Another Factor Besides Hydrophobicity Can Affect Signal Peptide Interaction with Signal Recognition Particle*

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Translocation of alkaline extracellular protease (AEP) into the endoplasmic reticulum of Yarrowia lipolytica is cotranslational and signal recognition particle (SRP)-dependent, whereas translocation of P17M AEP (proline to methionine at position 17, second amino acid in the pro-region) is posttranslational and SRP-independent. P17M signal peptide mutations that resulted in more rapid SRP-dependent translocation of AEP precursor were isolated. Most of these mutations significantly increased hydrophobicity, but the A12P/P17M mutation did not. The switch from SRP-dependent to SRP-independent translocation without a decrease in hydrophobicity (wild type to P17M) and restoration of SRP-dependent translocation without an increase in hydrophobicity (P17M to A12P/P17M) indicate that some factor(s) in addition to hydrophobicity determines selection of targeting pathway. Models of extended forms of wild type and A12P/P17M signal peptides are kinked, whereas the P17M signal peptide is relatively straight. Possibly the conformation/orientation of signal peptides at the ribosomal surface affects SRP binding and consequently the targeting route to the endoplasmic reticulum. Kinked signal peptides might approach SRP more closely more often. Most likely, these effects were only detectable because of the short length and low average hydrophobicity of the AEP signal peptide.

Whereas signal peptides have been extensively studied, the molecular details of their interactions with components involved in targeting, such as SRP, are still unclear (Ref. 1, and for reviews, see Refs. 2 and 3). They are generally 15–30-amino acids long (for eukaryotes) and located at the N terminus. Signal peptides distinguish proteins that will enter the secretory pathway from those that will not. Although there is no sequence conservation between signal peptides, they have definite similarities and can be divided into regions. There usually is a net positive charge near the N-terminal end (n-region) followed by a hydrophobic stretch of amino acids (h-region) and a more polar C-terminal region usually containing the signal peptide cleavage site.

Studies of mutated and synthetic signal peptides show that the degree of hydrophobicity affects the efficiency of translocation (for examples, see Refs. 4–6). For carboxypeptidase Y from Saccharomyces cerevisiae (7, 8), the wild type signal peptide did not interact in vitro with mammalian SRP, but a mutated form in which both glycines in the hydrophobic stretch were converted to leucines did. Recently, it was demonstrated that the average hydrophobicity of S. cerevisiae signal peptides is an important determinant of whether a protein is targeted by the SRP-dependent or -independent pathway (9). For proteins targeted by an SRP-independent pathway, the average hydrophobicity for 12 residues after the last positively charged residue of the n-region (HB12) is around 2.0 or less. For proteins targeted primarily by an SRP-dependent pathway, the HB12 values are significantly higher, around 3.0 or more.

A kinetic partitioning model has been proposed to explain SRP-dependence of protein translocation (1). One important factor in the partitioning is the affinity of the signal peptide for SRP which depends upon the primary sequence, hydrophobicity, and conformational propensities favoring formation of a productive complex with SRP (1). For isolated signal peptides, β-structure and random or unordered structure are favored in aqueous solution and α-helix in nonpolar environments (see Ref. 3). However, it is likely that short polypeptides are conformationally flexible (3). To our knowledge, there is no example where a change of conformation/orientation, independent of average hydrophobicity, affects choice of targeting pathways.

AEP secreted by the yeast Yarrowia lipolytica is primarily translocated by a cotranslational SRP-dependent pathway (10, 11). The first AEP precursor (55 kDa) detected in pulse-chase immunoprecipitation experiments is already translocated. Synthesis of this precursor is preferentially decreased at nonpermissive temperature in a strain containing the scr1–1 SRP RNA mutation, suggesting that at a minimum SRP has a role in AEP synthesis and probably in targeting. However, no untranslocated AEP precursor is detected in srp54Δ cells (12). These differing results may be explained by a longer time for adaptation for srp54Δ cells and possibly by the instability of the untranslocated wild type AEP precursor. In any case, it appears that AEP preferentially uses the SRP-dependent pathway, but it can be targeted by the SRP-independent pathway.

The P17M AEP mutation (Pro to Met at the second amino acid after the signal peptide cleavage site) results in SRP-independent posttranslational translocation (11). For P17M, the earliest precursor (53 kDa) detected in pulse-chase immunoprecipitation experiments is not translocated. Levels of P17M AEP precursors are not affected by the scr1–1 mutation, suggesting that P17M AEP does not interact with SRP. This change in targeting pathway was unexpected given that the P17M mutation does not affect signal peptide length or average hydrophobicity.

In this manuscript, we confirm that the rate of translocation can be increased and SRP-dependent targeting at least partially restored by increasing the hydrophobicity of a signal peptide. Our novel finding is that targeting pathway preference...
can be altered by mutations that have little or no effect on signal peptide hydrophobicity. Thus, some factor(s) other than hydrophobicity affects interactions of signal peptides with SRP. Computer models of the signal peptides are consistent with both the conformation/orientation and hydrophobicity of a signal peptide affecting its interactions with SRP and consequently the targeting pathway for translocation. Probably, these effects were observable only because the AEP signal peptide is short and has low average hydrophobicity for a yeast protein using the SRP-dependent pathway.

**EXPERIMENTAL PROCEDURES**

**Materials**—Sources for reagents, oligonucleotides, and radioactive compounds used for strain construction, DNA sequencing and pulse-chase immunoprecipitation experiments (10, 11), and preparation of AEP antiserum (10) have been described.

**Strains**—Wild type was CX61-1B A ade1 L. Constructions of P17M A ade1 ura3-D XPR2 (P17M), DY66 leu2 ura3 scr1:ADE1 scr2:LYS5 pIMR63 (SC1 LEU2 ARS18), DY63-XPR2 leu2 ura3-xpr2-ΔA scr1:ADE1 scr2:LYS5 pIMR63 (SC1 LEU2 ARS18), DY66 leu2 ura3 scr1:ADE1 scr2:LYS5 pIMR66 (scr1-1 LEU2 ARS18) have been described (11). SYM2 A ade1 ura3-D xpr2-ΔA was constructed as previously described (13), and construction of T8L T11L A12P/P17M between Leu 6 and Leu 7.

**9**

**primer 5**

elsewhere. A12P P17M

URA3

**For site-directed mutagenesis altered versions of the 1.7-kilobase fragment in pUC13 were mutagenized and cloned into SpalXhoI fragments of pUC13 were mutagenized and cloned into SpalXhoI cut pIMR100 (URA3 in pUC13 and XPR2 in EcoRV I/HindIII sites of pBR322), and the plasmids cut with MluI to target in the promoter region of xpr2-ΔA in SMY2. A6L FY7 FY8 P17M and A6L FY7 P17M were constructed by

**DNA Sequencing**—Double-stranded DNA sequencing was done by the dideoxy method using the Sequenase Kit (U. S. Biochemical Corp.) and [35S]dATP (21).

**Pulse-chase Immunoprecipitation and Endo H Digestion**—Pulse-chase labeling with [3H]leucine, disruption of cells, immunoprecipitation of cell extracts with AEP antibody, chloroacetic acid precipitation of extracellular samples, SDS-PAGE, and fluorography are based on previously described procedures (10). Immunoprecipitated samples were digested with endo H as described previously (11).

**RESULTS**

**Introduction**—Unlike wild type AEP, P17M AEP is translated posttranslationally (6). Translocation was examined using pulse-chase immunoprecipitation. Cells were labeled for 45 s with [3H]leucine, cell extracts immunoprecipitated with AEP antibody, and samples were analyzed by SDS-PAGE and fluorography. Films were exposed for 3 days.

**Pulse-chase Immunoprecipitation and Endo H Digestion**—Pulse-chase labeling with [3H]leucine, disruption of cells, immunoprecipitation of cell extracts with AEP antibody, chloroacetic acid precipitation of extracellular samples, SDS-PAGE, and fluorography are based on previously described procedures (10). Immunoprecipitated samples were digested with endo H as described previously (11). DNA ADE1 was maintained on YM or YM D

**Secondary Structure Predictions and Molecular Modeling**—Secondary structure predictions were done using the Chou-Fasman (24) and Robson-Garnier (25) methods, as implemented by MacVector 5.0 (Oxford Molecular Group, Oxford, UK) and Predict Protein (26). Models of signal peptides were generated using the Biopolymer module of the InsightII (95.0.6) Molecular Modeling System from Biosym/Molecular Simulations (San Diego, CA). Model polypeptide structures were constructed in either the right-handed α-helix or extended forms using the CVFF force field. The Discovery module was used for energy minimization and dynamic simulations. First, a 1000 step dynamic simulation at 600 K, and finally another 1000 step energy minimization were done in vacuo. The polypeptide was SOAKed to simulate water as the solvent, and a final 1000 step energy minimization was performed.

**RESULTS**

**Introduction**—Unlike wild type AEP, P17M AEP is translated posttranslationally in an SRP-independent manner (11). For wild type AEP, the precursor detected at the earliest point in pulse-chase immunoprecipitation experiments with brief labeling times (45 s) is the 55-kDa translocated AEP precursor (Fig. 1a) (10). For P17M AEP, little or no 55-kDa precursor is detected at the earliest time point (Fig. 1b). The P17M AEP 53-kDa precursor contains the signal peptide and lacks N-linked carbohydrate (suggesting it is not translocated), and it quantitatively chases into the 55-kDa AEP precursor (11). The P17M AEP 55-kDa precursor, like wild type AEP 55-kDa precursor, begins with Ala16 (therefore it lacks the signal peptide), contains 2 kDa of N-linked carbohydrate, and is protected in protease-protection experiments, suggesting that it is translocated (11). Thus the relative amounts of the 53- and 55-kDa

**FIG. 1.** Wild type AEP is translocated cotranslationally (a), and P17M AEP is translocated posttranslationally (b). Translocation was examined using pulse-chase immunoprecipitation. Cells were labeled for 45 s with [3H]leucine, cell extracts immunoprecipitated with AEP antibody, and samples were analyzed by SDS-PAGE and fluorography. Films were exposed for 3 days.
AEP precursors at earliest time points reflect the relative usage of the posttranslational and cotranslational targeting pathways.

No mature AEP is detected intracellularly for P17M (Fig. 1a) and several other versions of AEP (see below) because it appears that transit of any protein containing the P17M mutation is delayed in the ER. Therefore, it takes significantly longer than for wild type for labeled mature AEP to appear intracellularly and then in the extracellular medium.2

Increasing the Hydrophobicity of a Signal Sequence Can Increase the Rate of Translocation—Hydrophobicity of the wild type AEP signal peptide is relatively low (HB12 of 2.15; Table I) and closer to HB12 values for S. cerevisiae proteins using an SRP-independent targeting pathway (9). We attempted to target P17M AEP by an SRP-dependent pathway by increasing the hydrophobicity of the signal peptide. The T8L P17M signal peptide has increased hydrophobicity (HB12 of 2.78), but it was still translocated posttranslationally.2 For A6L F7L T8L P17M (HB12 of 3.34), both 53-kDa untranslocated and 55-kDa translocated precursors were detected at the earliest time points with the 55-kDa precursor predominating,2 suggesting that increasing the hydrophobicity of the signal peptide resulted in primarily cotranslational translocation (see below). For T8L T11L P17M (HB12 of 3.41), the translocation pattern resembled that of wild type AEP, although AEP precursor levels were decreased significantly (see below). These results show, as shown previously by others, that hydrophobicity of the signal peptide is an important factor in determining the choice of targeting pathway.

A Signal Peptide Mutation That Does Not Increase Hydrophobicity Can Increase the Rate of Translocation—The reason for the change from SRP-dependent targeting for wild type AEP to SRP-independent for P17M AEP was unclear because the P17M mutation was not in the signal peptide, and the hydrophobicity of the region actually slightly increased (HB16 value changed from 2.15 to 2.26) (Table I). Because prolines are known to cause kinks and to disrupt secondary structure (27), the P17M mutation might have a significant effect on the signal peptide’s conformation/orientation. If P17M results in SRP-independent posttranslational translocation because it changes the conformation/orientation of the signal peptide, then it might be possible to restore SRP-dependent cotranslational translocation by reconfiguring/orienting the signal peptide. Therefore, we constructed A12P/P17M. The predominant precursor in A12P/P17M at the earliest time point in a pulse-chase immunoprecipitation experiment was 55 kDa (Fig. 2a). No 53-kDa untranslocated precursor was detected, but

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**Table I**

| Version of AEP  | HB (12) | HB (16) | Mode of translocation  |
|----------------|---------|---------|------------------------|
| Wild type      | 2.15    | 1.86    | CT, SRP-dependent       |
| P17M           | 2.15    | 2.26    | PT, SRP-independent    |
| T8L P17M       | 2.78    | 2.73    | PT, SRP-independent*   |
| T8L T11L P17M  | 3.41    | 3.20    | CT                      |
| L A6L F7L T8L P17M | 3.34 | 3.17    | CT > PT, SRP-dependent |
| L L A6L F7L T8L P17M | 3.37 | 3.15    | CT > PT                |
| A12P P17M      | 1.95    | 2.11    | CT                      |
| T8L T11L A12P P17M | 3.21 | 3.05    | PT, SRP-independent   |
| A6L F7L T8L T11L A12P P17M | 3.79 | 3.49    | CT > PT                 |
| L L A6L F7L T8L T11L A12P P17M | 3.88 | 3.57    | CT = PT               |

* S. Matoba and D. M. Ogrydziak, unpublished data.

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There was a 51-kDa precursor. The mobility of both bands was increased by treatment with endo H (Fig. 2b), indicating they contained N-linked carbohydrate, consistent with translocation into the ER. The HB12 for A12P/P17M AEP (1.95) is slightly less than for P17M AEP (2.15). Thus the effects of the A12P change cannot be explained by an increase in hydrophobicity.

The P17M mutation adversely affects production of AEP activity as shown by smaller zones of clearing on skim milk agar plates compared with wild type (Fig. 3). Although A12P/P17M AEP was translocated more rapidly than P17M AEP, production of AEP activity decreased (Fig. 3).
change from SRP-dependent translocation for wild type AEP to SRP-independent translocation for P17M AEP without a decrease in hydrophobicity and the change from SRP-independent translocation for P17M AEP back to SRP-dependent for A12P/P17M AEP without an increase in hydrophobicity prove that hydrophobicity cannot be the only factor determining the choice between these targeting pathways. Given that both changes involve deletion or addition of a proline, conformation/orientation may be an important factor in signal peptide SRP-interactions.

For A6L F7L T8L P17M in the SCR1 background at 23 °C, there was more 55-kDa than 53-kDa precursor at the earliest time point (Fig. 4b). The scr1–1 mutation slows growth at 23 °C, and a decrease in A6L F7L T8L P17M AEP precursor levels was found (Fig. 4b). After a shift to 33 °C in the scr1–1 background, the levels of A6L F7L T8L P17M AEP precursors were further decreased. Both results indicate that SRP-dependent targeting had been at least partially restored.

**Molecular Modeling**—Because prolines alter the direction of polypeptide chains, the original hypothesis was that the P17M mutation changed the orientation of the signal peptide so that it no longer interacted with SRP and that the A12P/P17M combination restored the wild type orientation to some extent. Wild type AEP, P17M AEP, A12P/P17M AEP, and A6L F7L T8L P17M AEP peptides were modeled. The PredictProtein program predicted α-helical structures—from Ala1 to Leu14 for wild type and extending to Leu19 or Ala19 for the three proteins lacking Pro17. The Robson-Garnier method predicted α-helical structure from Ile6 to Ala38 for all four peptides. Predictions by the Chou-Fasman method differed—α-helical structure from Met1 to Leu18 for wild type extending to Ala22 for P17M AEP and A6L F7L T8L P17M AEP. For A12P/P17M AEP, the α-helical structure only extends from Pro12 to Ala22, and sheet is predicted from Lys5 to Val13. The peptides modeled as α-helices did not show much difference in overall shape (not shown). However, shapes of peptides modeled in the extended form were clearly different. Wild type AEP and A12P/P17M AEP peptides are kinked, shaped like a bow, whereas P17M AEP and A6L F7L T8L P17M AEP peptides are more linear, i.e. they would fit in a cylinder with a smaller diameter (Fig. 5). Although wild type AEP and A12P/P17M AEP peptides are of similar shape, if the C- or N-terminal amino acids are aligned, the central sections of the peptides do not overlap but head off in different directions, like mirror images of each other. The correlation of targeting pathway choice with predicted peptide structure suggests a model in which (i) kinked polypeptides with a larger radius of gyration interact more readily with SRP and (ii) increases in average hydrophobicity increase the affinity for SRP sufficiently that interactions with more linear signal peptides can occur.

**Additional Signal Peptide Mutations**—For T8L T11L P17M (HB12 of 3.41), an AEP precursor with similar mobility on SDS-PAGE as wild type 55-kDa translocated precursor (data not shown) predominated at the earliest time point (Fig. 6a). It contained 2 kDa of N-linked carbohydrate, consistent with it being translocated (data not shown). Although total counts incorporated were similar to other strains, the levels of intracellular precursor were quite low. Little or no mature labeled AEP (data not shown) or AEP activity (Fig. 3) was secreted.

The model that signal peptides with relatively low average hydrophobicity must be kinked for interaction with SRP does not account for decrease in the level of T8L T11L P17M AEP, and there are some changes in targeting pathways that are difficult to explain using this model. One example is T8L T11L A12P/P17M. The addition of the A12P mutation to T8L T11L P17M increased synthesis (Fig. 6b) and restored production of AEP to P17M-like levels (Fig. 3). The average hydrophobicity decreased slightly (3.41 to 3.21), but the value was still above 3.0. With the combination of high average hydrophobicity and a kinked signal peptide, we expected SRP-dependent translocation. Instead, the translocation pattern changed from largely cotranslational to posttranslational. Additional changes A6L and F7L increase the average hydrophobicity to 3.79, and as expected, translocation of A6L F7L T8L T11L A12P/P17M AEP was largely cotranslational (Fig. 6c); AEP production decreased slightly (Fig. 3). Difficult to explain is why addition of two Leu amino acids between positions 5 and 6, which increased the average hydrophobicity to 3.88, resulted in an increase in posttranslational translocation, so that about half of L L A6L F7L T8L T11L A12P/P17M used this pathway (Fig. 6d), and in substantial decreases in AEP precursor levels (Fig. 6d) and in secretion of AEP activity (Fig. 3).
No untranslocated AEP precursor was detected in A12P/P17M. The AEP-related material running near mAEP in the very early time points in Fig. 2 are possibly degradation products suggesting that A12P/P17M AEP untranslocated precursor is unstable, but untranslocated precursors were readily detected for P17M AEP and several other precursors containing the P17M mutation. The 51-kDa AEP precursor found in A12P/P17M had not been detected previously. It contains N-linked carbohydrate suggesting that it has been translocated, and it probably is a degradation product of the 55-kDa translocated precursor. An important finding was that levels of translocated A12P/P17M AEP precursors were significantly reduced in the scr1–1 background. Decreases in translocated AEP precursor have been attributed to maintenance of translational arrest (11). However, they may be because of translocation defects (because of instability of scr1–1 SRP) combined with instability of the untranslocated precursor. In either case, decreases in the levels of translocated precursors show an interaction of SRP with the A12P/P17M AEP precursor.

**Fig. 5.** Models of the extended configuration of the first 24 amino acids show that the P17M AEP and A6L F7L T8L P17M AEP peptides are fairly linear, whereas the wild type AEP and A12P/P17M AEP peptides are kinked and shaped like a bow. The C terminus is on the left and the N terminus on the right. The peptides were rotated in 60° increments.
The major finding of this study is that some factor besides average hydrophobicity must explain the differences in ER targeting pathways of wild type and A12P/P17M peptides compared with P17M and A6L F7L T8L T11L A12P/P17M peptides. The identity of the additional factor(s) has not been firmly established. Some possibilities include amphiphilicity, hydrophobic moment, molecular hydrophobicity potential (28), or a slowing of the rate of synthesis of some of the signal peptides allowing a longer time for interaction with SRP. Interestingly, helical wheel representations of the signal peptides revealed that all the signal peptides relatively flexible? How does the signal peptide conformation/orientation change as protein synthesis progresses? SRP has some affinity for ribosomes in the absence of signal peptide, but it increases by orders of magnitude when the signal peptide appears (32). Current models have SRP cycling on and off of ribosomes during the translation elongation cycle and competing with NAC for sampling of nascent chains as they exit the ribosome (33, 34). Does SRP position itself precisely enough on the ribosome that the conformation/orientation (position in space) of the signal peptide can influence their interaction? How much of the AEP signal peptide must be exposed before there is an interaction, and at what lengths do interactions with SRP start to decrease and then become no longer significant? At physiological SRP concentrations, affinity of SRP for preprolactin signal peptide fell dramatically after chains reached a certain length (35).

As found in other systems, hydrophobicity is an important factor in the AEP signal peptide/SRP interaction. The shape of the A6L F7L T8L P17M peptide is similar to that of the P17M peptide, but it is much more hydrophobic and uses the SRP-dependent targeting pathway. The increase in average hydrophobicity may increase the affinity for SRP so that even if the signal peptide and SRP are only rarely in close proximity the high affinity is sufficient for functional interactions to occur.

**Other Mutations**—The very low level of T8L T11L P17M AEP detected is probably because of a posttranscriptional effect such as decreased stability or synthesis, perhaps because of altered mRNA secondary structure. The most difficult result to explain by our model is for T8L T11L A12P/P17M AEP, which was translocated primarily posttranslationally, whereas T8L T11L P17M AEP and A12P/P17M AEP were translocated primarily co-translationally. Why addition of leucines to produce LLA 6L F7L T8L P17M (HB12 of 3.37) were much like those for A6L F7L T8L P17M (HB12 of 3.42) translocation is predominately posttranslational. 2

Implications for Secretion of Heterologous Proteins—Usually for secretion of heterologous proteins from *Y. lipolytica*, parts or all the XPR2 prepro-region have been used to direct the protein into the secretory pathway and to provide for accurate

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**Fig. 6. Translocation of AEP-related polypeptides in additional signal sequence mutants.** Shown are the results of pulse-chase immunoprecipitation experiments. Cells were labeled for 45 s with [3H]leucine, and cell extracts were immunoprecipitated. Samples were analyzed by SDS-PAGE and fluorography. T8L T11L P17M incorporated 15% more counts than the average for wild type (a), and the film was exposed for 11 days. For T8L T11L A12P/P17M (b), A6L F7L T8L T11L A12P/P17M (c), and L L A6L F7L T8L T11L A12P/P17M (d), the films were exposed for 7 days.
processing. How the efficiency of secretion is affected by the mode-of-translocation is unknown. Less P17M AEP is secreted than wild type AEP (Fig. 3), but this could be because of folding problems in the ER.\textsuperscript{2} If a wild type AEP signal peptide/mature foreign protein fusion is constructed and if, unlike AEP, the foreign protein does not have a proline near its N terminus, then the prediction of our model is that this fusion would be targeted posttranslationally as for P17M. In this case, how readily the foreign protein can be translocated posttranslationally might determine the productivity of the construct. The rice amylase signal peptide was more efficient than the AEP signal peptide for rice amylase secretion (37). We have produced a bovine \textit{\textbeta}-lactoglobulin using the wild type AEP signal peptide\textsuperscript{3} but did not try using the \textit{\textbeta}-lactoglobulin signal peptide. Neither mature \textit{\textbeta}-lactoglobulin nor rice amylase has a proline near the N terminus, but \textit{\textbeta}-lactoglobulin is much smaller (18 \textit{versus} 42 kDa) and may be more readily translocated posttranslationally.

Prolines and \textit{S. cerevisiae} SRP-independent Signal Peptides—If our model holds for \textit{S. cerevisiae} SRP/signal peptide interactions, then for signal peptides with low hydrophobicity (targeted by the SRP-independent pathway) the sequences just downstream should lack prolines. Otherwise these signal peptides would be predicted to interact to some extent with SRP. The prediction holds for four of the five \textit{S. cerevisiae} proteins for which this information is available: PDI (no prolines in 22 amino acids), CPY (0/20), Gas1p (0/22), and Suc2p (0/19). MFa-factor (HB <2.0) with an internal proline at position 4 and one at position 21, the second amino acid after the cleavage site, does not fit the prediction.

Conclusions—The short length and relatively low hydrophobicity of the AEP signal peptide may have made it possible to see changes in choice of targeting pathways without changes in hydrophobicity. If the AEP signal peptide had an HB12 >3.0 like SRP-dependent signal peptides in \textit{S. cerevisiae}, then most likely one would not see the P17M effect (change from SRP-dependent to SRP-independent targeting) because the resulting signal peptide would probably behave like the A6L F7L T8L P17M signal peptide which was targeted largely by the SRP-dependent pathway. The model that signal peptides with low hydrophobicity must be kinky to be recognized by SRP is attractive but not proven. What has been proven is that some other factor(s) besides average hydrophobicity is also important in choice of the targeting pathway for translocation.

\textsuperscript{3} M. Huang and D. M. Ogrydziak, unpublished data.

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\textsuperscript{3} M. Huang and D. M. Ogrydziak, unpublished data.