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

$\text{gal}^+$

$\text{epimerase}$

$\text{transferase}$

$\text{kinase}$

$\text{galT}::\text{IS}$

$\text{epimerase}$

Replicative transposition and DNA rearrangements

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

Stress-induced \textit{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:**

Germ line configuration

**LIGHT CHAIN:**

Germ line configuration
Junctional Flexibility: Augmenting Diversity


Antigen stimulation/selection: a rapid evolution system
Post-selection (antigen stimulation): antibody improvement and functional diversification.
Transcriptional Targeting of Class Switch Recombination

Nature Reviews Molecular Cell Biology 2; 493-503 (2001)
LINKING CLASS-SWITCH RECOMBINATON 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.