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Hypothesis

Prothymosin α is a nuclear protein

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Prothymosin α, a protein first isolated from rat thymus and widely distributed in animal tissues, has an attributed role in the stimulation of the immune system. Its structure contains thymosin α_1, a Glu-rich domain and a putative nuclear location signal. Furthermore, the amount of this protein seems to be associated with the relative size of the nucleus and is inducible during cell growth. We postulate that prothymosin α is located inside the cell nucleus and that its activity might be to organize some protein complexes.

Prothymosin α; Karyophilic signal; Acidic peptide; Nuclear protein

1. INTRODUCTION

Thymosin fraction 5 is a mixture of peptides extracted from calf thymus which has been reported to correct some immunodeficiencies and to induce T-lymphocyte differentiation in animal models. From this fraction, thymosin α_1 was isolated, which is a 28-amino-acid peptide found to be active in several in vitro assays used for thymosin fraction 5. Thus, it was considered to be one of the modulating factors in T-lymphocyte maturation (review [1]). Later, Horecker et al. [2], using a procedure designed to avoid any proteolytic modification, isolated a polypeptide from rat thymus, named prothymosin α, which contains the thymosin α_1 sequence at its N-terminus. Moreover, the existence of two other peptides in fraction 5, differing from thymosin α_1 only in their C-terminus [thymosin α_{11} and des-(25–28)-thymosin α_1] and also being contained in prothymosin α, led to the idea that the small peptides arose by proteolysis from prothymosin α during the extraction procedures (review [2]). Sequence analysis of cDNA for human prothymosin α [3,4] showed that it is not synthesized as a larger precursor molecule, and that its acidic properties (pI = 3.55) result from a large content of Glu/Asp residues clustered in the central region of the protein [4].

The assays for biological activity of thymic peptides were based on their ability to restore certain parameters of immune function [1]. Nevertheless, some proteins exhibiting activity in these tests and therefore with a proposed role in the immune system were shown not to carry out an immunity-related function. The proteins concerned were polypeptide β_1, identical to ubiquitin [5], thymosin β_4, a structural peptide [6], homeostatic thymus hormone, identical to histones H2A and H2B [7] and a serum thymic hormone, identical to serum prealbumin [8]. Prothymosin α was also found to be active in a mouse protection assay [9] but its biological role still remains unclear.

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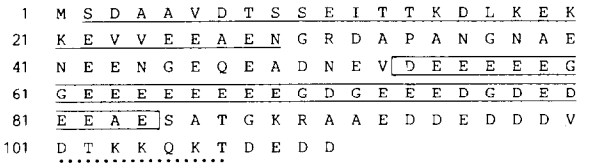

Fig.1. Amino acid sequence (single-letter code) of human prothymosin α as deduced from its cDNA [4]. The sequence of thymosin α_1 is underlined. The box contains the glutamic acid-rich cluster. The dotted line indicates the proposed nuclear location signal.

2. SIMILARITIES OF PROTHYMOSIN α WITH THE ACIDIC NUCLEAR PROTEINS

The overall acidic character and the presence of an acid-rich domain, 31 residues long, in the prothymosin α sequence (fig.1) are two significant similarities with certain nuclear peptides. Large Glu or Asp stretches were identified in the nucleus-located proteins nucleoplasmin [10], cyclin [11], nucleolin [12], NO38 [13], N1/N2 [14], the product of gene *rad6* of *Saccharomyces cerevisiae* [15] and several HMG chromosomal proteins [16], among others. These highly negatively charged regions have been related to nucleosome formation, since it has been found that polyglutamate and polyaspartate facilitate nucleosome assembly in vitro [17], as is the case for nucleoplasmin [18]. It has been suggested that acidic proteins could also be involved in nucleosome disassembly by competition with DNA for histone binding [18]. Moreover, in nucleolar proteins, the acid domains may function in the maturation of pre-ribosomes, such a role having been attributed to nucleolin [12] and NO38 [13].

In several nuclear proteins an amino acid sequence involved in nuclear location was identified. This sequence, as was demonstrated in SV40 large T antigen, consists of a short basic stretch in which the presence of Lys-128 (table 1) is critical for protein transport into the nucleus [19]. Similar karyophilic signals were recognized in other nuclear proteins (table 1). We have found a sequence (DTKKQKT) homologous to SV40 large T signal and located in the C-terminal region of prothymosin α, where Lys-103 would be equivalent to Lys-128. Based on comparison of these sequences we propose a consensus for nuclear location signals (table 1).

3. TISSUE DISTRIBUTION, LEVELS AND EXPRESSION OF PROTHYMOSIN α

Prothymosin α has a wide tissue distribution, having been detected by radioimmunoassay in several rat tissues (thymus, spleen, lung, kidney, liver, and brain) [9]. Using Northern blot analysis with prothymosin α cDNA as a probe, its mRNA was found in lymphoid and non-lymphoid mouse and human tissues [4]. It is remarkable that a direct relation exists between the level of prothymosin and the nucleus/cytoplasm ratio in each tissue, e.g. 414 μg per g thymus vs 58 μg per g brain [9]. In human blood, prothymosin α was found mainly in the leukocyte fraction (90%) while it was virtually absent from plasma (10%) and non-nucleate cells (1–2%) [20].

The expression of prothymosin α mRNA as

Table 1

Comparison of nuclear location signals

Protein	Organism	Segment	Sequence							Reference
Large T antigen	SV40	126–132	Pro	Lys	Lys	Lys	Arg	Lys	Val	19
N1/N2	*Xenopus laevis*	531–537	Val	Arg	Lys	Lys	Arg	Lys	Thr	14
Nucleoplasmin	*Xenopus laevis*	175–181	Pro	Thr	Lys	Lys	Gly	Lys	Gly	10
NO38	*Xenopus laevis*	153–159	Pro	Arg	Lys	Lys	Thr	Lys	Leu	13
Nucleolin	hamster	292–298	Glu	Ala	Lys	Lys	Gln	Lys	Val	12
Lamins A/C	human	415–421	Val	Thr	Lys	Lys	Arg	Lys	Leu	21
Prothymosin α	human	101–107	Asp	Thr	Lys	Lys	Gln	Lys	Thr	4
Proposed consensus[a]			X	B/Thr	Lys	Lys	Z	Lys	X	this paper

[a] X, any residue; B, basic residue; Z, polar residue

determined by blot-hybridization analysis showed low mRNA levels in resting lymphocytes, increasing 15-fold in quantity after treatment of the cells with several mitogens [4]. When quiescent NIH 3T3 cells were stimulated with serum, the amount of prothymosin α mRNA increased within 12 h of cell proliferation [4]. A similar pattern was found in the nuclear proteins nucleolin [12] and cyclin [11], whose synthesis correlates with cell growth [11]. A role in nucleosome or pre-ribosome formation is expected to demonstrate mRNA expression similar to that displayed by prothymosin α.

4. CONCLUSION

All the pieces of evidence presented above (putative nuclear signal, acidic stretch, wide distribution, inducible expression) have led us to propose that prothymosin α is a non-immune peptide located within the cell nucleus. Since certain unusual characteristics of prothymosin α are similar to those of acid nuclear proteins, its role in the nucleus might involve nucleosome assembly and/or disassembly.

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