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Mechanisms of Membrane Assembly: General Lessons from the Study of M13 Coat Protein and *Escherichia coli* Leader Peptidase†

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Each living cell is divided into multiple compartments that separate and organize the varied cellular functions. Isolation of organelles has revealed that each compartment, which may be a membrane or an aqueous space bounded by a membrane, has its own unique complement of "permanent resident" proteins. Even so-called simple bacteria have two to four compartments; for *Escherichia coli*, these are the cytoplasm, inner or plasma membrane, periplasm, and outer membrane. Eukaryotic cells, from yeast to man, have over 20 distinct compartments. Nevertheless, almost all protein synthesis begins in the cytoplasm, and noncytoplasmic proteins must be targeted to the appropriate organelle and insert into, or entirely across, one or even two membranes. This problem of protein targeting and membrane translocation has attracted increasing attention in the last 15 years. Since its essential features are present in all living cells, the problem can be best studied in microorganisms. In the true reductionist spirit, my co-workers and I have studied this problem in *E. coli*, and our focus has been on a particularly simple membrane protein, M13 coat protein.

M13 coat protein spans the plasma membrane of virus-infected *E. coli* (Wickner, 1975; Ohkawa & Webster, 1981). Its small size (50 residues) and ease of isolation from the virus in gram quantities led to its being the first integral membrane protein to be sequenced (Asbeck et al., 1969). It is comprised of an acidic, N-terminal domain of 20 residues that faces the periplasm, 20 entirely apolar residues that span the plasma membrane, and a C-terminal, basic cytoplasmic region of 10 residues. Coat protein is probably the simplest integral membrane protein, yet its structure suggests fundamental questions of membrane assembly. How does the polar amino terminus cross the bilayer? Since the purified protein is not water soluble, how does it avoid aggregation in the cytoplasm after its synthesis is complete? It is made with a typical N-terminal leader peptide; what is the role of this leader region in membrane insertion? During the last 14 years, these questions have guided studies of its membrane assembly. These studies have also led to an intensive investigation of leader peptidase, first for its catalytic properties and then for its own complex membrane assembly. Studies of coat protein biosynthesis (summarized in Figure 1) have yielded three major findings: (1) Energy is required for membrane assembly. (2) Membrane assembly is not coupled to polypeptide chain growth. (3) The leader peptide is vital for membrane assembly but so are the structural features of the mature protein. Examination of the membrane assembly of leader peptidase has shown that major conformational change accompanies translocation, and it has revealed new and unexpected topogenic elements throughout its structure. This review will summarize our current knowledge of the membrane assembly of these proteins and attempt to place them in the perspective of studies of membrane assembly and protein secretion.

The first clue as to the membrane assembly of coat protein was the discovery that it is made as a precursor, termed procoat, with a 23-residue N-terminal leader peptide (Sugimoto et al., 1977; Konings et al., 1975). The leader peptide is basic and apolar, as are the leader peptides of essentially all proteins targeted to the bacterial cell surface and the eukaryotic rough endoplasmic reticulum (von Heijne, 1983; Perlman & Halvorson, 1983). In vitro studies of the assembly of procoat into inverted bacterial inner membrane vesicles (Chang et al., 1978; Mandel & Wickner, 1979) showed that membrane-bound leader peptidase removes the leader peptide, which is then rapidly hydrolyzed. This assay of procoat cleavage allowed the isolation of leader peptidase (Zwizinski & Wickner, 1980; Wolfe et al., 1982) and its gene (Date & Wickner, 1981). In vitro assembly studies initially suggested that procoat could not assemble into the plasma membrane posttranslationally (Chang et al., 1979); however, procoat was later shown to have this capacity (Goodman et al., 1981). The difference between these studies was explained by the obser-

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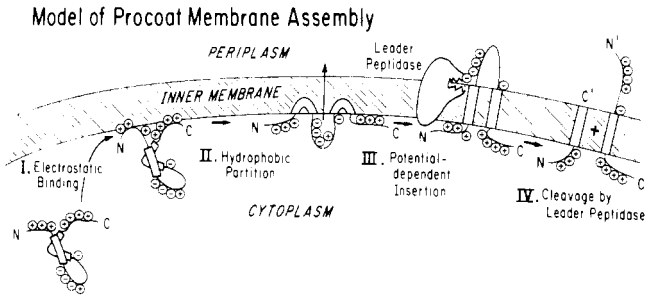

FIGURE 1: Membrane assembly of M13 coat protein [adapted from Kuhn et al. (1986a,b)]. Rectangles represent hydrophobic regions of procoat and coat.

vation that procoat aggregates and denatures during prolonged incubation without membranes. In vivo studies, in which M13-infected cells were pulse-labeled with radioactive amino acids to monitor the fate of pulse-labeled procoat, showed that procoat was first completely synthesis and then assembled posttranslationally across the plasma membrane (Ito et al., 1979, 1980; Date & Wickner, 1980). This fortuitous kinetic separation between synthesis and membrane assembly allowed a separate examination of the energy requirements of the membrane assembly step. The membrane electrochemical potential was found to be required for translocation of the central, acidic region of procoat across the membrane (Date et al., 1980a,b). This requirement for a membrane potential was later shown to be shared by essentially all exported bacterial proteins (Enequist et al., 1981; Daniels et al., 1981) as well as by proteins imported into the inner mitochondrial compartments (Hallermayer & Neupert, 1976; Daum et al., 1982). The functional of the potential in protein translocation across membrane is, however, unknown.

The in vitro assembly of procoat into inverted plasma membrane vesicles prompted the isolation of procoat (Silver et al., 1981; Zwizinski & Wickner, 1982) and leader peptidase and led to the reconstitution of this assembly event (Watts et al., 1981). Procoat has been shown to assemble into liposomes without the aid of any integral membrane proteins (Ohno-Iwashita & Wickner, 1983; Geller & Wickner, 1985). However, it is clear that other presecretory proteins will not by themselves assemble into liposomes. In vivo studies reinforced the idea that procoat membrane assembly is particularly simple. Conditional-lethal mutants in *sec* (for *sec*retion) genes affect the in vivo export of many bacterial proteins but do not affect the membrane assembly of procoat (Wolfe et al., 1985). What is special about procoat? To test whether the leader peptide of procoat might be special, we exchanged the leader peptide regions between pro-OmpA, which requires *sec*-encoded proteins for its export, and procoat, which does not. This study revealed that it is the mature sequence rather than the leader that specifies *sec*-protein dependence (Kuhn et al., 1987).

Features of the mature, coat protein region of procoat have as important a role in its membrane assembly as the leader peptide. Mutants that change the acidic residue at +2 to an uncharged residue slow membrane assembly (Russel & Model, 1981) yet allow it to proceed in the absence of a membrane electrochemical potential (Zimmermann et al., 1982). Mutants that interrupt the apolar character of the membrane-spanning region of coat, residues 20–40, by inserting an arginyl residue at position +26 or +30, completely block the membrane insertion event (Kuhn et al., 1987). We refer to this function as a "hydrophobic helper"; it is an apolar region of the protein, which is not part of a leader peptide yet is needed for protein translocation across the membrane. Even the basic

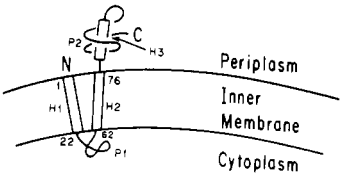

FIGURE 2: Orientation of leader peptidase across the plasma membrane of *E. coli* [adapted from Moore and Miura (1987)].

character of the very carboxy terminus of procoat, a polar region that never leaves the cytoplasm during membrane assembly, is essential for the translocation of other parts of the molecule (Kuhn et al., 1986b). Deletion of the basic charges in this region blocks procoat membrane assembly, and restoration of charges, albeit in a different order, restores translocation.

These studies show that procoat has a typical leader peptide and that the mature coat region has several features which are also vital for assembly. To determine whether this might be unique to procoat, or may be shared with larger proteins that are *sec*-dependent, we have analyzed the membrane assembly properties of leader peptidase. This enzyme is a 37 000-dalton integral protein of the plasma membrane. Structural and genetic studies (Wolfe et al., 1983; Moore & Miura, 1987) have shown that it spans the membrane with the topology shown in Figure 2. Its membrane assembly requires the membrane electrochemical potential and the products of *sec* genes A and Y (Wolfe et al., 1984). As with procoat and pro-OmpA, uncouplers could be used to divide the membrane assembly of leader peptidase into two steps (Wolfe et al., 1984). In the first step, leader peptidase is synthesized and binds to the inner surface of the plasma membrane. At this point, it is in a relatively open or unfolded conformation, as assayed by protease digestion in lysates. The second step, when the uncoupler is removed and the cells regenerate a potential, is the translocation of leader peptidase across the membrane and its folding into a relatively protease-resistant conformation. Recent studies have shown that an open, unfolded conformation is needed for other proteins to assemble across the bacterial and mitochondrial membranes as well (Randall & Hardy, 1986; Eilers & Schatz, 1986).

Since leader peptidase is much larger than procoat, and is *sec*-dependent, we hoped that a study of its membrane assembly might reveal different or additional themes to those found with procoat. This has largely been achieved through mutational analysis of leader peptidase. The second apolar region of leader peptidase (H2 in Figure 2) is an internal, uncleaved signal sequence (Dalbey & Wickner, 1987). When it is deleted, the membrane assembly of leader peptidase is blocked (Dalbey & Wickner, 1986). When H2 is genetically joined to OmpA and M13 coat protein, which have been deprived of their own normal leader peptides, it can promote their translocation across the membrane (Dalbey & Wickner, 1987). The deletion of other large blocks of residues flanking H2, e.g., residues 4–50 (H1 and P1), 82–98 (H3), 141–222 (the first half of P2), or 222–323 (the second half of P2), has little effect on the membrane assembly rate or yield of leader peptidase (Dalbey & Wickner, 1985, 1986). It is commonly assumed that regions which can be deleted without affecting membrane assembly have little or no role in the assembly process (Freudl et al., 1987). However, a more detailed analysis has shown this not to be true for leader peptidase.

Deletion of residues 2–22, which removes the first apolar region (H1 in Figure 2), completely prevents the translocation of the large C-terminal domain of leader peptidase (von Heijne et al., 1987). This region is a second example, in addition to

that in coat protein cited above, of a hydrophobic helper, a hydrophobic sequence that is not a leader or signal region but is essential for translocation. However, the requirement for the hydrophobic helper H1 is bypassed when the cytoplasmic domain (P1 in Figure 2) is also deleted (Dalbey & Wickner, 1986). We refer to this highly charged, cytoplasmic region as a "translocation poison" sequence. It is not itself essential for translocation in a positive sense; translocation of the C-terminal domain of leader peptidase is normal when the poison sequence is deleted. However, in the absence of the hydrophobic helper (residues 2–22), the translocation poison sequence (residues 30–50) blocks the action of the internal, uncleaved signal region, H2, which follows it. A poison sequence can also prevent translocation when the leader or signal region precedes the poison sequence (von Heijne et al., 1988). Why would such a region be retained during the evolution of leader peptidase? Although deletion of the poison sequence allows normal membrane assembly of leader peptidase, the assembled protein is catalytically inactive. This raises yet another interesting question: How do structural changes in the cytoplasmic domain of leader peptidase affect the catalytic site, which is in the periplasmic domain of the enzyme? Answers to this question might provide insights about other proteins, such as mammalian cell-surface receptors, which transmit information across the bilayer via a single stretch of polypeptide chain.

Even the large, periplasmic region of leader peptidase has features relevant to its own membrane translocation. When the major hydrophobic helper (H1) and the poison sequence are deleted, the periplasmic apolar region H3 becomes important for membrane assembly (Dalbey & Wickner, 1987). In the presence of H1, this region is not needed and is assumed to have only a minor role in assembly; it is therefore termed the minor hydrophobic helper. Finally, a large deletion, which removes residues 141–323 of the protein, prevents its translocation, while assembly is normal if either residues 141–222 or residues 222–323 are singly deleted (Dalbey et al., 1985). This suggests that the size of the protein (rather than a specific sequence near the carboxy terminus) is critical to its membrane assembly. Such a size requirement for export was first shown by Linda Randall in elegant studies of the export of pre-maltose binding protein (Randall, 1983). This protein must reach a "critical molecular weight" before it is translocated across the plasma membrane. The C-terminal portion of leader peptidase is therefore referred to as the critical molecular weight domain.

Comparison of the assembly of M13 procoat and *E. coli* leader peptidase into the plasma membrane shows several salient similarities. Neither assembly reaction is coupled to polypeptide chain growth, but both require an energy imput in the form of a membrane electrochemical potential. Each requires the function of a leader peptide; in the case of procoat, it is N-terminal and cleaved, while that of leader peptidase remains with the mature protein. In addition to the leader, other parts of each protein have equally vital roles in the membrane assembly process. The mechanistic roles of individual regions—leader (signal) sequences, hydrophobic helpers, poison sequence, etc.—are not known and await further studies.

An Emerging Consensus Mechanism for Protein Translocation across Membranes. Progress in our understanding the membrane assembly of these proteins has required that energies be focused for years in a rather narrow area. It is relevant to ask how this effort, focused on procoat and leader peptidase, relates to membrane assembly and protein translocation events in other organelles and organisms. As recently as 2 years ago, it appeared that distinctly different mechanisms governed protein assembly into the eukaryotic endoplasmic reticulum, into mitochondria, and into the bacterial cell surface (Wickner & Lodish, 1985). However, recent results point to a more satisfying unity (Zimmermann & Meyer, 1986). It now appears that the major themes of procoat and leader peptidase membrane assembly—(1) translocation not coupled to protein synthesis, (2) required energy input, (3) conformational change, as proposed in the membrane trigger hypothesis (Wickner, 1979), and (4) assembly information in the mature sequence—are also applicable to protein assembly across the membranes of other organelles and organisms.

Timing of Translocation. It is now clear that all translocation-competent membranes can function posttranslationally, or at least after the polypeptide has achieved a substantial size. This has long been appreciated for protein uptake into mitochondria (Harmey et al., 1977), nuclei (Dingwall et al., 1982), and chloroplasts (Chua & Schmidt, 1978). Bacterial protein export has been clearly shown to proceed posttranslationally. This was shown in early studies by Ito et al. (1977), by the studies of M13 coat protein biogenesis cited above, and by studies of β-lactamase (Koshland & Botstein, 1980). Uncouplers were also used to create two-step export of procoat (Date et al., 1980b) and pro-OmpA (Zimmermann & Wickner, 1983). Randall's analysis of nascent chains (Randall, 1983) clearly showed that translocation, whether occurring late in the growth of a polypeptide chain or posttranslationally, was not coupled in any way to ongoing protein synthesis. Recently, in vitro translocation across bacterial inner membrane vesicles has also been shown to proceed posttranslationally (Muller & Blobel, 1984; Chen et al., 1985) and has been reconstituted with highly purified pro-OmpA (Crooke & Wickner, 1987). However, until recently the similarity of bacterial and eukaryotic leader peptides and the strong belief that translocation into endoplasmic reticulum was firmly coupled to translation led many investigators to believe that bacterial export must be coupled to translation.

Although posttranslational translocation into the endoplasmic reticulum was first reported for M13 procoat (Watts et al., 1983), it has recently become apparent for other proteins as well. A receptor mechanism has been described for proteins targeted to the endoplasmic reticulum (Walter et al., 1984). This receptor consists of signal recognition particle (SRP), a peripheral membrane protein, and docking protein, its integral membrane protein receptor. In wheat germ extracts, SRP will bind to nascent chains of presecretory proteins and arrest their growth. When the polysome/SRP complex encounters the membrane-bound docking protein, the SRP is displaced, polypeptide growth resumes, and translocation can occur. It was suggested that this not only targets proteins to the endoplasmic reticulum but also prevents the synthesis of presecretory proteins in the cytoplasm. This work (Walter & Blobel, 1981) was strong evidence in support of the signal hypothesis. In the signal hypothesis, the problem of translocating folded, hydrated, polar regions of a polypeptide through a membrane is solved by extruding the nascent chain across the membrane as it emerged from the ribosome (Blobel & Dobberstein, 1975; Blobel, 1980), perhaps before it has a chance to fold. However, recent results have significantly revised our view of the role of SRP. Walter and associates have shown that SRP which is reconstituted without its 9/14K subunit is fully active in protein translocation yet will not induce translation arrest (Siegel & Walter, 1986). Several presecretory proteins do not show a translation arrest at all; SRP may, at most, slow their synthesis. Meyer has shown that up to 18 000 daltons of

immunoglobulin light chain can be synthesized in the absence of SRP and then translocated upon the addition of SRP and microsomal membranes (Meyer, 1985). The glucose transporter (Mueckler & Lodish, 1986) and pre-human placental lactogen (Caulfield et al., 1986) have been shown to translocate entirely posttranslationally in an SRP-dependent manner, while small proteins such as prepromellitin and M13 procoat translocate posttranslationally in an SRP-independent fashion (Zimmermann & Mollay, 1986). Perara et al. (1986) showed that ribosomes are essential for the translocation of rhodopsin or a β-lactamase/globin fusion protein into dog microsomes, but polypeptide chain growth is not needed. They suggested that the posttranslational translocation of these large polypeptide chains uses the ribosome either to participate in membrane recognition or to keep the polypeptide chain from misfolding.

The most striking evidence for posttranslational translocation into endoplasmic reticulum comes from studies of *Saccharomyces cerevisiae*. Recently, three groups have developed extracts that will synthesize yeast preproteins and translocate them into yeast microsomal membranes (Rothblatt & Meyer, 1986; Waters & Blobel, 1986; Hansen et al., 1986). Prepro-α-factor, preprocarboxypeptidase Y, and a fragment of pre-invertase assemble posttranslationally into these vesicles. In addition, there is evidence that proteins can assemble posttranslationally into yeast endoplasmic reticulum in vivo. Deshaies and Schekman (1987) have shown that prepro-α-factor will enter the endoplasmic reticulum posttranslationally in a *sec*61 strain at the permissive temperature. Blachly-Dyson and Stevens (1987) and Lolle and Bussey (1986) have shown that preprocarboxypeptidase Y and prepro-killer toxin can enter the endoplasmic reticulum posttranslationally.

Energetics. A second general theme of protein transit across membranes is that energy is required. Bacteria require both ATP and the membrane electrochemical potential for protein translocation (Chen & Tai, 1985; Geller et al., 1986), though the role of each energy source has remained elusive. Mitochondria also require both ATP and an electrochemical potential. Elegant studies in the laboratories of Neupert (Pfanner et al., 1987) and Schatz (Eilers et al., 1987; Verner & Schatz, 1987) have shown that ATP is needed to maintain premitochondrial proteins in an unfolded state, while the electrochemical potential is required for transit across the inner membrane. ATP is also required for protein import into chloroplasts (Grossman et al., 1980), nuclei (Newmeyer et al., 1986), and endoplasmic reticulum (Hansen et al., 1986; Rothblatt & Meyer, 1986; Waters & Blobel, 1986; Wiech et al., 1987).

The *conformation* of preproteins may be vital to their translocation. The membrane trigger hypothesis (Wickner, 1979) proposed that proteins would have to undergo a conformational change to pass from the polar cytoplasm into the apolar environment of a membrane. Leader peptidase was shown to undergo such a major conformational change during its membrane assembly, from an unfolded form that is readily digested by proteases to a folded, protease-resistant structure (Wolfe et al., 1984). Pre-maltose binding protein undergoes a similar conformational transition (Randall & Hardy, 1986). Mutants in the leader peptide of pre-maltose binding protein that inhibits its membrane transit promote a premature transition to the compact, folded structure, which is incompetent for assembly. In vitro uptake of pro-OmpA into inner membrane vesicles requires trigger factor, a protein that complexes with the pro-OmpA and maintains it in an unfolded conformation (Crooke & Wickner, 1987). Similarly, the uptake of a fusion protein between a cytochrome oxidase leader peptide and dihydrofolate reductase into mitochondria requires an unfolded conformation. This uptake is blocked when the folded state is stabilized by binding methotrexate (Eilers & Schatz, 1986). ATP is required for unfolding the protein prior to uptake, and this ATP requirement is bypassed when the protein is unfolded (Verner & Schatz, 1987). A cytosolic factor is thought to be involved in the ATP-dependent unfolded state of mitochondrial proteins. It is tempting to speculate (Rothman & Kornberg, 1986) that the role of ATP in each translocation system is to maintain an open conformation of the protein prior to translocation and that a cytosolic trigger factor may participate. This may be one of the roles of SRP for the endoplasmic reticulum.

Assembly Elements. Finally, in addition to the leader region, the mature region of proteins contains information that is important for bacterial protein secretion and membrane assembly. In addition to studies of procoat and leader peptidase, a mutant in the mature region of pre-maltose binding protein (Cover et al., 1987) can suppress or bypass the effects of a leader sequence mutation, and a region in the mature part of lamB protein is essential for the translocation of that protein across the plasma membrane (Rasmussen & Silhavy, 1987). The match between leader and mature sequences can determine the efficiency of export (Kuhn et al., 1987). Cloning eukaryotic presecretory proteins into *E. coli* does not guarantee their secretion, even when placed behind a normal bacterial leader sequence. Further studies will be needed to determine how the mature and leader sequences influence bacterial protein export and whether the mature regions of protein affect translocation rates in eukaryotic systems (Kohorn et al., 1987).

This brief review shows that the important lessons learned from the study of procoat and leader peptidase membrane assembly—translocation not coupled to translation, energy requirements, importance of conformation to membrane assembly, and information throughout the protein sequence—have their counterparts in other proteins, organelles, and organisms. However, we do not know the role of energy in protein translocation or whether translocation proceeds directly through the lipid bilayer or via a pore or transport system. Our current understanding of membrane assembly and secretion resembles a set of "boundary conditions", fragments of knowledge that will eventually have to be fit into a coherent picture. I suspect that the key to this picture, whether for bacteria, for endoplasmic reticulum, or for mitochondria, will be the solubilization and reconstitution of the relevant proteins in a translocation-competent membrane.

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REFERENCES

Ainger, K. J., & Meyer, D. I. (1986) *EMBO J. 5*, 951–955.
Asbeck, v. F., Beyreuther, K., Kohler, H., von Wettstein, G., & Braunitzer, G. (1969) *Hoppe-Seyler's Z. Physiol. Chem. 350*, 1047–1066.
Blachly-Dyson, E., & Stevens, T. H. (1987) *J. Cell Biol. 104*, 1183–1191.
Blobel, G. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1496–1500.
Blobel, G., & Dobberstein, B. (1975) *J. Cell Biol. 67*, 852–862.
Caulfield, M. P., Duong, L. T., & Rosenblatt, M. (1986) *J. Biol. Chem. 261*, 10953–10956.
Chang, C. N., Blobel, G., & Model, P. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 361–365.

Chen, L., & Tai, P. C. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4384–4388.
Chen, L., Rhoads, D., & Tai, P. C. (1985) *J. Bacteriol. 161*, 973–980.
Chua, N.-H., & Schmidt, G. W. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 6110–6114.
Cover, W. H., Ryan, P., Bassford, P. J., Jr., Walsh, K. A., Bollinger, J., & Randall, L. L. (1987) *J. Bacteriol. 169*, 1794–1800.
Crooke, E., & Wickner, W. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5216–5220.
Dalbey, R. E., & Wickner, W. (1986) *J. Biol. Chem. 261*, 13844–13849.
Dalbey, R. E., & Wickner, W. (1987) *Science (Washington, D.C.) 235*, 783–787.
Dalbey, R. E., Kuhn, A., & Wickner, W. (1987) *J. Biol. Chem. 262*, 13241–13245.
Daniels, C. J., Bole, D. G., Quay, S. C., & Oxender, D. L. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 5396–5400.
Date, T., & Wickner, W. (1980) *J. Virol. 37*, 1087–1089.
Date, T., & Wickner, W. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 6106–6110.
Date, T., Zwizinski, C., Ludmerer, S., & Wickner, W. (1980a) *Proc. Natl. Acad. Sci. U.S.A. 77*, 827–831.
Date, T., Goodman, J. M., & Wickner, W. (1980b) *Proc. Natl. Acad. Sci. U.S.A. 77*, 4669–4673.
Daum, G., Gasser, S. M., & Schatz, G. (1982) *J. Biol. Chem. 257*, 13075–13080.
Deshaies, R. J., & Schekman, R. (1987) *J. Cell Biol. 105*, 633–645.
Dingwall, C., Sharnick, S. V., & Laskey, R. A. (1982) *Cell (Cambridge, Mass.) 30*, 449–456.
Eilers, M., & Schatz, G. (1986) *Nature (London) 322*, 228–232.
Eilers, M., Oppliger, W., & Schatz, G. (1987) *EMBO J. 6*, 1073–1077.
Enequist, H. G., Hirst, T. R., Harayama, S., Hardy, S. J. S., & Randall, L. L. (1981) *Eur. J. Biochem. 116*, 227–233.
Freudl, R., Schwarz, H., Degen, M., & Henning, U. (1987) *J. Bacteriol. 169*, 66–71.
Geller, B. L., & Wickner, W. (1985) *J. Biol. Chem. 260*, 13281–13285.
Geller, B. L., Movva, N. R., & Wickner, W. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 4219–4222.
Goodman, J. M., Watts, C., & Wickner, W. (1981) *Cell (Cambridge, Mass.) 24*, 437–442.
Grossman, A., Bartlett, S., & Chua, N.-H. (1980) *Nature (London) 285*, 625–628.
Hallermayer, G., & Neupert, W. (1976) in *Genetics and Biogenesis of Chloroplasts and Mitochondria* (Buchler, Th., Ed.) Elsevier, Amsterdam.
Hansen, W., Garcia, P. D., & Walter, P. (1986) *Cell (Cambridge, Mass.) 45*, 397–406.
Harmey, M. A., Hallermayer, G., Korb, H., & Neupert, W. (1977) *Eur. J. Biochem. 81*, 533–538.
Ito, K., Sato, T., & Yura, T. (1977) *Cell (Cambridge, Mass.) 11*, 551–559.
Ito, K., Mandel, G., & Wickner, W. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1199–1203.
Ito, K., Date, T., & Wickner, W. (1980) *J. Biol. Chem. 255*, 2123–2130.
Kohorn, B. D., Harel, E., Chitnis, P. R., Thornber, P., & Tobin, E. M. (1987) *J. Cell Biol. 102*, 972–981.
Konings, R. N. H., Hulsebos, T., & van den Hondel, C. A. (1975) *J. Virol. 15*, 570–584.
Koshland, D., & Botstein, D. (1980) *Cell (Cambridge, Mass.) 20*, 749–760.
Kuhn, A., Kreil, G., & Wickner, W. (1986a) *EMBO J. 5*, 3681–3685.
Kuhn, A., Wickner, W., & Kreil, G. (1986b) *Nature (London) 322*, 335–339.
Kuhn, A., Kreil, G., & Wickner, W. (1987) *EMBO J. 6*, 501–505.
Lolle, S. J., & Bussey, H. (1986) *Mol. Cell. Biol. 6*, 4274–4280.
Mandel, G., & Wickner, W. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 236–240.
Meyer, D. I. (1985) *EMBO J. 4*, 2031–2033.
Moore, K. E., & Miura, S. (1987) *J. Biol. Chem. 262*, 88106–88113.
Mueckler, M., & Lodish, H. (1986) *Nature (London) 322*, 549–552.
Muller, G., & Zimmermann, R. (1987) *EMBO J. 6*, 2099–2107.
Muller, M., & Blobel, G. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 7737–7741.
Nakashima, Y., & Konigsberg, W. (1974) *J. Mol. Biol. 88*, 598–600.
Newmeyer, D. D., Lucocq, J. M., Burglin, T. R., & DeRobertis, E. M. (1986) *EMBO J. 5*, 501–510.
Ohkawa, I., & Webster, R. E. (1981) *J. Biol. Chem. 256*, 9951–9958.
Ohno-Iwashita, Y., & Wickner, W. (1983) *J. Biol. Chem. 258*, 1895–1900.
Perara, E., Rothman, R. E., & Lingappa, V. R. (1986) *Science (Washington, D.C.) 232*, 348–352.
Perlman, D., & Halvorson, H. O. (1983) *J. Mol. Biol. 167*, 391–399.
Pfanner, N., Tropschug, M., & Neupert, W. (1987) *Cell (Cambridge, Mass.) 49*, 815–823.
Randall, L. L., & Hardy, S. J. S. (1986) *Cell (Cambridge, Mass.) 46*, 921–928.
Rasmussen, B. A., & Silhavy, T. J. (1987) *Genes Dev. 1*, 185–196.
Rothblatt, J. A., & Meyer, D. I. (1986) *EMBO J. 5*, 1031–1036.
Rothman, J., & Kornberg, R. D. (1986) *Nature (London) 322*, 209–210.
Rottier, P., Armstrong, J., & Meyer, D. I. (1985) *J. Biol. Chem. 260*, 4648–4652.
Russel, M., & Model, P. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1717–1721.
Schaller, H., & Takanami, M. (1978) in *Single-Stranded DNA Phages* (Denhardt, D. T., Dressler, D., & Ray, D. S., Eds.) Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.
Schlenstedt, G., & Zimmermann, R. (1987) *EMBO J. 6*, 699–703.
Siegel, V., & Walter, P. (1986) *Nature (London) 320*, 81–84.
Silver, P., Watts, C., & Wickner, W. (1981) *Cell (Cambridge, Mass.) 25*, 341–345.
Sugimoto, K., Sugisaki, H., & Takanami, M. (1977) *J. Mol. Biol. 110*, 487–507.
Verner, K., & Schatz, G. (1987) *EMBO J. 6*, 2449–2456.
von Heijne, G. (1983) *Eur. J. Biochem. 133*, 17–21.
von Heijne, G., Wickner, W., & Dalbey, R. E. (1988) *Proc. Natl. Acad. Sci. U.S.A.* (submitted for publication).
Walter, P., & Blobel, G. (1981) *J. Cell Biol. 91*, 551–556.
Walter, P., Gilmore, R., & Blobel, G. (1984) *Cell (Cambridge, Mass.) 38*, 5–8.

Waters, M. G., & Blobel, G. (1986) *J. Cell Biol. 102*, 1543-1550.
Watts, C., Silver, P., & Wickner, W. (1981) *Cell (Cambridge, Mass.) 25*, 347-353.
Watts, C., Wickner, W., & Zimmermann, R. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 2809-2813.
Wickner, W. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4749-4753.
Wickner, W. (1979) *Annu. Rev. Biochem. 48*, 23-45.
Wickner, W., & Lodish, H. (1985) *Science (Washington, D.C.) 230*, 400-407.
Wiech, H., Sagstetter, M., Muller, G., & Zimmermann, R. (1987) *EMBO J. 6*, 1011-1016.
Wolfe, P. B., Silver, P., & Wickner, W. (1982) *J. Biol. Chem. 257*, 7898-7902.
Wolfe, P. B., Wickner, W., & Goodman, J. M. (1983) *J. Biol. Chem. 258*, 12073-12080.
Wolfe, P. B., Rice, M., & Wickner, W. (1985) *J. Biol. Chem. 260*, 1836-1841.
Zimmermann, R., & Wickner, W. (1983) *J. Biol. Chem. 258*, 3920-3925.
Zimmermann, R., & Meyer, D. I. (1986) *Trends Biochem. Sci. (Pers. Ed.) 11*, 512-515.
Zimmermann, R., & Mollay, C. (1986) *J. Biol. Chem. 261*, 12889-12895.
Zimmermann, R., Watts, C., & Wickner, W. (1982) *J. Biol. Chem. 257*, 6529-6536.
Zwizinski, C., & Wickner, W. (1980) *J. Biol. Chem. 255*, 7973-7977.
Zwizinski, C., & Wickner, W. (1982) *EMBO J. 1*, 573-578.

Articles

A Description of Conformational Transitions in the Pribnow Box of the trp Promoter of *Escherichia coli*†

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ABSTRACT: Selective changes in the NMR parameters of the sequence of CGTACTAGTTAACTAGTACG, which corresponds to the trp operator of *Escherichia coli*, were observed as a function of temperature. The changes were localized to the sequence TTAA in the Pribnow box (underlined). Differential changes in chemical shift were analyzed in terms of a three-state model (states I, II, and III) to give the equilibrium constants, enthalpy changes, and populations. The midpoints of the first and second transitions were 9 and 30 °C, with enthalpy changes of 58 and 35 kcal/mol, respectively. Measurement of the spin-lattice and cross-relaxation rate constants at different temperatures allowed some structural conclusions to be drawn about the nature of the transitions. The line width of the H2 of A11 goes through a maximum at about 30 °C, indicating moderately fast exchange between the states. The rate constants for exchange at the midpoints were about 200 (I ↔ II) and 250 (II ↔ III) s^{-1}. Taking these findings into account, we propose a mechanism for the interaction between RNA polymerase and the promoter. This mechanism can explain the temperature dependence observed for the initiation of transcription.

The initiation of transcription by prokaryotic RNA polymerase is for many purposes adequately described by the two-step reaction scheme (Chamberlin, 1974) depicted in Scheme I, where R is RNA polymerase, P is the promoter, RPc is the initial "closed" complex which is thought to involve recognition of a sequence [consensus TTGACA (Rosenberg & Court, 1979; Hawley & McClure, 1983)] found near the −35 region, and RPo is the active "open" complex (Siebenlist, 1979) from which transcription occurs. It is generally thought that the isomerization of RPc to RPo occurs in the Pribnow box [consensus sequence TATAAT (Rosenberg & Court, 1979; Hawley & McClure, 1983)] located at −10. The rate constant of this process is relatively small, ranging from 0.01 to 1 s^{-1}, depending on the strength of the promoter. The formation of the open complex may involve a number of mechanisms which have not yet been fully elucidated but is probably a multistep process (Bertrand-Burgraff et al., 1984; Buc & McClure, 1985). The formation of the open complex is strongly dependent on the temperature, having an apparent midpoint in the range 15–25 °C, depending on the promoter (Chamberlin, 1974; Richardson, 1975; Dausse et al., 1976; Suzucki et al., 1976). Studies of the temperature dependence of the formation of the open complex have led to the conclusion that an intermediate state becomes significantly populated at low temperatures. This additional state should be inserted between RPc and RPo in Scheme I (Kadesh et al., 1981; Roe et al., 1984; Buc & McClure, 1985).

Scheme I

$$R + P \leftrightarrow RPc \leftrightarrow RPo \rightarrow \text{transcription}$$

We have been studying the structure and solution properties of the trp operator/promoter (sequence CGTACTAGTTAACTAGTACG) by NMR[1] (Lefèvre et al., 1985a,b;

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