The collective data from studies performed with T cells and 3T3 cells indicate that cell cycle commitment is associated with expression of c-*fos* and c-*myc* proto-oncogenes, whereas c-*myb* expression is confined to G_1 progression. Thus, future experiments will surely focus on the critical functional consequences of nuclear binding by the products of the c-*fos*, c-*myc*, and c-*myb* genes during both normal and neoplastic cell proliferation.

Note added in proof. Subsequent to the submission of this manuscript for publication, a report by Thompson *et al.* (*24*) appeared, which confirms our findings and extends them to another species and other cell types; c-*myb* is expressed maximally during the midpoint of G_1 progression by chicken embryo fibroblasts, synchronized leukemic T cells, and bursal cells. An additional cell cycle–independent mechanism appears to result in an even more marked expression of c-*myb* in thymocytes.

REFERENCES AND NOTES

1. P. C. Nowell, *Cancer Res.* **20**, 462 (1960).
2. K. A. Smith, *Annu. Rev. Immunol.* **2**, 319 (1984).
3. S. C. Meuer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1509 (1984).
4. D. A. Cantrell and K. A. Smith, *Science* **224**, 1312 (1984).
5. ———, *J. Exp. Med.* **158**, 1895 (1983).
6. K. A. Smith, and D. A. Cantrell, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 864 (1985).
7. M. Gullberg and K. A. Smith, *J. Exp. Med.* **163**, 270 (1986).
8. A. B. Pardee, B.-Z. Shilo, A. L. Koch, *Hormones and Cell Culture*, G. H. Sato and R. Ross, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1979), vol. 6, pp. 373–392.
9. W. J. Pledger, C. D. Stiles, H. N. Antoniades, C. D. Scher, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4481 (1977).
10. ———, *ibid.* **75**, 2839 (1978).
11. K. Kelly, B. H. Cochran, C. D. Stiles, P. Leder, *Cell* **35**, 603 (1983).
12. M. Kronke, W. J. Leonard, J. M. Depper, W. C. Greene, *J. Exp. Med.* **161**, 1593 (1985).
13. K.-H. Klempnauer, G. Symonds, G. I. Evan, J. M. Bishop, *Cell* **37**, 537 (1984).
14. E. H. Westin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2194 (1982).
15. J. D. Mountz, A. D. Steinberg, D. M. Klinman, H. R. Smith, J. F. Mushinski, *Science* **226**, 1087 (1984).
16. J. H. Chen, *J. Virol.* **36**, 162 (1980).
17. T. J. Gonda, D. K. Sheiness, J. M. Bishop, *Mol. Cell. Biol.* **2**, 617 (1982).
18. J. Campisi, H. E. Gray, A. B. Pardee, M. Dean, G. F. Sonenshein, *Cell* **36**, 241 (1984).
19. M. E. Greenberg and E. B. Ziff, *Nature (London)* **311**, 433 (1984).
20. W. Kruijer, J. A. Cooper, T. Hunter, I. M. Verma, *ibid.* **312**, 711 (1984).
21. R. Muller, R. Bravo, J. Burckhardt, T. Curran, *ibid.*, p. 716.
22. J. C. Reed, D. E. Sabath, R. G. Hoover, M. B. Prystowsky, *Mol. Cell. Biol.* **5**, 3361 (1985).
23. J. C. Reed, P. C. Nowell, R. G. Hoover, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4221 (1985).
24. C. B. Thompson, P. B. Challoner, P. E. Neiman, M. Groudine, *Nature (London)* **319**, 374 (1986).
25. K. A. Smith, M. F. Favata, S. Oroszlan, *J. Immunol.* **131**, 1808 (1983).
26. G. Franchini, F. Wong-Staal, M. A. Baluda, C. Lengel, S. Tronick, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7385 (1983).
27. A. K. Sood, D. Pereira, S. M. Weissman, *ibid.* **78**, 616 (1981).
28. D. K. Watson, M. C. Psallidopoulos, K. P. Samuel, R. Dalla-Favera, T. S. Papas, *ibid.* **80**, 3642 (1983).
29. A. D. Miller, T. Curran, I. M. Verma, *Cell* **36**, 51 (1984).

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Conformations of Signal Peptides Induced by Lipids Suggest Initial Steps in Protein Export

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Despite the requirement for a functional signal sequence in protein export, little is known of the conformational properties and membrane interactions of these highly hydrophobic amino terminal extensions on nearly all exported proteins. The *Escherichia coli* λ phage receptor signal sequence was studied in phospholipid monolayers by circular dichroism and Fourier transform infrared spectroscopy; the signal peptide was shown to prefer an α-helical conformation when inserted into the lipid phase. However, interaction with the lipid surface without insertion induced the signal sequence, which is unstructured in bulk aqueous solution, to adopt a β structure. These observations are combined in a model for the initial steps in signal sequence-membrane interaction in vivo.

THE NEARLY UNIVERSAL REQUIREMENT in protein export for an amino terminal extension, termed a "signal peptide," has focused attention on the roles that these highly hydrophobic sequences might play (*1*). While many essential details of the secretion process are still poorly understood, it is clear that the involvement of the signal sequence in both prokaryotic and eukaryotic protein secretion includes recognition by proteins, interactions with membranes or membrane-resident components, facilitation of translocation, and specific cleavability (*2, 3*). Little is known, however, of the physical characteristics that must influence their participation in the export process, such as conformational preferences or potentials for membrane and protein interaction.

Our biophysical studies on a family of chemically synthesized signal sequences from an *Escherichia coli* outer membrane protein, the λ phage receptor protein (LamB protein) (*4*), show that two physical properties of the isolated signal sequences, their tendency to adopt an α-helical conformation in hydrophobic environments and their tendency to insert into phospholipid monolayers, correlate with their in vivo export function (*5–7*). These properties may be involved in the same step in the secretion process or in different steps. An α-helical conformation may be required to generate a sufficiently hydrophobic structure to allow membrane insertion. Alternatively, the α helix may be necessary for binding to a protein, while the ability to interact with lipids may be important for translocation. To analyze further the relationships between these properties of signal sequences, we have designed experiments that enable conformational characterization of signal peptides in lipid monolayers.

Monolayers containing 65% 1-palmitoyl-2-oleoyl phosphatidylethanolamine (PE) and 35% 1-palmitoyl-2-oleoyl phosphatidylglycerol (PG) (a ratio chosen to mimic the *E. coli* membrane) and the wild-type LamB signal peptide were prepared for spectroscopic examination by transferring them to a solid support (Fig. 1). A miniature Teflon Langmuir/Wilhelmy trough with a dipping well was constructed to minimize the volume of subphase required. The lipid monolayers were formed by spreading of aliquots from chloroform solution until the desired initial pressure was achieved. Peptide was then immediately injected into the subphase. The film pressure was monitored for an equilibration period, after which the monolayer was transferred either to quartz plates [for circular dichroism (CD)] or to Ge crystals [for Fourier transform–infrared (FT-IR)]. Monolayer transfer and spectroscopic investigations fol-

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lowed a previously developed protocol (*8, 9*), and a detailed discussion of the techniques will be presented separately (*10*).

Previous surface tensiometry experiments (*6, 7*) had indicated that the synthetic LamB signal peptides can interact with phospholipid monolayers in two ways: by binding electrostatically to the head groups of the lipids and by inserting into the hydrocarbon region of the monolayer. By selection of the surface pressures at which the monolayer CD and IR samples are prepared, it is possible to determine the signal peptide's conformation in each of these two binding modes. Two sets of samples were made. One set, in which the monolayer was spread at a surface pressure higher than the peptide's critical insertion pressure (*11*), allows electrostatic binding to the lipid head groups, but prevents insertion into the lipid monolayer. The other set of samples is prepared with the monolayer initially at a surface pressure lower than the critical insertion pressure; these conditions allow both insertion and electrostatic interactions. Injection of the signal peptide into the subphase below a low-pressure monolayer resulted in an immediate rise in the film pressure, confirming peptide insertion. A control film, prepared at high pressure with only zwitterionic lipid [distearoyl phosphatidylcholine (DSPC)] in the monolayer, contained only peptide attached to the solid support. Contributions from this source were excluded from the spectral analysis.

The spectral studies show the signal peptide to have marked conformational sensitivity to its environment. The CD spectrum of the wild-type LamB signal peptide electrostatically bound to the monolayer indicates a predominance of β structure (Fig. 2), based on comparisons to reference data for polypeptide samples in solution (*12*). While some effects of orientation are expected in the CD of monolayer samples, past studies have shown that the spectra are qualitatively quite similar to spectra normally associated with polypeptides in the α-helix and β-sheet conformations in solution, and can be interpreted in terms of these familiar structures (*8, 9*). The minimum at 215 nm and the crossover at 205 nm are both characteristic of β-structure CD's in solution (*12*). FT-IR data, obtained with attenuated total reflectance (ATR) infrared spectroscopy of the lipid-peptide monolayer transferred onto germanium ATR crystals at high pressure, also demonstrate the presence of β conformation; bands characteristic of this structure can be seen in the amide I region: strong absorption at 1630 cm^{-1}, with a small higher frequency shoulder at about 1690 cm^{-1} (Fig. 3A) (*13, 14*). The presence of a small amount of α-helical or random conformation or both is also indicated by intensity at 1660 cm^{-1} (*14, 15*). It is noteworthy that previous studies of this signal peptide in aqueous buffer had shown no evidence of a preferred secondary structure, and we had observed a strongly α-helical spectrum in micellar SDS and phospholipid vesicles (*5, 6*).

Signal peptide–lipid films, prepared under conditions where the peptide is both inserted into the monolayer and electrostatically bound to the head groups, show a substantial contribution to their CD spectra from an α-helical conformation in addition to β structure (Fig. 2). This is indicated by an increased negative ellipticity at 208 and 222 nm. Note that the spectrum shown reflects a sum of adsorbed and inserted peptide; hence, the inserted peptide is enriched in α-helical conformation. This conclusion is further supported by CD spectra obtained for signal peptide in PE/PG sonicated vesicles, which correspond to 60% α helix [based on mathematical curve-fitting to reference spectra (*6, 12*)]. The FT-IR data for the peptide inserted into the lipid monolayer (Fig. 3B) show enhanced intensity at 1660 cm^{-1}, indicative of an increased content of α helix or random structure or both, and the intensity of the 1630 cm^{-1} band (β structure) is much reduced. These data support the conclusions from CD that the inserted peptide is less likely to adopt a β conformation than the adsorbed peptide. The band at 1660 cm^{-1} in the amide I region can be assigned either to random or α-helical peptide segments; these particular conformations cannot be distinguished on

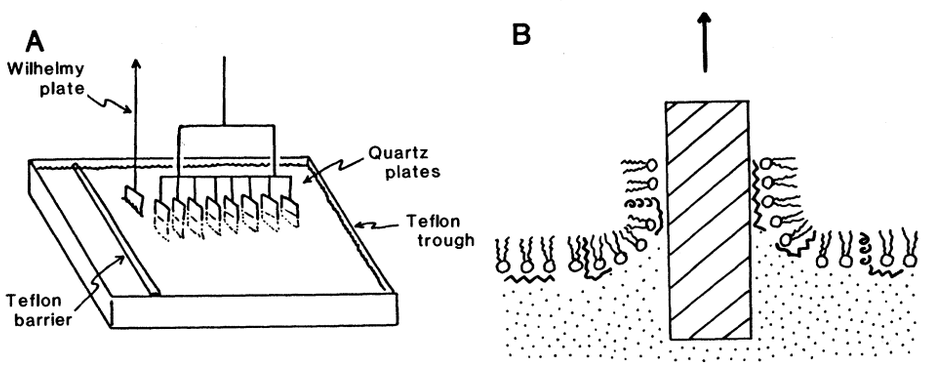

Fig. 1. (A) Transfer of a phospholipid monolayer to a solid support. A Teflon trough (10 by 8 by 0.7 cm) was constructed with a dipping well to accommodate plates for monolayer transfer. Its capacity is about 100 ml, and its surface area is about 80 cm^2. To transfer a monolayer, the trough was filled with 5 m*M* tris buffer (*p*H 7.3). Quartz or germanium plates were submerged in the buffer as shown. Lipids (either DSPC or 65% PE plus 35% PG) were spread from chloroform to the desired surface pressure, which was measured using a Wilhelmy plate. High-pressure monolayers were spread at 40 dyne/cm, and low-pressure monolayers were spread at 25 dyne/cm. These values are above and below, respectively, the peptide's critical pressure of insertion, which is 38 dyne/cm (*6*). Then, 500 μl of a peptide solution (1 mg/ml) in 5 m*M* tris (*p*H 7.3) was injected through the monolayer, and the subphase was stirred gently for 5 minutes. Final peptide concentration was about 3 μ*M*, a saturating concentration (*6*). The peptide was allowed to interact with the monolayer for 30 minutes. After this time the surface pressure had stabilized. (B) The film was then transferred to the quartz or germanium plates at constant surface pressure. The amount of peptide on the plates was estimated by ultraviolet absorption. The plates were stored at room temperature in an atmosphere of constant 80% humidity until spectra were obtained. The transfer shown corresponds schematically to a low-pressure monolayer experiment, in which some peptide would be inserted, some electrostatically adsorbed to the head groups, and some bound to the solid support.

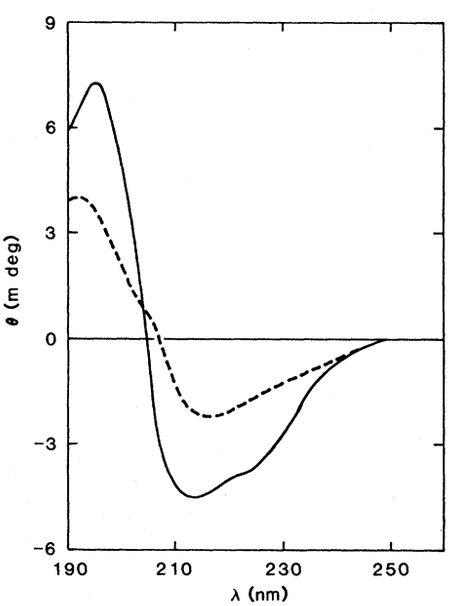

Fig. 2. CD spectra of the peptide interacting with a high-pressure (------) or low-pressure (——) monolayer on quartz plates. The cell holder for positioning the plates in the circular dichrometer sample compartment has been described (*8, 9*). The spectra were recorded on a Jasco J-41C dichrometer. The spectra are of 8 or 16 plates (16 or 32 monolayers) arranged in series, and are sums of 128 scans each. A control spectrum of DSPC transferred to the plates from a peptide-containing subphase at 40 dyne/cm was subtracted from the experimental spectra. Data were digitized every 2 nm.

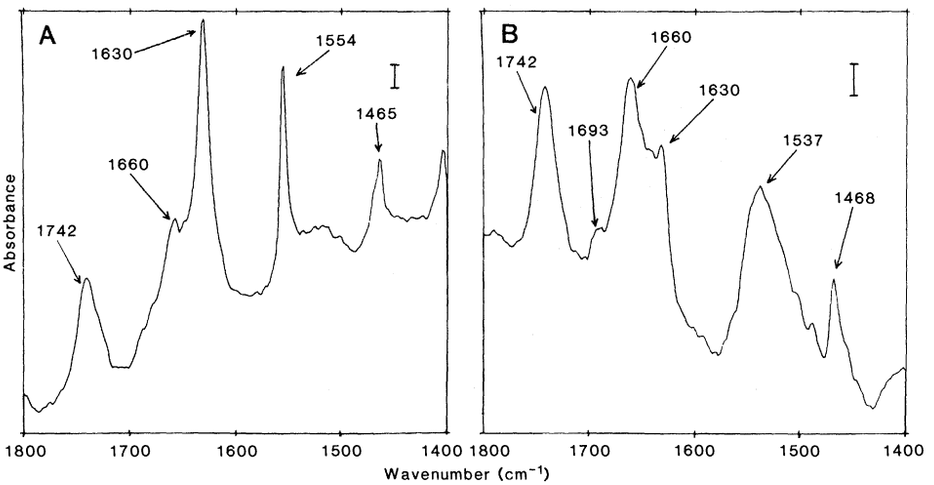

Fig. 3. FT-IR spectra of the peptide interacting with a PE/PG (65:35) monolayer transferred to a 45° germanium ATR crystal (50 by 10 by 2 mm; Harrick Scientific, Ossining, NY). Spectra were obtained on a Digilab FTS-10 FT-IR spectrometer equipped with a narrow band, liquid N_2-cooled HgCdTe detector at 4-cm^{-1} resolution with triangular apodization and one level of zero filling. Spectra were obtained using a 6× ATR beam condenser accessory (Harrick Scientific) at an incoming angle of incidence of 45°. Typically, 4096 scans were collected to improve the signal-to-noise ratio; the spectra presented here have not been smoothed. (A) FT-IR spectrum of the peptide interacting with a phospholipid monolayer above the critical insertion pressure of the peptide. The infrared bands at 1742 and 1465 cm^{-1} correspond to the C=O stretching and methylene scissoring vibrations of the phospholipid, respectively. The bands between 1600 and 1700 cm^{-1} are due to the amide I vibration of the peptide, while the band between 1500 and 1600 cm^{-1} is due to the amide II vibration of the peptide. (B) FT-IR spectrum of the peptide interacting with a phospholipid monolayer below the critical insertion pressure of the peptide. Band assignments are as in (A). The insert marks in both (A) and (B) indicate an intensity of 1.0×10^{-3} absorbance units.

the basis of their infrared spectra in the amide I region alone (*16, 17*). Random and α-helical conformations can be differentiated, however, on the basis of their vibrations in the amide III spectral region (1300 to 1200 cm^{-1}) (*18–22*). For the wild-type signal peptide inserted into the PE/PG monolayer, an infrared band is observed at 1280 cm^{-1} that is not observed in the spectrum of the peptide adsorbed onto the monolayer. Based on literature correlations (*23, 24*), we assign this vibration to the amide III band characteristic of an α-helical conformation for the peptide when inserted into the phospholipid monolayer.

These results demonstrate that isolated signal sequences may adopt several different conformations under specific environmental conditions. These conformational interconversions may facilitate the initiation of the export process in vivo. The present data provide the first direct evidence for various conformational states and interconversions of signal peptides that have been proposed to play a role in secretion (*25*). Specifically, our results suggest that signal sequences in the aqueous milieu of the cytoplasm would be unstructured (*5, 6*). Upon approaching a lipid surface (either subsequent to interaction of the signal recognition particle with the docking protein or under the influence of other factors, such as membrane potential), the signal sequence may adopt a β structure. This form could be the conformation that initially inserts into the membrane. The length of the hydrophobic regions in known signal sequences [10 to 12 residues (*26*), which could span a hydrophobic region of 35 Å in a β structure] argues for this possibility. Insertion should lead to a preference for an α-helical conformation, as indicated by the data presented here, and by previous studies of signal peptides in micellar media and in vesicles (*5, 6*). The consequence of the conformational change, if the NH_2-terminus stays anchored on the cytoplasmic side [as supported by recent studies (*27*)], is entry of the first 10 to 15 residues of the mature protein into the membrane. These steps are shown in Fig. 4; in this model, the signal sequence functions to establish a productive arrangement of the nascent chain such that its subsequent translocation is facilitated.

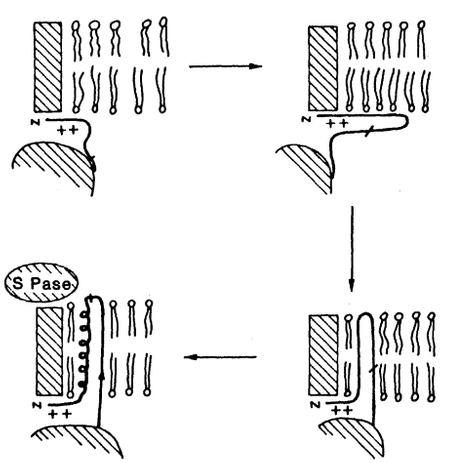

Fig. 4. Summary of steps suggested for the initial interactions of a signal sequence with a membrane based on conformations taken up by synthetic signal peptide in lipid monolayers.

REFERENCES AND NOTES

1. M. S. Briggs and L. M. Gierasch, *Adv. Protein Chem.*, in press.
2. P. Walter, R. Gilmore, G. Blobel, *Cell* **38**, 5 (1984).
3. T. J. Silhavy, S. A. Benson, S. D. Emr, *Microbiol. Rev.* **47**, 313 (1983).
4. The signal sequence of the λ receptor protein is: Met Met Ile Thr Leu Arg Lys Leu Pro Leu Ala Val Ala Val Ala Ala Gly Val Met Ser Ala Gln Ala Met Ala.
5. M. S. Briggs and L. M. Gierasch, *Biochemistry* **23**, 3111 (1984).
6. M. S. Briggs, thesis, Yale University (1986).
7. ———, L. M. Gierasch, A. Zlotnick, J. D. Lear, W. F. DeGrado, *Science* **228**, 1096 (1985).
8. D. G. Cornell, *J. Colloid Interface Sci.* **70**, 167 (1979).
9. ———, *ibid.* **88**, 536 (1982).
10. ———, R. A. Dluhy, M. S. Briggs, L. M. Gierasch, in preparation.
11. R. Verger and F. Pattus, *Chem. Phys. Lipids* **30**, 189 (1982); M. C. Phillips and C. E. Sparks, *Ann. N.Y. Acad. Sci.* **328**, 122 (1980).
12. N. Greenfield and G. D. Fasman, *Biochemistry* **13**, 4108 (1969).
13. S. Krimm and Y. Abe, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2788 (1972).
14. T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc.* **83**, 712 (1961).
15. N. A. Nevskaya and Y. N. Chirgadze, *Biopolymers* **15**, 637 (1976).
16. R. D. B. Fraser and T. P. MacRae, *Conformation in Fibrous Proteins and Related Synthetic Peptides* (Academic Press, New York, 1973).
17. J. H. Keighly, in *Introduction to the Spectroscopy of Biological Polymers*, D. W. Jones, Ed. (Academic Press, New York, 1976), pp. 17–80.
18. The amide III is a mixed-mode vibration [about 40% C-N stretching and 30% N-H in-plane bending (*19*)] and has been shown to be highly sensitive to side chain vibrations, especially the CH_2 wagging and twisting vibrations (*20*). However, vibrational analysis of model compounds has shown the amide III region to be qualitatively useful for discerning polypeptide chain conformation (*21, 22*).
19. T. Miyazawa, in *Poly-α-Amino Acids*, G. D. Fasman, Ed. (Dekker, New York, 1967), pp. 69–103.
20. S. L. Hsu, W. H. Moore, S. Krimm, *Biopolymers* **15**, 1513 (1976).
21. W. H. Moore and S. Krimm, *ibid.*, p. 2465.
22. J. F. Rabolt, W. H. Moore, S. Krimm, *Macromolecules* **10**, 1065 (1977).
23. B. G. Frushour, P. C. Painter, J. L. Koenig, *J. Macromol. Sci.* **C15**, 29 (1976).
24. T. G. Spiro and B. P. Gaber, *Annu. Rev. Biochem.* **46**, 553 (1977).
25. M. Inouye and S. Halegoua, *CRC Crit. Rev. Biochem.* **7**, 339 (1980); D. F. Steiner, P. S. Quinn, S. J. Chan, J. Marsh, H. S. Tager, *Ann. N.Y. Acad. Sci.* **343**, 1 (1980); B. M. Austen and D. H. Ridd, *Biochem. Soc. Symp.* **46**, 235 (1981); D. M. Engelman and T. A. Steitz, *Cell* **23**, 411 (1981); M. Rosenblatt, N. V. Beaudette, G. D. Fasman, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3983 (1980).
26. G. von Heijne, *J. Mol. Biol.* **184**, 99 (1985).
27. S. Hayashi, S.-Y. Chang, S. Chang, C.-Z. Giam, H. C. Wu, *J. Biol. Chem.* **260**, 5753 (1985); J. Coleman, M. Inukai, M. Inouye, *Cell* **43**, 351 (1985); B. Dobberstein, J. Lipp, M. Th. Haeuptle, I. Ibrahimi, *Microbiology*, in press.

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