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Topical Review

The Signal Peptide

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Introduction

In both prokaryotic and eukaryotic cells, proteins are allowed entry into the secretory pathway only if they are endowed with a specific targeting signal: a signal peptide (SP). The SP is in most cases a transient extension to the amino terminus of the protein and is removed by one of a small class of enzymes known as signal peptidases once its targeting function has been carried out. Many integral membrane proteins remain anchored to the membrane by an uncleaved SP, or by a succession of SP-like "start-transfer" and "stop-transfer" sequences. A number of reviews dealing with protein secretion in general and signal peptides in particular have been published in the last coupled of years (Briggs & Gierasch, 1986; von Heijne, 1988*a,b*; Gierasch, 1989); here, I will concentration on the most recent data on signal peptide structure and function.

Signal Peptides Have a Three-Domain Structure

Early on, comparisons of known SPs indicated that they typically have three distinct domains (Fig. 1): an amino-terminal positively charged region (n-region, 1–5 residues long); a central, hydrophobic part (h-region, 7–15 residues); and a more polar carboxy-terminal domain (c-region, 3–7 residues). Beyond this overall pattern, no precise sequence conservation could be found, and it soon became obvious that SPs are highly variable, rapidly evolving structures. Indeed, "idealized" SPs with h-regions consisting of strings of typically eight to ten Leu residues have been found to function efficiently both in *Escherichia coli* (Kendall, Bock & Kaiser, 1986), *Saccharomyces cerevisiae* (Yamamoto et al., 1987), and the eukaryotic Semliki Forest virus (M. Lobigs, *personal communication*).

More recently it has become possible to detect slight variations in the mean lengths and amino acid compositions of the three regions between different groups of organisms (von Heijne & Abrahmsén, 1989). Thus, SPs from eukaryotes tend to have slightly shorter n-, h-, and c-regions than SPs from Gram-negative bacteria, which in turn have shorter regions than SPs from Gram-positive bacteria.

The "positive-hydrophobic-polar" design immediately suggested that SPs might bind efficiently to lipid bilayers, possibly in a loop structure with the basic amino terminus bound to acidic lipid headgroups on the cytoplasmic face of the membrane (DiRienzo, Nakamura & Inouye, 1978; von Heijne & Blomberg, 1979; Engelman & Steitz, 1981). Up to this day, however, there is still no consensus as to whether the SP interacts primarily with receptor proteins or with the lipid bilayer itself; judging from the available data, it is probably involved in both kinds of interactions.

Signal Peptides Partition into Lipid Bilayers

SPs are potent surfactants, and their behavior in various kinds of detergent micelles and lipid mono- and bilayers has been studied in detail.

Briggs, Gierasch and co-workers have used synthetic analogues of the wildtype LamB SP and some of its genetically and biochemically characterized mutant versions in an attempt to correlate the biophysical properties of their interactions with detergents and lipid monolayers with their in vivo properties (Briggs & Gierasch, 1984; Briggs et al., 1985, 1986; Cornell et al., 1989). They found that the nonfunctional mutant peptides have less α-helix content in apolar environments than the wildtype

Key Words Signal peptide · signal sequence · protein secretion · protein export · membrane protein

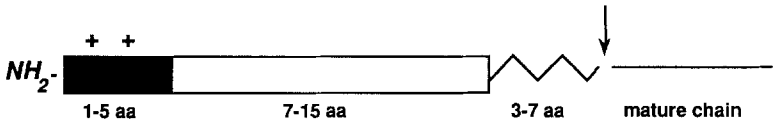

Fig. 1. The basic design of signal peptides

peptide. When exposed to a lipid monolayer, the wildtype SP binds to the surface of the monolayer at high surface pressure in a predominantly β-sheet rich structure, whereas it inserts as an α-helix perpendicularly to the monolayer at physiological surface pressures.

Similar results have also been obtained by the de Kruijff group. They have studied synthetic analogs of the PhoE SP, and have demonstrated that negatively charged phospholipids enhance the binding of the SP to the monolayer. Interestingly, in monolayers containing negatively charged cardiolipin molecules, the initial lipid-bond structure seems to be a "helical hairpin" with a short connecting loop that subsequently straightens out to make a single α-helix across the monolayer (Batenburg et al., 1988*a*,*b*).

Using an in vitro system for protein import into *E. coli* inner membrane vesicles, the same group recently showed that truncated SPs retaining the n- and h-regions could compete efficiently for protein import into the vesicles, with the n-region providing surface binding (probably to anionic phospholipids) and the h-region being primarily responsible for the inhibitory effect (De Vrije et al., 1989). The importance of anionic phospholipids for protein translocation in *E. coli* has also been demonstrated in vivo (De Vrije et al., 1988).

A Positively Charged n-region is Required for Efficient Translocation

The presence of positively charged lysine or arginine residues in the n-region is universal among bacterial SPs and is much preferred in SPs from higher organisms. Possibly the nonformylated and hence positively charged amino group on the initiation Met of the latter can compensate for a lack of lysine or arginine in some cases (von Heijne, 1984*a*).

SPs that lack basic residues or even have acidic residues in their n-region have been constructed by site-specific mutagenesis. Bacterial SPs with a negatively charged n-region have generally been found to be exported more slowly (Vlasuk et al., 1983; Iino, Takahashi & Sako 1987; Lehnhardt et al., 1988; Bosch et al., 1989; Puziss, Fikes & Bassford, 1989), but no absolute requirement for positive charge is apparent.

The n-regions of eukaryotic SPs have not been as extensively mutagenized. One study on yeast invertase failed to find any effect on secretion when various amino acid insertions were made in the n-region (Brown et al., 1984), whereas results on amino acid transpositions in the yeast prepro-α-factor n-region suggest that, in this case at least, the precise order of amino acids may be important (Green, Kramer & Shields, 1989).

A more critical role for amino-terminal positively charged residues in integral membrane proteins with uncleaved SPs have recently been uncovered by the observation that the balance of positive charge across the hydrophobic, membrane-spanning region can determine the final transmembrane topology (Szczesna-Skorupa et al., 1988; von Heijne et al., 1988; Szczesna-Skorupa & Kemper, 1989). This is more fully discussed below in the section on membrane protein assembly.

The h-region is Critical for Translocation

The first export-defective mutant SPs that were isolated all had acquired charged residues or deletions in their h-regions (*see* Oliver, 1985, for a review). Subsequent experiments have suggested that a disruption of the h-region either by charged or helix-breaking residues in most cases lead to a more-or-less severe kinetic defect in translocation, but rarely block export altogether (Michaelis, Hunt & Beckwith, 1986; Stader, Benson & Silhavy, 1986; Fikes et al., 1987; Lehnhardt, Pollitt & Inouye, 1987; Freudl et al., 1988). These results are corroborated by data on pseudo-revertants of export-defective SPs: second-site mutations that increase the hydrophobicity or extend the length of the h-region generally increase the rate of export (Emr & Silhavy, 1983; Bankaitis, Rasmussen & Bassford, 1984; Iida et al., 1985; Ryan et al., 1986).

A second, very interesting class of intragenic revertants involves residues in the mature portion of the protein. Thus, a Gly→Cys change in position 19 of the mature MalE protein partially restores export to a mutant with a defective SP (Ryan et al.,

1986), and a Tyr→Asp substitution in position 283 of the same protein has a similar phenotype (Cover et al., 1987). These results have been interpreted in terms of the concept of "translocation-competent conformations," i.e., the idea that a precursor protein can only be translocated when it is (partially) unfolded (Randall, Hardy & Thom, 1987). Pseudorevertants of the above type may thus be folding mutants that allow the SP more time to interact with the secretory machinery before the precursor folds into an export-incompetent structure.

Although only a handful of h-region mutations have been studied in eukaryotic cells, the effects that have been observed are very similar to what has been found for bacterial SPs (Kaiser & Botstein, 1986; Allison & Young, 1988).

The c-region Specifies the Signal Peptidase Cleavage Site

Early statistical analyses of the sequences surrounding SP cleavage sites led to the postulate that positions −1 and −3 were particularly important for specifying the site of cleavage—the so-called "(−3,−1)-rule" (von Heijne, 1983, 1984*b*, 1986; Perlman & Halvorson, 1983).

Genetical and biochemical studies have largely corroborated this rule (von Heijne, 1988*b*). In addition, one recent work suggests that turn-promoting residues immediately downstream of the cleavage site may enhance the rate of processing by bacterial signal peptidase I (Duffaud & Inouye, 1988).

In the last two years, signal peptide cleavage in eukaryotic cells has been examined in some detail by a number of groups. Although the early statistical data suggested that prolines were not allowed in the −3 to +1 region, it is now clear, both from subsequently sequenced wildtype proteins and from re-designed eukaryotic SPs, that Pro can be tolerated in position −1 (Nagahora, Fujisawa & Jijami, 1988). In addition, prolines in positions −6 to −4 (where they are statistically overrepresented) apparently have a positive effect on the rate of processing (Yamamoto, Taniyama & Kikuchi, 1989), and the optimum distance between a proline and the processing site appears to be 4–5 residues (Notwehr & Gordon, 1989)

A particularly thorough set of studies have been carried out by Gordon and colleagues (Folz & Gordon, 1986, 1987; Folz, Notwehr & Gordon, 1988). Their initial observation was that when the pro-peptide of preproapolipoprotein A-II was deleted, signal peptidase cleavage was redirected to a site two residues downstream of the normal one: from . . . Ser-Leu-Glu-Gly_{18} ↓ Ala-Leu-Val to . . .Ser-Leu-Glu-Gly-Gln-Ala_{20} ↓ Lys-Gln (a similar observation was made by Wiren, Potts and Kronenberg (1988) for preproparathyroid hormone). Since the original cleavage site was left intact in the deletion mutant, the effects of amino acid substitutions around the new cleavage site could be assayed by determining which cleavage site was used when different amino acid replacements were made. Thus, Ala_{20} was systematically replaced with 13 other residues, and the results were that residues "forbidden" by the (−3, −1) rule caused the processing site to revert to the original one, whereas when "allowed" residues were present in position 20, cleavages occurred to varying extents both after residues 18 and 20.

Activation of cryptic cleavage sites have also been reported in two instances when the charged amino terminus of an uncleaved signal peptide of a class I (*see below*) membrane protein has been deleted. Both the Ig class II histocompatibility antigen and the asialoglycoprotein receptor H1 become susceptible to what appears to be signal peptidase-mediated cleavage of their N-terminal signal-anchor domains when the preceding charged region is deleted (Lipp & Dobberstein, 1986; Schmid & Spiess, 1988). Possibly when there are no charges to "fix" the N-terminal end of the transmembrane segment, it may be able to move more freely in the membrane and eventually bind productively to signal peptidase.

The ultimate fate of the SP appears to be complete degradation catalyzed by a number of peptidases, at least in *E. coli*, where both protease IV and oligopeptidase A have been implicated (Novak & Dev, 1988).

Signal Peptides Cleaved by Signal Peptidase II Have a Different c-region

A small number of bacterial lipoprotein SPs are processed by signal peptidase II (Lsp) rather than signal peptidase I (Lep). Cleavage involves, as a first step, addition of a glyceryl moiety to a Cys residue, whereupon the SP is cleaved on the amino-terminal side of the modified cysteine.

A comparative study of SPs cleaved by signal peptidases I and II suggests that the main difference between the two classes resides in the c-region (von Heijne, 1989*a*). A rather well-conserved consensus sequence, Leu-Ala-(Gly,Ala) ↓ Cys, defines the signal peptidase II cleavage site (Duffaud et al., 1986), which is also, in essence, a continuation of the h-region. Thus lacking a polar c-region, lipoprotein SPs are on average 5 residues shorter than SPs cleaved by signal peptidase I. Interestingly, when the normal signal peptidase II cleavage site is

blocked by mutations, nearby cryptic signal peptidase I sites are sometimes uncovered (Ghrayeb et al., 1985).

Positively Charged Residues Downstream of Signal Peptides Can Block Translocations in Bacteria

The first few residues of mature exported bacterial proteins tend to lack positively charged amino acids and have a greater-than-normal content of negatively charged ones (von Heijne, 1986). A first hint that this may reflect functional constraints was provided by Liss, Johnson and Oliver (1985), who found that the PhoA SP could promote export of Staphylococcal nuclease only if an Arg residue immediately following the SP was removed.

The detrimental effect of positively charged residues downstream of bacterial SPs has now been documented in a number of cases, both in vivo and in vitro (Li, Beckwith & Inouye, 1988; Yamane & Mizushima, 1988). In addition, a hydrophobic segment in signal peptidase I from *E. coli* could be engineered to function as an uncleaved SP only when a stretch of predominantly positively charged residues on its C-terminal side was removed (von Heijne, Wickner & Dalbey, 1988; Laws & Dalbey, 1989).

Positively charged residues seem to be more easily tolerated behind eukaryotic SPs, since no comparable lack of them is apparent in the N-terminal part of the mature proteins. The reason for this difference between prokaryotic and eukaryotic SPs is unclear; an attractive idea is that it is related to the presence of an electrochemical potential across the bacterial inner membrane that is lacking in the ER membrane of higher cells.

Signal Peptide-Like Sequences Are Used for Intraorganellar Targeting

Close relatives of secretory SPs have recently been found in the targeting peptides of proteins imported into the inter-membrane space of mitochondria and the lumen of chloroplast thylakoids. These targeting peptides seem to have a modular design, with an amino-terminal piece that serves the primary function of targeting to the appropriate organelle followed by an "SP-like" sequence: a couple of positively charged residues, a hydrophobic stretch, and a more polar region with an apparent (−3,−1)-rule cleavage site (von Heijne et al., 1989). Upon import into the organelle (mitochondrial matrix; chloroplast stroma) the first part is removed, exposing the second part which then assumes the responsibility for further intra-organellar routing (Hartl et al., 1989).

Signal Peptides May Function by Interacting with Both Proteins and Lipids

As the foregoing discussion has shown, SPs are highly variable structures with only a minimal amount of sequence conservation. This was dramatically demonstrated by Kaiser et al. (1987), who found that some 20% of random sequences cloned in front of an SP-lacking invertase gene could promote measurable levels of secretion in yeast. The "functional" sequences found in this way were reminiscent of degenerate SPs, with an enrichment of positively over negatively charged residues and some stretches of hydrophobic or uncharged residues. In most cases, the "SP" was found to be noncleaved, which is not surprising in view of the more highly constrained pattern of amino acids normally present in the region surrounding the signal peptidase cleavage site.

The low degree of sequence conservation and the ability of synthetic SPs to the interact with lipids has led many workers to suggest that the SP interacts directly with the membrane at some critical step in the targeting pathway (von Heijne & Blomberg, 1979; Wickner, 1979; Gierasch, 1989; De Vrije et al., 1989). The other obvious alternative, i.e., binding of the SP to a protein receptor, has received some support from the successful crosslinking of the amino terminus of a eukaryotic SP to the signal recognition particle (SRP) Kurzchalia, Wiedmann & Rapoport, 1986) and to the signal sequence receptor (SSR), an integral protein of the ER membrane (Wiedmann et al., 1987). A third possibility is that one role of the SP is to retard or otherwise modulate the folding of the precursor to allow it more time to interact productively with the export apparatus before it folds into a translocation-incompetent conformation; this idea also has some experimental support (Liu et al., 1988; Laminet & Plückthun, 1989).

In *E. coli,* a number of genes (*sec* genes) have been implicated in the export process (Beckwith & Ferro-Novick, 1986). The SecA protein can bind to newly synthesized preproteins, but not to unfolded proteins lacking their SP (Cunningham et al., 1989). The SecB, GroEL, and "trigger factor" proteins can also bind to unfolded preproteins (Lecker et al., 1989; Kumamoto, 1989), but their precise roles in the targeting process have not yet been defined; most likely, they act as "chaperonins" (Hemmingsen et al., 1988; Ellis & Hemmingsen, 1989) that prevent preproteins from folding into non-translocatable structures.

At any rate, it is clear that the initial interaction between the SP and the membrane/receptor apparatus normally results in a hairpin-like insertion of the SP into the membrane with the positively charged amino terminus left on the cytoplasmic side and the signal peptidase cleavage side positioned close to the extra-cytoplasmic membrane face (Kuhn, 1987; Shaw, Rottier & Rose, 1988). Many studies have shown that cleavage is not essential for translocation of the preprotein, and accumulation of uncleaved but translocated precursors bound to the periplasmic face of the inner membrane of *E. coli* has been obtained in a strain where the production of signal peptidase I was placed under the control of an inducible promoter (Dalbey & Wickner, 1985).

It thus seems that the n- and h-regions are responsible for the targeting function of the SP, with the c-region only being needed for proper removal of the SP from the mature chain. Further sorting information, such as for inner membrane/periplasm/outer membrane sorting in *E. coli,* or for sorting within the secretory pathway of eukaryotic cells, apparently does not reside in the SP (Jackson et al., 1985; MacIntyre et al., 1987; Moore & Kelly, 1986), in spite of the fact that slight statistical differences between SPs from periplasmic and outer membrane proteins have been detected (Sjöström et al., 1987).

Cleaved and Uncleaved Signal Peptides Determine Membrane Protein Topology

Integral membrane proteins come in a variety of transmembrane topologies (Fig. 2). Proteins with one transmembrane region (class I–III) have been called bitopic, and those with two or more are called polytopic (class IV). Class I proteins are made with a cleavable SP and a stop-transfer sequence (a stretch of typically 18–25 hydrophobic residues followed by a cluster of positively charged amino acids); their final orientation after removal of the SP is N_{out}-C_{in}. Class II proteins have an uncleaved signal peptide, and consequently end up with an N_{in}-C_{out} topology. Only a small number of class III proteins with an amino-terminal hydrophobic region oriented N_{out}-C_{in} are known. This class of proteins have an inverted charge polarity across their hydrophobic region compared to normal SPs, with a cluster of positively charged residues flanking it on its carboxy-terminal side. Class IV proteins, finally, have multiple hydrophobic regions spanning the membrane.

As shown schematically in Fig. 2, there is a strong correlation between the transmembrane topology of integral membrane proteins of all classes

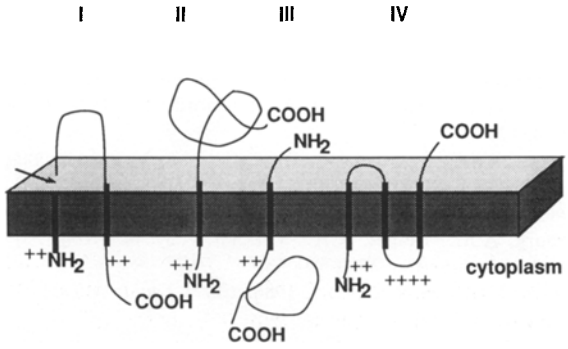

Fig. 2. Classification of integral membrane proteins

and the distribution of positively charged residues around the hydrophobic transmembrane regions. This "positive-inside" rule is observed both for prokaryotic and eukaryotic proteins, although it seems to be slightly stronger for the former group (von Heijne, 1986; von Heijne & Gavel, 1987). It has recently been suggested that the charge distribution around the *first* transmembrane region may be particularly important for setting up the topology of eukaryotic membrane proteins (Hartmann, Rapoport & Lodish, 1989), but the fact remains that a positive-inside charge bias is apparent also for the more C-terminal regions of polytopic eukaryotic proteins.

At any rate, a number of successful attempts at manipulating the transmembrane topology of both bitopic (Szczesna-Skorupa et al., 1988; von Heijne et al., 1988) and polytopic (von Heijne, 1989*b*) proteins on the basis of the "positive-inside" rule have been reported.

Conclusion

It seems safe to conclude that SPs are simply a slightly more "highly evolved" variety of a basic transmembrane peptide design that most likely is very ancient. During its evolutionary refinement, the length of the h-region has become shorter to the point where, in a typical SP, it can probably not reach all the way across the apolar interior of the lipid bilayer in an α-helical conformation. In addition, a "cleavage-cassette" has been attached to the C-terminus of the h-region, making it possible for the cell to remove the translocation signal once it has served its purpose. The most amazing aspect of SP-triggered protein export is perhaps this: that such an exquisite machinery for translocating proteins across membrane has been able to evolve around such a simple piece of polypeptide.

References

Allison, D.S., Young, E.T. 1988. *Mol. Cell. Biol.* **8**:1915–1922

Bankaitis, V.A., Rasmussen, B.A., Bassford, P.J. 1984. *Cell.* **37**:243–252

Batenburg, A.M., Brasseur, R., Ruysschaert, J.M., van Scharrenburg, G.J., Slotboom, A.J., Demel, R.A., de Kruijff, B. 1988*a*. *J. Biol. Chem.* **263**:4202–4207

Batenburg, A.M., Demel, R.A., Verkleij, A.J., de Kruijff, B. 1988*b*. *Biochemistry* **27**:5678–5685

Beckwith, J., Ferro-Novick, S. 1986. *Curr. Topics Microbiol. Immunol.* **125**:5–27

Bosch, D., de Boer, P., Bitter, W., Tommassen, J. 1989. *Biochim. Biophys. Acta* **979**:69–76

Briggs, M.S., Cornell, D. G., Dluhy, R. A., Gierasch, L. M., 1986. *Science* **233**:206–208

Briggs, M.S., Gierasch, L.M. 1984. *Biochemistry* **23**:3111–3114

Briggs, M.S., Gierasch, L.M. 1986. *Adv. Protein Chem.* **38**:109–180

Briggs, M.S., Gierasch, L.M., Zlotnick, A., Lear, J.D., DeGrado, W.F. 1985. *Science* **228**:1096–1099

Brown, P.A., Halvorson, H.A., Raney, P., Perlman, D. 1984. *Mol. Gen. Genet.* **197**:351–357

Cornell, D.G., Dluhy, R.A., Briggs, M.S., McKnight, J., Gierasch, L.M. 1989. *Biochemistry* **28**:2789–2797

Cover, W.H., Ryan, J.P., Bassford, P.J., Walsh, K.A., Bollinger, J., Randall, L.L. 1987. *J. Bacteriol.* **169**:1794–1800

Cunningham, K., Lill, R., Crooke, E., Rice, M., Moore, K., Wickner, W., Oliver, D. 1989. *EMBO J.* **8**:955–959

Dalbey, R.E., Wickner, W. 1985. *J. Biol. Chem.* **260**:15925–15931

De Vrije, T., Batenburg, A.M., Jordi, W., De Kruijff, B. 1989. *Eur. J. Biochem.* **180**:385–392

De Vrije, T., De Swart, R.L., Dowhan, W., Tommassen, J., De Kruijff, B. 1988. *Nature (London)* **334**:173–175

DiRienzo, J.M., Nakamura, K., Inouye, M. 1978. *Annu. Rev. Biochem.* **47**:481–532

Duffaud, G., Inouye, M. 1988. *J. Biol. Chem.* **263**:10224–10228

Duffaud, G.D., Lehnhardt, S.K., March, P.E., Inouye, M. 1986. *Curr. Topics Membr. Transp.* **24**:65–104

Ellis, R.J., Hemmingsen, S.M. 1989. *Trends Biol. Sci.* **14**:339–342

Emr, S.D., Silhavy, T.J. 1983. *Proc. Natl. Acad. Sci. USA* **80**:4599–4603

Engelman, D.M., Steitz, T.A. 1981. *Cell* **23**:411–422

Fikes, J.D., Bankaitis, V.A., Ryan, J.P., Bassford, P.J. 1987. *J. Bacteriol.* **169**:2345–2351

Folz, R.J., Gordon, J.I. 1986. *J. Biol. Chem.* **261**:14752–14759

Folz, R.J., Gordon, J.I. 1987. *J. Biol. Chem.* **262**:17221–17230

Folz, R.J., Notwehr, S.F., Gordon, J.I. 1988. *J. Biol. Chem.* **263**:2070–2078

Freudl, R., MacIntyre, S., Degen, M., Henning, U. 1988. *J. Biol. Chem.* **263**:344–349

Ghrayeb, J., Lunn, C.A., Inouye, S., Inouye, M. 1985. *J. Biol. Chem.* **260**:10961–10965

Gierasch, L.M. 1989. *Biochemistry* **28**:923–930

Green, R., Kramer, R.A., Shields, D. 1989. *J. Biol. Chem.* **264**:2963–2968

Hartl, F.U., Pfanner, N., Nicholson, D.W., Neupert, W. 1989. *Biochim. Biophys. Acta* **988**:1–45

Hartmann, E., Rapoport, T.A., Lodish, H.F. 1989. *Proc. Natl. Acad. Sci. USA* **86**:5786–5790

Hemmingsen, S.M., Woolford, C., van der Vies, S.M., Tilly, K., Dennis, D.T., Georgopoulos, C.P., Hendrix, R.W., Ellis, R.J. 1988. *Nature (London)* **333**:330–334

Iida, A., Groarke, J.M., Park, S., Thom, J., Zabicky, J.H., Hazelbauer, G.L., Randall, L.L. 1985. *EMBO J.* **4**:1875–1880

Iino, T., Takahashi, M., Sako, T. 1987. *J. Biol. Chem.* **262**:7412–7417

Jackson, M.E., Pratt, J.M., Stoker, N.G., Holland, I.B. 1985. *EMBO J.* **4**:2377–2383

Kaiser, C.A., Botstein, D. 1986. *Mol. Cell. Biol.* **6**:2382–2391

Kaiser, C.A., Preuss, D., Grisafi, P., Botstein, D. 1987. *Science* **235**:312–317

Kendall, D.A., Bock, S.C., Kaiser, E.T. 1986. *Nature (London)* **321**:706–708

Kuhn, A. 1987. *Science* **238**:1413–1415

Kumamoto, C.A. 1989. *Proc. Natl. Acad. Sci. USA* **86**:5320–5324

Kurzchalia, T.V., Wiedmann, M., Girshovich, A.S., Bochareva, E.S., Bielka, H., Rapoport, T.A. 1986. *Nature (London)* **320**:634–636

Laminet, A.A., Plücktuhn, A. 1989. *EMBO J.* **8**:1469–1477

Laws, J.K., Dalbey, R.E. 1989. *EMBO J.* **8**:2095–2099

Lecker, S., Lill, R., Ziegelhoffer, T., Georgopoulos, C., Bassford, P.J., Kumamoto, C.A., Wickner, W. 1989. *EMBO J.* **8**:2703–2709

Lehnhardt, S., Pollitt, N.S., Goldstein, J., Inouye, M. 1988. *J. Biol. Chem.* **263**:10300–10303

Lehnhardt, S., Pollitt, S., Inouye, M. 1987. *J. Biol. Chem.* **262**:1716–1719

Li, P., Beckwith, J., Inouye, M. 1988. *Proc. Natl. Acad. Sci. USA* **85**:7685–7689

Lipp, J., Dobberstein, B. 1986. *Cell* **46**:1103–1112

Liss, L.R., Johnson, B.L., Oliver, D.B., 1985. *J. Bacteriol.* **164**:925–928

Liu, G.P., Topping, T.B., Cover, W.H., Randall, L.L. 1988. *J. Biol. Chem.* **263**:14790–14793

MacIntyre, S., Freudl, R., Degen, M., Hindennach, I., Henning, U. 1987. *J. Biol. Chem.* **262**:8416–8422

Michaelis, S., Hunt, J.F., Beckwith, J. 1986. *J. Bacteriol.* **167**:160–167

Moore, H.H., Kelly, R.B. 1986. *Nature (London)* **321**:443–446

Nagahora, H., Fujisawa, H., Jigami, Y. 1988. *FEBS Lett.* **238**:329–332

Notwehr, S.F., Gordon, J.I. 1989. *J. Biol Chem.* **264**:3979–3987

Novak, P., Dev. I.K. 1988. *J. Bacteriol* **170**:5067–5075

Oliver, D. 1985. *Annu. Rev. Microbiol.* **39**:615–648

Perlman, D., Halvorson, H.O. 1983. *J. Mol. Biol.* **167**:391–409

Puziss, J.W., Fikes, J.D., Bassford, P.J. 1989. *J. Bacteriol.* **171**:2303–2311

Randall, L.L., Hardy, S.J., Thom, J.R. 1987. *Annu. Rev. Microbiol.* **41**:507–541

Ryan, J.P., Duncan, M.C., Bankaitis, V.A., Bassford, P.J. 1986. *J. Biol. Chem.* **261**:3389–3395

Schmid, S.R., Spiess, M. 1988. *J. Biol. Chem.* **263**:16886–16891

Shaw, A.S., Rottier, P.J.M., Rose, J.K. 1988. *Proc. Natl. Acad. Sci. USA* **85**:7592–7596

Sjöström, M., Wold, S., Wieslander, Å., Rilfors, L. 1987. *EMBO J.* **6**:823–831

Stader, J., Benson, S.A., Silhavy, T.J. 1986. *J. Biol. Chem.* **261**:15075–15080

Szczesna-Skorupa, E., Browne, N., Mead, D., Kemper, B. 1988. *Proc. Natl. Acad. Sci. USA* **85**:738–742

Szczesna-Skorupa, E., Kemper, B. 1989. *J. Cell Biol.* **108**:1237–1243

Vlasuk, G.P., Inouye, S., Ito, H., Itakura, K., Inouye, M. 1983. *J. Biol. Chem.* **258:**7141–1748
von Heijne, G. 1983. *Eur. J. Biochem.* **133:**17–21
von Heijne, G. 1984*a*. *EMBO J.* **3:**2315–2318
von Heijne, G. 1984*b*. *J. Mol. Biol.* **173:**243–251
von Heijne, G. 1986*a*. *Nucl. Acids Res.* **14:**4683–4690
von Heijne, G. 1986*b*. *J. Mol. Biol.* **192:**287–290
von Heijne, G. 1986*c* *EMBO J.* **5:**3021–3027
von Heijne, G. 1988*a*. *ISI Atlas Sci* **1:**205–209
von Heijne, G. 1988*b*. *Biochim. Biophys. Acta* **947:**307–333
von Heijne, G. 1989*a*. *Protein Engin.* **2:**531–534
von Heijne, G. 1989*b*. *Nature (London)* **341:**456–458
von Heijne, G., Abrahmsén, L. 1989. *FEBS Lett.* **244:**439–446
von Heijne, G., Blomberg, C. 1979. *Eur. J. Biochem.* **97:**175–181
von Heijne, G., Gavel, Y. 1988. *Eur. J. Biochem.* **174:**671–678
von Heijne, G., Steppuhn, J., Herrmann, R.G. 1989. *Eur. J. Biochem.* **180:**535–545
von Heijne, G., Wickner, W., Dalbey, R. 1988. *Proc. Natl. Acad. Sci. USA* **85:**3363–3366
Wickner, W. 1979. *Annu. Rev. Biochem.* **48:**23–45
Wiedmann, M., Kurzchalia, T.V., Hartmann, E., Rapoport, T.A., 1987. *Nature (London)***328:**830–833
Wiren, K.M., Potts, J.T., Kronenberg, H.M. 1988. *J. Biol. Chem.* **263:**19771–19777
Yamamoto, Y., Taniyama, Y., Kikuchi, M. 1989. *Biochemistry* **28:**2728–2732
Yamamoto, Y., Taniyama, Y., Kikuchi, M., Ikehara, M. 1987. *Biochem. Biophys. Res. Commun.* **149:**431–436
Yamane, K., Mizushima, S. 1988. *J. Biol. Chem.* **263:**19690–19696

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