
Meeting Report

Flux in the horizontal gene pool

(Report on the 3rd Workshop of the European Science Foundation Network on Molecular Biology and Ecology of Plasmid-Mediated Gene Spread, Cuenca, Spain, 12–16 September 1997)

As we accumulate DNA sequence information about an increasing number of bacterial genomes, new ways of looking at the information are required in order to understand the significance of this plethora of detail. The relatively small size of the minimalistic genomes of bacteria such as the mycoplasmas suggests that little more than a few hundred genes are necessary to specify bacterial structure and multiplicity. Additional genes have accumulated in particular species to adapt them to specific niches. Ecological niches are not static, they are highly dynamic. This is not just because of the seasonal variations and the march of geological time, but also because of the action of the organisms themselves which inhabit the environment. The interplay between organisms continually generates new opportunities for variant offspring to do better than their parents.

The last 50 years have demonstrated, in the most dramatic way possible, the enormous potential of mobile genetic elements (plasmids, phage and transposable elements) to allow rapid evolution of bacterial populations. Antibiotics which were regarded as the miracle of modern medicine have been rendered of limited use in many contexts because of the spread of plasmidborne resistance genes. Detailed study of plasmids has identified the replication functions, the stable inheritance mechanisms and the conjugal transfer processes that they encode and these genes, especially the ones for conjugal transfer, are starting to make some sense of the apparent plasticity of the bacterial genome. Werner Pansegrauf (Leiden, The Netherlands) reported that genes already associated with the conjugal transfer of plasmid DNA between bacteria and from bacteria to plant cells have now turned up in association with 'pathogenicity islands' in Helicobacter pylori. This provides further evidence for the underlying unity of genetic exchange/genome plasticity processes in bacteria. That bacteria can evolve very quickly, not just by plasmid transfer, was demonstrated by Larry Forney (East Lansing, MI, USA), who described how adaptation of Alcaligenes to degradation of 2,4-dichlorophenoxyacetic acid occurs rapidly and involves both genomic rearrangements and morphological changes. The genetic changes are likely to involve mismatch repair or recombination and create alternative ways of achieving the same growth advantage. To help understand the role of genetic exchange in this sort of bacterial evolution when the environment changes was one of the main purposes of this workshop, the third in a series organized with ESF support. The aim of the ESF Network has been to bring together molecular biologists and microbial ecologists to create a much richer and broader context in which to explore this topic. A major success illustrated at the meeting was the disappearance of the boundary between these two disciplines.

One of the ways in which barriers have been broken down is by explanation of the fundamental techniques and intellectual approaches of each discipline, prompting dialogue. One of the key issues described by Liz Wellington (Warwick, UK) is the significance of the fact that more than 90% of bacteria from the environment are unculturable. Does this mean that the genes they carry play no role in the gene flux or are they a source of genetic diversity by release or transfer of DNA to the actively growing sector? Techniques are needed to separate culturable from non-culturable bacteria and to then assess whether genes can transfer into or out of the non-culturable component. If there is no genetic exchange, then one may be justified in setting up model systems consisting of just the cultivable organisms. Monitoring the changes in these organisms without culturing them can be facilitated by total DNA extraction from environmental samples. Kony Smalla (Braunschweig, Germany) described a range of techniques which allow non-available data ( Gram, Austria) and reducing inorganic and organic material (such as humic acids) which interfere with most DNA manipulations, so that PCR can be used to detect the presence of specific mobile genetic elements. However, to isolate a mini replicon it is still important to purify DNA of the plasmid in question, create a library of DNA fragments and then determine which one is capable of self-replication in the appropriate host. Martine Couturier (Brussels, Belgium) presented a neat positive selection vector for cloning fragments which could then be screened for replicons or transfer origins. Since the transfer origins are normally dependent on many other genes it is best to screen them in the host carrying the parental plasmid. Ellen Zechner (Graz, Austria) showed how the nicks introduced into one strand at the replication or transfer origin can be detected in vivo by extension from a primer hybridized to total DNA so that the origin can be mapped under physiological conditions and the factors controlling origin activity can be determined. Such an extension of molecular techniques into more complex environments was also illustrated by Soren Molin (Lyngby, Denmark), who described the use of fluorescent probes for in situ identification of species, determination of growth rate and assessment of plasmid-mediated gene spread in mixed populations. Grazyna Jagura-Burdzy (Warsaw, Poland) and Guenther Koraimann (Graz, Austria) talked about techniques for studying protein–DNA interactions. A challenge will be to detect such interactions in more complex environments in vivo or to use the specific recognition of DNA sequences by these proteins as a means of probing events in the bacterial cell. Technical advances will surely develop from this dialogue.

Plasmids of Gram-positive bacteria were a major topic, which prompted examination of the concept 'cryptic' as applied to plasmids. From the molecular studies reported by Sierd Bron (Groningen, The Netherlands) and Jacques Mahillon (Louvain La Neuve,
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Belgium), it is clear that while replication and maintenance functions are highly conserved between Bacillus plasmids from many different locations, the associated genes are very host- and niche-specific, indicating an adaptation of the plasmid to help its host in its particular environment. Thus plasmids from the Japanese fermentation industry tend to carry genes which suppress sporulation, a wasteful activity in rich medium, allowing continuous growth. The advantage of plasmids for bacteria found in saline soils or arid deserts is yet to be determined. Although replication determinants are highly conserved they still do not fully understand how lagging strand origin determinants work or why they are host-specific. They may play a role in plasmid promiscuity (Gloria del Solar, Madrid, Spain) but why it is possible to delete all such sequences in some species without disastrous consequences is still a mystery. Despite being transformable, Bacillus species do not show evidence of extensive exchange of plasmids: host lineages follow plasmid lineages rather closely. On the other hand, Streptomycetes plasmids appear to spread easily and need only a very simple system to transfer from strain to strain as a result of hyphal fusion (Guenter Muth, Tubingen, Germany). Some plasmids of the actinomycete Amycolatopsis methanolica integrate into the genome of their host and increase mutation rate, allowing more rapid adaptation of their host under stress conditions by carrying variants of DNA repair enzymes (Harm Kloosterman, Groningen, The Netherlands). They thus promote evolution without the need for genetic exchange. Conjugative transposons have been described extensively in Gram-positive bacteria and Bacteroides but it now appears that they are also prevalent in Gram-negatives. Joseph Lengeler (Osnabrueck, Germany) described a family of elements apparently responsible for spread of metabolic transposons which carry, for example, components of the carbohydrate uptake and utilization system. Examination of DNA sequences has revealed properties related to both phage and plasmids. Tony Pembroke (Limerick, Ireland) described how the conjugative IncJ elements preferentially integrate into the chromosome at tRNA genes but that when one element is already present and in a recA context these elements can replicate freely in the cytoplasm. In other words, recombination is the dominant event for these elements on entry into a new host, overriding autonomous replication. Arianne Toussaint (Grenoble, France) described conjugative transposons carrying biphenyl degradation but then showed how a variety of phage, transposons and plasmid functions can be combined to create a continuum of mobile elements which contribute to gene flux. The common theme for this network is genetic exchange via cell-cell (conjugative) contact, although clearly transformation and transduction could also be important. While this concept was not presented as new it takes on fresh meaning as we consider the sequences of genomes, the range of genes which may be mobile and the complex communities in which bacteria exist.

Material in other talks and posters served to illustrate these points. Plasmid flux in marine environments (Ruth-Anne Sandaa, Bergen, Norway), the phytosphere (Mark Bailey, Oxford, UK) or model microcosms (Bjarke Christensen, Lyngby, Denmark) clearly exists but the factors which influence it and the balance between plasmid transfer and growth of transconjugants as a means of propagating mobile traits need further investigation. Although new collaborations have already arisen from this series of workshops, its seems that only after 2 years of dialogue are we learning to ask questions which can be tackled with a combined ecological and molecular biological approach. The challenge now is to harness these collaborations to add greatly to our understanding of microbial genetics, and microbial adaptation to their environments.

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