Secretory protein translocation across membranes—the role of the ‘docking protein’

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On emergence of the signal sequence of nascent secretory proteins from the large ribosomal subunit, translation is stopped by a cytoplasmic protein complex. A specific membrane protein of the endoplasmic reticulum, the ‘docking protein’, releases this block and allows further synthesis to be directly coupled to transfer across the membrane.

TABLE 1

<table>
<thead>
<tr>
<th>Microsomes/components</th>
<th>Activity in:</th>
<th>Reticulocyte lysate</th>
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<tr>
<td>RM</td>
<td>+</td>
<td>+</td>
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<tr>
<td>RM&lt;sub&gt;K&lt;/sub&gt;</td>
<td>-</td>
<td>+</td>
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<tr>
<td>RM&lt;sub&gt;EK&lt;/sub&gt;</td>
<td>-</td>
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<tr>
<td>RM&lt;sub&gt;EK&lt;/sub&gt; + DP&lt;sub&gt;F&lt;/sub&gt;</td>
<td>-</td>
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<tr>
<td>RM&lt;sub&gt;K&lt;/sub&gt; + DP&lt;sub&gt;F&lt;/sub&gt;</td>
<td>-</td>
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<td>RM&lt;sub&gt;EK&lt;/sub&gt; + DP&lt;sub&gt;F&lt;/sub&gt; + SRP</td>
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<tr>
<td>RM&lt;sub&gt;EK&lt;/sub&gt; + SRP</td>
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<td>-</td>
</tr>
<tr>
<td>RM&lt;sub&gt;EK&lt;/sub&gt; + DP&lt;sub&gt;F&lt;/sub&gt; + SRP</td>
<td>-</td>
<td>+</td>
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Rough microsomes (RM) in combination with isolated factors (DP<sub>F</sub> or SRP) were tested for their ability to translocate nascent proteins in wheat germ and reticulocyte lysate systems. To exclude the possibility that reconstitution was being mediated by contaminating proteins in DP<sub>F</sub> preparations, controls were done using a specific anti-DP<sub>F</sub> antibody. In these controls the antibody was used to specifically remove DP<sub>F</sub> from the preparations. Such controls were performed in every case where DP<sub>F</sub> was used in this report, and each time the preparation treated in this way was unable to reconstitute activity in inactive rough microsomes. RM<sub>K</sub>, KCl-washed RM; RM<sub>EK</sub>, elastase-treated and KCl-washed RM; SRP, signal recognition protein; DP<sub>F</sub>, cytoplasmic fragment of docking protein released by elastase and high salt. Microsomes and factors were prepared and assays carried out as described previously.

We report here that this protein is indeed the molecule which relieves the SRP-induced block. Translation can then proceed when the initiated ribosomal complex has made contact with the correct membrane, that is, the one containing the 72K protein. In accordance with this function, we shall henceforth refer to this molecule as the ‘docking protein’ of the ER. We also report the presence in the cell cytoplasm of a functional equivalent of the microsomal signal recognition protein.

Signal recognition protein interacts with docking protein

Previously published data suggested that the source of the cell-free translation system affected greatly the translocation capability observed for salt-washed microsomes (compare refs 14, 15 with 19, 20). Whole rough microsomes (RM) were fully active in the wheat germ and reticulocyte lysate systems (Fig. 1, lanes 1 and 2). Salt-washed microsomes (RM<sub>EK</sub>), however, were only active in the reticulocyte lysate (compare Fig. 1, lane 3 with lane 4). Microsomes treated with protease and high salt (RM<sub>EK</sub>) were inactive in either system (Fig. 1, lanes 5 and 6).
Salt washing exerts its effect by removing the 250K complex (SRP) from rough microsomes, whereas salt washing in conjunction with proteolysis removes from RMk an active 60K cytoplasmic domain from the 72K docking protein referred to as 'docking protein fragment', or DPk. To define the requirements of translocation more precisely, we recombined the two factors with the various inactive rough microsomes and tested these in the two translation systems. The addition of the 60K fragment conferred activity on RMk in the reticulocyte lysate system alone; this combination is hence functionally equivalent to RMk (see Table 1). It should then be possible to add SRP to RMk+DPk and restore activity in the wheat germ system. This was indeed the case, suggesting a functional identity between RMk+DPk+SRP and whole rough microsomes. We therefore conclude that two components are required to restore full activity to RMk; the 60K DPk fragment and the 250K complex (SRP). It is clear also that reticulocyte lysate contributes the functional equivalent of the SRP.

The fact that the SRP and the DPk could be removed sequentially from rough microsomes suggested a possible interaction between them. To test this, membranes (RMk) were incubated with either SRP or DPk. After washing, the membranes were assayed in the wheat germ system in the presence of DPk or SRP, respectively. In agreement with the results given above (Fig. 1), neither RMk (Fig. 2a, lane 1) nor RMk charged with

![Diagram of translocation activity](image)

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**Fig. 2** Sequential reconstitution of translocation activity from isolated components. Inactive rough microsomes (RMk) were allowed to bind DPk or SRP, and assayed in wheat germ in the presence of the component that was omitted from the preincubation. a, Lane 1, RMk; lane 2, RMk charged with DPk; lane 3, RMk charged with SRP in the presence of SRP; 4, RMk charged with SRP assayed in the presence of DPk.5, intact rough microsome control. RM, DPk, and SRP were prepared as described elsewhere. Activity is defined as described in Fig. 1 legend. b, RMk were treated with anti-DPk IgG, washed and assayed for translocation activity in the presence of SRP in dithiothreitol-free wheat germ system. Lane 1, activity of RMk pretreated with PBS; 2, activity of RMk pretreated with normal rabbit (NR) IgG; 3, activity of RMk pretreated with an affinity-purified anti-Er glycophosphatase specific for antigens on the surface of RMk vesicles; 4, activity of RMk pretreated with anti-DPk IgG. In the membrane-affinity-based translocation assay, 2.5 µl rough microsomes (A260= 60 m-1) were incubated in the presence of the fraction being tested at 0°C for 30 min in conditions where KCl concentration = 50 mM. The membranes were pelleted by centrifugation at 12,500 g for 20 min, then the rough microsome pellets were washed and suspended in the complete wheat germ cell-free translation system and assayed for translocation activity.

DPk (lane 2) were active. When the RMk+DPk membranes were assayed in the presence of SRP (Fig. 2a, lane 3) the activity was restored to the level of whole rough microsomes (Fig. 2a, lane 5). No reconstitution was observed (Fig. 2a, lane 4) for RMk pre-incubated with SRP and assayed in the presence of DPk. These data demonstrate that DPk must be present on the membrane for SRP to interact functionally with rough microsomes.

Further evidence for the functional interaction of DPk with SRP is provided by data obtained using a specific anti-DPk antibody. When RMk were charged with this antibody (by the method described in Fig. 2 legend), the membranes were no longer able to interact with SRP (Fig. 2b). RMk incubated with either pre-immune IgG or an ER-specific antibody directed against other antigens on the cytoplasmic surface of rough microsomes were still fully active when assayed in the wheat germ system in the presence of SRP.

A model summarizing these findings is shown in Fig. 3. High salt concentration removes the 250K complex (SRP), yielding RMk which are inactive in the wheat germ system. Proteolysis and high salt remove the 60K portion of a larger membrane protein (DPk), yielding RMk which are inactive in either system. Reconstitution occurs in the reticulocyte lysate when DPk is added to RMk and in the wheat germ system when DPk and then SRP are added to RMk.

![Diagram of reconstitution](image)

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**Fig. 3** Dissection and reconstitution of rough microsomes using protease and high salt. When rough microsomes are treated with 0.5 M KCl, translocation activity is lost in wheat germ (due to the salt-mediated removal of SRP) and RMk are generated which are nonetheless active in reticulocyte lysate. Treatment of salt-washed microsomes (RMk) with elastase and high salt yields RMkK which are inactive in either system. This is due to the loss of a 60K peptide fragment from the docking protein (DPk). Reconstitution can be achieved sequentially by re-addition of DPk to RMk (thus restoring activity in reticulocyte lysate) followed by re-addition of SRP to RMk+DPk membranes. In this way full reconstitution is achieved in both cell-free systems.

![Diagram of SRP-induced block](image)

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**Fig. 4** SRP-induced block of translation of secretory proteins is removed by DPk. Translations in the wheat germ system were blocked by the addition of SRP. We examined the ability of different membrane preparations to overcome this block. Lane 1, products of light chain mRNA-dependent cell-free translation (15 min) in wheat germ in the presence of SRP. Note the large accumulation of 8-9K material labelled 'block'. Lane 2, addition at 15 min of RMk to a 'blocked' translation, observed at 40 min. 3, as in lane 2 except that RMk was added instead of RMk. 4, Same as lane 1. 5, As in lane 2 except that RMk+DPk was added instead of RMk. 6, As in lane 2 except that DPk was added instead of RMk (note the increase in plI as opposed to Li due to the absence of membranes). Synchronous translation assays were carried out as described by Walter and Blobel.
DP fragment releases SRP-induced translation block

Walter et al. have recently demonstrated that the SRP arrests the translation of preproactin in the wheat germ cell-free system. This phenomenon is characterized by an accumulation of incomplete nascent peptides having a molecular weight of 7–8K. A similar result was obtained for IgG light chain (Fig. 4, lanes 1 and 4). This block is removed by the addition of RMK (lane 2). Then the question arises: with which membrane component does SRP interact to result in the resumption of translation? DP, RMK, or both could be required. As shown in Fig. 4, the block (lane 1) was removed by adding RMK (lane 2), but not by addition of RMK alone (lane 3). When DP was added to the RMK, the block was removed (lane 5). The same effect was obtained when DP alone was added to the blocked system (lane 6). Thus we conclude that DP is necessary for the resumption of translation and translocation, while the residual membrane (RMK) is not.

Translation of secretory proteins blocked by soluble protein in reticulocyte lysate

The ability of RMK to translocate nascent peptides in the reticulocyte lysate system, but not in wheat germ, suggested that a molecule equivalent to SRP must exist in the reticulocyte lysate. Such a component was characterized by making use of its ability to interact functionally with the membrane in low salt conditions. Lysate fractions were tested by allowing the component to bind to RMK and assaying for reconstitution of translocation activity in the wheat germ system. The use of such an affinity-based assay was necessary, as direct addition of reticulocyte lysate inhibited the wheat germ cell-free system. Furthermore, it proved extremely sensitive. Several properties of the reticulocyte factor were determined; the results (Table 2) indicate that this factor has several of the basic features of SRP. It is an acidic protein of MW ~250,000, has a hydrophobic character, and blocks translation of nascent secretory proteins. The reticulocyte factor-induced block can be removed by adding RMK, as was observed in the case of SRP. The presence of a block-inducing substance such as the reticulocyte factor in reticulocyte lysate may explain why the cytoplasmically-located nucleocapsid proteins of certain viral mRNAs were translated well and the membrane proteins only poorly. Due to the small quantity of the reticulocyte factor in reticulocyte lysate, it has not been possible to determine whether its subunit composition is the same as that of SRP.

The cytoplasmic location of reticulocyte factor suggested that SRP may also be only transiently associated with the membrane, and that it is present in the cell as a soluble, cytoplasmic factor. Using the membrane-affinity assay described in Fig. 2 legend, we found that post-microsomal supernatants of dog pancreas contained a substance capable of restoring activity to RMK when assayed in the wheat germ system. It possessed all the characteristics of reticulocyte factor (see Table 2) and SRP. In addition, a fivefold higher level of SRP-like activity was found in dog pancreas supernatant when compared with reticulocyte lysate (Fig. 5). These findings are consistent with the functional role of SRP in translocation. The delay in translation of secretory proteins, that is, until contact with the proper membrane has been made, would be optimally localized in the cytoplasmic compartment.

Implications and conclusions

A model consistent with all the data reported here, and with data obtained previously, is shown in Fig. 6. The initial events in protein translocation can be described as follows. Synthesis of a secretory protein is initiated on free ribosomes. After 60–70 amino acids have been polymerized and the signal sequence has emerged from the large ribosomal subunit, further translation is interrupted by SRP. This block persists until contact is made with the 72K fragment (DP) which represents the cytoplasmic domain. At this point translation, coupled with translocation, proceeds.

Such a sequence of events ensures optimal co-translational processing of secretory proteins, even when synthesis commences on free ribosomes. The presence of a cytoplasmic form of

<table>
<thead>
<tr>
<th>Property</th>
<th>Method of determination</th>
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<tr>
<td>Proteinaceous</td>
<td>Proteolysis</td>
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<tr>
<td>Salt-linked to rough microsomes</td>
<td>Microassay</td>
</tr>
<tr>
<td>Acidic pH</td>
<td>DEAE binding*</td>
</tr>
<tr>
<td>Hydrophobic</td>
<td>α-CN, pentyl agarose*</td>
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<tr>
<td>MW ~250,000</td>
<td>Sucrose gradient†</td>
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<td>Blocks translation</td>
<td>Translation in</td>
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<td>of secretory proteins</td>
<td>wheat germ†</td>
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The ability to reconstitute translocation activity of RMK in the wheat germ system was tested by adding various lysate-derived fractions using the method described in Fig. 2 legend. For proteolysis, the reticulocyte lysate was treated with trypsin (20 µg ml⁻¹) for 20 min at 25°C and then tested in the assay system described in Fig. 2 legend. In the microassay, the reticulocyte lysate was brought to 0.5 M in KCl and tested as above. * Ref. 20. † Ref. 21.
the signal recognition protein which blocks translation would guarantee that pre-secretory proteins are not completed in the cytoplasm. Efficient transfer of proteins can occur, therefore, only after the correct membrane has been contacted. Such a membrane can be operationally defined as one which contains the docking protein. This protein has been shown to be restricted to the endoplasmic reticulum. The 72K docking protein is the first site of interaction between nascent secretory proteins and the ER membrane. An understanding of the function of this protein and SRP explains the specificity but not the mode of translocation. To determine the actual mechanism whereby proteins cross the membrane, further dissection of the membrane, including disassembly of the lipid bilayer, is necessary. It is also conceivable that other soluble components provided by the cell-free translation system function in this process. Their identification would require further fractionation of the lysates.

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Inducibility of human β-interferon gene in mouse L-cell clones

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Transfer of a 36-kilobase piece of human DNA containing the β-interferon (IFN-β) gene into mouse Ltk- cells leads to transient expression of human interferon even without an exogenous inducer. A low level of human interferon expression is also found in most stable clones containing the transferred DNA. With double-stranded RNA or Newcastle disease virus (NDV) as inducer, human interferon expression is greatly increased. The induced transcript is identical to normal human IFN-β mRNA. Neighboring genes contained on the transferred DNA are co-induced but are not essential for the production of human interferon in mouse L cells.

The interferons (IFNs) are a class of proteins capable of inducing an antiviral state in many cell types against a wide range of viruses. Almost all cell types produce one or more interferons upon induction with virus or a synthetic inducer such as double-stranded RNA (dsRNA), or a synthetic inducer such as double-stranded RNA (dsRNA), a primary human fibroblast, produces IFN-β (ref. 2) and several other proteins of unknown biological function in response to the inducer poly(I)poly(C). In these cells interferon is normally synthesized for a limited period of several hours during which the IFN-β mRNA accumulates and then rapidly disappears. The shut-off occurs in the continuous presence of inducers and can be delayed by treatment with inhibitors of protein synthesis. This treatment then allows the accumulation of IFN-β mRNA (superinduction)2. It has been suggested that continuous protein synthesis supplies the cell with IFN-β mRNA degrading protein(s), thus involving the participation of other genes in IFN-β regulation. We have analysed the induction of human IFN-β and interferon-β-associated genes in human fibroblast and lymphoblastoid cells3,4 and have previously reported the isolation and physical characterization of a 36-kilobase (kb) region of the human genome including and surrounding an intact IFN-β gene5. The discovery that other poly(I)poly(C)-inducible genes are located in this cloned region presented the possibility that regulatory functions were acting through a common pathway on this family of genes.

We describe here the production of human interferon in mouse L-cell clones containing defined fragments from the human genome surrounding and including the human IFN-β (HuIFN-β) gene. The human IFN-β gene is clearly inducible with a spectrum of induction protocols which reflect the responsiveness of the mouse cells’ own interferon genes rather than those of human fibroblasts. Because a 1.9-kb DNA fragment carrying only the structural gene of HuIFN-β is sufficient to allow induction, the neighbouring genes located on the human chromosomal DNA are not essential for the human interferon induction in mouse L cells.

Isolation of mouse L cells containing HuIFN-β cosmID DNA

We have co-transferred S. comisid pCosIFN-β DNA containing the human β-interferon gene7 (Fig. 1) together with plasmid pHCT72 cosk/k DNA containing the herpes simplex virus thymidine kinase gene7 into mouse Ltk- cells. More than 20 hypoxanthine–aminopterin–thymidine (HAT)-resistant clones were isolated. The single clones were maintained in culture for over 50 generations of HAT medium before further studies at the molecular level were carried out. During this period the properties of individual clones with respect to interferon production remained unaltered. High molecular weight DNA was prepared from these cell clones and compared with DNA from human diploid fibroblasts (FS4 cells), mouse Ltk- DNA and cosmID clone pCosIFN-β DNA by Southern blotting10 after restriction enzyme digestion. Figure 2 shows the result for two