Previous studies have demonstrated that signal peptides bind to the signal recognition particle (SRP) primarily via hydrophobic interactions with the 54-kDa protein subunit. The crystal structure of the conserved SRP ribonucleoprotein core, however, raised the surprising possibility that electrostatic interactions between basic amino acids in signal peptides and the phosphate backbone of SRP RNA may also play a role in signal sequence recognition. To test this possibility we examined the degree to which basic amino acids in a signal peptide influence the targeting of two Escherichia coli proteins, maltose binding protein and OmpA.

Whereas both proteins are normally targeted to the inner membrane by SecB, we found that replacement of their native signal peptides with another moderately hydrophobic but unusually basic signal peptide (\(\Delta\)EspP) rerouted them into the SRP pathway. Reduction in either the net positive charge or the hydrophobicity of the \(\Delta\)EspP signal peptide decreased the effectiveness of SRP recognition. A high degree of hydrophobicity, however, compensated for the loss of basic residues and restored SRP binding. Taken together, the data suggest that the formation of salt bridges between SRP RNA and basic amino acids facilitates the binding of a distinct subset of signal peptides whose hydrophobicity falls below a threshold level.

The signal recognition particle (SRP)\(^1\) is a ribonucleoprotein complex that targets proteins to the eukaryotic endoplasmic reticulum (ER) as well as the bacterial inner membrane (IM). Although a core domain of SRP is highly conserved throughout evolution, both the size of the particle and its substrate specificity vary considerably (for review, see Ref. 1). Mammalian SRP is a relatively large particle comprised of six polypeptides and a 300-nucleotide RNA. In the first phase of the targeting reaction, the SRP 54-kDa subunit (SRP54) binds to both N-terminal signal sequences and transmembrane segments (TMSs) of integral membrane proteins (which often lack cleaved signal peptides) as they emerge during translation (2–4). Subsequently the ribosome-nascent chain complex migrates to the ER, where an interaction between SRP54 and a membrane-bound receptor catalyzes release of the nascent chain and its insertion into a protein translocation channel (5–7). At the other extreme, Escherichia coli SRP consists of only a homolog of SRP54 (Ffh) and an \(-100\) nucleotide RNA (4.5 S RNA) that is closely related to helix VIII of mammalian SRP RNA. Despite the difference in size, bacterial and mammalian SRPs share many biochemical properties (8, 9). The substrate specificity of \(E.\ coli\) SRP, however, is more restricted in that it primarily targets inner membrane proteins (IMPs) to the IM (10–12). Most periplasmic and outer membrane proteins, which contain cleaved signal peptides, are targeted to the membrane by molecular chaperones such as SecB (13). Unlike SRP, chaperones do not recognize signal sequences. Instead, they bind to the mature region of presecretory proteins late during translation or post-translationally to maintain translocation competence and to ensure that signal peptides are accessible to gate open translocation channels (14).

Biochemical studies showed 20 years ago that SRP recognizes the 7–13-amino acid hydrophobic core (“H region”) that is a universal feature of signal peptides (15). More recently, cryo-electronmicroscopy and X-ray crystallographic analysis of mammalian SRP54 and its bacterial homologs revealed the presence of a large hydrophobic groove in the “\(M\) domain” that likely represents the signal peptide binding pocket (16, 17). Mammalian SRP appears to interact with signal peptides that vary widely in hydrophobicity. In bacteria and the yeast \(S\)accharomyces \(c\)erevisiae, which also has multiple targeting pathways; however, SRP discriminates between different targeting signals that vary only slightly in hydrophobicity. In those organisms presecretory proteins that contain moderately hydrophobic signal peptides are bypassed by SRP and targeted by molecular chaperones by default. In \(E.\ coli\), maltose binding protein (MBP) and OmpA are normally targeted to the IM by SecB, but increasing the net hydrophobicity of their signal peptides reroutes both proteins into the SRP pathway (18). Furthermore, the biogenesis of M13 procoat protein, a small IMP whose insertion normally does not require any targeting factor, becomes SRP-dependent when it contains an unusually hydrophobic signal peptide (19). Likewise, yeast SRP binds preferentially to signal peptides that have a high hydrophobicity index (20). The data suggest that different SRP54 homologs are calibrated to bind to a different range of targeting signals. Indeed the observation that the putative binding pockets of evolutionarily distant \(M\) domains differ considerably in size and shape (16, 17) might account at least in part for the variation in substrate specificity.

The recent solution of the crystal structure of the \(E.\ coli\) Ffh \(M\) domain bound to a fragment of 4.5 S RNA raised the unexpected possibility that SRP RNA may also play a role in substrate recognition (21). The x-ray data show that a portion of the phosphate backbone of 4.5 S RNA lies adjacent to the hydrophobic groove in the Ffh \(M\) domain and appears to create an extended signal peptide binding pocket. The structure suggests that electrostatic interactions between the phosphates...
and basic amino acids that often reside at the N terminus ("N region") of signal peptides and that flank TMSs might contribute to substrate recognition. Curiously, biochemical studies have not provided any evidence that SRP interacts with basic amino acids. Mutation of basic amino acids in model signal peptides does not significantly affect recognition by mammalian SRP in cell-free assays (22, 23). By contrast, alteration of the charged amino acids and the H region can profoundly affect signal peptide cleavage, interaction with components of the translocation machinery, and translocation into ER vesicles (22–26). 

In E. coli, basic amino acids that flank TMSs influence IMP topology but are not required for membrane integration (27). A screen for mutations in the MBP signal sequence that improve topology but are not required for membrane integration (27). A signal peptide derived from the signal peptide of the presecretory proteins. Consistent with our hypothesis, we expected to make a relatively small contribution to SRP recognition or that SRP RNA interacts with only a subset of basic amino acids. Mutation of basic amino acids in model signal region would be expected to make a relatively small contribution to SRP recognition because only about a third of the putative extended binding site contained 100 such sites (and that probably reroute MBP into the ER). SRP RNA and basic amino acids play a minor role in substrate recognition on the basis of hydrophobicity alone. However, that basic residues only promote the binding of SRP to a distinct subset of signal peptides that barely escape detection on the basis of hydrophobicity alone.

**EXPERIMENTAL PROCEDURES**

Reagents, Media, and Bacterial Strains—Polynuclear rabbit antisera against MBP and influenza hemagglutinin epitope HA 11 (HA) were purchased from New England Biolabs and Covance, respectively, and a polyclonal antiserum against Ffh has been described (8). Selective media contained 100 μg/ml ampicillin and 30 μg/ml chloramphenicol as indicated. All bacterial cultures were grown at 37°C except where indicated. The bacterial strains used in this study were MC4100 (F- araD139 Δ(lacY1) gyrA96 thi-1 endA1 hsdR17 (rK- mK-)) (28) and DH5α (MC4100 ΔΔlacY1 Δ(lacZΔM15) (28)). Bacteriophage λgt11 and λgt11 was used as the helper phage. Bacterial cultures were grown to OD600 = 0.5 before IPTG was added. In all experiments aliquots were removed from each culture 20–30 min after IPTG addition. Cells were then pulse-labeled with [35S]methionine and [35S]cysteine (18, 29). To construct plasmid pJH28, a construct that carries the signal peptide of EspP was first amplified by PCR using the oligonucleotides TTTGAGCGGATCCACCCATGCGGTCGTGTGCCCAGAA-3′ and either 5′-TTGAGGCGATCCCAATCGGCTGTTGCGCCAGAAA-3′ or 5′-TTGAGGCGATCCCAATCGGCTGTTGCGCCAGAAA-3′ and then cloned into the NdeI site of pJH65. In the N region of signal peptides in targeting pathway selection. As expected, SRP recognition required the presence of multiple basic amino acids. Several lines of evidence indicated, however, that basic residues only promote the binding of SRP to a distinct subset of signal peptides that barely escape detection on the basis of hydrophobicity alone.

**Effect of Signal Peptide Charge on SRP Recognition**

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The Highly Basic $\Delta$EspP Signal Peptide Reroutes E. coli Presecretory Proteins into the SRP Pathway—In considering the hypothesis that basic amino acids in signal peptides play a role in targeting pathway selection, we reasoned that naturally occurring presecretory proteins that contain signal peptides as defined in von Heijne (39) are shown. An Eagl site that was introduced into pHL36 to facilitate cloning created a glutamine to proline mutation near the end of the OmpA signal peptide. Versions of MBP that contain the $\Delta$EspP signal peptide are underlined. Amino acids that are identical in all of the MBP and $\Delta$EspP variants are shown $\approx$. An Eagl site that was introduced into pHL36 to facilitate cloning created a glutamine to proline mutation near the end of the OmpA signal peptide. Versions of MBP that contain the $\Delta$EspP signal peptide or a truncated version that lacks the N-terminal extension of the EspP signal peptide, isogenic secB- and secE- strains in which $\text{ffh}$ is under the control of the araBAD promoter (HDB51 and HDB52, respectively) were transformed with a plasmid encoding MBP or $\Delta$EspP-MBP and grown in medium supplemented with arabinose. Ffh was then depleted from half of the cells by switching the carbon source to glucose, and protein export was assayed as described above. Ffh depletion did not measurably affect the export of $\Delta$EspP-MBP in HDB51 but caused a significant export defect in the secE- strain (Fig. 2B, lane 4). The results suggest that $\Delta$EspP-MBP is targeted by SRP in wild-type E. coli but can also be targeted effectively by molecular chaperones when the SRP pathway is impaired. Indeed given that the $\Delta$EspP signal peptide is only moderately hydrophobic, this interpretation of the data is consistent with other results showing that SRP dependence correlates with an unusual degree of signal peptide hydrophobicity (see below and Ref. 18). We next obtained direct evidence that SRP can interact with the $\Delta$EspP signal peptide in chemical cross-linking experiments. Cell-free translation reactions were programmed with mRNAs that encode the first 94 amino acids of MBP or $\Delta$EspP-MBP, radioactive nascent chains were synthesized, and the homobifunctional cross-linker disuccinimidyl suberate was added to isolated ribosome-nascent chain complexes in the presence or absence of 50 nm E. coli SRP. When $\Delta$EspP-MBP (but not wild-type MBP) nascent chains were synthesized, a prominent radiolabeled band of $\sim$55 kDa (the combined molecular mass of Ffh and the nascent chain) was observed in the presence of SRP (Fig. 3A, lanes 1–4). Immunoprecipitation with an anti-Ffh antiserum confirmed that the band corresponded to a cross-linked complex of Ffh and the nascent chain (Fig. 3B, lane 4). Ffh was cross-linked to the $\Delta$EspP signal peptide considerably less efficiently than to the highly hydrophobic MBP+1 signal peptide (data not shown), but the reason for this discrepancy is unclear.

The results of a different set of experiments strongly suggested that SRP also targets $\Delta$EspP-OmpA to the IM. Presumably because SecB targets wild-type OmpA post-translationally, a variable amount of pro-OmpA was reproducibly observed in pulse-labeled MC4100 and related secB+ strains (Figs. 2, A and C, lane 1). This effect was particularly pronounced when cells were grown at 22°C (Fig. 2C, lane 1, top panel). Interestingly, the precursor form of $\Delta$EspP-OmpA was not observed in pulse-labeled MC4100 (Fig. 2A, lane 7; Fig. 2C, lane 3, top panel). When a strain harboring an $\text{ffh}$ Ts mutation (SKP1101) and an isogenic $\text{ffh}^+$ strain (SKP1102) were shifted to 42°C, however, the $\Delta$EspP-OmpA precursor was observed in the mutant strain (Fig. 2C, lane 3, bottom panel). These results suggest that $\Delta$EspP-OmpA is targeted rapidly to the IM by the co-translational SRP pathway in wild-type cells but routed by default into a slower post-translational pathway when SRP function is impaired. We obtained further evidence that SRP targets $\Delta$EspP-OmpA to the IM in experiments in which we overproduced TF, a chaperone that binds promiscuously to comparing the relative amounts of precursor and mature forms of MBP or OmpA at each time point. Consistent with previous results, the wild-type proteins were exported much less efficiently in the secB– strain than in MC4100 (Fig. 2A, lanes 1–6). By contrast, $\Delta$EspP-MBP or $\Delta$EspP-OmpA was exported equally well in both strains (Fig. 2A, lanes 7–12). These results imply that the presence of the highly basic signal peptide reroutes the proteins from the SecB pathway to another targeting pathway or abolishes the need for a targeting factor altogether.

Further investigation indicated that the $\Delta$EspP signal peptide directs presecretory proteins into the SRP targeting pathway. To test the effect of depleting SRP on the export of proteins containing the $\Delta$EspP signal peptide, isogenic secB- and secE- strains in which $\text{ffh}$ is under the control of the araBAD promoter (HDB51 and HDB52, respectively) were transformed with a plasmid encoding MBP or $\Delta$EspP-MBP and grown in medium supplemented with arabinose. Ffh was then depleted from half of the cells by switching the carbon source to glucose, and protein export was assayed as described above. Ffh depletion did not measurably affect the export of $\Delta$EspP-MBP in HDB51 but caused a significant export defect in the secE- strain (Fig. 2B, lane 4). The results suggest that $\Delta$EspP-MBP is targeted by SRP in wild-type E. coli but can also be targeted effectively by molecular chaperones when the SRP pathway is impaired. Indeed given that the $\Delta$EspP signal peptide is only moderately hydrophobic, this interpretation of the data is consistent with other results showing that SRP dependence correlates with an unusual degree of signal peptide hydrophobicity (see below and Ref. 18). We next obtained direct evidence that SRP can interact with the $\Delta$EspP signal peptide in chemical cross-linking experiments. Cell-free translation reactions were programmed with mRNAs that encode the first 94 amino acids of MBP or $\Delta$EspP-MBP, radioactive nascent chains were synthesized, and the homobifunctional cross-linker disuccinimidyl suberate was added to isolated ribosome-nascent chain complexes in the presence or absence of 50 nm E. coli SRP. When $\Delta$EspP-MBP (but not wild-type MBP) nascent chains were synthesized, a prominent radiolabeled band of $\sim$55 kDa (the combined molecular mass of Ffh and the nascent chain) was observed in the presence of SRP (Fig. 3A, lanes 1–4). Immunoprecipitation with an anti-Ffh antiserum confirmed that the band corresponded to a cross-linked complex of Ffh and the nascent chain (Fig. 3B, lane 4). Ffh was cross-linked to the $\Delta$EspP signal peptide considerably less efficiently than to the highly hydrophobic MBP+1 signal peptide (data not shown), but the reason for this discrepancy is unclear.

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nascent polypeptides early in biosynthesis. Previous work showed that TF overproduction strongly retards the export of OmpA, $\beta$-lactamase, and alkaline phosphatase (a protein that does not require a chaperone for export) but does not affect the biogenesis of proteins targeted by SRP (29). This effect can be explained by the observation that the binding of SRP and TF to nascent polypeptides is mutually exclusive (35). We transformed HDB37 (MC4100 ara+) with plasmids expressing the TF gene under the control of the araBAD promoter and either OmpA or $\Delta$EspP-OmpA. As expected, the addition of arabinose greatly delayed the export of OmpA (Fig. 2D, lanes 1–4). TF overproduction, however, only very slightly affected the export of $\Delta$EspP-OmpA (Fig. 2D, lanes 5–8). Taken together with the results described above these data provide strong evidence that the presence of the $\Delta$EspP signal peptide routes presecretory proteins into the SRP pathway.

SRP Recognizes the $\Delta$EspP Signal Peptide on the Basis of Both Charge and Hydrophobicity—We next wished to determine whether the basic amino acids in the N region of the $\Delta$EspP signal peptide are required for SRP binding. To this end we mutagenized the basic residues in various combinations to glutamine. Mutants that contained glutamine in place of the first two lysines and the histidine ($\Delta$EspP(3)), the lysine and arginine adjacent to the H region ($\Delta$EspP(2)), and all five of the charged and partially charged residues ($\Delta$EspP(5)) were produced (see Fig. 1). MC4100 and HDB55 were transformed with plasmids that encode the modified versions of $\Delta$EspP-MBP, and export was assessed as described above. Decreasing the net charge of the N region of the signal peptide did not affect export in MC4100 but led to progressively severe export defects in the secB strain (Fig. 4, top three panels). Interestingly, mutation of either the first two or the last two charged amino acids in the $\Delta$EspP signal peptide partially restored the SecB requirement. These results imply that complete rerouting of MBP into the SRP pathway requires the presence of basic amino acids at multiple positions within the N region of the $\Delta$EspP signal peptide.
In considering the features of a signal peptide that promote SRP binding, we were struck by the fact that the H regions of the EspP and MBP signal peptides are curiously similar in sequence (see Fig. 1). Five amino acids in the respective H regions are identical, and two others are closely related. Although both H regions contain seven large and two small hydrophobic amino acids, a calculation based on a standard hydrophathy scale indicates that the EspP H region has a higher average hydrophobicity. We conjectured that this relatively small difference between the two signal peptides might help to explain their differential ability to interact with SRP.

To test this possibility we attached versions of the EspP signal peptide that contain single point mutations (F12A and L15T) to MBP. These mutations were chosen because they introduced the less hydrophobic amino acids found at specific positions in the MBP signal peptide. The single amino acid substitutions had no effect on MBP biogenesis in MC4100 but created export defects in MBP signal peptide.

**Fig. 3. Cross-linking of SRP to the EspP signal peptide.** Ribosome-nascent chain complexes containing the first 94 amino acids of MBP or EspP-MBP (94-mer) were isolated and incubated with disuccinimidyl suberate either in the absence (−) or presence (+) of 50 nM SRP. Proteins were resolved by SDS-PAGE either before (A) or after (B) immunoprecipitation with an anti-Ffh antiserum.

**Fig. 4. Interaction of SRP with the EspP signal peptide requires a minimum level of charge and hydrophobicity.** MC4100 and HDB55 were transformed with a plasmid that produces the indicated variant of EspP-MBP. After IPTG was added, protein export was analyzed by pulse-chase labeling and immunoprecipitation with an anti-MBP serum. The length of the chase is shown. p, precursor; m, mature.
HDB55 that were at least as severe as those produced by the ΔEspP(−5) mutant (Fig. 4, bottom two panels). Indeed the export of MBP containing the ΔEspP(L15T) signal peptide showed essentially the same degree of SecB dependence as wild-type MBP (compare Figs. 4 and 2A). These results strongly suggest that the H region of the ΔEspP signal peptide barely surpasses a threshold level of hydrophobicity that is essential for SRP recognition. Moreover, by showing that targeting pathway selection is far more sensitive to small changes in the H region than to neutralization of the entire N region, the data suggest that signal peptide hydrophobicity is the primary parameter that governs SRP binding.

We obtained additional evidence that SRP recognition requires a minimum level of signal peptide hydrophobicity in experiments in which we increased the net positive charge of the N region of the wild-type MBP signal peptide. Our mutagenesis strategy involved changing 2 or 3 amino acids to arginine or lysine. The most highly charged signal peptide variant (MBP(+3)) contains a stretch of five consecutive basic amino acids that is nearly identical in sequence and location to the basic motif found in the ΔEspP signal peptide (see Fig. 1). Pulse-chase experiments conducted in MC4100 and HDB55 cells showed that attachment of a signal peptide containing two extra positive charges (MBP(+2)) to MBP had no effect on the rate of export or the SecB requirement (Fig. 5, top two panels). The export of MBP containing the MBP(+3) signal peptide was then analyzed at 22 °C as well as 37 °C since electrostatic interactions are likely to be stronger at low temperature. Remarkably, attachment of the MBP(+3) signal peptide appeared to actually increase dependence on SecB at both temperatures and slow export at 22 °C (Fig. 5, last three panels). These data confirm that a high net positive charge of a signal peptide does not promote SRP binding if the hydrophobicity falls even slightly below a sharply defined threshold.

A High Degree of Signal Peptide Hydrophobicity Is Sufficient to Promote SRP Recognition—Given that the targeting of ΔEspP-MBP appeared to be more sensitive to changes in hydrophobicity than net positive charge, we hypothesized that basic amino acids might be superfluous for SRP recognition provided that a signal peptide is sufficiently hydrophobic. To test this idea, we first systematically increased the hydrophobicity of the ΔEspP(−5) signal peptide by mutating C11 and G14 and increasing the length of the H region and examined the export of MBPs containing the mutant signal peptides. Although some of the mutations slightly delayed MBP export in MC4100 (Fig. 6A, lanes 1–3), it was clear that increases in the overall hydrophobicity and length of the H region progressively reduced the SecB dependence of export (Fig. 6A, lanes 4–6). Elevating the hydrophobicity of the signal peptide concomitantly increased the severity of export defects in HDB51 after Ffh depletion (Fig. 6B, lanes 3 and 4). This enhanced SRP dependence likely reflects protein aggregation in the absence of a co-translational targeting mechanism. A signal peptide that contained leucines in place of Cys-11 and Gly-14 (ΔEspP*2(−5)) appeared to confer partial dependence on both
the SecB and SRP pathways and therefore probably interacts with SRP only marginally. The data demonstrate that a high degree of signal peptide hydrophobicity is sufficient to route a presecretory protein into the SRP pathway. To corroborate this conclusion, we subsequently reexamined the export of MBP*1, an MBP derivative containing three amino acid substitutions that increase the hydrophobicity of the signal peptide (Fig. 1). Previous studies showed that MBP*1 is targeted to the IM by SRP (18). Because the MBP*1 signal peptide is nearly as hydrophobic as the most hydrophobic ΔEspP signal peptide derivatives described above, we surmised that the three basic amino acids in the N region might be dispensable for SRP recognition. Consistent with this prediction, we found that like MBP*1, MBP*1(1–3) was exported efficiently from secB− cells (Fig. 6A, bottom panel). Moreover, both proteins showed similar export defects in cells that lack Fh (Fig. 6B, bottom panel). Taken together the results provide additional evidence that SRP recognizes signal peptides primarily on the basis of hydrophobicity.

**DISCUSSION**

In this report we describe evidence that basic amino acids in the N region of signal peptides can play a significant role in promoting signal peptide recognition by SRP. Initially we found that the unusually basic ΔEspP signal peptide suppresses the SecB requirement in the export of MBP and OmpA under physiological conditions and that this effect was dependent on the presence of multiple basic amino acids in the N region. Taken together, several observations strongly suggest that the elimination of the SecB requirement was due to a rerouting of the proteins into the SRP pathway. First, the export of ΔEspP-MBP was inhibited by Ffh depletion in secB− cells. The simplest interpretation of this result is that the protein can be targeted effectively by both SRP and chaperone-based pathways and that export defects are detected only when multiple pathways are impaired. Because SRP acts at a very early stage of protein biosynthesis, this explanation implies that it provides the primary targeting pathway for ΔEspP-MBP. Second, cross-linking experiments showed directly that SRP can interact with the ΔEspP signal peptide. Third, the presence of the ΔEspP signal peptide accelerated OmpA export except when the SRP pathway was impaired. Fourth, the ΔEspP signal peptide prevented the delay in OmpA export that is associated with TF overproduction. Based on previous studies (29, 35), the most likely explanation of this result is that interaction with SRP prevents the binding of TF to the mature region of ΔEspP-OmpA. Finally, several experiments showed that the presence of a highly basic N region is necessary but not sufficient to explain the strong effect that the ΔEspP signal peptide exerts on targeting pathway selection. The data strongly suggest that the basic amino acids in the ΔEspP signal peptide contribute to eliminating the SecB requirement by promoting a specific macromolecular interaction rather than by affecting the folding of presecretory proteins.

Although we found that signal peptide charge can influence targeting pathway selection in *E. coli*, our results clearly show that signal peptide hydrophobicity is the primary criterion for SRP recognition. We found that SRP recognizes signal peptides that are devoid of basic amino acids provided that they are atypically hydrophobic. Furthermore, single point mutations that slightly change the hydrophobicity of the H region profoundly affect SRP recognition (see also Ref. 18), whereas mutations that alter the charge of the N region have much smaller effects. Indeed one of our most intriguing observations is that a threshold level of signal peptide hydrophobicity is absolutely essential for SRP recognition. Taken together with the finding that a 20-fold overproduction of SRP does not alter the targeting of MBP (18), this observation suggests that SRP has dramatically different affinities for signal peptides that vary only slightly in hydrophobicity. Given that SRP binds to a diverse range of substrates, such an exquisite degree of specificity seems surprising. The ability of *E. coli* SRP to interact with signal peptides may be very limited, however, because it is probably designed to interact primarily with the extended stretches of hydrophobic residues found in the TMSs of IMPs. In this regard it is interesting to note that SRP recognizes the MBP*1 signal peptide but not ΔEspP*1(1–5) signal peptide. The H domain of the former peptide is longer but has a lower average hydrophobicity. Indeed it makes sense that the number of hydrophobic amino acids in a targeting signal would be an important factor in SRP recognition since few TMSs have an average hydrophobicity equivalent to that of signal peptides such as MBP*1.

Our results also imply that basic residues promote the binding of SRP to only a subset of signal peptides whose hydrophobicity falls slightly below a critical level. The contribution of signal peptide charge to SRP recognition may not have been detected in previous studies precisely because it was not significant for the recognition of the small number of model signal peptides that were examined. Our data predict that basic residues promote the recognition of only relatively few naturally occurring signal peptides in *E. coli* because the hydrophobicity threshold for SRP interaction is set extremely high. If the threshold is set closer to the hydrophobicity of an average signal peptide in other species due to differences in the structure of SRP54/Ffh or the interaction of SRP with the translation machinery, however, then the composition of the N region may be relevant for the binding of a much greater number of substrates.

In light of the crystallographic analysis of the SRP ribonucleoprotein core (21), it is very likely that basic residues in signal peptides promote SRP binding by forming electrostatic interactions with the phosphate backbone of SRP RNA. It is doubtful that basic residues form salt bridges with SRP54/Ffh because the protein does not have any significant negatively charged surfaces (16, 37). We cannot completely exclude the possibility, however, that basic amino acids in signal peptides facilitate SRP binding by an indirect mechanism, perhaps by affecting the length or helical structure of the H region. Given that arginine- and lysine-to-glutamine substitutions perturb the interaction of the ΔEspP signal peptide with SRP significantly but presumably alter its biophysical properties only very minimally (38), this scenario seems unlikely. A simple model that emerges from our data is that electrostatic interactions involving SRP RNA help to stabilize the binding of signal peptides that bind to SRP54/Ffh with only moderate affinity. Factors such as the size and hydrophobicity of the signal peptide binding pocket in the M domain and the relative position of the M domain with respect to the phosphate backbone of SRP RNA may influence the range of substrates that are effectively engaged via these stabilizing interactions. Indeed a two-part binding surface that has the capacity to form two distinct types of chemical bonds with potential ligands may have evolved to fine tune the limits of SRP recognition to meet the needs of different organisms.

**Acknowledgments**—We thank Greg Phillips for providing bacterial strains and Manu Hegde for critical reading of the manuscript.

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J. Biol. Chem. 2003, 278:46155-46162.
doi: 10.1074/jbc.M309082200 originally published online August 29, 2003

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