implications for the view on the interaction of the electron carriers and, as a consequence, on the mechanism of electron transfer. The situation at the left hand side leads to forced contacts between the redox carriers, which could implicate electron transfer occurring in an (ordered)[12] multi-enzyme complex, while that at the right hand side would allow a mechanism based on random collisions between (possibly partially complexed)[13] redox carriers[9]. However, for the latter it should be realized that the periplasm also harbours binding proteins, hydrolases and polysaccharides so that in fact all available space left might be occupied. Recent work on the nature of the periplasm[5,14] suggests indeed that this cellular compartment has to be regarded as a highly viscous layer. Thus, although the periplasmic space seems much bigger than generally assumed, the yet compact structure might imply that transport processes of substrates and electrons proceed by (semi-ordered) chains of associated carrier proteins (see Ref. 14).

The issue of the structure and size of the periplasmic space of Gram-negative bacteria is far from being resolved. In an effort to shed more light on this issue we have investigated the structure of the periplasmic-localized electron-transport chain [components randomly distributed in the periplasm or organized in (semi) ordered chains][9]. Preliminary results suggest that the components are distributed randomly in the periplasm. Overall electron-transfer rates by whole cells could be described by rate constants for electron transfer (measured *in vitro*) and the concentrations of the components in the periplasm assuming a periplasmic space which occupied 30% of the total cell volume (equivalent with a periplasmic layer of ~50 nm). Thus from these unpublished experiments it seems that the periplasmic layer is much wider than assumed in most textbooks.

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# A kit generation?

We read with interest the letter of Müller-Hill (*TIBS* 14, 444, 1989) in which he commented upon a published example of the flagrant and widespread failure of a large section of the modern generation of molecular biologists to understand even the simplest principles of the techniques they use. The root cause of this lamentable and embarrassing situation seems to be the increasing use of pre-packaged kits and reagents, in preference to consulting standard laboratory manuals (the 'Bibles' of yore) and using know-how and protocols developed and adapted independently in different laboratories through the understanding and years of continual refinement of published procedures. All one needs to do now to clone (into a pre-prepared vector) and sequence a gene is to open a packet (most kits even contain buffers) and read an instruction leaflet (the new Bible!). This 'TV dinner' approach may be highly successful (more so than instant cuisine, for sure) but is putting power and all creativity in the packaging consultants, and banishing years of genetics and genius into '*lac*' kits. Many students no longer know or want to know how *lac* can be red, white or blue. We are surely well on the way to becoming a 'kit generation', where experiments are guaranteed to work or, you get your money back!


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# Thermodynamics and life

In his essay entitled 'Do the laws of chemistry apply to living cells?' P. J. Halling[1] brings attention to a question that has long been ignored by biologists and biochemists. As far as I know, this question was first asked by F. G. Donnan[2] more than 60 years ago in a paper entitled 'Concerning the applicability of thermodynamics to the phenomena of life'. His main theme, similar to that of Halling, was a warning to biologists that the laws of classical thermodynamics, being statistical in nature, might not be applicable to very small biological systems.

I have recently argued[3] that a thorough investigation of how cells deal with the fluctuations expected in the numbers and distributions of molecules present in very small numbers may lead to a better understanding, at a cellular level, of what life is and how it originated on earth. I have indicated that a small cell has at least three mechanisms by which it could decrease the fluctuations in the numbers of important molecules. These are (not surprisingly) growth, internal organization and reproduction, three of the basic structural and functional attributes of cells. Growth increases the number of molecules available and the internal organization, in the form of a complex cytoskeletal matrix, may help decrease Brownian motion and consequently prevent the molecules from diffusing away from the areas where they are needed for cellular reactions. Reproduction produces many copies of a cell. If a critical molecule (such as the *lac* repressor in Halling's example) normally exists in small numbers, statistically, some of the cells are expected to have more and some less than an average number of this molecule. If the cells have a mechanism to transfer these molecules from those cells that have a surplus of them to those that need them, in the long run, all the cells in a population will benefit from the general process of

reproduction. This argument is partially supported by the recent proposals that bacterial populations may actually behave like multicellular organisms, often growing as connected chains or mats and exhibiting intercellular exchange of metabolic products and genetic material[4,5]. Therefore, an isolated bacterium, especially a relatively small one, is likely to be an unstable system whose survival depends on the outcome of the statistical fluctuations governing its metabolism.

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## Chance phenotypic variation

It is refreshing to see discussion of chance phenotypic variation resulting from Poisson distributions of regulatory molecules present in small numbers[1,2]. Two points deserve clarification.

First, the volume of a bacterial cell depends considerably on its growth conditions[3]; *E. coli* cells growing in rich medium have a volume of about $10^{-15}$ l, which gives a concentration of a little over 1 nM for one molecule per cell, a convenient number usually adopted by workers in the field, rather than the 10 nM calculated by Halling. The difference is important if one is to estimate numbers of molecules per cell from concentrations cited by others. Cells which grow slowly have a smaller volume, suggesting that these types of phenomena would be more likely to be seen in such cells.

Second, Müller-Hill[2] recounts experimentation and discussion of these issues from the 1960s. However, one of his examples, spontaneous phage production by λ lysogens, almost certainly does not result directly from chance fluctuations in the level of λ repressor in a way analogous to *lac* repressor. Rather, two lines of evidence suggest it is an indirect consequence of chance induction of the host SOS response (reviewed in Ref. 4). Spontaneous induction is almost totally blocked by host *recA* mutations[5], which prevent the cleavage of λ cI repressor, and by *ind*$^-$ mutations in the cI gene[6,7], which make repressor resistant to cleavage. The few phage which are released from *recA*$^-$ hosts or *ind*$^-$ lysogens of *recA*$^+$ hosts are largely cI mutants. These data suggest that the SOS response is triggered in a small proportion of the cells in a growing culture, giving rise to induction of a λ prophage. How this rare SOS induction comes about is mysterious. It is unlikely to result from wide fluctuations in the level of expression of LexA repressor, since this gene is autoregulated[4] and there are about 1000 LexA molecules per cell[8]. More likely it results from random activation of RecA protein, leading to destruction of LexA and λ repressors. Hence, this system may be another example of phenotypic variation of the type discussed, but the molecular basis remains unknown.

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