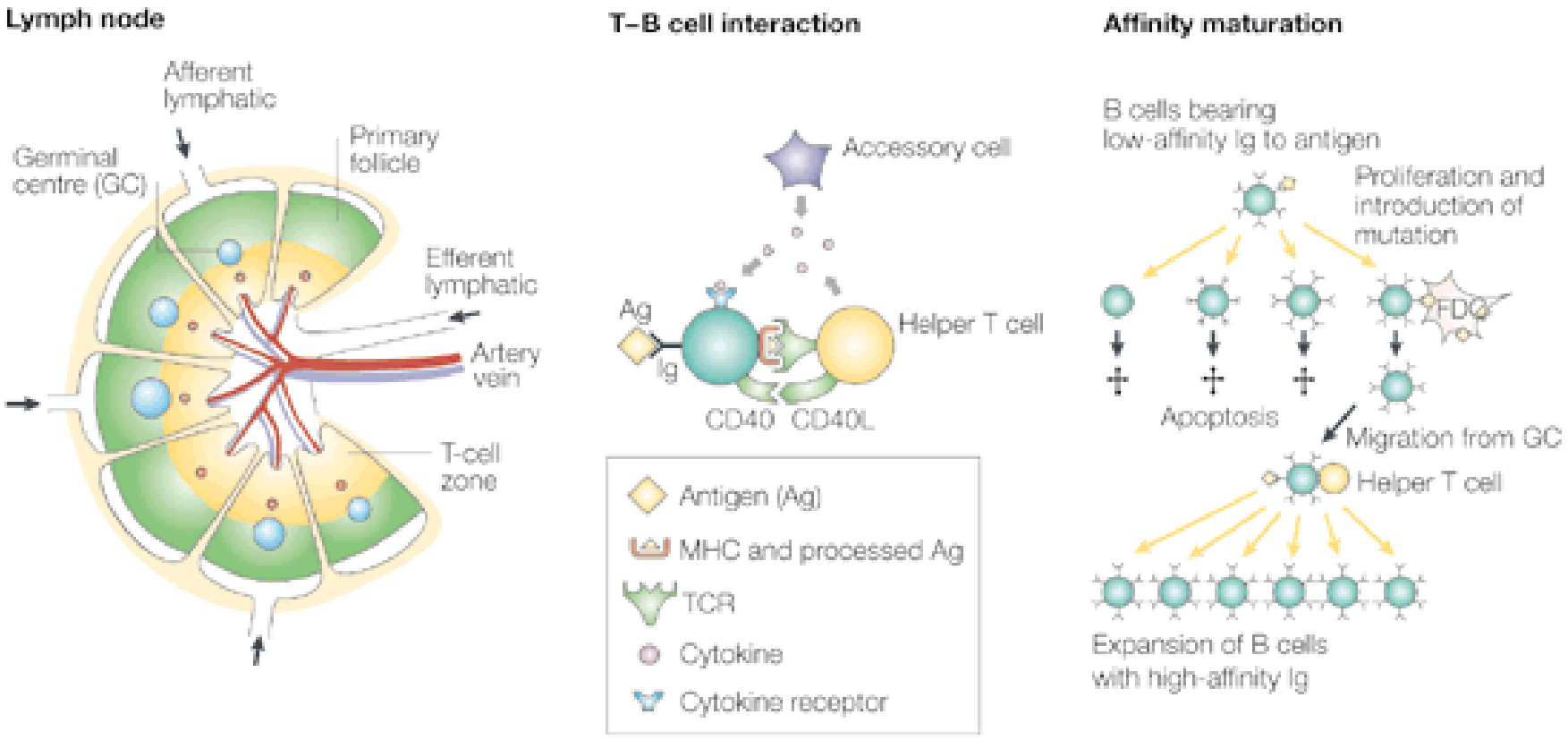
Antigen stimulation/selection: a rapid evolution system



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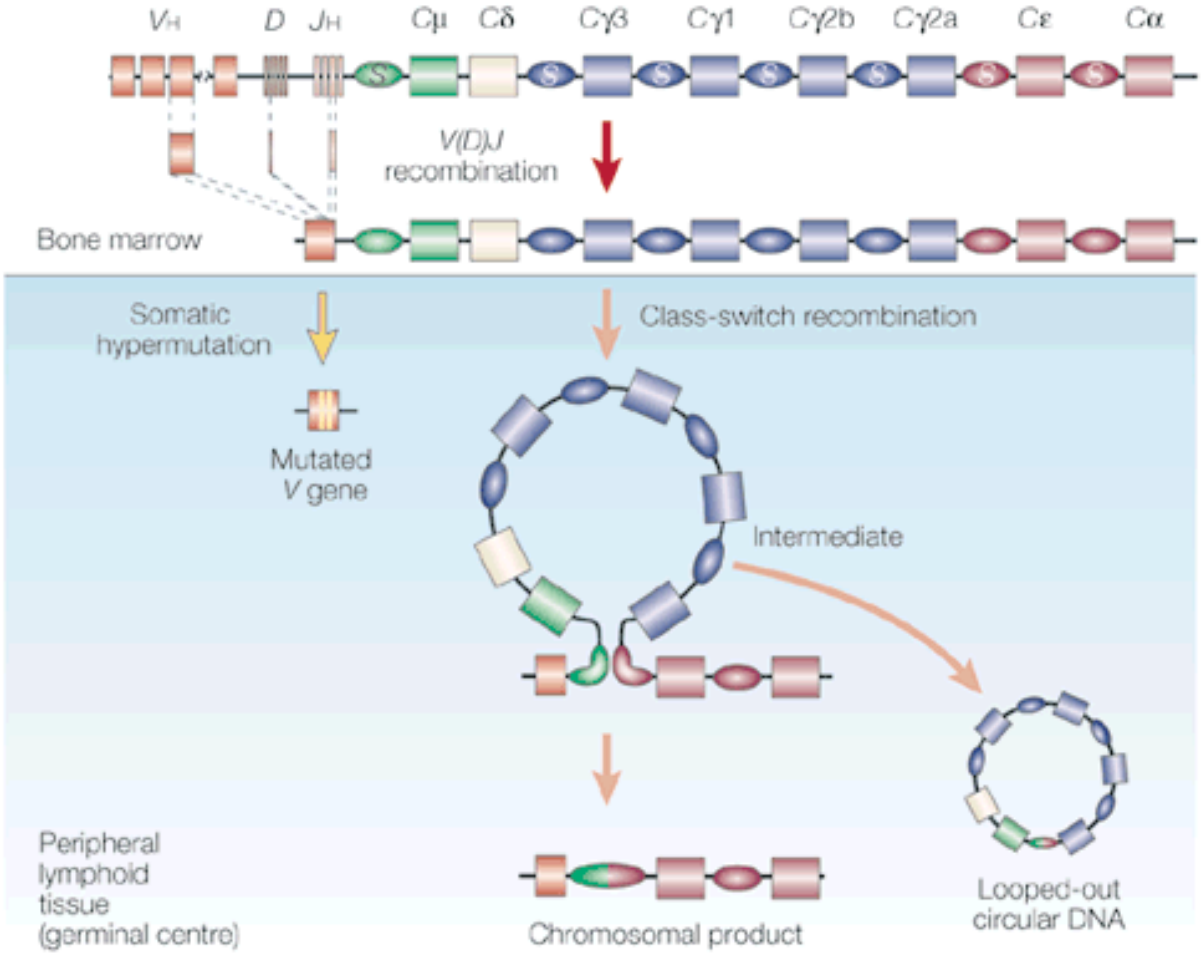
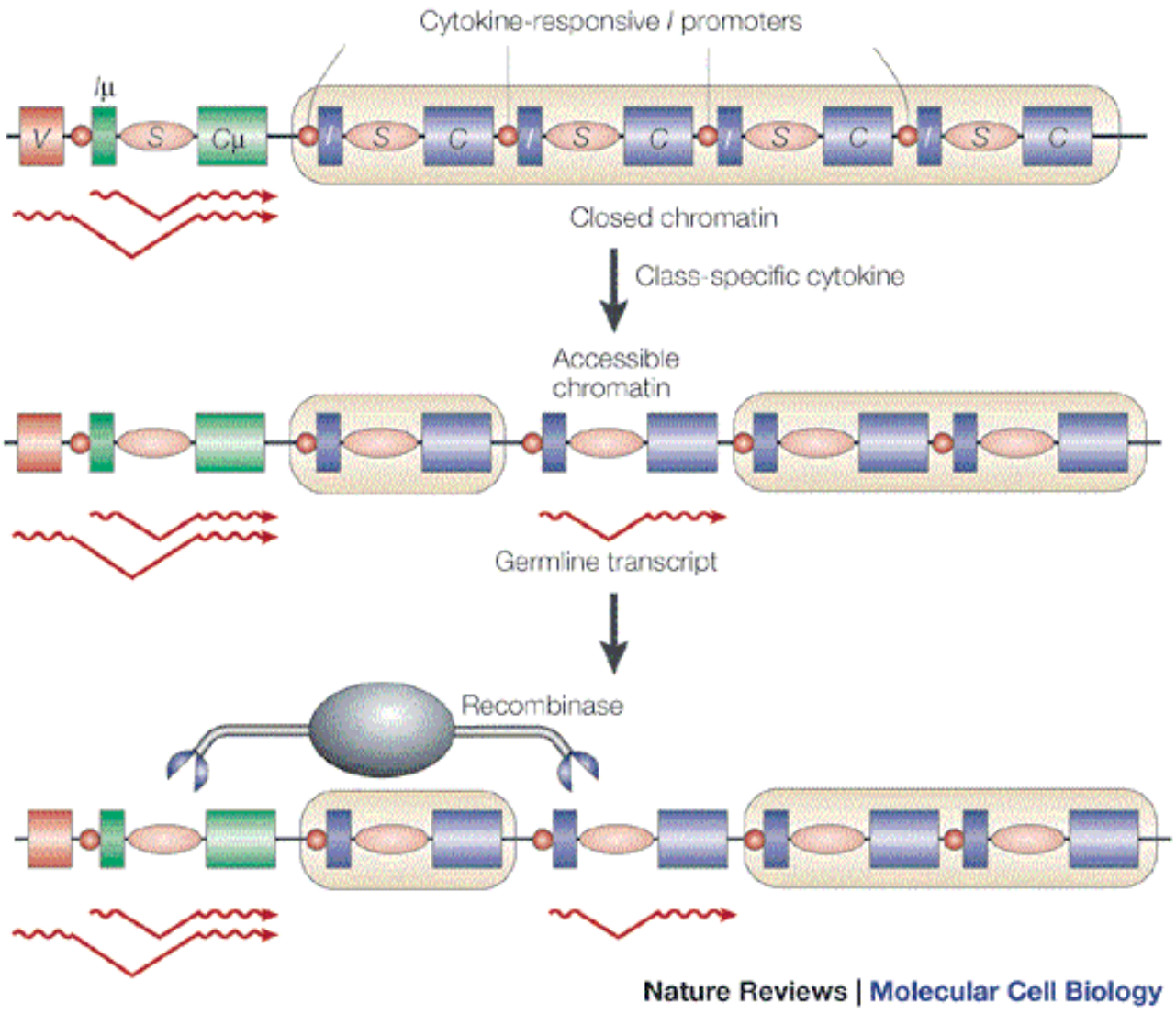
Post-selection (antigen stimulation): antibody improvement and functional diversification



Nature Reviews Molecular Cell Biology 2; 493-503 (2001) LINKING CLASS-SWITCH RECOMBINATION WITH SOMATIC HYPERMUTATION

Transcriptional Targeting of Class Switch Recombination

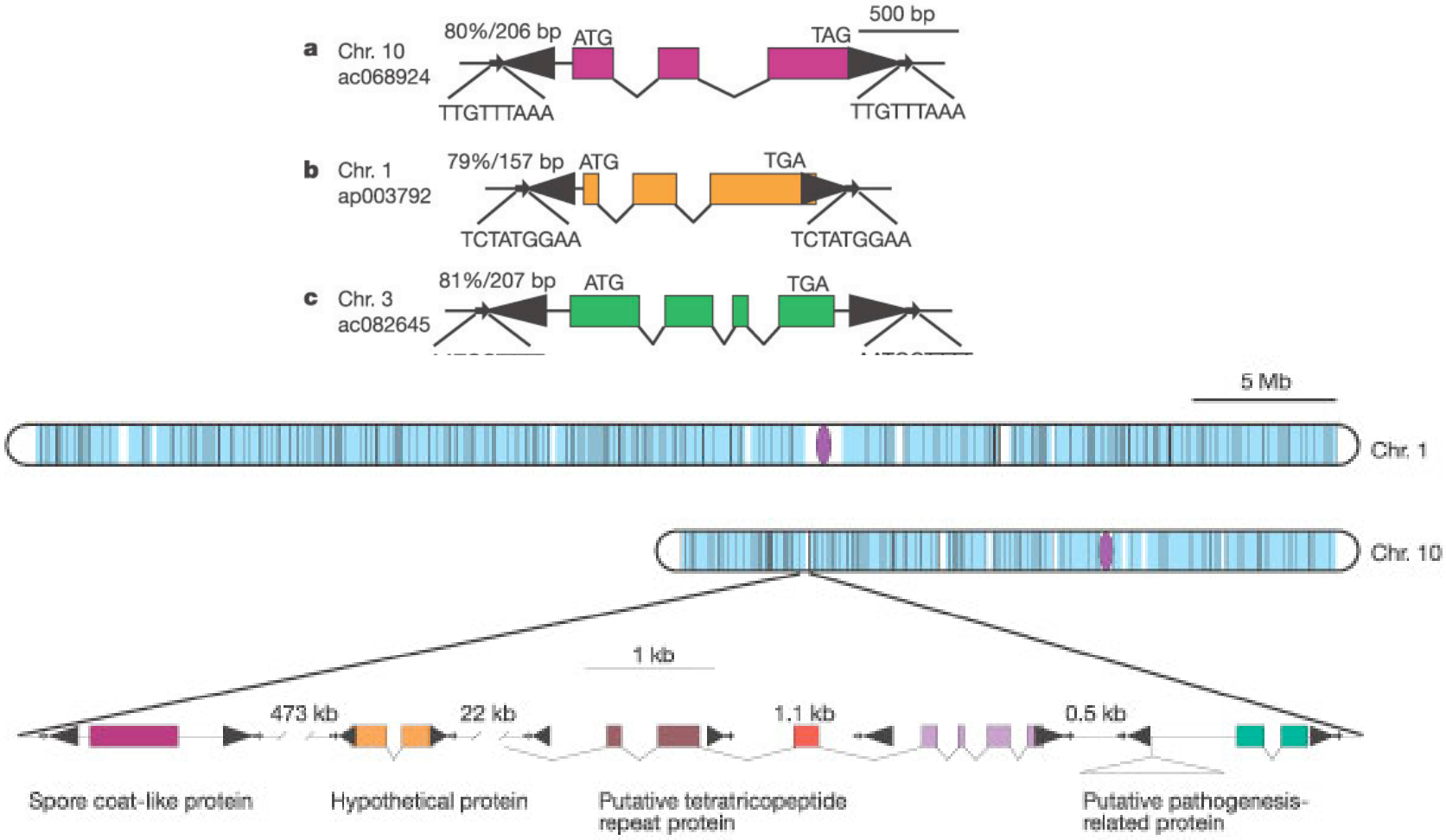


Nature Reviews Molecular Cell Biology 2; 493-503 (2001) LINKING CLASS-SWITCH RECOMBINATION WITH SOMATIC HYPERMUTATION

Immune System Lessons: cellular capabilities for controlled but non-determined DNA restructuring

- Tight regulation of complex set of events as to cell type, sequence of particular DNA changes, and linkage to selection & cellular proliferation
- Capacity for multiple types of DNA changes, including ability to incorporate untemplated sequences
- Targeting of VDJ joining events to particular locations within coding regions while maintaining flexibility of novel sequences formed
- Transcriptional activation and targeting of somatic hypermutation (base changes) to V regions of Ig coding sequences
- Lymphokine-directed transcriptional activation and targeting of class switch recombination (breakage and rejoining)

Natural genetic engineering of sequenced genomes - Pack-MULEs

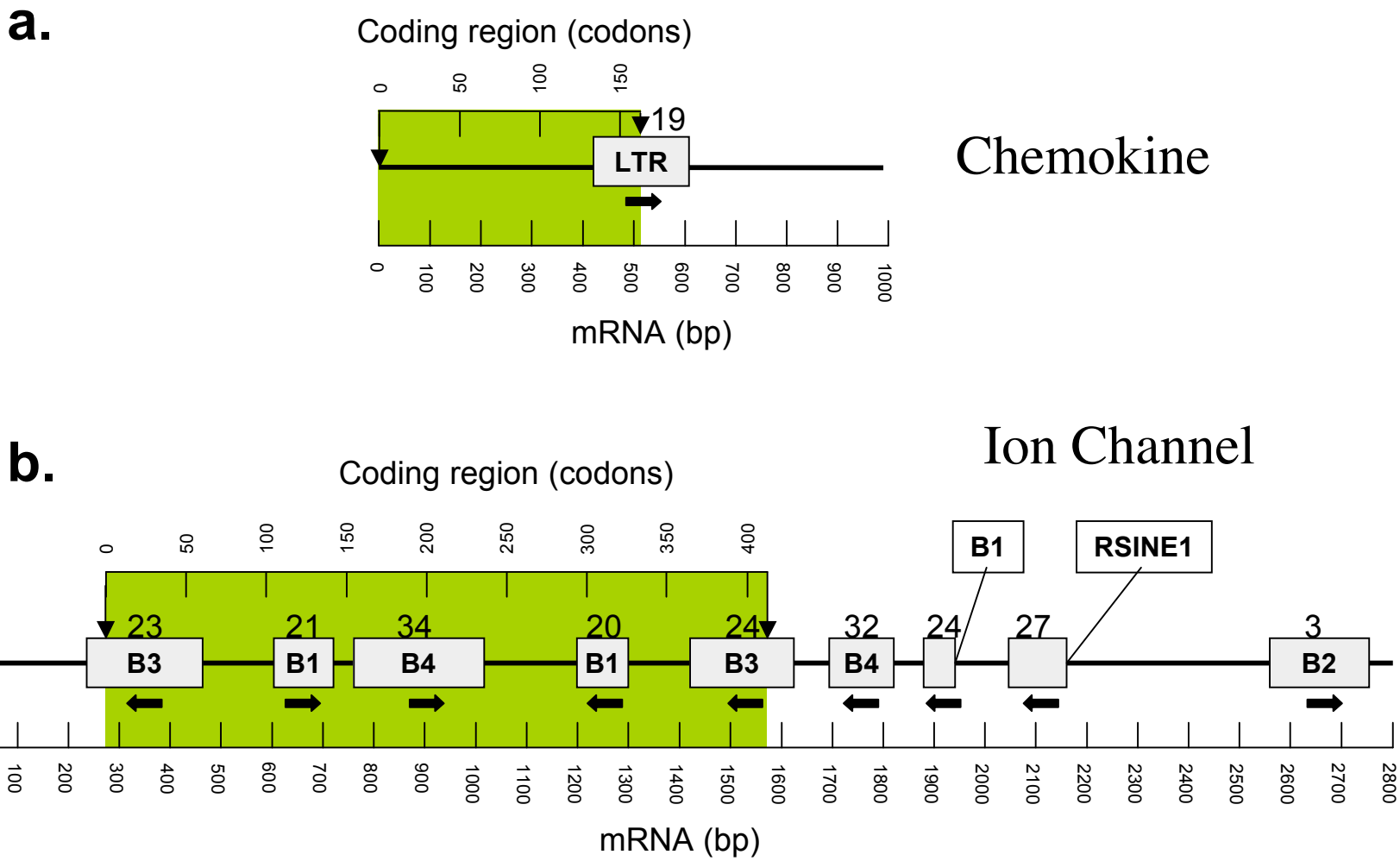


Ning Jiang, Zhirong Bao, Xiaoyu Zhang, Sean R. Eddy and Susan R. Wessler. 2004. Pack-MULE transposable elements mediate gene evolution in plants. Nature 431, 569-573.

Natural Genetic Engineering Modalities

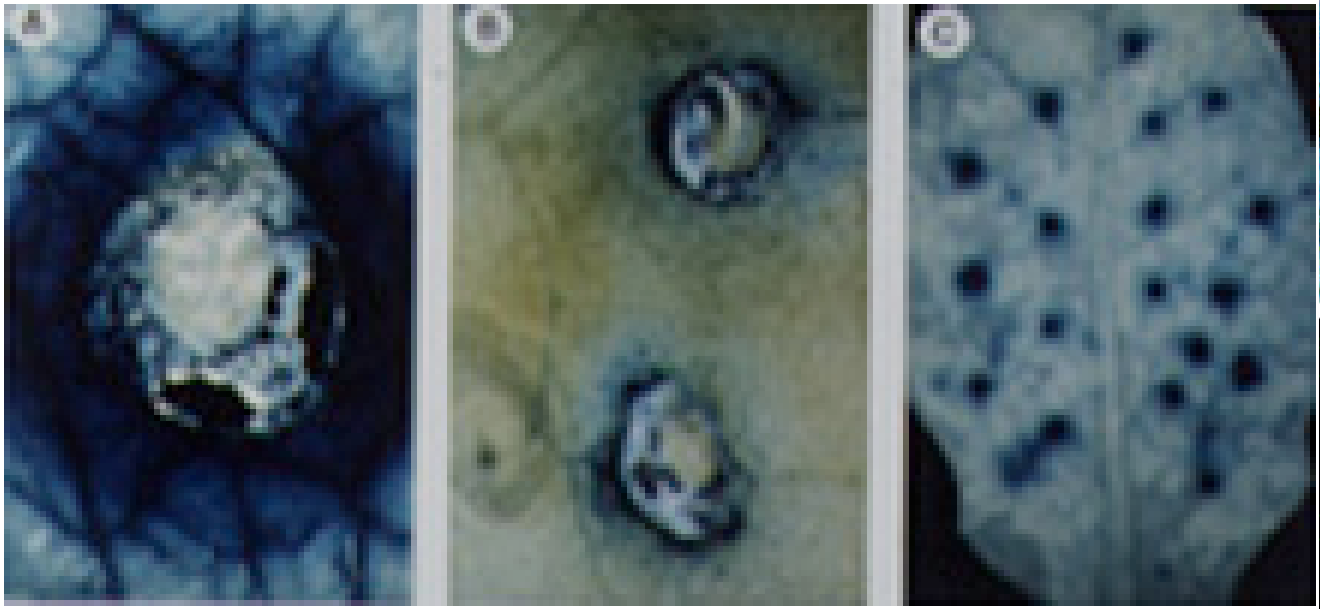
- Homology-dependent exchange & gene conversion:
  - DS break repair
  - Rearrangements by crossover at dispersed homologies
  - Cassette exchange, protein diversification
- Non-homologous end joining (NHEJ)
  - DS break repair
  - Targeted and untargeted rearrangements
- Mutator polymerases
- Terminal transferase - insertion of novel sequences
- Site-specific recombinases
  - Integration of horizontally transferred DNA
  - Regulation of protein synthesis, protein diversification
- DNA transposons (replicative, cut-&-paste, rolling circle helitrons)
  - Amplification and insertion of repeat elements
  - Large-scale rearrangements (in particular, duplications)
- Reverse transcription-dependent retrotransposons (retroviral-like, LINEs, SINEs)
  - Amplification and insertion of repeat elements
  - Integration of processed RNA cDNA copies
  - Small-scale movement of genomic segments (e.g. exon shuffling)
- Homing and retrohoming introns

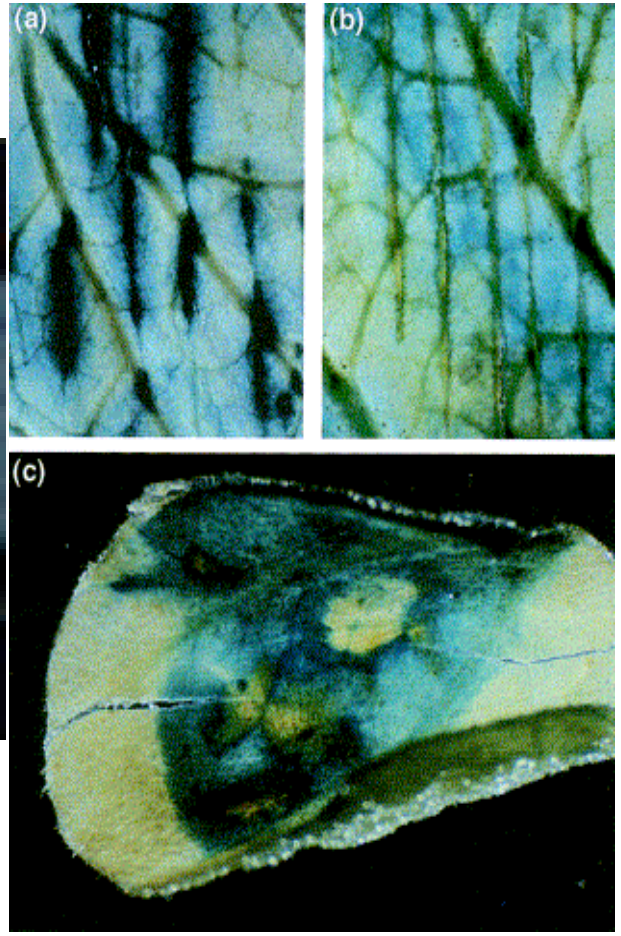
Natural genetic engineering of sequenced genomes - protein coding sequences



Nekrutenko, A. and W.-H. Li. 2001. Transposable elements are found in a large number of human protein coding regions. Trends in Genetics **17**: 619-625

Leaf wounding and retrotransposon transcription





http://www-biocel.versailles.inra.fr/Anglais/03Transposon.html

The expression of the tobacco Tnt1 retrotransposon is induced by wounding : the expression of the LTR-GUS construct is detected by a blue staining surrounding injury points in transgenic tomato (A), tobacco (B) and Arabidopsis (C) plants.

M.-A. Grandbastien et al. Stress activation and genomic impact of Tnt1 retrotransposons in Solanaceae. Cytogenetic and Genome Research 2005;110:229-241

Targeting of natural genetic engineering

Known molecular mechanisms:

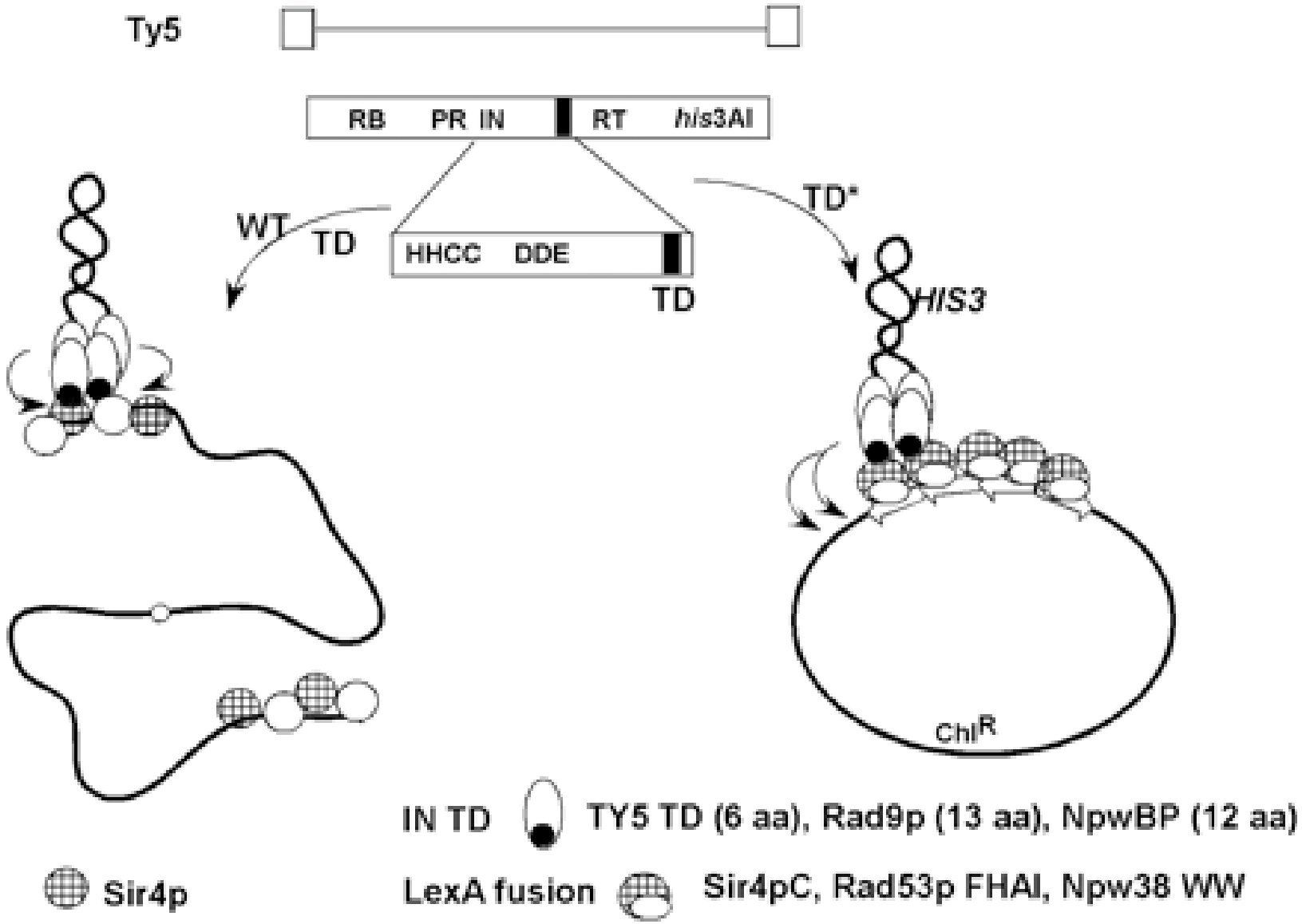
- Sequence recognition by proteins (yeast mating-type switching, ribosomal LINE elements, homing introns, VDJ joining);
- Protein-protein interaction wth transcription factors or chromatin proteins (Ty retrotransposon targeting);
- Sequence recognition by RNA (reverse splicing of group II retrohoming introns);
- Transcriptional activation of target DNA (somatic hypermutation; class-switch recombination).

Unknown mechanisms:

- Telomere targeting of certain LINE elements in insects;
- HIV & MLV targeting upstream of transcribed regions;
- P factor homing directed by transcription, chromatin signals;
- P factor targeting to heat-shock promoters.

Shapiro, JA. 2005. A 21st Century View Of Evolution: Genome System Architecture, Repetitive DNA, And Natural Genetic Engineering. Gene **345**: 91-100

Yeast Ty5 targeting



S. Sandmeyer. Integration by design. PNAS, May 13, 2003; 100(10): 5586 - 5588.

Advantages of non-random searches of genome space at evolutionary crises

- Genome changes occur under stress or other conditions, when they are most likely to prove beneficial;
- Multiple related changes can occur when a particular natural genetic engineering system is activated;
- Rearrangement of proven genomic components increases the chance that novel combinations will be functional;
- Targeting can increase the probability of functional integration and reduce the risk of system damage (ensure syntactically correct changes in the program architecture, as in GP);
- Rearrangements followed by localized changes provide opportunities for fine tuning once novel function has been achieved.