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Microbial Gene Transfer: An Ecological Perspective

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Abstract

Microbial gene transfer or microbial sex is a means of exchanging loci amongst prokaryotes and certain eukaryotes. Historically viewed as a laboratory artifact, recent evidence from natural populations as well as genome research has indicated that this process may be a major driving force in microbial evolution. Studies with natural populations have taken two approaches- either adding a defined donor with a traceable gene to an indigenous community, and detecting the target gene in the indigenous bacteria, or by adding a model recipient to capture genes being transferred from the ambient microbial flora. However, both approaches usually require some cultivation of the recipient, which may result in a dramatic underestimation of the ambient transfer frequency. Novel methods are just evolving to study *in situ* gene transfer processes, including the use of green fluorescent protein (GFP)-marked plasmids, which enable detection of transferrants by epifluorescence microscopy. A transduction-like mechanism of transfer from viral-like particles produced by marine bacteria and thermal spring bacteria to *Escherichia coli* has been documented recently, indicating that broad host range transduction may be occurring in aquatic environments. The sequencing of complete microbial genomes has shown that they are a mosaic of ancestral chromosomal genes interspersed with recently transferred operons that encode peripheral functions. Archaeal genomes indicate that the genes for replication, transcription, and translation are all eukaryotic in complexity, while the genes for intermediary metabolism are purely bacterial. And in eukaryotes, many ancestral eukaryotic genes have been replaced by bacterial genes believed derived from food sources. Collectively these results indicate that microbial sex can result in the dispersal of loci in contemporary microbial populations as well as having shaped the phylogenies of microbes from multiple, very early gene transfer events.

Introduction

Gene transfer can generally be regarded as prokaryotic sex, or a mechanism to "mix genes" between prokaryotic community members. However, the process of prokaryotic gene transfer is dramatically different from sex as it occurs in dioecious higher organisms. First, it is usually not a component of the cellular life cycle in prokaryotes. This might be contested in the case of transformation, as the genes for competence development undergo activation at discreet times in the growth cycle of batch cultures (Smith *et al.*, 1981). Secondly, the frequency of transfer is usually quite low. The frequency of transfer is expressed as the number of transferrants (*i.e.* cells which represent the successful acquisition of a gene) divided by the number of total recipients (or donors) present. Very high transfer frequencies (usually conjugation) are 10^{-1} to 10^{-3} transferrants per recipient, while transfer in the environment can be as low as 10^{-8} to 10^{-9} (Frischer *et al.*, 1994, Jiang and Paul, 1998). Even though the transfer frequencies observed may be low, this does not mean that gene transfer has had very little impact on microbial communities. Low frequencies of transfer over geologic time is believed to be a major driving force in microbial evolution (Pennisi, 1998).

Unlike sex in higher organisms, only one or a very limited number of closely linked loci are transferred at any one particular time. Entire genomes are never recombined, as for diploid organism. This leads to "micro-evolution of loci". And certain loci are known to transfer more frequently than others.

Lastly, the limit on the nature or genetic relatedness of the participants does not have the same restrictions as that of dioecious organism. The "species concept" or producing fertile offspring has no meaning in prokaryotic sex. Indeed, the diversity in partners participating in prokaryotic sex is not even limited by kingdoms.

Gene transfer was regarded as a laboratory artifact for many years. The potential use of recombinant microbes in the environment stimulated gene transfer research in the 1980's. This required development of methods to try to detect gene transfer, both in the lab and in the environment. As a result, there have been three general approaches to investigating the how, when, and why of environmental gene transfer. The first is to add an easily detected gene to the environment and investigate acquisition by the indigenous flora (Frischer *et al.*, 1994). This is perhaps most satisfying in that one can detect the exogenous gene by probing or PCR (Williams *et al.*, 1997). The only caveat is to be certain that signal detected is from the gene in a recipient and not in the donor that was added (termed counter selection against the donor). The second is to add a recipient and detect transfer of an indigenous marker to the recipient. This is clearly more difficult because one often doesn't know what to look for in terms of phenotype or genotype. The third approach is to detect acquired genes in the genomes of environmental isolates. This is usually done by sequence analysis of loci in question and finding divergent sequences. This is satisfying because it affirms the importance of gene transfer. However, the questions of when, how, and by what mechanism are then merely the subject of speculation. None the less, it is in

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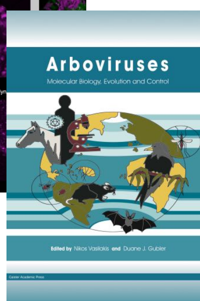

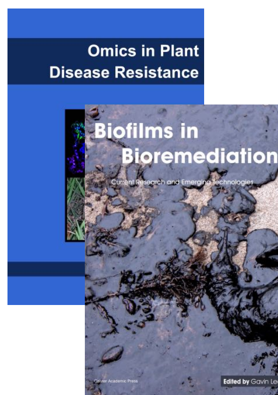

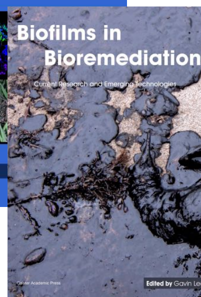

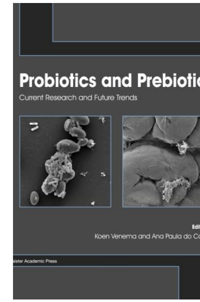

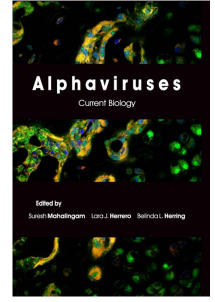

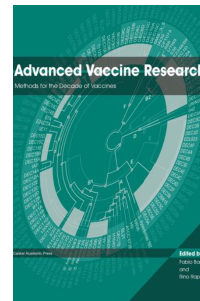

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this arena that gene transfer is experiencing a revival in interest. The sequencing of entire microbial genomes (Fleischman *et al.*, 1995; Frazer *et al.*, 1995; Bult *et al.*, 1996) has shown that microbes are mosaics of acquired genes. In fact, the rooting of the universal tree is highly problematic because of multiple transfer events between the kingdoms.

In this review I have focused on gene transfer to indigenous organisms in the environment, paying less attention to laboratory and microcosm studies. Because much work has been done on conjugation and less on transduction, I emphasize some of the more startling findings coming from the latter field. Finally, how gene transfer has impacted our understanding of molecular phylogeny will be examined.

Mechanisms of Transfer

The three recognized mechanisms of prokaryotic gene transfer are transformation, conjugation, and transduction. These mechanisms should be viewed as a framework for conceptualizing gene transfer in the environment. However, other novel mechanisms may exist, and combinations of mechanism may occur in the environment.

Transformation was the first mechanism of gene transfer to be recognized (Griffith, 1928) and its discovery paved the way for understanding that DNA was the genetic material in all cells. Transformation involves the uptake and expression of genes encoded in extracellular DNA. Unlike conjugation and transduction, transformation is a normal, physiological function of certain bacteria and is mediated by chromosomal genes (Smith *et al.*, 1981). A distinction is made between natural transformation and artificial transformation in regard to competence, or the capability to uptake DNA. Naturally competent bacteria express competence at some time in their life cycle, whereas artificially induced competence, as used in plasmid transformation in *E. coli*, is the result of chemical or physical perturbation of the cell membrane/wall to enable covalently close plasmid penetration (Mandel and Higa, 1970).

Conjugation is a plasmid or transposon encoded mechanism of transfer that requires cell contact, first described by Lederburg and Tatum (1946). Because of their location on plasmids or transposons, the genes transferred in conjugation generally encode accessory functions such as antibiotic, UV, and heavy metal resistance or expanded metabolic capabilities (*i.e.* Xenobiotic degradation; Willets and Wilkins, 1984). Conjugation has often been viewed as the most promiscuous of the three transfer mechanisms because of the lesser restriction on similarity of recipient to donor imposed by transformation and transduction.

Transduction, which literally means "carrying over', is the process of gene transfer whereby a phage mistakenly packages some host DNA in the capsid and transfers it to another bacterium upon subsequent infection (Zinder and Lederburg, 1952). This process can result in either the transfer of a random fragment of the host genome or plasmid (termed generalized transduction) or, when a temperate phage is employed, specific genes which flank the place of prophage integration (Ackermann and DuBow, 1987). Although perhaps the least understood mechanism of environmental gene transfer, recent findings indicate that this process may be occurring more frequently in the marine environment than previously thought.

Other methods of gene transfer include capsduction, or gene transfer by a small phage-like structure (Joset and Guepsin-Michel, 1993), protoplast fusion (Matsushima and Baltz, 1986) and transposition.

Transformation

The potential for gene transfer by transformation in the environment was first demonstrated by Graham and Istock (1978) using chromosomal loci of *Bacillus subtilis* in sterile soil. Since these early studies, many reports have described transformation in sand/seawater microcosms, although most often with sterile sand and *B. subtilis* as recipient (Aardema *et al.*, 1983; Lorenz and Wackernagel, 1987; Lorenz and Wackernagel, 1994). These studies have indicated that DNA binds to sand, becoming protected from DNase, but still capable of causing transformation.

Copious amounts of high MW dissolved DNA in the marine environment (DeFlaun *et al.*, 1987; Paul *et al.*, 1987) suggested that transformation could be a viable mechanism of gene transfer. Gene transfer to a High Frequency of Transformation *Vibrio* was demonstrated using plasmid multimers and seawater microcosms (Paul *et al.*, 1991) The presence of the ambient microbial community in water column simulations either had no effect on the rate of transfer or inhibited transfer. Nutrients stimulated the transfer rate. These results suggested that nutrients facilitated transfer, probably by stimulating recipient growth, and by providing a preferred carbon source (over the transforming DNA) from the indigenous community. At low nutrient conditions, we hypothesized that the DNA was used as a C/N/P source by the indigenous flora, and was not available to transform the recipients. In sediments, the presence of the indigenous flora inhibited gene transfer, whereby sterile sediments enabled transfer to occur (Paul *et al.*, 1991). In soil, *Acinetobacter calcoaceticus* could only be transformed in nonsterile microcosms when excess nutrients were present (Nielsen *et al.*, 1997). Thus, it sees that studies with sterile sediments or soil do not yield relevant information concerning transfer in the marine environment.

The facilitation of natural transformation by cell contact which was DNase sensitive was demonstrated by Stewart *et al.* (1983) in *Pseudomonas stutzeri*. The intergeneric transfer of a small, non- conjugative plasmid from *E. coli* to *Vibrio* JT-1 (later identified as a *Pseudomonas* species) occurred and was shown to be DNase-sensitive (Paul *et al.*, 1992). Heat -killed donor cells were as efficient as living cells, and interestingly transfer in liquid yielded a 10-fold higher frequency of transfer than filter matings.

Environmental conditions in microcosms may reveal gene transfer properties of bacterial strains not revealed by culture studies using rich media. For example, *E. coli* has been known for some time not to be naturally competent, only being transformable by chemical treatment, osmotic shock, and electroporation. Using river water, spring water and mineral water, *E. coli* was shown to be naturally competent for the uptake of pUC18 DNA (Baur *et al.*, 1996). Competence seemed to be internally regulated, being greatest in exponential phase and least in stationary phase. This work argues for the potential for *E.coli* (and perhaps other coliform bacteria) to be transformed in natural environments.

Transfer to the Indigenous Community

The capability to measure transformation of DNA in natural microbial communities has challenged investigators for years. Nearly all such attempts have used closed microcosms. However, the work of Day and Fry (Williams *et al.*, 1992; Williams *et al.*, 1996) is unique in that an indigenous mercury resistance plasmid (pQM17) was used in the river from which it was isolated in open filter matings. Stones with filters containing the donors or DNA were placed next to stones containing recipients. Transfer occurred with frequencies ranging from 10^{-6} to 10^{-4} per recipient. A significant effect of temperature was observed, with no transfer below 10 °C.

Natural transformation of indigenous marine bacteria has been demonstrated by Frischer *et al.* (1994) using plasmid multimers of pQSR50. Plasmid multimers were used to ensure internal homology and thus provide a site for self-homologous recombination. The ambient microbial communities in water column samples (20 L) or coral mucus (1 L) were first concentrated to approx. 50 ml and 1 ml used in filter transformation assays. For sediments and bacteria in sponge tissue, the bacteria were extracted mechanically and then used in filter transformation assays. Transformation was assessed as expression of the antibiotic resistance genes encoded by the plasmid in combination with confirmation by molecular probing. However, certain environments contained indigenous marine bacteria that possessed sequences that hybridized with the probe used and any such environments were not considered further. Positive transformation was found in 5 of 13 experiments, with transfer frequencies ranging from 3.6×10^{-6} to 1.13×10^{-9}. In all cases of plasmid transfer to the natural community, restriction profiles from transformants were altered when compared to the parent plasmid. Differences in the recovered transformant plasmids were accounted for by difference in methylation compared to the parent plasmid, as well as some genetic rearrangement (Williams *et al.*, 1997). Thus, transfer to the indigenous flora, at least when considering plasmid DNA, can result in rearrangement and alteration of the DNA, contributing to plasmid and recipient evolution.

Conjugation

As mentioned, conjugation is often viewed as the most promiscuous of the gene transfer mechanisms, showing the least restriction in relatedness of host/recipients. Although conjugation has been observed in soils, insect larvae (Vila-Boas *et al.*, 1998) seed surfaces (Lilley and Baily, 1997; Lilley *et al.*, 1996), in raw salmon on a cutting board (Kruse and Sorum, 1994), in porcine feces (Kruse and Sorum, 1994), crown gall tumors (Zhang *et al.*, 1993) and a variety of other environments, few studies have focused on the indigenous flora, particularly in the marine environment.

A problem in the study of gene transfer in aquatic or novel environments is that conjugative plasmids used in such studies are of terrestrial origin and may not be representative of or behave like those indigenous to the environment in question. Dahlberg *et al.* (1997) isolated 95 mercury resistance conjugative plasmids from a variety of marine environments by transfer to a model recipient. There was a tremendous diversity in plasmids and 12 general structural groups were differentiated. Plasmids from different environments showed little similarity as determined by RFLP's. The plasmids were further-tested for similarity to known plasmid incompatibility groups using inc/rep probes (Couturier *et al.*, 1988). Hybridization studies showed no similarity between any of the twelve groups of naturally occurring marine plasmids and the well characterized plasmids often used in gene transfer studies. This study shows the need to develop more environmentally relevant plasmids and probes for the study of conjugation *in situ*.

Sandaa and Enger (1994) noted transfer of the highly promiscuous plasmid pRAS1 from the fish pathogen *Aeromonas salmonicida* to the indigenous flora in marine sediments. If oxytetracycline was added as a selective pressure, transfer frequencies increased to 3.4×10^{-1} transconjugants per recipient and 3.6 transconjugants per donor, perhaps the highest reported gene transfer frequency for any environmental gene transfer study. The plasmid was transferred to a variety of biotypes as determined by phenotypic characterization of the recipients. The transfer frequencies expressed per recipient are undoubtedly overestimates, because recipients were enumerated as cultivatable CFU's and not direct counts. None-the-less, this work illustrates the promiscuity of certain conjugative plasmids, and the ease at which antibiotic resistances can spread through natural marine bacterial communities.

Perhaps one of the most elegant demonstrations of conjugation to the indigenous marine flora was the work of Dahlberg *et al.* (1998). These investigators used the conjugative plasmid pBF1 which contains the green fluorescent protein (GFP) gene in *Pseudomonas putida* as a donor. This gene is not expressed in the donor because of a chromosomal repressor. Gene transfer was noted when cultivated recipients were used as well as the natural marine population. Transfer to the indigenous population could be observed directly by epifluorescence microscopy, without the need of cultivation of transconjugants. Transfer frequencies ranged from 2×10^{-6} to 1.4×10^{-4}, with transfer highest in filter mating compared to bulk matings. The plasmid used also encoded for mercury resistance, but selection in mercury did not facilitate or enhance transfer frequencies. Thus, selective pressure may or may not enhance transfer to the indigenous population.

Transduction

The discovery of the tremendous number of viruses in aquatic environments (Berg *et al.*, 1989; Proctor and Fuhrman, 1990; Paul *et al.*, 1991) has raised the issue of these as potential gene transfer vectors. The elegant studies of Miller and coworkers with microcosms in lakes using the *P. aeruginosa* system (Miller *et al.*, 1977; Saye *et al.*, 1987; Ripp and Miller, 1995) has demonstrated the potential for transduction of both plasmid and chromosomal markers. Frequency of transduction was approximately 10^{-7} per recipient.

Jiang and Paul (1998) described plasmid transduction of a lysogenic marine phage host system, ϕHSIC/HSIC. Concomitant with acquisition of plasmid DNA was the acquisition of a portion of the viral genome, as indicated by molecular probing with a 100 bp gene fragment of the transducing phage. Transfer frequencies were quite low (10^{-9} to 10^{-7}).

To investigate transfer to the indigenous marine population by transduction, Jiang and Paul (1998) produced lysates from host bacteria that contained the plasmid pQSR50. The lysates were then added to concentrated indigenous marine microbial communities. One phage, ϕD1B, yielded transductants of the natural population. Transductants were verified by probing with the Tn5 sequences. As found for natural transformation of plasmid DNA, restriction profiles were modified in transductants. Amplification of the transductants using plasmid specific primers indicated correct amplification of the kanamycin resistance gene but that a restriction site was missing from another portion of the plasmid. This indicates that , as with natural plasmid transformation, some rearrangement/ recombination of the plasmid DNA occurred by the natural community.

Perhaps the most compelling and unique evidence for transduction comes from the work of Chiura and coworkers (1997, 1998). These investigators observed VLP (virus-like particle) production in five marine bacterial cultures, with the ratio of viruses to host approximately 1.0. These VLP's could transfer amino acid prototrophy to an auxotrophic *E. coli* strain. The viruses were lethal to the *E. coli*. The gene transfer frequency was between 2.6×10^{-3} to 3.58×10^{-5} per VLP. These results suggested that some type of generalized transduction was occurring, and that such gene transfer could occur using a very broad host range of donors. Unfortunately, because there were no probes available for the loci transferred, it was not possible to verify gene acquisition by probing.

These results were quite surprising because the viral-host interaction is viewed to be quite specific. However, recent findings on the host range of marine Myoviridae indicates that some phages can be quite broad host range (Wichels *et al*., 1998). Therefore, broad host range transduction may be an important mechanism of horizontal transfer in the marine environment. Supporting this concept were other findings of Chiura *et al*. (1998) using VLP's that were produced by natural communities of geothermal hot spring bacteria. These VLP's could transduce *E. coli* to prototrophy, with an average transfer frequency of 10^{-6}/ VLP. VLP's were purified from natural populations without cultivation and these also caused transfer to prototrophy. The mechanism of transfer to prototrophy was not known but believed to involve simple complementation of the mutant. Collectively these results indicate that gene transfer by transduction can occur across wide taxonomic boundaries in the marine and hot spring environments.

Gene Transfer and Microbial Evolution

Not too long ago, attributing an unusual genomic finding to a lateral transfer event would have been met with general skepticism. Irregularities in phylogenetic trees often were thought to be caused by errors in tree construction. However, complete genome analysis has made the concept of gene transfer actually indispensable to understanding microbial evolution and phylogeny in general.

"Lateral gene transfer is not just a molecular phylogenetic nuisance supported in evidence by a few anecdotal cases. Instead, it is a major force, at least in prokaryotic evolution." Doolittle and Logsdon, 1998.

The molecular evidence for gene transfer is overwhelming and leading to a general acceptance of this concept. For example, Lawrence and Roth (1996) concluded from the examination of the bacterial genome that it was a mosaic of ancestral chromosomal genes interspersed with some recently transferred operons that encode peripheral functions. The successful transfer of complete functions is facilitated by genes which are in clusters, opposed to those that are spread out throughout the genome. Thus, functions encoded by genes within close proximity of one another have a greater likelihood of being transferred than those which are on different parts of the chromosome.

The sequencing of complete genomes of archaea, bacteria, and eukaryotes has clearly "shaken the tree of life" (Pennisi, 1998). The concept that organelles in eukaryotes arose from endosymbiotic capture (Margulis, 1970) actually represents a large scale gene transfer event. rRNA phylogeny had endorsed a tree that included a primordial progenitor that gave rise to bacteria, and then later a second branch which diverged into eucarya and archaea. However, new evidence suggests that both bacterial and archeal genes are showing up in eukaryotes (Pennisi, 1998). Additionally, archaea have many eukaryotic traits (Doolittle and Logsdon, 1998). It now appears that early organisms stole genes from branch to branch, either from food or swapping genes with neighbors. Thus, it is difficult to make sense of phylogenies because of all the gene swapping, and different phylogenies are obtained depending on what genes are examined. For example, the archaea look fairly homogeneous in terms of 16S rRNA phylogenies, but the CTP synthetase gene spread archaea amongst all other organisms evaluated (Penninsi, 1998). The spirochaete *Treponema palladium* contains two Archeal ATPases.

Archaea are now recognized as having mixed heritage. The genes for replication, transcription, and translation are all eukaryote-like in complexity (Doolittle and Logsdon, 1998). However, the genes of intermediary metabolism are purely bacterial. The Archaeal genome sequencing papers indicate that the genes for replication, transcription, and transduction form an enduring cellular hardware, whereas the other genes for biochemical functions are a type of software, and prone to change.

A major mechanism for acquisition of genes by eukaryotes may have been grazing on prokaryotes and/or eukaryotes ("you are what you eat"; Doolittle, 1998). That is, over time, there has been a tendency to replace true eukaryotic or archeal genes in eukaryotes with those coming in from the food source. Although such transfer may be infrequent, there is a constant influx of genes, and once a eukaryotic gene is lost, it is lost for good. The replacement of archaeal genes in eukaryotes was thought to occur quite early in the evolution of eukaryotes, and that archaeal genes are found at all is surprising (Doolittle, 1998).

Perhaps one of the most unique horizontal transfer events to have occurred was from bacteria to vertebrates. The rearrangement of antibody and T-cell receptors is indispensable to the vertebrate immune response, and is conferred by the recombination activating genes I and II (RAG I and II) (Bernstein *et al*., 1996) An examination of one of the most primitive jawed vertebrates, the carnachine sharks, showed that the sequence of RAG I and RAG II were most closely related to the integrase family of genes

and the integration factors, respectively, of bacterial site-specific recombination systems. The apparent "Big Bang" of ancestral marine systems that occurred in jawed vertebrates was apparently due to transfer of microbial site specific recombinases.

Concluding Remarks

Our understanding of microbial gene transfer has evolved through the use of the three approaches described in the introduction. If understanding the mechanism of gene transfer is important, then using a defined donor system and the indigenous population as recipient is the best approach. Gene transfer can be confirmed by detection of the transferred DNA by probing. If the capability for transfer to occur is important and the presence of donors needs to be known, then adding a universal recipient to the system is the best approach. If one doesn't care at all about the mechanism but that only a transfer has occurred throughout the evolutionary history of the organism, then genome analysis is the appropriate approach.

The mechanisms of gene transfer, described in detail for type strains, have provided a framework for the understanding of environmental gene transfer. Sterile microcosm studies with known donors and recipients have provided some valuable information on the potential for transfer to occur, but may be misleading because of the complications that the indigenous flora impose. Additionally, terrestrially-derived models of gene transfer may have no validity in marine or other environments. There is a need for the isolation and description of marine gene transfer systems, which may reveal novel mechanisms of transfer. Indeed, the division of the three mechanisms of gene transfer (transformation, conjugation, and transduction) may diffuse into grey areas of overlap when describing transfer in natural environments. It can be envisioned that a phage may lyse a bacterium containing a conjugative plasmid, which may transform a bacterium, and then be transferred by conjugation to second recipient. Transducing phages could conceivably carry conjugative plasmids or transposons.

In view of all the evidence for gene transfer, perhaps the focus now should not be on "does gene transfer occur?" or "what genes are the result of lateral transfer" but rather "what prevents certain loci from being transferred?" How does an evolutionary anchor get established, or what determines what becomes genetic hardware vs. genetic software? These are all questions worthy of study in the understanding of microbial sex in the new millenium.

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