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Cotranslational Processing and Protein Turnover in Eukaryotic Cells†

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Translation of the genetic code is only one step in the sequence of events that determines the steady-state level of each of the multitude of mature proteins found in cells. Most proteins are also subjected to a wide variety of co- and posttranslational modifications (Wold, 1981). The structural alterations resulting from these modifications induce or control biological activity, direct intracellular and/or extracellular translocation, and affect stability and turnover (degradation). While some modifications are readily reversible, e.g., phosphorylation, the majority represent permanent changes and are "reversed" only by replacement of the modified molecule.

Almost certainly, the first opportunity for modification of a polypeptide occurs at the amino terminus when the nascent chain is only 20–40 amino acids in length (Jackson & Hunter, 1970; Palmiter et al., 1978). Two enzymes, methionine aminopeptidase (MAP) and N^{α}-acetyltransferase (NAT), can act individually or can act in concert to remove the initiator methionine residue and add an N^{α}-acetyl group (from acetyl-CoA). The effect of these actions (or lack thereof) is to produce four classes of proteins (with respect to the amino-terminal structure), as illustrated in Figure 1. Thus, proteins will or will not retain N-terminal methionine and will or will not have an N^{α}-acetyl group. Of course, subsequent modifications, such as the existence of a signal peptide, can obscure these initial changes and create new subclasses. However, the majority of mature intracellular eukaryotic proteins possess amino-terminal structures that still apparently reflect the actions of these two enzymes.

Terminal modifications are not restricted to the actions of MAP and NAT. Acylases and acyl amino acid hydrolases have been identified from a variety of sources, and their actions, at least in theory, could also affect the N-terminal structure of eukaryotic proteins either co- or posttranslationally. An alternative scheme that would produce the same classes of proteins as MAP and NAT (Figure 1) is shown in Figure 2. This hypothetical pathway utilizes NAT and an acyl amino acid hydrolase specific for N-Ac-Met residues but does not require MAP (Wold, 1984). However, as described below, the removal of N-Ac-Met is probably primarily a posttranslational event. In this regard, there are several well-defined posttranslational modifications of the α-amino group of proteins, ranging from nonenzymatic glycosylations (Bunn et al., 1975) to the non-mRNA-directed addition of amino acids (Soffer, 1980), and these include acetylations presumably catalyzed by an enzyme(s) distinct from that involved in cotranslational modifications (Rubenstein et al., 1981; Rubenstein & Martin, 1983). These or other acetylases can also modify ϵ-amino groups of lysine (Allfrey, 1977).

The physiological significance of NAT and MAP and the cotranslational modifications they do (or do not) catalyze have been variously attributed to functional and/or stabilizing roles (Jornvall, 1975; Driessen et al., 1985). However, there is only limited evidence bearing on this point and not all of it is compelling. For example, attempts to establish that proteins normally N^{α}-acetylated are more unstable without this modification have been unconvincing (Brown, 1979; Driessen et al., 1983). Nonetheless, the ubiquitous distribution of these enzymes in eukaryotic cells and their highly conserved substrate specificity (Persson et al., 1985; Sherman et al., 1986) argue for a significant role; one such possible function, directing the early routes of protein turnover, is described below.

NAT and MAP: Properties and Specificity. Both NAT and MAP are associated noncovalently with ribosomes (Driessen et al., 1985; Kerwar et al., 1971) and are presumably positioned to modify each nascent polypeptide as it emerges at the ribosomal surface. It is likely that each ribosome has both activities, but this has not been rigorously demonstrated. Both enzymes can be removed by 0.15 M KCl washings of polysome preparations although the inclusion of detergents such as deoxycholate greatly improves the efficiency and recovery (R. L. Kendall, R. Yamada, and R. A. Bradshaw, unpublished observations). It is presently unclear whether there are also soluble forms of these enzymes, an issue that is confused by the presence of multiple acetylase and aminopeptidase activities in eukaryotic tissues (Allfrey, 1977; Freitas

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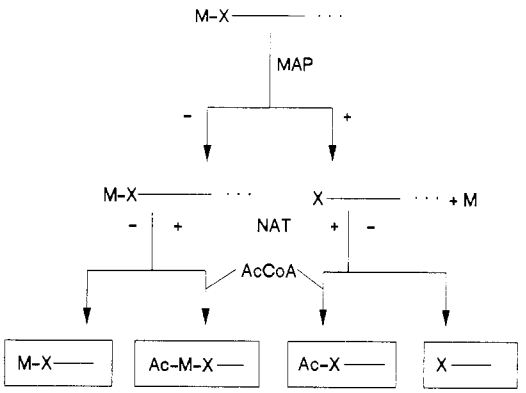

FIGURE 1: Proposed pathway for the cotranslational modification of eukaryotic proteins by methionine aminopeptidase (MAP) and N^{α}-acetyltransferase (NAT). M represents initiator methionine and X, the penultimate residue. Dots indicate nascent polypeptide chains; (+) and (-) indicate positive or negative actions of the enzymes.

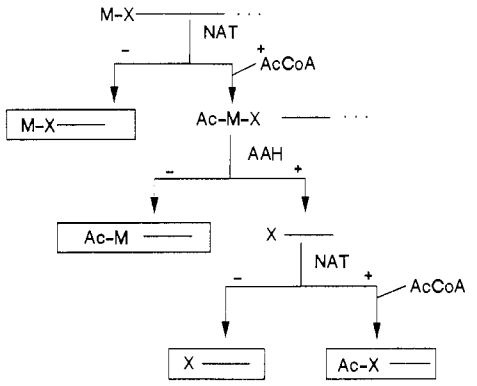

FIGURE 2: Alternative pathway for the cotranslational modification of eukaryotic proteins by N^{α}-acetyltransferase (NAT) and acyl amino acid hydrolase (AAH). Symbols as in Figure 1. Taken from Wold (1984).

et al., 1981; Glembotski, 1982; Yoshida & Lin, 1972; Barnea & Cho, 1983; Pease & Dixon, 1981).

Homogeneous preparations of an eukaryotic form of either enzyme have not been reported although two prokaryotic MAPs have been isolated and/or cloned (Ben-Bassat et al., 1987; Miller et al., 1987). Preliminary characterization of partially purified preparations of NAT and MAP suggests both enzymes are large, multimeric molecules. In addition, rat liver NAT preparations display extraordinary lability, as has been noted for NAT preparations from other sources (Driessen et al., 1985), that is only partially stabilized by EDTA and deoxycholate (Yamada et al., 1987).

The specificity of both enzymes has been surmised from an examination of the germane, directly determined structures found in the protein sequence data base (Persson et al., 1985; Driessen et al., 1985; Sherman et al., 1986) and by an examination of a number of mutant proteins, primarily in yeast (Tsunasawa et al., 1985). These analyses, insofar as the data allow, suggest that MAP is inhibited by an adjacent charged or bulky residue (Burstein & Schechter, 1978; Sherman et al., 1986) and that N^{α}-acetyl groups tend to be added, with only a few exceptions, to terminal amino acids with relatively small side chains (Persson et al., 1985; Bloemendal, 1977). The primary specifying residue appears to be the penultimate amino acid (which becomes the N-terminal in instances where the initiator methionine is removed). However, computer analyses have also suggested that additional specifying influences may be found in regions as far as 40 residues from the amino terminus (Persson et al., 1985; Augen & Wold, 1986).

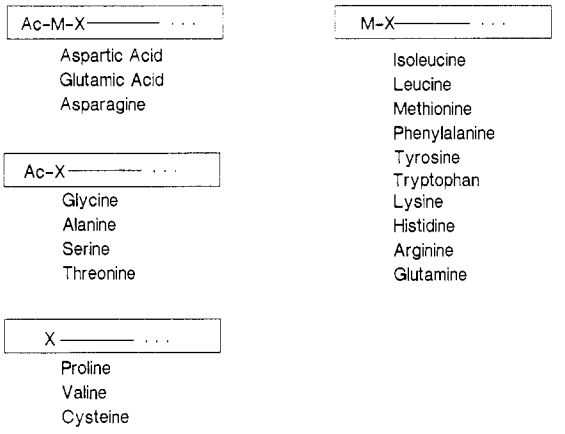

FIGURE 3: Categories of proteins produced by cotranslational modifications of eukaryotic proteins as a function of the penultimate residue. Taken from Huang et al. (1987).

Table I: Distribution of N^{α}-Acetyl Groups in Eukaryotic Proteins of Known Sequence[a]

sequence	no. of proteins	%		
Ac-Gly-	4	5		
Ac-Ala-	28	33	77	
Ac-Ser-	30	35		
Ac-Thr-	3	4		
Ac-Val-	2	2		95
Ac-Asp-	1	1		
Ac-Met-Asp-	9	10		
Ac-Met-Glu-	5	6	18	
Ac-Met-Asn-	2	2		
Ac-Met-Lys-	1	1		
total	85	99		

[a] Compiled from the listing of Driessen et al. (1985). Redundancies introduced by multiple species entries were eliminated.

A more systematic study of the effect of the penultimate residue on the catalytic selectivity of these two enzymes was carried out in yeast by using a synthetic gene construct that was altered in the second codon by site-directed mutagenesis (Huang et al., 1987). The effect of each of the 20 genetically directed amino acids was tested by examining the status of the amino terminus of the cytoplasmically expressed protein. As shown in Figure 3, all four expected categories (see Figure 1) were found. When the substituted amino acid was alanine, glycine, serine, or threonine, the initiator methionine was removed and an N^{α}-acetyl group added. Valine, cysteine, and proline resulted in the removal of methionine without the N^{α}-acetyl addition. Similarly, aspartic acid, glutamic acid, and asparagine prevented the removal of methionine, which was subsequently acetylated, while leucine, isoleucine, methionine, phenylalanine, tyrosine, tryptophan, lysine, arginine, histidine, and glutamine blocked the removal of methionine as well as N^{α}-acetylation.

These results confirm the specificity of MAP, based on side-chain size, proposed by Sherman et al. (1986) and extend the view that the penultimate residue is the primary influence in MAP specificity. However, there is a somewhat greater range in specificity reported for NAT, and the results of this study are at some variance with other data from yeast proteins, particularly studies with mutant isocytochromes *c* (Tsunasawa et al., 1985). However, they are in quite reasonable agreement with the eukaryotic protein sequence data base as a whole. As shown in Table I, when the data are adjusted for multiple entries of the same protein found in different species, there is a 95% agreement. Recently, Boissel et al. (1988) have reported a similar study using β-globin expressed in cell-free

systems with essentially the same results as those of Huang et al. (1987). This suggests that the specificity of these enzymes, determined in this yeast paradigm, is generally applicable to eukaryotic systems and that the specificity of these enzymes is quite narrowly conserved from yeast to man.

Intracellular Protein Degradation. The specific structural features of a protein that determine intracellular stability are largely unknown. Gross structural alterations such as those induced by the incorporation of amino acid analogues (Hershko et al., 1982) or caused by mutations (Wahl et al., 1974) frequently lead to an accelerated intracellular degradation of the protein; however, the structural basis for the wide variation in the half-lives of normal proteins is just beginning to be investigated. Recently, a number of hypotheses have been put forward that focus attention on specific features of the primary sequence of a protein as determinants of intracellular stability.

Dice and co-workers [see Dice (1987)] have identified a pentapeptide sequence that seems to target proteins to lysosomes for rapid degradation when human fibroblasts are subjected to serum withdrawal. Rechsteiner and co-workers (Rogers et al., 1986) have noted the presence in rapidly degraded, but not long-lived, proteins of known amino acid sequences, of regions enriched for proline, glutamic acid, serine, and threonine (PEST sequences) flanked by basic amino acid residues. Both of these putative proteolysis signal sequences are encoded in the structural genes of the proteins. Neither sequence is known to be a target for co- or posttranslational events, although it is possible that phosphorylation (or other modification) of serine or threonine residues in PEST sequences is important for the turnover of proteins.

Several lines of evidence suggest that the N-terminal amino acid of proteins may also play a major role in determining intracellular stability. Cell-free studies of ubiquitin-mediated proteolysis, in which substrates were chemically modified so as to selectively block either the α-amino group at the N-terminus or the ϵ-amino groups of internal lysines, indicated a significant role for an unblocked α-amino terminus (Hershko et al., 1984). N^{α}-Carbamoylation of lysozyme or globin largely prevented the formation of ubiquitin–protein conjugates and the subsequent degradation even though internal ϵ-amino groups of lysine were unblocked. If a new free α-amino terminus was created by polyalanylation of N^{α}-carbamoyl lysozyme, then degradation by the ubiquitin system was restored. Conversely, blockage of ϵ-amino groups, but not the α-amino group, by guanidination of lysozyme with *O*-methylisourea reduced, but did not abolish, ubiquitin-dependent degradation.

Further support for the importance of a free α-amino group for ubiquitin-mediated proteolysis comes from a comparison of the degradation of naturally blocked (N^{α}-acetyl) proteins such as cytochrome *c* and enolase from mammalian sources with the homologous unblocked yeast proteins. While the yeast proteins are readily conjugated to ubiquitin and degraded by enzymes present in a rabbit reticulocyte lysate, the acetylated mammalian proteins are neither conjugated nor degraded (Hershko et al., 1986).

Bachmair et al. (1986) implicated the identity of the N-terminal amino acid as a structural feature important in intracellular turnover. They created a series of plasmids encoding ubiquitin–*Escherichia coli* β-galactosidase fusion proteins in which the normally occurring N-terminal methionine of the bacterial enzyme was replaced by one of 16 different amino acids. When the fusion protein was expressed in *Saccharomyces cerevisiae*, the ubiquitin entity was rapidly cleaved, exposing the substituted amino acid as the new N-terminus of the β-galactosidase. The half-lives of these proteins were determined by pulse-labeling, immunoprecipitation, and SDS gel electrophoresis. For six different amino acids, including the normally occurring methionine, the half-life was greater than 20 h. For the remaining amino acids, the half-lives of the modified β-galactosidases varied from 2 to 30 min (Table II). An arginine residue gave the shortest half-life. These findings led Bachmair et al. (1986) to propose the "N-end" rule, suggesting protein stability was directly linked to the amino-terminal residue of intracellular eukaryotic proteins.

Table II: Correlations of Amino-Terminal Substitutions on the Stability of β-Galactosidase Expressed in Yeast and the Specificity of Methionine Aminopeptidase[a]

N-terminal amino acid, X	side-chain radius of gyration of X (Å)	half-life (X-β-Gal)[b]	initiator Met cleavage in yeast[c]
Gly	0.00	>20 h	+
Ala	0.77	>20 h	+
Ser	1.08	>20 h	+
Thr	1.24	>20 h	+
Val	1.29	>20 h	+
Met	1.80	20 h[d]	(−)[d]
Ile	1.56	30 min	−
Glu	1.77	30 min	−
Gln	1.75	10 min	−
Tyr	2.13	10 min	−
Asp	1.43	3 min	−
Leu	1.54	3 min	−
Phe	1.90	3 min	−
Lys	2.08	3 min	−
Arg	2.38	2 min	−

[a] Stability of β-galactosidase was measured following cleavage of N-terminal ubiquitin from ubiquitin–β-galactosidase fusion proteins by endogenous ubiquitinase. X represents the amino acid released as the N-terminus of the β-galactosidase. [b] Taken from Bachmair et al. (1986). [c] Taken from Huang et al. (1987). [d] Methionine is not removed from Met-Met sequences; however, stabilization is clearly afforded by only a single methionine.

Consistent with these findings, Reiss et al. (1988) have provided evidence that ubiquitin–protein ligase, which catalyzes the conjugation of ubiquitin to proteins, contains binding sites that interact with the N-terminal amino acid of at least some proteins. Binding of protein substrate to the ligase was assayed by chemical cross-linking or by the extent of conversion of enzyme-bound labeled proteins to ubiquitin–protein conjugates in pulse–chase experiments. The binding of some substrates could be inhibited by simple amino acid derivatives. Amino acids modified at the carboxyl group (hydroxamates, methyl esters or dipeptides) were inhibitory but those modified at the amino group were not inhibitory, consistent with previous findings that proteins with free amino termini bound much more strongly to the ligase than their N^{α}-acetylated homologues (Hershko et al., 1986).

The binding of proteins containing basic amino acids at the N-terminus to the ligase was specifically inhibited by derivatives of arginine, histidine, or lysine but not by derivatives of other amino acids. Derivatives of leucine, phenylalanine, tyrosine, and tryptophan inhibited the binding of proteins containing bulky, hydrophobic amino termini. The binding of a third class of proteins, those containing neither basic nor bulky, hydrophobic N-terminal amino acids, was not inhibited by derivatives of any amino acid including those of the amino acid occurring at the N-terminus. Thus, the ubiquitin–protein ligase may contain separate specific sites at which binding of N-terminal basic or N-terminal bulky, hydrophobic residues

occurs as well as other sites ["body" sites (Reiss et al., 1988)] at which interactions occur with other regions of potential substrates for the ubiquitin system. That such interactions are important, even for proteins having amino termini for which an N-terminal binding site exists, is indicated by the greatly enhanced binding of RNase A (N-terminal lysine) in which methionine residues have been oxidized compared to unmodified RNase A.

Studies by Ciechanover et al. (1985) and Ferber and Ciechanover (1986) established a requirement for tRNA for the formation of ubiquitin–protein conjugates and subsequent proteolysis of some proteins by the rabbit reticulocyte lysate cell-free system. The discovery that those proteins whose conjugation and degradation were tRNA-dependent all contained N-terminal aspartic or glutamic acid residues suggested a possible function for arginyl-tRNA:protein transferase. This enzyme, which catalyzes the ribosome-independent transfer of arginine from arginyl-tRNA to mature proteins containing acidic amino termini, is widely distributed in eukaryotes (Soffer, 1980). Indeed, a tRNA-dependent arginylation of appropriate substrates was demonstrated in reticulocyte extracts, and the arginylated proteins were rapidly conjugated to ubiquitin and degraded (Ferber & Ciechanover, 1987). More recently, a protein required (along with tRNA) for the conjugation and degradation of substrates containing acidic amino termini has been purified from reticulocyte extracts and shown to be identical with arginyl-tRNA:protein transferase (Ciechanover et al., 1988). tRNA-dependent, ribosome-independent transfer of other amino acids to proteins has also been observed (Shyne-Athwal et al., 1986). The relationship of these activities to proteolysis is unknown.

Cotranslational Processing and Protein Turnover. Ubiquitin-dependent proteolysis is believed to be important in the turnover of abnormal proteins and cytoplasmic (or other intracellular) proteins with short half-lives (Hershko & Ciechanover, 1986). The coincidence of the catalytic specificities of MAP and NAT (Huang et al., 1987) and the destabilizing residues identified by Bachmair et al. (1986) suggests that cotranslational modification of the N-termini of nascent polypeptides and their eventual turnover are linked events (Table II). More precisely, the four categories of proteins created by the combined action (or inaction) of MAP and NAT can be viewed as substrate pools for the subsequent action of other enzymes, acting posttranslationally, that lead directly to proteolytic degradation by one or another mechanism(s). The situation that arises with aspartic acid, glutamic acid, and, potentially, asparagine illustrates this point. The addition of arginine to the existing N-terminus of a protein by arginyl-tRNA:protein transferase requires the presence of aspartate or glutamate in that position. In fact, substrates normally used to assay this enzyme are extracellular proteins, such as bovine serum albumin, whose N-terminal aspartic acid (or glutamic acid, in other cases) arises from the excision of a signal peptide during translocation across the ER membrane. The fact that proteins containing N-terminal aspartate and glutamate are not readily found among intracellular proteins is explained by the specificities of MAP and NAT; such proteins would normally retain *N*-acetylmethionine at the amino terminus. Clearly, such a structure would keep these proteins quite stable, at least from ubiquitin-based turnover mechanisms directed to the N-terminus. However, removal of *N*-acetylmethionine by an appropriate acyl amino acid hydrolase (see Figure 2) would provide an appropriate substrate for the arginyl-tRNA:protein transferase. The resulting arginylated protein would in all likelihood be rapidly degraded by ubiquitin-mediated processes. This proposed pathway is depicted in Figure 4. Clearly, aminopeptidases acting posttranslationally that are capable of removing methionine from proteins with isoleucine, leucine, etc. (see Figure 3) could also lead to unstable proteins (by the N-end rule). This destabilization could also be enhanced by the N-terminal transfer of other amino acids, in a fashion analogous to the transfer of arginine to exposed glutamic and aspartic acid residues. Such additional modifications may not be necessary since bovine β-lactoglobulin, which has an N-terminal leucine, is a good substrate for ubiquitin-dependent proteolysis in rabbit reticulocyte lysates (Reiss et al., 1988).

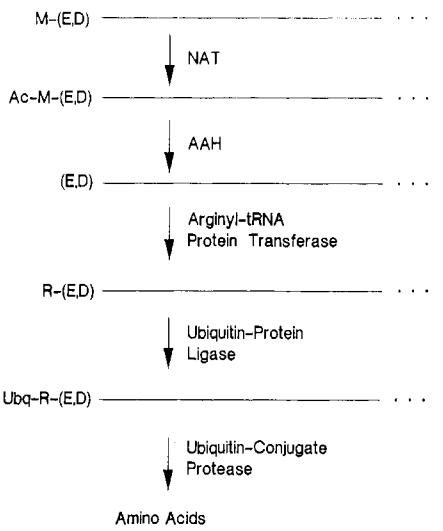

FIGURE 4: Proposed pathway for the degradation of eukaryotic proteins containing aspartic acid (D) or glutamic acid (E) adjacent to the initiator methionine. R = arginine; Ubq = ubiquitin; other symbols as in Figures 1 and 2.

In contrast to the proteins that retain the initiator methionine, the desmethionyl population, which apparently makes up a large percentage of intracellular proteins, is quite stable. Proteins containing (initially) penultimate valine, cysteine, and proline, which have unmodified α-amino groups, could be susceptible to ubiquitin-based degradation although they were not identified as destabilizing by Bachmair et al. (1986). In vitro, both rabbit globin (N-terminal valine) and cytochrome *c* from *Candida krusei* (N-terminal proline) are good substrates for ubiquitination and proteolysis (Reiss et al., 1988). The proteins containing alanine, serine, threonine, and glycine are extensively, if not completely, N^{α}-acetylated and are, in this form, apparently quite stable. Their turnover could be mediated by unblocking with acylases or acyl amino acid hydrolases, examples of which have been reported, although none have been convincingly tied to any turnover mechanisms. Endopeptidases, such as calpain (Pontremoli & Melloni, 1986), may also expose new, destabilizing sites to trigger turnover.

A recent study by Johnson et al. (1988) bears on this point. Two alleles of the gene encoding hypoxanthine phosphoribosyltransferase (HPRT), *Hprt a* and *Hprt b*, have been identified in subspecies of *Mus musculus*. Mice expressing the *a* allele have 25–70-fold higher levels of HPRT in their erythrocytes than mice expressing the *b* allele. The elevated levels result from the fact that the HPRT A protein is degraded much more slowly than the HPRT B protein during maturation of reticulocytes to erythrocytes (Johnson et al., 1985; Johnson & Chapman, 1987). On the basis of a comparison of the cDNA sequences and N-terminal amino acid sequence analysis of the purified proteins, amino acid differences between HPRT A and HPRT B were found at positions 2 and 29 (Johnson et al., 1988). The HPRT A protein has

an alanine residue following the initiator methionine and is blocked, presumably by acetylation following cleavage of the methionine by MAP. In fact, the inability to sequence the HPRT A protein and its lesser net positive charge relative to HPRT B are consistent with this structure. The HPRT B protein has proline as the penultimate amino-terminal residue. The prediction that the initiator methionine would be cleaved by MAP but that the newly exposed proline residue would not be acetylated by NAT was borne out by the N-terminal sequencing studies. Thus, the greater in vivo stability of HPRT A in mouse erythroid cells may be due to blockage of the amino-terminal residue in this protein. It is of interest to note that the stabilities of HPRT A and HPRT B are similar in nonerythroid cells of the mouse. Whether this reflects differences in the nature of the degradation systems in erythroid and nonerythroid cells is unclear, but reticulocytes are known to have a particularly active ubiquitin-dependent proteolytic system. In this regard, hemoglobin A, with unblocked valine residues on both α and β chains, that are clearly related to function (through 2′,3′-diphosphoglycerate binding), is quite stable. Thus, the free amino group appears to be a necessary but not sufficient feature for regulatory turnover of some proteins.

It is noteworthy that not all proteins of this group are (or remain) acetylated. N-Terminal glycine residues have been reported as unacetylated (Schroeder et al., 1962; Stegink et al., 1971) and myristoylated (Sefton & Buss, 1987) in addition to being acetylated. Whether all glycine residues are initially acetylated and then further modified, after selected deacylation, or are only selectively acetylated by the polysome NAT, leaving a subgroup available for further modification, is unknown.

Conclusions and Future Directions. The apparently ubiquitous eukaryotic distribution of NAT and MAP is, at least in part, explained by the view that cotranslational processing of the N-termini of nascent polypeptides marks the protein for further modification, eventually leading to degradation. The 13 amino acids that direct retention of the initiator methionine clearly protect these proteins from premature destruction while at the same time provide the opportunity for rapidly inducing instability by the simple removal of this residue (or its N^{α}-amino-substituted derivative) at a time and place consistent with the physiological needs of the cell. While it is unlikely that all proteins that retain initiator methionine will ultimately be degraded by a ubiquitin-based mechanism, many are likely to be. Furthermore, it provides the means, in the case of overproduction, of rapidly clearing an excess of the protein. For example, a trans-acting transcription regulating protein, which is synthesized in the cytoplasm but must travel to the nucleus to function, would be expected to have substantial stability in the cytoplasm but be subject to quick turnover in the nucleus in order to remain an effective modulator. At the same time, there needs to be an easily activated mechanism for clearing any surfeit of molecules in the cytoplasm that may accrue for whatever reason. The retention of methionine (thus blocking a destabilizing residue) and its removal by a cytoplasmic aminopeptidase would satisfy the first and last requirements. The existence of a nuclear aminopeptidase operating to provide for turnover in that compartment remains a possibility. However, such molecules may just as easily be degraded by other mechanisms, unrelated to the ubiquitin system, as well.

The connection between cotranslational processing, protein turnover, and the ubiquitin-based degradation system provides new perspectives on the regulation of protein metabolism and cell physiology. More than likely, several additional components must be identified before a complete picture of protein turnover is realized. However, the appreciation of the role of NAT and MAP as one of the earliest influences represents an important step forward.

Acknowledgments

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Accelerated Publications

Site-Directed Mutagenesis and High-Resolution NMR Spectroscopy of the Active Site of Porphobilinogen Deaminase†

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ABSTRACT: The active site of porphobilinogen (PBG)[1] deaminase (EC 4.3.1.8) from *Escherichia coli* has been found to contain an unusual dipyrromethane derived from four molecules of 5-aminolevulinic acid (ALA) covalently linked to Cys-242, one of the two cysteine residues conserved in *E. coli* and human deaminase. By use of a *hemA*⁻ strain of *E. coli* the enzyme was enriched from [5-^{13}C]ALA and examined by ^{1}H-detected multiple quantum coherence spectroscopy, which revealed all of the salient features of a dipyrromethane composed of two PBG units linked head to tail and terminating in a CH_2–S bond to a cysteine residue. Site-specific mutagenesis of Cys-99 and Cys-242, respectively, has shown that substitution of Ser for Cys-99 does not affect the enzymatic activity, whereas substitution of Ser for Cys-242 removes essentially all of the catalytic activity as measured by the conversion of the substrate PBG to uro'gen I. The NMR spectrum of the covalent complex of deaminase with the suicide inhibitor 2-bromo-[2,11-$^{13}C_2$]PBG reveals that the aminomethyl terminus of the inhibitor reacts with the enzyme's cofactor at the α-free pyrrole. NMR spectroscopy of the ES_2 complex confirmed a PBG-derived head-to-tail dipyrromethane attached to the α-free pyrrole position of the enzyme. A mechanistic rationale for deaminase is presented.

Previous work from this laboratory (Scott et al., 1988a,b) and elsewhere (Jordan & Warren, 1987; Hart et al., 1987) has defined the active site of PBG[1] deaminase to contain a dipyrromethane (DPM) cofactor linked covalently to the enzyme. It was further shown by ^{13}C NMR spectroscopy of deaminase biosynthetically enriched from [5-^{13}C]ALA that

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[1] Abbreviations: PBG, porphobilinogen; NMR, nuclear magnetic resonance; ALA, 5-aminolevulinic acid; HMB, (hydroxymethyl)bilane; DPM, dipyrromethane; LB, Luria broth; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside; SDS, sodium dodecyl sulfate; PE, phosphate–EDTA; EDTA, ethylenediaminetetraacetic acid.

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