PROTEIN TRANSLOCATION

Travelling by TRAM

Peter Walter

THE mechanism by which proteins move across the membrane of the endoplasmic reticulum (ER) is still obscure. Not only do soluble, hydrophilic proteins need to be moved completely across the lipid bilayer, but membrane proteins need to be integrated in their proper, asymmetric orientation. For proteins that have many membrane-spanning domains, the process requires the correct insertion of each transmembrane domain. These events are catalysed by ER membrane proteins which are thought to interact during their functional cycle and to constitute a multicomponent translocation apparatus, termed translocon. On page 47 of this issue[1], Rapoport and colleagues describe their identification of a hitherto unknown ER membrane protein and provide evidence that it is a component of the translocon.

The translocon mediates the binding of ribosomes engaged in synthesizing secretory or membrane proteins that have been targeted to the ER membrane by the signal recognition particle and its receptor. After targeting, translocation of the nascent proteins presumably proceeds through an aqueous pore[2] that may be shielded from the hydrophobic core of the membrane by the translocon components.

It is likely that the translocon is a multifunctional and perhaps dynamic assembly. Beyond a basic set of components required for translocation itself, different translocating proteins have different requirements, such as signal peptide cleavage or glycosylation, that may necessitate the incorporation of additional components into the translocon. Furthermore, elements in the translocating proteins, termed topogenic sequences, can regulate steps in translocation[3]. Stop transfer sequences, for example, will halt the translocation of nascent protein chains and thus lead to their integration into the membrane. These sequences appear to be recognized by translocon components and, interestingly, remain in contact with the translocon until protein synthesis is terminated[4]. Translocon components must also promote the integration of signal anchor sequences in the proper orientation which can give rise to either type I or type II membrane proteins.

To understand this functional complexity, much effort is currently being spent to identify the protein components of the translocon. One powerful approach has been to identify molecules that can be crosslinked to nascent chains during their transit across the ER membrane. It has been shown that the crosslinking patterns are complex, indicating that the nascent chains lie close to a number of different glycosylated and nonglycosylated ER membrane proteins[5–8].

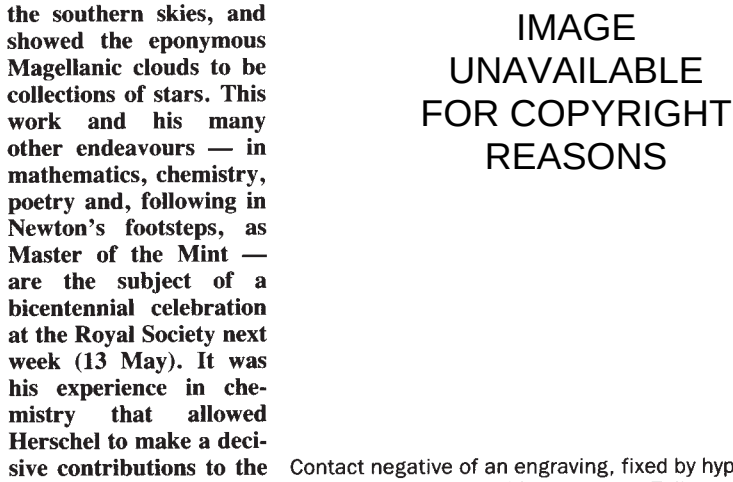
In pioneering studies, Krieg *et al.*[5] and Wiedmann *et al.*[8] identified ER membrane proteins to which short nascent chains could be crosslinked. Prominent among these were membrane glycoproteins with a relative molecular mass of 35,000 to 39,000. Wiedmann *et al.* termed a major crosslinking target SSRα (SSR standing for signal sequence receptor), on the assumption that the crosslinks were specific to the signal sequences of translocating chains. They proceeded to purify a major ER membrane glycoprotein and, assuming that this protein was the only ER membrane glycoprotein of this size range, referred to it as SSRα. Both assumptions, however, quickly proved to be problematic. First, not only the signal sequence, but also distant parts of the translocating nascent chains can be crosslinked to the membrane glycoprotein[5]. Thus, the crosslinked protein does not function as a 'signal sequence receptor'. Second, antibodies to SSRα precipitate only a very small proportion of the crosslinked products, suggesting that another glycoprotein, not SSRα, is crosslinked to the nascent chains[5]. Finally, in a carefully controlled study, Migliaccio *et al.*[9] showed that reconstituted ER vesicles that have been immunodepleted of the SSRα and its associated subunits show no detectable defect in protein translocation of both secretory and membrane protein substrates.

Taken together, these findings make it unlikely that SSRα plays a vital role in ER protein translocation. As SSRα can be crosslinked to longer nascent chains protruding into the ER lumen[1,10], it remains possible, however, that SSRα is involved in aspects of the maturation of the nascent chain which are not monitored or which are not rate limiting in the *in vitro* translocation assay.

In a remarkably complete study, Görlich *et al.*[1] now provide evidence that a different ER membrane glycoprotein, TRAM, participates in protein transloca-

Herschel and hypo

LIKE the best of his era, John Herschel (1792–1871) was a polymath. The son of the astronomer William Herschel (discoverer of Uranus) he founded the observatory in Cape Colony, South Africa, to map the southern skies, and showed the eponymous Magellanic clouds to be collections of stars. This work and his many other endeavours — in mathematics, chemistry, poetry and, following in Newton's footsteps, as Master of the Mint — are the subject of a bicentennial celebration at the Royal Society next week (13 May). It was his experience in chemistry that allowed Herschel to make a decisive contributions to the development of photography — the fixing process. Fox Talbot had developed a 'photogenic drawing' method using paper treated with silver salts. The unexposed areas could be only partially protected by sodium chloride solution, which slowed down but did not halt the light-induced darkening of the silver salts. By showing that sodium thiosulphate ('hypo') dissolved away the unexposed areas, Herschel made permanent images possible. He also coined the terms positive and negative, invented the blueprint process (cyanotypes) and introduced photography to astronomy. R.P.



Museum of the History of Science, Oxford.

Contact negative of an engraving, fixed by hypo, made by Herschel on 5 August 1839 (the year Talbot and Daguerre each described their photographic methods.

tion. TRAM was identified and purified as a crosslinking target of nascent protein chains that are translocating across the ER membrane (hence its name: translocating chain-associating membrane protein). It is likely that TRAM is identical to the main crosslinking target identified in the initial studies[5,8], but may have been confused with crosslinks to SSRα which is of similar size. Görlich *et al.* isolated and sequenced a complementary DNA clone and raised antibodies against a peptide predicted to comprise a portion of the cytoplasmic tail of TRAM. The authors then demonstrated that these antibodies efficiently precipitated the crosslinked product containing the nascent chain. This shows convincingly that the cDNA clone encodes the protein identified by the crosslinking approach.

To address the functional significance of TRAM during translocation, Görlich *et al.* depleted TRAM from ER vesicles. Study of protein translocation across the ER was revolutionized by Nicchitta and Blobel[11], who showed that reconstituted artificial lipid vesicles containing protein from a detergent extract of completely solubilized ER membranes are competent for translocation. Görlich *et al.* modified this procedure to improve the yield of sealed vesicles after detergent removal. Using this modified protocol, they removed TRAM by immunoabsorption from the detergent extract and then reconstituted TRAM-depleted vesicles. Translocation of two secretory proteins, β-lactamase and prepro-α-factor, was completely blocked in TRAM-depleted vesicles; surprisingly, however, translocation of a third substrate, preprolactin, was still observed, albeit at reduced efficiency. Readdition of purified TRAM to the immunodepleted extract restored the activity of the resulting membrane vesicles. So it seems that TRAM is required for ER translocation of at least a subset of proteins.

At present, TRAM is the most convincing candidate for a component of the mammalian ER translocon. Because the initial crosslinking studies showed that the environment in which a nascent chain traverses the ER membrane is complex, it is likely that many more translocon components will be discovered. Genetic studies have led to the proposal that three interacting ER membrane proteins, Sec61p, Sec62p and Sec63p, are involved in protein translocation in yeast[12]. One might predict that translocon components have been evolutionarily conserved, as is true for the signal recognition particle and its receptor[13]. Mammalian homologues of Sec61p, Sec62p and Sec63p and a yeast homologue of TRAM may therefore be forthcoming. To date, different experimental approaches may only have revealed the tip of an iceberg in either system. □

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MICROBIAL GENETICS

Sexual identity and smut

Richard Egel

THE patterns of sexual reproduction in eukaryotic microorganisms are full of odd nooks and crannies. Yet, bit by bit, common schemes with lesser variations are emerging. Two remarkable papers in *Cell*[1,2], produced by Regine Kahmann's group in Berlin, take that process a stage further. They describe the molecular organization of mating-type genes in *Ustilago maydis*, the common corn smut, and show that one locus specifies complementary pairs of pheromones and receptors, whereas another locus with several alleles encodes two correlated subfunctions for each allele. These alleles are involved in self/nonself recognition.

Sexual differentiation need not be different from other kinds of differential gene expression, in which cytoplasmic states and environmental stimuli feed back on various controlling signals upstream of particular genes. Quite often, though, genomic differences mark the very top of regulatory cascades that govern sexual identity. This development can culminate in recognizable sex chromosomes, or heterosomes (as opposed to the remaining autosomes). But in primitive organisms more limited differences at the so-called mating-type loci are the norm.

In many fungi, such as *Saccharomyces*[3], *Schizosaccharomyces*[4] and *Neurospora*[5], the mating-type regions encode regulatory proteins, which, on their own or in combination, control three sets of other genes; these genes are responsible more directly for the physiological activities during zygote formation or meiosis, or both. Two sets of such subordinate gene functions are limited to the haploid phase, one for either mating type, whereas the third set depends on heterozygosis and is therefore specific for the diploid stage.

In *U. maydis*, allelic differences are required at two loci (*a* and *b*) for the fungus to proceed through an entire life cycle. The mating-type locus in a strict sense (*a*) has two alleles, but there is no recognizable DNA homology between the mating-type-specific segments of about five and eight kilobases, respectively[6]. Bölker *et al.*[1] now show that each one of these *a* alleles carries a gene for a mating pheromone and another gene for the receptor that is responsive to the opposite pheromone (see figure). (Such genes in yeast, for example, are common to both mating types, but their expression is separately controlled by the mating-type genes.)

Mutual stimulation by the pheromone response precedes fusion of two haploid, yeast-like cells of *Ustilago*. Thereafter, at the functionally diploid level, one might expect there to be no further need for the pheromones, but they nonetheless remain necessary for proper development. Cell fusion in most fungi, however, does not lead to a proper diploid stage; rather, the still-haploid nuclei exist in a common cytoplasm and establish a so-called dikaryon, their concerted mitotic divisions following a stereotyped and somewhat contorted pattern. Each cell division at a hyphal tip is accompanied by retrograde fusion of a tiny branch cell with the newly arisen penultimate cell, forming a so-called clamp connection and conveying one of the daughter nuclei backwards. In this way, both the growing tip and the new penultimate cell retain pairs of nuclei with different mating types, and an ordered pheromone response is probably required for the formation of each clamp connection.

The *b* locus, on the other hand, is important only after the initial fusion event, for maintenance of the dikaryotic state. This is also the infectious phase of smut as a plant pathogen. Some 30 different *b* alleles are known, and all heterozygous combinations of them are compatible whereas all homozygous combinations are not. It has long been a puzzle how molecular interactions at the protein level could account for such a