The rules of sperm-mediated gene transfer

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Abstract

Sperm cells from echinoids to man under certain conditions take up foreign DNA and internalize it into the genome. This evidence was enchased in labs. The question– Can sperm cells take up the foreign DNA in nature? – is open. Some mobile DNA elements transpose from genome to genome. Sperm cells can bee seen as an ideal vehicle for the selfish DNA. Dual role of sperm cells for transport of the own haploid genome and the selfish DNA is conceded. The set of rules for bottom-up modeling are proposed.

Vocabulary

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)

Vertical and horizontal gene transfer

Gene transfer from parents to progeny has been defined in biology as a vertical inheritance. Gene transfer between different organisms was determined as a horizontal or lateral transfer (Fig.1).

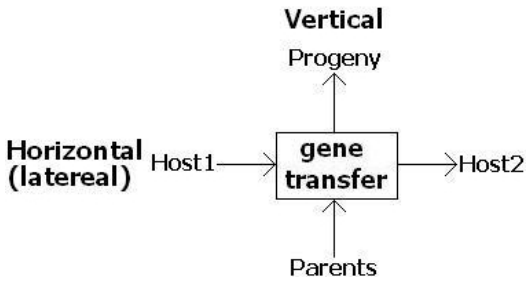

Fig.1 Vertical inheritance within host and horizontal transfer between hosts.

Study of lateral as well as vertical gene transfer has a long hard history. In this paper only the horizontal inheritance is discussed, which is divided into three categories: gene transfer from bacteria to bacteria, from bacteria to eukaryotes, and from eukaryotes to eukaryotes. The last two ones are most speculative.

Gene transfer from bacteria to bacteria

Transformation – gene transfer by uptake and incorporation of exogenous DNA. In 1928, Griffith showed that genetic information can be transferred from the capsule strain of *Streptococcus pneumonia* to the noncapsule strain *in vivo*. In 1944, Avery, Macleod and McCarty discovered that the transforming agent is DNA.

Conjugation – a 'sex' process in bacteria was discovered by Lederberg and Tatum in 1946. They observed the transfer of genetic properties from one auxotroph strain of *Escherichia coli* K12 to another.

Transduction – bacteriophage-mediated gene transfer. In 1956, Morse and coworkers described the transduction phages, which can transfer a fragment of host chromosome from one bacterium to another.

Competence – ability of cell to take up DNA, i.e. Gram-negative bacteria, (*Haemophilus*, *Neisseria*, *Helicobacter* and *Acinetobacter*) as well as Gram-positive bacteria (*Bacillus*, *Mycobacterium* and *Streptomyces*). In case of *Bacillus subtilis*, DNA binds to the cell surface, probably via a type IV pilus. One of the two DNA strands is taken up while the other strand is degraded on the external surface of the cell. The uptake system of *Bacillus subtilis* consists of ComEA, ComEC and ComFA proteins. ComEA is a dsDNA-binding receptor that extracts the DNA molecule from the type IV pilus and feeds it into the transmembrane channel. It exhibits no sequence specificity. The rate of transport is about 200 nucleotides/sec.

The uptake and stable maintenance of extracellular DNA – *genetic transformation* – is a major force in microbial evolution.

Gene transfer from bacteria to eukaryotes

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

Sequencing of the human genome and lateral gene transfer into eukaryotes

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 (Lander et al., 2001).

Howard M. Temin (1934-1994) investigated Rous Sarcoma Virus (RSV); he proposed the retroviral origin of cancer. 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 other mechanisms for lateral gene transfer into eukaryotes?

Mobile elements are Selfish DNA

Mobile DNA elements are widespread in bacteria, plants and animals. The study of bacterial genomes has revealed an amazing plasticity that is mediated by mobile DNA, contributions to pathogenicity and to bacterial antibiotic resistance. The mobile DNA provides rearrangements – 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.

Many kinds of mobile DNA were discovered. Here are some examples of related mobile elements: for bacteria – IS-elements (insertion sequences) and ampicillin resistance transposon Tn3, both in gram-negative and gram-positive bacteria; for *Saccharomyces cerevisiae* – Ty1-element; for *Drosophila melanogaster* – *copia*-element; for plants – *Ac* in maize; for mammals – LINE (long interspersed nuclear elements) and SINE (short) elements (Craig et al., 2002).

Analysis of bacterial genomes has revealed an extraordinary amount of gene transfer between bacteria. The canonical example of lateral transfer for eukaryotes is movement of P-element from *Drosophila willistoni* to *D.melanogaster*, an event that might have occurred last century (Daniels et. al., 1990). Transmission of *D.melanogaster* δ-element through spermatozoa was established (Minamori, 1971, 1972).

There are some theoretical questions and paradoxes concerning with the mobile DNA elements. Here are some of them.

Bottle neck Effect

Imagine a situation. How can I transform a big multicellular organism? Of cause, I have to introduce DNA on the single cell stage, into ova or spermatozoon. How could a selfish DNA attack the genome? Possibly, the selfish DNA does so am I! Selfish DNA attacks the germ cells.

C-value Paradox

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?

Red Queen Effect

Next question – What about the relationship between the selfish DNA and genome? Is it the arms raise or cooperation? I do not know the answer this question.

Experimental data

History of sperm-mediated gene transfer

In 1971, Brackett, Baranska, Sawicki, and Koprowski from Pennsylvania University described the phenomenon of transfer of foreign DNA in ova by spermatozoa. The property of SV40 viral DNA to infect CV-1 cells in culture was used. Rabbit spermatozoa were treated with SV-40 DNA and used for artificial insemination. Zygotes and 2-cell embryos were removed from oviducts and CV-1 cell were cocultivated as a test. Cytopathic effect was found. It showed the SV-40 DNA transmission. Unfortunately this pioneering work was ignored by scientific community.

In 1989, Lavitrano and coworkers rediscovered this phenomenon and generated transgenic mice. Epididymal sperm cells were incubated with pSV2*cat* plasmid followed by *in vitro* fertilization and implantation of 2-cells embryos into foster mothers. Transgenic progeny were obtained, which then transmitted transgene to the next generation.

This paper was received with skepticism and generated much debate, because the experiment was unsuccessful in other laboratories. Brinster et al. (1989) had published summarized negative results from 8 laboratories.

In this time and later new data appeared for other species, i.e. sea urchin (Arezzo, 1989), bee (Milne et al., 1989), fish (Muller et al., 1992). In some papers spermatozoa were treated DNA with lipofection or electroporation. However the transgene integration into genome is not proved in many cases. Experiments showed that exogenous DNA can be eliminated in the course of embryo development. If the stable integration of foreign DNA in genome had been found, the transformed sperm cells could be used for production of transgenic animals, birds and fish.

In 1992, Khoo et al. published the results of gene transmission into F3 zebrafish generation. In 1997, Tsai at al. used the shellfish Japanese abalone in the experiment. Next in 1998, Spadafora published the first revue and explained some results. Perry at al. (1999) proofed sperm-mediated gene transfer by intracytoplasmic sperm injection. Some biologists began to realize the role of sperm cells in general biological processes (Smith. 2002). At last in 2002, new results on pigs appeared from two laboratories (Chang et al., 2002; Lavitrano et al., 2002).

Sperm cells are ideal vehicle for selfish DNA, because SP can take up foreign DNA

Spermatozoa move to ova, which release an attractant. They compete for the ova. In a matter of fact, HIV-1 binds with CD4 protein on the surface of sperm cells that next can lead to infection. In addition, the reverse transcription activity was discovered in SP (Giordano et al., 2000).

Sperm cells from echinoids to man under certain conditions can take up the foreign DNA. Motile spermatozoa 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 come up 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 the lab condition

There are my own experiments on rabbits and fish – loach (*Misgurnus fossilis* L.), which was firstly described by J.B. Lamarck. Our team studied the expression of CMV-*lacZ* gene in loach fry after SMGT (Andreeva et al., 2003).

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.

The β-galactosidase was expressed as blue spots, dots and dashes. The expression was observed in the covering sells of the axial organs, fin edge, and yolk-sac wall, because CMVe-promoter acts in the tissues of ectodermal origin. This spotted pattern of expression probably has been as a result of transgene excision from the cell lines.

Fig.2 CMV*lacZ*-gene expression after sperm-mediated gene transfer into loach *Misgurnus fossilis* L. **A**) mock-treated control; **B, C**) experimental group. The β-gal-positive 72 h fry of loach after fertilization by pcDNA3-*lacZ* transfected sperm cells with subsequent electroporation.

Supposed mechanism of foreign DNA penetration into SP, and DNA integration into genome

Mechanism of interaction of SP with DNA was discovered by Corrado Spadafora and coworkers (Spadafora, 1998). In Fig.3 is a scheme of natural and artificial ways for exogenous DNA into sperm cells. An 'Inhibition'-factor prevents DNA binding to B-protein. In the absence of this factor, DNA interacts with the 'Binding'-protein moving it then to C-protein. The 'Capture'-protein internalizes DNA into spermatozoon due to endocytosis. Later, a portion of the DNA is released from a vesicle into cytoplasm to enter the nucleus. Probably, the DNA comes into contact with the nuclear matrix to integrate then into the foot of chromatin loop domain. The remaining portion of the DNA is eliminated outside from the vesicle. By electroporation or with added DMSO, the DNA overcomes cell's barriers to bind then to chromatin (Kuznetsov et al., 1998).

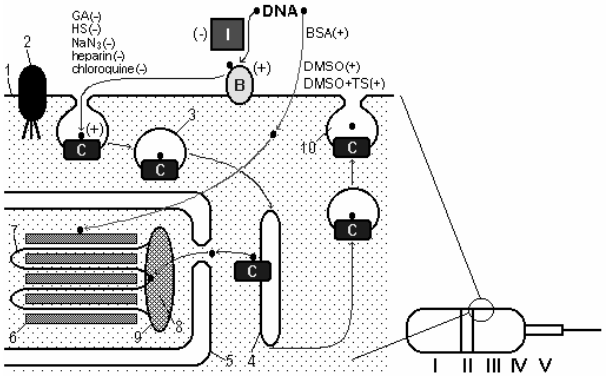

Fig.3 Mechanism of foreign DNA penetration into spermatozoon;
I – IF-1,
B – MHCII,
C – CD4.

Evidence of foreign DNA integration into sperm genome was described by Zoraqi and Spadafora (1997). They rescued plasmid pSV2*cat* from mouse sperm genome. Two mouse DNA sequences, identical in two independent clones (T3 and RV8), flanked unidentified DNA, within which the plasmid had been integrated. As a result of hybridization the same sequences were observed in 14 randomly selected clones.

C. Spadafora wrote 'Mouse epididymal sperm cells have the spontaneous ability to take up exogenous DNA. A proportion of the sperm-bound DNA is further internalized into sperm nuclei. We have found that the internalized plasmid DNA becomes tightly associated with the nuclear scaffold, is extensively rearranged, and undergoes recombination with the sperm genomic DNA. Sequence analysis of two randomly selected clones independently recovered by plasmid rescue from pSV2CAT plasmid-challenged sperm cells shows that DNA fragments from the plasmid are integrated into the mouse sperm genome. The sites of integration are identical in both clones, suggesting that these events do not occur randomly, but take place at preferential sites. A topoisomerase II consensus sequence is found adjacent to one end of the integration site, suggesting a possible role of this enzyme in the process of nonhomologous recombination'.

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

Information for the modeling

The data above show that sperm cells can provide the integration of DNA fragments into genome. These evidences were defined as 'DNA percolation' (Kuznetsov et al., 1998). Possibly, sperm-mediated gene transfer can be massive and parallel in populations. Experiments have revealed some elemental rules of SMGT and the molecular mechanism of DNA uptake by SP that allow us to simulate this process 'bottom-up' on computer. Some analogies between Biology and Computer Science may be useful for imagination, i.e. Genetic transformation and Programming, DNA integration and Program concatenation, DNA manipulation and String operators, Gene expression and Program execution.

SMGT rules

- 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

Identified 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

Conclusion – SMGT as a global net

Presented data lead to consider the SMGT as a global net for transport of selfish DNA. Its role for biology is not understood. The behavior of this system will be investigated. Could we extrapolate the principles of sperm-mediated gene transfer onto technical devices and artificial systems? What brings us the analogies between biology and mathematics? That about 'SP-algorithm' (stream programming, not sperm), which means a massively parallel transfer of information between hosts?

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