10 Trumpower, B. L. (1981) *Biochim. Biophys. Acta* 639, 129–155
11 Miller, B. and Cumsky, M. G. (1993) *J. Cell Biol.* 121, 1021–1029
12 Cyr, D. M., Stuart, R. A. and Neupert, W. (1993) *J. Biol. Chem.* 268, 23751–23754
13 Gärtner, F. *et al.* (1995) *J. Biol. Chem.* 270, 3788–3795
14 Lodi, T. and Ferrero, I. (1993) *Mol. Gen. Genet.* 238, 315–324
15 Schulze, M. and Rödel, G. (1989) *Mol. Gen. Genet.* 216, 37–43
16 Nobrega, F. G., Nobrega, M. P. and Tzagoloff, A. (1992) *EMBO J.* 11, 3821–3829
17 Fölsch, H., Guiard, B., Neupert, W. and Stuart, R. A. (1996) *EMBO J.* 15, 479–487
18 Adrian, G. S., McGammon, M. T., Montgomery, D. L. and Douglas, M. G. (1986) *Mol. Cell. Biol.* 6, 626–634
19 Pfanner, N., Hoeben, P., Tropschug, M. and Neupert, W. (1987) *J. Biol. Chem.* 262, 14851–14854
20 Koll, H. *et al.* (1992) *Cell* 68, 1163–1175
21 Beasley, E. M., Müller, S. and Schatz, G. (1993) *EMBO J.* 12, 2303–2311
22 Schwarz, E., Seytter, T., Guiard, B. and Neupert, W. (1993) *EMBO J.* 12, 2295–2302
23 Glick, B. S., Beasley, E. M. and Schatz, G. (1992) *Trends Biochem. Sci.* 17, 453–459
24 Hartl, F-U., Ostermann, J., Guiard, B. and Neupert, W. (1987) *Cell* 51, 1027–1037
25 Glick, B. S. *et al.* (1992) *Cell* 69, 809–822
26 Ono, H. *et al.* (1995) *J. Biol. Chem.* 270, 16932–16938
27 Höhfeld, J. and Hartl, F-U. (1994) *J. Cell Biol.* 126, 305–315
28 Rospert, S., Müller S., Schatz, G. and Glick, B. S. (1993) *J. Biol. Chem.* 269, 17279–17288
29 Rospert, S. *et al.* (1996) *EMBO J.* 15, 764–774
30 Hwang, S. T. and Schatz, G. (1989) *Proc. Natl Acad. Sci. USA* 86, 8432–8436
31 Glick, B. S., Wachter, C., Reid, G. A. and Schatz, G. (1993) *Protein Sci.* 2, 1901–1917
32 Voos, W. *et al.* (1993) *J. Cell Biol.* 123, 119–126
33 Stuart, R. A. *et al.* (1994) *Eur. J. Biochem.* 220, 9–18
34 Gärtner, F., Bömer, U., Guiard, B. and Pfanner, N. (1995) *EMBO J.* 14, 6043–6057
35 Gruhler, A. *et al.* (1995) *EMBO J.* 14, 1349–1359
36 Ungermann, C., Neupert, W. and Cyr, D. M. (1994) *Science* 266, 1250–1253
37 Blobel, G. (1980) *Proc. Natl Acad. Sci. USA* 77, 1496–1500
38 Kaput, J., Goltz, S. and Blobel, G. (1982) *J. Biol. Chem.* 257, 15054–15058
39 van Loon, A. P. G. M. and Schatz, G. (1987) *EMBO J.* 6, 2441–2448
40 Hartl, F-U. and Neupert, W. (1990) *Science* 247, 930–938

PEST sequences and regulation by proteolysis

Martin Rechsteiner and Scott W. Rogers

In 1986, we proposed that polypeptide sequences enriched in proline (P), glutamic acid (E), serine (S) and threonine (T) target proteins for rapid destruction. For much of the past decade there were only sporadic experimental tests of the hypothesis. This situation changed markedly during the past two years with a number of papers providing strong evidence that PEST regions do, in fact, serve as proteolytic signals. Here, we briefly review the properties of PEST regions and some interesting examples of the conditional nature of such signals. Most of the article, however, focuses on recent experimental support for the hypothesis and on mechanisms responsible for the rapid degradation of proteins that contain PEST regions.

THE CONTINUAL CYCLE of synthesis and degradation of proteins within organisms has been known for more than half a century[1]. More than 30 years ago, Schimke[2] demonstrated that intracellular proteolysis can rapidly extinguish the activity of certain enzymes. However, it has only recently been appreciated that proteolysis is a widespread regulatory mechanism. Several factors have contributed to this increased recognition. It is now clear that rapid turnover is not restricted to a few metabolic enzymes. Rather, it is a property of many proteins, including a host of transcription factors and important cell-cycle regulators, such as cyclins[3]. Numerous studies on the ubiquitin-mediated proteolytic pathway have provided insight into the biochemical mechanisms responsible for rapid degradation[4]. In addition, peptide motifs that target proteins for destruction have been identified; these include PEST regions[5], KFERQ motifs[6] and the cyclin destruction box[7]. These findings have led to increased awareness that proteolysis plays an important role in biochemical regulation. Here, we will review evidence that PEST regions serve as signals for proteolytic degradation.

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The nature of PEST regions

In the early 1980s, we were trying to answer a question formulated so elegantly by Schimke[8]. Namely, what properties of intracellular proteins determine their more than 1000-fold range in metabolic stability? Our approach was to measure the half-lives of proteins of known X-ray structure after their microinjection into HeLa cells. This attempt to correlate protein structure and metabolic stability was producing complicated answers, so we turned to the inferred sequences of a small number of rapidly degraded proteins. It became apparent that sequences from each of these proteins (e.g. Myc, Fos, Jun, p53, etc.) contained regions enriched in proline (P), glutamate (E), serine (S) and threonine (T), and that these regions were uninterrupted by positively charged residues.

To objectively determine whether a protein contains a PEST region, we developed a computer program called PEST-FIND. The algorithm, which is available in PC/GENE or from the Biomedical Informatics Unit at http://www.biu.icnet.uk/projects/pest/, defines PEST sequences as hydrophilic stretches of amino acids greater than or equal to 12 residues in length. Such regions contain at least one P, one E or D and one S or T. They are flanked by lysine (K), arginine (R) or histidine (H) residues, but positively charged residues are disallowed within the PEST sequence. PEST-FIND produces a score ranging from about –50 to +50. For example, the sequence KLLPLLELLLSK generates a PEST score of –33, and the sequence KEEEESPEEEEK scores +33. By definition, a score above zero denotes a possible PEST region, but a value greater than +5 sparks real interest.

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Table I. PEST sequences known to function as proteolytic signals

Protein	Sequence	PEST-FIND score
GCN4	KTVLPIPELDDAVVESFFSSSTDSTPMFEYENLEDNSK	5.3
	KEWTSLFDNDIPVTTDDVSLADK	1.7
	KAIESTEEVSLVPSNLEVSTTSFLPTPVLEDAK	3.5
IκBα	RIQQQLGQLTLENLQMLPESEDEESYDTESEFTEFTEDEL-PYDDCVFGGQR	5.9
Fos	KVEQLSPEEEEK	10.1
	KIPDDLGFPEEMSVASLDLTGGLPEVATPESEEAFT-LPLLNDPEPK	5.7
	GSSSNEPSSDSLSSPTLLAL	4.6
Ornithine decarboxylase	KEQPGSDDEDESNEQTFMYYVNDGVYGSFNCILYDH	3.7
	HGFPPEVEEQDDGTLPMSCAQESGMDR	5.2
Cactus	KEFAVPNETSDSGFISGPQSSQIFSEEIVPDSEEQDK	7.7
	KEQPVVLDSGIIDEEEDQEEQEK	10.4
	RGAETVTPPDSDYDSSDIEDLDDTK	17.9
CLN2	KLTISTPSCSFENSNSTSIPSPASSSQSH	7.0
CLN3	KDSISPPFAFTPTSSSSSPSPFNSPYK	8.2
	KTSSSMTTPDSASH	10.6
NIMA	HSSQMSSSNSEDSDFPSSTDISQLSLESPTNK	12.6
	KFEPTLAYSDDEDDTPELPSPTR	16.4

The sequences of several PEST regions known to be proteolytic signals are presented in Table I. Clearly, they can vary considerably in sequence and in length. Secondary structure algorithms do not produce a consistent pattern for PEST regions. For example, the sequences from GCN4, ornithine decarboxylase (ODC) and IκBα shown in Table I are predicted to be α-helical; by contrast, PEST sequences from CLN2, CLN3 and NIMA score largely as β-turns. The X-ray structures of several PEST-positive proteins are available. Unfortunately, the PEST regions are not resolved, presumably because of their conformational flexibility even in the crystal state. It seems quite likely the PEST sequences are solvent-exposed loops or extensions, based on their hydrophilic nature.

The distribution of PEST sequences

It can be claimed, only half jokingly, that PEST sequences are found in all interesting cellular proteins. They are present in key metabolic enzymes, transcription factors, protein kinases, protein phosphatases and cyclins. They are also abundant among proteins that give rise to immunogenic peptides presented on MHC I molecules (Realini, C. *et al.*, unpublished). Schimke has shown that proteins exhibiting rapid changes in concentration must be short-lived[8]. The presence of a PEST region surely meets this biological requirement for rapid degradation. Some proteins contain multiple PEST regions, which can be distributed anywhere along the polypeptide chain (see Fig. 1). They are, however, often present as carboxy-terminal extensions, and their enrichment at this position is both interesting and statistically significant. A few proteins, such as protein phosphatase inhibitor I and the yeast transcriptional regulator GCN4, contain extensive PEST regions.

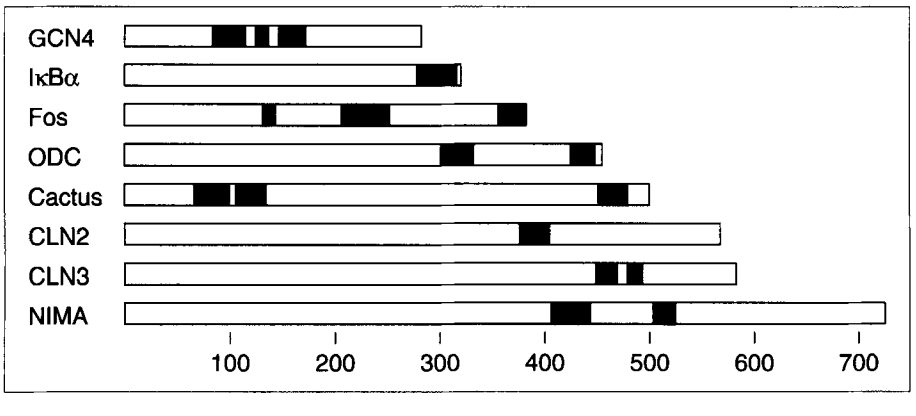

Figure 1

Distribution of sequences enriched for proline, glutamate, serine and threonine in rapidly degraded proteins. The sequences of eight proteins discussed in the text are presented as long open bars. The location and length of PEST sequences are indicated by red boxes within each open bar. The numbers at the bottom denote amino acid residues.

PEST sequences are often conditional proteolytic signals

Some PEST sequences appear to be constitutive proteolytic signals, for example, the carboxyl terminus of mouse ODC. However, many PEST sequences are conditional signals, and there are a number of ways to activate them (see Table II). As discussed in a previous review[9], one of the most striking examples is provided by the photoperiod protein, phytochrome. In its dark form, Pfr_{670}, the protein has a half-life of about 100 hours; upon light absorption the photoconverted Pfr_{730} is degraded with a $t_{1/2}$ of one hour. Light causes *cis–trans* isomerization in a linear tetrapyrrole moiety anchored at Cys321. The only PEST region in the 1000-residue phytochrome is located between amino acids 323 and 361. Based on this remarkable proximity, we have proposed that light absorption exposes the adjacent PEST region, thereby initiating degradation[9]. Cyclic-AMP-dependent kinase provides another possible example of conditional PEST regions. Both regulatory (R) and catalytic (C) subunits of cAMP kinase contain PEST sequences. The half-life of the R_2C_2 tetramer is about ten hours, whereas the dissociated R and C subunits are degraded with a half-life of about one hour[9]. It seems reasonable to assume that PEST regions are masked in the assembled holoenzyme and exposed upon cAMP-mediated dissociation. Similar conformational unmasking of a PEST motif might explain the rapid downregulation of protein kinase C upon phorbol ester binding[9]. There are several examples in which phosphorylation controls the metabolic stability of a protein, and it seems likely that phosphate addition to serines and threonines will prove to be a widespread mechanism for activating a latent PEST signal. This is discussed further under pathways responsible for degrading PEST proteins.

Support for the PEST hypothesis

Three kinds of evidence can be presented in favor of the PEST hypothesis: statistical, mutational and experimental transfer of PEST sequences. PEST-FIND has proved remarkably successful at identifying rapidly degraded proteins.

By 1991, the sequences of 47 short-lived proteins were known to us and 43 of these contained PEST regions. Less than 10% of the mammalian proteins in SWISS-PROT were PEST-positive. Hence, the significant enrichment of PEST sequences among rapidly degraded proteins provides excellent statistical support for the hypothesis.

Phil Coffino and his colleagues[10] provided initial experimental support for the PEST hypothesis by truncating mouse ODC (mODC) at residue 424; just before the carboxy-terminal PEST region (see Fig. 1). They found that the shortened protein was at least tenfold more stable than the native enzyme. Naturally or experimentally produced deletions of PEST regions from other proteins have also resulted in increased metabolic stability. Deletion of carboxy-terminal residues from both yeast and mammalian cyclins implicate PEST regions in proteolytic targeting, as the truncated products are metabolically much more stable[11–14].

NIMA, a cell cycle regulated protein kinase from *Aspergillus*, is also stabilized by removal of a large carboxy-terminal segment that contains two PEST regions[15]. Likewise, IκBα and cactus, which are cytoplasmic inhibitors of NF-κB, turn over much slower in the absence of their carboxy-terminal PEST regions[16–19]. The deletion of a 27-residue PEST sequence from the carboxyl terminus of c-Fos increases its stability considerably[20]. Less drastic changes in a PEST region can also stabilize a protein. Kornitzer *et al.*[21] observed that single amino acid changes in the highest scoring PEST region of GCN4 significantly inhibited degradation with mutation of Thr105 or Pro106 having the most dramatic effect . The importance of Thr–Pro or Ser–Pro pairs has also been observed for the degradation of recombination activator protein RAG2 (Ref. 22) and the yeast G1 cyclin CLN3 (Ref. 23), two proteins in which phosphorylation precedes degradation. Conceivably, Thr–Pro or Ser–Pro pairs are general targets for protein kinases that initiate degradation.

Four studies have demonstrated that PEST regions are transplantable proteolytic signals. Ghoda *et al.*[24] transferred the carboxy-terminal PEST region from mODC onto the stable *Trypanosoma* ODC and converted it to a rapidly degraded protein. Loetscher *et al.*[25] transferred the same 37 residues onto the amino or carboxyl terminus of dihydrofolate reductase (DHFR) and found that resulting fusion proteins were degraded as much as 40-fold faster than the parental DHFR enzyme or DHFR molecules bearing non-PEST extensions of equal length.

Table II. PEST sequences as conditional signals

Protein	Inducing agent	Proteolytic pathway
Phytochrome	Light	Ubiquitin–26S protease
Fructose-1,6-bisphosphatase	Phosphorylation	Ubiquitin–26S protease
Ornithine decarboxylase	Spermidine induces antizyme which binds ODC	26S protease
cAMP dependent protein kinase	cAMP results in dissociation	?
nProtein kinase Cε	Thyrotropin releasing hormone	Calpain
IκBα	Many	Ubiquitin–26S protease
G1 cyclins	Phosphorylation	Ubiquitin–26S protease

Two more-recent papers involve transfer of PEST regions from yeast cyclins to metabolically stable proteins. Salama *et al*[26] appended the carboxyl terminus of CLN2 onto thymidine kinase and found the chimera to be rapidly degraded throughout the yeast cell cycle. Furthermore, they showed that removal of the most prominent PEST region in the CLN2 tail stabilized the fusion protein, but the same PEST region, by itself, did not impart rapid degradation to thymidine kinase. Yaglom *et al.*[23] grafted 180 amino acids from the carboxyl terminus of CLN3 onto the amino terminus of β-galactosidase from *Escherichia coli* and found that the resulting chimeric protein was rapidly degraded. In addition, these authors demonstrated that deletions of the residues 441–478 of CLN3 resulted in significant stabilization of the chimeric protein. Furthermore, mutation of Ser468 in the CLN3 tail resulted in a fivefold increase in the half-life of CLN3–β-galactosidase. The two PEST regions in the carboxyl terminus of CLN3 lie between residues 445 and 484. Thus, within the 180-residue tail of CLN3, two contiguous PEST regions were pinpointed as the major determinants of rapid degradation.

Pathways for degrading PEST proteins

In our original paper[5], we speculated that proline residues within PEST sequences might induce degradation by proline endopeptidase. We also suggested that calpains might be the proteases responsible, reasoning that if negatively charged PEST regions bind Ca^{2+}, they could provide both peptide bonds and a necessary co-factor for proteolysis by the Ca^{2+}-activated proteases. Both ideas have recently been tested. It has been shown that α-casein and mODC are impervious to prolyl oligopeptidase, indicating that these two PEST-positive proteins, at least, are not substrates for this protease[27]. A correlation between the presence of PEST sequences and calpain susceptibility provided circumstantial evidence in favor of calpain as the enzyme that degrades PEST proteins[28]. However, two direct tests have recently shown that PEST regions are not responsible for calpain-mediated degradation. Site-directed mutagenesis of plasma membrane Ca^{2+}-ATPase, an excellent calpain substrate, demonstrated that a calmodulin-binding site is crucial for proteolysis, but two nearby PEST regions could be changed markedly with little effect on rates of proteolysis by calpain[29]. A similar approach using c-Fos showed that deletion of three PEST regions did not reduce its proteolysis by calpain[30]. However, Eto *et al.*[31] provide considerable evidence that calpain is responsible for the intracellular degradation of nuclear protein kinase C, an enzyme that contains a strong PEST region. Although it might be premature to exclude a role for calpains in the degradation of PEST-containing proteins, the available evidence does not favor their involvement.

A considerable body of evidence supports the idea that PEST sequences target proteins for degradation by the 26S proteasome. Because the ubiquitin–26S proteasome-mediated degradation of the PEST-containing proteins, phytochrome, MATα2 repressor and p53, was covered in an earlier review[3], we will just consider recent reports implicating the 26S proteasome. Using highly purified components, Murakami *et al.*[32] observed that degradation of mODC required the 26S proteasome and antizyme[33], but not ubiquitination. Similarly, it has been shown that unmodified c-Jun is degraded by the 26S proteasome[34]. Most studies

Table III. Peptide motifs that target intracellular proteins for rapid degradation

	Example	Protein[a]	Reference
Confirmed			
PEST	.. HGFPPEVEEQDDGTLPMSCAQE ...	mODC	24, 25
KFERQ	KETAAAKFERQHMDSS ...	RNase A	6
Cyclin destruction box	.. RTALGDIGN ...	Cyclin B	7
Carboxy-terminal tag	.. AANDENYALAA	many	45
Proposed			
KEKE	.. EEEKKKMEESKAK ...	Hsp90	48
AQP	.. PPPSGAGQHPQPQPP ...	EBNA	—
pentaleu	MHLLLLLGSG ...	—	47
PB29	MHSWNFKLYVMGSG ...	—	47

[a]Abbreviations used: mODC, mouse ornithine decarboxylase; EBNA, Epstein–Barr virus nuclear antigen; Hsp, heat shock protein.

report, however, that degradation of PEST-positive substrates requires their prior ubiquitination or depends upon a functional ubiquitin pathway. For example, growth of yeast on glycerol induces the gluconeogenic enzyme, fructose-1,6-bisphosphatase (FBPase), which contains a low scoring amino-terminal PEST sequence. Addition of glucose to the growth medium causes phosphorylation within the PEST region and rapid destruction of FBPase soon follows. This occurs by the ubiquitin–26S proteasome pathway, as shown by the transient appearance of FBPase attached to multi-ubiquitin chains and by its dependence on three ubiquitin-conjugating enzymes (Ubc1, Ubc4 and Ubc5) and a functioning 26S proteasome[35]. Two groups have demonstrated that degradation of c-Fos occurs by the ubiquitin–26S proteasome pathway, although there are differences in the ubiquitin components identified[20,36].

A number of laboratories have contributed to our detailed knowledge of IκBα destruction, which is, by far, the most extensively characterized proteolytic substrate containing a PEST sequence. IκBα and cactus bind the transcriptional activator NF-κB and prevent it from entering the nucleus. A variety of signaling events can induce rapid degradation of IκBα thereby liberating NF-κB as a transcriptional factor (for review, see Ref. 37). Excess IκBα molecules that do not form complexes with NF-κB are also subjected to proteolysis. It is not clear if the same proteolytic pathway is responsible for both constitutive and signal-induced degradation of IκBα.

Moreover, controversy surrounds the role of the carboxy-terminal PEST region of IκBα in signal-induced degradation; it seems clear, however, that the PEST sequence is important for the constitutive pathway. Signal-induced degradation requires IκBα phosphorylation on the amino-terminal Ser32 and Ser36 (Refs 17, 38). These modifications, in turn, are necessary for the attachment of multi-ubiquitin chains to Lys20 or Lys21 of IκBα (Refs 39, 40). Presumably, the multi-ubiquitinated NF-κB inhibitor is then recognized by subunit 5a of the 26S proteasome and rapidly degraded[41]. This rather detailed picture of IκBα destruction illustrates the potential complexity of proteolytic-targeting reactions.

Recognition of PEST sequences

We still do not know how PEST sequences are recognized. The common properties identified by the PEST-FIND algorithm might be sufficient to elicit binding to a single proteolytic factor. Alternatively, individual PEST regions might be recognized by specific members within a family of proteolytic factors. In this regard, an expanding number of HECT-domain proteins of the ubiquitin-mediated degradative pathway could serve as PEST-recognition factors[42]. Although it seems logical to look among ubiquitin pathways for components that recognize PEST sequences, one cannot exclude the possibility that PEST regions are recognized by protein kinases. Phosphorylation could either result in direct recognition of the substrate by the 26S protease or the attachment of multi-ubiquitin chains and subsequent binding of the ubiquitinated protein to the proteolytic complex. Alternatively, phosphorylation of PEST sequences might induce conformational changes that expose other regions of the substrate for interaction with proteolytic components.

Other proteolytic signals

Peptide motifs besides PEST sequences can target proteins for rapid degradation (see Table III). Glotzer *et al.*[7] identified a short region in cyclin B that is required for its rapid degradation at mitosis. Sequences similar to the cyclin destruction box have been identified in several rapidly degraded proteins, and mutations in or deletion of the destruction box stabilized these proteins[43,44]. Sauer and his colleagues[45] have recently described a remarkable peptide-targeting motif in *Escherichia coli*. A hydrophobic 11-residue peptide tail is added to the carboxyl terminus of nascent polypeptide chains by co-translational switching of ribosomes from damaged messenger RNAs to a 362-nucleotide RNA called the ssrA transcript. The tagged protein

is then degraded by a tail-specific protease[46]. Sadis *et al.*[47] have reported two amino acid motifs that function as synthetic signals for ubiquitin-dependent proteolysis. One of these is similar to sequences in the short-lived MATα2 repressor, whereas the other is pentaleucine. Realini and colleagues[48] have proposed that a highly charged peptide motif consisting of alternating lysine and glutamic acid residues, a KEKE region, is important for presentation of antigenic peptides on MHC I molecules. Sequence examination of 260 full-length precursors to presented peptides has revealed a striking enrichment for PEST sequences (28%), KEKE motifs (16%) and AQP motifs (23%). It seems reasonable to suppose that rapidly degraded, rather than stable, proteins are more likely to generate immunogenic peptides. If this is true, each motif might function as a proteolytic signal.

Future directions

So, after ten years, experimental support for the PEST hypothesis is robust and growing. Still, there are a number of unresolved issues. Foremost among them is whether the 26S proteasome is the only enzyme that degrades PEST proteins. Should the calpains really be dismissed as participants? A second mechanistic question revolves around the recognition of PEST sequences. How many PEST proteins are recognized directly by the 26S proteasome? Which proteasome subunits are involved? Which components of the ubiquitin-marking pathway bind PEST sequences? E2s? HECT-domain proteins? Others?

An exact description of what constitutes a PEST region is a second broad area of uncertainty. For example, would a single lysine or arginine in the midst of numerous negatively charged residues extinguish the signal? Do internal histidines influence recognition? Must a PEST region contain a glutamate/aspartate or would phosphorylation of serine and threonine be sufficient to convert a PST region lacking E to a proteolytic signal? At present, answers to the questions posed above are not available. To some this might be considered a sad state of affairs; for those who love to experiment, so much the better!

References

1 Schoenheimer, R. (1942) *The Dynamic State of Body Constituents*, Harvard University Press
2 Schimke, R. T. (1964) *J. Biol. Chem.* 239, 3808–3817
3 Rechsteiner, M. (1991) *Cell* 66, 615–618
4 Hershko, A. and Ciechanover, A. (1992) *Annu. Rev. Biochem.* 61, 761–807
5 Rogers, S., Wells, R. and Rechsteiner, M. (1986) *Science* 234, 364–368
6 Dice, J. F. (1990) *Trends Biochem. Sci.* 15, 305–309
7 Glotzer, M., Murray, A. W. and Kirschner, M. W. (1991) *Nature* 349, 132–138
8 Schimke, R. T. (1973) *Adv. Enzymol.* 37, 135–185
9 Rechsteiner, M. (1990) *Semin. Cell Biol.* 1, 433–440
10 Ghoda, L. *et al.* (1989) *Science* 243, 1493–1495
11 Cross, F. R. (1988) *Mol. Cell. Biol.* 8, 4675–4684
12 Richardson, H. E., Wittenberg, C., Cross, F. and Reed, S. I. (1989) *Cell* 59, 1127–1133
13 Tyers, M., Tokiwa, G., Nash, R. and Futcher, B. (1992) *EMBO J.* 11, 1773–1784
14 Bai, C., Richman, R. and Elledge, S. J. (1994) *EMBO J.* 13, 6087–6098
15 Pu, R. T. and Osmani, S. A. (1995) *EMBO J.* 14, 995–1003
16 Belvin, M. P., Jin, Y. and Anderson, K. V. (1995) *Genes Dev.* 9, 783–793
17 Brown, K. *et al.* (1995) *Science* 267, 1485–1488
18 Rodriguez, M. S., Michalopoulos, I., Arenzana-Seisedos, F. and Hay, R. T. (1995) *Mol. Cell. Biol.* 15, 2413–2419
19 Whiteside, S. T. *et al.* (1995) *Mol. Cell. Biol.* 15, 5339–5345
20 Tsurumi, C. *et al.* (1995) *Mol. Cell. Biol.* 15, 5682–5687
21 Kornitzer, D., Raboy, B., Kulka, R. G. and Fink, G. R. (1994) *EMBO J.* 13, 6021–6030
22 Lin, W-C. and Desiderio, S. (1993) *Science* 260, 953–959
23 Yaglom, J. *et al.* (1995) *Mol. Cell. Biol.* 15, 731–741
24 Ghoda, L. *et al.* (1990) *J. Biol. Chem.* 265, 11823–11826
25 Loetscher, P., Pratt, G. and Rechsteiner, M. (1991) *J. Biol. Chem.* 266, 11213–11220
26 Salama, S. R., Hendricks, K. B. and Thorner, J. (1994) *Mol. Cell. Biol.* 14, 7953–7966
27 Goossens, F. *et al.* (1995) *Eur. J. Biochem.* 233, 432–441
28 Wang, K. K. W., Villalobo, A. and Roufogalis, B. D. (1989) *Biochem. J.* 262, 693–706
29 Molinari, M., Anagli, J. and Carafoli, E. (1995) *J. Biol. Chem.* 270, 2032–2035
30 Carillos, S. *et al.* (1996) *Biochem. J.* 313, 245–251
31 Eto, A. *et al.* (1995) *J. Biol. Chem.* 270, 25115–25120
32 Murakami, Y. *et al.* (1992) *Nature* 360, 597–599
33 Hayashi, S-I., Murakami, Y. and Matsufuji, S. (1996) *Trends Biochem. Sci.* 21, 27–30
34 Jariel-Encontre, I. *et al.* (1995) *J. Biol. Chem.* 270, 11623–11627
35 Schork, S. M., Thumm, M. and Wolf, D. H. (1995) *J. Biol. Chem.* 270, 26446–26450
36 Stancovski, I. *et al.* (1995) *Mol. Cell. Biol.* 15, 7106–7116
37 Verma, I. M. *et al.* (1995) *Genes Dev.* 9, 2723–2735
38 Traenckner, E. B-M. *et al.* (1995) *EMBO J.* 14, 2876–2883
39 Scherer, D. C. *et al.* (1995) *Proc. Natl Acad. Sci. USA* 92, 11259–11263
40 Baldi, L., Brown, K., Franzoso, G. and Siebenlist, U. (1996) *J. Biol. Chem.* 271, 376–379
41 Deveraux, Q., Ustrell, V., Pickart, C. and Rechsteiner, M. (1994) *J. Biol. Chem.* 269, 7059–7061
42 Huibregtse, J. M., Scheffner, M., Beaudenon, S. and Howley, P. M. (1995) *Proc. Natl Acad. Sci. USA* 92, 2563–2567
43 Galan, J. M., Volland, C., Urban-Grimal, D. and Haguenauer-Tsapis, R. (1994) *Biochem. Biophys. Res. Commun.* 201, 769–775
44 Kaplon, T. and Jacquet, M. (1995) *J. Biol. Chem.* 270, 20742–20747
45 Keiler, K. C., Waller, P. R. H. and Sauer, R. T. (1996) *Science* 271, 990–993
46 Keiler, K. C. and Sauer, R. T. (1995) *J. Biol. Chem.* 270, 28864–28868
47 Sadis, S., Atienza, C., Jr and Finley, D. (1995) *Mol. Cell. Biol.* 15, 4086–4094
48 Realini, C., Rogers, S. W. and Rechsteiner, M. (1994) *FEBS Lett.* 348, 109–113