Human prothymosin α inhibits division of yeast *Saccharomyces cerevisiae* cells, while its mutant lacking nuclear localization signal does not

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Abstract Effect of human prothymosin α and its mutant overproduced in *S. cerevisiae* on yeast cell division was studied. Wild-type prothymosin α appeared to block division of yeast cells. Its inhibitory action could be abolished by deletion of the last nine carboxy-terminal amino acids of prothymosin α containing nuclear localization signal, thus pointing to the nucleus as a compartment, where prothymosin α performs its action.

Key words: Prothymosin α; Cell division; Nuclear localization signal; *S. cerevisiae*

1. Introduction

Prothymosin α (ProTα) is a highly acidic protein of 13 kDa, which is thought to be a precursor of thymosin α1, a putative thymic hormone [1]. However, accumulating evidence suggests that, apart from its immunomodulating activity, ProTα provides some general function within the cell, most probably related to cell division [2–5]. In accord with this notion is the presence of the karyophilic sequence in the ProTα molecule [6,7] responsible for the nuclear targeting of the protein, and ubiquity of ProTα in various tissues and species, including unicellular organisms such as yeast [8].

Here, human ProTα was produced in yeast *S. cerevisiae*, and its effect on yeast cell division was studied. Wild type human ProTα appeared to block division of yeast cells. Most notably, deletion of the carboxy-terminal nuclear localization signal of the protein abolished this inhibitory effect.

2. Materials and methods

Escherichia coli JM109 and *Saccharomyces cerevisiae* SKY594 [MATa, *leu2-3, 112, lys7, ura3-52 (cir°)*] and 2805 [MATa, *pep4::His3, prb 1-δ*, can1, Gal2, his3δ, ura3-52] strains were used throughout this work.

Yeast shuttle vector pYeDP1/8-2 [9] was kindly provided by D. Pompon. Construction of pYeHT1 containing human ProTα cDNA inserted into *Bam*HI and *Kpn*I sites of pYeDP1/8-2 was described in [10]. To obtain DNA fragment coding for ProTα lacking karyophilic signal, a PCR was performed on pYeHT1 with primers (5′-dATGTCAGACGCAGCCGTAGA-3′) and (5′-dCTAGGTATCGACATCGTCATC-3′) corresponding to the very amino-terminus of ProTα and to the carboxy-terminal region of the protein excluding the last nine amino acid residues, respectively. A 303 bp long PCR product was inserted into the *Sma*I site of pUC19, sequenced, and then recloned into the *Bam*HI–*Kpn*I sites of pYeDP1/8-2 yielding pYeKHT1. Construction of the plasmid encoding ProTα with three point mutations [Ser[1]Thr, Asn[39]Asp, Lys[87]Glu] will be described elsewhere (Yu.R. and A.V., manuscript in preparation).

Yeast cells were transformed with the plasmids by electroporation [11]. For induction of GAL10-CYC1 promoter, cultures grown in glucose-containing SD medium (2% glucose, 0.67% yeast nitrogen base, 0.1% casamino acids) supplemented with 30 μg/ml L-lysin and 60 μg/ml L-leucine were washed twice with the same medium containing 2% galactose instead of glucose, resuspended in this medium, and grown at 30°C.

The percentage of cells that survive galactose treatment was determined by the colony-forming-unit test essentially as described in [12].

Procedure for ProTα isolation was as follows. The cells were lysed in lysis buffer containing 6 mM Tris-HCl (pH 6.8), 10% SDS, 2% β-mercaptoethanol at 100°C for 3 min. An equal volume of hot (100°C) phenol saturated with 20 mM ammonium acetate (pH 4.5), 10 mM EDTA, 0.5% SDS was added, vortexed and incubated at 100°C for 2 min and then at 0°C for 10 min. After centrifugation the precipitate of cell debris was discarded and approx. one-third volume of chloroform was added to the supernatant for phase separation. The upper aqueous phase was re-extracted with saturated phenol and mixed with one-fifth volume of 3 M sodium acetate (pH 5.2). This resulted in a new separation of phases. ProTα was precipitated from the lower phenol phase with 3 volumes of ethanol, washed with ethanol, dried and dissolved in water. The sample was extracted twice with phenol/chloroform/TE and precipitated with ethanol. Electrophoresis of ProTα in 8% polyacrylamide gels containing 7 M urea was performed according to the standard technique employed for nucleic acid analysis [13]. The gels were stained with 0.2% Methylene blue in 50 mM ammonium acetate (pH 4.5) for 5 min, briefly destained with water, and immediately photographed.

3. Results

3.1. Production of human ProTα in S. cerevisiae

In order to test the effect of human ProTα on division of yeast *S. cerevisiae* cells, the protein-coding regions of cDNAs coding for the wild type human ProTα and its mutated form lacking nine C-terminal amino acids including nuclear localization signal were cloned into the pYeDP1/8-2 shuttle vector under the control of GAL10-CYC1 promoter, which could be induced by growing yeast cells in the galactose-containing medium [9].

Yeast cells bearing the appropriate plasmids were grown in glucose-containing medium and then shifted to the galactose-containing medium to induce transcription of ProTα cDNA. ProTα was isolated from these cells 18 hours after induction and subjected to analysis. Polyacrylamide gel electrophoresis commonly used for nucleic acid sequencing in conjunction with ProTα staining with nucleic acid-specific dye Methylene blue appeared to be an ideal means for ProTα analysis due to the impressive negative charge of this highly acidic protein. Fig. 1 demonstrates that both wild type ProTα and ProTα lacking nine C-terminal amino acids including nuclear localization signal were produced in *S. cerevisiae*, their electrophoretic mobilities being dependent upon the size of the respective protein. Identity of the overproduced protein with ProTα was con-

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Abbreviations: PCR, polymerase chain reaction; ProTα, prothymosin a.

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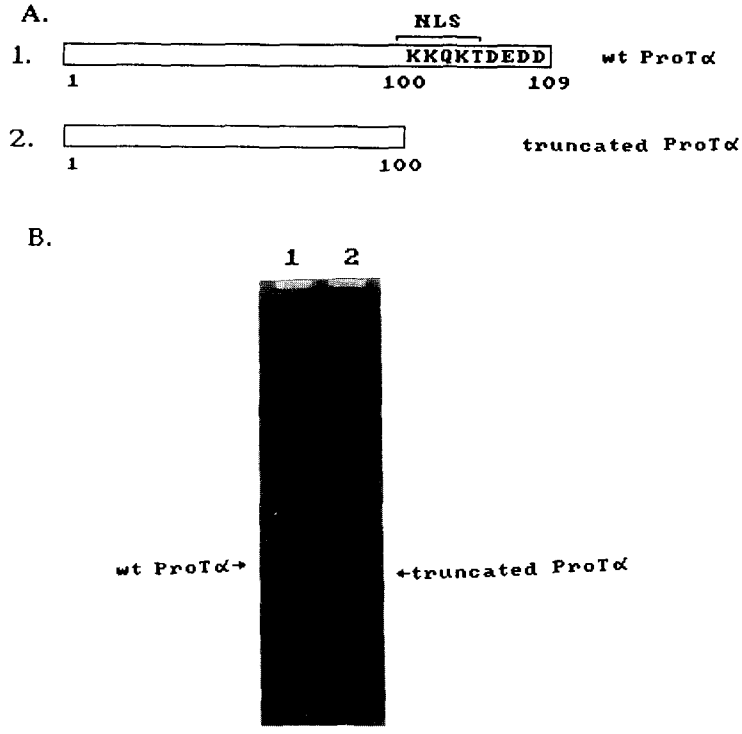

Fig. 1. Synthesis of human ProTα (1) and its truncated form (2) in yeast. (A) Schematic diagram of overproduced proteins; ProTα sequence which is absent in its truncated form is shown in the single-letter amino acid code; amino acids are numbered from the N-terminus; NLS, nuclear localization signal. (B) Electrophoretic analysis of wild type and truncated human ProTα isolated from equivalent amounts of yeast cells.

firmed by its amino acid analysis. No ProTα was observed when the cells were grown in the absence of the inducer (not shown) due to the low level of the endogeneous protein in yeast [8]. Thus both wild type human ProTα and its truncated form could be synthesized in *S. cerevisiae* in regulatable fashion. Besides, a yeast strain producing human ProTα with three point mutations [Ser[1]Thr, Asn[39]Asp, Lys[87]Glu] was obtained and proved to synthesize mutated human protein with high efficiency (not shown).

3.2. Fate of S. cerevisiae *cells producing human prothymosin* α

Behaviour of *S. cerevisiae* cells synthesizing ProTα was assessed by growing yeast cells bearing the respective plasmids in the galactose-containing medium. While in the absence of inducer the cells transformed with pYeHT1, pYeKHT1 and the vector pYeDP1/8-2 grew equally well, production of the wild type human ProTα resulted in severe inhibition of cell growth (Fig. 2). The inhibitory action of human ProTα on yeast cell growth appeared to be reversible: withdrawal of the inducer from the medium abolished transcription of the human ProTα gene and restored cell division. Thus, overproduction of human ProTα caused arrest of yest cell growth but not cell death, as was additionally confirmed by colony-forming- unit test (not shown).

Surprisingly, block of yeast cell division observed with wild type ProTα was almost completely abrogated by deletion of nine carboxy-terminal amino acids bearing the nuclear localization signal. Indeed, yeast cells synthesizing truncated ProTα produced even more protein as compared to the cells synthesizing wild type ProTα (see Fig. 1) and yet grew quite well (Fig. 2). Also, no growth inhibition was observed in case of the cells producing triple ProTα mutant (not shown).

4. Discussion

Although function of ProTα is obscure, evidence is emerging that this protein is involved in proliferation of mammalian cells [2–5]. Since ProTα is highly evolutionary conserved and a homologous protein exists in yeast [8], we attempted to address ProTα function using yeast *S. cerevisiae* as an alternative experimental system. In the absence of cloned yeast ProTα gene, we employed human ProTα cDNA for production of human ProTα in yeast. We argued that if yeast ProTα were related to cell division as well, interference of homologous but probably somewhat different human ProTα should impair division of yeast cells. This indeed turned out to be the case: production of human ProTα reversibly blocked yeast cell division. This inhibitory effect was not due to production of a significant amount of the plasmid-encoded protein per se, since synthesis of truncated ProTα at the level exceeding that of wild type protein exerted no effect on cell growth. Further evidence for the specificity of action of human ProTα came from the behaviour of yeast cells producing triple ProTα mutant, which still retained characteristic acidity of the protein and its nuclear localization signal, and yet turned out to be inactive in suppression of yeast cell growth.

Most interestingly, inhibitory action of human ProTα on yeast cell growth was abrogated by deletion of just nine carboxy-terminal amino acids of the protein. This fact has interesting implications because the region in question contains karyo-

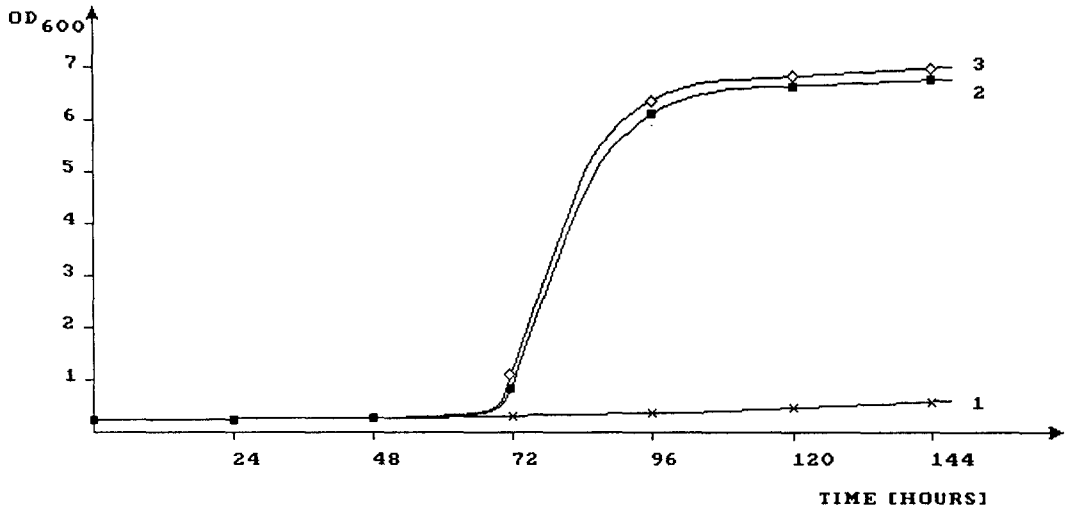

Fig. 2. Growth of yeast cells (SKY594) producing: 1, wild type human ProTα, 2, truncated ProTα, 3, no ProTα (vector alone) in the galactose-containing medium.

philic sequence targeting ProTα to the nucleus. The machinery for nuclear import is believed to be well conserved in eukaryotes including yeasts [14]. Thus, the most straightforward explanation of the observed cell behaviour implies that nuclear targeting of human ProTα is prerequisited for its interference with cell division.

Our results point to the nucleus as a potential place of action of ProTα, in agreement with its probable relation to cell proliferation. Further experiments will be required to determine the molecular mechanisms through which ProTα exerts its activity. We believe, however, that the possibility to investigate the role of ProTα in cell division process in a relatively simple experimental system now becomes feasible.

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