{ Alife Mutants Hackingsession on Systems and Organisms, Bielefeld 2004 }

The rules of sperm-mediated gene transfer

- ***Vertical and horizontal gene transfer***
- ***Sperm cell as vehicle for selfish DNA***
- ***Elemental rules***
- ***Bottom-up modeling***

How I say

Abbreviations and definitions:

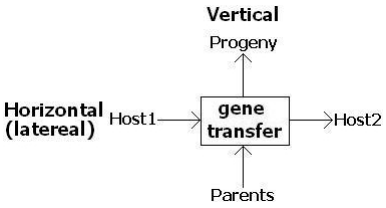
- SP – sperm cells, SMGT – sperm-mediated gene transfer
- DNA integration – inclusion of the DNA molecule into the single site of chromosome
- 'Percolation' – multiple integration of DNA-fragments into different parts of genome
- 'Mobile (transposable) DNA element – DNA, which able to copy into the new sites (actually parasite, selfish DNA)
- Retrotransposone, retrovirus – mobile elements transmitted by reverse transcription (RNA→DNA)

Analogies between Biology and Computer Science:

- Genetic transformation ↔ Programming
- DNA integration ↔ Program concatenation
- DNA manipulation ↔ String operators
- Gene expression ↔ Program execution

Vertical and horizontal gene transfer. Selfish DNA

- Vertical inheritance within the host and horizontal transfer between hosts



- Selfish DNA:
- Mobile DNA elements are widespread. They provide rearrangements in genes, which are exception to the general principle that genes transmit with great fidelity from parents to progeny. Rearrangements are typically rare, but are sometimes maintained by selective pressure. About 45% of human genome is composed of mobile DNA
- The canonical example of lateral transfer is movement of P-element from *Drosophila willistoni* to *D.melanogaster*, an event that might have occurred last century (Daniels et. al., 1990)

Well established lateral gene transfer

Gene transfer from bacteria to bacteria:

- Transformation – gene transfer by uptake and incorporation of exogenous DNA (Griffith, 1928; Avery et al., 1944)
- Conjugation – a 'sex' process in bacteria (Lederberg, Tatum, 1946)
- Transduction – bacteriophage-mediated gene transfer (Morse et al., 1956)
- Competence – ability of cells to take up DNA, i.e. Gram-negative bacteria (*Haemophilus*, *Neisseria*, *Helicobacter* and *Acinetobacter*) as well as Gram-positive bacteria (*Bacillus*, *Mycobacterium* and *Streptomyces*)
- The uptake and stable maintenance of extracellular DNA – genetic transformation – is a major force in microbial evolution

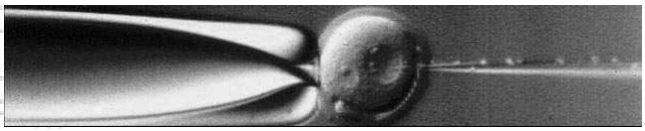
Transfer of octopine-type Ti-plasmid from *Agrobacterium tumefaciens* to plants results in tumor growth to develop bacteria in infected plants (Montagu, Schell, 1982)

Lateral gene transfer into human genome?

- The sequenced human genome contains 223 bacterial genes. Probably multiple independent gene transfers from different bacteria occurred
- Some introduced genes appear to be involved in important physiological functions and have been fixed during evolution, because of the selective advantage they provide
- Howard M. Temin (1934-1994) proposed the retroviral origin of cancer (Rous Sarcoma Virus - RSV). In theory, retrovirus can transfer the oncogene from one individual to another that may lead to oncogenic transformation
- The available carrying capacity for retroviral vectors is ~7.5 kb (Verma, Somia, 1997), which is too small for most genes. Are there any others mechanisms for lateral gene transfer into eukaryotes?

Lander et al., 2001

Bottle neck Effect, C-value Paradox, Red Queen Effect



- How can I transform a big multicellular organism? I have to introduce DNA on the single cell stage, into ovum or spermatozoon. How could a selfish DNA attack the genome? Possibly, the selfish DNA does so am I!
- C-value (Thomas, 1971) is a term for the DNA content of a cell. The greatest range of variation occur in unicellular eukaryotes: the range from yeast to amoeba is 80000-folds, and within green algae is 3000-fold. Why is the amount of non-coding DNA so great, and so variable?
- What about a relationship between the selfish DNA and genome? Is it the arms raise or cooperation?

Brief history of sperm-mediated gene transfer

Brackett at al.	Uptake of heterologous genome by mammalian spermatozoa and its transfer to ova through fertilization	1971
Lavitrano et al.	Sperm cells as vectors for introducing foreign DNA into eggs: genetic transformation of mice	1989
Brinster at al.	No simple solution for making transgenic mice	1989
Khoo et al.	Sperm cells as vectors for introducing foreign DNA into zebrafish	1992
Tsai at al.	Sperm as a carrier to introduce an exogenous DNA fragment into the oocyte of Japanese abalone (*Haliotis divorsicolor suportexta)*	1997
Spadafora C.	Sperm cells and foreign DNA: a controversial relation	1998
Perry et al.	Mammalian transgenesis by intracytoplasmic sperm injection	1999
Lavitrano et al.	Efficient production by sperm-mediated gene transfer of human decay accelerating factor (hDAF) transgenic pigs for xenotransplantation	2002
Chang et al.	Effective generation of transgenic pigs and mice by linker based sperm-mediated gene transfer	2002

Sperm cells are ideal vehicle for selfish DNA

- Spermatozoa move to ova, which release an attractant. They compete for the ova
- HIV-1 binds with CD4 protein on the surface of sperm cells
- Sp from echinoids to man in certain conditions can take up foreign DNA. Motile SP capture the DNA better than nonmotile ones (Horan et al., 1991), but high DNA concentrations inhibit SP motility (Schit et al., 1998)
- More active spermatozoa arrive the ova
- Sperm nucleases are activated in response to the internalization of foreign DNA by sperm cells and cleave the DNA; the activity increases with the DNA concentration, i.e. critical amounts of DNA are 10 ng/10^6 epididymal mouse spermatozoa, 40 ng/10^6 epididymal boar spermatozoa, and 500 ng/10^6 ejaculated boar spermatozoa (Maione et al., 1997)

SMGT in lab condition

- Control loach fry (*Misgurnus fossilis L.*) – mock analysis
- Experimental β-gal-positive fry 72 h after the eggs fertilization by sperm cells transfected with pcDNA3-*lacZ*

Expression observed in the covering sells of the axial organs, fin edge, and yolk-sac wall

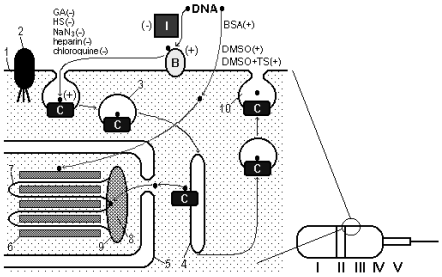
Sperm cells were squeezed out of the loach testis and intensively washed. Electric discharge (V=150 V, R=150 Ω, C=20 μF) was passed through the cell's suspension containing 0.5 μg/ml DNA. Transfected sperm was added to eggs for fertilization. After development the embryos were fixed with 2.5% glutaraldehyde and stained by X-gal

Andreeva et al, 2003

Proposal mechanism of DNA penetration into SP

Blue line - natural way, red line - artificial way; 1 - spermatozoon plasmalemma, 2 - cytoskeleton-bound protein to separate membrane domains, 3 - vesicle, 4 - DNA release from vesicle into cytoplasm, 5 - nuclear envelope, 6 - protamines, 7 - chromosomal DNA loop, 8 - scaffold, 9 - nuclear annulus, 10 - DNA release from vesicle out of cell; I - inhibition factor, B - binding protein (30-35 kD, MHCII), C - capture protein (CD4); +/- - facilitation / retardation of DNA uptake

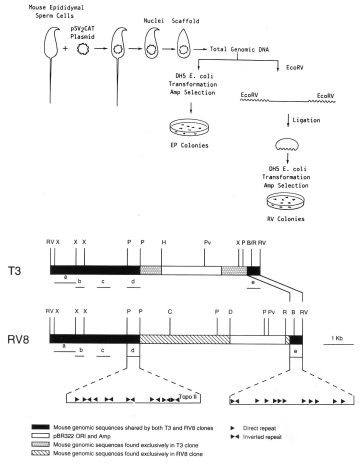
I - acrosomal cap, II - equatorial segment, III - head postacrosomal region, IV - posterior ring, V - middle piece



Kuznetsov et al., 1998

Integration of foreign DNA into mouse sperm genome

- pSV2*cat* is rescued from mouse sperm genome (EP – episome, RV – EcoRV digestion and ligation)
- Two mouse DNA sequences, identical in T3 and RV8 clones (solid), flank unidentified DNA (dotted, hatched), within which the plasmid (open) has been integrated (Amp^R and *ori* of pSV2*cat*: 707-2542 in T3, 751-2620 in RV8). TopoII consensus sequence adjacent to one end of the integration site; b,c,e – Alu-like repeats.
- As a result of hybridization the same (solid) sequences present in 14 randomly selected clones!



Zoraqi, Spadafara, 1997

Conclusion for experiments

- Sperm cells take up DNA, giving them the double function of acting as a vehicle for transmitting not only their own but also foreign DNA
- Rescued plasmids were heavily rearranged, because sperm cells have enzymes, able to mediate DNA rearrangements
- pSV2*cat* plasmid integrated into 'acceptor' genomic site in the sperm DNA
- Random chromosomal DNA sequences appeared to integrate together with the plasmid DNA in the same genomic site

Set of rules for SMGT

- Spermatozoon looks for ova by chemical gradient
- Actually DNA is transmitted by Mendel's rules
- Sperm cells can take up any DNA from environment
- 2 rules for SP movement:
  - high amounts of DNA inhibit SP movement
  - low amounts of DNA activate its movement
- 2 rules for DNA integration:
  - high amounts of DNA lead to it fragmentation by sperm nucleases
  - low amounts of DNA don't activate those nucleases
- After host death, DNA are fragmented and released into environment

- Observed structure:
  - DNA-uptake switch consist at least of 2 proteins, which bind to DNA, and 1 protein, which prevents DNA interaction
  - DNAs integrate into preferential sites of genome

Bottom-up modeling

- SMGT is a global net! I don't know the result of modeling. It's OK!

- What brings us the analogies between biology and mathematics?
- That about 'SP' (StarLogo Programming, not sperm :), which means the simulation of massively parallel systems?

?