Chapter 1

Signals for Protein Targeting into and across Membranes

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1. INTRODUCTION

How can a newly synthesized protein navigate its way through the maze of organelles found inside a cell to reach its intended destination? This basic question has fascinated workers in cell and molecular biology for a long time; yet, we still do not have all the final answers. Some principles are clear, however. The targeting information is encoded within the nascent protein itself in the form of distinct "signals," either in the form of stretches of amino acids or as surface patches made from discontinuous parts of the protein. These signals are recognized by receptors, which guide the protein to import machineries located in the appropriate organelle. The biochemistry of the various import pathways is covered in other chapters in this book; this chapter presents an overview of the sorting signals themselves, their sequences and structures, and possible modes of function.

2. SIGNALS IN THE SECRETORY PATHWAY

The great majority of secretory proteins produced by eukaryotic cells are made on ribosomes bound to the cytoplasmic surface of the endoplasmic reticu-

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lum (ER) membrane (see Muesch *et al.*, 1990, for a review of an alternative pathway). The growing polypeptide is co-translationally translocated into the lumen of the ER, where folding of the chain is catalyzed by ER-resident proteins such as BiP, prolyl isomerase, and protein disulfide isomerase (Gething and Sambrook, 1992). Once properly folded (Braakman *et al.*, 1992), the protein is transported through the Golgi and post-Golgi compartments to the plasma membrane and is finally secreted from the cell.

To control the passage through the secretory pathway, a number of signals are used. The default is secretion from the cell; in this case, an N-terminal signal peptide is required to target the protein to the ER, and no additional signals are needed. Proteins designed to be retained in one or other of the compartments along the pathway are provided with specific retention signals in addition to the signal peptide. Finally, some membrane proteins cycle continuously between the plasma membrane and endosomal compartments, and thus need endocytosis signals.

2.1. Signal Peptides

Signal peptides are found both in prokaryotic and eukaryotic proteins and seem to have been largely conserved throughout evolution. A functional tripartite design has been defined both through statistical sequence analyses and molecular genetic studies: a positively charged N-terminal end (n-region), a central, hydrophobic stretch (h-region), and a more polar C-terminal segment (c-region) (Gierasch, 1989; von Heijne, 1990). Targeting information is contained in the n- and h-regions, whereas the c-region serves as the recognition site for a signal peptidase that removes the signal peptide once membrane translocation has been initiated.

2.1.1. The n-Region

In bacterial signal peptides, at least one positively charged lysine or arginine is always found in the n-region. Not surprisingly, when these charged residues are removed or replaced with negatively charged ones, the mutant signal peptide functions less efficiently (Lehnhardt *et al.*, 1988; Puziss *et al.*, 1992). It is not clear exactly why this is so, though *in vitro* it has been shown that positively charged residues in the n-region enhance the affinity of the SecA protein for the signal peptide (Akita *et al.*, 1990). Certain mutations in the *secY* gene can suppress the secretion defect caused by an acidic n-region (Puziss, *et al.*, 1992).

Eukaryotic signal peptides also tend to have a positively charged n-region, but this is not an absolute rule (von Heijne, 1984) and quite a few signal peptides with acidic n-regions are known. The role of the n-region has not been exten-

sively studied in eukaryotic systems, although n-region mutations that affect secretion have been described (Green *et al.*, 1989).

2.1.2. The h-Region

To function well, the h-region must be of a certain minimal length and hydrophobicity. Recent studies on a range of h-region mutants (Hoyt and Gierasch, 1991) suggest that the average hydrophobicity in this region must be $\geq$ 2.4 on the Kyte-Doolittle scale (Kyte and Doolittle, 1982). Homopolymeric h-regions composed only of leucine, isoleucine, or phenylalanine residues function well, whereas valine, alanine, and tryptophan h-regions work less well or not at all (Chou and Kendall, 1990; Rusch and Kendall, 1992). The minimum length of a functional h-region seems to be $\sim$ 7 residues (von Heijne, 1985; Yamamoto and Kikuchi, 1989; Chou and Kendall, 1990; Hikita and Mizushima, 1992). If, on the other hand, the h-region becomes too long the c-region may be moved out of reach of the signal peptidase enzyme, and the protein becomes permanently anchored to the membrane (Chou and Kendall, 1990).

It appears that the h-region may interact both with proteins and lipids at different stages during the initiation of translocation. In bacteria, the signal peptide can bind to the SecA protein (Akita *et al.*, 1990; Schatz and Beckwith, 1990; Wickner *et al.*, 1991), and in eukaryotic cells it interacts with the 54 kDa subunit of the signal recognition particle (SRP) (High and Dobberstein, 1991; Lutcke *et al.*, 1992). There is strong circumstantial evidence, mainly from biophysical studies of synthetic signal peptides, that signal peptides also interact directly with the lipid bilayer (Demel *et al.*, 1990; Hoyt and Gierasch, 1991).

2.1.3. The c-Region

The c-region is not necessary for targeting to the secretory pathway; rather, its function is to provide a cleavage site for the signal peptidase enzyme. The cleavage site is defined by the "(−3,−1)-rule" (Perlman and Halvorson, 1983; von Heijne, 1983; von Heijne 1986d), which states that only small, uncharged residues are allowed in positions −1 and −3 relative to the cleavage site. The critical importance of these two positions has now been amply documented (Folz *et al.*, 1988; Fikes *et al.*, 1990; Shen *et al.*, 1991). A helix-breaking residue often provides a point of demarcation between the h- and c-regions (von Heijne, 1985) and appears, at least in some signal peptides, to help present the cleavage site in the correct conformation (Yamamoto *et al.*, 1989; Shen *et al.*, 1991).

Prolines are nearly completely absent from position +1, and it has recently been found not only that Pro_{+1} prevents signal peptide cleavage in *E. coli*, but also that expression of proteins with this mutation competitively inhibits the signal peptidase I (Lep) enzyme (Barkocy-Gallagher and Bassford, 1992;

Nilsson and von Heijne, 1992). Presumably, the Pro_{+1} does not influence the binding of the signal peptide to the signal peptidase but only prevents cleavage, leaving the signal peptide stuck on the enzyme.

Signal peptidases do not appear to belong to any of the standard groups of proteases (the serine, cysteine, aspartic acid, and metallopeptidase), and may constitute a new protease family with a possible mechanistic similarity to the β-lactamases (Black *et al.*, 1992; Sung and Dalbey, 1992).

2.1.4. The "Charge-Block Effect"

Although not part of the signal peptide proper, a region encompassing the first 10–20 residues of the mature protein is also critical for the initiation of membrane translocation, at least in *E. coli* (Andersson and von Heijne, 1991). This region normally contains few positively charged amino acids (von Heijne, 1986c), and the introduction of only one or two extra lysines or arginines can dramatically affect secretion (Li *et al.*, 1988; Yamane and Mizushima, 1988; Boyd and Beckwith, 1989; Laws and Dalbey, 1989; Summers *et al.*, 1989; Zhu and Dalbey, 1989; Boyd and Beckwith, 1990; MacIntyre *et al.*, 1990). A similar blocking effect has also been demonstrated in eukaryotic secretory proteins, although only with much higher numbers of charged residues (Kohara *et al.*, 1991; Johansson *et al.*, 1992). One implication of these observations is that many eukaryotic proteins may be difficult to export from bacteria simply because the N-terminal region of the mature chain carries too many positively charged amino acids.

2.2. ER-Retention Signals

A number of proteins are specifically retained in the ER through a retrieval mechanism where molecules that escape toward the Golgi are recognized by a recycling receptor and delivered back to the ER (see Chapter 4). Two distinct retention signals have been discovered: a C-terminal tetrapeptide sequence, Lys-Asp-Glu-Leu (KDEL, or HDEL in yeast) normally found on soluble proteins in the ER lumen (Pelham, 1990), and a cytoplasmically exposed C-terminal tail with two or more apparently critical lysines and possibly some additional important characteristics found on proteins that span the ER membrane (Gabathuler and Kvist, 1990; Jackson *et al.*, 1990). Recently, a yeast membrane protein with a lumenally exposed C-terminus was found to end with a typical HDEL-signal (Sweet and Pelham, 1992), suggesting that membrane proteins with the appropriate topology can also be retrieved through the KDEL (or HDEL) receptor system.

2.3. Golgi-Retention Signals

Golgi-retention signals have been localized to transmembrane domains, including short cytoplasmic and lumenally exposed flanking regions, in at least

three different proteins (Hurtley, 1992). The coronavirus E1 protein has three transmembrane segments, but only the N-terminal segment can confer Golgi retention onto non-Golgi fusion partners (Swift and Machamer, 1991). Some point-mutations in this transmembrane segment abolish retention. Retention signals in two single-spanning Golgi proteins, the α-2,6-sialyltransferase (Munro, 1991; Colley *et al.*, 1992) and β-14-galactosyltransferase (Nilsson *et al.*, 1991; Teasdale *et al.*, 1992), have also been found in the transmembrane and polar flanking segments. Interestingly, in the case of α-2,6-sialyltransferase, it has been shown that the hydrophobic segment can be replaced by 17 leucines with no adverse effects on retention; when the hydrophobic segment is lengthened to 23 leucines, retention is no longer observed (Munro, 1991), suggesting that there are cytoplasmic and lumenal determinants that must be kept at a fixed distance apart for efficient retention.

2.4. Lysosomal-Targeting Signals

Soluble lysosomal proteins are diverted from the main secretory pathway in the *trans*-Golgi network. This is accomplished by the recognition of mannose-6-phosphate residues on the protein. The critical step in lysosomal protein biogenesis is the action of the *N*-acetylglucosamine-1-phosphotransferase enzyme that is responsible for the first step in the conversion of mannose residues to mannose-6-phosphate (Kornfeld and Mellman, 1989). This enzyme recognizes a "patch" on the surface of the protein rather than a linear string of amino acids (Baranski *et al.*, 1990; Baranski *et al.*, 1991), although it is not known precisely what features of the patch are critical (see Chapter 7).

2.5. Endocytosis Signals

Many plasma membrane proteins are endocytosed in response to a signal located in their cytoplasmic domains. This signal has been shown to contain a critical tyrosine residue (Peters *et al.*, 1990) located in a short segment that by NMR (nuclear magnetic resonance) analysis (Bansal and Gierasch, 1991; Eberle *et al.*, 1991) appears to form a reverse turn. Some lysosomal membrane proteins are first directed to the plasma membrane, then endocytosed in response to a tyrosine signal, and finally delivered to the lysosome (Peters *et al.*, 1990), although other pathways for lysosomal targeting of membrane proteins also exist (Vega *et al.*, 1991).

3. SIGNALS FOR MITOCHONDRIAL PROTEIN IMPORT

The signals and import machinery responsible for the import of proteins into mitochondria have been extensively studied (Glick and Schatz, 1991; Pfanner *et*

al., 1992; see Chapter 5). Four different intramitochondrial locations can be defined: the outer and inner membranes, the intermembrane space, and the matrix; a matching set of targeting signals has evolved to ensure proper routing of the imported protein.

3.1. Matrix-Targeting Peptides

The most well-characterized mitochondrial import signal is the matrix-targeting variety. As in the case of secretory signal peptides, there is no conserved "consensus" sequence; rather, the basic feature is the presence of a positively charged amphiphilic α-helix (Roise *et al.*, 1986; von Heijne, 1986b). The importance of the amphiphilic helix has been demonstrated both by extensive mutational analysis (Bedwell *et al.*, 1989; Lemire *et al.*, 1989) and directly through structure determination by NMR (Endo *et al.*, 1989). It is thought that translocation of the positively charged presequence is driven by the electrical component of the proton motive force across the inner membrane (Martin *et al.*, 1991).

After import, the matrix-targeting signal is cleaved either once or twice by matrix proteases. The first cleavage is catalyzed by a two-subunit enzyme (Pollock *et al.*, 1988; Yang *et al.*, 1988). Although not strictly conserved, the cleavage-site is usually defined by an arginine in position −2, or by an Arg-X-Tyr motif (with cleavage taking place immediately after the tyrosine) (Gavel and von Heijne, 1990a). About a third of all imported proteins are cleaved a second time by a distinct protease (Kalousek *et al.*, 1988), which removes an additional eight (or, in some cases, nine) N-terminal amino acids (Hendrick *et al.*, 1989; Gavel and von Heijne, 1990a). The octa-peptide can only be removed if it has a hydrophobic N-terminal amino acid (typically Phe), and if some other less well understood criteria are met by the sequence downstream of the octa-peptide (Isaya *et al.*, 1992).

3.2. Outer Membrane-Targeting Signals

The targeting of two proteins of the outer mitochondrial membrane, porin and Mas70p, has been studied rather extensively. Porin does not have a cleavable targeting signal, and does not require a potential across the inner membrane. It does, however, require ATP for import, and utilizes at least a part of the same machinery as matrix proteins (Kleene *et al.*, 1987; Pfanner *et al.*, 1987; Hwang and Schatz, 1989). It is not clear what portion or portions of the chain contain the targeting information.

Mas70p, on the other hand, has a clearly demarcated N-terminal targeting signal, although it is not cleaved upon import. The first 12 residues have a net positive charge, and can, by themselves, mediate import into the matrix (Hurt *et al.*, 1985; Nakai *et al.*, 1989). The following ~ 25 residues are quite apolar, and apparently provide a stop-transfer function, anchoring the protein to the outer

membrane with the large C-terminal domain projecting outside the organelle (Reizman *et al.*, 1983; Hase *et al.*, 1984).

3.3. Intermembrane Space-Targeting Signals

There are at least three different pathways for import into the intermembrane space, exemplified by cytochrome b_2, cytochrome *c* heme lyase, and cytochrome *c*. Cytochrome b_2 has a bipartite targeting signal, with a typical N-terminal matrix-targeting part immediately followed by a second signal with many of the characteristics of a signal peptide for protein secretion (von Heijne *et al.*, 1989). The import pathway dictated by this combination of signals is first into the matrix, where the matrix-targeting part is removed, and then re-export across the inner membrane (Koll *et al.*, 1992). Because the latter step is similar to bacterial protein export (and thus presumably an evolutionary remnant from the pre-endosymbiont days of the mitochondrion), this pathway has been termed "conservative sorting" (Hartl and Neupert, 1990).

Cytochrome *c* heme lyase lacks a cleavable presequence, but is nevertheless targeted to the normal import machinery in the outer membrane. However, it never reaches the matrix compartment, but rather appears to deviate from the standard path after passage through the outer membrane, and it ends up in the intermembrane space (Lill *et al.*, 1992). It is possible that the lack of a typical matrix-targeting signal prevents its import into the matrix; it is not known what other feature or features of the nascent chain guide its targeting.

Cytochrome *c*, finally, is imported directly through the outer membrane into the intermembrane space. Apo-cytochrome *c* binds specifically to outer membrane lipids and appears to partly penetrate the membrane spontaneously (Jordi *et al.*, 1989). Full import is catalyzed by cytochrome *c*, and thus converts it to the soluble holo-enzyme (Nargang *et al.*, 1988; Nicholson and Neupert, 1989). In this case, no specific signal seems to be involved, and both targeting and import depend on global properties of the apo-cytochrome *c* molecule.

4. SIGNALS FOR CHLOROPLAST PROTEIN IMPORT

In terms of intra-organellar targeting, protein import into chloroplasts is even more complex than for mitochondria. With its two envelope membranes and the thylakoid stacks, there are at least six different compartments within this organelle (see Chapter 6).

4.1. Stromal Transit Peptides

Stromal transit peptides vary greatly in both length and sequence, yet some common characteristics can be defined (von Heijne *et al.*, 1989). Transit peptides

are notably rich in hydroxylated amino acids (Ser in particular), and contain few or no acid residues. They do not appear to be designed to form well-defined secondary structures such as amphiphilic α-helices, and are in fact mostly predicted as random coils (von Heijne and Nishikawa, 1991). Model studies have shown that transit peptides can bind to lipid monolayers containing acidic phospholipids and galactolipids typically found in the chloroplast envelope (van't Hof *et al.*, 1991), although the binding is weaker than for mitochondrial targeting peptides. Competition experiments using an *in vitro* import system and synthetic peptides corresponding to different parts of a transit peptide have suggested that the central region may be involved in the initial binding to the chloroplast surface, and that the N-terminal and C-terminal ends rather affect a later step in the import pathway (Perry *et al.*, 1991).

No highly conserved cleavage-site motif for the stromal processing peptidase has been found, although ~ 1/3 of all known transit peptides end with a loosely conserved VX(A/C) ↓ A pattern (Gavel and von Heijne, 1990b).

4.2. Thylakoid Transfer Domains

Similar to the mitochondrial intermembrane space proteins, proteins destined for the lumen of the thylakoids have a bipartite targeting signal composed of an N-terminal stromal transit peptide followed by a thylakoid transfer domain with all the characteristics of a secretory bacterial signal peptide (von Heijne *et al.*, 1989; Bassham *et al.*, 1991). Indeed, thylakoid transfer domains have been shown to function as signal peptides in *E. coli* (Meadows and Robinson, 1991). Upon import into the thylakoid, the transfer domain is cleaved by a peptidase with a substrate specificity very similar to the *E. coli* signal peptidase I (Halpin *et al.*, 1989; Anderson and Gray, 1991; Shackleton and Robinson, 1991). Whether the stromal transit peptide has to be removed prior to thylakoid import is somewhat controversial (Bauerle *et al.*, 1991; Bauerle and Keegstra, 1991; Mould *et al.*, 1991).

4.3. Envelope-Targeting Signals

Two proteins of the outer envelope membrane have been cloned, and their import has been partly characterized (Salomon *et al.*, 1990; Li *et al.*, 1991). Neither has a cleavable presequence, nor do they require ATP for proper membrane integration. The chloroplasts can be treated with thermolysine with no effect on the import, suggesting that insertion is "spontaneous" and independent of proteinaceous factors in the outer envelope membrane.

Two inner membrane proteins, the phosphate translocator and a 37-kDa protein of unknown function, have also been cloned (Flügge *et al.*, 1989; Dreseswerringloer *et al.*, 1991; Flügge *et al.*, 1991). These proteins appear to use the

normal import pathway, although their presequences do not look like typical stroma-targeting transit peptides.

5. SIGNALS FOR NUCLEAR PROTEIN IMPORT

Nuclear import is mechanistically very different from the other import processes discussed so far (Silver, 1991; see Chapter 9). The nuclear pore complex allows passage of small macromolecules by passive diffusion, and only larger proteins require active, signal-sequence-dependent transport across the nuclear envelope. Nuclear-targeting sequences are integral parts of the fully folded, mature protein, can act cooperatively, and presumably need to be exposed on the surface of the molecule. They may bind to cytosolic receptors that in turn mediate the interactions with the nuclear pore (Adam and Gerace, 1991), and then piggy-back into the nucleus.

Nuclear-targeting sequences have long been known to be composed of clusters of basic amino acids (Lys and Arg), and it has recently been proposed that a common consensus motif is defined by a dibasic pair, a spacer segment of any ten amino acids, and a second basic cluster with at least three out of five basic residues (Dingwall and Laskey, 1991; Robbins *et al.*, 1991). About half of all known nuclear proteins contain this motif; in contrast, it is found in only 4% of all nonnuclear proteins.

6. SIGNALS FOR PEROXISOMAL PROTEIN IMPORT

The mechanism of protein import into peroxisomes is largely unknown, although at least three import-deficient mutants have been found that map to peroxisomal membrane proteins (Erdmann *et al.*, 1991; Hohfeld *et al.*, 1991; Tsukamoto *et al.*, 1991). Some genetic evidence also shows that at least two independent import pathways exist (Walton *et al.*, 1992); this conclusion is strengthened by the recent identification of two very different kinds of targeting signals: one C-terminal and one N-terminal (see Chapter 8).

6.1. The SKL Signal

A C-terminal Ser-Lys-Leu (SKL) motif is found on many peroxisomal proteins and has been shown to act as a targeting signal when transplanted onto reporter proteins (Gould *et al.*, 1989; Miyazawa *et al.*, 1989; Gould *et al.*, 1990a; Gould *et al.*, 1990b). Some conservative substitutions are allowed (Gould *et al.*, 1989), although the C-terminal Leu seems to be absolutely required. Furthermore, the signal only works from a C-terminal and not from internal positions.

6.2. Amino-Terminal Import Signals

Many peroxisomal proteins do not have SKL signals, and hence must be targeted in some other fashion. An N-terminal, cleavable-targeting signal was recently identified in rat 3-ketoacyl-CoA thiolase (Osumi *et al.*, 1991; Swinkels *et al.*, 1991). Deletion analysis suggested that the necessary information is carried by the 11-residue stretch MHRLQVVLGHL. Related N-terminal extensions are present in peroxisomal thiolases from other organisms, as well as in a plant glyoxysomal malate dehydrogenase. It is perhaps noteworthy that two tripeptides related to the SKL-motif (HRL and GHL) are found in the thiolase presequence, although the SKL pathway is probably not used by these proteins (Swinkels *et al.*, 1991).

7. SIGNALS FOR MEMBRANE PROTEIN ASSEMBLY

Just like soluble proteins, integral membrane proteins must be targeted to the appropriate organelle and suborganellar compartment, but in addition they must contain signals that guide their integration into the target membrane. These signals define the topology of the molecule, i.e., the number of transmembrane segments and the overall orientation relative to the membrane.

7.1. Topological Signals

Most membrane proteins depend on the cell's secretory machinery for their insertion, and hence use signal peptides for targeting and for triggering the initial translocation event. This is true in bacteria, where both inner and outer membrane proteins are made with (cleavable or uncleavable) N-terminal signal peptides, as well as in eukaryotic cells. Proteins of the plasma membrane and the membranes of the exocytic pathway are inserted into the ER membrane and further transported to their intended compartment. Mitochondrially encoded inner membrane proteins also seem to use signal peptide-like targeting peptides, as do thylakoid membrane proteins (see the relevant sections above).

The transmembrane topology is primarily determined by two features of the amino acid sequence: the number and positions of the stretches of apolar amino acids that form the transmembrane segments proper, and the distribution of positively charged amino acids (Lys and Arg) in the loops connecting the transmembrane segments (von Heijne and Manoil, 1990).

Typical transmembrane segments are 15–20 residues long and highly enriched in apolar amino acids. A stretch of as little as 8–10 consecutive leucines is sufficient to impart a transmembrane topology, whereas a much longer segment is needed if alanines are used instead (Kuroiwa *et al.*, 1991). Thus, overall

hydrophobicity seems to be the main factor that distinguishes transmembrane from nonmembrane segments.

The orientation of the transmembrane segments is largely controlled by flanking positively charged amino acids. This was first suggested by a statistical analysis of bacterial inner membrane proteins, where it was shown that Arg and Lys residues were many times more abundant in cytoplasmically exposed parts compared to periplasmic parts (von Heijne, 1986a)—the "positive inside-rule." Similar observations have since been made for eukaryotic plasma membrane proteins (von Heijne and Gavel, 1988; Hartmann *et al.*, 1989), for thylakoid membrane proteins (Gavel *et al.*, 1991), and for mitochondrially encoded proteins of the inner mitochondrial membrane (Gavel and von Heijne, 1992).

The positive inside-rule has been tested experimentally in a number of systems. In *E. coli*, positively charged amino acids have a decisive influence on the orientation of inner membrane proteins (Boyd and Beckwith, 1989; von Heijne, 1989; Boyd and Beckwith, 1990; Nilsson and von Heijne, 1990; McGovern *et al.*, 1991; Andersson *et al.*, 1992), and they clearly play a role also for eukaryotic membrane proteins, although the results obtained so far are less clear-cut than for *E. coli* (Haeuptle *et al.*, 1989; Szczesna-Skorupa and Kemper, 1989; Sato *et al.*, 1990; Parks and Lamb, 1991; Andrews *et al.*, 1992).

7.2. Degradation Signals

The ER is a site of "quality control" that only allows the further transport along the exocytic pathway of proteins that have folded and oligomerized properly (Desilva *et al.*, 1990). Recently, it has been found that isolated subunits of oligomeric membrane proteins may have "degradation signals," possibly in the form of a charged residue, in their transmembrane domains; these are signals that become masked upon oligomerization (Bonifacino *et al.*, 1990a,b; Klausner and Sitia, 1990; Bonifacino *et al.*, 1991). The way in which these signals are recognized and the proteolytic system responsible for degradation have not yet been characterized.

8. CONCLUSIONS

Protein trafficking in the prokaryotic and eukaryotic cell depends on an intricate system of signals, receptors, and membrane-translocation machineries. Some of the signals are well characterized and can often be rather easily recognized in the primary amino acid sequence.

A measure of our understanding of these signals can be obtained by trying to formulate rules or patterns that discriminate among different signals, and then let a computer "direct" proteins to different compartments. This approach was re-

cently tried on a sample of bacterial proteins, with a "sorting" algorithm based on a number of published (and unpublished) methods for detecting various targeting signals (Nakai and Kanehisa, 1991). The result was rather encouraging: About 80% of the proteins in each class (cytoplasm, inner membrane, periplasm, and outer membrane) were assigned to the correct compartment. A similar study focusing on eukaryotic protein sorting with a much larger number of possible compartments (nucleus, cytoplasm, ER membrane, ER lumen, Golgi, lysosome membrane, lysosome lumen, plasma membrane, peroxisome, mitochondrial matrix, inner membrane, intermembrane space, and outer membrane, and the extracellular medium) reached almost 60% correct predictions (Nakai, 1991).

Statistical and experimental studies of integral membrane proteins have also led to improvements in our ability to predict the transmembrane structure from the amino acid sequence; a recent method employing hydrophobicity analysis coupled with a screening procedure based on the positive inside-rule, in fact, managed to identify correctly the transmembrane segments and predict the correct orientation for 23 out of 24 bacterial inner membrane proteins of known sequence and topology (von Heijne, 1992).

In summary, then, we know quite a lot about the signals of protein sorting, and we are beginning to unravel the mechanisms of signal recognition and membrane translocation. Beyond their biological significance, these studies have also made it clear that we have a long way to go before our own mail services and overnight courier deliveries work as efficiently as Mother Nature's own inventions.

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