tivity and accumulation began 30 years before the decrease in the $\delta^{18}O$ record.

Increases in particulates may have resulted from increased atmospheric impurities, decreased accumulation, or both. The accumulation history from Quelccaya (*1*) suggests that the increase in particulates at the onset of the Little Ice Age must be due to increased atmospheric loading, not decreased accumulation. The 1500–1720 interval was the wettest in the 1000-year record, whereas the 1720–1860 interval was very dry; nonetheless, the dry-period microparticle concentration and conductivity values are similar to those from the preceding wet period. Preliminary scanning electron and light-microscopic analyses show no significant changes in the types of particles deposited during the Little Ice Age. Therefore, the observed increase in particulate deposition is attributed to increased wind velocities across the high altiplano of southern Peru.

Ice core data provide a record of several facets of past variations in the earth's atmosphere, although that record, like all proxy climate records, represents a complex integration of both local and large-scale processes. The Quelccaya ice core records provide a well-dated climatic record of the Little Ice Age in the tropics of South America and support the growing body of evidence that the Little Ice Age was a global event.

REFERENCES AND NOTES

1. L. G. Thompson, E. Mosley-Thompson, J. F. Bolzan, B. R. Koci, *Science* **229**, 971 (1985).
2. L. G. Thompson, S. Hastenrath, B. Morales Arnao, *ibid.* **203**, 1240 (1979); L. G. Thompson, *J. Glaciol.* **25**, 69 (1980).
3. L. G. Thompson, E. Mosley-Thompson, P. M. Grootes, M. Pourchet, S. Hastenrath, *J. Geophys. Res.* **89**, 4638 (1984).
4. L. G. Thompson *et al.*, *J. Glaciol.* **28**, 57 (1982).
5. L. G. Thompson and W. Dansgaard, *Antarct. J. U.S.* **10**, 24 (1975).
6. T. Simkin *et al.*, *Volcanoes of the World* (Hutchinson Ross, Stroudsburg, PA, 1981).
7. Anonymous, *Moquegua, Informe del Peru* (Promotora Editorial Latinoamericana, Newton-Cortazar, Lima, 1976), vol. 7, part 8; F. M. Bullard, *Bull. Volcanol.* **24**, 443 (1962); P. Francis, *Volcano News* 7, 4 (1981).
8. A. Vazquez de Espinosa, *Smithson. Misc. Collect.* (C. U. Clark, transl.) **102**, 497 (1942).
9. J. R. Bray, *Science* **171**, 1242 (1971); A. Robock, *ibid.* **206**, 1402 (1979); V. Cermak, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **10**, 1 (1971); H. Flohn and R. Fantechi, *The Climate of Europe: Past, Present and Future* (Reidel, Dordrecht, 1984); H. H. Lamb, *Climate: Present, Past and Future*, vol. 2, *Climatic History and the Future* (Methuen, London, 1977); C. J. E. Schuurmans, in *Climatic Variations and Variability: Facts and Theories*, A. Berger, Ed. (Reidel, Dordrecht, 1981), pp. 245–258; R. G. Barry, in *Climatic Change and Variability: A Southern Perspective*, A. B. Pittock, L. A. Frakes, D. Jenssen, S. A. Peterson, J. W. Zillman, Eds. (Cambridge Univ. Press, Cambridge, England, 1978), pp. 150–166; C. D. Schonwiese, *Klimaschwankungen* (Springer-Verlag, Berlin, 1978).
10. J. Gribben and H. H. Lamb, in *Climatic Change*, J. Gribben, Ed. (Cambridge University Press, Cambridge, England, 1978), pp. 68–82.
11. H. H. Lamb, in *Climatic Changes on a Yearly to Millennial Basis*, N. A. Mörner and W. Karlén, Eds. (Reidel, Dordrecht, 1984), pp. 225–240; H. H. Lamb, *ibid.*, pp. 309–329.
12. I. Borzenkova, K. Ya. Vinnikov, L. P. Spirina, P. I. Stechnowskii, *Meteorol. Gidrologiva* 7, 27 (1976).
13. B. S. Groveman and H. E. Landsberg, *Geophys. Res. Lett.* **6**, 767 (1979).
14. H. E. Landsberg, in *Paleoclimate Analysis and Modeling*, A. D. Hecht, Ed. (Wiley, New York, 1985), pp. 27–70.
15. A. M. Johnson, *World Survey of Climatology, Climates of Central and South America*, W. Schwerdtfeger, Ed. (Elsevier, Amsterdam, 1976), vol. 12, p. 147.
16. As a measure of the linear relation between the $\delta^{18}O$ records in summit core and core 1, the slope and the SE of the slope were calculated (slope = 0.89 ± 0.04, n = 40). A perfectly linear relation would have a slope of unity and SE of zero. For the past 21 decades (1770–1980), the better-documented part of the temperature record, the slope is 0.95 ± 0.06 (n = 21), and the elimination of the decade with a prominent departure (1810–1820) makes little difference (slope = 0.94 ± 0.08, n = 20). The linear relation between each of the two ice-core $\delta^{18}O$ records and temperatures in the Northern Hemisphere has been characterized by the slope; however, the difference in the units of measure and the lack of an empirical relation between $\delta^{18}O$ and temperature makes the slope more difficult to interpret. Nevertheless, the nonzero slope and the low SE attest to the similarity between the ice core $\delta^{18}O$ records and the Northern Hemisphere temperature history (summit core: slope = 0.17 ± 0.03, n = 40; core 1: slope = 0.14 ± 0.03, n = 40). For the better-documented part of the temperature record (1770–1820), the linear relations are (i) summit core (slope = 0.22 ± 0.04, n = 21) and (ii) core 1 (slope = 0.20 ± 0.04, n = 21).
17. R. B. Stothers, *Science* **224**, 1191 (1984).
18. All x-ray energy-dispersive spectra were obtained under identical instrumental conditions. Accelerating potential was 20 kV, count rates were 2000 counts/sec, and the spectra were accumulated for 100 seconds.

19. Supported by NSF grants ATM75-15513A02, ATM78-1609A01, ATM81-05079A02, and ATM82-13601A02. The NSF Division of Polar Programs (NSF-DPP) supported 1974 field investigations (GV41411) and development of the solar-powered drill. The Quaternary Isotope Laboratory (University of Washington) oxygen isotope study was supported by NSF grants DPP-8019756 and DPP-8400574, and that of the Geophysical Institute (University of Copenhagen) by the Danish Natural Science Research Council. We thank the Polar Ice Coring Office, Electroperu, and the Inter-American Geodetic Survey for their support. We thank the many people who have participated in the field programs; M. E. Moseley and R. A. Feldman for the Huaynaputina ash sample from Cerro Trapiche and for many valuable discussions, M. Davis for assistance in the laboratory and energy-dispersive analysis, J. Bolzan for comments on the manuscript, A. Leonardi for energy-dispersive analysis, J. Paskievitch for assistance in data analysis, K. Doddroe for typing, and R. Tope for the illustrations. Contribution 548 of the Institute of Polar Studies, Ohio State University.

23 January 1986; accepted 27 June 1986

Amino Acid Sequences Common to Rapidly Degraded Proteins: The PEST Hypothesis

SCOTT ROGERS, RODNEY WELLS, MARTIN RECHSTEINER

The amino acid sequences of ten proteins with intracellular half-lives less than 2 hours contain one or more regions rich in proline (P), glutamic acid (E), serine (S), and threonine (T). These PEST regions are generally, but not always, flanked by clusters containing several positively charged amino acids. Similar inspection of 35 proteins with intracellular half-lives between 20 and 220 hours revealed that only three contain a PEST region. On the basis of this information, it was anticipated that caseins, which contain several PEST sequences, would be rapidly degraded within eukaryotic cells. This expectation was confirmed by red blood cell–mediated microinjection of ^{125}I-labeled caseins into HeLa cells where they exhibited half-lives of less than 2 hours. The rapid degradation of injected α- and β-casein as well as the inverse correlation of PEST regions with intracellular stability indicate that the presence of these regions can result in the rapid intracellular degradation of the proteins containing them.

ONE OF THE GENERALIZATIONS TO emerge from studies on protein secretion and organelle biogenesis is that portions of the amino acid sequence comprising a protein can specify its ultimate location within the cell (*1–3*). Selective degradation of intracellular proteins—the sorting of a protein out of existence—might be considered the ultimate in targeting. Here we report an observation that emerged from a survey of the literature: proteins that are rapidly degraded within eukaryotic cells contain regions rich in proline (P), glutamic acid (E), serine (S), and threonine (T).

Table 1 contains a list of the most rapidly degraded eukaryotic proteins of known sequence, their half-lives, and the amino acid regions (PEST) whose features are shared among the members of the set. Common to all PEST regions are high local concentrations of P, E, S, or T and to a lesser extent aspartic acid. To identify PEST regions by computer, we combined specific enrichment for these amino acids with several other features in an algorithm, PEST-SCORE, for ranking stretches of protein sequence (*4*). Scores for each PEST region in rapidly degraded proteins are listed in Table 1.

PEST regions begin and end with positively charged residues (Fig. 1), but internal lysine, arginine, and histidine residues are

Departments of Biology and Biochemistry, University of Utah, Salt Lake City, UT 84112.

not present. Although this restriction was imposed for ease of computer scanning, it eliminates very few potential PEST regions among rapidly degraded proteins. Instead, long PEST regions are divided into subregions (such as c-*myc* protein residues 10–51, 52–65; p53, 39–62, 62–98). PEST regions are also characterized by a clustering of negatively charged residues. This feature, particularly evident in E1A, c-*myc* protein, ornithine decarboxylase (ODC), and phytochrome, may extend to all PEST regions since Ser, Thr, and Tyr residues can be phosphorylated. In fact, the sequences of α- and β-casein shown in Fig. 1 are heavily phosphorylated at Ser residues.

Two of the proteins in Table 1, *myc* and E1A, are thought to have common origins (*5*); the rest share no obvious evolutionary relationship. Although many of the proteins do accumulate within nuclei, ODC, hydroxymethylglutaryl coenzyme A (CoA) reductase, and phytochrome (*6*) are usually found in the cytoplasm. Thus, PEST regions correlate far better with rapid degradation than with specific compartmentation, functional activity, or common ancestry. Recognizing that caseins contain regions rich in P, S, and E, we suspected that they would be rapidly degraded within cultured cells. Therefore, we introduced ^{125}I-labeled caseins into HeLa cells by means of red blood cell–mediated microinjection (*7*) and determined their half-lives as described (*8*). In three experiments, the half-lives of α- and β-casein were found to be less than 5 hours.

A notable feature of PEST sequences is the enrichment for Glu and Gln relative to Asp and Asn. From compilations of sequenced proteins (*9*), ratios of about 1.0 are expected for Glu:Asp and Gln:Asn. However, in those sequences with PEST scores greater than 4.0, Glu outnumbers Asp 77 to 34, and there are similarly almost 2 Gln for every Asn. There may be a specific requirement for Glu since its replacement by Asp is common among evolutionarily related proteins (*9*). Furthermore, if PEST sequences are located on the protein surface, as seems likely given their hydrophilicity, the observed Glu:Asp and Gln:Asn asymmetries are more striking since constraints on amino acid replacement are less rigid for solvent-exposed residues (*10*).

The combined frequencies of prolines, negatively charged, and hydroxylated amino acids ranges from 0.31 to 0.46 in the proteins listed in Table 1. From these data it can be calculated that ten contiguous PEST residues are expected once in every 6,570 residues to once in 18,000 residues (*11*). Since *myc* protein and E1A contain tracts of 13 and 14 PEST residues, respectively, the probability that such regions arise randomly in these two proteins is exceedingly remote. At an average frequency of 0.35, even five contiguous PEST residues are expected only once in every 300 amino acids, yet half of all PEST regions, which are at most 50 amino acids in length, contain five or more contiguous PEST residues.

The distribution of hydrophobic and hydrophilic regions along a polypeptide chain can be assessed by computing the average hydrophobicity of amino acids over short lengths of the protein chain (*12*). Such analyses, termed hydropathy plots, further indicate that PEST defines regions of proteins with similar properties. First recognized by examining residues near prolines for enrichment of glutamic acid and serine, PEST regions are also evident on hydropa-

Table 1. Amino acid sequences common to rapidly degraded proteins. The partial sequences are presented with the one letter amino acid code as follows: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine. Other abbreviations: ODC, ornithine decarboxylase; P_{730}, phytochrome; HSP 70, heat shock protein 70; HMG-CoA, hydroxymethyl glutaryl–CoA; TAT, tyrosine aminotransferase.

Protein*	PEST score	Residues	Sequence	Half-life (hours)
E1A	4.0	44–94	HELYDLDVTAPEDPNEEAVSQIFPDS-VMLAVQEGIDLLTFPPAPGSPEPPH	0.5
	11.6	125–149	HEAGFPPSDDEDEEGEEFVLDYVEH	
	11.8	177–202	RTCGMFVYSPVSEPEPEPEPEPEPAR	
	13.3	223–244	RECNSSTDSCDSGPSNTPPEIH	
c-*myc*	2.3	10–51	RNYDLDYDSVQPYFYCDEEEN-FYQQQQQSELQPPAPSEDK	0.5
	−3.9	52–65	KFELLPTPPLSPSR	
	−1.7	83–126	RGDNDGGGGSFSTADQLEMVTELLGGDM-VNQSFICDPDDETFIK	
	−1.3	168–206	HSVCSTSSLYLQDLSAAASECIDPSVVF-PYPLNDSSSPK	
	8.6	206–241	KSCASQDSSAFSPSSDSLLSSTESSPQG-SPEPLVLH	
	25.4	241–269	HEETPPTTSSDSEEEQEDEEEIDVVSVEK	
	−0.6	276–287	RSESGSPSAGGH	
p53	−3.7	39–62	HCMDDLLLPQDVEEFFEGPSEALR	0.5
	1.7	62–98	RVSGAPAAQDPVTETPGPVAPAPA-TPWPLSSFVPSQK	
	0.1	213–232	HSVVVPYEPPEAGSEYTTIH	
c-*fos*	−2.8	31–91	HSPADSFSSMGSPVNAQDFCTDLAV-SSANFIPTVTAISTSPDLQWLVQPA-LVSSVAPSQTR	0.5
	10.1	128–139	KVEQLSPEEEEK	
	5.7	205–250	KIPDDLGFPEEMSVASLDLTGGL-PEVATPESEEAFTLPLLNDPEPK	
	−3.3	265–279	KTEPFDDFLFPASSR	
	−1.8	307–358	HSGSLGMGPMATELEPLCTPVVTC-TPSCTAYTSSFVFTYPEADSFPSCAAAH	
	4.4	360–380	KGSSSNEPSSDSLSSPTLIAL	
ODC	0.0	298–333	KEQPGSDDEDESNEQTFMYYVNDGVYG-SFNCILYDH	0.5
	5.2	423–449	HGFPPEVEEQDDGTLPMSCAQESGMDR	
v-*myb*	1.9	4–16	KVEQEGYPQESSK	0.5
	−4.5	174–186	HGCLPEESASPAR	
P_{730}	−2.1	323–361	HLQYMENMNSIASLVMAVVVNENEED-DEAESEQPAQQQK	1.0
HSP 70	0.8	33–46	RTTPSYVAFTDSER	1–2
	−4.0	125–152	KETAEAYLGESITDAVITVPAYFNDSQR	
	−3.1	424–445	KTFSTYSDNQPGVSIQVYEGER	
HMG-CoA reductase	4.2	381–395	KLSSVEEEPGVSQDR	1.5–3
	5.5	429–442	RTQELEIELPSEPR	
	−4.4	442–456	RPNEECLQILESAEK	
TAT	0.3	382–395	HFPEFENDVEFTER	2
α-Casein	6.6	58–79	KEMEAESISSSEEIVPNSVQEK	2–5
	−1.9	151–193	RQFYQLDAYPSGAWYYVPLGTQYTDA-PSFSDIPNPIGSENSEK	
β-Casein	6.6	1–25	RELEELNVPGEIVESLSSSEESITR	2–5
	−2.8	113–134	KYPVQPFTESQSLTLTDVENLH	

*References for the sequences and half-lives are as described (*41*).

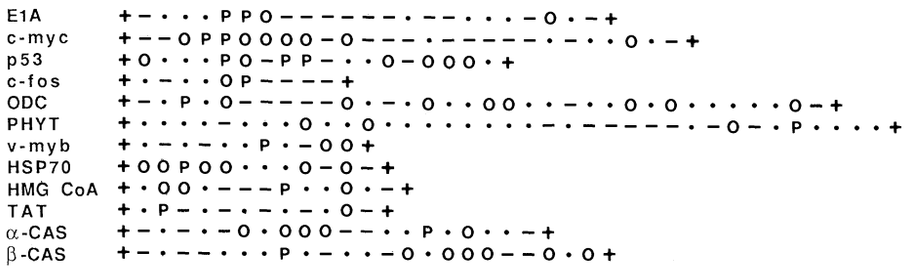

Fig. 1. Schematic representation of PEST regions. Several PEST sequences are presented in schematic form to display similarities among them. + Denotes Arg, Lys, or His; − denotes Glu or Asp; ○ denotes Ser or Thr; P denotes Pro; and all other amino acids are denoted by ●. It can be seen that PEST regions are flanked by positively charged amino acids and that negatively charged amino acids tend to be clustered.

thy plots as tracts of extreme hydrophilicity followed or occasionally preceded by a hydrophobic peak (Fig. 2A). While this feature does not uniquely identify PEST regions, 12 of the 21 positive PEST sequences identified in this study approximate the pattern (Fig. 2C).

The short sequence, Asn-x-Ser/Thr, identifies potential glycosylation sites in secretory and transmembrane proteins (*3*). We have not found a similar consensus sequence within PEST, although Pro-x-Glu/Asp is frequently present. The absence of a unique arrangement of amino acids suggests that PEST regions are more analogous to leader peptides where biochemical specificity is imparted by structural features of the sequence rather than recognition of specific residues (*13*).

A schematic comparison of the frequencies and distributions of PEST regions within the 12 rapidly degraded proteins and 35 "stable" proteins (*14*) is presented in Fig. 3. The enrichment of PEST regions among the rapidly degraded proteins was confirmed by statistical analysis ($P < 0.001$, Mann-Whitney U test). Two other features of amino acid sequences are noted in Fig. 3; rapidly degraded proteins contain an abundance of Arg-Arg pairs ($P < 0.05$) and a marginally significant enrichment of (Cys, His) (with the order Cys-His or His-Cys) pairs ($P < 0.06$). However, both caseins and ODC lack Arg-Arg or (Cys, His) pairs indicating that PEST regions are the primary determinants for rapid proteolysis.

If as we have suggested, PEST sequences confer rapid degradation, how might they do so? This is a difficult question because our understanding of intracellular proteolytic pathways remains limited despite the progress made by analyzing ubiquitin-dependent proteolysis in reticulocyte lysates (*15*). Degradation of p53 requires adenosine triphosphate (ATP) (*16*) and the breakdown of newly synthesized, rapidly degraded proteins in cultured cells proceeds with an Arrhenius activation energy (E_a) between 10 and 15 kcal/mol (*17*). This contrasts with an E_a approaching 30 kcal/mol for both ubiquitin-dependent proteolysis in reticulocyte lysates and degradation of longer lived proteins injected into HeLa cells (*18*). These divergent values can be interpreted as evidence for the existence of two degradative pathways with different rate-limiting steps. The E_a for proteolysis of ^{125}I-labeled caseins within HeLa cells is 13 kcal/mol (*19*), an activation energy low enough to suggest that the major ubiquitin-mediated pathway is not responsible for the degradation of this injected protein.

Another mechanism could involve proline endopeptidase, which has been identified in a variety of tissues from several species (*20*). Direct cleavage by this enzyme could explain the presence of Pro within PEST sequences since (under certain conditions) polyproline and polyglutamic acid form extended left-handed helices (*21*) and enrichment of these two amino acids in PEST regions might impart unusual conformations important for proteolysis.

The extreme instability of caseins, proteins known to bind calcium, suggests a more complicated pathway. The PEST region in murine ODC encompassed by residues 293 and 313 contains possible phosphorylation sites for casein kinase II (*22*). In fact, because casein kinases phosphorylate serines and threonines adjacent to negatively charged residues (*23*), many PEST sequences contain potential sites for phosphorylation by these enzymes. An interesting model can be developed by assuming that phosphorylation in PEST regions generates Ca^{2+}-binding sites. Calcium-activated proteases, the calpains, have long been known to reside in the cytoplasm of eukaryotic cells (*24*). In view of the extremely low Ca^{2+} concentration in the cytosol (*25*), the relatively high Ca^{2+} concentrations, 40 μ*M* or

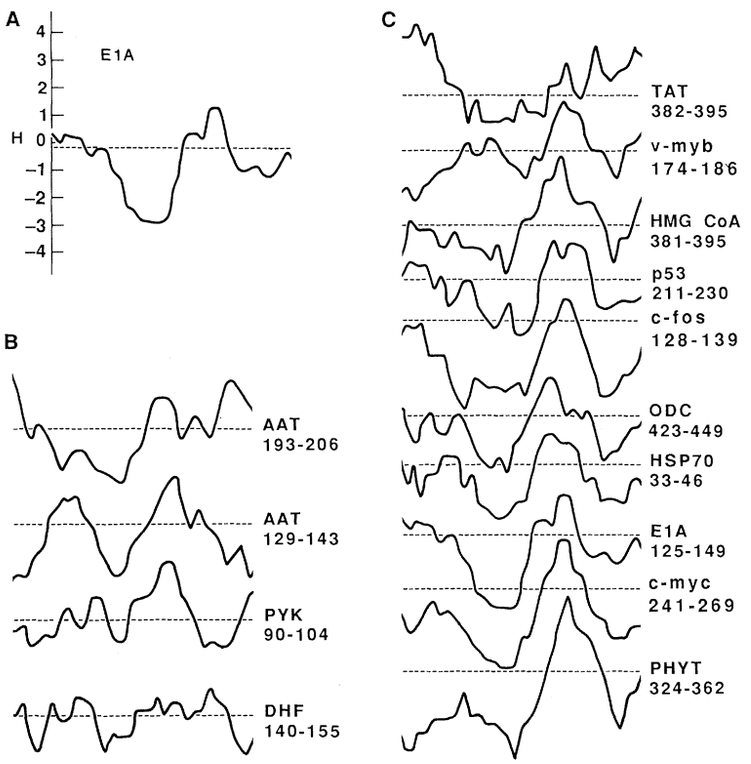

Fig. 2. Hydrophobicity (H) plots of selected PEST regions. Residue hydrophobicities were averaged over windows of seven residues. The vertical H scale denotes the average free energy in kilocalories per mole per amino acid, and the dashed line represents polyglycine. The plots were generated on an IBM-portable computer with the "SOAP" program of Kyte and Doolittle (*12*) written in Pascal by J. S. Parkinson. (A) Hydrophobicity plot for residues 125–149 in the adenovirus early protein, E1A. (B) Hydrophobicity plots for the four PEST regions observed in the stable set. (C) Hydrophobicity plots for a subset of PEST regions from rapidly degraded proteins. The sequences have been aligned to emphasize the common features of a deep hydrophilic valley followed by a rather sharp hydrophobic peak.

greater, required to demonstrate Ca^{2+}-activated proteolysis in vitro have presented some conceptual problems regarding the physiological roles of these enzymes. Recent sequence information confirms that calpains contain Ca^{2+}-binding domains (*26*), but the source of the Ca^{2+} that fills the domain is unknown. If potential calpain substrates were capable of sequestering Ca^{2+} within PEST regions, peptide bond hydrolysis might occur at low apparent Ca^{2+} concentrations. Thus, Ca^{2+}-binding to appropriate substrates might elicit degradation by Ca^{2+}-activated proteases just as ubiquitin conjugation to suitable substrate proteins results in proteolysis by a large, ATP-dependent reticulocyte protease (*27*).

To our knowledge, all rapidly degraded eukaryotic proteins of known sequence contain one or more PEST regions. However, since there are stable proteins that contain PEST regions, we assume that other factors, such as intracellular location or masking due to association with other macromolecules, also influence the degradation of intracellular proteins (*14*). This is supported by the conditional proteolysis of some rapidly degraded proteins, dependent on such events as formation of a complex [p53 and T antigen (*28*)], changes in spectral state [phytochrome (*29, 30*)], and developmental stage [cyclin (*31, 32*)]; cyclin contains a strong PEST region (PEST-FIND = 4.8). These three examples, which show that PEST regions do not invariably confer rapid degradation on proteins containing them, may explain why truncated HMG CoA reductase is stabilized even though it retains PEST regions (*33*).

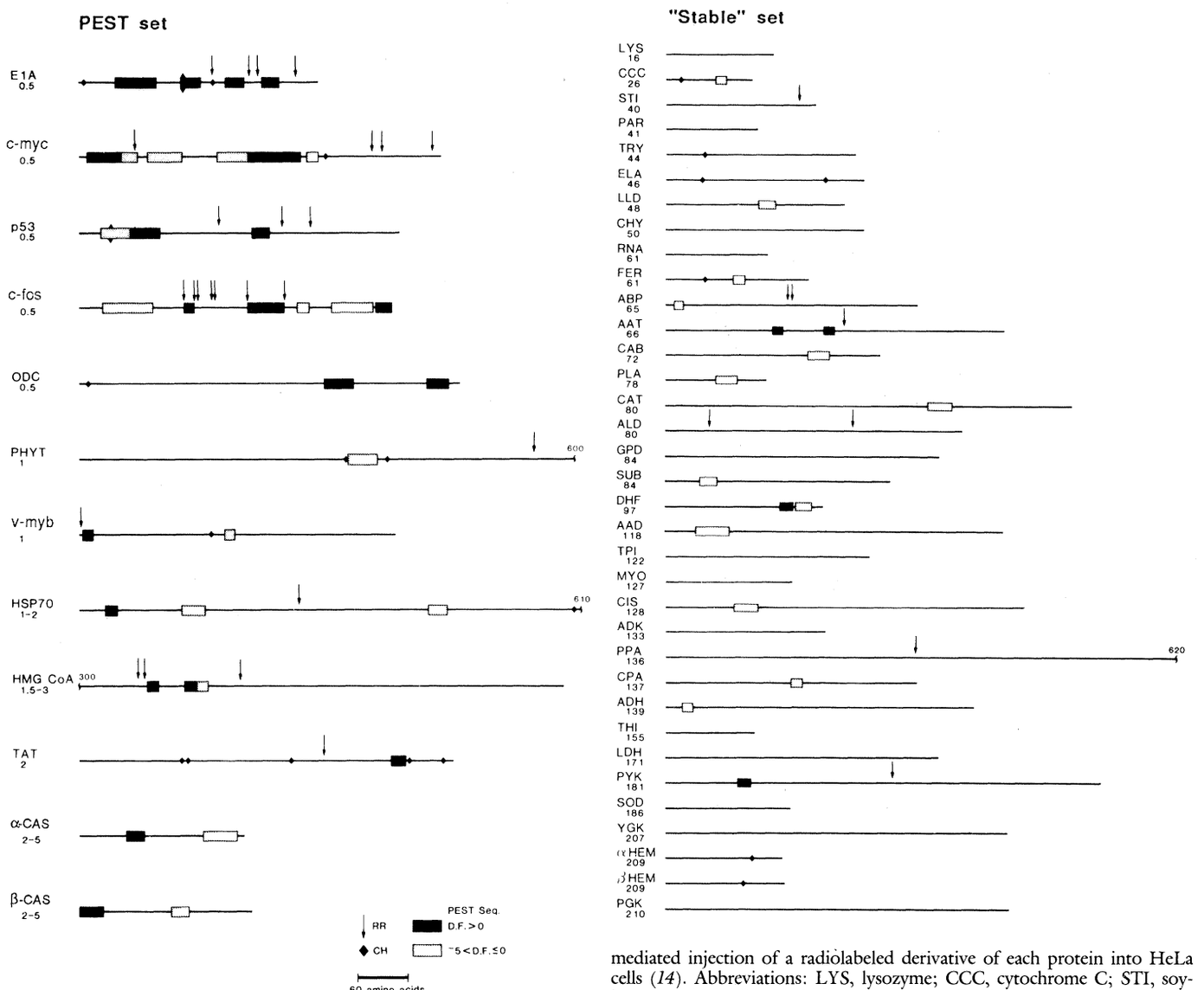

Fig. 3. Diagrammatic representation of the positions of PEST regions, Arg-Arg pairs, and Cys, His pairs among 12 rapidly degraded proteins and 35 stable proteins. Each protein is represented by a thin horizontal line proportional to the length of its amino acid sequence except where numbers denote truncation at a specific residue. PEST regions with PEST-FIND scores greater than 0 are shown in black; those scoring between −5 and 0 are gray. Arrows identify adjacent Arg-Arg pairs and diamonds denote adjacent Cys/His residues. The half-life in hours of each protein is listed under its abbreviation. Excluding α- and β-casein (CAS), half-lives for proteins in the PEST set were obtained from the literature (see Table 1). Half-lives of all proteins in the stable set and the caseins were determined by red blood cell–mediated injection of a radiolabeled derivative of each protein into HeLa cells (*14*). Abbreviations: LYS, lysozyme; CCC, cytochrome C; STI, soybean trypsin inhibitor; PAR, parvalbumin; TRY, trypsinogen; ELA, elastase; LLD, light chain dimer McG; CHY, chymotrypsinogen; RNA, ribonuclease A; FER, ferritin; ABP, arabinose binding protein; AAT, aspartic aminotransferase; CAB, carbonic anhydrase B; PLA, phospholipase A2; CAT, catalase; ALD, aldolase; GPD, glyceraldehyde 3-phosphate dehydrogenase; SUB, subtilisin; DHF, dihydrofolate reductase; AAD, D-amino acid oxidase; TPI, triose phosphate isomerase; MYO, myoglobin; CIS, citrate synthase; ADK, adenylate kinase; PPA, phosphorylase A; CPA, carboxypeptidase A; ADH, alcohol dehydrogenase; THI, thioredoxin; LDH, lactate dehydrogenase; PYK, pyruvate kinase; SOD, superoxide dismutase; YGK, yeast phosphoglycerate kinase; HEM, oxyhemoglobin; PGK, phosphoglycerate kinase.

Prokaryotic cells also contain rapidly degraded proteins, and several with known sequence were surveyed for PEST. Lambda proteins N and CII have half-lives less than 5 minutes (*34*); the *Escherichia coli* heat shock σ factor, htpr, is also highly unstable (*35*). Since none of these proteins contains a PEST region, other structural features must elicit their rapid proteolysis. Indeed, the recent observation that E1A and *myc* protein can be overproduced in *E. coli* (*36*) suggests that PEST does not signal rapid proteolysis in bacteria.

The PEST hypothesis is one of several proposed relationships between protein structure and intracellular stability. Dehlinger and Schimke suggested that larger proteins are degraded faster than smaller ones (*37*), and they also speculated that dissociation of multimeric proteins would lead to rapid proteolysis of the subunits (*37*). Dice and Goldberg, on the other hand, presented evidence that negatively charged proteins are degraded faster than positively charged proteins (*38*). Although the small number of PEST proteins limits comparison to previous hypotheses, certain features of PEST proteins provide some support for two of the proposed correlations. In general, PEST proteins are acidic and should, therefore, contribute to any relationship between negative charge and instability. The behavior of adenosine 3′,5′-monophosphate (cAMP)–dependent kinase, which contains PEST sequences, supports the dissociation hypothesis. The cAMP-dependent kinase is a tetrameric enzyme composed of two regulatory (R) and two catalytic (C) subunits (*39*). The enzyme dissociates into R_2 and C_2 dimers in the presence of cAMP, and the dimers are degraded much faster than the holoenzyme (*40*). We recently found that both R and C subunits contain strong PEST sequences, so we suggest that dissociation exposes these regions as Schimke hypothesized.

In summary, we have proposed that PEST regions confer susceptibility to rapid intracellular proteolysis, and we have speculated on possible mechanisms. The rapid degradation of injected casein provides direct experimental support for the hypothesis, but further experiments are needed to test these ideas rigorously. One expects that attaching PEST sequences to stable proteins, either chemically or genetically, will produce rapidly degraded hybrid proteins. By the same reasoning, removal of PEST regions should stabilize proteins within animal cells. If such experiments support the hypothesis, it should be possible to vary sequences within PEST regions and thus assess the contribution of individual amino acids to rapid turnover.

REFERENCES AND NOTES

1. D. D. Sabatini, G. Kreibich, T. Morimoto, T. M. Adesnik, *J. Cell. Biol.* **92**, 1 (1982).
2. G. Schatz and R. A. Butow, *Cell* **32**, 316 (1983).
3. D. Kalderon, W. D. Richardson, A. F. Markham, A. E. Smith, *Nature (London)* **311**, 33 (1984); M. N. Hall, L. Hereford, I. Herskowitz, *Cell* **36**, 1057 (1984); P. A. Silver, L. P. Keegan, M. Ptashne, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5951 (1984).
4. The algorithm for identifying PEST regions is based on enrichment in proline (P), glutamic acid (E), aspartic acid (D), serine (S), and threonine (T). This parameter is combined with hydrophilicity to obtain a PEST-FIND score as follows. Stretches of amino acid sequence containing ten or more residues between positive flanks (His, Lys, or Arg) are selected and the mole percent (MP) of PEDST is calculated after subtracting one mole equivalent of P, E/D, S/T. At the same time, an average hydrophobicity value (H_0) is computed for the sequence by summing the product of the MP of each residue and its normalized hydrophobicity value. The latter was obtained by converting the hydrophobicity values of Kyte and Doolittle (*12*) to positive integers ranging from 0 for Arg to 90 for Ileu. The two parameters, MP and H_0, are then combined in the linear equation PEST-FIND = 0.5 (MP) − 0.5 (H_0). The PEST-FIND score, which can range from −45 for polyileu to about +50 for polyasp plus one Pro and one Ser, is the value listed in Table 1. A computer program written in IBM-BASIC allows automatic searches for PEST regions, and it is available upon request. The use of positive residues as start and stop signals during computer searches, though objective and convenient, can produce PEST scores lower than intuitively expected due to dilution by "non-PEST" sequence between the flanks. See Table 1, residues 323–361 in phytochrome and residues 298–333 in ODC as examples.
5. R. Ralston and J. M. Bishop, *Nature (London)* **306**, 803 (1983).
6. ODC—S. Anehus *et al.*, *Eur. J. Cell. Biol.* **35**, 264 (1984); HMG CoA reductase—D. A. Brown and R. D. Simoni, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1674 (1984); phytochrome—L. H. Pratt, *Annu. Rev. Plant. Physiol.* **33**, 557 (1982).
7. M. Rechsteiner, *Techniques in Somatic Cell Genetics*, G. Shay, Ed. (Plenum, New York, 1983), pp. 385–398.
8. T. McGarry, R. Hough, S. Rogers, M. Rechsteiner, *J. Cell. Biol.* **96**, 338 (1983).
9. M. O. Dayhoff, L. T. Hunt, S. Hurst-Calderone, in *Atlas of Protein Sequence and Structure*, M. O. Dayhoff, Ed. (National Biomedical Research Foundation, Silver Spring, MD, 1978), vol. 5, p. 363.
10. M. Go and S. Miyazawa, *Int. J. Pept. Protein Res.* **15**, 211 (1980).
11. The mean frequency of success runs (μ) in Bernoulli trials is given by $\mu = 1 - P^R/qP^R$ where P = probability of success, q = probability of failure and R = run length. J. J. Hunter, *Mathematical Techniques of Applied Probability* (Academic Press, New York, 1983), p. 239.
12. J. Kyte and R. F. Doolittle, *J. Mol. Biol.* **157**, 105 (1982).
13. G. Von Heijne, *ibid.* **173**, 243 (1984).
14. S. Rogers and M. Rechsteiner, in *Intracellular Protein Catabolism*, E. Khairallah, J. Bond, J. W. Bird, Eds. (Liss, New York, 1985), p. 405.
15. A. Hershko and A. Ciechanover, *Annu. Rev. Biochem.* **51**, 335 (1982).
16. R. M. Gronostajski, A. L. Goldberg, A. B. Pardee, *Mol. Cell. Biol.* **4**, 442 (1984).
17. N. T. Neff, G. N. DeMartino, A. L. Goldberg, *J. Cell. Physiol.* **101**, 439 (1979); P. Bates, G. A. Coetzee, D. R. Van der Westhuyzen, *Biochim. Biophys. Acta* **719**, 377 (1982).
18. R. Hough and M. Rechsteiner, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 90 (1984).
19. M. Rechsteiner, unpublished results.
20. P. C. Andrews, C. D. Minth, J. E. Dixon, *J. Biol. Chem.* **257**, 5861 (1982); M. Orlowski, *Mol. Cell. Biochem.* **52**, 49 (1983).
21. K. H. Forsythe and A. J. Hopfinger, *Macromolecules* **6**, 423 (1973).
22. C. Kahana and D. Nathans, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1673 (1985).
23. G. M. Hathaway and J. A. Traugh, *Curr. Top. Cell Regul.* **21**, 101 (1982).
24. T. Murachi, K. Tanaka, M. Hatanaka, T. Murakami, *Adv. Enzymol.* **19**, 407 (1981); G. N. DeMartino and D. E. Croall, *Arch. Biochem. Biophys.* **232**, 713 (1984).
25. A. P. Somlyo, *Nature (London)* **309**, 516 (1984).
26. S. Ohno *et al.*, *ibid.* **312**, 566 (1984).
27. R. Hough, G. Pratt, M. Rechsteiner, *J. Biol. Chem.* **261**, 2400 (1986).
28. P. T. Mora, K. Chandrasekaran, J. C. Hoffman, V. W. McFarland, *Mol. Cell. Biol.* **2**, 763 (1982); M. Oren, N. Reich, A. J. Levine, *ibid.*, p. 443; N. C. Reich and A. J. Levine, *Nature (London)* **308**, 199 (1984); R. M. Gronostajski, A. L. Goldberg, A. B. Pardee, *Mol. Cell. Biol.* **4**, 442 (1984).
29. L. H. Pratt, G. Kidd, R. Coleman, *Biochim. Biophys. Acta* **365**, 93 (1974).
30. H. P. Hershey, R. F. Barker, K. B. Idler, J. L. Lissemore, P. H. Quail, *Nucleic Acids Res.* **13**, 8543 (1985).
31. T. Evans, E. T. Rosenthal, J. Youngblom, D. Distel, T. Hunt, *Cell* **33**, 389 (1983).
32. J. Ruderman, personal communication.
33. G. Gil, J. R. Faust, D. J. Chin, J. L. Goldstein, M. S. Brown, *Cell* **41**, 249 (1985).
34. S. Gottesman, M. Gottesman, J. E. Shaw, M. L. Pearson, *ibid.* **24**, 225 (1981).
35. C. Georgopoulos, personal communication.
36. B. Ferguson, N. Jones, J. Richter, M. Rosenberg, *Science* **224**, 1343 (1984); R. A. Watt, A. R. Shatzman, M. Rosenberg, *Mol. Cell. Biol.* **5**, 448 (1985).
37. P. J. Dehlinger and R. T. Schimke, *Biochem. Biophys. Res. Commun.* **40**, 1473 (1970).
38. J. F. Dice and A. L. Goldberg, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3893 (1975).
39. J. A. Beavo, P. J. Bechtel, E. G. Krebs, *Adv. Cyclic Nucleotide Res.* **5**, 241 (1975).
40. R. A. Steinberg and D. A. Agard, *J. Biol. Chem.* **256**, 10731 (1981); B. A. Hemmings, *FEBS Lett.* **196**, 126 (1986).
41. *Adenovirus early protein E1A*: sequence—H. van Ormondt, J. Maat, C. P. van Beveren, *Gene* **11**, 299 (1980); half-life—K. R. Spindler and A. J. Berk, *J. Virol.* **52**, 706 (1984). *c-myc*: sequence—R. Watt *et al.*, *Nature (London)* **303**, 725 (1983); half-life—G. Ramsey, G. I. Evan, J. M. Bishop, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 7742 (1984); R. N. Eisenman, C. Y. Tachibana, H. D. Abrams, S. K. Hann, *Mol. Cell. Biol.* **5**, 114 (1985). *p53*: sequence—R. Zakut-Houri *et al.*, *Nature (London)* **306**, 594 (1983); D. Pennica *et al.*, *Virology* **134**, 477 (1984); half-life—(*28*). *c-fos*: sequence—F. van Straaten, R. Müller, T. Curran, C. Van Beveren, I. M. Verma, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3183 (1983); half-life—T. Curran, A. D. Miller, L. Zokas, I. M. Verma, *Cell* **36**, 259 (1984). *ODC*: sequence—(*22*) and M. Gupta and P. Coffino, *J. Biol. Chem.* **260**, 2941 (1985); half-life—L. Persson, J. E. Seeley, A. E. Pegg, *Biochemistry* **23**, 3777 (1984); T. Minaga, L. J. Martin, W. N. Piper, E. Kun, *Eur. J. Biochem.* **91**, 577 (1978). *v-myb*: sequence—K.-H. Klempnauer, T. J. Gonda, J. M. Bishop, *Cell* **31**, 453 (1982); half-life—W. J. Boyle, M. A. Lampert, A. C. Li, M. Baluda, *Mol. Cell. Biol.* **5**, 3017 (1985). *Phytochrome*: sequence—(*30*); half-life—(*29*). *Heat shock protein*: sequence—T. D. Ingolia, E. A. Craig, B. J. McCarthy, *Cell* **21**, 669 (1980); half-life—K. B. Palter, M. Watanabe, L. Stinson, A. P. Mahowald, E. A. Craig, *Mol. Cel. Biol.* **6**, 1187 (1986). *HMG-CoA reductase*: sequence—D. J. Chin *et al.*, *Nature (London)* **308**, 613 (1984); half-life—J. R. Faust, K. L. Luskey, D. J. Chin, J. L. Goldstein, M. S. Brown, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5205 (1982); D. J. Chin *et al.*, *Mol. Cell. Biol.* **5**, 634 (1985). *Tyrosine amino transferase*: sequence—T. Grange *et al.*, *J. Mol. Biol.* **184**, 347 (1985); half-life—F. Auricchio, D. Martin, G. Tomkins, *Nature (London)* **224**, 806 (1969). α-*Casein*: sequence—J.-C. Mercier, F. Grosclaude, B. Ribadeau-Dumas, *Eur. J. Biochem.* **23**, 41 (1971); half-life—this study. β-*Casein*: sequence—B. Ribadeau-Dumas, G. Brignon, F. Grosclaude, J.-C. Mercier *ibid.* **25**, 505 (1972); half-life—this study.

42. We would like to thank P. Coffino for providing us with the sequence of ODC prior to publication, E. Craig for the half-life of *Drosophila* HSP70, C. Georgopoulos for the half-life of htpr, A. Levine for discussions on p53 and relationships among oncogene products, J. S. Parkinson, M. Capecchi, B. Olivera, and K. V. Rote for helpful suggestions on the manuscript, and M. Vaughan for manuscript preparation. Supported by grant GM27159 from the National Institutes of Health.

27 March 1986; accepted 3 July 1986