Basic Molecular and Cell Biology

Sorting signals and cellular membranes

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Cells use a surface receptor protein to trap and internalise the cholesterol they need for membrane synthesis. The receptor protein is synthesised on ribosomes, which become bound to the membrane of the endoplasmic reticulum (fig 1). The receptor protein is then transported via vesicles through the Golgi stack, where it undergoes a variety of modifications before appearing on the cell surface. Here the receptor protein binds circulating low density lipoprotein, a cholesterol carrier, and the two are internalised by surface invaginations called coated pits. These pinch off to form coated vesicles, and uncoating is followed by fusion with endosomes. Low density lipoprotein is parted from the receptor, delivered to lysosomes, and degraded; the cholesterol so released passes to the endoplasmic reticulum, where membrane synthesis takes place. The receptor is recycled from the endosomes back to the cell surface, where it participates in roughly a hundred more rounds of internalisation. It too is then degraded in lysosomes.

Cellular compartments

The life cycle of the low density lipoprotein receptor is characterised by movement from one membrane bound compartment to the next in a highly ordered and efficient manner. Compartmentation is a major feature distinguishing eukaryotes from prokaryotes and enables them to carry out many different cellular functions under optimal conditions. Since the focus of this review is proteins I shall define a compartment as a collection of soluble proteins surrounded by a membrane. Different compartments have different collections of soluble and membrane proteins, though some proteins may be common to both compartments. The function of the endosome, for example, is to separate complexes such as low density lipoprotein from their receptor. Binding of the two is sensitive to acid so the membrane of the endosome contains proton pumps which keep the inside acidic, causing the low density lipoprotein to dissociate from the receptor. The lysosome also has proton pumps in the membrane to keep the inside acidic, but this is to allow the soluble degradative enzymes that it contains to work optimally. One might ask why there is a need for both endosomes and lysosomes. Since lysosomes are acidic low density lipoprotein could be both released from the receptor and then degraded. The answer is that the receptor would also be degraded. The endosome serves to protect the receptor for further rounds of internalisation.

The sorting problem

What is true for endosomes and lysosomes is also true for all other cellular compartments. They have each evolved to carry out a

FIG 1—Synthesis and cycling of the low density lipoprotein receptor. Messenger ribonucleic acid (mRNA) coding for the receptor leaves the nucleus and is translated on ribosomes, which become attached to membrane of the endoplasmic reticulum. After transport to the cell surface the receptor binds low density lipoprotein (LDL) and delivers it to the endosomes, recycling back to the cell surface to bind more low density lipoprotein. Degradation of low density lipoprotein in lysosomes releases the cholesterol needed for membrane synthesis.

particular set of functions which confer unique advantages on the eukaryotic cell. These specialisations, however, create a major problem, which is the focus of this review. The problem is in two parts, the first concerning the growth and division of eukaryotic cells. These must be accompanied by the growth and division of the membrane bound compartments, which in turn necessitates the synthesis of the membrane and soluble proteins that make up each compartment. The largest compartment in the cell, the cytoplasm (bounded by the plasma membrane), is specialised for protein synthesis probably because most of the proteins synthesised in the

cell are for use in the cytoplasm. But this specialisation means that the proteins needed for all of the compartments illustrated in figure 1 must also be synthesised in the cytoplasm. In other words, they are synthesised at a site different from the site at which they carry out their cellular function. This explains why the low density lipoprotein receptor is not inserted directly into the plasma membrane; synthesis starts in the cytoplasm, and the endoplasmic reticulum is specialised for the assembly of compartmental proteins. This also means that the low density lipoprotein receptor is synthesised alongside proteins destined for other compartments. Therefore, the mechanisms that transport them to their correct location in the cell must also separate them en route from proteins destined for other compartments.

Sorting signals

Synthesis begins on free ribosomes and differs from the synthesis of cytoplasmic proteins in that the first 20 or so amino acids constitute a signal specifying the endoplasmic reticulum as the first destination (fig 2). This signal is interpreted by a signal recognition particle, which binds to the signal and temporarily halts synthesis of the protein. The ribosomal complex then docks with a docking protein, found only on the endoplasmic reticulum, ensuring that further synthesis is coupled to transfer across the membrane of the endoplasmic reticulum and no other. The soluble endoplasmic reticulum protein and the lysosomal enzyme pass completely across the membrane and end up freely soluble inside; the low density

FIG 2—Synthesis and transport of proteins destined for different cellular compartments. Protein signals that have been characterised include those that enable a protein to leave the cytoplasm, those that retain it in the endoplasmic reticulum, and those that direct it to lysosomes.

The second part of the problem arises once the protein has reached its destination. Proteins such as the low density lipoprotein receptor can function only if they move from compartment to compartment. Low density lipoprotein receptors can enter endosomes, but there are many plasma membrane proteins which function at the cell surface and spend their lifetime there. They, unlike the low density lipoprotein receptor, must be prevented from entering coated pits. Similarly, during receptor recycling endosomal proteins must be prevented from entering the recycling vesicles. Both parts of this problem together constitute what is referred to as the sorting problem; perhaps the easiest way to think about it overall is as a problem of protein purification. Each compartment must maintain its identity and does so by regulating the proteins which enter and leave. Proteins which are allowed to enter must be purified away from proteins that are not allowed to enter. Similarly, proteins that are allowed to leave must be freed from those that make up the compartment's collection of proteins. The endoplasmic reticulum, for example, does not allow cytoplasmic proteins to cross the membrane and prevents endoplasmic reticulum proteins from leaving when exported proteins such as the low density lipoprotein receptor leave. How does it do this? Obviously, the mechanism must be able to distinguish between proteins, which implies that they are tagged in such a way as to identify their correct location in the cell. I would now like to illustrate what little we know about these tags and the mechanisms which recognise them with the examples of the low density lipoprotein receptor, a lysosomal enzyme, and a soluble protein of the endoplasmic reticulum.

lipoprotein receptor stops part of the way across, ending up as a membrane protein. As each protein crosses, the signal sequence, its job done, is removed and oligosaccharides containing many mannose residues are added to the protein. These oligosaccharides have considerable importance for the subsequent sorting of the lysosomal enzyme.

The next step depends on the protein. Soluble endoplasmic reticulum proteins have an invariant sequence of four amino acids at the carboxy terminal end, which somehow acts as a retention signal stopping the protein from leaving the endoplasmic reticulum with proteins destined for export. In contrast, both the low density lipoprotein receptor and the lysosomal enzyme move to the transitional elements of the endoplasmic reticulum, the region responsible for selecting proteins for export. Selection has to be efficient so as to keep the concentrations of the receptor and lysosomal enzyme low. If, for example, lysosomal enzymes were allowed to accumulate the endoplasmic reticulum might start to resemble the lysosome, and this would compromise its identity. Proteins selected for export are enclosed within vesicles which bud from the transitional elements and fuse with the nearest Golgi cisterna, termed the *cis* cisterna. It is here that the address tag for the lysosomal enzyme is constructed. Specific enzymes recognise a sequence found only in proteins destined for lysosomes and then add phosphate to some of the terminal mannose residues in the bound oligosaccharide side chains. These mannose-6-phosphate residues are recognised by a specific receptor that is also present in the *cis* cisterna. The mannose-6-phosphate receptor interprets the

signal carried by the lysosomal enzyme and becomes responsible for carrying the protein to its correct destination.

Both the bound lysosomal enzyme and the low density lipoprotein receptor then move in vesicles to the *medial* and then to the *trans* cisterna. Here they part company, each being separately packaged into budding vesicles. The lysosomal enzyme is carried to lysosomes, where the mannose-6-phosphate tag is hydrolysed. This releases it from the receptor, which recycles back to the *cis* Golgi cisterna for further rounds of lysosomal transport. It is not known how the low density lipoprotein receptor is directed to the cell surface. Many argue that this is the default pathway—that is, everything that leaves the endoplasmic reticulum is sent to the cell surface unless it is either removed or retained on the way.

Once on the cell surface the low density lipoprotein receptor begins to cycle. To do that it must contain information that causes it to be trapped selectively in coated pits. The tag for this selective trapping resides in that part of the receptor exposed to the cytoplasm. Mutations in this part yield receptors which are expressed on the cell surface and bind low density lipoprotein, but they are not efficiently internalised. Cholesterol cannot be taken up, and children with this defect suffer from familial hypercholesterolaemia. The tag is extremely efficient, allowing a

5000-fold purification of the receptor in a single step. This ensures that only those proteins that are meant to go to the endosomes are delivered there. All others are excluded from coated pits. The mechanism which carries out this selection is still unknown.

In summary we should remember that the three dimensional organisation of the cell demands two types of information: information to transfer the protein to that part of the cell where it is to function, and information which allows it to carry out its function. In the simplest case this entails merely keeping it in a particular compartment. In the more complicated case information is needed to move it specifically between compartments. Future work will uncover more of the signals which encode this information and determine the nature of the mechanisms which decode it.

General references

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Logic in Medicine

An outline of formal logic and its applications in medicine—II

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III— Logic and life: the application of formal systems

Any science—physical, biomedical, or any other—has as its outcome theories. Theories may, as suggested in the last section, be considered as sets of statements closed under logic. Thus logic is the abstract theory of theories. A system of logic works on theories in two ways. Firstly, it issues permissions to argue in certain ways. It offers guarantees that certain forms of argument—the ones that it delivers as valid—will never introduce falsehood into any body of theory. Arguing in the permitted ways may bring hidden falsehoods to light but will not itself be the source of any error. Secondly, it prohibits certain sorts of theory. Specifically, it prohibits theories that do not contain all their own logical consequences. The significance of this fact will become apparent in the next section. In that it provides a characterisation of the concept of “theory,” logic relates to medical science in just the same way as to other sciences, no more and no less closely.

A second way in which logic impinges on medicine is by imposing constraints on actual passages of argument. All rational activity is subject to rules; it is a virtue of the way in which it is governed by

rules that it counts as rational. The rules of formal logic are culled from those of natural discourse and must be measured against such discourse to assess their adequacy. It is clear that valid and accurate reasoning is needed not only in research but also in the day to day practice of medicine—for instance, in diagnosis and in the decision making that goes with treatment. Much of this reasoning, where it is complex enough to need doing consciously, will require far more elaborate parts of language than are covered by the example of & and → calculus given previously. It will be partly mathematical, particularly probabilistic, for instance. But its basic structure will still be logical, and there is no reason why reflective awareness of the qualitative logical structure of inference should not add some clarity to the thought processes required. At the very least, fluency with formal languages often confers a facility in grasping and manipulating intricate or convoluted statements or reasonings, keeping track of several layers of hypothesis, and so on.

Counterexample

A system of formal logic is a catalogue of valid forms of inference, but it is not going to be used as a straightforward diagnostic tool like a chart or handbook. Appeals to logic occur in more complicated dialectical contexts. Suppose, for example, someone makes a claim C, of which you are unconvinced. You may well reply by asking why C is thought to be true. Its proponent might then produce an argument with C as its conclusion. At this point you have several rational options. One option is to challenge the validity of the argument, perhaps backing up your challenge with a counterexample