RÉSUMÉ ————————————————————————————————————

Trapped exons

A difficulty in isolating genetic loci by 'reverse genetics' is how to identify the gene of interest from the surrounding DNA. A new technique described by G. Duyk and colleagues, called exon trapping, allows the direct recovery of transcribed DNA sequences (*Proc. natn. Acad. Sci. U.S.A.* **87**, 8995–8999; 1990). The DNA is cloned into a retroviral vector, downstream of a splice-donor site and an intron that contains a portion of the β-galactosidase gene. DNA fragments which contain a splice-acceptor site can generate spliced RNA, which after packaging can be identified because of the loss of β-galactosidase activity. Although the method is time-consuming and cannot detect genes without introns, it should become a powerful weapon in the molecular geneticist's arsenal.

Hard stuff

IN trying to understand how diamond films can be grown at low pressure, researchers at the Ford Motor Company may have stumbled on a crystal that could harder still than diamond. Using a reliable semi-empirical formula, M.A. Tamor and K.C. Hass (*J. Mater. Sci.* **5**, 2273–2276; 1990) calculated the lattice parameters for a structure which they term H6 and which can be likened to graphite with one bond in each planar hexagon twisted by 60° to bind covalently to the next plane. The structure is less dense than diamond, as befits a low-pressure allotrope, and metallic, as the surfaces of growing diamond films appear to be. Moreover, as required, it can be deformed continuously to give the diamond structure. But it remains to be seen whether the suggestion that the structure is 50 per cent harder than diamond will be borne out by more detailed calculations.

The right size

MANY vertebrate lineages show a tendency to extreme smallness that imposes constraints on their anatomy. In the Palaeozoic amphibian *Quasicaecilia texana* from the Permian of Texas, discussed by R. L. Carroll (*Palaeontology* **33**, 893–909, 1990), the back of the skull is dominated by an enormous otic (inner ear) capsule that forces the jaw articulation forwards, with consequent alteration of jaw musculature and, presumably, lifestyle and diet. The 15-mm-long skull of *Quasicaecilia* is by no means the smallest: modern salamanders of the genus *Thorius* can have skulls as tiny as 3.3 mm long, of which the eye sockets occupy a relatively enormous 27 per cent. The enigmatic caecilians, burrowing worm-like amphibians, have skulls very similar to *Quasicaecilia* — hence the name — but the similarity may be largely due to convergent responses to small size rather than common ancestry.

minor wild-type form of the invariant chain, with an extended N terminus due to the use of an alternative site of translation initiation, has an additional endoplasmic-reticulum retention signal that dominates other signals[3].

Using immunoelectron microscopy, Bakke and Dobberstein[4] found intracellular forms of the invariant chain in both early and late endosomes. Lotteau *et al.*[3] propose that the perinuclear vesicles to which the main wild-type form of invariant chain localizes in the absence of class II molecules are autophagosomes, because they are characterized by a lysosomal marker but are reached directly from the endoplasmic reticulum. They find that coexpression of class II molecules and the invariant chain results in the targeting of both molecules to a different population of more peripheral intracellular vesicles with endosomal characteristics. Class II α and β chains must obscure the perinuclear signal and allow the peripheral vesicle targeting signal to dominate. Coexpression of the α chain alone with the invariant chain results in α chain localizing with each form of invariant chain, confirming that the invariant chain can influence the transport of class II molecule subunits and that it must also be combined with the β chain for selection of its secondary intracellular targeting signal. Class II α and β chains could influence transport of the invariant chain with signals of their own, although the invariant chain alone has signals capable of targeting the complex.

Procedures

The slight differences between the behaviour of invariant chains introduced into HeLa cells (Lotteau *et al.*) and CV1 cells (Bakke and Dobberstein) can be attributed to variation in experimental procedures, and to presumable differences between the transport pathways in the transfected cell types. It is notable that both HeLa cells and CV1 cells, which do not normally express class II molecules or invariant chains, have the recognition machinery to respond to the transport signals of these molecules. Data from Salamero *et al.*[11] suggest that mouse L cells are not capable of this recognition. In L-cell transfectants, the presence or absence of the invariant chain does not seem to influence the degree to which the class II molecules intercept the endocytic pathway.

Although the invariant chain can influence the intracellular transport of class II molecules, it is not required for the export of class II molecules from the endoplasmic reticulum[12], nor is it absolutely required for class II presentation of antigenic peptides derived from exogenous proteins[2,13]. It is also not required for class II presentation of peptides from endogenous antigens[12], which may be

acquired by class II molecules from the class I peptide pool, provided that the invariant chain is not bound. In addition, class II molecules can gain access to the endocytic pathway by endocytosis after surface expression and may be able to exchange bound peptides in the endosome[14]. Given these options for class II molecules, why is the invariant chain needed at all? And how can its presence influence antigen presentation with so many alternative modes of peptide binding for class II molecules?

Influence

The invariant chain's chaperone-like qualities, so unusual for an escort service, may account for its further influence on the function of class II molecules. The synthesis of class II molecules in the absence of the invariant chain alters the folding of the peptide-binding domains[13], so that the binding ability of some peptides is enhanced and that of others is lost[15]. In helping to fold the antigen-binding site, the invariant chain should enable class II molecules to bind to a wider range of antigenic peptides while blocking peptide binding in the endoplasmic reticulum. The chain's targeting to the endocytic pathway and its removal, which occurs under conditions that also generate antigenic peptides, allows class II molecules to acquire these peptides directly rather than relying on exchange. So where a particular antigenic peptide is generated and how well it can bind to a class II molecule will determine whether its presentation requires the invariant chain. In turn, the invariant chain increases the versatility of class II molecules, maximizing their exposure to exogenous antigen and allowing them to have roles different from those of class I molecules. Perhaps the best way to think of the invariant chain is neither as a chaperone nor an escort, but rather as a dating service which provides a wide range of pre-selected partners but does not restrict which of them you take home. □

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