Nodulin-24 Follows a Novel Pathway for Integration into the Peribacteroid Membrane in Soybean Root Nodules*

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Nodulin-24 is a nodule-specific protein of the peribacteroid membrane (PBM) in soybean. It has an apparent molecular mass of 33 kDa while its full-length cDNA encodes a polypeptide of only 24 kDa. In vitro transcription of nodulin-24 cDNA followed by translation resulted in a peptide translocated into microsomal membranes with cleavage of a signal sequence. The cleavage site of the signal sequence in nodulin-24 was determined to be between Ala (A25) and Arg (R26) by microsequencing of the [3H]leucine-labeled processed peptide. Fusion of the signal sequence of nodulin-24 with the β-glucuronidase peptide prevented co-translational cleavage of the signal sequence although the translocation of the fused protein into microsomes occurred co-translationally. Trypsin treatment of membrane-translocated nodulin-24 did not result in any alteration in size suggesting that the newly synthesized peptide is fully protected in the membrane vesicle. Fusion of nodulin-24 with β-glucuronidase also showed no change in size following trypsin treatment, suggesting that nodulin-24 has no membrane-spanning region. In addition, in vitro synthesized nodulin-24 was present in the supernatant fraction after sonication of microsomal membranes. Mature nodulin-24, on the other hand, is not solubilized from PBM by sodium carbonate (pH 11) or EGTA and is soluble only in detergent. These data suggest that nodulin-24 is synthesized as a luminal protein in the endoplasmic reticulum and post-translationally attached to the membranes en route to the PBM. This processing results in a significant increase in the apparent molecular mass of nodulin-24 which may be due to the attachment of membrane lipids as this protein shares characteristics with membrane lipoproteins of many pathogenic bacteria.

Successful invasion of legume roots by rhizobia, followed by endocytosis of the bacteria into the host cytoplasm, results in the development of root nodules effective in nitrogen fixation (Verma, 1992; Brewin, 1991). Rhizobia are segregated inside the host cell into a subcellular compartment surrounded by a membrane, the peribacteroid membrane (PBM), of host origin (Verma et al., 1978). The PBM plays critical roles in symbiosis as all metabolic exchanges between the two partners occur through this membrane (Verma and Fortin, 1989).

Many nodule-specific host proteins (nodulins) have been isolated (Delauney and Verma, 1988) and some of them, e.g. nodulin-24 and nodulin-26, have been localized to the PBM. Nodulin-26 is an intrinsic membrane protein and lacks a cleavable signal sequence (Miao et al., 1992). We have demonstrated that both amino and carboxyl ends of nodulin-26 face the host cell cytoplasm (Miao et al., 1992). On the other hand, nodulin-24 appears to be associated with the PBM and was suggested to be located on the surface facing the bacteroids (Fortin et al., 1987). The nodulin-24 gene contains five exons, three of which (exons 2–4) encode a repeated amphipathic domain (Fortin et al., 1985; Katinakis and Verma, 1985). In vitro translation of nodulin-24 mRNA in the presence of microsomal membranes suggested that nodulin-24 is processed co-translationally into a 20-kDa polypeptide (Katinakis and Verma, 1985). However, when PBM proteins were reacted with antibody against nodulin-24, the size of native nodulin-24 was found to be about 33 kDa, suggesting that this nodulin undergoes a significant post-translational modification (Fortin et al., 1985). Nodulin-24 has homologies to nodulin-16 and a few glycine-rich plant proteins in signal peptide and COOH-terminal regions (Nirunaksiri and Sengupta-Gopalan, 1990; Sandal et al., 1992).

The nature of the PBM is unique as it possesses properties common to both plasma membrane and tonoplast (Verma et al., 1978; Miao et al., 1992). The biogenesis of the PBM compartment requires extensive vesicular transport (Cheon et al., 1993) and specific targeting of PBM and peribacteroid fluid nodulins. It is not known how PBM nodulins are specifically targeted to this de novo formed subcellular compartment. All PBM nodulins are synthesized on membrane-bound polysomes (Jacob et al., 1987) and co-translationally inserted into the membrane irrespective of whether they carry a cleavable signal sequence or not. Co-translationally cleavable signal sequences of proteins contain a positively charged region and a hydrophobic core (von Heijne, 1983, 1986), but the downstream domain flanking the cleavage site is not well characterized. The machinery to cleave the signal sequence is conserved between animals and plants (Chrispeels, 1991).

In this study, we examined synthesis, processing, and topology of nodulin-24 using in vitro translated nodulin-24 peptides and isolated PBM. In addition, the cleavage site of the signal sequence in nodulin-24 was determined by microsequencing of [3H]leucine-labeled in vitro translation product. The requirements for signal peptide cleavage were tested by protease-protection assay of nodulin-24 and β-glucuronidase fusion proteins. The results suggest that nodulin-24 is synthesized on the ER-bound ribosomes and is released into the lumen of the ER. Further post-translational processing, presumably in Golgi, attaches this protein to the membrane. Its overall hydrophobic and amphipathic character and the presence of a characteristic lipid-binding domain may allow this nodulin to be buried in the lipid of the PBM surface facing the bacteroids.
**Experimental Procedures**

Materials—Soybean (Glycine max L. cv. Prize) root nodules were obtained as described (Cheon et al., 1993). *In vitro* transcription and translation systems were from Promega Corp. (Madison, WI). [*H]Leucine (142 Ci/mmol) and [*35S]methionine (1000 Ci/mmol) were from Amersham Corp.

Plasmid Constructions—For *in vitro* transcription, a series of fusion constructs of nodulin-24 and β-glucuronidase were made as follows: a HindIII-PstI restriction fragment from pN24, a PUC19 derivative containing the nodulin-24 cDNA insert from pNod20 (Katinakis and Verma, 1985), was cloned downstream of the bacteriophage T7 promoter in PGEM2 (Promega Corp., Madison, WI). Constructs T1 and T2 were made by cloning cDNA fragments containing sequences corresponding to exon 1, and exons 1–2, respectively, of the nodulin-24 gene (see sequence accession no. M10595) into XbaI and Smal-cut phiB221. The fragment containing exon 1 was obtained by digestion of pN24 with XbaI and DraI, followed by elution of a 0.1-kb band from an agarose gel, while the fragment containing exons 1–2 was obtained by digestion of pN24 with PstI and mungbean nuclease followed by digestion with XbaI. The entire length of the nodulin-24 cDNA, except the termination codon, was amplified by polymerase chain reaction and fused with the β-glucuronidase gene, resulting in the formation of construct T0. All the above constructs were subcloned into PGEM2 for *in vitro* transcription.

*In Vitro Transcription—Plasmids were linearized by cutting with appropriate restriction enzymes downstream of the gene of interest and transcribed with T7 RNA polymerase according to the manufacturer's instructions.

*In Vitro Translation and Processing of the Products—*In vitro synthesized transcripts were translated in a rabbit reticulocyte lysate using either [*35S]methionine or [*3H]leucine and canine microsomal membranes (Promega Corp., Madison, WI). The protease protection assay was carried out as described (Spies and Lodish, 1986), and the labeled peptides were immunoprecipitated (Anderson and Blobel, 1983) with nodulin-24 or β-glucuronidase antibody. *In vitro* translation, the reaction mixture was centrifuged at 356,000 g for 20 min at 4 °C. The pellet was dissolved in STBS buffer (0.25 M sucrose, 10 mM Tris-HCl, 150 mM NaCl). Protease digestion with either trypsin, tosylomethyl-2-phenylthyl chloromethyl ketone-treated trypsin or proteinase-K (final concentration of 100 µg/ml) was carried out on ice for 1 h in the absence or presence of Triton X-100 (TX-100) at a final concentration of 1%. ConA-Sepharose 4B (Pharmacia LKB Biotechnology Inc.) binding was performed following immunoprecipitation protocol and replacing protein A with ConA-Sepharose. *In vitro* translated products were subjected to ten 5-s pulses of sonication (Sonifier 450, Branson Sonic Power Co., Danbury, CT) at maximum output and then centrifuged at 356,000 g for 30 min at 4 °C. Samples were resolved in 10% or 14% SDS-polyacrylamide gels. Following electrophoresis, labeled peptides were subjected to tryptic digestion with and without the addition of TX-100. Microsomal membranes, and immunoprecipitated with poly- conal antibody against nodulin-24 (see "Experimental Procedures"). As expected, nodulin-24 was processed into a 20-kDa polypeptide in the presence of microsomal membranes. Tryptic digestion of the reaction mixture did not change the size of this polypeptide (Fig. 1, lane 3), although trypsin together with a nonionic detergent digested the processed nodulin-24 completely (Fig. 1, lane 4). This suggests that no part of the processed nodulin-24 protrudes from the ER into the cytoplasm.

In order to define the cleavage site of the signal sequence, the most probable cleavage site in nodulin-24 was first identified using a procedure described by von Heijne (von Heijne, 1986). Fig. 2A shows *S(i)* values at each potential cleavage site which were obtained by summing the weights of residues including positions −13 and +2 relative to each site. The highest *S(i)* value assign the cleavage site between Ala (A25) and Arg (R26). This site also satisfies the criteria of the (−3, −1) rule for cleavage (von Heijne, 1983; Perlman and Halvorson, 1983) with Val in the relative position −3 and Ala in the relative position −1. For experimental determination of the cleavage site, the [*3H]leucine-labeled co-translationally processed product of nodulin-24 was subjected to Edman degradation. Radioactive peaks were found at the 3rd and the 21st cycles of sequential degradation which correspond to the positions of leucine residues in the deduced sequence of nodulin-24, if the cleavage occurred as predicted above. The data suggest that nodulin-24 is co-translationally processed into a mature polypeptide of 122 residues with arginine at the amino terminus.

The *Entire Nodulin-24 Sequence Is Required for Signal Cleavage*—The putative signal peptide of nodulin-24 identified above was tested for its role in targeting a protein to the ER. Different lengths of nodulin-24 cDNA were fused in-frame with the β-glucuronidase gene (Fig. 3A). Construct T1 contained the exon-1 sequence encoding the putative signal peptide plus 5 more residues fused with the β-glucuronidase gene; T2 had, in addition to the T1 sequence, the exon-2 sequence encoding 18 residues (repetitive domains in nodulin-24, Katinakis and Verma, 1985) while T3 had the whole nodulin-24 coding region fused with β-glucuronidase. The *In vitro* translation product of
stead, the size of the band increased due to co-translational glycosylation of β-glucuronidase (see Fig. 4; Itturriaga et al., 1989). The same results were obtained in the case of T2 which had 23 additional amino acids after the signal peptide. When a longer segment of nodulin-24 (containing exons −1, −2, and −3) was used, similar results were obtained (data not shown).

Translocation of the fusion products occurred in all cases as detergent addition to trypsin-protection assay showed complete digestion of the products. However, cleavage occurred only in the case of the T3 translation product. These results indicate that although the signal peptide is sufficient for translocation across microsomal membranes, the co-translational processing of nodulin-24 requires almost full-length of nodulin-24 flanking the cleavage site.

**Nodulin-24 Is a Lumenal Protein in the ER and Is Modified Post-translationally to Become Membrane-associated**—If nodulin-24 had any membrane-spanning region, construct T3 (Fig. 3A) should have a stop transfer domain which would be detectable in the protease-protection assay. In that event, the entire β-glucuronidase protein should be located outside the membrane vesicles and thus become accessible to trypsin. However, no digestion of T3 product was observed (Fig. 3B). When microsomal membranes were solubilized by TX-100, the fusion protein was completely digested. Similar results were obtained using proteinase-K in place of trypsin (data not shown). These results suggest that nodulin-24 may be located inside the microsomal membrane or in the lumen of the ER. To test its location, we subjected microsomal membranes following in vitro translation, to sonication and centrifugation, and separated the microvesicles from the supernatant (Fig. 4, lanes 3 and 4). Most of the nodulin-24 was found in the supernatant, indicating that nodulin-24 is not associated with the ER membranes but is present in the lumen. However, mature nodulin-24 isolated from root nodules is tightly attached to the membrane (Fortin et al., 1985). Sodium carbonate (pH 11) (Fujiki et al., 1982) or EGTA treatment (van Renswoude and Kempf, 1984) of soybean PBM did not remove nodulin-24 from the membrane (Fig. 5), while TX-100 solubilized it along with the PBM (data not shown). These data suggest that nodulin-24 is a lumenal protein in the ER, but becomes modified post-translationally and attached to the membrane during its passage to the PBM.

No co-translational glycosylation of nodulin-24 was detected using ConA (Fig. 4, lanes 5 and 6). Treatment of intact or sonicated PBM proteins with proteinase-K did not alter the size of nodulin-24 while nodulin-26 was cleaved (Fig. 6). This suggests that nodulin-24 is buried in the lipid layer and is not accessible to protease digestion. Addition of TX-100 to the reaction mixture resulted in complete digestion of nodulin-24 (data not shown), confirming that the protein is masked by lipids. Furthermore, nodulin-24 was found to have a homolo-
gous domain to the lipid attachment site of prokaryotic membrane lipoproteins at carboxy-terminal region (Table I; Bairoch, 1991; Sankaran and Wu, 1993). These data indicate that nodulin-24 may be post-translationally anchored to lipid, possibly through this lipid attachment site, and become a membrane protein (see below) although no direct evidence is available for this phenomenon in eukaryotes.

**DISCUSSION**

Nodule development involves extensive membrane proliferation in the infected cells to enclose invading bacteria. Although the PBM is derived from the plasma membrane, progressive changes in composition of the PBM have been observed (Verma et al., 1978; Fortin et al., 1985) including the acquisition of several new proteins such as nodulin-26 and nodulin-24. The expression of nodulin-26 and nodulin-24 is altered in ineffective nodules or nodules devoid of bacteria (Morrison and Verma, 1987). If the vesicular transport of PBM proteins is retarding using antisense expression of rob7 gene, the late endosomes accumulate in the perinuclear region and these compartments become lytic, degrading endocytosed bacteria (Cheon et al., 1993).

Nodulin-24 and nodulin-26 are targeted to the PBM via different mechanisms since nodulin-26 has no cleavable signal sequence (Miao et al., 1992) while nodulin-24 was shown to have a cleavable signal sequence. The amino acid sequences of nodulin-23 (Mauro et al., 1985) and nodulin-24 were analyzed using a weight-matrix approach (von Heijne, 1986), and potential cleavage sites were identified between amino acid residues Ala20 and Glu21 for nodulin-23, and between Ala26 and Arg26 for nodulin-24. This suggests that nodulin-23 and nodulin-24 may be directed to the PBM by a similar sorting mechanism.

We addressed the question of how nodulin-24 becomes attached to the PBM and increases its molecular mass from 20 to 33 kDa (Fortin et al., 1985). Based on a hydropathy plot, the repeated domains at the amino-terminal region of processed nodulin-24 are highly amphipathic while the carboxyl half of this protein is hydrophobic. No membrane spanning region, which in most cases is a hydrophobic stretch of about 20 amino acid residues (Jennings, 1989), was observed in this protein (Katinakis and Verma, 1985). To test for the presence of a transmembrane domain using a protease-protection assay (Garoff, 1985), various regions of the nodulin-24 cDNA were fused with the β-glucuronidase reporter gene. When the entire cDNA of nodulin-24 was fused with the β-glucuronidase and the membrane-translocated product was treated with trypsin or proteinase-K (Fig. 3B (d)), neither nodulin-24 nor β-glucuronidase was digested by the protease. This result suggested that either nodulin-24 has hydrophobic interactions with membrane lipids and is attached to the membrane or that it is a lumenal protein in the ER and is subsequently modified to become associated with the membrane. The experimental data suggest the latter possibility. Nodulin-24 was found in the supernatant fraction when the in vitro translation product, obtained in the presence of microsomal membranes, was sonicated and centrifuged (Fig. 4). These data suggest that nodulin-24 in the ER is not membrane bound but becomes attached to the membrane post-translationally along the sorting route to PBM. Proteinase-K treatment of PBM vesicles after sonication (Fig. 6) resulted in partial digestion of nodulin-26, while nodulin-24 was unaffected. Since nodulin-24 does not have any membrane spanning region, it may be covalently bound to and embedded in the membrane lipids which may be also responsible for the increase of its molecular mass. These lipids may also protect it from proteolytic digestion.

There are a few examples of lipids that act as membrane anchors. The variant surface glycoprotein of the parasitic protozoan, Trypanosoma brucei, is linked to the membrane via the glycosyl-phosphatidylinositol (GPI) moiety (Low, 1989). Many

**Fig. 5. Tight association of nodulin-24 with the PBM.** Lane 1, PBM without any treatment; lane 2, PBM washed with sodium carbonate (pH 11); lane 3, PBM washed with EGTA. Each pellet from different treatments was resolved by SDS-PAGE, blotted, and probed with nodulin-24 antibody.

**Fig. 6. Insensitivity of nodulin-24 in PBM to protease digestion.** Panel A is a SDS-PAGE gel of PBM after proteinase-K digestion, and panel B is a Western blot of the gel in panel A reacted with nodulin-24 antibody. Nodulin-26 was also identified by Western blotting (data not shown). Proteinase-K was added to the PBM fraction, and the mixture was sonicated and incubated as described under "Experimental Procedures." M, molecular markers in kDa; lane 1, PBM; lane 2, PBM with 0.3 μg/ml of proteinase-K; lane 3, PBM with 3 μg/ml of proteinase-K. N24, nodulin-24; N26, nodulin-26; arrowhead, partially digested nodulin-26.

**Table 1**

**Presence of a consensus lipid attachment site in nodulin-24**

A prokaryotic membrane lipoprotein lipid attachment site was found in nodulin-24 sequence with no mismatch. In this motif (Bairoch, 1991) the 9th residue should be one of the following: I, V, M, S, T, A, G, or Q. The 10th position can be any of A, G, or S. A cysteine residue in the last position of this motif is invariant and serves as the lipid attachment site.

| Accession no. | Membrane lipoproteins | Lipid attachment motif |
|----------------|------------------------|------------------------|
| M12163         | *E. coli* lipoproteins-28 | LLLAGILLLAC           |
| X57402         | *E. coli* lipoprotein-34  | GVSLLVLLAAC           |
| M22859         | *E. coli* osmB lipoprotein | ATTALSALSAC           |
| XG1213         | *E. coli* pal lipoprotein  | ILAFLPMIAAC           |
| X51393         | Pseudomonas lpp, lipopeptide | LLAGIGIATAC           |
| M84922         | Pseudomonas endoglucanase | ASVAAMLAAC           |
| X17337         | *Streptococcus* amA protein | VLLAAAGVLAC           |
| M10595         | Glycine max nodulin-24    | FPSSLGGVSC           |

Consensus: (D, E, R, K)6(L, I, V, M, F, S, T, A, G)2(I, V, M, S, T, A, G, Q)(A, G, S,C)
proteins in the GTPase superfamily are isoprenylated or myristoylated or palmitoylated and become membrane-bound (Hancock et al., 1989; Spiegel et al., 1991; Magee and Newman, 1992). Nodulin-24 does not carry any consensus sequence for these modifications. However, it has a region homologous to the lipid-binding domain (Table I) of bacterial lipoproteins (Bairloch, 1991; Sankaran and Wu, 1993). Diglyceride is attached to a cysteine residue in the lipid-binding domain before processing of prolipoprotein by a signal peptidase II followed by N-acylation at the cysteine residue, suggesting that it is buried in the lipid membrane. Likewise, translocation and signal peptidase processing using human N-acylation at the cysteine residue. This modification of membrane proteins with diacylglycerol and palmitate is unique to prokaryotes. It is interesting to find this characteristic lipid-binding domain in prokaryotic membrane proteins in nodulin-24. Furthermore, Coleman et al. (1985) showed that lipid binding still occurs in the absence of propeptide processing, raising a possibility that nodulin-24 may have the same mode of lipid binding even though no cleavage occurs at the carboxyl-terminal region which contains the lipid-binding domain.

The role of a signal sequence in targeting protein to the ER has been demonstrated by constructing fusion proteins with reporters (Garoff, 1985; see for review, Chrispeels, 1991). Fusion of the nodulin-24 signal sequence with β-glucuronidase (construct T1) did not allow cleavage of the signal sequence in in vitro translation with microsomal membranes, although the fusion protein was targeted to the ER and translocated across the membranes. Even when an additional fragment of 18 amino acids of nodulin-24 was added to the construct, the cleavage still did not occur (Fig. 3B). Studies of co-translational translocation and signal peptidase processing using human preproapolipoprotein-A (Folz and Gordon, 1987; Nothwehr et al., 1989) suggested that the NH2-terminal propeptide may affect the cleavage site of signal peptidase, and there may exist structural characteristics for recognition and cleavage. In the case of the F1-ATPase β-subunit, a mitochondrial protein, deletion of 17 residues distal to the cleavage site of the targeting sequence resulted in mitochondrial import without the cleavage of targeting sequence (Vassarotti et al., 1987). It was concluded that the deleted protein may not have a common structure to other mitochondrial precursors that are recognized by the matrix protease of mitochondria. Likewise, fusion constructs of nodulin-24 with the β-glucuronidase may lack a structure recognized by signal peptidase.

Thus, nodulin-24 appears to follow a unique path to become associated with the inner surface of the PBM. Despite the lack of any transmembrane domain, this protein is still protected from protease digestion, suggesting that it is buried in the lipid facing the bacteroids. The intriguing presence of a lipid-binding domain analogous to the membrane proteins of many pathogenic bacteria raises the possibility that a gene encoding this protein may have been transferred from bacteria into the plant genome during recent evolution.

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REFERENCES
Anderson, D. J., and Blobel, G. (1983) Methods Enzymol. 96, 111-120
Bairloch, A. (1991) Nucleic Acids Res. 19, 2241-2245
Brewin, N. J. (1991) Annu. Rev. Cell Biol. 7, 191-226
Burnette, W. N. (1981) Anal. Biochem. 112, 195-203
Cheon, C.-J., Lee, N.-G., Siddeque, A.-M. B., Bal, A. K., and Verma, D. P. S. (1993)EMBO J. 12, 4125-4135
Chrispeels M. J. (1991) Annu. Rev. Plant Physiol. Plant Mol. Biol. 43, 21-53
Coleman, J., Inouye, M., and Inouye, M. (1985) Cell 43, 351-360
Delauney, A. D., and Verma, D. P. S. (1985) Plant Mol. Biol. Report 6, 279-285
Folz, R. J., and Gordon, J. I. (1987) J. Biol. Chem. 262, 17221-17230
Fortin, M. G., Zelechowska, M., and Verma, D. P. S. (1985) EMBO J. 4, 3041-3046
Fortin, M. G., Morrison, N. A., and Verma, D. P. S. (1987) Nucleic Acids Res. 15, 513-524
Fujiki, Y., Hubbard, A. L., Fowler, S., and Lazarow, P. B. (1982) J. Cell Biol. 93, 97-102
Garoff, H. (1985) Annu. Rev. Cell Biol. 1, 403-445
Hancock, J. F., Magee, A. L., Childs, J. E., and Marshall, C. J. (1989) Cell 57, 1167-1177
Iurriaga, G., Jefferson, R. A., and Bevan, M. W. (1989) Plant Cell 1, 381-390
Jacobs, F. A., Zhang, M., Fortin, M. G., and Verma, D. P. S. (1987) Nucleic Acids Res. 15, 1271-1280
Jennings, M. L. (1989) Annu. Rev. Biochem. 58, 999-1027
Katunisik, F., and Verma, D. P. S. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4157-4161
Low, M. G. (1989) FASEB J. 3, 1600-1608
Magee, T., and Newman, C. (1992) Trends Cell Biol. 3, 318-323
Mauro, V. P., Nguyen, T., Katunisik, F., and Verma, D. P. S. (1985) Nucleic Acids Res. 13, 239-249
Miao, G.-H., Hong, Z., and Verma, D. P. S. (1992) J. Cell Biol. 118, 481-490
Morrison, N., and Verma, D. P. S. (1987) Plant Mol. Biol. 9, 185-196
Nirunsuksiri, W., and Sengupta-Gopalan, C. (1991) Planta, 185, 384-396
Nothwehr, S. F., Folz, R. J., and Gordon, J. I. (1989) J. Biol. Chem. 264, 4642-4647
Perlman, D., and Halvorson, H. O. (1985) J. Mol. Biol. 187, 391-409
Sandil, N. N., Bojesen, K., Richter, H., Sengupta-Gopalan, C., and Marcker, K. A. (1992) Plant Mol. Biol. 18, 607-610
Sankaran, K., and Wu, H. (1993) in Lipid Modification of Proteins (Schlesinger, M. J., ed) pp. 183-181, CRC Press, Boca Raton
Spiegel, A. M., Backlund Jr., P. S., Butrynaki, J. E., Jones, T. L. Z., and Simonds, W. F. (1991) Trends Biochem. Sci. 16, 329-341
Spieß, M., and Lodish, H. F. (1986) Cell 44, 177-185
van Renswoude, J. V., and Kempf, C. (1984) Methods Enzymol. 104, 329-339
Vassarotti, A., Chen, W.-J., Smagula, C., and Douglas, M. G. (1987) J. Biol. Chem. 262, 411-415
Verma, D. P. S. (1992) Plant Cell 4, 373-382
Verma, D. P. S., and Fortin, M. G. (1989) in The Molecular Biology of Plant Nuclear Genes (Schell, J., and Vasil, I. K., eds.) pp. 329-353, Academic Press, San Diego
Verma, D. P. S., Kanzania, V., Zegbi, V., and Bal, A. K. (1978) J. Cell Biol. 78, 918-936
von Heijne, G. (1983) Eur. J. Biochem. 132, 17-21
von Heijne, G. (1986) Nucleic Acids Res. 14, 4683-4690