Volume 291, number 1, 37–40 FEBS 10222 October 1991

ADONIS 001457939100932A

Cloning and sequence analysis of pituitary cDNA encoding the β-subunit of *Xenopus* proteasome

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Received 23 July 1991

The proteasome is a multicatalytic proteinase composed of a number of non-identical subunits. A *Xenopus* pituitary cDNA was isolated and found to code for the β-subunit of proteasome. The amino acid sequence deduced from the open reading frame consisted of 215 amino acid residues with a calculated molecular weight of 23 979. A comparative structural analysis indicated that the proteasome subunits can be divided into two groups with the same evolutionary origin. One group consists of subunits with an N-terminally blocked residue and includes components C2, C3, C8 and C9, while the second group of non-blocked proteins includes component C5 and the β-subunit.

Proteasome; Multicatalytic proteinase; β-Subunit; cDNA sequence; *Xenopus* pituitary

1. INTRODUCTION

The proteasome is a symmetrical, cylinder-shaped particle which is present in both nucleus and cytoplasm of a variety of eukaryotic cells [1]. This particle with a sedimentation coefficient of approximately 20 S (molecular mass about 750 kDa) is composed of a characteristic set of at least 13 non-identical polypeptide subunits with molecular masses between 20 and 35 kDa [2–4]. The individual subunits may each have a unique catalytic property, i.e. cleavage of peptide bonds on the carboxyl side of basic, hydrophobic or acidic amino acid residues [5]. The cellular function of the proteasome is, however, still unknown although recent evidence suggests that this highly-conserved particle is a multifunctional proteinase involved in the degradation of proteins (and possibly also RNA) in a non-lysosomal pathway [6–8].

For the elucidation of the role of the proteasome it is important to determine the primary structures of all subunits of this proteinase complex. Recently, cloning and sequence analyses of cDNAs revealed the structures of a number of subunits of human, rat, *Drosophila*, yeast and archaebacterial proteasomes [9–17]. Here we report the cloning of a *Xenopus* pituitary cDNA encoding another component, the β-subunit, of the proteasome.

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2. MATERIALS AND METHODS

A cDNA library in the vector λZAP-II (Stratagene) was constructed with RNA isolated from intermediate pituitary glands of black-background-adapted *Xenopus laevis*. From this library single plaques were picked at random and the recombinant pBluescript SK-phagemids were rescued from the bacteriophage (λZAP) clones by in vivo excision according to the instructions of the manufacturer. Sequencing on both strands and with pBluescript subclones was performed with single-stranded and double-stranded DNA using T7 DNA polymerase and the dideoxy chain termination method [18]. For Northern blot analysis, poly(A)$^+$ RNA was isolated from *Xenopus* liver with guanidineisothiocyanate and oligo(dT) cellulose, and RNA samples (10 μg) were fractionated on a 1.2% agarose gel in 2.2 M formaldehyde. After transfer [19], the filter was hybridized in standard hybridization buffer [20] in the presence of 50% formamide with insert DNA of cDNA clone X7355 ^{32}P-labelled by random priming to 5×10^8 cpm/μg DNA. Homology searches were performed with the CAOS/CAMMSA computer facility of the University of Nijmegen which includes the sequence alignment program Clustal [21].

3. RESULTS AND DISCUSSION

3.1. *Cloning and sequence analysis of pituitary cDNA encoding the β-subunit of* Xenopus *proteasome*

The prohormone proopiomelanocortin (POMC) is expressed to very high levels in the melanotrope cells of the intermediate pituitary of *Xenopus* adapted to a black background [22]. During our search for proteins co-expressed with POMC we isolated from a *Xenopus* intermediate pituitary cDNA library a cDNA clone (X7355) with an insert of about 900 bp (which includes a poly(A)-tail of 35 nucleotides) (Fig. 1). A putative polyadenylation signal (ATTAAA) is located 29 nucleotides upstream from the polyadenylation site. The longest open-reading frame of clone X7355 codes for a protein of 215 amino acids, giving a protein with a

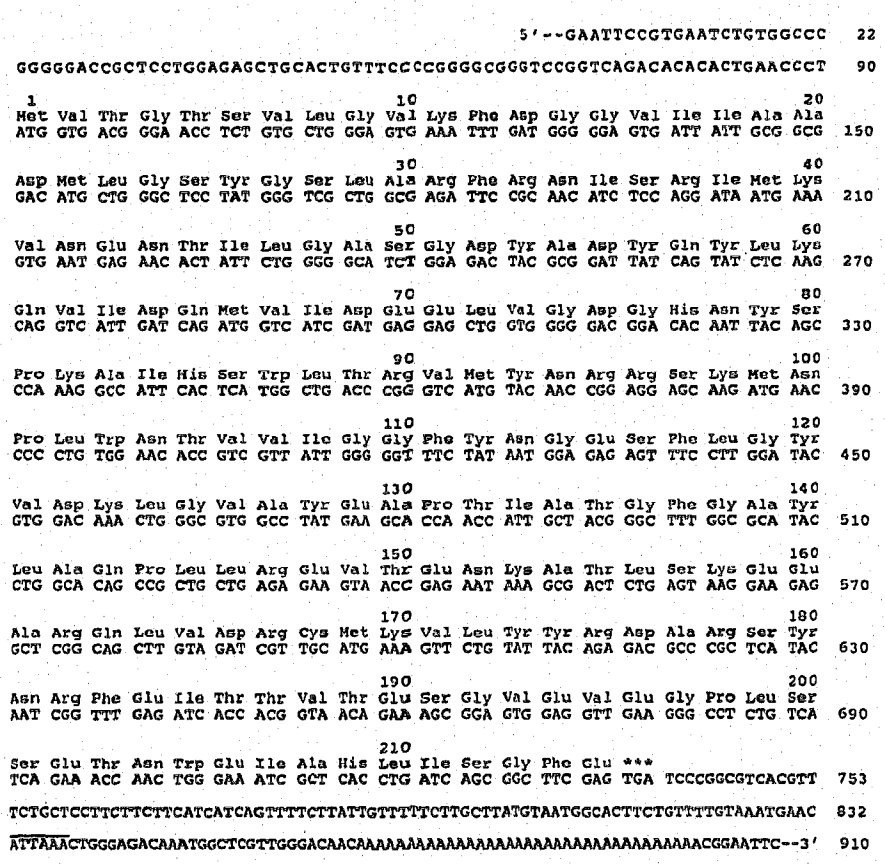

Fig. 1. Nucleotide sequence and deduced amino acid sequence of pituitary cDNA encoding the β-subunit of *Xenopus* proteasome. The putative signal for polyadenylation (ATTAAA) is overlined.

calculated molecular weight of 23 979. A computer homology analysis at the protein level (NBRF protein database; release 28) revealed that the N-terminal 24 amino acid residues of this protein are nearly identical (92% identity) to the N-terminal region that has been sequenced for the β-subunit of proteasome isolated from human erythrocytes [23] (Fig. 2); no significant identity with any other protein was found. A database search at the nucleotide sequence level (EMBL nucleotide database; release 27) showed no significant identity with any nucleotide sequence. We conclude that clone X7355 encodes the β-subunit of *Xenopus* proteasome. Northern blot analysis of *Xenopus* liver poly(A)$^+$ RNA with clone X7355 as a probe revealed only one hybridizing band of about 1050 nucleotides (data not shown), indicating that X7355 is a nearly full-length cDNA clone.

3.2. *Comparison between the amino acid sequences of proteasomal proteins*

Purification of the human proteasome from erythrocytes revealed the existence of at least 13 subunits of different primary structures [23]. Most of these subunits have been found to be N-terminally blocked [23], a situation also reported for rat proteasome subunits [10]. Molecular cloning of cDNAs has revealed the structures of four of the N-terminally blocked proteasome subunits (components C2, C3, C8 and C9) [9–11,13,14]. In addition, for five of the non-blocked subunits the N-terminal sequences have been determined by Edman degradation and these subunits have been named α, β, γ, δ and ε in order of their decreasing apparent molecular weights (from 30 to 20 kDa) [23]. Cloning of one of the non-blocked subunits (C5) [9,12] has indicated that it corresponds to the γ-subunit of the proteasome. Alignment of the structures of components C2, C3, C5 (γ), C8 and C9 has shown that the amino acid sequences of C2, C3, C8 and C9 are similar while the sequence of C5 (γ) has diverged markedly from those of the other subunits. It has therefore been concluded that C5 (γ) is a new type of subunit of the proteasome complex [9,12]. The predicted amino acid sequence of the *Xenopus* β-subunit shows a low but significant identity with all subunits of human proteasome although the highest identity is with component C5 (γ) (Fig. 2). From the above it appears that the proteasome subunits form a multigene family which can be classified into at least two groups, one group consisting of the α-, β-, γ- (C5), δ- and ε-subunits with a free N-terminus and a second group including components C2, C3, C8 and C9 with

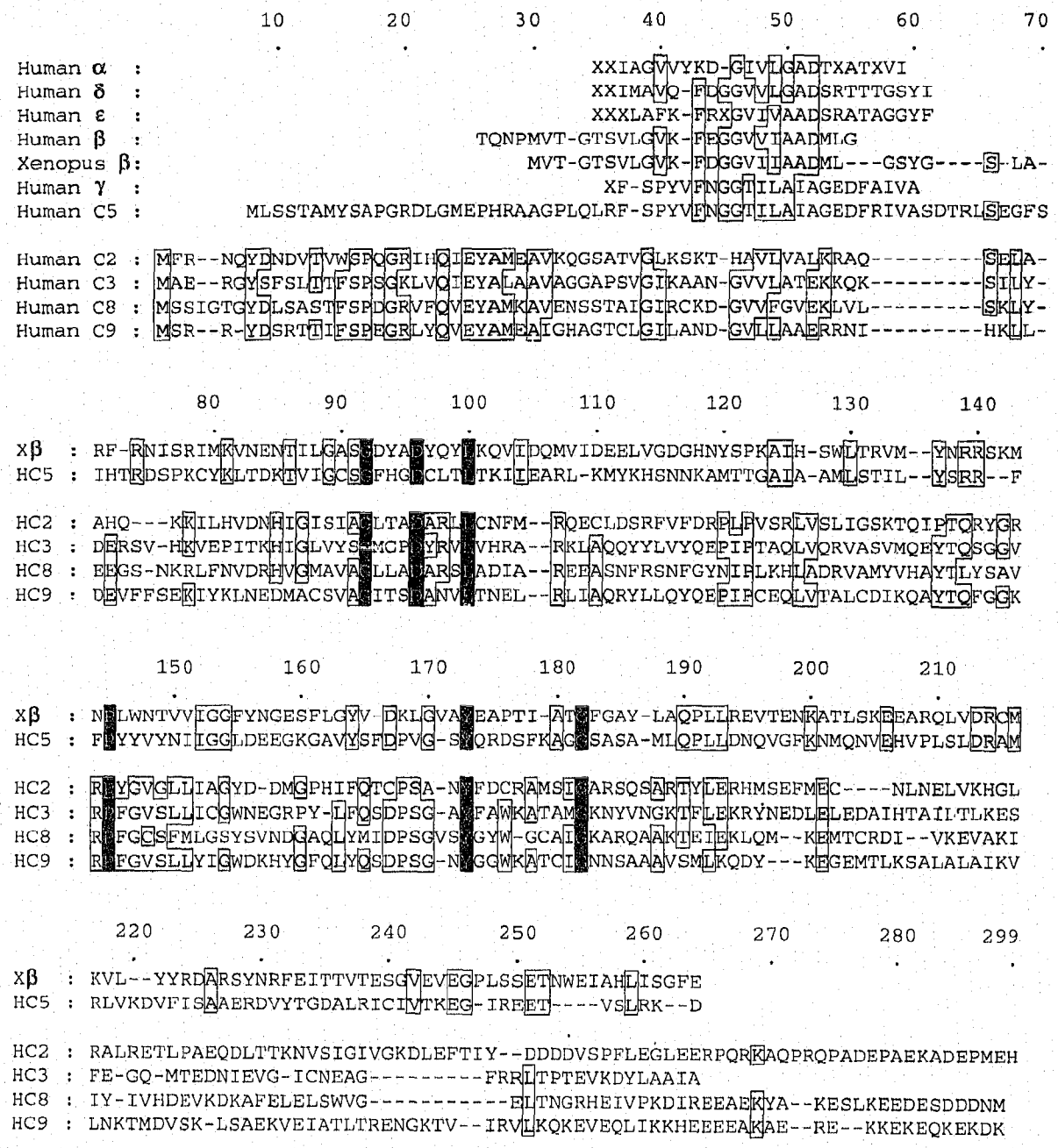

Fig. 2. Alignment of the amino acid sequences of proteasomal proteins. The one-letter amino acid notation is used. Gaps (–) have been introduced to achieve maximum homology. Residues are boxed in black if they are identical in all sequences at the respective position. Residues identical between component C5 and the β-subunit are boxed as are residues identical in all or in all but one of the sequences of components C2, C3, C8 and C9. Sets of four or more identical residues in the N-terminal regions of the α-, β-, γ-, δ- and ε-subunits are also boxed. X indicates residues for which identifications were uncertain in the N-terminal amino acid sequencing [23]. The human sequences have been taken from [9,23].

a blocked N-terminus. The degree of sequence identity between the cloned subunits indicates that all components of the proteasome may have originated from a common ancestral proteinase.

Acknowledgements: This work was supported by the Netherlands Organization for Scientific Research (NWO).

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