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Synthetic leader peptide modulates secretion of proteins from microinjected *Xenopus* oocytes

(signal peptide/membrane segregation/cotranslational interference/translocational block/secretion enhancement)

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ABSTRACT **To investigate the role of the leader peptide in modulating secretion from living cells, we injected a synthetic peptide into *Xenopus* oocytes. The peptide consisted of the NH_2-terminal leader sequence of mouse immunoglobulin light chain precursor. We found that the leader peptide has two different roles in regulating secretion from the oocytes. First, it competitively inhibits the synthesis of secretory and membrane proteins but not of cytoplasmic proteins. The inhibition occurs both with oocyte proteins and with proteins directed by coinjected myeloma mRNA. The inhibition reaches a maximum 2 hr after injection and decays within 3 hr. It appears to be mediated through the cell membrane, because ^{125}I-labeled leader peptide segregates into the membrane fraction of microinjected oocytes simultaneously with the interference with methionine incorporation. A second role of the microinjected leader peptide is to induce a rapid acceleration in the rate of export of secretory proteins from the oocyte. The maximal enhancement effect is obtained upon injection of 50 ng of leader peptide per oocyte. It is not merely due to the small size, negative charge, or hydrophobicity of the peptide, because enhanced secretion does not occur when glucagon, poly-L-glutamic acid, or Triton X-100 is injected. Furthermore, immunoreaction of the peptide with specific antibodies prior to microinjection prevents the accelerated export. Our observations indicate that in *Xenopus* oocytes, the leader peptide is involved in both translocation and later step(s) in the secretory pathway.**

Most secretory proteins are processed in the living cell from nascent polypeptide chains, extended at their NH_2 terminus by the leader, or signal, peptide (1–3). The leader peptide is involved in the vectorial translocation of nascent proteins across the membrane of the endoplasmic reticulum (1, 4–8). The emergence of the leader peptide from the large ribosomal subunit is accompanied by a translation block, mediated by a cytoplasmic signal recognition protein complex (9, 10). This block is only released upon interaction of the complex with the "docking protein," a component of the endoplasmic reticulum membrane (10, 11). Thus, the number of nascent chains for secretory proteins cannot exceed the number of available routes for translocation, and the leader peptide operates as a regulatory element at this step in the pathway for secretion.

After the release of the translation block, continued synthesis of secretory proteins resumes, coupled to translocation of the processed chains across the membrane (10). The leader peptide is then cleaved off the forming chains and is rapidly degraded (12). The processed proteins are transferred into the cysterna of the rough endoplasmic reticulum and via small vesicles to the Golgi apparatus, to be packaged into secretory granules, which accumulate until secretion occurs (13). One may postulate that additional regulatory mechanism(s) may also operate at these later steps in the secretory pathway. These would control the amount of sequestered proteins within the cell, so that it would not exceed the capacity of the cell to secrete proteins. One possibility for such regulation could be by acceleration of the rate of secretion of already processed and sequestered proteins. Therefore, we were interested in investigating the possibility that the leader peptide itself might be involved in such regulation(s). To examine this working hypothesis, one must determine the nature and the time course of the effect(s) exerted by the leader peptide on the processing and translocation of secretory proteins and in affecting the rate of secretion of already processed and sequestered secretory proteins.

Several steps of the processing–translocation phenomenon have been reconstituted *in vitro*, by using isolated microsomes (5, 6). Synthetic leader peptide (SLP) of the preproparathyroid hormone has been shown to compete with the cell-free processing of various prehormones (14). This implied that the interaction sites on the microsomal membranes are saturable and can recognize all leader peptides. However, the *in vitro* system could not provide conditions for following the time course of the leader peptide's interaction with these binding sites. In addition, the later steps in the secretion process of already sequestered proteins cannot be reconstituted under cell-free conditions.

Microinjected *Xenopus* oocytes offer an attractive surrogate system for such experiments. The oocytes are huge (1 mm in diameter) cells, secreting specific proteins via an exocytotic release of vesicle contents. This occurs both with oocyte endogenous proteins and with translation products of various microinjected mRNAs (15). Protein secretion from the oocytes appears to be highly selective but neither cell type nor species specific (16). Nascent polypeptide chains for secretory proteins become compartmentalized within vesicles during their synthesis in the oocytes (17–19) and are subsequently secreted in a processed form (19, 20). When the mature proteins, rather than their mRNAs, are injected into the oocytes, they may become degraded in the oocyte and are not re-exported. This implies that secretion from the oocyte involves cotranslational events (16, 20). Scarcity of putative membrane binding sites for such cotranslational events has been suggested as the limiting factor for translation of membrane-associated mRNAs in the oocytes (21).

We now report that in microinjected *Xenopus* oocytes, SLP of mouse immunoglobulin light chain precursor segregates into the membrane fraction and modulates secretion of proteins in two ways. It inhibits secretion of nascent chains for secretory proteins. In addition, it selectively accelerates the rate of se-

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Abbreviation: SLP, synthetic leader peptide.

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cretion of already processed and sequestered secretory proteins in a concentration- and time-dependent manner.

MATERIALS AND METHODS

Adult *Xenopus laevis* females were obtained from the South African Snake Farm (Fish Hoek, South Africa). The frogs were anesthetized by cooling in ice, ovarian lobes were removed, and individual oocytes were manually dissected. Wherever indicated, collagenase was used to remove the follicle cell layer. Stage 6 oocytes were microinjected, as described (22).

SLP was prepared by a modification (23) of the solid-phase technique and was purified by gel filtration and reversed-phase liquid chromatography (unpublished data). The peptide was 26 amino acids long and consisted of residues −1 to −19 of the NH_2-terminal leader peptide of MOPC-321 light chain precursor (24), the adjacent two NH_2-terminal residues of the mature light chain (Asp-Ile), one leucine, three glutamic acid residues, and one glycine as the COOH-terminal residue. The synthesis and isolation of the SLP will be published elsewhere. Iodination of the SLP was performed according to Bolton and Hunter (25), and the iodinated peptide was purified by gel filtration using Sephadex G-25 in 50 mM ammonium bicarbonate.

Antibodies against the purified SLP were elicited in rabbits and were purified by affinity chromatography on a Sepharose 4B column, to which the SLP was covalently bound (26).

RNA was extracted from TEPC-15 mouse myeloma cells according to Kirby (27). Poly(A)-containing mRNA was separated by oligo(dT)-cellulose chromatography (28). Electrophoretic separation of proteins on polyacrylamide gradient gels in the presence of $NaDodSO_4$ was according to Laemmli (29).

RESULTS

We examined the effect of SLP on protein synthesis by first injecting 50 ng of leader peptide into an oocyte and subsequently incubating the oocyte in medium containing [^{35}S]methionine and found a reproducible and marked inhibition of accumulation of labeled protein in the membrane of the oocyte (Fig. 1*A*). Leader peptide also reduced the rate of methionine incorporation into proteins secreted from the oocytes to the medium (Fig. 1*B*). The absolute rate of protein synthesis in *Xenopus* oocytes has been reported to be about 20 ng/hr (31). Therefore, it appears that the amounts of SLP that inhibit the synthesis of secretory and membrane proteins are close to those that can be expected to exist in the oocytes under physiological conditions. The effect is rapid and time-dependent. It reaches a maximum of 50% inhibition in the membrane fraction at 30 min after injection. The SLP-induced interference with secretion is delayed, compared to that in the membrane fraction, reaching a 40% maximum by 1 hr after injection. The SLP-induced inhibition of membrane-associated protein synthesis and secretion of the oocyte proteins decays by 3 hr after injection, and after 6 hr the effect is no longer apparent (Fig. 2*B*). Similar observations were obtained when 500 ng of SLP was injected per oocyte (not shown). When ^{125}I-labeled SLP was injected into the oocytes, almost half associated with the membrane fraction. It then disappeared from the membranes, possibly by degradation, in a time-dependent manner that coincided with the SLP-induced inhibition effect (Fig. 2*A*). The post-injection inhibition exerted by ^{125}I-labeled SLP, which was similar to that caused by unlabeled SLP, is specific to membrane-bound and secreted proteins. No decrease was observed in the rate of synthesis of soluble proteins in the microinjected oocytes.

To determine whether the SLP-induced inhibition results from competition with the nascent NH_2 terminus of polypeptide chains, we injected SLP together with [^{35}S]methionine and

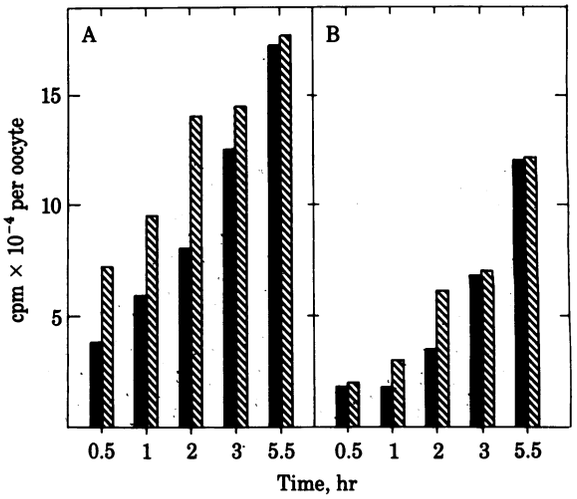

FIG. 1. Leader peptide-induced inhibition of post-injection incorporation of [^{35}S]methionine into oocyte proteins. (*A*) Membrane fraction; (*B*) medium. ■, Leader peptide; ▨, control. Oocytes were injected, each with 50 μl of Barth medium (30) or with leader peptide (50 ng per oocyte), and incubated in groups of 10 in 100 μl of Barth medium containing 30 μCi (1 Ci = 3.7×10^{10} Bq) of [^{35}S]methionine. Incubation was at 19°C for the indicated times. Incubation medium was separated and oocytes were homogenized (five strokes, A pestle, Teflon-glass homogenizer) in 100 μl per group of homogenization buffer [20 mM Tris·HCl, pH 7.6/50 mM KCl/10 mM $Mg(OAc)_2$] containing 10% sucrose. Homogenates were layered over discontinuous sucrose gradients, of 0.34 ml of 50% and 20% sucrose in homogenization buffer, and centrifuged in an Eppendorf centrifuge for 30 min at 0°C. Membrane fractions, at the interphase between the 50% and the 20% sucrose layers, were collected. Total and CCl_3COOH-insoluble radioactivity in oocyte homogenates, incubation medium, and membrane fractions was determined in 2-μl aliquots. Data represent average values of two separate experiments (different frogs); results varied between 7 and 18%.

with poly(A)-containing RNA from TEPC-15 mouse myeloma cells. Under these conditions, SLP completely blocks the incorporation of radioactivity into secreted proteins. It also greatly reduces methionine incorporation into membrane-bound pro-

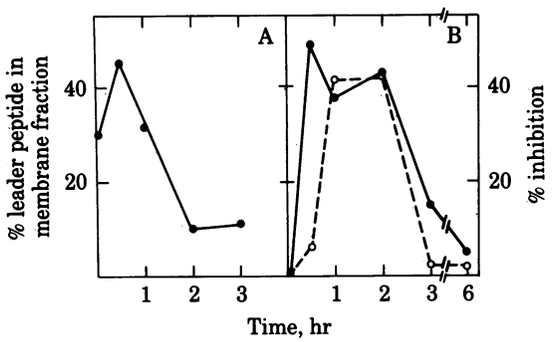

FIG. 2. Time dependence of inhibition of post-injection methionine incorporation into oocyte proteins coincides with leader peptide segregation into oocyte membrane fraction. (*A*) ^{125}I-Labeled leader peptide (25×10^5 cpm/μg) was injected into duplicate groups of oocytes (50 ng per oocyte). Incubation was at 19°C for the indicated times. Membrane fractions were prepared as detailed in the legend to Fig. 1. The ^{125}I-labeled peptide was precipitated by CCl_3COOH from 100-μl aliquots of total oocyte homogenates and of separated membrane fractions, and CCl_3COOH-insoluble radioactivity was determined in a Packard gamma counter. Data show the % of total CCl_3COOH-precipitable radioactivity in the oocytes that appears in the membrane fraction. (*B*) % inhibition of post-injection methionine incorporation into proteins in the membrane fraction (●) and in the oocytes' incubation medium (○) was calculated from the data presented in Fig. 1.

Table 1. Leader peptide blocks incorporation of microinjected methionine into membrane-bound and secreted translation products of TEPC-15 mouse myeloma mRNA

Injected material, 50 nl per oocyte	Total injected [^{35}S]methionine, cpm × 10^{-6} per oocyte	CCl_3COOH-Precipitable radioactivity, cpm/10^6 cpm in oocytes		
		Secreted	Membrane bound	Cytoplasmic
TEPC-15 mRNA (60 ng)	1.25	22,818	11,604	12,225
+ Leader peptide (250 ng)	1.20	1,023	7,103	16,300
Barth medium	1.30	3,727	12,855	15,500
Leader peptide (250 ng)	1.20	2,774	4,903	15,387

Oocytes were separated from dissected ovaries of mature *X. laevis* females by incubation with 0.2% type I collagenase (Sigma) in 10 ml of Ca^{2+}-deficient Barth medium for 16 hr at 19°C. Stage 6 mature oocytes were then selected and washed with regular Barth medium. [^{35}S]Methionine (600–1,000 Ci/mmol, Amersham) was lyophilized to dryness in aliquots of 30 μCi and redissolved in 1-μl aliquots of the solutions to be injected. Oocytes were injected, each with about 1.5 μCi of lyophilized methionine in the injected solution. Injected oocytes were incubated in groups of 10 in 100 μl of Barth medium for 2 hr at 19°C. Separation of subcellular fractions was as in the legend to Fig. 1.

teins. Both the rate of secretion and the SLP-induced interference with secretion were much more apparent in the mRNA-injected than in the control oocytes (Table 1). In both cases, the inhibition effect exerted by the injected SLP was specific to membrane-bound and secreted proteins. Thus, no decrease was observed in the rate of synthesis of soluble proteins in the control or the mRNA microinjected oocytes (Table 1). Under these experimental conditions, the injected SLP appeared to interfere primarily with processes necessary for the secretion of myeloma proteins. This conclusion has been confirmed by $NaDodSO_4$/polyacrylamide gel electrophoresis, which showed various distribution patterns for proteins secreted from control and mRNA-injected oocytes, in the presence and absence of coinjected SLP (Fig. 3).

In the presence of mRNA, the block of secretion is most apparent when 250 ng of SLP is injected per oocyte. Twenty-five nanograms per oocyte does not affect secretion. The inhibition of secretion caused by 250 ng of SLP per oocyte increases from 62% at 1 hr after injection to 95% at 2 hr and decreases to 63% by 4 hr after injection. Thus, it appears that SLP competes both with endogenous and mRNA-directed incorporation of methionine into secreted and membrane proteins in microinjected oocytes.

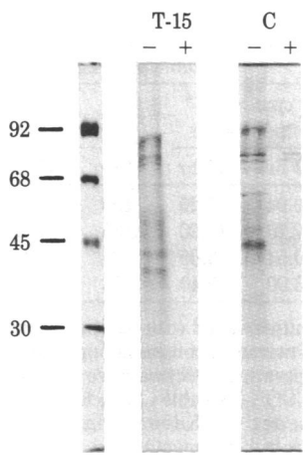

FIG. 3. Electrophoretic analysis of translation products secreted from microinjected oocytes reveals leader peptide-induced block of myeloma secretory proteins. Collagenase-treated oocytes were injected as described in the legend to Table 1. Aliquots of 15 μl from the oocytes' incubation medium were separated by gradient (5–15%) $NaDodSO_4$/polyacrylamide gel electrophoresis. Oocytes were injected with TEPC-15 total mRNA (T-15) or Barth medium (control, C) in the presence (+) or in the absence (−) of added SLP. Molecular weights are shown as $M_r \times 10^{-3}$.

In contrast to this inhibition, which manifests itself by interfering with the post-translational processing or compartmentalization (or both) of newly formed polypeptide chains, SLP enhances the rate of secretion of already synthesized and sequestered proteins, which are destined for eventual secretion. This stimulation was apparent when increasing quantities of SLP were injected into oocytes that were preincubated with [^{35}S]methionine for 18 hr. The rate of secretion of endogenous labeled, CCl_3COOH-insoluble proteins from the SLP-injected oocytes was reproducibly accelerated in a dose-dependent manner. In oocytes injected with as little as 10 ng of SLP per oocyte, the rate of secretion was 2-fold higher than that of control oocytes at 2 hr after injection. The accelerated secretion increased up to 10-fold over control in oocytes injected with 50 ng of SLP per oocyte. Further increase of the SLP quantity injected, up to 500 ng per oocyte, did not affect the enhanced secretion (Fig. 4). The absolute rate of protein synthesis in *Xenopus* oocytes has been reported to be about 20 ng/hr (31). Therefore, it appears that the amounts of SLP that induce accelerated secretion are close to those that can be expected to exist in the oocytes under physiological conditions. Polyacrylamide gel electrophoresis of the secreted labeled proteins, followed by autoradiography of the dried gels, revealed a general increase in the intensity but no apparent differences between the size distribution patterns of the proteins secreted from SLP-injected or control oocytes (Fig. 4 *Inset*). Furthermore, we failed to see any morphological differences between control and SLP-injected oocytes, both by light or electron microscopy (not shown). Thus, it appears that microinjected SLP selectively speeds up the release into the incubation medium of polypeptides that are destined to be secreted but does not alter the morphological features of the oocytes nor does it affect the natural segregation pattern of proteins within the subcellular oocyte compartments.

[^{35}S]Methionine-labeled polypeptides continue to accumu-

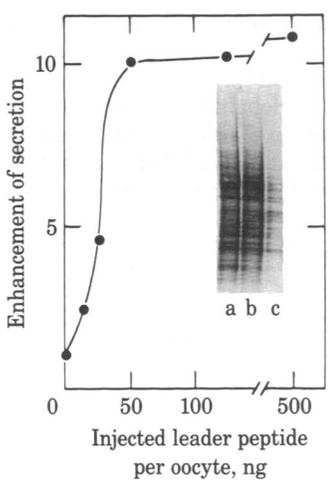

FIG. 4. Enhancement of secretion of prelabeled oocyte proteins depends on dose of injected leader peptide. Oocytes were preincubated in groups of 10 in 100 μl of Barth medium containing 50 μCi of [^{35}S]methionine for 18 hr at 19°C. Injection was with 50 μl per oocyte of increasing concentrations of leader peptide, into duplicate groups of oocytes. Injected oocytes were incubated in fresh Barth medium for 2.5 hr. CCl_3COOH-Insoluble radioactivity was determined in 2-μl samples of oocyte medium. Data represent average values calculated from three experiments (different frogs). The amount of radioactive proteins secreted by oocytes injected with Barth medium alone was arbitrarily designated as 1.0. (*Inset*) Aliquots (5 μl) of the incubation medium of oocytes injected with 500 ng (a) or 50 ng (b) of SLP or with Barth medium (c) were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis.

late at a constant rate in the incubation medium of preincubated control oocytes for over 50 hr (Fig. 5 *A* and *B*). This indicates that the oocytes remain fully viable after the preincubation period, as it has been shown that active secretion of both oocyte proteins (15) and protein products of microinjected mRNAs (20, 22) continues to occur for many hours. Oocytes injected with 50 ng of SLP per oocyte display a rapidly induced secretion enhancement, and the concentration of secreted proteins in their incubation medium remains much higher than that of control oocytes for over 80 hr (Fig. 5*A*). The initially high rate of protein secretion from these oocytes decreases exponentially, and by 20 hr after injection it already appears to be rather close to the secretion rate displayed by control oocytes (Fig. 5*B*). The initial exponential decrease in the rate of secretion from SLP-injected oocytes indicates a rate of decay with a $t_{1/2}$ of about 2 hr for the secretion enhancement effect.

Amino acid residues 2–15 in SLP are all hydrophobic, and the COOH terminus of the peptide is composed of negatively charged amino acids. To find out whether these properties are sufficient to induce accelerated secretion, we injected oocytes with 0.1% Triton X-100 or with 44 μM poly-L-glutamic acid. Neither of these controls affected the rate of secretion from the injected oocytes, which displayed levels of secreted proteins similar to those obtained from noninjected or from Barth medium-injected oocytes (Fig. 6). Injection of increasing quantities of glucagon, a peptide of a similar molecular weight to that of SLP, ruled out the possibility that the small size of the peptide is a major element in inducing the accelerated secretion. Injection of Ala-Gly-Ser-Glu peptide has shown that this short peptide is not sufficient to induce the accelerated secretion by itself (not shown). Taken together, these control experiments confirm that the accelerated rate of secretion from SLP-injected oocytes may be due to the entire SLP molecule.

To further investigate the biological specificity of the enhancement effect, we reacted SLP with anti-SLP antibodies and then injected the mixture into the oocytes. Immunoreacted SLP failed to accelerate secretion. The loss of this biological function is not due to the presence of immunoglobulins in the injected material, because SLP preincubated and coinjected with normal rabbit immunoglobulins does not lose its capacity to induce accelerated secretion (Table 2). This result indicates that the domain(s) in SLP that interacts with the anti-SLP antibodies is essential for inducing the secretion enhancement effect.

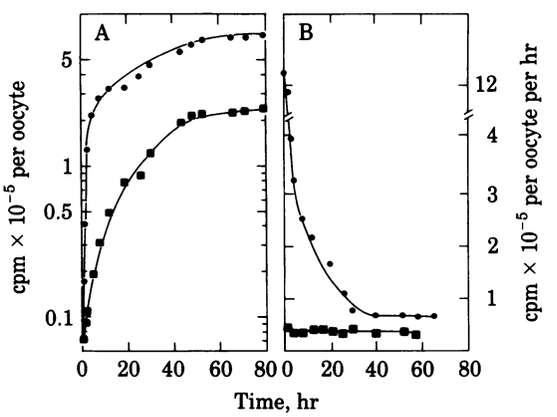

FIG. 5. Time dependence of secretion enhancement. Oocytes were preincubated as in the legend to Fig. 4. Microinjection was in duplicate groups with Barth medium (■) or with 50 ng of leader peptide (●) in 50 nl per oocyte. Medium was changed at the indicated intervals and CCl_3COOH-insoluble radioactivity was determined in 2-μl samples of the incubation medium. Cumulative radioactivity (*A*) and rate of secretion (*B*) were calculated for each time point.

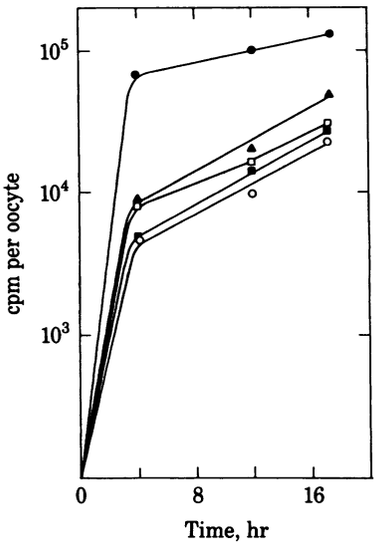

FIG. 6. Specificity of leader peptide-induced enhancement of secretion. Preincubated oocytes were microinjected in two groups of 10 with SLP (50 ng per oocyte, ●), Barth medium (50 μl per oocyte, ▲), poly-L-glutamic acid (16 ng per oocyte, ○), or 0.1% Triton X-100 (50 μl per oocyte, □). CCl_3COOH-Insoluble radioactivity in oocyte incubation medium was determined at the indicated times in 2-μl aliquots. Noninjected oocytes (■) served as controls. Data represent average values of three separate experiments.

DISCUSSION

The use of *Xenopus* oocytes as an *in vivo* surrogate secretory system was combined in our experiments with the use of a synthetic, biologically functional leader peptide. Our observations indicate that in *Xenopus* oocytes the leader peptide is involved in at least two different steps along the secretory pathway.

The translocation of nascent polypeptide chains through the membrane of the rough endoplasmic reticulum has been shown to be blocked *in vitro* in the presence of the SLP of preproparathyroid hormone (14) and of MOPC-321 immunoglobulin light chain precursor (32). In microinjected *Xenopus* oocytes, the SLP of mouse immunoglobulin light chain precursor prevents secretion of proteins directed by the oocyte mRNAs, as well as by exogenous coinjected mRNAs. Both observations appear to reflect a single competitive inhibition phenomenon. The inhibitory effect of SLP is most clearly visualized when [^{35}S]-

Table 2. Enhancement of secretion is blocked by immunoreaction of leader peptide with antibodies

Injected material, 50 nl per oocyte	[^{35}S]Methionine incorporated into secreted CCl_3COOH-insoluble proteins			
	cpm × 10^{-4} per oocyte		% of control	
	4 hr	17 hr	4 hr	17 hr
Control (Barth medium)	0.94	5.20	100	100
Leader peptide (50 ng)	7.10	13.20	755	253
+ Anti-leader peptide (1.25 μg)	0.54	3.60	57	69
+ Normal rabbit Ig (1.25 μg)	3.90	9.40	414	181

Leader peptide (1 mg/ml) was immunoreacted with purified rabbit anti-leader peptide antibodies or with normal rabbit immunoglobulins (25 mg/ml) for 15 min at room temperature. Oocytes were preincubated, as in the legend to Fig. 1. CCl_3COOH-Insoluble radioactivity was determined in 2-μl samples of the oocytes' incubation medium. Data represent average values of two separate experiments.

methionine is injected together with mRNA and the SLP into the oocytes. This could be due to better accessibility of SLP to polysomes occupied by the injected mRNAs, as compared to those including oocyte mRNAs.

We have also found that SLP accelerates the export of already synthesized and sequestered secretory proteins from the oocytes. This enhancement effect is best seen when SLP is injected into oocytes, in which the synthesized proteins have been previously labeled. The block of secretion of simultaneously translated chains cannot be detected under these conditions, because newly formed labeled chains represent a very small proportion of labeled proteins. Similarly, the accelerated secretion of unlabeled sequestered proteins cannot be seen when newly formed chains are labeled, as in the first experimental approach. A third approach—namely, SLP microinjection followed by uptake of [^{35}S]methionine from the incubation medium—results in a partial arrest of translocation and secretion of oocyte proteins. This can be explained by incomplete segregation of the microinjected SLP in the oocyte.

The observed acceleration of secretion by SLP was specific, as shown by several lines of experimental evidence. (*i*) Microinjection of equimolar quantities of poly-L-glutamic acid, of glucagon, and of Triton X-100 failed to accelerate secretion. These were used as controls for SLP properties such as negative charge, small molecular weight, and hydrophobicity. (*ii*) No morphological differences could be found between sections from oocytes that were injected with SLP and control oocytes. (*iii*) The acceleration of secretion only occurred with proteins that are naturally secreted by the oocytes. (*iv*) No acceleration occurred when SLP was immunoreacted with anti-SLP antibodies prior to microinjection.

The specificity of the secretion block exerted by SLP on simultaneously translated nascent secretory polypeptide chains is indicated from the observation that the synthesis of cytoplasmic proteins remained totally unaffected under conditions in which protein secretion and incorporation into the membrane were profoundly inhibited. This is in agreement with the differential capacity for translation and lack of competition between mRNAs that segregate to free and membrane-bound polysomes in the oocytes (21).

The mechanisms by which SLP inhibits the translocation and secretion of simultaneously translated proteins and accelerates the secretion of sequestered proteins have not been determined. One or more of the steps required for translocation of nascent chains might be blocked by SLP. SLP might competitively inhibit (either or both) (*a*) the binding of nascent polypeptides to the cytoplasmic signal recognition protein (33) or (*b*) the interaction of the signal recognition protein complex with the docking protein in the membrane of the rough endoplasmic reticulum (10). The rate at which ^{125}I-labeled SLP segregates into the oocyte membrane coincides with the rate of inhibition of translocation and secretion. This indicates that the peptide operates at the cell membrane and favors the second possibility. When accelerating secretion of sequestered proteins, SLP might affect (*c*) the rate of later post-translational processing events, required for the formation of mature proteins. These probably occur on the membrane of the rough endoplasmic reticulum, as suggested by the *in vitro* glycosylation of vesicular stomatitis virus protein (34). Alternatively, or in addition, SLP might enhance (*d*) the rate at which processed polypeptides reach the secretory vesicles or the movement of secretory vesicles towards the plasma membrane and their fusion with it (or both). Each of these steps might involve additional regulatory proteins, which may be pursued by further use of SLP-microinjected oocytes as a surrogate system.

The saturable translocation sites on microsomal membranes appear to recognize various leader peptides (14). The mechanism by which protein secretion from the oocytes operates is neither cell type nor species specific (35). Indeed, we find the secretion of both endogenous oocyte proteins and proteins directed by injected mRNAs to be modulated by SLP. However, it is not yet clear whether the bifaceted role of SLP in modulating secretion from oocytes represents a general phenomenon that operates in all eukaryotic cells.

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1. Milstein, C., Brownlee, G. G., Harrison, T. M. & Mathews, M. B. (1972) *Nature (London) New Biol.* **239**, 117–120.
2. Kreil, G. (1981) *Annu. Rev. Biochem.* **50**, 317–348.
3. Sabatini, D. D., Kreibich, G., Morimoto, T. & Adesnik, M. (1982) *J. Cell Biol.* **92**, 1–22.
4. Blobel, G. & Sabatini, D. D. (1971) in *Biomembranes*, ed. Manson, L. A. (Plenum, New York), Vol. 2, pp. 193–195.
5. Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67**, 835–851.
6. Szczesna, E. & Boime, I. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1179–1183.
7. Bedouelle, H., Bassford, P. J., Fowler, A. V., Zabin, I., Beckwith, J. & Hofnung, M. (1980) *Nature (London)* **285**, 78–81.
8. Talmadge, K., Stahl, S. & Gilbert, W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3369–3373.
9. Walter, P. & Blobel, G. (1981) *J. Cell Biol.* **91**, 551–556.
10. Meyer, D. I., Krause, E. & Dobberstein, B. (1982) *Nature (London)* **297**, 647–650.
11. Meyer, D. I., Louvard, D. & Dobberstein, B. (1982) *J. Cell Biol.* **92**, 579–583.
12. Habener, J. F., Rosenblatt, M., Dee, P. C. & Potts, J. T., Jr. (1979) *J. Biol. Chem.* **254**, 10596–10599.
13. Farquar, M. G. & Palade, G. E. (1981) *J. Cell Biol.* **91**, 77s–103s.
14. Majzoub, J. A., Rosenblatt, M., Fennick, B., Mannus, R., Kronenberg, H. M., Potts, J. T., Jr., & Habener, J. F. (1980) *J. Biol. Chem.* **255**, 11478–11483.
15. Mohun, T. J., Lane, C. D., Colman, A. & Wylie, C. C. (1981) *J. Embryol. Exp. Morphol.* **61**, 367–383.
16. Lane, C. D., Colman, A., Mohun, T., Morser, J., Champion, J., Kourides, I., Craig, S., James, T. C., Appelbaum, S. W., Ohlsson, R. I., Paucha, E., Houghton, M., Matthews, J. & Miflin, B. J. (1980) *Eur. J. Biochem.* **111**, 225–235.
17. Zehavi-Willner, T. & Lane, C. D. (1977) *Cell* **11**, 683–693.
18. Lane, C. D., Shanon, S. & Craig, R. (1979) *Eur. J. Biochem.* **101**, 485–495.
19. Rapoport, T. A. (1981) *Eur. J. Biochem.* **115**, 665–669.
20. Colman, A. & Morser, J. (1979) *Cell* **17**, 517–526.
21. Richter, J. D. & Smith, L. D. (1981) *Cell* **27**, 183–191.
22. Soreq, H., Parvari, R. & Silman, I. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 830–834.
23. Li, C. H., Yamashiro, D. & Lemaire, S. (1975) *Biochemistry* **14**, 953–956.
24. Burstein, Y. & Schechter, I. (1978) *Biochemistry* **17**, 2393–2399.
25. Bolton, A. E. & Hunter, W. M. (1973) *Biochem. J.* **133**, 529–539.
26. Cuatrecasas, P. (1970) *J. Biol. Chem.* **245**, 3059–3065.
27. Kirby, K. S. (1968) *Methods Enzymol.* **12**, 87–89.
28. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
29. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
30. Lane, C. D., Marbaix, G. & Gurdon, J. B. (1971) *J. Mol. Biol.* **61**, 73–91.
31. Richter, J. D., Wasserman, W. J. & Smith, L. D. (1982) *Dev. Biol.* **89**, 159–167.
32. Koren, R. (1983) Dissertation (The Weizmann Institute of Science, Rehovot, Israel).
33. Walter, P., Ibrahimi, I. & Blobel, G. (1981) *J. Cell Biol.* **91**, 545–550.
34. Katz, F. N., Rothman, J. E., Lingappa, V. R., Blobel, G. & Lodish, H. F. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3278–3282.
35. Colman, A., Lane, C. D., Craig, R., Boulter, A., Mohun, T. & Morser, J. (1980) *Eur. J. Biochem.* **113**, 339–348.