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16. After the present work was completed, we became aware of a very recent publication on epitope mapping in protein antigens by R. Jemmerson and Y. Paterson [*Science* **232**, 1001 (1986)]. These investigators based their analysis of two epitopes of horse cytochrome c on a similar phenomenon, namely, on the different rate of proteolysis of peptide bonds of an epitope in free and antibody-bound cytochrome c. By this approach peptides contributing to the epitope could be revealed. The analysis of Jemmerson and Paterson is limited by the specificity of the protease and by the resistance of the antibody or Fab fragment to proteolysis. The focus of the present procedure is on individual residues of an epitope, the analysis being limited by the distribution of modifiable side chains. Thus, the two procedures complement each other and should, in combination with conventional cross-reactivity measurements, greatly contribute to a more comprehensive analysis of epitopes.
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Leader Peptidase of *Escherichia coli*: Critical Role of a Small Domain in Membrane Assembly

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Leader peptidase spans the *Escherichia coli* plasma membrane with its amino-terminal domain facing the cytoplasm and its carboxyl terminus facing the periplasm. It is made without a cleavable leader sequence. The three apolar domains near the amino terminus of the peptidase are candidates for internal "signal sequences" and they anchor the protein to the lipid bilayer. Oligonucleotide-directed deletion was used to show that only the second domain has an essential function in membrane assembly. While this second apolar domain is crucial for membrane assembly, its continued function when disrupted by arginine suggests that its apolar character per se is not its only important feature.

MANY MEMBRANE PROTEINS ARE synthesized without a cleavable, amino-terminal leader peptide (*1*). Their membrane assembly has been proposed (*2*) to require internal, uncleaved "signal sequences," short amphipathic domains which initiate the translocation of other regions of the protein. Bos *et al.* (*3*) have shown that a viral hemagglutinin contains such a sequence. It has also been suggested (*4*) that membrane proteins may form spontaneous insertion domains which integrate into the bilayer. The sequence and structural characteristics of such domains have yet to be determined.

Like many proteins of the inner membrane of *Escherichia coli*, leader peptidase has no cleaved leader peptide (*5*). This enzyme spans the plasma membrane, with a short domain near the amino terminus exposed to the cytoplasm and a large, polar carboxyl-terminal domain exposed to the periplasm (*5*). The active site of the enzyme is at the periplasmic membrane surface (*6*). However, its amino-terminal domain, which faces the cytoplasm, is essential for its in vitro catalytic activity (*7*). We have used drugs and mutants to study its membrane assembly in vivo. Leader peptidase requires both the electrochemical membrane potential (*8*) and the function of the *sec*A and *sec*Y genes (*9*) for membrane assembly. As with essentially all exported bacterial proteins, its membrane insertion is not coupled to ongoing polypeptide chain growth (*8*). Prior to membrane assembly, leader peptidase is in a conformation which is more readily degraded by protease than the final, transmembrane form (*8*). Deletion of 182 amino acids from the carboxyl-terminal end of the protein (*10*) blocks its membrane assembly.

Our working model of the orientation of leader peptidase across the plasma membrane (Fig. 1A) is based on the sequence of the protein (*5*) and on its topology (*11*). Early studies (*5*) revealed that trypsin can only remove approximately 60 residues from the amino terminus of leader peptidase in sealed, inverted plasma membrane vesicles. More recently (*11*), it was shown that all but the amino-terminal part of leader peptidase, including the first two apolar domains, is accessible to digestion by proteinases added to intact spheroplasts. To test which apolar domains of leader peptidase form an internal, uncleaved "signal," which are essential for stable anchoring to the membrane, and which affect catalytic activity, we have inserted arginyl residues into these regions, or deleted them entirely, by oligonucleotide-directed mutagenesis (Fig. 1B). These mutant leader peptidase genes are called "XR," where X is the amino acid residue which is replaced by arginine, or ΔX-Y, indicating deletion of residues X to Y. Of the ionizable amino acids, arginine was chosen because its pK_a (dissociation constant) is the furthest from neutrality. Each mutant leader peptidase gene was sequenced in M13mp8 and transferred into a plasmid where its expression was regulated by the *ara* promoter. The plasmids bearing these mutants under arabinose promoter regulation are termed pRDXR or pRDΔX-Y.

The membrane assembly of each mutant leader peptidase was assayed in intact cells. Addition of arabinose to *E. coli* pRD9R/HJM114 induces the synthesis of leader peptidase 9R. Synthesis was assayed by labeling cells with [^{35}S]methionine, immunoprecipitation with antiserum to leader peptidase, and SDS-PAGE (polyacrylamide gel electrophoresis) and fluorography (Fig. 2B, first lane). To determine whether leader peptidase 9R can assemble across the plasma membrane, labeled cells were treated with tris, sucrose, and EDTA to permeabilize the outer membrane, then incubated with trypsin. Leader peptidase was accessible to trypsin digestion (Fig. 2B), indicating that it had assembled across the plasma membrane. Most cell proteins (Fig. 2A) and, in particular, an abundant cytoplasmic marker protein (the amino-terminal fragment of ribulokinase) (Fig. 2B) remained inaccessible to digestion unless the inner membrane permeability barrier was abolished by ultrasound. This confirmed that the plasma membrane remained intact during these studies.

Three further criteria were used to ascertain that leader peptidase 9R assembles normally into the plasma membrane: (i) Disruption of the most apolar segment of the protein might have led to its complete export into the periplasm. To test this, cells (Fig. 3A, lane 4; total protein) were treated with tris, sucrose, and EDTA to permeabilize their outer membrane and release the periplasmic contents (Fig. 3A, lane 6). Leader peptidase 9R was entirely recovered with the treated cells (Fig. 3B, lane 5) and not with the periplasm (Fig. 3B, lane 6). (ii) Trypsin treatment of right-side-out inner membrane vesicles from such cells not only degraded the leader peptidase 9R (Fig. 4,

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lane 3), but left a protected fragment of approximately 11,000 daltons (arrowhead, lane 4). Detailed mapping of the topology of wild-type leader peptidase (*11*) has shown that this corresponds to the amino terminus of the enzyme (Fig. 4, lane 2), including all three of the apolar domains. This fragment is not seen in digests of membranes from cells that were not induced for leader peptidase synthesis, and it is not protected from protease digestion when the membranes are disrupted by detergent (*11*). (iii) Leader peptidase 9R was shown by immunoblotting to be stable (Fig. 5A), allowing assay of the enzyme activity in detergent extracts (Fig. 5B). In this experiment, radiochemically pure ^{35}S-labeled M13 procoat (*12*) was used as substrate. Leader peptidase cleaves procoat to coat plus leader peptide. The plasmid pRD8 expresses the wild-type leader peptidase under arabinose control. In a wild-type strain of *E. coli*, such as HJM114, induction of the pRD8 plasmid causes a substantial overproduction of the enzyme (*13*). Fortyfold diluted extracts of HJM114 with pRD8 catalyzed comparable cleavage of procoat as undiluted extracts of HJM114 without plasmid (Fig. 5B). Cell extracts containing leader peptidase 9R showed full enzyme specific activity (Fig. 5B). To assay the activity of leader peptidase 9R in intact cells, we have used the fact that overproduction of leader peptidase accelerates the conversion of M13 procoat to coat in vivo (*14*). Wild-type *E. coli* HJM114, bearing either no plasmid, pRD8 (expressing wild-type leader peptidase), or the plasmids described above, were infected with M13 am7 virus. Each culture was labeled for 15 seconds with [^{35}S]methionine, with subsequent addition of an excess of unlabeled methionine. Samples were harvested 5, 30, or 60 seconds after the addition of unlabeled methionine and analyzed by immunoprecipitation, SDS-PAGE, and fluorography. Although the conversion of procoat to coat is quite slow in HJM114 (Fig. 5C), it is rapid in cells overproducing wild-type leader peptidase. Leader peptidase 9R was as active in vivo as the wild-type enzyme. Taken together, these assays provide conclusive evidence that leader peptidase 9R assembles normally into the plasma membrane.

Similar experiments were performed to find out whether leader peptidase 68R (Fig. 6A) or leader peptidase 91R (Fig. 6B)

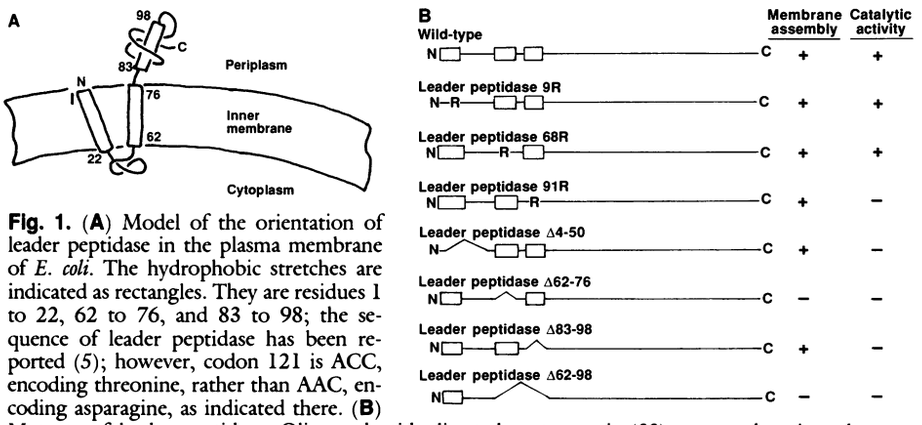

Fig. 1. (**A**) Model of the orientation of leader peptidase in the plasma membrane of *E. coli*. The hydrophobic stretches are indicated as rectangles. They are residues 1 to 22, 62 to 76, and 83 to 98; the sequence of leader peptidase has been reported (*5*); however, codon 121 is ACC, encoding threonine, rather than AAC, encoding asparagine, as indicated there. (**B**) Mutants of leader peptidase. Oligonucleotide-directed mutagenesis (*22*) was used to introduce an arginine into the apolar regions or to delete individual segments of leader peptidase. The seven mutagenic primers, and the changes they encoded, are: GCCCTGATTCGGGTGATTGCC, Leu^{9} to Arg; TCTGTTTTTCGGGTACTGGCT, Pro^{68} to Arg; TCAGGTTCGAGGATGCCGACT, Met ^{91} to Arg; GGCATGGCGAATAAAAAGGTT, deletion of residues 4 to 50; GGCTGGCTGGAACGTTCGTTT, deletion of 62 to 76; TTTATTTATGAAGATTTTATT, deletion of 83 to 98; GGCTGGCTGGAAGATTTTATT, deletion of 62 to 98. The mutant oligonucleotides as well as the universal primer were annealed to single-stranded M13mp8 DNA containing the leader peptidase gene. This template DNA was prepared from phage grown on an *ung*$^{-}$ host. This procedure (*23*) increased the efficiency of mutagenesis tenfold. The large fragment of *E. coli* DNA polymerase I and T4 ligase was used to make the viral DNA double stranded. After transformation into *E. coli* JM103 (*ung*$^{+}$), the phage was isolated from single plaques and its DNA was sequenced (*24*) to identify the mutants. For the isolation of deletion mutants, plaques were first screened by hybridization with the ^{32}P-labeled oligonucleotide. Digestion of plasmid DNA with Sma I and Sal I yielded a 1300-bp fragment containing the leader peptidase gene. This fragment, containing each desired mutation, was inserted into the pING-1 vector, which had been cleaved with Sma I and Sal I. The same protocol was followed to construct the plasmids pRD9R, pRD68R, pRD91R, and pRDΔ4-50, pRDΔ62-76, pRDΔ83-98, and pRDΔ62-98. The mutation at codon 68 in plasmid pRD68R was confirmed by showing that an Hpa II restriction site was lost. As was expected, the 163-bp Hpa II fragment of wild-type leader peptidase was missing and was replaced by a 180-bp fragment. Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs. The T4 kinase was obtained from Bethesda Research Laboratories. Bacterial alkaline phosphatase and the large fragment of DNA polymerase I were from Boehringer. Transformations were performed with calcium phosphate (*26*). All enzyme reactions and DNA preparations were performed as described (*27*).

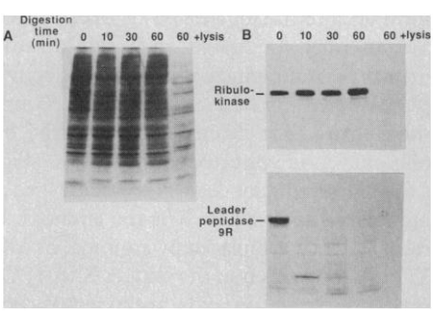

Fig. 2. Protease-accessibility of leader peptidase 9R. Cells (pRD9R/HJM114) were grown at 37°C to mid-log phase in M9 minimal medium (*28*) with 0.5% fructose and all amino acids (each at 50 μg/ml) except methionine and incubated with arabinose (0.2%) for 1 hour. Samples (0.75 ml) were labeled with 250 μCi of [^{35}S]methionine for 1 minute and then added to an equal volume of chilled buffer [40% (w/v) sucrose, 20 m*M* EDTA, 60 m*M* tris-HCl, *p*H 8.0]. Portions of the cell suspension were incubated at 0°C without further addition or with trypsin (250 μg/ml). Where indicated, cells were disrupted by ultrasound prior to incubation for 1 hour with trypsin. After the addition of trypsin inhibitor (2.5 mg/ml) and phenylmethylsulfonyl fluoride (PMSF) (5 m*M*), samples were acid-precipitated or immunoprecipitated (*29*), and then analyzed by SDS-PAGE and fluorography (*30*). (**A**) Acid precipitates. (**B**) Immunoprecipitation with antiserum to ribulokinase, a cytoplasmic marker, or antiserum to leader peptidase.

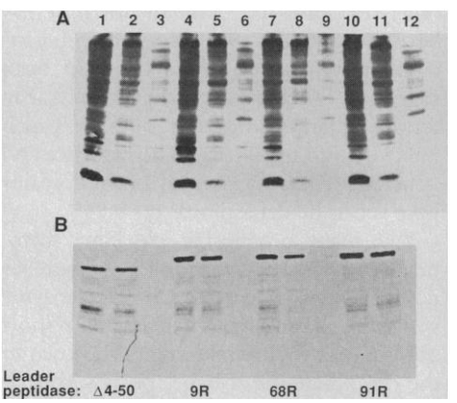

Fig. 3. Mutant leader peptidases are not secreted into the periplasm. Cultures of *E. coli* HJM114 with plasmid pRDΔ4-50, pRD9R, pRD68R, or pRD91R were grown at 37°C to $A_{600\ nm}$ of 0.2, then supplemented with arabinose (0.2%) for 1 hour. Portions (10 ml) were labeled with 250 μCi of [^{35}S]methionine for 1 minute, then cells were isolated by centrifugation (16,000*g*, 0°C, 5 minutes). Osmotic shock fluid and shocked cells were prepared as described (*31*). Total protein and immunoprecipitates prepared with antiserum to leader peptidase were analyzed by SDS-PAGE and fluorography. Samples were derived from unfractionated cell protein (lanes 1, 4, 7, and 10), shocked cells (lanes 2, 5, 8, and 11), and osmotic shock fluid (lanes 3, 6, 9, and 12).

assemble across the plasma membrane. Each was accessible to protease added to the periplasmic surface of the inner membrane, while ribulokinase was inaccessible to digestion in the same cells. Therefore, these mutants of leader peptidase were still capable of membrane assembly. Neither mutant protein was secreted into the periplasm (Fig. 3B). Proteolysis of inner membrane vesicles bearing leader peptidase 68R yielded a protected amino-terminal fragment of the same molecular size as the wild-type enzyme (Fig. 4, lane 6), while the corresponding fragment from leader peptidase 91R is slightly smaller. This is consistent with the fact (*11*) that the third hydrophobic region of leader peptidase is part of the periplasmic domain of the enzyme (Fig. 1A) and suggests that trypsin cleaves at this new arginine site. The lower intensity of the fluorographic image of the protected amino-terminal fragment in leader peptidase 91R and leader peptidase Δ83–98 (see below) is consistent with the fact that two methionines (at positions 91 and 92) of the four in the wild-type amino-terminal region are removed by these mutations. Leader peptidase 68R showed full enzyme activity in both in vivo and in vitro assays (Fig. 5), confirming that its membrane assembly was correct. In contrast, leader peptidase 91R was inactive (*15*), which again suggests that this region is part of the periplasmic active site (Fig. 1A).

Since systematic insertion of arginine into the three hydrophobic domains failed to prevent membrane assembly, we turned to oligonucleotide-directed deletion analysis. Residues 4 to 50 were deleted from the leader peptidase molecule. In addition to the first apolar segment of the protein, this deletion removed most of the polar, cytoplasmic domain. Nevertheless, leader peptidase Δ4–50 assembled across the plasma membrane and was accessible to digestion by external protease (Fig. 7A). Since 46 residues were deleted from the amino terminus of this mutant, an 11,000-dalton protected fragment was not observed after proteolysis of inner membrane vesicles (Fig. 4, lane 10). Although leader peptidase Δ4–50 showed little enzyme activity in detergent extracts, it promoted the in vivo conversion of procoat to coat (Fig. 5C) to a significant extent (though less than for the wild-type enzyme). This is in agreement with the inference from protease mapping (Fig. 7A) of the correct membrane assembly of leader peptidase Δ4–50. Together with the results shown in Fig. 2, these data show that the first apolar domain of leader peptidase is not essential for its membrane assembly. Similarly, deletion of residues 83–98, the third apolar domain, had no measurable effect on leader peptidase membrane assembly. Leader peptidase Δ83–98 was accessible to digestion by external protease (Fig. 7B) and a somewhat smaller amino-terminal peptide was protected from degradation by the plasma membrane (Fig. 4, lane 12), consistent with the deletion of the third hydrophobic region from this peptide. However, deletion of either residues 62 to 98 (Fig. 7C) or 62 to 76 (Fig. 7D) prevented leader peptidase membrane assembly, indicating that the second hydrophobic domain is important for the assembly process. In these experiments, outer membrane protein A (OmpA) was degraded. Since OmpA can only be digested by proteinase from the periplasmic side of the outer membrane, this confirmed that the outer membrane had been permeabilized in our assay procedure.

Our studies are applicable to questions of how a protein without a cleaved leader sequence assembles across a membrane, and what is required to anchor it to the membrane. A protein without a cleaved leader sequence may have an internal, uncleaved

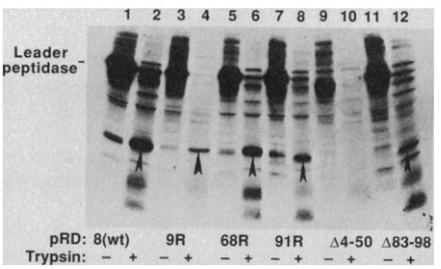

Fig. 4. Protected amino-terminal fragments of leader peptidase. Cultures (50 ml) of *E. coli* MC1061 bearing plasmids encoding wild-type or mutant leader peptidases were grown to early log phase, induced for leader peptidase synthesis with 0.2% arabinose for 2 hours, and then labeled with [^{35}S]methionine (250 μCi) for 2 minutes. Cells were converted to spheroplasts (*32*) and disrupted by ultrasound; the membrane fraction was isolated by ultracentrifugation (143,000*g*, 2 hours, 2°C). Membranes were further purified by isopycnic sucrose gradient centrifugation (*32*), and fractions were analyzed by SDS-PAGE and fluorography. The inner membrane fraction (20-μl samples) prepared from MC1061 containing pRD8, pRD9R, pRD68R, pRD91R, pRDΔ4-50, and pRDΔ83-98 were incubated for 1 hour at 37°C with either no further addition or with trypsin (200 μg/ml). After addition of soybean trypsin inhibitor (1.25 mg/ml) and PMSF (5 m*M*), samples were analyzed by SDS-PAGE and fluorography.

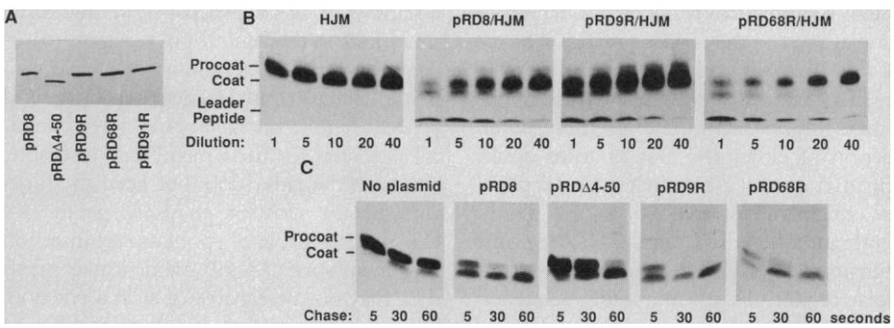

Fig. 5. Leader peptidase activity. (**A**) The chemical level of leader peptidase was assayed by immunoblotting (*33*) with an antiserum to leader peptidase. (**B**) Enzyme activity in crude extracts of *E. coli* HJM114 and this strain with plasmid pRD8, pRD9R, or pRD68R. Cultures (1 ml) were incubated at 37°C to $A_{600\ nm}$ of 0.25, then diluted with an equal volume of a solution containing 10 m*M* tris-HCl (*p*H 8.0), 10 m*M* EDTA, 1% Triton X-100, lysozyme (1 mg/ml), deoxyribonuclease (5 μg/ml), ribonuclease (1 μg/ml), and PMSF (5 m*M*). The lysates were incubated for 30 minutes at room temperature and either used undiluted or diluted 1:5, 1:10, 1:20, or 1:40 with 50 m*M* tris, *p*H 8.0, 0.1% Triton X-100. Leader peptidase activity was assayed by posttranslational conversion of procoat to coat protein plus leader peptide (*12*). (**C**) Effect of leader peptidase overproduction on the in vivo conversion of procoat to coat protein. HJM114 and this strain with plasmid pRD8, pRDΔ4-50, pRD9R, or pRD68R were grown to $A_{600\ nm}$ of 0.25, then infected with M13 amber 7 virus at a multiplicity of 100. After a 1-hour infection period, the cells were labeled with [^{35}S]methionine for 15 seconds, then mixed with nonradioactive methionine (0.5 mg/ml, final concentration) and incubated for 5, 30, or 60 seconds. Samples were lysed, immunoprecipitated with an antiserum to coat protein, and analyzed by SDS-PAGE and fluorography.

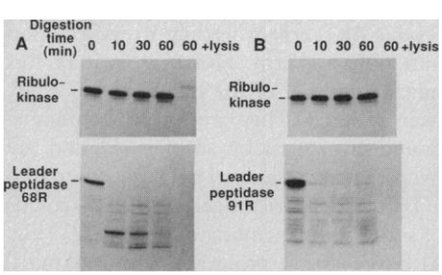

Fig. 6. Protease-mapping of leader peptidase 68R and 91R. *Escherichia coli* strain HJM114 containing pRD68R (**A**) or pRD91R (**B**) was grown, labeled, and analyzed as described in Fig. 2. The samples were immunoprecipitated with antiserum to either ribulokinase or leader peptidase.

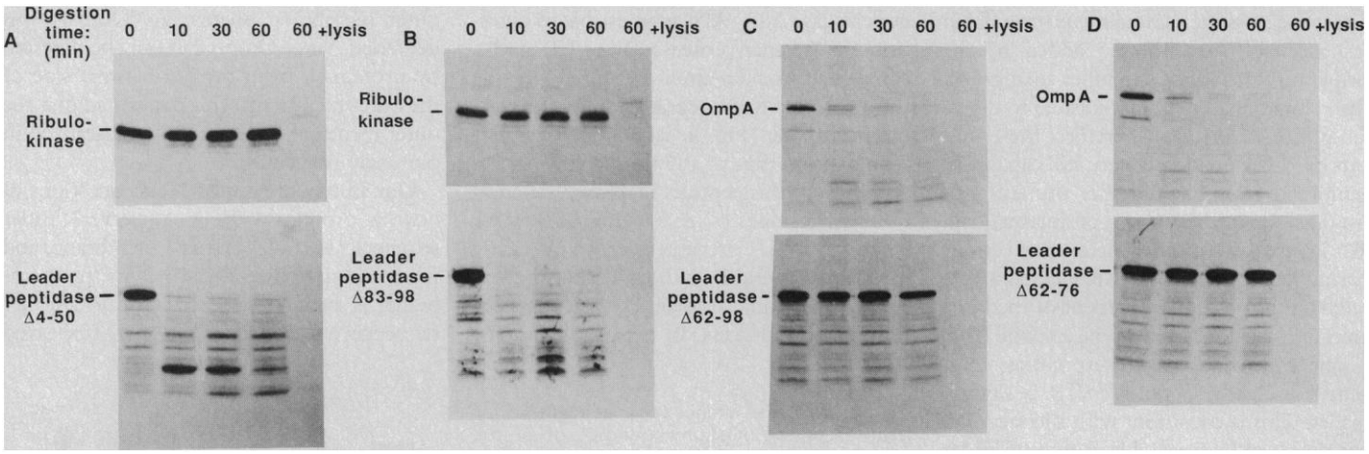

Fig. 7. Effects of deletions on leader peptidase membrane assembly. *Escherichia coli* strain HJM114 bearing plasmids (**A**) pRDΔ4-50, (**B**) pRDΔ83-98, (**C**) pRDΔ62-98, or (**D**) pRDΔ62-76 were grown, labeled with [^{35}S]methionine, and analyzed by protease accessibility as described in Fig. 2. In (A), the culture was incubated with nonradioactive methionine (500 μg/ml) for 5 minutes prior to incubation with tris, sucrose, and EDTA. Although immunoprecipitations were performed with antisera to leader peptidase, ribulokinase, and OmpA in each case, only the relevant control data are shown.

signal sequence (*2*). Such sequences would be expected to have a similar character to their cleavable, amino-terminal counterparts. One salient feature of cleaved leader sequences is an apolar domain of approximately 15 residues (*16*). Residues 62 to 76 are an obvious candidate for such a role in leader peptidase, especially since the region amino terminal to residues 62 to 76 is cytoplasmic and the residues carboxyl terminal to 62–76 are periplasmic. Furthermore, we show that the deletion of this apolar domain blocks membrane assembly, while deletion of either the first or third apolar domain does not. Nevertheless, leader peptidase efficiently assembles into the plasma membrane when this second apolar region is interrupted by arginine. Interruption of the apolar region of cleavable leader sequences by substitution of ionizable residues almost always dramatically inhibits protein translocation (*17*). We suggest that the uncleaved signal sequence (or sequences) of this protein may have structural features different from their cleaved counterparts. Clearly, mutations inactivate the enzymatic activity of leader peptidase more often than they prevent its membrane assembly for the following reasons. (i) Each of the more than 100 enzymatically inactive mutants of leader peptidase, generated by bisulfite mutagenesis, assembles into the plasma membrane (*15*). (ii) Deletion of 102 residues from the carboxyl terminus eliminates enzyme activity, but does not prevent membrane assembly (*10*). (iii) Although leader peptidase Δ83–98 and 91R are enzymatically inactive, they readily assemble into the membrane. Further studies are clearly necessary to define those features of the protein which govern its membrane assembly.

In addition to leader peptidase, our laboratory has also studied the membrane assembly properties of M13 coat protein. Although both are integral, transmembrane proteins of the plasma membrane of *E. coli*, our current studies underscore the differences in their membrane assembly properties. Insertion of arginine into the hydrophobic segment of either the leader or the membrane anchor prevents the membrane assembly of M13 procoat (*16*) and even mutations in the central polar region (*6*) or near the carboxyl terminus (*18*) have dramatic effects. Both leader peptidase and procoat require the membrane electrochemical potential for their membrane assembly, although the latter does not need the function of the *sec*A or *prl*A/*sec*Y genes (*9*). Procoat is capable of spontaneous insertion into membranes (*7, 19*), while leader peptidase may not be capable of such a reaction. It has been suggested (*4*) that different domains of large, multispanning membrane proteins may assemble by either catalyzed or spontaneous mechanisms.

Once assembled into the membrane, leader peptidase is firmly bound and behaves in all respects as an integral membrane protein. The amino-terminal domain is exposed to the cytoplasm, and the large, polar carboxyl-terminal domain is exposed to the periplasm (*5*). Protease mapping studies (*11*) indicate that residues 62 to 76 span the membrane. We expected that the stable binding of leader peptidase to the membrane should have been affected by insertion of arginine at residue 68, perhaps leading to its secretion into the periplasm. However, neither this mutant nor any of the others in our study were secreted. They are firmly membrane-bound at neutral or alkaline *p*H and are hydrophobic, as judged by partitioning into a Triton X114–rich phase (*15*). Proteolysis of sealed inner membrane vesicles (*11*) (Fig. 4) leaves similar protected, amino-terminal fragments in each mutant as in the wild-type protein. Other membrane proteins that have polar or ionizable residues within membrane spanning domains span the membrane at least six times, forming a polar channel that may conduct a polar solute (*20*). However, leader peptidase has no known transport function, is a monomeric protein, and does not span the membrane enough times to shield an arginine at residue 68. Adams and Rose (*21*) have shown that arginine may also be accommodated in the center of the membrane anchor of the vesicular stomatitis virus G protein. Perhaps several charged residues will be needed to effectively disrupt a membrane anchor domain. Computer modeling studies (*20*) suggest that insertion of arginine at residue 68 could shift the precise location of the membrane-spanning domain (*22*).

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Avian v-*myc* Replaces Chromosomal Translocation in Murine Plasmacytomagenesis

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Deregulation of c-*myc* expression in association with chromosomal translocations occurs in over 95% of murine plasmacytomas, rat immunocytomas, and human Burkitt lymphomas. Infection with a murine retrovirus (J-3) containing an avian v-*myc* rapidly induced plasmacytomas in pristane-primed BALB/cAn mice. Only 17% of the induced plasmacytomas that were karyotyped showed the characteristic chromosomal translocations involving the c-*myc* locus. Instead, all of the translocation-negative tumors demonstrated characteristic J-3 virus integration sites that were actively transcribed. Thus, the high levels of v-*myc* expression have replaced the requirement for chromosomal translocation in plasmacytomagenesis and accelerated the process of transformation.

PLASMACYTOMAS CAN BE REGULARLY induced in inbred BALB/cAn mice by the intraperitoneal (i.p.) injection of pristane (2,6,10,14-tetramethylpentadecane) (*1*). The incidence of plasmacytomas varies from 20% to 60% depending on the dose of pristane used (*1*). More than 95% of pristane-induced plasmacytomas have chromosomal translocations involving chromosome 15 (*2*) and an associated deregulation of *myc* gene transcription (*3*). Plasmacytomas can be induced with much shorter latent periods when the mice are given 0.5 ml pristane followed by infection with Abelson murine leukemia virus (A-MuLV) (*4*), and these plasmacytomas also have chromosome 15 translocations (*5*). In an analogous manner, deregulation of *myc* expression occurs in 100% of human Burkitt lymphomas along with translocations involving the *myc* chromosome (*6, 7*). In spite of this nearly universal association of plasmacytomas and Burkitt lymphomas with *myc* gene deregulation, this oncogene's causative role in either tumor has never been directly demonstrated.

The present study was designed to determine whether an activated avian v-*myc* gene can participate in plasmacytomagenesis. Thus, we conditioned BALB/c mice with a single i.p. injection of 0.5 ml pristane and then infected them with defective J-2 or J-3 viruses and Moloney murine leukemia virus (Mo-MuLV) as helper. J-2 and J-3 are murine retroviral constructs that contain a hybrid avian v-*myc* and a hybrid avian and mouse v-*raf* oncogene between long terminal repeats (LTRs) and other elements from Mo-MuLV (*8*). The J-3 virus contains the same elements as J-2 but has a 200-bp deletion from the 3′ end of *gag* and the 5′ end of *raf*, creating a frame shift that prevents synthesis of complete *raf* protein.

During an observation period of 153 days no tumors developed in the 45 mice infected with J-3 virus that had not been primed previously with pristane or in 13 mice injected with pristane alone. Helper virus alone injected intraperitoneally did not produce any tumors in primed mice in 150 days (*9*). By comparison, 22% of 114 BALB/cAn mice injected with pristane and then infected with J-3 developed plasmacytomas, about the same frequency as plasmacytomas induced by a single dose of 0.5 ml pristane and no virus (*1*). One significant difference is that in the presence of J-3 virus, plasmacytomas developed in less than half the latent period (mean of 68 days) for mice treated with pristane alone (*1*). Another significant difference resulting from including J-3 virus in the induction regimen is that a few plasmacytomas were also induced in BALB/cJ and (BALB/cAn × DBA/2)F_1 (CDF_1) mice, which are resistant to plasmacytoma induction by pristane alone (*1*).

Myeloid tumors were the next most common neoplasms, arising in 8% of BALB/cAn, in 25% of BALB/cJ, and in 2% of CDF_1 mice within 153 days after pristane and J-3 virus. Myeloid tumors were very common (57% of the animals) in BALB/cAn mice injected with J-2 virus 8 or 33 days postpristane, but no mice on this regimen developed plasmacytomas (Table 1, experiment IB). Three pristane-treated mice (10%) developed lymphocytic neoplasms af-

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