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

Replicative transposition and DNA rearrangements

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

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

Immune Systems Receptors: How to generate virtually infinite diversity with finite coding capacity
Combinatorial Diversity: assembling immunoglobulin coding sequences from cassettes

**HEAVY CHAIN:**

- \(V_{Hi} \to V_{Hi} \to V_{Hx} \to D_{Hi} \to D_{Hj} \to D_{Hx} \to J_{H1} \to J_{H2} \to J_{H3} \to J_{H4} \to C_{\mu}\)
- Germ line configuration
- \(V_{Hi}-D_{Hi} \) joining product plus N region untemplated nucleotides
- \(D_{Hi}-J_{H2} \) joining product

**LIGHT CHAIN:**

- \(V_{\lambda i} \to V_{\lambda j} \to V_{\lambda x} \to J_{\lambda 1} \to J_{\lambda 2} \to J_{\lambda 3} \to J_{\lambda 4} \to C_{\lambda}\)
- Germ line configuration
- \(V_{\lambda j}-J_{\lambda 3} \) joining product
Junctional Flexibility: Augmenting Diversity


Antigen stimulation/selection:
a rapid evolution system
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

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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

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
Leaf wounding and retrotransposon transcription

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.

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 with 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.

Yeast Ty5 targeting

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.