

PROKARYOTIC CELL STRUCTURE AND FUNCTION

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Keywords: cytoplasm, eps, fimbria, flagellum, gram-positive, gram-negative lipopolysaccharide, nucleoid, outer membrane, peptidoglycan, periplasm, pili, plasmid, prokaryote, ribosome, structure.

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1. Introduction

Any account of prokaryote structure and function is limited by the exclusion of information on detailed physiology and growth. This problem is partially addressed by the inclusion of detail beyond the scope of this article as defined by the title. The reader is referred to chapters in EOLSS where prokaryotic growth and physiology (*Prokaryote Physiology in Anaerobic and Aerobic Atmospheres*), genetics (*Prokaryote Genetics*) and diversity (*Prokaryote Diversity*) are discussed, as well as to *Bioenergetics* and *Biochemistry*. What follows is therefore a relatively simplistic view of prokaryotic structure and function, which in many cases relies on generalizations. Where possible, examples of significant exceptions to structural norms or functional roles, and the organisms exhibiting these, have been included.

Prokaryotic cells typically range in size from 0.2 μm to 2.0 μm in diameter and from 1 to over 6 μm in length. Certain *Spirochaeta* may be as long as 250 μm (although they may be as narrow as 100 nm), while rare exceptions such as *Epulopiscium fishelsoni* may be as large as 600 x 80 μm , and certain ultramicrobacteria (or nanobacteria) as small as 0.05 μm in diameter have been observed microscopically. The cell size of bacteria appears to be limited by rates of diffusion within the cell. Cell shapes vary from uniform spherical or coccoid cells such as *Streptococcus pyogenes*, to rod shaped cells (bacilli) which may have either blunt ends as with *Bacillus subtilis* or tapered ends such as *Fusobacterium nucleatum*, to irregularly shaped cells which may be curved as is the case with *Vibrio cholerae*, or distinctly pleomorphic in that they lack a characteristic shape (such as *Corynebacterium diphtheriae*, or the Mycoplasma's which lack cell walls). Rigid spiral (spirillum) cells such as those of *Spirillum volutans*, flexible spirals (*Spirochaete*) for example *Treponema pallidum*, trichomes such as the cyanobacterium *Oscillatoria limnetica*, or branched filaments, as are found in organisms such as *Streptomyces albus*, are also typical bacterial cell shapes. Appendaged bacteria such as *Hyphomicrobium facilis* have irregular but characteristic shapes.

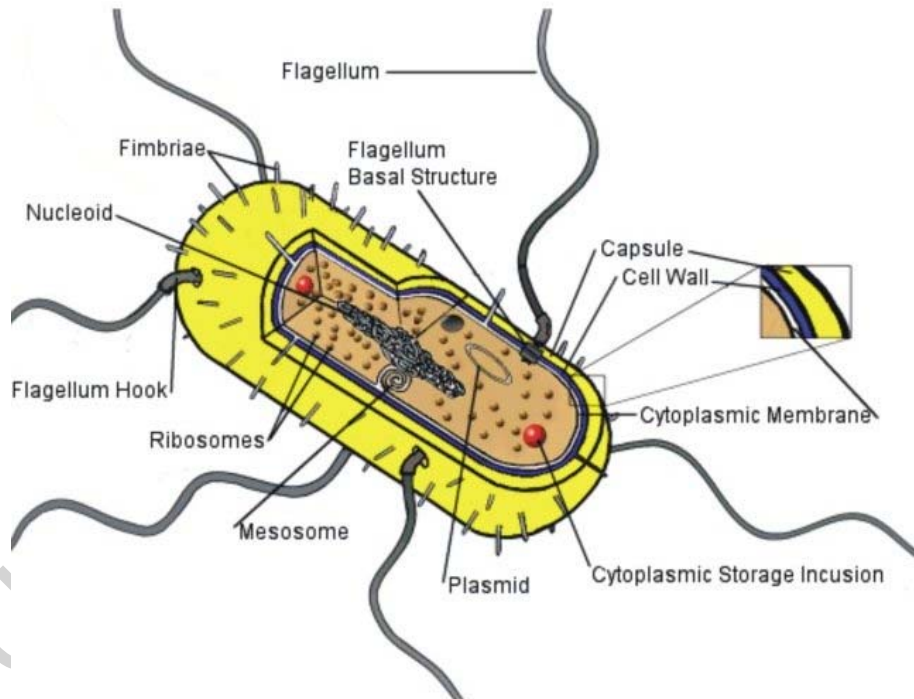


Figure 1. Generalized bacterium (diagrammatic representation)

A variety of structures and structural variations are found within prokaryotic cells. Not all structures are common to all genera, and major differences occur between higher-level groupings. Despite the variations, a common structural motif is apparent in that almost all bacteria have a chemically complex cell wall separated from the cell membrane by a periplasmic space. Invaginations of the plasma membrane to form simple membranous structures in the cell may occur, since prokaryotes contain no membrane bound organelles. The interior of the cell appears morphologically homogenous with the exception of a discrete region in which the genetic material is located, termed the nucleoid, and contains the ribosomes, inclusion bodies, and various

cellular components such as RNA's and enzymes. Exterior to the cell wall, flagella, fimbriae and pili may be present, and some bacteria possess a capsule or slime layer composed primarily of polysaccharide. Figure 1 shows a diagrammatic representation of a generalized bacterium.

2. Nucleoid

The genome of the bacterium is usually a single covalently closed circular molecule of dsDNA, supercoiled and highly folded to fit into the cell. Although typically not membrane bound, exceptions such as the genus *Pirellula*, which has a single membrane surrounding the nucleoid region, and *Gemmata obscuriglobus* in which the nuclear body is surrounded by two membranes, do occur. In some bacteria, notably species of *Streptomyces*, the genome may be linear. The single chromosome may contain all the necessary genetic information, or additional small circular dsDNA plasmids may be present in the cytoplasm. Actively reproducing bacteria contain additional complete or partial copies of the genome (two to four copies per cell) presumably to allow for the slower chromosomal replication rate relative to the cell doubling time during phases of maximal growth rate. In bacteria where multiple chromosomes are apparent, such as *Rhodobacter sphaeroides* 2.4.1T, Chromosome II appears to have no functional difference to chromosome I. The DNA typically constitutes between 1 and 2% of the dry cell mass. The *Escherichia coli* genome is approximately 4.2×10^6 bp in length, but chromosome size varies from as small as 7.9×10^5 bp in *Mycoplasma pneumoniae* to 9.5×10^6 bp in *Myxococcus xanthus*. The packaging of bacterial chromosomes in the nucleoid into nucleosome-like structures appears to be facilitated by histone-like proteins, the most abundant of which (at least in *E. coli*) is the protein HU. This is present in the cell predominantly as a 9 kDa heterodimer, $\alpha\beta$, capable of introducing negative supercoiling in the presence of topoisomerase I, and condensing the DNA into nucleosome-like structures, suggesting a histone-like role for the protein. It has been suggested that the mesosome is the point of attachment for the chromosome, and that this may facilitate segregation during cell division.

3. Cytoplasmic Matrix

The cytoplasm is the aqueous solution containing all cytoplasmic components and constitutes the region within the cytoplasmic membrane, excluding the nucleoid. The cytoplasmic matrix is about 70% water and lacks a cytoskeleton. Despite the apparent homogeneity, the matrix is highly organized with respect to protein location.

3.1 Ribosomes

Presumed function	Protein
mRNA binding	S1, S2, S3, S5, S9, S14
tRNA binding	S3, S5, S9, S10, S14, S19
30S folding	S4, S7, S8, S15, S16, S17
Peptidyl transfer	L2, L5, L6, L11, L15, L16, L18, L25, L27
50S folding	L3, L4, L20, L24

Translocation and binding of protein factors	L7/L12
Unknown function	S6, S11, S13, S18, S20/L26, S21, L1, L9, L13, L14, L17, L19, L21, L22, L23, L28 – L34

Table 1. Presumed functions of ribosomal proteins in *Escherichia coli*

The ribosome is the platform and active site of protein synthesis. Prokaryotic ribosomes constitute up to 10% of the dry cell mass, have a sedimentation coefficient of 70S, and are composed of a large (50S) and small (30S) ribosomal subunit. The *E. coli* 50S ribosomal subunit consists of 34 ribosomal proteins, and the 5S and 23S rRNA molecules, which, together with the 30S subunit consisting of 21 ribosomal proteins and the 16S rRNA, give a combined molecular mass of 2.7×10^6 . Table 1 lists the polypeptide components by function.

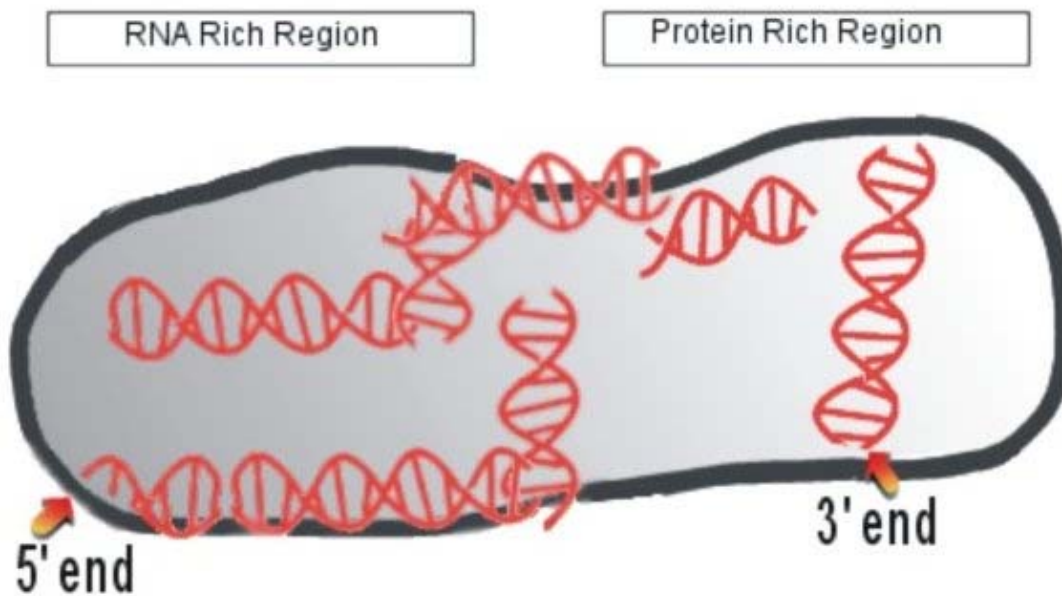


Figure 2. Diagrammatic representation, in two dimensions, of the 30S ribosomal subunit and 16S rRNA.

The rRNAs form stable three dimensional structures which serve as a structural scaffold for ribosomal protein attachment, as depicted in Figure 2. In addition to their structural role, the 16S rRNA has a region complementary to the Shine-Delgarno region of mRNA, which facilitates mRNA binding to the ribosome for translation. The structure of the small and large subunits, as revealed by electron microscopy, is diagrammatically represented in Figure 3. The larger subunit is more spherical than the small subunit. The region between the head and the base of the small subunit appears to fit over the edge of the base on the stalk side of the large subunit, such that the platform lies between, but displaced from the central protuberance of the large subunit (see Figure 3c).

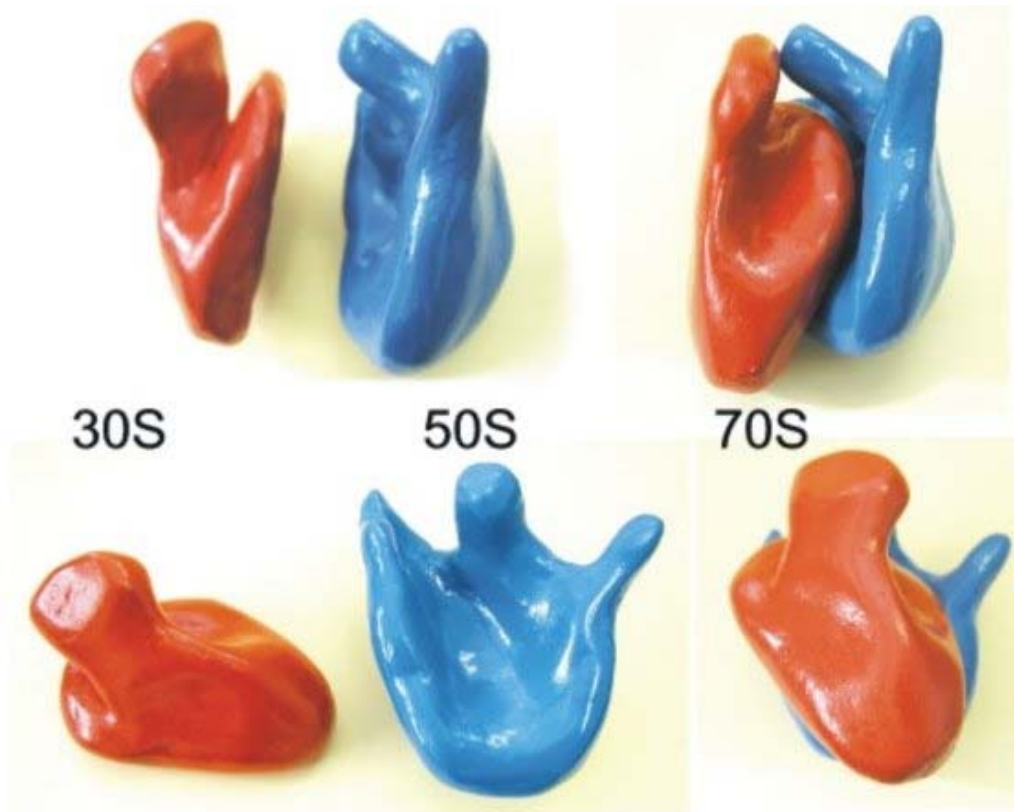


Figure 3. Diagrammatic representation of the ribosomal subunits of the 70S ribosome, in two orientations, independently and as a complete ribosome.

The 50S subunit is not involved in the initiation of translation, which requires the free 30S subunit to bind an N-formylmethionyl tRNA^{fMet} (fMet-tRNA) under the direction of IF2 which has bound GTP, followed by an mRNA molecule which is positioned correctly by the fMet-tRNA and the 16SrRNA. IF3 ensures the independence of the subunits not actively busy with translation, by binding to the 30S subunit when no tRNA is bound. IF1 one appears to play a role in 50S subunit binding as well as the concomitant release of IF2 and GDP. The ribosome has two binding sites for aminoacyl-tRNA and peptidyl-tRNA, the A site and P site respectively. Elongation of the peptide occurs in cycles. Initially the P site contains the fMET-tRNA and the A site is empty. The aminoacyl-tRNA corresponding to the codon positioned at the A site is then inserted. This is facilitated by EF-Tu which has bound GTP, thereby allowing the binding of the aminoacyl-tRNA. The Ef-Tu-GDP which is released, is reactivated by EF-Ts which facilitates the release of GDP and binding of GTP to EF-Tu. The transpeptidation reaction is initiated by the binding of an aminoacyl-tRNA to the A site, and is a function of the peptidyl transferase activity of the large subunit. The 23S rRNA appears to play a significant role in the peptidyl transfer. The peptide chain is thereby transferred to the tRNA at the A site. This is followed by translocation of the ribosome along the mRNA during which the P site tRNA moves to the exit site and is released, and the tRNA containing the growing peptide chain is moved to the A site. This process is facilitated by the translocase protein, EF-G, with energy provided by GTP hydrolysis. During translocation the ribosome undergoes some conformational changes. The cycle repeats until termination (nonsense codon at A site which is recognized with the aid of

release factors RF-1, Rf-2 and RF-3) results in the hydrolysis of the peptide from its tRNA by the peptidyl transferase, and release of the tRNA. Finally, ribosomal dissociation and mRNA release occurs.

3.3 Plasmids, episomes and bacteriophage

Plasmids are autonomous extrachromosomal replicons, which are widespread amongst the bacteria and offer metabolic and physiological flexibility of the organism's response to environmental changes and stresses. With rare exceptions, plasmids exist within the cell as highly supercoiled circular dsDNA molecules of a few kilobases, such as ColV-K30 which is 2 kbp in length, to over a hundred kilobases in length, for example the CAM plasmid of *Pseudomonas*. It should however be noted that the predominance of circular dsDNA plasmids in the literature may be due to the routine isolation methods and the relative difficulty of identifying large linear plasmids or ssDNA plasmids, and that the linear plasmids that have been identified in *Streptomyces*, *Rhodococcus*, and the spirochaete *Borrelia* may represent examples of plasmids that are more common than is appreciated. Plasmids are present in host cells as either single copies or multiple copies in excess of 40 per cell. Plasmids may encode one or more phenotypic features, which may in turn be expressed by the host. These features include antibiotic resistance (e.g. R1, R6, R100, pSH6 and pSJ23a), toxin production (e.g. pZA10 and Ent(p307)), substrate degradation including xenobiotics (e.g. pJP4, pWWO and TOL), adherence antigens such as encoded by the K88 plasmid of *E. coli*, bacteriocin production (e.g. Col E1, ColE2 and CloDF13), and sex pilus and conjugation (e.g. R1, F factor and RP4). A representation of R100 is included as a typical plasmid in Figure 4. In addition to the genes encoding these features, plasmids contain the necessary regulatory and replicative elements to ensure replication to correct copy number and segregation into daughter cells.

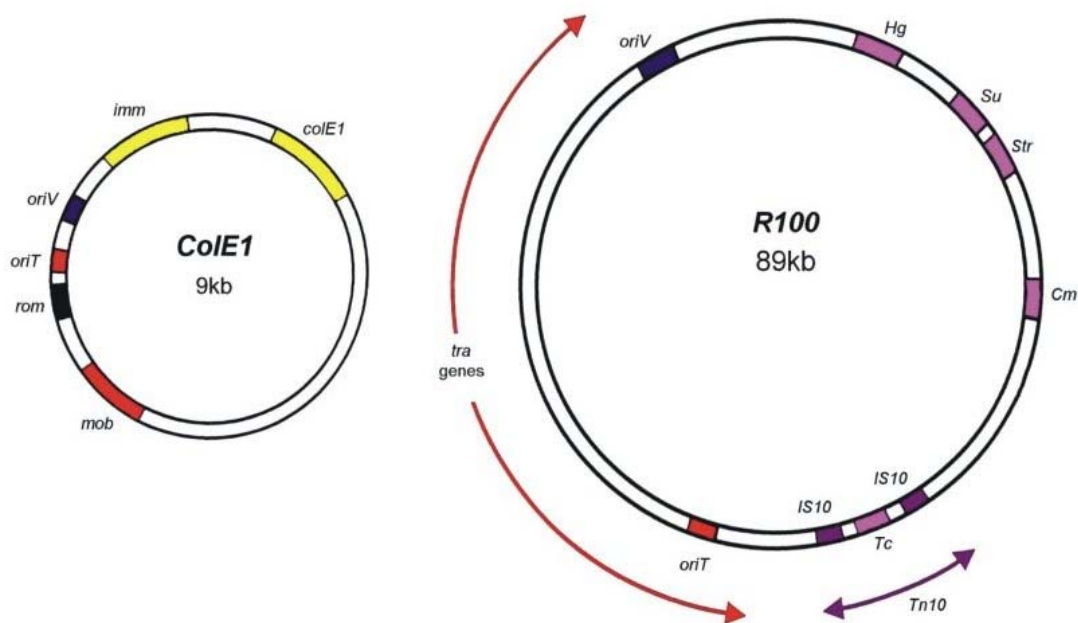


Figure 4. The plasmids R100 and ColE1. *tra* are transfer genes, *oriT* is the origin of

transfer, *oriV* is the origin of replication, *rom* product is involved in copy number control, *colE1* encodes colicin E1 and *imm* codes for immunity to the colicin, *mob* codes for the nuclease necessary for mobilization. Resistance genes are *Tc* (tetracycline), *Cm* (chloramphenicol), *Str* (Streptomycin), *Su* (Sulphonamides), and *Hg* (Mercuric ions).

Plasmids are commonly classified according to their molecular characteristics, gene functions (particularly antibiotic resistance patterns which they confer), incompatibility groups, host range, and bacteriophage susceptibility of hosts. Molecular characterization is based on features such as plasmid size, the plasmid's restriction endonuclease map, or DNA sequence. The presence and diversity of antibiotic resistance genes provides a rapid and easy method of characterizing a plasmid, but suffers the same problems of molecular characterization in that plasmids are dynamic and subject to gene rearrangements, deletions and insertions, particularly by transposition. Incompatibility grouping provides a more fundamental differentiation of plasmid types. The characterization is based on the inability of closely related plasmids to coexist in a host, and is easily established by transformation of hosts already carrying a particular plasmid. Incompatibility of plasmids is based on the inability to distinguish between plasmids during a particular stage of either replication or partitioning of plasmids during host division. For single copy plasmids this may be understood by the presence of an incompatible plasmid mimicking a second copy of the original plasmid and by following the negative control theory whereby some form of replication repressor is synthesized. Similarly, the inability to distinguish between the plasmids by the system ensuring segregation, for example similar *cis* acting partition sites, could result in incompatibility. The ColE1 multicopy plasmid (~20 copies per cell) replicates by means of an RNA primer, the transcription of which is initiated 555 bp upstream of the *oriV*, which is cleaved by RNase H at the origin generating a free 3'-OH which acts as a primer for replication. Replication is regulated by an antisense RNA of approximately 108 bp, RNA I, which prevents replication presumably by alteration of the primer molecule's secondary structure. The RNA I therefore functions as a repressor for any newly introduced plasmid with the same replication strategy. Iterons, as are found in the F plasmid and RK6, are small repeated sequences also cause incompatibility by virtue of their regulatory effect on replication and the potential for cross regulation. F has nine repeats of a 17 bp sequence, while RK6 has eight repeats of a 22 bp sequence. Iterons function in regulation of plasmid copy number by binding the plasmid encoded Rep protein, which in turn is regulated by CopB-*copA* interactions (the basis for the IncFII observed for example between R100 and R1). In F, which has several iterons, plasmid copy number is tightly controlled, while in RK6, which has only one iteron, copy number is less tightly controlled. It is postulated that inter and intramolecular associations facilitated by Rep result in reduced ability to replicate.

Plasmids may also be characterized by the colicins or bacteriocins encoded. One such example, ColE1 is of particular importance because of the detailed knowledge on its replication and control. The presence of virulence or pathogenicity components is also of use in the characterisation of plasmids, and includes toxins such as those produced by *Clostridium botulinum* and *C. tetani*, the enterotoxins and adhesion mediators of *E. coli*, and in some cases such as the *Ti* plasmid of *Agrobacterium tumefaciens*, completely functionally define the plasmid. Although not pathogenicity or virulence factors, the plasmids of the genus *Rhizobium* carry the genes for nodulation and nitrogen fixation

necessary for symbiosis with leguminous plants, and are often grouped with the *Agrobacterium* plasmids as plasmids of plant associated bacteria. Metabolic features, some of which were referred to earlier, provide yet another means of identifying and or classifying plasmids. In general terms these metabolic features should be expressed as intracellular proteins conferring a unique metabolic activity such as a particular fermentation on the host. Bacteriophage susceptibility as a function of plasmids is yet another mechanism of characterization of plasmids. For example, the F plasmid codes for the F pilus, which is the attachment site for bacteriophages M13 and MS2.

The host range of a particular plasmid is defined by the nature of the plasmid origin of replication and the ability of the host to recognize the *oriV* and may be classified as *narrow host range*, such as those containing the PMB1 origin of replication, or *promiscuous*, that is having a broad host range and being conjugative e.g. RP4. Plasmids may replicate bidirectionally from the origin of replication, for example ColE1 and F, or unidirectionally, for example R100. Alternatively, as in many gram-positive plasmids, replication might occur via a single stranded intermediate. Regulation of plasmid replication by iterons, or RepA type systems may be supplemented by systems such as the *rom* mediated control of ColE1. *rom* appears to mediate the interaction of RNA I and RNA II, where RNA II is the primer molecule and RNA I the antisense molecule, as described earlier. ColE1 is replicated by DNA polymerase III, the principle replication polymerase, DNA polymerase I and RNA polymerase.

It should be noted that numerous artificially constructed plasmids are available for cloning and various other purposes. Although every attempt is made to contain these plasmids, typically by use of hosts that are unable to survive in the natural environment, plasmids can and do escape and become stably maintained in feral populations. In addition to this, constructs are used to confer particular phenotypes on bacteria specifically for release into the environment, and these are thus designed to survive.

The significance of bacteriophages in the genetic compliment of bacteria is often underestimated. Although not part of the prokaryotic structure *per se*, their prevalence imparts a not insignificant functionality via transduction. EHEC express two phage encoded toxins, the shiga-like toxins. Similarly, transposons may impart distinctive metabolic features to hosts and should be considered equally with plasmids in terms of their role in bacterial phenotype.

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