# Plasmids and bacterial pathogens

*from J.R. Saunders*

THE way in which plasmids confer pathogenicity characteristics on host bacteria is of considerable concern in both developed and developing countries, and provided the central theme at a recent conference* in the Dominican Republic. Various aspects of plasmid biology were covered, ranging from the mechanisms of genetic transposition and plasmid replication to the epidemiology of antibiotic-resistant bacteria in human and animal populations.

Plasmids probably determine only a small proportion of all bacterial virulence traits. However, they may provide sufficient additional genetic information to convert relatively innocuous organisms into pathogens. This is particularly true of enteropathogenic strains of *Escherichia coli*, the prime cause of travellers' diarrhoea and other economically important diseases of humans and domesticated animals. The distressing symptoms of these conditions are largely produced by one or more plasmid-specified enterotoxins, which include a single type of heat-labile toxin (LT) and probably at least two distinct heat-stable toxins (ST). S. Falkow (Stanford) reported a striking similarity between the gene for LT and that for the cholera toxin, despite the fact that the former is always plasmid-borne in *E.coli* and the latter chromosomally determined in *Vibrio cholerae*. The difference in disease patterns, apparently specified by the same virulence determinant, may reflect the different methods by which the two causative agents actually deliver the toxin to their human hosts. This emphasizes how heavily the expression of pathogenicity determinants depends on the genetic and physiological infrastructure into which they become inserted. It is interesting in this context that certain characteristics, such as particular chromosomally determined somatic (O) antigen types, are found habitually among enterotoxigenic strains of *E.coli*.

Haemolytic strains of *E.coli* can frequently be isolated from infected body fluids. However, the exact role of haemolysins in the pathogenicity of these bacteria is not clear. In *E.coli*, $\alpha$-haemolysins are determined either by the chromosome or by Hly plasmids. Such plasmids isolated from different strains have been shown by W. Goebel (Wurzburg) to share a common haemolysin determinant. This consists of three cistrons, *cisB* (1,500 base pairs) determining a protein located in the outer membrane and probably responsible for the transport of haemolysin, *cisA* (2,500 base pairs) encoding a precursor of $\alpha$-haemolysin, and *cisC* (about 500 base pairs) which is apparently required for the conversion of the *cisA* product to active $\alpha$-haemolysin. Despite the wide distribution of the Hly determinant it does not seem to constitute part of a transposon (see De La Cruz *et al. J. Bact.* **143**, 825; 1980). This contrasts with one of the ST genes whch is known to be transposable (So *et al. Nature* **277**, 453; 1979).

The ability of enteric bacteria to adhere to the gut wall is a primary requirement for their pathogenicity. The best studied of such plasmid-mediated determinants are those for the filamentous surface protein antigens K88 and K99, which enable enteropathogenic *E.coli* to adhere to the mucosa of the small intestine of pigs and calves respectively. Similar pilus-like structures, termed colonization factors (CFA I and II), are plasmid-encoded in human enteropathogenic *E.coli*. Two groups (J. van Embden, Bilthoven and G. Dougan, Dublin) reported the cloning and genetic analysis of the K88 and K99 antigens. Dougan has found that at least four genes, organized into two operons, are involved in the expression of the K88 antigen in *E.coli* minicells. Operon I comprises three genes transcribed from *adhA* (encoding a protein of molecular weight 70,000 which may be the basal structure for membrane attachment of the antigen) through *adhB* (29,000-MW protein) to *adhC* (17,000-MW protein). *adhC* may encode a positive regulator for operon II containing a single gene *adhD* determining the 23,000-MW subunit of the K88 antigen itself.

In addition to adhering to the intestine, some enteric pathogens are able to invade the gut tissues. D. Kopecko (Washington) described plasmids of about 120 megadaltons in *Shigella sonnei*. Loss of these plasmids results in transition of host strains from the virulent, smooth colonial form I to a virulent, rough colonial form II. Such plasmids apparently determine the synthesis of the form I somatic (O) antigen required for invasion of colonic epithelium.

The potential of bacteria as invasive pathogens is obviously enhanced by their ability to withstand the antibacterial properties of serum. A number of unrelated antibiotic-resistance plasmids and temperate bacteriophages, such as $\lambda$, confer on host bacteria resistance to complement (activated by either classical or alternative pathways). K. Timmis (Berlin) showed that serum resistance conferred by the conjugative plasmid R6-5 is determined by the product of the *traT* gene. This is a 25,000-MW protein responsible for the surface exclusion (prevention of conjugative mating with related donors) and present as 20,000 copies in the outer membrane of *E.coli* carrying R6-5. M. Binns (St Louis) has demonstrated that the *traT* product of another conjugative plasmid, R100, impairs the formation of the terminal complement complex. Furthermore, *traT*-containing cells remain resistant to complement even if they have been pretreated with antibodies to *traT*. This suggests that the *traT* protein does not actively protect bacteria against serum but has a passive effect, possibly involving subtle alteration of the architecture of the cell envelope. In addition, studies by Timmis of mutants in the *traT* gene indicate that the serum-resistance and surface-exclusion properties of *traT* protein may be distinct. Whatever the mechanisms involved, serum-resistance determinants, such as *traT*, may provide a selective advantage leading to the maintenance of conjugative resistance plasmids in pathogenic bacteria.

An important prerequisite for invasive pathogens is the ability to proliferate in the low free-iron conditions prevailing in animal tissues. A significant proportion of *E.coli* strains causing bacteraemia in humans or animals are found to carry ColV plasmids. In addition to encoding colicin V production and serum resistance, such plasmids specify accessory iron-sequestering mechanisms. P. Williams (Leicester) has shown that they determine an extracellular iron-chelating compound of low molecular weight which competes successfully with transferrin for iron *in vivo*. In addition, they specify, together with chromosomally determined components, an active iron uptake system. The possession of these extra mechanisms for acquiring free iron is thus sufficient to allow growth of *E.coli* in the tissues of infected animals.

Knowledge gained from *E.coli* should facilitate the analysis of more complex, chromosomally determined pathogenic mechanisms in less tractable organisms. And cloning of virulence determinants provides the promise of effective vaccines against enteric and other infections.

*J.R. Saunders is in the Department of Microbiology, University of Liverpool.*

*On the 'Molecular Biology, Pathogenicity and Ecology of Bacterial Plasmids' (organized by S.B. Levy, R.C. Clowes and E. Koenig), held from January 5th to 9th, 1981 in Santo Domingo, Dominican Republic.


## 100 years ago

### *PHYSICAL NOTES*

M. Plantamour continues to study with his sensitive levels the phenomena of periodic rise and fall of the ground which he has observed in Switzerland. He believes he has established a connection between these periods and those of the changes of temperature of the earth's surface, there being an annual change of level in an east-west direction corresponding with the mean temperatures of the surface during the year.

From *Nature* **23**, 31 March, 517, 1881.

0028-0836/81/140362-01$01.00