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THE RELATIONSHIP BETWEEN SIZE OF A PRESECRETORY PROTEIN AND EXTENT OF SIGNAL-RECOGNITION-PARTICLE-MEDIATED ARREST OF ITS TRANSLATION

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Small eukaryotic presecretory proteins, such as preprocecropinA, prepromelittin, and prepropeptideGLa, are transported into mammalian microsomes both with the aid of ribosome and signal recognition particle (SRP) and independently of these ribonucleoprotein particles. Typically, synthetic extensions of these precursor proteins show the phenotype of naturally occuring large presecretory proteins. However, it was shown that small truncated forms of naturally occuring large presecretory proteins are not transport competent or transport competent only in the presence of the two ribonucleoprotein particles. In order to directly address this apparent paradox, we studied the sensitivities of nascent polypeptide chains, related to preprocecropinA and prepromelittin, to SRP-mediated arrest of elongation and compared them with synthetic extensions of various length.

In protein export from mammalian cells one can distinguish between signal peptide-dependent and -independent mechanisms. The signal peptide-dependent mechanism (operating for the vast majority of newly-synthisized proteins) is initiated at the level of the membrane of the endoplasmic reticulum. The respective secretory proteins are synthesized as precursor proteins with aminoterminal signal peptides (1). In general, transport involves these signal peptides as well as a still putative signal peptide receptor on the cytosolic surface and signal peptidase on the luminal side of the microsomal membrane (1-3). There are two classes of precursor proteins with respect to their mechanism of transport into mammalian microsomes (2,3). One class of presecretory proteins consists of precursor proteins which contain more than 70 amino acid residues and involve the two ribonucleoprotein particles (RNPs), ribosome and signal recognition particle (SRP), and their respective receptors on the microsomal surface (RNP-dependent

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transport). The other class consists of precursor proteins which comprise less than 70 amino acid residues (including the signal peptide) and do not strictly depend on the RNPs (RNP-independent transport) (4-11). The microsomal membrane proteins TRAMp and sec61αp (12-15) appear to be involved in transport of RNP-dependent as well as -independent precursor proteins.

The distinction between these two classes of presecretory proteins was based on our observations that i) naturally occuring small presecretory proteins (i.e. precursors with a content of between 64 and 70 amino acid residues), such as preprocecropinA (ppcecA) (8), prepropeptideGLa (pppGLa) (7) and prepromelittin (ppm) (4,5) are efficiently transported into mammalian microsomes in the absence of the two RNPs and their receptors (Table 1), and that ii) synthetic extension of these precursor proteins in size at the carboxyl terminus typically leads to the phenotype of naturally occuring presecretory proteins with a content of more than 70 amino acid residues (5,8,9).

On the other hand, it was shown that truncated forms of naturally occuring large presecretory proteins (i.e. precursors with a content of more than 70 amino acid residues), such as preprolactin (ppl), prelysozyme (plsm) and preproinsulin (ppi), i.e. related peptidyl-tRNAs (in case of ppl and plsm) or proteins (in case of ppi) with a content of between 45 and 86 amino acid residues, are either not transport competent (ppl-55-mer, plsm-51-mer, ppi-45-mer) or transport competent only in the presence of the two RNPs and their receptors (ppl-86-mer, plsm-74-mer, ppi-64-mer) (Table 1) (16-19). On the basis of the latter observations it was concluded that the phenotype which we had described for natural small presecretory proteins does not reflect a physiological situation (18-20). A similar interpretation of our data was put forward on the basis of observing that ppm can be transported in a signal recognition particle- and docking protein-involving manner (21) (as we have observed for ppcecA (8)).

Here we studied the sensitivities of nascent polypeptide chains which are related to ppm and ppcecA to SRP-mediated arrest of elongation and compared them with the derivatives which contain synthetic extensions of various length at the carboxyl terminus. Thus, the presecretory proteins to be compared had identical signal sequences but varried in length. The arrest assays with mammalian SRP were carried out in the wheat germ translation system under conditions where i) globin (a cytosolic protein which has previously been shown to be incapable of interaction with SRP (22)) was coexpressed and served as an internal negative control for the action of SRP, i.e. a control for

variations in the overall translation efficiency, ii) the concentrations of mRNA were low (i.e. ribosomes were not limiting) and comparable for the precursor proteins to be compared, and iii) the number of ribosomes per mRNA, i.e. the concentrations of nascent chains of precursor proteins to be compared were constant (synchronized translations). The sensitivity of nascent polypeptide chains which are related to preprolactin served as a positive control for the action of SRP (22-24).

MATERIALS AND METHODS

Materials

The wheat germ translation system, rabbit globin mRNA and the restriction enzymes were purchased from Boehringer Mannheim. The methylated dinucleotide 7mG(5`)ppp(5`)G, used for capping of the transcripts, and all nucleotides for transcription were obtained from Pharmacia/LKB. The inhibitory nucleotide 7mG(5´)p, used for synchronization of translation, was from Sigma. SP6 polymerase and human placental ribonuclease inhibitor (RNasin) were purchased from Promega. [^{35}S]-methionine (1000 Ci/mmol) was from Amersham.

Methods

The construction of plasmids, coding for ppcecA and related proteins, were described previously (8,9). Plasmids coding for ppm and its derivatives were described previously (5). Plasmids were linearized with appropriate restriction enzymes and transcribed with SP6 polymerase as described (5,8). Translation in wheat germ lysates were performed as described (8). Samples were boiled in sample buffer and subjected to gel electrophoresis (8). Gels were treated with sodium salicylate, dried and exposed to X-ray films (Kodak X-Omat AR) at -80 ^{o}C. Densitometric analysis of X-ray films was performed with an LKB Ultrascan XL laser densitometer.

RESULTS AND DISCUSSION

In a first series of experiments titrations of the levels of the various capped transcripts were carried out in the wheat germ translation system. For visualisation of the [^{35}S]-methionine labeled translation products, separation by gelelectrophoresis and subsequent fluorography were carried out. Quantitation of the translation efficiency was based on the densitometry of the fluorographs. The concentration range of the transcripts where a twofold increase of RNA resulted in a twofold increase of translation products was determined in order to be employed in the following experiments (data not shown).

Within this linear range of mRNA concentrations the minimal incubation time was determined for each protein which led to completion of chain growth (data not shown). A synchronisation time of 2 min was found to be way below the minimal time required for completion of translation. Therefore, synchronisation took place after 2 min in the following experiments and was used in order to allow an equal number of ribosomes on the various mRNA strands. On the basis of the results of these experiments and taking into account the number of methionines in the various translation products, the concentrations of mRNA were fine tuned with respect to giving rise to equal amounts of translation products within a protein family and to amounts of products which were within one order of magnitude when comparing the two protein families, ppl and globin, respectively (data not shown). The optimization was repeated under conditions of globin coexpression.

Under these latter conditions SRP arrest assays were carried out for different concentrations of mammalian SRP. The two protein families were ananlyzed under conditions where SRP was present in the translation mixture when it was shifted from 0 to 30 °C. After 2 min the cap analog 7-methylguanosine 5`-monophosphate was added as an initiation inhibitor and incubation was continued for 30 min in order to allow completion of initiated polypeptide chains. The amount of precursor protein and of the coexpressed globin in the SRP-free aliquot were set to 100% of translation products. Also, the globin amounts of each aliquot of the same experiment were set to 100% and the percentage of precursor protein was calculated in regard to 100% globin for each aliquot. Thus, it was possible to exclude variations in translation between the various aliquots.

As expected, there was no effect of SRP on translation efficiency of globin but there was an effect on translation of ppl (Figure 1A). Furthermore, the efficiency of ppl synthesis decreased with increasing concentrations of SRP. Figure 1B shows the effect of SRP on the translation of ppm and related precursors. Each of these presecretory proteins showed a decreased translation efficiency in the presence of SRP. The most pronounced inhibitory effect was observed for ppm-DHFR/1. Figures 1C and D show the effect of SRP on the translation of ppcecA and related precursor proteins. We observed the strongest reduction of translation for ppcecDHFR. However, small precursor proteins like ppcecA and ppcecD were not inhibited in translation. As a matter of fact, translation was stimulated for ppcecA and for ppcecD, i.e. their translation was less affected as compared to the translation of globin (Figure 1D). The signal peptide deletion mutant of ppcecDHFR, pcecDHFR, showed no SRP-effect (Figure 1E).

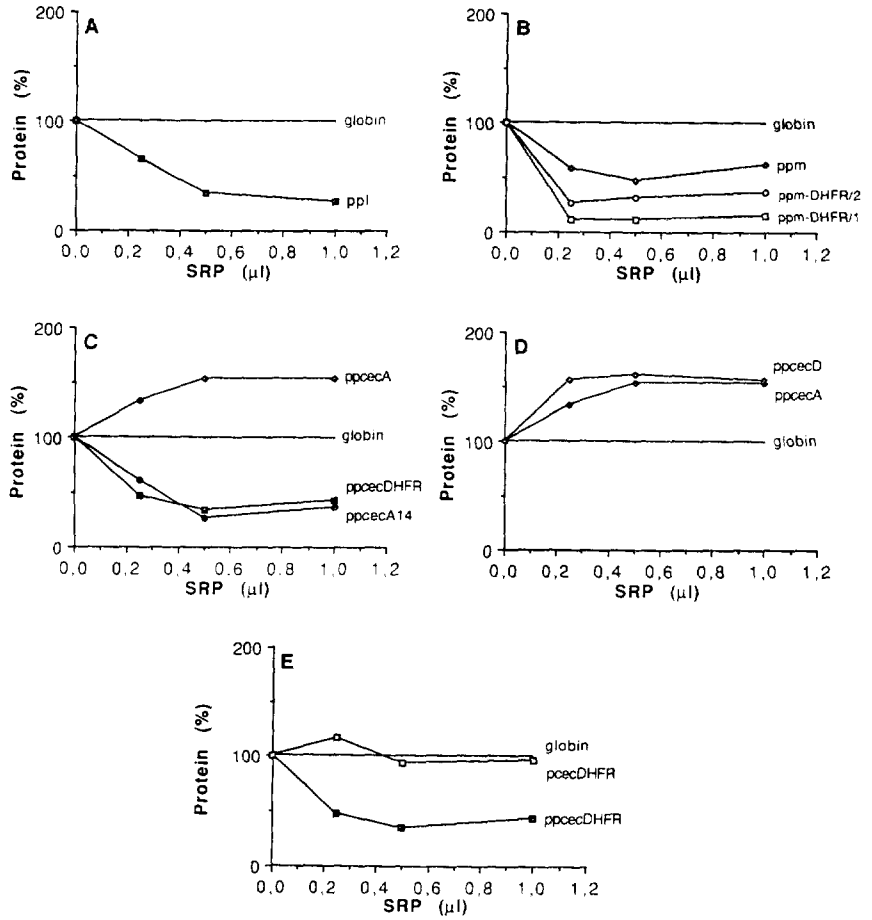

Figure 1. Relationship between SRP concentration and efficiency of expression of precursors related to ppm and ppcecA.

Various wheat germ translation reactions were programmed simultaneously with an in vitro transcript, coding for a certain protein, and a mixture of α- and ß-globin mRNA. The translation mixtures were divided into four aliquots and were supplemented with SRP-buffer or increasing amounts of mammalian SRP in buffer. Translation in the presence of ^{35}S-methionine was initiated by transfer of the samples from 0° to 30° C and was carried out for 2 min. After addition of 7-methylguanosine 5´-monophosphate and elongation for 28 min translation was stopped by the addition of sample buffer. The various aliquots were analysed by gel electrophoresis and fluorography. The fluorographs were subjected to densitometric analysis. Note that the molar ratios of expression in the absence of SRP were globin : ppl : ppm and related proteins : ppcecA and related proteins = 1 : 2 : 2 : 6.

SRP binds to signal peptides of nascent chains of large presecretory proteins emerging from the ribosome (22,25,26). This leads to a subsequent SRP/ribosome-interaction (22). After binding of signal

recognition particle to docking protein (23,24) the nascent chain is handed over to TRAM-protein and sec61α-protein (12-14), and the ribosome binds to the ribosome receptor (27). Since the SRP/signal peptide-interaction can occur only as long as the signal peptide is presented to SRP by the ribosome (28,29), the transport appears to be coupled to translation. For small presecretory proteins transport is not coupled to translation and does not depend on ribosome and SRP and their receptors (Table 1). This may be due to the facts between 30 to 40 amino acid residues of a nascent polypeptide are burried within the ribosome (30-32) and that a signal peptide contains about 20 amino acid residues (33). Thus in theory protein synthesis is terminated and the polypeptide is released from the ribosome before an interaction with SRP had a chance to occur (34).

We had proposed that the ability of a precursor protein for RNP-independent transport is not just a function of the size of a precursor protein and that in natural small presecretory proteins this ability was selected as a result of the inefficiency to use the RNP-independent transport mechanism which is a result of the size. This view was supported by the finding that a truncated nascent chain of a hybrid between a signal peptide and globin did not depend on the ribosome for translocation into microsomes (Table 1) (35). On the other hand, it was observed that truncated forms of naturally occuring large presecretory proteins are either not transport competent or transport competent only in the presence of the two RNPs and their receptors (Table 1). On the basis of these observations it was concluded that the phenotype which we had described for natural small presecretory proteins does not reflect a physiological situation.

Here, we directly addressed this controversy. In order to do so, we studied the sensitivities of nascent polypeptide chains which are related to natural occuring small presecretory proteins (ppm and ppcecA) to SRP-mediated arrest of elongation end compared them with the derivatives which contain synthetic extensions of various length at the carboxyl terminus. Thus, the presecretory proteins to be compared had identical signal sequences, i.e. potentially identical affinities for SRP, but varried in length. In summary, we observed i) that for the naturally occurring small precursor proteins ppm, ppcecA and ppcecD the remaining level of translation in the presence of SRP was most pronounced, and ii) that within a protein family the inhibitory effect of SRP increased with increasing length of the precursor proteins. Despite of the presence of identical signal sequences, precursors of the same family showed different SRP-mediated arrest effects in our experiments, i.e. we observed a clear dependence of the SRP-signal

Table 1. Summary of the abilities of various presecretory proteins for ribonucleoprotein-particle-dependent and -independent transport and for SRP mediated arrest of their translation

protein	content of amino acid residues	transport ribonucleoprotein-particle dependent	transport ribonucleoprotein-particle independent	SRP arrest	references
ppm	70	+	+	+	this study[1)]
ppm-DHFR/2	106	+	-	+	this study
ppm-DHFR/1	257	+	-	+	this study
ppcecA	64	+	+	-	this study[2)]
ppcecA14	85	+	+	+	this study
ppcecDHFR	252	+	+	+	this study
ppcecD	62	+	+	-	this study
pcecDHFR	231	-	-	-	this study
ppl	229	+	-	+	this study[3)]
globin	140/145	-	-	-	this study[3)]
ppl-55-mer-tRNA		-	-	-	(16)
ppl-86-mer-tRNA		+	-	+	(16)
plsm-51-mer-tRNA		-	-	-	(17)
plsm-74-mer-tRNA		+	-	+	(17)
ppi-45-mer		-	-	-	(18,19)
ppi-64-mer		+	-	+	(18,19)
pglobin-101-mer-tRNA		+	+	n.d.	(35)
pglobin-140-mer-tRNA		+	-	n.d.	(35)

Refer to the text for details. 1) The transport phenotypes of precursor proteins which are related to ppm have been described previously (5,6). SRP-mediated arrest has been observed previously for ppm (21). 2) The transport phenotypes of precursor proteins which are related to ppcecA have been described previously (8,9). Note that SRP-mediated arrest has been observed previously for ppcecA under conditions where higher concentrations of transcript were employed (8). 3) The transport phenotype of ppl as well as SRP-mediated arrest have been described previously (22). Furthermore, the lack of a SRP-mediated arrest has been described previously for globin (22). n.d., not determined.

sequence-interaction on the precursor's length. But it is not the binding itself, but the chance for binding that is influenced by precursor length. Therefore, natural small presecretory proteins are inefficient in their

ability to use the RNP-dependent transport mechanism which is a result of the size. Their ability for RNP-independent transport was selected as a result of this inefficiency. Apparently, this ability is not just a function of the size of a precursor protein.

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REFERENCES

1) Zimmermann, R. and Meyer, D.I. (1986) Trends Biochem. Sci. 11, 512-515.
2) Wiech, H., Klappa, P. and Zimmermann, R.(1991) FEBS Lett. 285, 182-188.
3) Klappa, P., Zimmermann, M., Dierks, T. and Zimmermann, R. (1993) Subcell. Biochem. 21, 17-40.
4) Zimmermann, R. and Mollay, C. (1986) J. Biol. Chem. 261, 12889-12895.
5) Müller, G. and Zimmermann, R. (1987) EMBO J. 6, 2099-2107.
6) Müller, G. and Zimmermann, R. (1988) EMBO J. 7, 639-648.
7) Schlenstedt, G. and Zimmermann, R. (1987) EMBO J. 6, 699-703.
8) Schlenstedt, G., Gudmundsson, G.H., Boman, H.G. and Zimmermann, R. (1990) J. Biol. Chem 265, 13960-13968.
9) Schlenstedt, G., Gudmundsson, G.H., Boman, H.G. and Zimmermann, R. (1992) J. Biol. Chem 267, 24328-24332.
10) Wiech, H., Sagstetter, M., Müller, G. and Zimmermann, R. (1987) EMBO J. 6, 1011-1016.
11) Zimmermann, R., Sagstetter, M., Lewis, J.L. and Pelham H.R.B. (1988) EMBO J., 7, 2875-2880.
12) Görlich, D. and Rapoport, T.A. (1993) Cell 75, 615-630.
13) Görlich, D., Hartmann, E., Prehn, S. and Rapoport, T.A. (1992) Nature 357, 47-52.
14) Görlich, D., Prehn, S., Hartmann, E., Kalies, K.-U. and Rapoport, T.A. (1992) Cell 71, 489-503.
15) Klappa, P., Zimmermann, M. and Zimmermann, R. (1994) FEBS Lett. 341, 281-287.
16) Siegel, V. and Walter, P. (1988) EMBO J. 7, 1169-1175.
17) Ibrahimi, I.M., Cutler, D., Stueber, D. and Bujard, H. (1986) Eur. J. Biochem. 155, 571-576.
18) Okun, M.M., Eskridge, E.M. and Shields, D. (1990) J. Biol. Chem. 265, 7478-7484.
19) Okun, M.M. and Shields, D. (1992) J. Biol. Chem. 267, 11476-11482.

20) Hull, J.D., Gilmore, R. and Lamb, R.A. (1988) J. Cell Biol. 106, 1489-1498.
21) Ibrahimi, I. (1987) J. Cell Biol. 104, 61-66.
22) Walter, P., Ibrahimi, I. and Blobel, G. (1981) J. Cell. Biol. 91, 545-550.
23) Meyer, D.I., Krause, E. and Dobberstein, B. (1982) Nature 297, 647-650.
24) Gilmore, R., Blobel, G. and Walter, P. (1982) J. Cell Biol. 95, 463-469.
25) Kurzchalia, T.V., Wiedmann, M., Girshovich, A.S., Bochkareva, E.S., Bielka, H. and Rapoport, T.A. (1986) Nature 320, 634- 636.
26) Krieg, U.C., Walter, P. and Johnson, A.E. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 8604-8608.
27) Savitz, A.J. and Meyer, D.I. (1993) J. Cell Biol. 120, 853-863.
28) Ainger, K.J. and Meyer, D.I. (1986) EMBO J. 5, 951-955.
29) Wiedmann, M., Kurzchalia, T.V., Bielka, H. and Rapoport, T.A. (1987) J. Cell Biol. 104, 201-208.
30) Malkin, L.I. and Rich, A. (1967) J. Mol. Biol. 26, 329-346.
31) Blobel, G. and Sabatini, D.D. (1970) J. Cell Biol. 45, 130-145.
32) Bernabeu, C. and Lake, J.A. (1982) Proc. Natl. Acad. Sci. USA 79, 3111-3115.
33) von Heijne, G. (1984) EMBO J. 3, 2315-2318.
34) Rapoport, T.A., Heinrich, R., Walter, P. and Schulmeister, T. (1987) J. Mol. Biol. 195, 621-636.
35) Perara, E., Rothman, R.E. and Lingappa, V.R. (1986) Science 232, 348-352.