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Do myc, fos and E1A function as protein phosphatase inhibitors?

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The oncogenic proteins myc, fos and E1A bear striking resemblance to protein phosphatase inhibitors 1 and 2. Both sets of proteins possess several regions rich in proline (P), glutamic acid (E), serine (S) and threonine (T). In addition to PEST sequences four of the five proteins contain clusters of arginine-arginine pairs. On the basis of these similarities, I suggest that myc, fos and E1A are protein phosphatase inhibitors.

In a recent review in Nature (1), Tony Hunter noted that "except for their localization to the nucleus, there has been paucity of clues as to the functions of myc and fos proteins." Upon examining the sequences of rapidly degraded intracellular proteins, myc and fos included, we observed that they contain one or more PEST sequences--stretches of 14 to 50 amino acids rich in proline, glutamic acid, serine and threonine (2). We postulated that PEST regions confer a short half-life on proteins that contain them. During a follow-up survey of additional proteins for the presence of PEST sequences, I discovered (Table 1) that the protein phosphatase inhibitors 1 and 2 contain multiple PEST regions (3, 4). Because myc, fos and E1A similarly contain

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TABLE 1. PEST Sequences in myc, fos, E1A and phosphatase inhibitors 1 and 2

Protein	Residues	Sequence[a]	PEST Score[b]	Sequence Reference
Adenovirus early protein E1A	44-94	HELYDLDVTAPEDPNEEAVSQIFPDS-VMLAVQEGIDLLTFPPAPGSPEPPH	4.0	13
	125-149	HEAGFPPSDDEDEEGEEFVLDYVEH	11.6	
	177-202	RTCGMFVYSPVSEPEPEPEPEPEPAR	11.8	
	223-244	RECNSSTDSCDSGPSNTPPEIH	13.3	
c-myc	10-51	RNYDLDYDSVQPYFYCDEEEN-FYQQQQQSELQPPAPSEDK	2.3	14
	52-65	KFELLPTPPLSPSR	-3.9	
	83-126	RGDNDGGGGSFSTADQLEMVTELLGGDM-VNQSFICDPDDETFIK	-1.7	
	168-206	HSVCSTSSLYLQDLSAAASECIDPSVVF-PYPLNDSSSPK	-1.3	
	206-241	KSCASQDSSAFSPSSDSLLSSTESSPQG-SPEPLVLH	8.6	
	241-269	HEETPPTTSSDSEEEQEDEEEIDVVSVEK	25.4	
	276-287	RSESGSPSAGGH	-0.6	
c-fos	31-91	HSPADSFSSMGSPVNAQDFCTDLAV-SSANFIPTVTAISTSPDLQWLVQPA-LVSSVAPSQTR	-2.8	15
	128-139	KVEQLSPEEEEK	10.1	
	205-250	KIPDDLGFPEEMSVASLDLTGGL-PEVATPESEEAFTLPLLNDPEPK	5.7	
	265-279	KTEPFDDFLFPASSR	-3.3	
	307-358	HSGSLGMGPMATELEPLCTPVVTC-TPSCTAYTSSFVFTYPEADSFPSCAAAH	-1.8	
	360-380	KGSSSNEPSSDSLSSPTLIAL	4.4	
Protein phosphatase inhibitor 1	33-54	RPTPATLVLTSDQSSPEVDEDR	11.4	
	91-126	HLGQQEQGEEPEGAAEGTGAQESQ-PPGTPGTGAESR	10.4	3
Protein phosphatase inhibitor 2	75-101	HSMIGDDDDAYSDTETTEAMTPDTLAK	11.1	
	116-133	REQESSGEEDSDLSPEER	24.4	4
	165-197	HDDEEDEEMSETADGESMNTEES-NQGSTPSDQR	23.1	

[a]The partial sequences are presented using the one letter amino acid code in which 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.

[b]The algorithm for identifying PEST regions is based on enrichment in proline (P), glutamic acid (E), aspartic acid (D), serine (S) and threonine (T) combined with hydrophilicity as described in reference 2.

tandemly repeated PEST regions, I suggest that these three oncogene products may also function as protein phosphatase inhibitors.

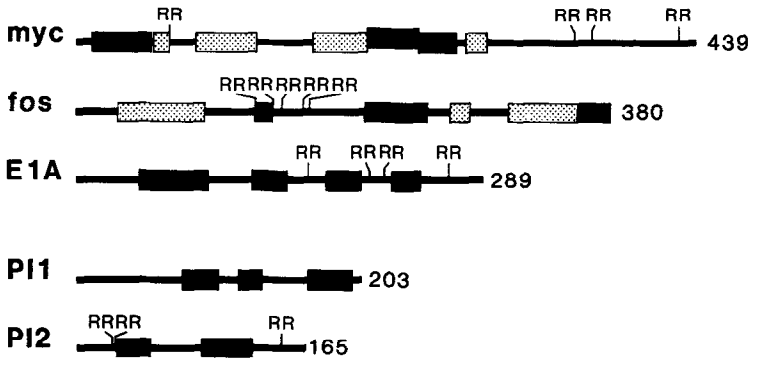

Figure 1. Diagrammatic representation of the positions of PEST regions and arg-arg pairs. Each protein is represented by a horizontal line proportional to the length of its amino acid sequence. PEST regions with scores greater than 0 are shown in black, those scoring between -5 and 0 are grey. The number of amino acids is given at the right margin of each protein, and arg-arg pairs are denoted RR.

Several observations support this hypothesis. First, PEST sequences generally comprise a small fraction of the polypeptide chain even in rapidly degraded proteins (2). However, myc, fos, and E1A, like protein phosphatase inhibitors 1 and 2, are composed almost entirely of PEST regions (Figure 1). If PEST served only to signal rapid turnover, myc, fos, and E1A would seem to have no function other than to be destroyed. Second, when the Protein Identification Resource Sequence Library was examined using the FASTP search program (5), residues 110 to 145 in protein phosphatase inhibitor 2 showed some homology to sequences in the gag polyprotein of simian sarcoma virus, polyoma large T antigen and myc ($3 < Z < 6$). Holmes et al. (4) already noted the overlap between residues 128 to 137 in protein phosphatase inhibitor 2 and residues 131 to 140 in fos. Similar searches using protein phosphatase inhibitor 1 did not reveal significant homology to myc, fos or E1A. Nevertheless, PEST regions are probably flexible, surface loops based on their

extreme hydrophilicity. In fact, X-ray data are available on three proteins that contain weak PEST regions (RNAse T1, dihydrofolate reductase and the Klenow fragment of DNA pomyerase I), and the PEST regions do not contribute to the electron density. Consequently, PEST sequences may, like signal sequences (6), perform similar functions despite the apparent absence of sequence homology. Third, cAMP-dependent kinase provides an additional connection between PEST sequences and phosphatase activity. The rapidly-degraded, regulatory subunit (R) of cAMP kinase contains 2 PEST regions near the amino terminus (7); the R2 dimer also inhibits protein phosphatase (8, 9). Fourth, inhibition of protein phosphatase activity is equivalent to enhancement of kinase activity, and the latter is a well-documented mode of oncogene action (10). Taken together these findings raise the distinct possibility that myc, fos and E1A function as phosphatase inhibitors.

An attractive feature of my suggestion is the ease with which it can be tested by those with purified oncogene products. Since E1A and myc have been overproduced in E. coli (11, 12), and there are in vitro assays for protein phosphatase inhibitor activity, (3, 4) a decisive test of the hypothesis is possible.

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