Paper presented at International Symp. on Large Marine Ecosystems : Exploration and Exploitation for Sustainable development and Conservation or ganised by Fishery Survey of India, Mumbai at Cochin 25-27 Nov. 1998



# ACCESSORY DNA ELEMENTS AS BIODIVERSITY MARKERS

**Arnab Sen, A A.Zynudheen, K.Ashok Kumar, C.N.Ravishankar, R.Badonia and K.K.Solanki**

*Research Centre of Central Institute of Fisheries Technology, Veraval.*

## ABSTRACT

Accessory DNA elements whether they be virus, plasmids or transposable elements have in common the ability to over-replicate the DNA, with respect to their hosts. The sophisticated bio-chemical and regulatory mechanisms of many accessory elements suggest that they are the products of long term selection. Bacterial euchromosomal genes are generally co-adapted complexes, so any genetic inter change is mostly deleterious. Accessory genes on the other hand are transferable among the different strains or species of bacteria and are selected to function successfully in all of them. Accessory DNA could serve as mapping zones of biodiversity and could reflect on the nature of survival and adaptation of bacteria in an ever changing environment.

## Introduction

Accessory DNA elements resemble the accessory chromosomes of higher eucaryotes in two respects viz. (i) the elements generally include no genes that are unconditionally required for the organism that harbours them and (ii) they are all able to replicate autonomously or else to over-replicate their own DNA relative to the typical chromosomal DNA of the cell. Over replication is one of the hall marks of accessory elements. Viruses and plasmids can replicate as autonomous replicons, physically separate from the chromosome. In steady state growth, plasmid DNA doubles at the same average rate as chromosomal DNA even for a multicopy plasmid. Over replication occurs either when the number of plasmid copies is increasing to its steady state value or during intercellular transfer.

Transposons and insertion sequences are defined as elements able to move from one location to another within the bacterial genome. Insertions sequences are short (usually less than 2 kbp) and confer no known phenotype on the cell, other than the effect on the target genes of the insertion. Transposons are larger elements; frequently including insertions sequences and their termini

that carry genes that effect cellular phenotypes. Transposons and insertion sequences are not known to replicate autonomously.

The retention of accessory DNA in nature during evolution is puzzling. Another aspect of accessory DNA is whether over replication is required for survival in nature. Viruses were considered as agents of diseases, whose most obvious activities were deleterious or lethal to the host. Their autonomous nature was hence manifested right at the outset. One view of the virus host relationship treats the virus as predator and host as prey. One may ask what are the properties that determine whether the stable equilibrium between the predator and prey is possible and what selective forces favour the emergence of predator resistance mutants. It was however generally presumed that in the long run, evolution should favour balanced complexes in which the virus does the minimal damage to the host and the highly virulent viruses that cause devastating epidemics are biological dead ends, seemingly outside the mainstream of evolution. Lysogenic bacteria might seem the ultimate in such evolution towards a state of reduced virulence, because they allow the perpetuations of the viral genome at a minimal deleterious cost to the host.

Impressed by the fact that prophages, situated in the chromosome, behave as bacterial genes, lead Campbell (1961) to hypothesis that they should be subjected to the same selective forces, as any other genes, and in the long run survival requires not only that harm be minimized but that some positive benefit should flow from them. Related considerations have lead to propose that the retroviruses of vertebrates evolved from normal genomic constituents of cells (protoviruses) that serve some function in the biology of the host. With other accessory DNA's, the discussion has progressed in the opposite direction from that with viruses. Both plasmids and transposons were considered as cellular constituents and not as infectious agents and their ability to over-replicate has gradually become apparent. They must constitute at least some metabolic burden to the cell. Their pathogenicity has however not been documented.

Because the elements were discovered as cellular constituents, it was frequently assumed that they serve some function useful to the host. And their ability to over-replicate has gradually become apparent, it was feasible to ask whether they might be considered as invaders or parasites; or in anthropomorphic terms as 'selfish DNA'. The same general consideration apply both to viral DNA and to other forms of accessory DNA. Unrestrained over-replication of either type of element may be deleterious to the host and hence, in the long run, to the element itself. In both cases long range selection should favour those element - host combinations where over-replication is restrained and ideally, where the element confers some benefit to the host.

## Genes involved in autonomous replication or over-replication

The genes of an accessory element can be divided into two categories. (a) Genes functioning directly in the replication or dissemination of the element itself and (b) genes whose function is to confer a phenotype on the host. A typical accessory element encodes one or more enzymes that recognise a specific DNA sequence on the element as a replication origin. A similar specific recognition is seen in processes that lead less directly to reproduction of the element, such as packaging of phage DNA into infectious particles or its non replicative insertion into the bacterial chromosome.

The reasons for focussing on specific genes involved in over-replication is that these genes are the genes on which natural selection must act and has acted in the past. The following general features are very important :

1. Over-replication mechanism are often quite complex and sophisticated indicating that they are products of a long history of selection.

2. Element-encoded enzymes generally recognise specific target sequences within the element. There is greater specificity for the site on the element than for that on the host.

3. There are specific interactions between host proteins and element encoded proteins.

## Origin and phylogeny of accessory elements

While recognising that selection will eventually eliminate neutral or harmful DNA, the authors of the selfish DNA hypothesis point out that the residence time may be long, so that the steady state level is high. The implication is that most accessory DNA currently in evidence is of relatively recent origin and the rate at which old elements are lost equals that at which new elements arise. Elements with new features could in principle originate by two mechanisms;

(a) By mutation of DNA sequences whose previous function, if any, was unrelated to over-replication.

(b) By changes in pre-existing elements, including rearrangements that incorporate euchromosomal genes into the element.

It has also to be considered that an analogy with spontaneous generation may be unfair, some accessory elements are quite small and therefore carry limited information, and their over-replication functions might in some cases arise by minor variations of euchromosomal functions. For example, new plasmids might sometimes originate by excision of bacterial replication origin. Second, a DNA sequence might change, not by becoming a new element but by developing a subsequence that resembles the target site of an element already present in the cell. Hence, we can conclude that over-replicating elements owe their survival to the ability to generate new gene combinations. The phenomenon of generating new gene combinations is regarded as the natural function of the element that is the function that accounts for its survival through evolutionary time.

**Limits on over-replication**

Extensive over-replication should be deleterious to be host and hence ultimately to the element. This is because both the element itself and its gene products involved in over-replication constitute a metabolic burden on the cell, even when they are not frankly deleterious like viruses. If the need for co-adaptation is generally significant, we might expect that the typical accessory element would regulate its function so as to minimize any negative effect on the phenotype.

The prototypic examples of such regulation are the temperate. Prophages such as the prophage encodes a repressor protein that specifically turns off transcription from the viral genome, so that viral replications are virtually unexpressed. Perpetuation of the prophage is thus accomplished at minimal expense to the host. Plasmid transfer into cells, already carrying a plasmid is also restrained. Conjugative plasmids also exert control over both transfer and replication. Transportation of transposons is also regulated. All these regulatory mechanisms reduce the burden on the host occasioned by excessive synthesis of accessory DNA or its products. Their existence provides yet another line of evidence that existing elements are highly evolved products of natural selection.

**Partitioning of the bacterial genome between accessory and euchromosomal DNA**

Given that accessory DNA exists and that some host phenotypic traits are determined by it, we may ask how natural selection might drive certain genes

into accessory DNA and others into euchromosomal DNA. An explanation to this idea is that this strategy expands the effective genome size of the species without imposing on each individual the burden of reproducting the entire genome.

The above hypothesis has certain implications viz., Firstly, genes of accessory DNA should typically be genes that are needed occasionally rather than continuously under natural conditions. Secondly, although we may speak of accessory DNA expanding the gene pool of a bacterial 'species'; the concept is hard to defend unless transfer can occur not only within an otherwise genetically homogeneous population of cells but also among different strains or species. Inter-strain or inter-specific transfer on the other hand allows certain strains to serve as permanent reservoirs for plasmids that can be temporally totally absent from others. A third implication follows from the second one. If genes of accessory DNA can be transferred among different strains and function in all of them, then evolution must limit the development of specific interaction between their product and those of euchromosomal genes for maintaining a degree of conservedness.

## Gene flow between accessory and euchromosomal DNA

To speak as we have of natural selection partitioning the bacterial gene pool between accessory and euchromosomal DNA implies that natural mechanisms exist to transfer genes from one fraction to the other. Laboratory studies indicate that any euchromosomal gene can be transferred into the accessory fraction and *vice versa*.

## Movement of genes from euchromosomal to accessory DNA

The first good examples of incorporation of euchromosomal genes into top accessory DNA were the formation of specialised transducing variants of phage λ and F variants of the F plasmid. Both λ and F are elements that can insert their DNA into the host chromosome. The reverse process (excision) is generally precise and element directed. However, rare instances of imprecise excision, presumably catalysed by host enzymes, generate variants in which contiguous host DNA is added to the elements. Sometimes replacing part of the elements own DNA.

In these variants, DNA of the element adjoin host derived DNA at two points; the insertion site of the element and a new "noval joint" formed by breakage and joining of heterologous DNA.

Transposable elements such as bacteriophage MU or IS 1 can also incorporate DNA from other sources, the mechanism is different from that observed with λ and F. Whilst the steps of the process are not known, the final result is equivalent to what would happen if two copies of the element inserted at two nearby sites of a target molecule, so that a segment of the molecule is now flanked by two copies of the element. These findings suggest that whenever two copies of a known transponsable element flank a DNA segment, the two elements plus intervening DNA; now constitute a new, compound element that can transpose as a unit, thus creating a new transposon.

## Movement of genes from accessory dna to euchromosomal DNA

While the possibility that all euchromosomal DNA is already transposable may pose a theoretical problem, there has not been much practical difficulty in distinguishing operationally between accessory DNA and Euchromosomal DNA. An inserted element maintains its separate identity because its termini are recognised by element, encoded enzymes that can excise it precisely. Any DNA between these termini belongs to the element and moves with it wherever it goes. By movement of a gene into euchromosomal DNA we mean not just insertion into the chromosome as part of an inserting element but insertion into the chromosome followed by some additional change that eliminates the possibility of subsequent excisions. This is easily accomplished by secondary events such as deletions or inversions that remove part of the accessory element, permanently welding some of its genes into the chromosome.

## Functions of accessory DNA in determining host phenotype

### *Phage determined traits*

Whereas lysogenic bacteria typically show few overt differences from their non lysogenic parents, a prophage can impart new traits to its bearer. A change in phenotype due to lysogenisation is termed lysogenic conversion. Many of the phenotypes are more readily interpreted as functioning to exclude competitors of the virus rather than to further host survival e.g. of some phage determined traits are surface polysaccharides, toxin production.

### *Plasmid determined traits*

The catalogue of plasmid determined traits is extensive and diverse. Conspicuous are resistance to antibiotics, heavy metals and other deleterious agents, production of bacteriocins, ability to ferment organic compounds.

### *Transposon determined traits*

Most known transposons determine resistance to antibiotics; other transposons carry the lac. Operon derived from a plasmid of Yersinia enterocolitica; an enterotoxin gene of *E. coli* and genes for hydrocarbon degradation in *Pseudomonas*.

## Conclusion

Accessory DNA is rich in genes determining resistance to various agents frequently by encoding enzymes that inactivate the enzyme in question. It has been suggested that the primary natural function of some of the *Pseudomonas* plasmids for hydrocarbon oxidation is not energy metabolism but detoxification.

If accessory DNA indeed tends to encode special kinds of traits we might explain that fact in two ways :

(a) Certain genes might typically and characteristically, over a long evolutionary period belong to accessory elements, even though they are apparently euchromosomal.

(b) The genes encountered most frequently on accessory elements might be reflective of selective pressures experienced recently and / locally by the population under study.

Thus the prevalence of antibiotic resistance genes on accessory elements might be attributable to the widespread use of antibiotics by man, rather than to the intermittent need for such genes in previous centuries. In this particular case, antibiotic resistance plasmids are found in bacteria isolated before use of human antibiotics was widespread and in isolated communities that have had little or no contact with antibiotics.

The simplest interpretation is that these determinants characteristically reside on accessory elements and that human intervention has only selected for an increase in number and perhaps variety of accessory elements carrying them.

It has been suggested that, whereas euchromosomal operons for synthesis or biosynthetic pathways have evolved stepwise by gene duplication and divergence, the plasmid operons for degradative pathways might have evolved instead by accretion, picking up genes for various steps from different hosts and placing them under some common control.

Finally, classification of bacterial genes in this manner is simplistic and categorical. Doubtless there are genes in accessory elements that are recently derived from euchromosomal DNA and owe their current location to recent or local selective factors or even to pure accidents. Nevertheless, natural selection should tend to fix euchromosomal genes as specialist and accessory genes as generalists, so the concept of the former as stable residents and the latter as permanent vagabonds should be natural rather than arbitrary.

## References

Abraham, J., D.Mascaren, R.Fischer, M.Benedik, A.Campbell and H.Echols, 1980. *Proc. Natl. Acad. Sci.* USA, *77* : 2477-81.

Ambler, R.P., T.C.Meyer and M.D.Kamen, 1979. Anomalies in amino acid sequences of small cytochromes C and cytochrome $C^{I}$ from two species of purple photosynthetic bacteria. *Nature, 278* : 661-62.

Anilionis, A. and M.Riley, 1980. *J. Bacteriol, 143* : 355-76.

Benzer,S., 1959. *Proc. Natl. Acad. Sci.* USA, *45* : 1607-20.

Burnet, F.M., 1950. Virus as organism. Harvard Univ., Press Publisher, Cambridge. Massachusetts, USA.

Campbell, A., 1961. Evolution, *15* : 153-56.

Campbell, A., 1977. Comprehensive Virology, editor H. Fraenkel, R.Conrat. R.Wagner, *8* : 259-328. New York Plenum.

Chakraborthy, A.M., D.A.Friello and L.H.Bopp, 1978. *Proc. Natl. Acad. Sci.* USA, *75* : 3109-12.