Stop-go proteins

from Graham Warren

SOME ten years ago the discovery of the signal sequence[1,2] showed how, in principle, the cell could 'tag' proteins destined for secretion. When a secretory protein is synthesized the first part of the growing polypeptide chain to emerge from the ribosomal complex is a 'signal' or 'leader' sequence that directs the ribosomal complex to the membrane of the endoplasmic reticulum (ER). There the signal sequence is removed and the rest of the protein synthesized as it is transferred across the membrane into the cisternal space.

Such discoveries were made possible by the development of cell-free systems that mimicked the *in vitro* transfer process. Signal sequences have subsequently been found on all nascent proteins initially inserted into the ER membranes. What has remained a mystery, however, is the way in which the signal sequence carries out its function. Other cellular proteins must be involved but their study requires a painstaking dissection and reconstitution of the ER membrane. Progress in this area has until recently been slow, but now two ER proteins have been characterized[4,5]: one stops synthesis of the protein until a suitable site for transfer across the ER membrane has been found and one, described in this issue of *Nature* (see p.647), 'docks' the protein at a suitable site.

It has been known for several years that microsomal vesicles (derived from dog pancreatic ER) lose the capacity to transfer secretory proteins *in vitro* when extracted with high salt solutions. Activity can be restored by a component of the salt extract — a protein complex containing six different polypeptide chains[9] — but the precise function of the protein complex was unknown. Peter Walter and Gunter Blobel have recently shown[4,5] that it binds strongly to those polysomes synthesizing secretory proteins and stops further translation, but binds only weakly to free ribosomes and has no effect at all on polysomes synthesizing cytoplasmic proteins. Translation of secretory proteins is stopped after the nascent chain has reached a length of about seventy amino acids. Forty of the amino acids will be buried within the large ribosomal complex and thirty will have emerged from the ribosomal complex — the same length as the signal sequence for the secretory protein under study. The signal sequence is rich in leucine which can be replaced in the cell-free system by a more hydrophilic analogue, β-hydroxyleucine. When this is done the protein complex can no longer effectively inhibit synthesis of the now-modified secretory protein. The authors therefore conclude that the complex recognizes the signal sequence and have termed it the signal recognition protein (SRP). Though much work needs to be done to determine the individual contribution of the six polypeptides to the inhibition of translation, the complex has been found to perform the same function with other secretory proteins[6] (and see p.647) and at least one membrane protein[9].

The block in translation can be released by addition of the salt-extracted microsomal vesicles and subsequent synthesis is then coupled to transfer. The ER proteins responsible for releasing this block were unknown but one protein essential for *in vitro* transfer[10] had been previously characterized by Meyer and Dobberstein. It is released from salt-extracted microsomal vesicles by protease treatment and has a molecular weight of 60,000. Antibodies to this proteolytic fragment[11] showed it to be derived from an ER membrane protein with a molecular weight of 72,000 that is entirely restricted to the ER *in vivo*. The work reported in this issue of *Nature* (p.647) now shows that the purified proteolytic fragment specifically releases the translation block induced by SRP. The membrane bound form of the protein has the same effect. This means that the block on synthesis produced by SRP will not normally be released until the SRP has bound to the membrane protein. The protein thus acts to 'dock' the ribosomal complex at a suitable site on the ER.

To accord with the overall scheme for the homing step proposed by Walter and Blobel[4,5] at least some of the SRP should be free in the cytoplasm ready to bind to polysomes synthesizing proteins initially destined for the ER. SRP was, however, characterized from a salt-extract of microsomal vesicles. The cytoplasmic form has now been detected (see p.647) using a sensitive assay and significant amounts of SRP have been found in the cytoplasm of reticulocytes and dog pancreatic cells.

The homing step explains how the ribosomal complex becomes specifically bound to the ER membrane. The signal sequence does not play a direct role in this step but acts through SRP. This may have an additional advantage since the hydrophobic signal sequence would not be free to interact with any cellular membranes other than the ER. Once it is free to interact with the lipid bilayer, the homing step will have ensured that this bilayer will be that of the ER. The inhibition of translation is clearly an important part of SRP's function; to

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SPEAKING on 27 May at a Royal Society discussion meeting on Cell Membranes and Glycoprotein Synthesis, Dr Günter Blobel of the Rockefeller University gave advance notice of a new and unexpected twist in the long running saga of the signal sequence. It now seems that the signal recognition protein, whose role is described in the accompanying article, has a specific ribonucleic acid associatied with it.

That the ribonucleic acid plays some, as yet undefined, part in the process of ensuring that secretory proteins are secreted is suggested by the inhibitory action of ribonuclease on the ability of the signal recognition protein to arrest the translation of secretory proteins (see accompanying article). The sedimentation coefficient and partial sequence of the ribonucleic acid associated with the signal recognition protein make it all-but certainly identical to 7S RNA, the sequence of which has just been described by two independent laboratories (E. Ullu, S. Murphy & M. Melli *Cell* **29**, 195; 1982 and W. Li, R. Reddy, D. Henning, P. Epstein & H. Busch *J. biol. Chem.* in the press). Intriguingly, 7S RNA is closely related to the '*Alu*' family of RNAs, the genes for which exist in many thousands of copies scattered throughout the mammalian genome. *Alu* RNAs, although present in abundance in most cells, are of unknown function. It could be that the role of one subset has now been unearthed.

At the same meeting Blobel acknowledged the demise of a different twist in signal sequences. His classic signal sequence is at the amino terminus of a secreted protein and is cleaved off as the protein traverses the membrane. Later it emerged that ovalbumin, although a secreted protein that competed with others for insertion into microsomes *in vitro*, did not subsequently lose an amino-terminal peptide. In 1979 Blobel's laboratory published a paper (V.R. Lingappa, J.R. Lingappa & G. Blobel *Nature* **281**, 117) claiming that the signal peptide of ovalbumin was internal, and suggesting a location around amino acid 250 of the 385-residue chain. Now, as Blobel acknowledged, it looks as if that result was in error. One is instead to believe the conclusion of William Braell and Harvey Lodish (*J. biol. Chem.* **257**, 4578; 1982) that the signal sequence of ovalbumin is on the amino-terminal side of residue 150 and thus not exceptional.

But, according to an earlier paper of Braell and Lodish (*Cell* **28**, 233; 1982), what is not true for ovalbumin is true for the erythrocyte anion transport protein which really does have an internal signal sequence somewhere around its middle.

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