Pakistan J. Pharm Sci. Vol.2 (No.2), 1989.

Review

EXTRACHROMOSOMAL GENETIC ELEMENTS

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Introduction

Plasmids or episomids are the cytoplasmic (extrachromosomal) double stranded DNA molecules that harbour specialized genes and have the ability to replicate autonomously in bacterial cells (Novick, 1969; Lin *et al.*, 1984). A plasmid can be easily and stably inherited without being linked to the chromosome (genophore). Thus, plasmids are the autonomous replicons that are stably inherited (Hardy, 1981). Plasmids may even be exchanged between cells of different species (Hardy, 1987). Extrachromosomal elements have also been reported in unicellular cyanobacteria (Lau and Doolittle, 1979; Schmetherer and Wolk, 1988). Plasmid like systems have been found in eukaryotes as well (Gangs, 1983; Oliver and Brown, 1985).

There are several different types of plasmids. Many have been identified which do not have any apparent effect on the phenotype of the host cell and are called as 'Cryptic plasmids'. The remaining plasmids can be subdivided depending upon their transmissibility from one host to another. Many of the transmissible plasmids are also capable of mobilizing the bacterial DNA of host and are causing it to be transferred into another cell (Baroda, 1979). Various criteria are used to classify plasmids. Most important characteristic is used to provide one type of classification. Thus, *R* plasmids confer resistance to one or more antibacterial drugs. *Col* plasmids code for antibacterial protein substances called colicins; while *degradative* plasmids code for a variety of catabolic enzymes, and *virulence* plasmids enhance the pathogenicity of bacteria (Hardy, 1981). Bacteria often contain two or more different plasmids which coexist and are said to be *compatible. Incompatible* plasmids cannot coexist together and after a few generations one or the other is lost by unilinear inheritance (lordanescu, 1976). Plasmids can be either *conjugative* or nonconjugative. *Conjugative* plasmids transfer their copies from one bacterium to another and many of them are known to code for conjugating protein tubes called sex pili and DNA passes through these rubes (Baroda, 1979). Similarly, *stringent* plasmids are found in single copy in a bacterial cell while some plasmids are found in multicopy number (Hardy, 1981).

Insertion sequences (IS) and transposons (Tn) are the genetic elements that also occur in bacterial and phage chromosomes and range in size from 10^3 to $2x10^4$ base pairs

(Schleif, 1986). Their interesting feature is that they are able to make replicas of themselves and to insert them into DNA molecules with which they have little or no sequence homology i.e. they are capable of illegitimate recombination (Kleckner, 1981; Shapiro, 1983). Transposons code for many of the important characteristics specified by plasmids such as antibiotic resistance (Kleckner *et. al.*, 1977). Bacteriophage Mu is a giant transposable element and does generate mutations in infected cell populations. These mutations are generated by its insertion into various bacterial genes. Mu does behave like a lambda phage but without sequence specificity in its choice of the bacterial *att* site (Bukhari *et. al.,* 1976). However, two facts indicate that Mu is more like a Iransposon than like phage lambda. First, Mu duplicates five bases of chromosomal DNA upon its insertion. Second, Mu does not excise from the chromosome and replicates in the cytoplasm (Bukhari *et. al*., 1976; Symonds *et at,* 1987). Transposable elements have been reported in *Eschenchia colt, Salmonella typhimunum, Saccharornyces* and *Drosophila* (Schlcif, 1986).

Following are some of the important properties that are mediated by plasmid genes:

1. Resistance to antimicrobial drugs and toxic metal ions

Clinically significant antibiotic resistance is determined by the genes located on extrachromosomal DNA (Novick, 1969; Falkow, 1975; Lacey, 1975; Hon and Horodniceanu, 1980; Khatoon and Mohammad, 1986; Hardy, 1987; Rasool et al., 1987). Such genes (factors) are known as resistance transfer factors (RTF) and have been a source of tremendous concern in the management of chemotherapy (Baroda, 1979). Resistance genes, incorporated into transposons have also contributed to the rapid dissemination of antibiotic resistance by providing efficient mechanism for incorporating resistance determinants into new vectors which can transfer to and stably replicate in diverse host. It is believed that R-plasmid specified antibiotic resistance may have evolved as self protection mechanism in antibiotic-producing microorganisms. It seems that bacteria will evolve resistance to most, if not all, antibiotics with which they are challanged (Foster, 1983).

Plasmid mediated genes have been reported to code for the synthesis of outer membrane protein components in bacteria that present impermeability of melathion (Ahmed *et. al,* 1987).

A number of plasmid associated genes have also been known to resist inorganic metals. Knowledge of metal ion resistance may prove helpful in understanding the antibiotic resistance mechanisms. Most metal resistance is plasmid mediated and has perhaps evolved in response to selective pressure (Summers and Silver, 1978; El Solh and Ehrlich, 1982).

2. Fertility (sex) factor controls

Transfer of plasmid borne genes from a donor (male or F^+) to the recipient (female or F^+) cells is under the control of fertility factor (Williams, 1978). Conjugative plasmids bear these factors and these plasmids actually transfer pieces of chromosomal DNA between bacteria and therefore, referred to as 'Sex factors'. Such plasmids (the

conjugative) are useful for mapping the position of chromosomal genes (Hardy, 1981).

3. Degradative plasmids

Degradative plasmids enable *Pseudomonas putida* and related bacteria to grow on substrates such as toluene, xylene, octane, camphor, naphthalene, salicylate and nicotinic acid. Plasmid encoded enzymes convert these growth substrates to metabolites including acetaldehyde, pyruvate and acetate which can then feed the metabolic pathways that are catalysed by chromosomal enzymes. Most degradative plasmids code for atleast 10 enzymes which are involved in the catabolism of a particular substrate (Chakrabarty, 1976; 1983). Methylotrophs (yeasts and bacteria) have also been reported that are able to row on methane and methanol. The products such as single cell protein (SCP), poly-3-hydroxybutyrate (a biodegradable polymer), propylene epoxide, vitamins, amino acids and carboxylic acids (e.g. citric acid) and acetic acid (produced by anaerobic fermentation) have been considered by biotechnologists (Anthony, 1987).

Degradative plasmids enable bacteria to grow on synthetic compounds and may therefore, contribute for the removal of substances that can act as pollutants. Plasmids also enable bacteria to degrade the widely used herbicide 2, 4-D (2, 4-dichlorophenoxyacetic acid), chlorinated hydrocarbons such as P-chlorbiphenyl and alkyl benzene sulphate. Attempts are being made to use the metabolic potential of degradative plasmids by constructing strains which can be used for pollution control or for chemical synthesis (Hardy, 1981). Degradative plasmids are usually conjugative and some have a wide host range (Chakrabarty, 1976).

Metabolic plasmids sometimes become apparent because they confer ability to ferment unusual substrates so that strains give unexpected results when being identified. Strains of *Salmonella* are almost always Lac^-, i.e. they are unable to ferment (hydrolyse) lactose. Lac^+ strains of *Salmonella typhi* and other *Salmonellae* have been isolated on rare occasions and have been found to harbour plasmids which confer the Lac^+ phenotype. Lac^+ plasmids have also been found in strains of *Serratia*, *Streptococcus* and *Proteus.* It is interesting that the lactose operon was found to be encoded by a transposon Tn 951 in *Yersinia enterocolitica* (Hardy, 1981).

An important group of metabolic plasmids are those which enable strains of *Rhizobiurn* to nodulate and fix nitrogen in the roots of leguminous plants (such as peas, beans and clover). Nitrogen fixation involves the conversion of molecular nitrogen into a form utilizable by plants. It is a very vital process for increasing the yield of crops. Nitrogen fixing genes/operon (*nif*) are located on the plasmids of not only of symbiotic *Rhizobium* (Nuti *et al.,* 1979) but also *of Klebsiella* (Cannedy and Dixon, 1978). There have been a number of attempts to transfer *nif* opeon of *Klebsiella pneumoniae* to different eukaryotes (Hinnen *et al.,* 1978).

4. Pathogenicity of animals, humans and plants

There are reports for the existence of plasmid mediated genes responsible for enterotoxins (Ent plasmids) and haemolysin (Hly plasmids) among Gram positive pathogenic cocci (Williams, 1978). Production of exfoliative toxin (causing scalded skin syndrome) by *Staphylococcus aureus* strains depends on the presence of a plasmid that

encodes the protein (Hardy, 1981). The enterotoxigenic strains of *Escherichia coli* have similarly been reported to harbour plasmids (Smith and Williams, 1976).

Ti (tumour inducing) plasmid has been found in *Agrobacterium tumefaciens* (a Gram negative rod belonging to *Rhizobiaceae).* Apart from causing tumours (crown galls) in dicotyledonous plants, *Ti* plasmid is also used as cloning vehicle/vector (Old and Primrose, 1988. However, the disease (tumour induction) has yet to be established in monocotyledonous plants. *Ti* plasmids are very large and can also be transferred between strains by conjugation. In addition to stimulating the growth of plant cells, *Ti* plasmids also cause them to synthesise compounds known as opines. Opines are not produced by normal plant tissue and have not been found elsewhere in nature other than in crown gall. The opines, in return are utilized by host bacteria as a source of carbon and nitrogen. Presumably, the catabolic enzymes and permeases are en-coded by plasmid genes. Thus, catabolic enzymes and permeases are encoded by plasmid genes. Thus, bacteria bearing *Ti* plasmids seem to have evolved an ingenious strategy for tapping the synthetic abilities of plants to provide them with exclusive source of carbon and nitrogen (Drummond, 1979). In fact, the ability of *Ti* plasmid to transfer and to maintain part of itself in plant cells have opened the way for inserting the genes into plants. These can be attached to the appropriate part of *Ti* plasmid, so that they can be transferred from *A. tumefaciens* to plant cells as part of the *Ti* plasmid. In one of the experiments, the transposon Tn7 was inserted into part of the *Ti* plasmid which is maintained by plant tumours (T-DNA) and was stably maintained by the tumourous plant cells induced by the plasmid (Nuti *et. al*, 1979).

5. Bacteriocin production

Bacteriocins are the protein antibiotics produced by a number of bacteria and are active mainly against closely related bacteria (Akhtar, 1987; Khan, 1989). Genes responsible for bacteriocin production are located on plasmids. Bacteriocin is the general term, and the individual types of bacteriocins are generally named according to the species of the organism which originally produced it (Birge, 1981). Thus, colicins are the plasmid determined bacteriocins produced by certain members of *Enterobacteriaceae,* which are lethal for closely related strains. About 40% of *E. coli* strains for humans or animals are colicinogenic (Col^+) (Helinski and Herschman, 1967). Substances analogous to colicins are produced by many Gram positive and Gram negative genera. For example, some *Streptococci, Staphylococci* and *Lactobacilli* produce streptoccins, staphyloccins and lactosins respectively. These bacteriocin proteins are associated with lipid and carbohydrates (Hardy, 1981). Similarly, pyocins or acruginocins are produced by many strains of *Pseudomonas aemginosa.* These bacteriocins are specified by chromosomal genes and have immunological cross reactions with temperate phages of *Pseudomonas* (Okhawa *et. al,* 1973). The activity spectra of bacteriocins of phytopathogenic *pseudomonads* have been reported by several workers (Vidaver *et. al,* 1972). Vidaver *(1976)* observed a dramatic decrease in disease (upto 99% of the control) that could be achieved by inoculating seeds and roots with nonpathogenic bacteriocinogenic Agrobactenum *radiobater.* Infact, this procedure did control crown gall of peaches in Australia (Htay and Kerr, 1974). Interesting feature of the biological control of crown gall is that the sensitivity to agrocin 84 (produced by *Agrobocterium radiobacter)* is

conferred by a gene on the nopaline type of *Ti* plasmid found in virulent strains of *Agrobactenum tumefaciens.* Strains which become agrocin resistant through loss of the *Ti* plasmid are therefore, avirulent (Hardy, 1975). Gamier and Cole (1986) isolated a bacteriocinogenic plasmid from *Clostridium perfringens* (perfringocin) and were able to clone in E. coli.

As bacteriocins are commonly produced by bacteria, it is assumed that they confer a selective advantage by killing other bacteria that are in competition with the producing strains. It may be presumed that narrow spectrum of action of bacteriocins examplifies the Darwin's proposal that competition between closely related species will be particularly intense because these species, which share many common features in terms of mutation and habitat will be competing in a similar environment (Hardy, 1981). It is better to call this phenomenon as `professional cohabitational antagonism'.

Plasmids and Genetic Engineering

Plasmids or extrachromosomal genetic elements are used as vectors or vehicles to clone DNA molecules. Restriction endunucleases, ligases and other enzymes can be used to add pieces of foreign DNA called `inserts, to plasmid molecules *in vitro.* The recombinant plasmid can then be inserted back into a suitable host bacterium, usually E. *coli* by transformation. The insert is added at a dispensable or non-essential site in the vector so that the recombinant plasmid can replicate in the bacterium (Maniatis *et al.*, 1982).

Main steps in using plasmid vector to clone DNA molecules are underlined below (Watosn *et. al., 1983;* Dillon *et al.,* 1985):

1. Isolation of circular plasmid DNA molecules and preparation of the DNA insert. The insert may be a piece of chromosomal DNA from an animal or a plant, a c DNA (Complementary DNA) molecule derived from RNA, or a chemically synthesized sequence.
2. Insertion of the foreign DNA into the plasmid. There are several ways of doing this, however, they all constitute breaking a circular plasmid at a specific point with a restriction endonuclease to cut it open into a linear molecule. The cleaved plasmid is mixed with the. DNA to be inserted (the ends of the insert are usually made homologous to the ends of the vector) and the two arc scaled together by DNA ligase to form a circular molecule.
3. Addition of the recombinant plasmids to host bacteria by transformation.
4. Identification of the transformants which have recombinant plasmids. Usually, only a fraction of all the transformants contain the cloned fragments of the insert.

Small recombinant plasmids (the synthetic ones) such as pBR 322 (Fig.1), which have genes coding for drug resistance are widely used as vectors for cloning DNA molecules. Natural plasmids such as pSC-101 (Fig.2) may also be used for this purpose. DNA is usually inserted at a site which inactivates one of the resistance genes so that recombinant plasmids can be detected (Fig. 3). Phages and cosmids, which are made by combining parts of the plasmids and phage DNA, are also used as vehicles (Garland and Williamson, 1979; Hardy, 1981; Watson and Tooze, 1981). Intact, Genetic Engineering

(recombinant DNA technology) has proved to be useful in the construction of tailor-made bacterial genotypes for the stable inheritance of the desired characteristics (Gianni et. al., 1987; Krishnapillai, 1987; Clara and Garbriela, 1988).

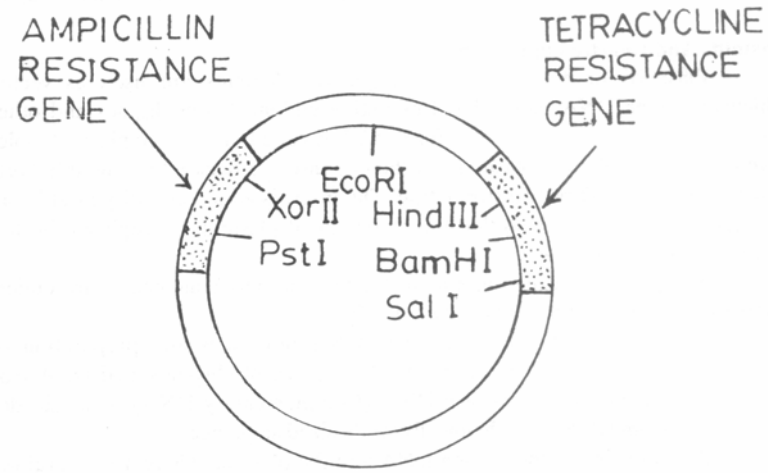

FIG.1 SYNTHETIC PLASMID BR 322 (4361 BASE PAIRS)

(Watson & Tooze, 1981)

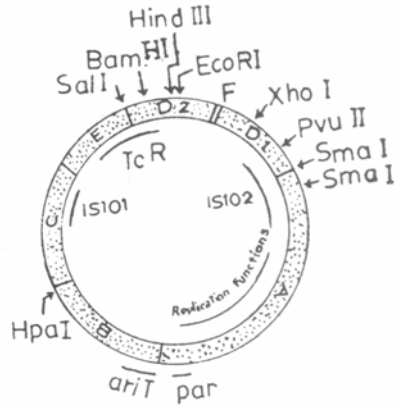

FIG.2 NATURAL PLASMID SC 101 (9.09 k b.)

(Old & Primrose, 1988)

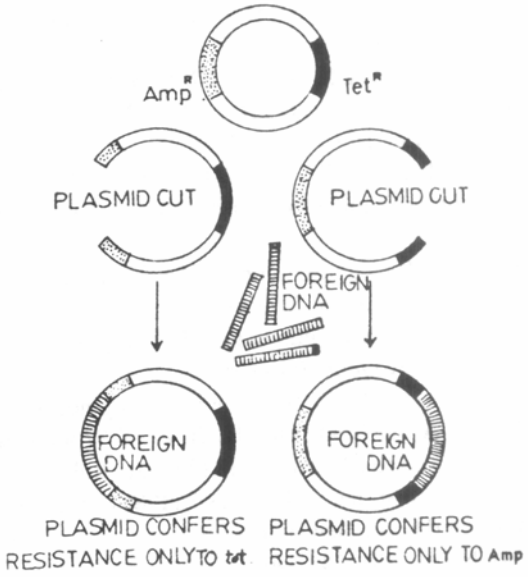

FIG.3 PLASMID ENGINEERING

(Watson & Tooze, 1981)

Conclusion

Many important genes are part of the separate circles of DNA called plasmids. Plasmids are important in medicine and in agriculture. They confer antibiotic resistance on animal and human pathogens. They can code for toxins and other proteins which increase the virulence of the pathogens. Some plasmid genes are beneficial e.g. plasmids enable species of *Rhizobium* to fix nitrogen in the nodules of leguminous plants. They can also code for antibiotics which can be used to control pathogenic bacteria. Plasmid genes also cede for a wide range of metabolic activities and enable bacteria to degrade compounds which would accumulate as pollutants if they were not degraded by microorganisms. Finally, plasmids are also used as reliable and stable tools in Genetic Engineering.

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