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STRUCTURE AND FUNCTION OF SIGNAL RECOGNITION PARTICLE (SRP)

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Secretory and membrane proteins are translocated across or inserted into the membrane of the endoplasmic reticulum (ER) in eucaryotic cells. This process is mediated by a signal sequence, is GTP dependent and involves several macromolecular components, among them signal recognition particle (SRP), dockingprotein (DP) or SRP-receptor, and signal sequence receptor (SSR). SRP interacts with the signal sequence of nascent proteins and arrests or retards their further elongation. This arrest is released, when SRP interacts with the DP in the ER membrane.

SRP is a ribonucleoprotein particle consisting of six different polypeptides of 9, 14, 19, 54, 68 and 72kD and a 7S RNA (SRP7S RNA). Reconstitution and cross-linking studies have revealed, that SRP can structurally and functionally be divided into at least three distinct domains. The 5′ and 3′ Alu like RNA segments together with the SRP9/14 proteins are required for the SRP-mediated arrest of polypeptide chain elongation; the SRP19 is required for the binding of the SRP54 to the SRP7S RNA and the SRP54 protein itself makes contact to the signal sequence. The SRP68/72 proteins are important for the interaction with the ER membrane (see Siegel and Walter, 1988). DP consists of a cytoplasmically exposed 72 kDα subunit (DPα) and a 30 kD membrane-integrated β subunit. In order to characterize the molecular interactions between signal sequence, SRP and DP we have cloned and characterized cDNAs coding for SRP proteins.

SRP19 and SRP54

cDNA clones coding for SRP19 and SRP54 protein have been isolated from a Madin-Darby canine kidney (MDCK) library and characterized by sequence analysis. The deduced protein sequence revealed that SRP19 is a basic protein of 144 amino acid residues. When synthesized in a cell-free system it specifically binds to SRP7S RNA (Lingelbach et al. 1988).

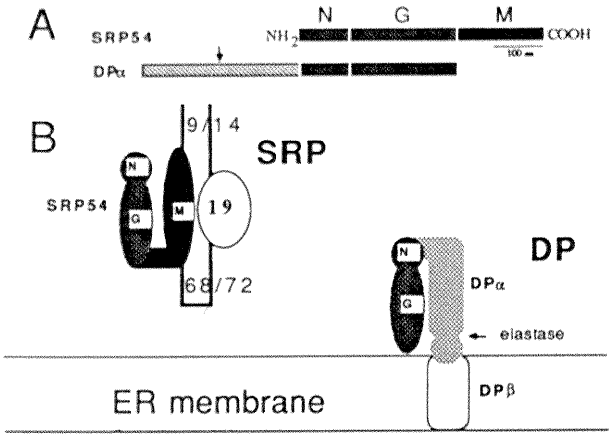

Figure 1.
A) Linear outline of the 54 kD protein of SRP (SRP54) and docking protein (DPα). Three domains can be distinguished in SRP54, N: (amino acid 1-100), G: putative GTP-binding domain, M: methionine rich domain.
B) Model outlining the interactions of SRP54 with 7S RNA/SRP19 and of DPα with DPβ. The U shaped thick line indicates 7S RNA.

SRP54 comprises 504 amino acid residues. It can be divided into three distinct regions, a central region (amino acids 100-300) which contains three highly conserved consensus elements indicative of guanine-nucleotide binding sites (G), an N-terminal region (aa 1-100) (N) and a C-terminal methionine-rich region (M) (Fig. 1). Known GTP-binding proteins (ras, EF-Tu and EF-G) constitute a family of proteins that use GTP-hydrolysis to drive the sequential binding of biological molecules to a series of reaction partners. We have suggested; that the G-domain of SRP54 is involved in the selection and binding of signal

sequences (Römisch et al. 1989). The M-domain is necessary and sufficient for the binding of SRP54 to the particle. This was deduced from reconstitution experiments using deletions of the SRP54 protein, SRP19 and 7S RNA (Römisch et al, in prep.).

When the SRP54 sequence was compared with sequences from data libraries we found striking similarities to three proteins: over its entire length SRP54 is similar to an E. coli 48 kD protein (P48) of unknown function (Byström et al., 1983). The extensive homology indicates that P48 might be the E. coli homologue of the SRP54 protein. The sequences of the N- and the G-domains of SRP54 were found to be highly similar to the C–terminal sequences of DPα and of the FTS Y, an E. coli protein of unknown function (Gill et al.,1986). The homologous G-domains in SRP54 and in DPα might point to a similar function of these two domains.

Recently it has been shown that E. coli 4.5S RNA is homologous to part of SRP7S RNA (Poritz et al., 1988). We found that SRP7S RNA can functionally replace 4.5S RNA and that SRP54 and E. coli P48 specifically bind to 4.5S RNA in vitro and in E. coli cells. Deletion of 4.5S RNA and over-expression of P48 or SRP54 lead to accumulation of precursors to at least one secretory protein. We propose that 4.5S RNA and P48 are assembled in a ribonucleoprotein particle that is the E. coli homologue of mammalian SRP (Ribes et al. submitted).

SRP68 and SRP72

SRP68 and SRP72 proteins have been suggested to play a role in the interaction of the SRP-arrested ribosomal complex with the ER membrane (Siegel and Walter, 1988). In order to elucidate the molecular structures involved in this interaction we have cloned cDNAs coding for SRP68 and SRP72. From the sequence of the cDNA we deduce that the SRP68 is a basic protein with 622 amino acid residues. It has a glycine rich region close to the N-terminus that is also found in some other RNA-binding proteins. Alone SRP68 does not bind to SRP7S RNA. No striking homology to other known proteins was found. (Herz, J., Flint, N., Frank, R. ,Stanley, K. and Dobberstein, B., in prep.). The sequence of the SRP72 cDNA is currently being determined (Lütcke et al.).

Functional analysis of SRP, DP and other components of the ER involved in protein insertion into the membrane.

In order to study the function of components involved in protein insertion into the membrane of the ER we are using a post-translational assay system. A nascent chain/ribosome/SRP complex is isolated and requirements for membrane insertion of secretory and membrane proteins can be tested (High, et al.).

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