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PLASMIDS AND THEIR HOSTS

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Summary. — Both viruses and plasmids are submicroscopic, biologically active elements characterized by their nucleic acids. Both contain either DNA or RNA; both display genetic continuity. Both are known to exist in procaryotic as well as in eucaryotic organisms and both may considerably influence the phenotypes of their hosts. Bacterial plasmids are circular, covalently closed double-stranded DNA molecules. The molecular weights of most plasmids fall within the range from 1×10^6 to 100×10^6, the mol. weight of 30×10^6 being a limit between the small and large ones. A single plasmid molecule is sufficient to realize the phenotypic expression of its genes in its host bacterium. A great variety of genes are localized in plasmids; most of the known genes are structural ones coding for proteins accomplishing various biological functions. In plasmids there are e. g. loci determining the donor mating character of the host bacterium, loci encoding bacteriocins (extracellular proteins of specific antibiotic activity) or enzymes either degrading or inactivating antibiotics (and thus responsible for resistance of the host bacterium to their action), loci coding for enzymes catabolizing various organic substrates (including some bizarre ones) and also for enzymes enabling the fixation of elementary nitrogen by the bacterium etc. The plasmid once acquired to bacterial cell by conjugation, transduction or transformation, becomes a stable component of its genetic material and is stably inherited by its progeny; thus a bacterial clone endowed with the new trait arises.

Most plasmids may recombine with each other and form stable and hereditary cointegrates. Like viral DNA, many plasmids may be transiently or permanently integrated into chromosomal DNA of the host cell and vice versa: segments of chromosomal DNA — with their genes — may get stably incorporated into plasmids. All plasmids harbouring *tra* genes may be autonomously transferred by bacterial conjugation, some of them not only in intraspecific, but also in intrageneric or intergeneric crosses.

It may be assumed that plasmids (like insertion sequences) have been natural gene vectors between bacteria, influencing their evolution. There are also convincing arguments that plasmids are autonomous organisms.

Key words: *plasmids*; *plasmid genes*; *virus-plasmid relationships*; *bacterial phenotypes*

Plasmids and Viruses

The borderline separating plasmids and viruses has been so far wiped off by recent years' research that it, as a matter of fact, does not exist any more. Both plasmids and viruses are submicroscopic, biologically active elements containing a nucleic acid, capable of autoreproduction in host cells and endowed with characteristic and outstanding heredity. A variety of DNA-plasmids can be integrated into chromosomal DNA of the bacterial host cells just as prophages of temperate bacteriphages or proviruses are integrated into chromosomal DNA of their respective host cells. Prophages of temperate bacteriophages (i. e. of bacterial viruses) which permanently exist in the cytoplasm of their bacterial hosts (e. g. prophage *E. coli P1*) are classical plasmids. Hosts of both plasmids and viruses are found among procaryotic cells just as among eucaryotic organisms. As expected, there exist DNA as well as RNA plasmids in analogy to DNA and RNA viruses. The most striking common feature of plasmids and viruses — from the theoretical and practical points of view — seems to be their ability to cause marked transformations of genotypes and phenotypes of their host cells.

On the other hand, the principal difference betwen viruses and plasmids resides in the fact that plasmids do not harbour genes coding for synthesis or morphogenesis of any capsid. They are not able to coat themselves into protein or even more complicated envelope layers and hence — under natural conditions — they are not capable to exist outside their hosts. (However, naked plasmids may also be kept in a native state under laboratory conditions.) The question arises, whether this single difference between viruses and plasmids is of such crucial importance as to be regarded for a plausible criterion of life.

Among others, very characteristic plasmids exist harbouring structural and regulatory genes for the synthesis of specific bactericidal proteins — the bacteriocins. Besides those bacteriocins acting as dispersed hydrophilic macromolecules in water solutions, there are also bacteriocins of particulate nature: e. g. certain (R type) pyocins, marcescins of group A, colicins (e. g. colicin 15), carotovoricins or bacteriocins of *Clostridia*. Particles of these bacteriocins are morphologically indistinguishable from phage tails (including core and contractile sheath, basal plate and even fibres) or — some of them — from still larger parts of the phage capsids including more or less complete protein envelopes of phage heads. These plasmids and bacteriocins — namely products of the expression of their genetic information — are lacking just one step to become bacterial viruses: they need to be integrated with each other into complete bacteriophages. A plasmid and a bacteriophage may even be in two forms of the same biological entity; e. g. bacteriophage *E. coli* λ can replicate its DNA not only as a linear prophage integrated into the chromosome of the host bacterium as a linear molecule, but also as an autonomous circular plasmid (λdr and λN). And analogous replication forms are used also by temperate bacteriophages φ 80, fd and f2.

At the present state of molecular biology, classical and model plasmids are the DNA-plasmids of bacteria (Meynell, 1973). Only these and their relationships to their bacterial hosts will be considered in this review.

Basic Characteristics of Plasmids

Physical chemistry of plasmids

The category of plasmids was established by Lederberg (1952). According to Hayes (1969), plasmids are cytoplasmic elements with a genetic function, physically separated from the chromosome and capable of continuous, autonomous replication in bacterial cells. They are maintained hereditarily in them, also without any specific selection. All bacterial plasmids have so far been isolated as covalently closed, circular molecules of double-stranded (ds)DNA, displaying a typical secondary and tertiary structure (supercoiled molecules — Fig. 1).

Naturally occurring plasmids vary greatly in their dimensions; their molecular weights fall within the scope from less than 1×10^6 to about 150×10^6. Each million (megadalton) of their molecular weight comprises 1.55 kilobase pairs, polymerized into ds-DNA segment of about 0.48 μm contour length (Clowes, 1972*a*). Thus a bacterial plasmid is about 17× to 2300× shorter than the bacterial chromosome. It follows that, in comparison with the chromosome capacity of about 4500 genes, plasmids manage to bear only about 2 to more than 300 genes. The smallest plasmids code for two proteins of an average size. The molecular weight of the largest stable plasmids described achieves 240×10^6, which is only 10× less than that of the chromosome of *Escherichia coli*. Owing to the wide extent of plasmid molecular weights, it is reasonable to distinguish small plasmids (mol. weight less than about 30×10^6) and large ones (mol. weight more than about 30×10^6). Both these physical categories of plasmids differ explicitly also in a biological respect: in their replication control.

Plasmid replication control

On the basis of this criterion, plasmids with a stringent and plasmids with a relaxed replication control may be distinguished (Arai and Clowes, 1975). Plasmids with the stringent replication control are replicated only during the chromosome replication, i. e. once in each cell cycle. Consequently, these plasmids are present in low and constant copy numbers (just 1 to 3 copies) per chromosome during the whole cell life. This replication control is typical for large plasmids.

On the other hand, plasmids with relaxed control of replication start replicating accidentally during any phase of cell cycle, so that at least some of their copies replicate several times in the course of it. At cell division, they also accidentally segregate into daughter cells. Some of them achieve high copy numbers per chromosome (up to 60 copies). In this way, nearly all small plasmids replicate. Their copy numbers per bacterial chromosome are thus not constant during the cell cycle and vary in individual plasmids.

While active proteosynthesis is needed for the initiation of the stringently controlled replication of large plasmids (just as for that of chromosome replication — Molin and Nordström, 1980), it is not needed for the initiation of the relaxed replication. Thus, small plasmids can replicate without an disturbance for 10-15 hours following a complete cessation of proteosynthesis, considerably accumulating their copies in host cells. In this way, it is possible to raise e. g. the plasmid *Col. E1* (mol .weight 4.2×10^6) copy number from the usual 20-30 copies up to about 3,000 copies per chromosome (Clewell, 1972). As an analogical accumulation is realized in all cell of the inhibited culture, the concentration of DNA of the plasmid in question in its lysates may be raised on a large scale; this is a great methodical advantage for transformation experiments with plasmid DNA. Col El plasmid itself is often used as a vector in such experiments, especially for purposes of gene engineering (Hershfield *et al.*, 1974).

The replication of each plasmid is directed from its own esential region; here, thereof, are the genes located responsible for plasmid DNA replication and regulation (Helinski, 1976). In the other, so-called nonessential region of each plasmid, genes are harboured, the expression of which contributes — often substantially — to the spectrum of phenotypic markers of bacterial hosts and to the traits of their cultures. In addition, every plasmid regulates the ability of the own host not to admit replication — and hence not to admit a hereditarily stable coexistence — of incompatible plasmids. Plasmids of Gram-negative eneteric rods have been classified into more than 30 incompatibility groups (Datta, 1975). DNA molecules of many plasmids bear extensive nucleotide pair sequences, the genotypical and phenotypical meaning of which has not been elucidated yet.

Plasmids as vectors of genetic information of bacteria

The essential plasmid region which secures its autonomous replication, secures — sensu stricto — only its autonomous start, comprising the so-called replication origin. (In the course of replication, plasmids take advantage of the enzymatic apparatus — DNA polymerases and replication factors — by means of which the host bacteria replicate their chromosomes.) The essential region is represented merely by a relatively small part of the plasmid genome. E. g. in Col El it occupies less than 8% of its all extent (i. e. about 500 nucleotide pairs — Tomizawa *et al.*, 1977). It means that more than 92% of the plasmid length are not only at the disposal of genes with phenotypic functions, but also serve for the inclusion of other DNA fragments with additional genes. The replication of these included genes is warranted in the course of every replication of the Col El plasmid itself (or of the plasmid constructed in this way). Even in the largest plasmids, the essential region does not exceed 8,000 nucleotide pairs (about 5×10^6 mol. weight). Thus any plasmid may become a vector of a great variety of additional genes: either of another plasmid genes and/or of typical chromosomal ones — but not only of bacterial genes. The host bacterium keeps such a plasmid cointegrate (Clowes, 1972*b*) and ensures its replication, being also able to transfer it to

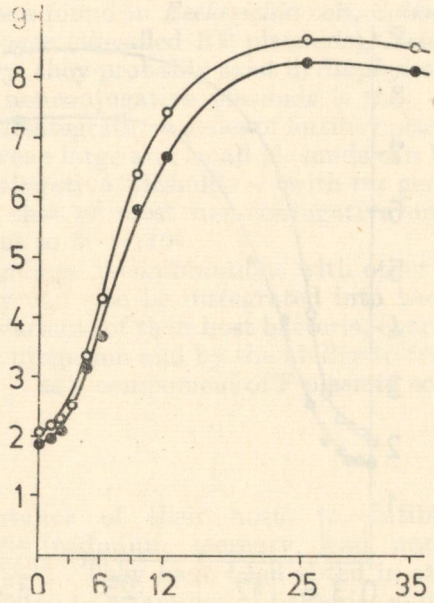

Fig. 2.
Growth curves of two clones of the strain *E. coli* 8 011 in Casamino-acids medium Cells containing (●) and lacking (○) the plasmid F'lac.
Abscissa: incubation at 37 °C in hr; ordinate: No. of living cells per ml (log values).

its daughter cells in corresponding copy numbers. A spontaneous loss of the plasmid almost does not exist and may be achieved experimentally ("plasmid curing") just in a small proportion of the cell population. A bacterium endowed with a plasmid cointegrate gives rise to a new clone or strain which — — together with its "natural" genes — goes on cloning the additional genes, no matter whether they are of bacterial, animal or even human origin.

Most of the "original" plasmid genes code for genetic information of their hosts, which is additional to their normal genotypes; the phenotypic markers of bacterial cells, arising by their expression, are not indispensable for bacteria under usual circumstances. However, they easily become selectively advantageous for those bacteria under special ecological conditions, ensuring their specific, adequate interactions with other microbes, with macroorganisms or with chemical components of their environment.

Regularly, bacteria must pay a tax for these possible advantages: for the replication of plasmids energy and enzymatic machinery of their cells are necessary. At a reduced availability of energetical sources, they expend energy even on the costs of more urgent needs, such as of cell multiplication E. g. Šmarda and Mládek (unpublished results) proved this by comparing growth curves of *Escherichia coli* and *Salmonella typhimurium* strains lacking plasmids with the same strains harbouring both large and small plasmids. Thus, the growth of *E. coli 8 011* in Casamino-acids medium is distinctly retarded and its colony forming ability is decreased in the presence of F'lac plasmid of mol. weight 78×10^6 (Fig. 2). Similar — and even much more marked — are the manifestations of the small multi-copy plasmid Col E2 (m. w. 3.8×10^6) in the *Salmonella typhimurium* LT 2 culture (Fig. 3).

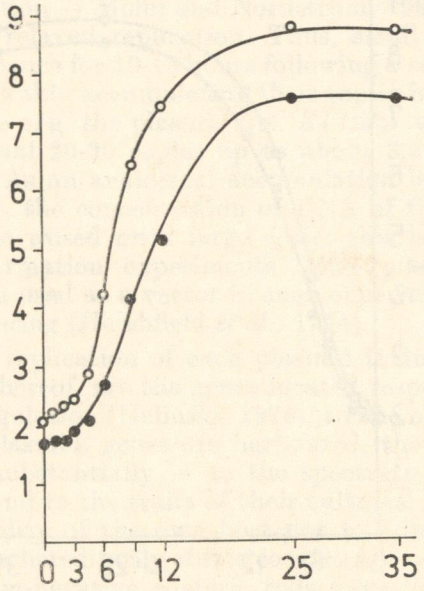

Fig. 3.
Growth curves of two clones of the strain **Salmonella** typhimurium LT 2 in Casaminoacids medium
Cells containing (●) and lacking (○) the plasmid Col E2.
Abscissa and ordinate as in Fig. 2.

Classification of Naturally Occurring Plasmids

Today, 10 clearly different basic phenotype classes of plasmids are known. Among these, plasmid cointegrates (constructed by gene engineering or natural ones) are not included, because of their unlimited variability.

F plasmids

Plasmids of the class F, fertility plasmids (formerly: sex-factors), have been detected as the first ones (Lederberg *et al.*, 1952). Their specific genes, *tra* genes, code for the synthesis of a specific protein — pilin — which is the subunit for formation of conjugative pili. In this way, *tra* genes promote the ability of their host bacteria to establish conjugation and to act as gene donors (Brinton, 1965). Hence, such bacteria are able autonomously to transfer parts of their genetic material to recipients (while reduplicating it). Such plasmids — or their *tra* genes — are very often spontaneously integrated into other plasmids, rendering them conjugative (see below).

The CCC (covalently closed, circular) molecule of F plasmid has the contour length of 31 μm and mol. weight of 64×10^6 (Clowes, 1972*a*). In F plasmid of *Escherichia coli*, the *tra* region comprises about 20,000—24,000 nucleotide pairs and harbours 21 structural genes (Rowburry, 1977). The biosynthesis of a functional F-like conjugative pilus requires the presence of at least 13 genes. *tra* genes, although usually tightly bound together in F plasmids, may get dispersed in recombinant plasmids.

Original fertility plasmids have been found in *Escherichia coli*, *Salmonella* (signed Δ), *Vibrio cholerae*, *Pseudomonas* (so-called EP plasmids), *Neisseria*, *Streptomyces* — and, most surprisingly, they probably exist in *Staphylococcus*. The distinction of conjugative and nonconjugative plasmids is thus determined by their ability or non-ability to integrate *tra* genes of fertility plasmids. In this way, the basic difference between large and small plasmids can be explained: the molecular weight of conjugative plasmids — with *tra* genes — — always exceeds 30×10^6, while that of most non-conjugative ones — without *tra* genes — does not amount to 30×10^6.

The capability of F plasmids to undergo recombinations with other DNA molecules reaches even further; they can also be intregrated into bacterial chromosomes, thus giving rise to Hfr variants of their host bacteria, characterized by the high frequency of their conjugation and by the ability to transfer parts of a copy of their chromosome — as a compoment of F plasmid copy — to recepients (Cavalli, 1950).

R plasmids

R plasmids code for the resistance of their hosts to antibiotics, chemotherapeutics, heavy metal ions (cadmium, mercury, lead, antimon, bismuth) and/or UV-rays (Falkow, 1975). They have been noted in at least 50 species of at least 26 genera belonging to 5 families of bacteria, and most probably they occur generally in bacteria of all species. Their spreading in nature is facilitated by their ability to integrate *tra* genes very easily; thus most R plasmids are conjugative ones (Vapnek *et al.*, 1971). At their conjugative transfer, boundaries separating species or genera are no obstacles. The limits of transfer possibilities for resistance to antibiotics genes — through R plasmid conjugation or transformation — cannot be foreseen at all. A single R plasmid, which may be a conjugative one, can bear genetic determinants of resistance to several antibiotics — and even to all antibiotics and chemotherapeutics routinely used in clinical practice — in a single DNA molecule (Watanabe and Fukasawa, 1961). E. g. the plasmid R 724 belonging to the incompatibility group O codes for resistance to chloramphenicol, tetracyclines, streptomycine, kanamycine and further aminoglycosides and to sulphonamides. Altogether, genes for resistance to more than 10 basic antibiotics have been found in plasmids. The R plasmid-coded resistance resides in various molecular mechanisms. In principle, it lies in the synthesis of specific enzymes either splitting or modifying antibiotcs so that they lose their activity (through acetylation, adenylation, phosphorylation, etc.). Alternatively, it depends of the modification of bacterial cell surface, blocking the drug passage into the cells; this is most frequent, e. g. in the case of resistance to sulphonamides and tretracyclines (Mitsuhashi and Hashimoto, 1976).

It is also worth noting that certain R plasmids markedly alter the sensitivity of their bacterial hosts to bacteriophages and bacteriocins. It is obviously due to a pleiotropic effect of their genes on the molecular composition and architecture of bacterial surface layers. Several mechanisms have been reported to prevent the lytic cycle of a bacteriophage in a bacterium

bearing a plasmid and thus to protect it: resistance (through alteration of phage receptors), abortive infection (due to the damage of the cell membrane), superinfection exclusion (where the virus DNA is prevented from enetering the bacterium) or — perhaps — restriction (phage DNA is broken down by restrictive endonucleases — Duckworth *et al.*, 1981).

Lachowicz *et al.* (1972) made the strain *Shigella flexneri* (serotype 2a) Azi^r lactose-positive by transferring a F'lac plasmid into it; eventually, by conjugation with type donors they transferred 16 various R plasmids (each representing an individual incompatibility group) into this lactose-positive Shigella, thus isolating 16 clones with various antibiotics resistance patterns. Some of these gained or lost their sensitivity to certain phages of the standard typification set. Šmarda (unpublished) analyzed the sensitivity of the same clones to 17 standard colicin types. In 32 combinations (out of 272 tested) the colicin-sensitivity pattern was changed; in 15 combinations colicin-sensitivity was established, in 17 it was lost. In back croses with *E. coli* recipients it was shown, however, that there was no complete linkage between any determinants of resistance to antibitotics and those of sensitivity to colicins.

B plasmids

Through possession of anyone of the class B plasmids, host bacteria gain the ability to produce specific bacteriocins and — at the same time — the immunity against them (Brandis and Šmarda, 1971). As mentioned above, bacteriocins are proteins of specific bactericidal effects on certain strains of the same species, genus or — at least — family of the bacteriocinogenic producer. Only some bacteriocins of Gram positive bacteria dispose of a wider activity spectrum. Molecular weights of most bacteriocins amount to 10^4. Bacteriocins are named according to the producer strain species: colicins (Fig. 4), cloacins, marcescins, megacins, pesticins, pyocins (aeruginocins) etc. Wrongly, some have been termed according to their producer genus: staphylococcins, enterocins, clostocins.

Bacteriocinogeny is a wide-spread and probably general marker of bacteria. As far as we are aware, the biochemical (molecular) mechanisms of the inhibitive action of various bacteriocins are manifold; many of them kill sensitive bacteria through the disruption of the energized state of their plasma membrane, through discharging its electrostatic potential (e. g. colicin K, cloacin DF 13). Other bacteriocins depolymerize and denature DNA, further ones break down 16S rRNA or cause point lysis of the cell wall etc. The biochemical targets for most bacteriocins are not known yet. Generally, bacteriocins act on sensitive bacteria via specific receptors. Interesting is the fact that also cells completely lacking walls are killed: procaryotic — protoplast-like stable L-forms of Gram-negative bacteria (Šmarda and Taubeneck, 1968) as well as eucaryotic — protists (Šmarda *et al.*, 1975), mammalian tissue cells (Šmarda *et al.*, 1978) and especially cancer cells (Šmarda and Drozdová, in preparation).

Some bacteriocinogenic plasmids bear also genes determining or, at least, markedly enhancing the virulence of their hosts (Col V) linked to those for bacteriocinogeny.

M plasmids

M plasmids, proved in bacteria of the genus *Salmonella*, *Proteus*, *Erwinia*, *Klebsiella*, *Streptococcus*, but also in *Escherichia coli*, are metabolic ones. They code for information of special metabolic pathways or for parts of them. In this way, they confer upon their hosts certain metabolic markers, often very characteristic of their species; these are used as taxonomic criteria, as the production of sulphure-hydrogen in *Proteus* and *Salmonella*.

The M class plasmids include also very important plasmids which ensure their hosts the metabolic outfit to fixation of air elemental nitrogen. Natural hosts of these plasmids are the genera *Rhizobium*, *Azotobacter* and others. The Nif plasmid harbours the *nif* group of 17 necessary genes, including the structural gene for a specific nitrogenase. In *Klebsiella pneumoniae*, all the *nif* genes are located in the chromosome. A plasmid carrying *nif* genes of *Klebsiella pneumoniae* has been artificially constructed (Dixon *et al.*, 1976). It belongs to the incompatibility group P, remarkable through its very wide spectrum of potencial hosts among Gram-negative bacteria. It has been successfully transferred and expressed in *E. coli*. Attempts were made to transfer the Nif plasmid into soil *Pseudomonas* bacteria, into ubiquitary soil *Bacilli*, as well as directly to cereals. By means of a Ti plasmid (see below) it could be integrated into plant cell chromosomes.

In various pseudomonads, but also in *E. coli*, there are plasmids which enable these bacteria to establish in quite new ecological niches, mostly through making metabolic use of unusual, even curious substrates: camphor (Cam plasmid), toluene (Tol plasmid), octanole, xylene, naphthalene or salicylic acid (Chakrabarty, 1976). For this group of plasmids, the denotation Deg has been proposed. In *Pseudomonas aeruginosa* or *Pseudomonas putida* strains can be found, equipped with plasmids coding for oxidative enzymes which enable the biodegradation of various aliphatic, aromatic, cycloparaphinic and pluricyclic aromatic hydrocarbons. Through transformation, DNAs of such plasmids may be combined in the hosts in such a way that they get e. g. the ability to exploit masterfully all linear aliphatic hydrocarbons of chain lengths from C_6 upto C_{18} as energy sources. Through introduction of a further plasmid cointegrate, the capacity of a strain with such an outfit to degrade hydrocarbons can be further extended to alicyclic hydrocarbons such as terpenes etc. Such strains can oxidate a variety of wasteproducts of oil refinement, which are not exploitable and decomposable in any other way, into carbon dioxide and water.

P plasmids

Class P plasmids bear the genetic information for various physiological conditions of their hosts, such as genes for sporulation, or for special extracel-

lular products of constructive metabolism, e. g. for pigmentation. Again, many of these physiological abilities serve as basic taxonomic criteria: in the genus *Bacillus*, *Streptomyces*, but also in *Erwinia* or in *Escherichia coli*. In various *Streptomycetes* it has been proved that their production of antibiotics is a plasmid-bound function; thus the production of methylenomycine A is coded by a transmissible plasmid of *Streptomyces coelicolor* unlike that of actinorhodine). Plasmid-coded or at least plamid-regulated is the synthesis of tetracycline and of the macrolide antibiotics (e. g. erythromycine). A question may be asked as to whether this is not a general situation in all *Streptomycetes*. At least the production of oxytetracycline in *Str. rimosus*, of chloramphenicol in *Str. venezuelae*, or kasugamycine and aureothricine in *Str. kasugaensis*, of homolycine in *Str. clavuligerus* and possibly also of turimycine in *Str. hygroscopicus* depends most probably on regulative functions of genes localized in particular plasmids (Hopwood, 1978).

V plasmids

Plasmids of the class V contribute substantially to the virulence of their host bacteria. They are among others: plasmid Vir of the *Salmonellae*, the above mentioned plasmid Col V, plasmids for superficial antigen K88 of enteropathogenic strains *E. coli* of piglets (Orskov and Orskov, 1966) and for antigen K99 of enteropathogenic strains of calves and lambs. These plasmids, signed also San, enable the adherence of their hosts to the surface of enteric mucosa epithelial cells. Also, virulence antigens of *Yersinia pestis* (phenotype Vwa^+) are coded by plasmids of this class.

T plasmids

Plasmids Hly of various bacteria, e. g. of *Streptococci* or *E. coli* (Smith and Halls, 1967) code for α-, β- and γ- haemolysins. These plasmids and those coding for toxicity, i. e. for production of thermo-stable and -labile enterotoxins (Ent plasmid — So *et al.*, 1975) or urease — form the class T. Such plasmids are well-known not only in *Streptococci*, but also in various Gram-negative enteropathogenic rods, mainly in *E. coli*, *Salmonella* and *Proteus*.

Ti plasmids

Remarkable, very peculiar plasmids are those of the class Ti. They harbour genes responsible for tumour transformation of plant tissue cells, i. e. for the induction of plant tumours. They are known in *Agrobacterium tumefaciens* and are easily transferable into root cells of higher plants, where they cause crown-gall tumour nods (Zaenen *et al.*, 1974). They were succesfully integrated into host chromosomes.

D plasmids

Plasmids of the class D are, as a matter of fact, free genomes of temperate or defective bacteriophages, which exist only in the cytoplasm of host cells. They are neither capable of integration into their chromosomes (and hence

to become prophages), nor are they able to accomplish a reproductive cycle (and thus cause cell lysis). They are known in *E. coli* and their type is DNA of the phage Pl. Also the DNA replicative forms of thread-like bacteriophages *E. coli* belong into this class; they control the continuous production and "secretion" of their mature forms, again without concomitant lysis.

Plasmids with molecular regulatory functions

Plasmids are known coding for bacterial DNA restriction (or ability to modify the DNA of host bacteria). Plasmid pKM 101 enhances the mutation rate of *Salmonella typhimurium* cells.

C plasmids

Last, plasmids of the class C have been proved in at least 17 bacterial species of various families. These are cryptic plasmids, both the gentotypic and phenotypic meanings of which for the host bacteria are not known yet. Modern separation techniques (gel electrophoresis, gradient ultracentrifugation and isoelectric focusation) frequently prove at least one DNA fraction corresponding to a low-molecular plasmid in a strain analyzed, the significance of which is still obscure.

Dynamics of Plasmid Constitution and Possible Consequences for the Evolution of Bacteria

Plasmids, first discovered in *Enterobacteriaceae*, have since been proved in bacteria of many species of at least 36 genera — and, most probably, it is possible to meet them in any species. The presence of a plasmid (or plasmids) in a bacterial cell is rather a rule than an exception. Many — perhaps most — — plasmids obviously dispose of a wide host spectrum; certain conjugative plasmids are capable of an autonomous transfer into strikingly many species (though the frequency of interspecific conjugative transfers of plasmids is usually substantially lower than that of intraspecific ones). In this way, the bacteria may easily acquire genes — and markers — not common for their species. Noncoliform enteric bacteria can transfer into *E. coli* germs a variety of genes, for which there are no locuses at disposal in their chromosome. The natural processes of conjugation and transduction undoubtedly take place in the intestinal tract. Through these ways, *E. coli* bacteria may acquire e. g. genes for urease, lysine-decarboxylase, phenylalanine-deaminase, sulphite-reductase (Reanney, 1976), for gelatine hydrolysis, for citrate utilization etc. Conclusively, plasmids warrant a far-reaching genetic flexibility to bacteria.

In the introduction, the capability of many plasmids to integrate themselves into chromosomes of their host bacteria was mentioned. This was first discovered for plasmids F, but many plasmids B, Col, M, R, T and P dispose of the same ability. The integration frequency of various plasmids varies in wide limits. Integrated plasmids can be released again from the chromosome and regain their extrachromosomal autonomy. During

this process, they may comprise adjacent chromosomal genes into their circular structure. In this way, plasmids of the "prime" type are formed: F′, B′, Col′, M′etc. The gene equipment of these varies greatly (e. g. Fredericq, 1969). The best known are plasmids F′lac, F′gal (in *E. coli*) and P′lac (in *Proteus*), containing complete lactose operons from their hosts' chromosomes. (F′lac has the length of 38 μm.) Owing to their capability of autonomous interspecific transfer, these plasmids may give rise also to lactose-positive clones of typical lactose-negative species.

It has been mentioned that resistance genes are located in certain segments — in the non-essential regions — of R plasmids DNA. Both ends of these segments are mostly delimitated by particular palindromatic nucleotide sequences ("inverted repeats" — Sharp *et al.*, 1973). These short symmetrical sequences (of the same DNA strand) being complementary to each other, enable pairing of both palindromes and forming a short double-strand stem of a single-strand loop. This structure is capable of insertion into various regions of plasmids, but also of chromosomes; therefore, such DNA formations are called insertion sequences (IS). A complete insertion sequence may easily and repeatedly "jump" from one plasmid into another, but also from a plasmid into a chromosome and vice versa, without losing anything of the intergrity of its structure (Starlinger, 1977). The best known genes of insertion sequences, identified until now, are — as indicated — genes for resistance to antiboiotics; IS furnished with resistance genes are called transposons (Cohen, 1976). 10 various transposons have been identified, some of them bearing resistance genes for two antibiotics in one loop. It may be assumed that insertion sequences and transposons play an important, though not yet sufficiently understood role not only in the evolution of plasmids, but also in the evolution of bacteria; they enable a variety of reorganisations of bacterial genomes through translocations, duplications, inversions, etc. (E. g. the plasmid F makes use of the IS2 for its integration into the bacterial chromosome.)

Are Plasmids Autonomous Organisms?

It has been shown that plasmids resemble DNA-viruses in all basic respects — except that they are not able to organize any protein coat around themselves and hence lack any extracellular form (which would be analogous to free virions). Thus disposing of a substantially simpler organization than viruses, plasmids may be poised at the very threshold of life — just on the borderline between the living and hon-living. But there are some important facts strongly supporting the view that they are independent subcellular organisms — more or less parallel to viruses (Novick, 1981).

First, each plasmid controls autonomously its characteristic copy number in any host bacterium (see Chapter 2), while copy numbers of different plasmids in the same host vary from 1 to more than 100. That means that each plasmid masterfully regulates its own reproduction. Second, through conjugational and transductional transfer plasmids may be exchanged

between different species and even genera of bacteria, which — on the other hand — are not able to exchange chromosomal genes. Third — and most significant: plasmids carry genes that enable them to survive, to reproduce and to spread horizontally regardless of the fate of their host cell and of their host species.

These observations indicate that plasmids are more than mere components of genomes of particular bacteria: that they are autonomous organisms, forming the lowermost element in the hierarchy of life. Within the system of subcellular organisms, viruses (animal, plant and bacterial ones) can be considered partly extracellular, partly intracellular parasites. Plasmids, on the other hand, can be regarded as endosymbionts: they coexist stably within the host organism and — at least on special occasions — they serve it by supplying it with hereditary genetic functions of high adaptive value and thus with selective advantages.

The hypothesis that naked circular DNA molecules are organisms, of course, does not follow the lines of the conventional cell-based concept of life. But there is no reason to deny that any nucleic acid system that controls its own reproduction (plasmids, viroids) reveals the most important and typical trait of life.

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Explanation of Figures (Plates XX and XXI):

Fig. 1. DNA molecules of the *Escherichia coli* plasmid pSF 2124. Native CCC (supersoiled) forms (arrows) and open OC forms (resulting by cleavage of one strand). × 56 100. Orig. Benada.

Fig. 4. Inhibition zones formed by colicins in agar cultures of sensitive bacteria. (Colicin is synthesized during the growth of colicinogenic bacteria in the centre of agar plate.)