The cisternal elements are usually tubular and form tortuous arrays in the peripheral cytoplasm and the juxtanuclear area. These cisternae branch repeatedly and are extensively interconnected with the vacuolar elements. Time-course studies using labelled tracers show that the elements in the peripheral cytoplasm are reached within a few minutes while those in the juxtanuclear area become labelled only after longer intervals. Acidification, ligand dissociation and the retrieval of many receptors such as those for transferrin is so rapid that most of it must take place within the peripheral elements.

The role of the juxtanuclear components is more obscure. However, it seems likely that they have a function distinct from that of lysosomes because, in several systems, a temperature-dependent step, operable only at 20°C and above, has been shown to be required for transfer of the ligands and receptors which they contain to the degradative environment of the lysosome²⁸. It is mainly within the juxtanuclear compartment that the limiting membrane boundary of the endosome appears to pinch off inwards to form multivesicular bodies: a process that may allow removal of receptors from the limiting membrane into the lumen of the system²⁷. In this way receptors can join the mainstream of 'content' travelling towards the lysosome. The retrieval of receptors from the juxtanuclear elements to the cell sur-

face has not so far been clearly demonstrated but there are strong suspicions that it is from this compartment that ligand-receptor complexes are transported to cell-surface domains other than that from which they were derived. The juxtanuclear compartment may thus operate as a redistribution centre for transcellular traffic as well as the site of transfer to lysosomes.

One structural aspect of the endosome that remains to be clarified is the extent to which its membrane boundaries are continuous. Two steps in the endosome pathway are strongly temperature sensitive: the plasma membrane-endosome and the endosome-lysosome transfers. Elsewhere, however, extensive connections between cisternal and vacuolar elements, although often difficult to demonstrate,

clearly exist, and it is possible therefore that much of the routing of integral membrane proteins within the system depends on transfers in the plane of the membrane rather than shuttling vesicles. Consideration of this possibility leads to the general question of what drives and directs the movement of integral membrane proteins within membranes and, for the endosome in particular, whether its receptors should be regarded as purposive migrant proteins²⁹, or passive aggregates carried by a directed membrane flow. The recent papers of Bretscher³⁰ and Ekblom³¹, which have given new impetus to the earlier ideas³² of a directed flow of membrane lipid, clearly suggest the latter. □

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Split genetics

Introns in archaeobacteria

from John Rogers

THE discovery of split genes in archaeobacteria¹ gives support to a pair of radical hypotheses about the earliest living organisms, the 'progenotes'. One of these hypotheses is that RNA splicing, far from being a eukaryotic invention, is a relic of the genetic instability of the progenotes. The other is that archaeobacteria represent a separate line of descent from the progenotes, alongside true bacteria (eubacteria) and eukaryotes.

RNA splicing occurs in all eukaryotes from yeast to man, but not in eubacteria such as *Escherichia coli*. Rather than seek a reason why such a disruptive process should have been newly selected in eukaryotes, Doolittle² proposed that it has always been with us. The progenotes, he suggested, used RNA splicing to link together such short meaningful stretches of RNA as their primitive polymerases could create, and the ancestors of eukaryotes retained this capability; it is the eubacteria which have lost it in 'streamlining their genomes'. (This scheme can also explain why eukaryotes lack the elegant bacterial operon system for coordinated gene expression; it is presumably another aspect of streamlined genomes.) RNA splicing does occur in mitochondria and chloroplasts — putative descendants of symbiotic bacteria — but these could arguably have acquired it from the eukaryotic nuclear genome.

The separate hypothesis that 'archaeobacteria' represent a third line of descent from the progenotes was proposed by Woese and Fox^{3,4}. Their RNA sequence analyses assigned these bizarre prokaryotes a taxonomic status equal with that of eukaryotes and eubacteria. The archaeobacteria combine features of both taxa along with other purely archaeobacterial features, and they could be the least evolved of all organisms.

Putting these two hypotheses together, an obvious corollary is that archaeobacteria, despite their small genomes, might retain RNA splicing. Now, Kaine *et al.*¹ have cloned a cluster of tRNA genes from *Sulfolobus solfataricus* — an archaeobacterium from acidic hot springs — and sequenced two of the genes. These are almost certainly genuine tRNA genes, with anticodons corresponding to serine and leucine, since they have all the characteristic features of tRNA sequences, and they are the only examples of these particular sequences in the genome. Both genes have two features previously seen only in eukaryotic tRNA genes: lack of the 3'-terminal CCA sequence (which must be added post-transcriptionally) and the presence of a short intron.

Each intron comes one nucleotide 3' to the anticodon and could base-pair with the anticodon, just as in eukaryotes. However, this base-pairing must be incomplete; instead, in a uniquely archaeobacterial feature, the boundaries of the introns seem to lie in repeated tetranucleotides (GACC) exposed in symmetrically placed loops.

So the admittedly specialized process of tRNA splicing apparently predates the origin of eukaryotes. One question now is whether the same applies to the much more widespread and versatile process of mRNA splicing, which is what the evolutionary problem is really about. □

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