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The *Su(Ste)* repeat in the Y chromosome and βCK2tes gene encode predicted isoforms of regulatory β-subunit of protein kinase CK2 in *Drosophila melanogaster*

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Abstract We report an exon-intron structure of the *Su(Ste)* repeat capable of encoding an isoform of the β-subunit of protein kinase CK2. The predicted *Su(Ste)* gene product contains a drastically changed amino acid sequence of the N-terminal fragment as compared to the earlier described bCK2tes gene considered to be an ancestor of the *Su(Ste)* repeats. The following peculiarities of molecular divergence of the *Su(Ste)* and βCK2tes genes are revealed: damages of the autophosphorylation site; usage of an alternative splicing site instead of a damaged one; conservation of the zinc finger domain in spite of local ORF alterations.

Key words: Casein kinase 2; Spermatogenesis; Zinc finger; *Drosophila melanogaster*

1. Introduction

Protein kinase CK2 participates in such important processes as regulation of the cell cycle, growth and development [1]. There are more than 100 proteins known to be its targets for phosphorylation [1]. Among them are topoisomerase II [2], elongation factors [3], p53 [4], and oncoproteins [5,6]. Protein kinase CK2 heterotetramer is composed of two catalytic subunits (αα or αα′) and two regulatory subunits (ββ or ββ′). It was shown that the β-subunit (βCK2) stabilizes holoenzyme and takes part in regulation of its activity and substrate specificity [7]. βCK2 is a highly conserved protein; particular amino acid motifs are conserved in βCK2 from yeast to higher eukaryotes [8–14]. The gene and cDNA of *Drosophila melanogaster* βCK2 were cloned earlier [9], and some properties of the encoded protein were studied [15,16]. In the genome of *D. melanogaster*, apart from the unique X-linked βCK2 gene, there are two clusters of repeated *Stellate (Ste)* genes on the X chromosome bearing open reading frames, highly homologous to βCK2 [17–19]. In addition, the Y-chromosome contains a cluster of repeats homologous to *Ste* genes, that are considered to act as suppressors of *Ste* expression (*Suppressor-of Stellate (Su(Ste))* [20]. In the absence of the *Su(Ste)* cluster, *Ste* genes are overexpressed resulting in the formation of protein crystals in spermatocytes, thus affecting gametogenesis and fertility of such males. The mechanism of interaction between the *Ste* and *Su(Ste)* loci, which provides the normal proceeding of spermatogenesis, is yet to be determined. It was recently shown that the euchromatic *SSL* gene may be considered an ancestor of amplified *Su(Ste)* repeats [21]. This gene was shown to be testis specific and here we renamed it βCK2tes (from 'testes'). In this report we show that *Su(Ste)* genes are transcribed and able to encode a βCK2 variant. The peculiarities of evolution of the coding sequences of βCK2tes and *Su(Ste)* genes as well as a mode of *Su(Ste)* gene action as a suppressor are discussed.

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Abbreviations: CK2, casein kinase 2; ORF, open reading frame

2. Materials and methods

2.1. Isolation of cDNA clones

Canton S testes cDNA library (Stratagene), kindly provided by Dr. T. Hazelrigg, was used. All procedures were performed according to the Stratagene protocol. The library was screened with a 650 bp 3-fragment of the *Su(Ste)* gene (Fig. 1) generated by PCR with primers 5′-TTGCAATTAAACAGATTC-3′ and 5′-CGGGGAAAATCTTTGGGGC-3′, using cosmid 18 [20] containing *Su(Ste)* genes (provided by M.D. Balakireva) as a template. DNA sequencing was done using the Sequenase 2.0. kit (United States Biochemicals) and standard M13 and specific primers [22].

2.2. Northern blot analysis

Total RNA was isolated by guanidinium thiocyanate extraction [23] from embryos, larvae, pupae, adult males, females, testes (*gt* w^a strain) and *Drosophila* cell culture 67j25 [24] (line D), fractionated by electrophoresis in denaturing formaldehyde-agarose gel and transferred to nylon filter HyBond-N (Amersham). The *Eco*RI fragment of βCK2 cDNA (gift of Dr. C. Glover) was subcloned into pBlueScript SK^- vector and used for preparing radiolabeled antisense RNA probe. Transcription in vitro was performed for 1 h at 37°C in buffer containing 40 mM Tris-HCl, pH 7.5; 60 mM $MgCl_2$; 5 mM NaCl; 10 mM DTT; 0.5 mM of each ATP, GTP, CTP; 100 ng of the linearized plasmid; 20–100 μCi [α-^{32}P]UTP; 2–5 units of T7 RNA polymerase (Gibco BRL); 25 units of RNAse inhibitor (Gibco BRL). Filter prehybridization, hybridization and washing were done under standard conditions [22]. As a control the hybridization of the same filter with the rp49 probe [25] was done.

3. Results

3.1. Transcription of βCK2 related genes

The Canton S testes cDNA library was used to detect transcripts of *Su(Ste)* genes. The library was screened with the probe from the *Su(Ste)* gene region, which is not homologous to *Ste* genes [19] (Fig. 1). The most extended (861 bp) cDNA clone, named pBS511, was sequenced (GenBank accession number L42288). Comparison of the nucleotide sequence of cDNA 511 with the sequences of *Su(Ste)* genes [20] reveals the exon-intron structure of the *Su(Ste)* gene encoding this transcript (Fig. 1). The 3′-splice site of the first intron is damaged in *Su(Ste)* repeats, due to G to C substitution of the last nucleotide in the intron sequence [17]. The cDNA 511 demonstrates the usage of an alternative 3′-splice site located 4 bp downstream. cDNA 511 clone contains an ORF, comprising

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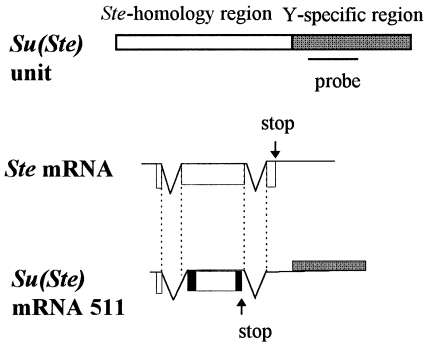

Fig. 1. Diagram of *Ste* and *Su(Ste)* processing. The *Stellate* ORF is indicated by open rectangles, fragments of other ORFs are presented by black rectangles. The probe using for cDNA library screening is indicated.

468 nucleotides. The sequence of N-terminal amino acids of the predicted protein product encoded by the region preceding the first intron is similar to the Ste protein. The usage of the alternative splice site for splicing the first *Su(Ste)* intron results in a shift of the Ste-protein ORF. However, as a result of two single nucleotide deletions, the Ste ORF is soon restored. The stop codon is located just before the second intron. The detected ORF implies the potential ability of *Su(Ste)* genes for protein expression.

Apart from testis expressed *Su(Ste)* repeats the earlier detected testis-specific *SSL* (*Su(Ste)*-like) gene, considered to be an ancestor of *Su(Ste)* repeats, was described [21]. Here we renamed the SSL gene βCK2tes. These observations raise the question concerning possible germinal specificity of expression of the genuine X-linked gene [9] encoding βCK2.

The developmental profile of genuine βCK2 gene expression was obtained using the riboprobe complementary to the βCK2 coding strand (see Section 2). Abundant transcripts of the βCK2 gene of the expected size (~1700 nucleotides) are revealed at the embryonic stage and also in the *Drosophila* cell culture of embryonic cells (Fig. 2). The abundance of transcription in females is probably due to the presence of ovarian tissue and large amounts of embryos. No (or very low) βCK2tes expression was detected in testes. *Ste* genes, as known, are expressed in testes of males lacking the Y-chromosome [17]. Thus, the βCK2tes gene can encode a tissue-specific isoform of βCK2 while the genuine βCK2 gene is widely expressed.

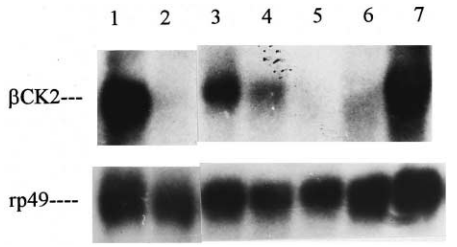

Fig. 2. Developmental expression of βCK2 gene. Upper panel: Northern blot analysis of RNA isolated from cell culture (1), testes from males of the *gt* w^a line (2), embryos (3), larvae (4), pupae (5), males (6) and females (7) of the same line. Hybridization with the single-stranded RNA probe complementary to the βCK2 coding chain. Lower panel: The same membrane hybridized with the rp49 probe for quantitative control. The size of RNA is indicated in nucleotides (nt).

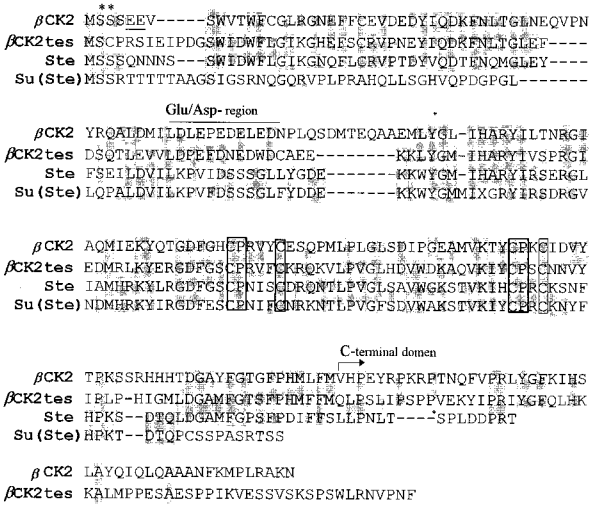

Fig. 3. Alignment of *D. melanogaster* βCK2 related proteins. Identical amino acid positions are shown as shadowed boxes. Regions enriched in Glu/Asp residues and the C-terminal domain are marked. Cysteine residues in a zinc finger motif are boxed. Serine residues representing sites of phosphorylation are marked by asterisks. Glu residues in the autophosphorylation site of βCK2 are underlined.

3.2. Comparison of amino acid sequences of βCK2, βCK2tes and Su(Ste)

Primary structures of the putative protein products of *Su(Ste)* and βCK2tes genes are aligned with the related *D. melanogaster* amino acid sequences βCK2 [9] and *Ste* [17] (Fig. 3). Despite the gaps in the sequence similarity, it continues through the whole length of the sequences. Comparison (Fig. 3) demonstrates that the Zn finger motif ($CPX^3CX^{12}CPX$) is conserved in all four related sequences. This Zn binding motif probably plays a role in the formation of the quaternary structure of the CK2 holoenzyme or in the interaction with protein targets or in DNA-protein interactions [26]. The Glu-Asp rich regions of the acidic amino acid region participating in the regulation of enzyme activity [27] and the C-terminal domain of genuine βCK2 necessary for binding to αCK2 [28] are highly conserved only in βCK2tes protein. It is known, however, that Ste protein is capable of in vitro formation of active complex with αCK2, in spite of the very shortened C-terminal domain and its low level of similarity to the C-terminus of βCK2 [29].

4. Discussion

In the genome of *D. melanogaster* there is a family of genes capable of encoding protein products that are highly homologous to the regulatory β-subunit of protein kinase CK2. Except for βCK2 itself and *Ste* repeats, this family also includes testes expressed *Su(Ste)* repeats and βCK2tes gene. The function of *Su(Ste)* repeats is puzzling; they may be designed to silence hyperexpression of *Ste* repeats leading to male sterility. The detection of *Su(Ste)* transcription allows us to speculate that suppression of homologous *Ste* repeats may be mediated by the widely extended but poorly understood mechanism of so-called cosuppression in plants [30]. The mechanism of cosuppression concerns the homology-dependent silencing and may operate at the transcription level as a result of hybridization of homologous RNA to the regulatory region as well as at the translation level. The *Su(Ste)*

genes may be considered silencers of the homologous *Ste* genes. However, the detected coding ability of *Su(Ste)* genes allows one to suppose other mechanisms of suppression of *Ste* transcription taking into account the peculiarities of the predicted Su(Ste) protein.

The *Su(Ste)* transcript may encode the putative product that has a significant homology to βCK2 (Fig. 3). Probably, the mechanism of suppression is determined by competition of the *Su(Ste)* product with Ste protein for the binding with the catalytic subunit of CK2 thus affecting, for example, the phosphorylation status of the transcription apparatus of *Ste* genes that make available *Ste* transcription inhibition. It is possible to speculate that the *Su(Ste)* product, which has a putative DNA binding Zn finger domain, can directly inhibit *Ste* transcription.

The βCK2tes gene and possibly the *Su(Ste)* repeats located on the Y-chromosome, unlike βCK2, are characterized by tissue-specific expression. A suggestion about the existence of several βCK2 species that may serve to provide substrate specificity or to target the enzyme was put forward [1]. The existence of two β genes is known only for *Saccharomyces cerevisiae* [8] and *Arabidopsis thaliana* [11]. βCK2tes protein may perhaps provide substrate specificity and a particular level of activity of CK2 during *Drosophila* spermatogenesis accompanied by complex regulation of cell division and differentiation. The site of autophosphorylation located in the N-terminus of βCK2 (Ser residue surrounded by acidic amino acids) is present in β-subunits from different organisms. It was suggested that this site plays a role in determining the functionally active state of the enzyme [31]. This site of autophosphorylation is altered in βCK2tes, Ste and Su(Ste) proteins as a result of Ser residue substitutions and/or elimination of adjacent acidic Glu residues (Fig. 3). These modes of divergence may have resulted in the evolution of specific ways of regulation of CK2 activity during spermatogenesis. Thus, proteins related to βCK2, although possessing extended regions of homology in ORFs and conservation of some functional motifs, may differ in regions that carry out some regulatory functions. The gene family related to βCK2 but having independent systems of regulation of their expression is a valuable material for studies of evolution of protein kinase subunits as well as cyclines, taking into account the shared ability of these proteins to modulate enzymatic activity of protein kinases [1].

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