

The *Drosophila* SSL gene is expressed in larvae, pupae, and adults, exhibits sexual dimorphism, and mimics properties of the β subunit of casein kinase II

Umesh Karandikar,¹ Stacey Anderson,¹ Neil Mason, Regina L. Trott, Clifton P. Bishop, and Ashok P. Bidwai*

Department of Biology, Life Sciences Building, West Virginia University, P.O. Box 6057, Morgantown, WV 26506-6057, USA

Received 23 December 2002

Abstract

Drosophila melanogaster casein kinase II (CKII) is composed of catalytic α and regulatory β subunits that generate the $\alpha_2\beta_2$ holoenzyme. A two-hybrid screen of a *Drosophila* embryo library using CKII α as bait has resulted in the isolation of multiple cDNAs encoding SSL, a CKII β -like polypeptide. We demonstrate that CKII β , β' , and SSL exhibit robust and comparable interaction with CKII α . Residues in SSL that mediate interaction with CKII α appear similar to those in CKII β , and SSL forms homodimers and heterodimers with CKII β or β' as well. We have tested all known *Drosophila* CKII β -like proteins for rescue of the ion-homeostasis defect of yeast lacking β subunits and find that CKII β and SSL complement, β' has marginal function, and Stellate appears non-functional. We have used real-time RT-PCR to assess developmental expression, and find that CKII β is robust and ubiquitous, whereas SSL is restricted to males (third-instar-larvae, pupae, and adults), but is nondetectable in females of the corresponding stages. These results indicate that SSL expression encompasses a greater developmental window than that previously suggested and may confer distinct functions to CKII in a sex-specific manner.

© 2003 Elsevier Science (USA). All rights reserved.

Keywords: Protein kinase; Phosphorylation; Sex-specific

CKII is a highly conserved Ser/Thr protein kinase that is ubiquitous in eukaryotes (reviewed in [1]). The enzyme is composed of catalytic (α) and regulatory (β) subunits, which associate to generate the $\alpha_2\beta_2$ holoenzyme. With the exception of *Drosophila melanogaster* [2], *Caenorhabditis elegans* [3], and *Schizosaccharomyces pombe* [4], CKII from most organisms contains two distinct isoforms of the catalytic subunits that are encoded by separate genes. On the other hand, β subunit heterogeneity has been documented only in *Saccharomyces cerevisiae* [5], *Arabidopsis thaliana* [6], and *D. melanogaster* [7]. CKII phosphorylates Ser/Thr within hyperacidic microdomains and its consensus for phosphorylation can best be described as (S/T)(D/E) X (D/E) [8]. Consistent with this, a number of proteins essential

for DNA-replication, transcription, translation, cell cycle regulation, and cell signalling contain such sites and are known to be phosphorylated in vitro and in vivo [9]. The enzyme is activated by polybasic compounds such as spermine, polylysine, and protamine which mediate these effects via the β subunit, whereas polyacidic compounds such as polyglutamate are believed to inhibit activity via competition with acidic microdomains of target proteins.

Studies using recombinant proteins suggest that when compared to the holoenzyme, monomeric CKII α exhibits approximately 20% of the activity [10]. In line with this observation, CKII β stimulates activity \sim 5-fold and full reconstitution of activity correlates with assembly of the holoenzyme. The stimulation of activity by CKII β involves both the affinity (k_m) for the substrate and its rate (V_{max}) of phosphorylation [11]. Additionally, CKII β is (auto)phosphorylated at its N-terminus, MS^PS^P SEE, (catalyzed by CKII α) and this reaction has

* Corresponding author. Fax: 1-304-293-6363.

E-mail address: abidwai@wvu.edu (A.P. Bidwai).

¹ These two authors contributed equally to this work.

been suggested to mediate turnover via ubiquitination and proteasome-mediated degradation [12]. Given the fact that CKII activity is messenger-independent and that no regulators have been identified to date, regulation by (auto)phosphorylation may represent a potential mechanism to downregulate CKII in vivo. Collectively these studies suggest that the β subunit serves a critical regulatory function.

Genetic analyses in a number of model systems have begun to clarify the roles of CKII β . While loss of this subunit is dispensable for viability in yeast, it elicits severe defects in ion-homeostasis in *S. cerevisiae*, and this phenotype is rescued by expression of *Drosophila* CKII β [13], demonstrating functional conservation. CKII β is recessive lethal in *Drosophila* [14] and, given the inordinately high conservation of this subunit, its essentiality may be applicable to all metazoan organisms. A hypomorphic allele of CKII β is associated with a reduction in the size of the mushroom body due to attenuation of cell proliferation and a decrease in the number of Kenyon cells [14], results that corroborate a requirement of this enzyme for cell cycle progression [9]. Surprisingly, the lethality associated with loss of CKII β is only 'partially' rescued by a non-autophosphorylating isoform, raising the potential importance of this reaction in regulation of CKII functions in vivo. *Drosophila* also harbors two autosomal genes that encode β -like proteins, i.e., β' [7] and SSL [15], and a multicopy locus on the X-chromosome known as *Stellate* (*Ste*) [16]. The *Ste* locus is potently repressed in XY males due to the Y-linked *Su(Ste)* locus [17]. Consistent with this, the absence of *Su(Ste)* in XO males elicits massive overproduction of *Ste* which forms crystalline aggregates in spermatocytes and elicits sterility [18]. It has been previously suggested that *Ste* may mimic CKII β [18], but structure/function studies argue against this possibility because this protein appears to lack residues critical for interaction with CKII α (see below). The relevance of *Ste* to CKII thus remains unclear. The functions of β' and SSL also remain enigmatic. Both proteins conserve motifs required for interaction with CKII α , but given the recessive lethality of CKII β , it is likely that these isoforms have distinct functions.

We report here the isolation of multiple cDNAs encoding SSL from an embryo two-hybrid library and demonstrate that interaction of SSL with CKII α is robust and equivalent to that observed with CKII β or β' . Deletion analysis confirms that residues mediating the CKII α –CKII β interaction are conserved in SSL. Additionally, SSL is capable of forming homodimers as well as heterodimers with CKII β or β' . Furthermore, we demonstrate that CKII β or SSL exhibits comparable efficiency in their ability to rescue the ion-homeostasis-defects of *ckb1/2* yeast. Finally, real-time RT-PCR analysis suggests that, unlike CKII β which is expressed throughout development, transcripts encoding SSL are

readily detectable in third-instar-larvae, pupae, and adults in a male-specific manner. These studies suggest that SSL may confer a sexually dimorphic modulation of CKII functions.

Materials and methods

Two-hybrid screen. The open-reading-frame encoding CKII α was amplified by PCR using primers that introduce *EcoRI* and *BamHI* sites at the 5' and 3' ends, respectively, and subcloned into the plasmid pGBT9 (Clontech) wherein the cDNA is expressed as a fusion with the DNA-binding (DB) domain of *S. cerevisiae* Gal4. The insert was sequenced on an Applied Biosystems DNA Sequencer 373A to confirm the correctness of the sequence. Yeast strain HF7c [19] expressing GAL4DB-CKII α fusion protein was used as the host strain to screen a 3–18 h *Drosophila* embryo cDNA library (provided by S.J. Elledge, Baylor College of Medicine, Houston). Briefly, the two-hybrid cDNA library ($\sim 1 \times 10^8$ total recombinants) inserted in the plasmid pACT [20] was transformed into the host strain, and an estimated 4×10^8 transformants were plated on medium lacking Trp, Leu, and His, and colonies (~ 650) showing robust growth were counterscreened for expression of β -galactosidase as described [21]. His+/LacZ+ clones (~ 90) were subjected to loss of the bait-encoding plasmid and those that also exhibited a concomitant loss of HIS3 and LacZ expression were chosen for further analysis. Library plasmids were recovered in *E. coli* and those encoding CKII β/β' were identified by PCR using specific primers. The remaining cDNAs were identified by DNA sequencing. From this analysis, 5 clones encoding SSL were recovered and their isolation forms the basis of this study.

Explicit two-hybrid interactions. None of the SSL clones isolated by us contained a full-length 5' end. We, therefore, selected clone DmA90-464 (that lacks only the start codon), and full-length SSL was reconstructed by PCR using SSL-specific primers that also introduce *BamHI* and *XhoI* sites at the 5' and 3' ends, respectively. Constructs that express CKII α , CKII β , β' , or SSL were generated in the plasmid pACT, where proteins are expressed as fusions with the activation domain (AD) of Gal4. Deletions in SSL, i.e., SSL-G148*, SSL-E173*, and SSL-K186*, were generated using the QuickChange Site-directed Mutagenesis Kit (Stratagene) according to the manufacturer's directions, and mutations were confirmed by sequencing using dideoxy-chain terminations on an Applied Biosystems DNA sequencer 373A. Full-length SSL and truncations were combined with pACT constructs expressing CKII α , CKII β , β' , or SSL, and interactions were assessed in yeast HF7C by growth in minimal media lacking histidine (His-) and by LacZ assays on two independent transformants each in triplicate.

Suppression of salt sensitivity of *ckb1* or *ckb2* *S. cerevisiae*. cDNAs encoding β' or SSL were subcloned into the plasmid, pESC-Ura (stratagene), where proteins are expressed with a Flag-epitope tag (at their C-terminus) under control of a synthetic *GAL* promoter. Construction of plasmids that similarly express CKII β or *Stellate* has been described previously [13]. Plasmids expressing CKII β , *Stellate*, β' , and SSL were introduced into yeast harboring a deletion of either the *CKB1* (YAPB7) or *CKB2* (JCR8) gene using lithium acetate [22], and transformants were selected on minimal medium lacking uracil. Subsequently, transformants were grown in rich medium containing galactose as the sole carbon source (YPGal), adjusted for cell numbers, and plated onto YPGal or YPGal+0.5 M NaCl essentially as described. Plates were incubated at 29 °C and photographed.

Real-time RT-PCR. Primer and probes for real-time RT-PCR of CKII β are as follows; forward primer, 5'-GGTTTAAATGAGCA GGTACCCA ACTA-3'; reverse primer, 5'-TCGTCCTCCGGT CCAAGT-3'; FAM (6-carboxyfluorescein)-labeled probe that spans Exons 1 and 2 of the CKII β gene, 5-CGGCCAAGCGTTGGAC ATGATCTTG-3'. Primer and probes for real-time RT-PCR of SSL

are as follows; forward primer, 5'-TCCGCCCGTGGAGAAGTAC-3'; reverse primer, 5'-GGACTTGCTGACCGAGGATTC-3'; FAM-labeled probe that spans Exons 1 and 2 of the SSL gene, 5'CCCCGTATCTATGGCTTCCAGTTGCAC-3'. Primers and probes were designed using the Primer Express software (Applied Biosystems). The optimal conditions for the primers and probes were identified on RNA isolated from pooled larvae and pupae using the High Pure RNA Isolation Kit (Roche) that was reverse transcribed using the Taqman Gold RT-PCR kit (Applied Biosystems). Based on this analysis, the optimal conditions that were used for analysis were as follows: SSL, forward primer (500 nM), reverse primer (250 nM), and the FAM-labeled probe (250 nM); CKII β , forward primer (200 nM), reverse primer (275 nM), and the FAM-labeled probe (250 nM).

Male crawling third-instar-larvae were identified by the presence of testis and RNA isolated from a single individual was used as a template. In addition, male third-instar-larvae were selected and allowed to undergo pupation, and RNA isolated from a single individual was used as a template. Females at similar stages were isolated in an analogous manner. RNA was isolated from single larvae, pupae, and adults (male and female) using the High Pure RNA Isolation Kit (Roche) that was reverse transcribed using the Taqman Gold RT-PCR kit (Applied Biosystems). Real-time RT-PCR was performed using ABI's Universal PCR master mix, and the lowest C_t value and highest ΔR_N define optimal conditions for the primers and probes. A 10- μ L aliquot of the cDNA was added to a 40- μ L reaction master mix containing all of the ingredients (forward and reverse primers, probe, and master mix), and reactions containing water and the master mix were run, in parallel, as negative controls. Real-time RT-PCR was performed on an ABI's 7700 PCR machine using default parameters. Fluorescence output results were captured and analyzed using Sequence Detection Software Version 1.7 (Applied Biosystems), and the threshold cycle (C_t) was used for assessing relative levels of CKII β vs SSL transcripts.

Results and discussion

Isolation of cDNAs encoding SSL from embryo libraries

The yeast strain HF7C expressing CKII α as a bait was used to screen a *D. melanogaster* 0–18 h embryo two-hybrid cDNA library. From $\sim 4 \times 10^8$ transformants, 90 clones that activate *HIS3* and *LacZ* in a bait-dependent manner were recovered. PCR analysis was used to eliminate clones that encode CKII β or β' , since these are likely to be isolated in a two hybrid hunt at a high frequency because of the high-affinity α - β or α - β' interactions [13]. The remaining clones were retested against Gal4DB-alone or Gal4DB-CKII α to ensure specificity, and those that induced reporter gene expression only in combination with CKII α were identified by DNA sequencing. Sequencing revealed that this screen has so far yielded 13 clones encoding rpL22 [23], 7 encoding E(spl)m7 [24], 5 (DmA90-35, -154, -279, -330, -464) encoding SSL, and the rest encoding novel proteins that will be described elsewhere. DNA sequences of the five SSL-encoding cDNAs revealed that three clones (DmA90-35, -154, and -330) are missing the first 18 nucleotides of the open-reading-frame, whereas two (DmA90-154 and -464) are lacking the initiation codon. Additionally, none of these clones contain a

poly(A)tail, suggesting that they are not of full-length with respect to their 3' untranslated regions.

Our isolation of cDNAs encoding SSL in a random two-hybrid screen of an embryo cDNA library was surprising because it has been recently suggested that SSL is testis-specific [25]. The *Drosophila* genome harbors three genes that encode CKII β -like proteins, i.e., β' [7], SSL [15], and Ste [16]. Sequence alignments and phylogenetic analysis suggest that β' is more closely related to CKII β than are SSL and Ste [7], and the relevance of Ste to CKII is unclear because it does not appear to interact with CKII α . We, therefore, deferred from parallel analysis with this isoform and focused on CKII β , β' , and SSL. To provide for a comprehensive and controlled analysis, identical constructs encoding these three proteins in the Gal4AD-vector, pACT, were tested against CKII α in the Gal4DB-vector, pGBT9 (see Materials and methods). As expected, expression of CKII α , CKII β , β' , or SSL by themselves did not elicit transcription of *HIS3* or *LacZ* (Fig. 1A). On the other hand, coexpression of CKII α + CKII β elicited robust induction of both reporter genes, indicative of a high-affinity interaction, and in this regard β' and SSL also exhibit a comparable affinity for CKII α (Fig. 1B). The reasons underlying the higher *LacZ* values for the CKII α + SSL combination, compared to those for CKII α + β/β' , are presently unclear, but it is worth noting that identical values are obtained when the orientation of CKII α and SSL with respect to Gal4DB or Gal4AD is reversed. Thus the SSL-CKII α interaction is orientation-independent, as is the case for interaction of CKII α with β/β' [7]. These results are somewhat analogous to those recently obtained by Kalmykova et al. [15], but the virtually identical induction of *LacZ* (Fig. 1B) would suggest that CKII β , β' , and SSL exhibit a comparable and high affinity interaction with CKII α . Based on the observation that CKII β is a dimer [26], we have also tested this property of the SSL protein. The robust growth of cells coexpressing Gal4DB-SSL + Gal4AD-SSL fusion proteins in His-media suggests that SSL can also form homodimers, although the levels of *LacZ* appear significantly attenuated compared to those with SSL + CKII α (Fig. 1B). Additionally, SSL also appears to be competent at forming heterodimeric complexes with CKII β or β' . We attribute the attenuated *LacZ* values, not to a weak interaction, but rather to a general instability of *Drosophila* β -like proteins in yeast. We have previously observed that expression of CKII β in yeast does not elicit significant protein accumulation unless coexpressed with CKII α [27], and in line with this observation, CKII α + CKII β versus CKII β + CKII β interactions assessed via two-hybrid analysis demonstrate that the latter combination exhibits a high attenuation of *LacZ* induction [7]. Consistent with these observations, *LacZ* values are substantially higher when these β -isoforms are coexpressed with DmCKII α (Fig. 1). Taken

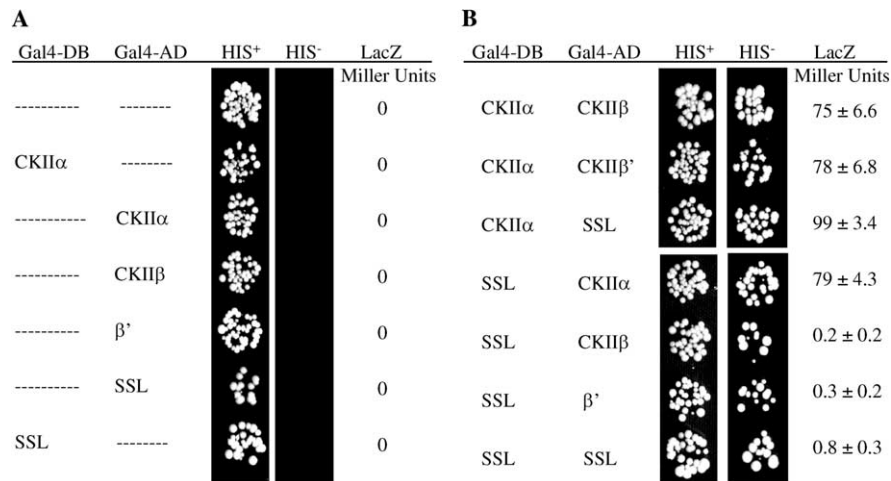


Fig. 1. Isolation of SSL and interaction with DmCK2 subunits. *S. cerevisiae* strain HF7C was transformed with plasmids expressing the indicated fusions with GAL4DB or GAL4AD. Transformants were selected, and following growth in glucose medium, cultures were tested for induction of *HIS3* expression by plating on complete minimal medium (His⁺) or minimal medium lacking histidine (His⁻). In parallel, two independent transformants were assessed for *LacZ* activity each in triplicate and values are expressed as Miller Units.

together, these results raise the likely possibility that holoenzyme isoforms can be built with equivalent efficiencies using a homodimeric core containing $\beta(\alpha_2\beta_2)$, $\beta'(\alpha_2\beta'_2)$ or SSL ($\alpha_2\text{SSL}_2$), and also suggest that tetramers containing distinct β -isoforms, e.g., $\alpha_2\beta\beta'$, $\alpha_2\beta$ SSL, etc. can also be generated. The identification of substrates that interact exclusively with the $\alpha_2\beta_2$ holoenzyme will be necessary to determine whether these isoforms confer distinct functions.

Mapping of the interaction domain

The domains of CKII β that are responsible for mediating homotypic (β – β) or heterotypic (β – α) interactions have been established by a combination of biochemistry, two-hybrid analysis, and structural analysis on individual subunits and the holoenzyme. These

studies demonstrate that a Cys4-zinc-finger motif in CKII β mediates homodimerization [26], whereas residues in the vicinity of the C-terminus mediate interaction with CKII α [28] (Fig. 2A). We, therefore, aligned the C-terminal CKII α -interaction domain of all of the *Drosophila* β -like proteins, relative to the human protein, to clarify the level of conservation. While this domain is remarkably conserved amongst human β , fly β , and β' , it is highly divergent in SSL and is virtually absent in Ste (Fig. 2B). We, therefore, generated deletions of the SSL protein to test whether this region also mediates interaction with CKII α . We find that, compared to full-length SSL, truncation at Lys186 (K186*) decreases the affinity of interaction with CKII α to \sim 30%, whereas truncations at either Glu173 (E173*) or Gly148 (G148*) virtually abolish interaction (Fig. 2C). The residual reporter gene activity may represent a

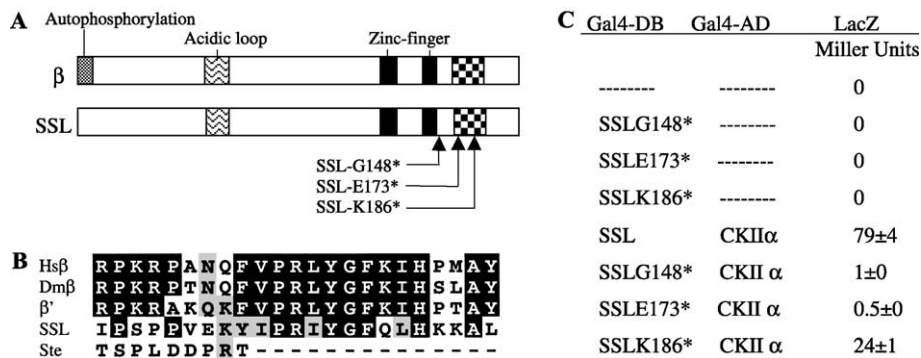


Fig. 2. Mapping of the SSL–CKII α interaction domain. (A) Schematic representation of the SSL protein illustrating motifs (not drawn to scale) conserved amongst all β -homologs; an N-terminal (auto)phosphorylation site, acidic loop, a Cys4-type zinc finger that mediates dimerization, and a domain (checkerboard) that mediates interaction with CKII α . (B) Alignment of the CKII α -interaction domain in CKII β like proteins. White letters, identical residues; black letters, non-conserved substitutions; and shaded letters, conservative substitutions. (C) Constructs expressing SSL or the indicated truncations were transformed in yeast HF7C by themselves or in combination with CKII α . Two independent transformants were each assayed in triplicate for *LacZ* activity and values are expressed as Miller Units.

bridge interaction mediated via yeast CKII subunits, which exhibit two hybrid interactions with their *Drosophila* homologs (Trott and Bidwai, unpublished). Control experiments where yeast expressed the truncation variants by themselves did not induce either reporter gene. These results demonstrate that, in spite of sequence divergence, the domain that mediates the SSL–CKII α interaction is “functionally” identical to that in β or β' , and the equivalent affinities of these three proteins for interaction with CKII α (Fig. 1) further underscore the likelihood that these proteins can generate alternative holoenzyme isoforms in vivo.

Rescue of ion-homeostasis defects of *ckb1* or *ckb2* yeast

We have previously observed that loss of either the β or β' subunit elicits defects in ion-homeostasis in *S. cerevisiae* [13], and this phenotype, elicited due to attenuated expression of the sodium-transport-pump [29], is complemented by expression of DmCKII β . This system thus affords a bioassay for assessing the functions of all *Drosophila* β -like proteins, i.e., β' , SSL, and Ste. Constructs that confer Gal-1/10 mediated expression of CKII β , β' , SSL, and Ste were generated as described (see Materials and methods), and tested for suppression of the ion-homeostasis defect of YAPB7 (deletion of *CKB1* encoding CKII β [13]) or JCR8 (deletion of *CKB2* encoding CKII β' [30]). As expected, transformation of *ckb1* or *ckb2* strains with the expression vectors lacking an insert (pBM272 or pESC-URA) did not elicit any suppression (Fig. 3). On the other hand, transformation with a plasmid expressing CKII β elicited suppression, albeit incomplete, and comparable results were observed with cells expressing SSL. On the other hand, complementation with β' was at best marginal, while none was observed with the Ste protein. Taken together, the rank order of efficacy of these proteins would appear to be (CKII β \sim SSL) $>$ β' .

We have previously demonstrated that Ste does not exhibit a two-hybrid interaction with CKII α , and the most likely reason is the absence of an α -interaction domain (Fig. 2B). These studies raise the obvious question why, in spite of comparable interaction with CKII α , β' exhibits the least functionality, and we believe that structural conservation of domains in metazoan β subunits provides a plausible explanation. Apart from residues mediating β – β or β – α interactions, all canonical β subunits conserve two “functional” domains; an N-terminal autophosphorylation site and an acidic domain (Fig. 3B). CKII β autophosphorylates at M¹SS^PSEE and this, in turn, elicits phosphorylation of Ser²(M¹S^PS^PSEE) because the consensus is S-D/E-x-D/E and Ser^{Phos} mimics Asp [26]. Interestingly, this site in β' is M¹TDSDE and the presence of Asp³ (instead of Ser³ in β) raises the possibility that β' is ‘constitutively phosphorylated’, whereas the corresponding site in SSL

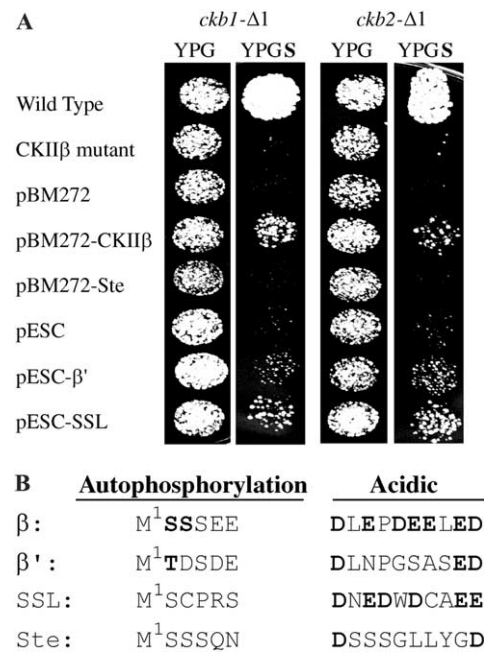


Fig. 3. Complementation of *ckb1* or *ckb2* yeast by *Drosophila* CKII β -like proteins. (A) Yeast strains lacking either *ckb1* (*ckb1*- Δ 1) or *ckb2* (*ckb2*- Δ 1) subunits were transformed with the expression vectors or vectors expressing CKII β , Ste, β' , or SSL. Transformants were selected, and following growth in rich medium, dilutions were plated to rich galactose medium (YPG) or on YPG supplemented with 0.5 M NaCl (YPGS). Plates were incubated at 29 °C for 3–4 days and photographed. (B) Alignment of the functional motifs in *Drosophila* CKII β -like proteins.

(M¹ SCPRS) is non-autophosphorylatable. It has been suggested that autophosphorylation stabilizes CKII β against ubiquitination and proteosomal degradation in mammalian cells [12], but somewhat conflicting results have been obtained in *Drosophila* [14], wherein non-autophosphorylating CKII β is equally competent at rescuing the lethality associated with a deletion of the *CKII β* gene. Our observation that CKII β and SSL, but not β' , appear to be functional in the yeast system would suggest that autophosphorylation may not account for the functional differences. A more likely reason for the observed behavior of these β -like proteins is, perhaps, the acidic domain (Fig. 3B). Analysis of the structure of the holoenzyme ([28,31] and references within) combined with kinetic analysis suggests that this domain modulates substrate recognition and mediates activating effects of polybasic compounds such as spermine. In line with this, 7/10 residues of this region of CKII β and 6/10 in SSL (both of which exhibit equivalent complementation, Fig. 3A) are either Asp/Glu, whereas that in β' is the least acidic (3/10, see Fig. 3B). If substrate recognition and/or targeting of CKII-activators is one of the functions of this domain, SSL would appear to be closest in function to CKII β and this is essentially reflected in the rank order of efficacy.

Real-time RT-PCR analysis of the expression of CKII β and SSL

It has recently been suggested, based on Northern blot analysis, that β' and SSL are expressed only in adult *Drosophila* in a testis-specific manner [25], a scenario that is at odds with our isolation of SSL cDNAs from embryo libraries. We have, therefore, used real-time RT-PCR, a more sensitive approach for detection of transcripts and one that also provides an assessment of relative transcript copy number. FAM-labeled probes specific to CKII β and SSL were designed as described in Materials and methods and used to probe RNA isolated from the indicated developmental stages and sexes. We find that CKII β transcripts do not display sexual dimorphism (Fig. 4), whereas transcripts encoding SSL are easily detected in males at all of these stages, but appear undetectable in females. The essentially similar C_t values for CKII β in males versus females at all developmental stages tested would argue against the possibility that our inability to detect SSL transcripts in females is an artifact, and reflect the observation that levels/activity of CKII appear constant throughout development and that CKII β is recessive lethal in males and females [14]. Our results clearly demonstrate that expression of SSL occurs across a wider developmental window than that recently suggested [25] and that SSL exhibits sexual dimorphism and thus is likely to be a male-specific gene. Our isolation of multiple cDNAs encoding SSL from an embryo-library would, at face value, also suggest expression at the earliest developmental timepoint. However, attempts to probe RNA isolated from embryos via real-time RT-PCR suggest that transcript levels at this stage are below detectable levels (data not shown). We believe that our successful

isolation of SSL transcripts from an embryo library is a reflection of the inordinately large number of primary two hybrid transformants (4×10^8), and that interaction of SSL with CKII α is the highest of any interacting partner in this two-hybrid hunt. Our two-hybrid screen may thus favor the isolation of relatively rare transcripts present at levels lower than those detectable via other means.

These studies raise obvious questions about the functions of SSL in a male specific manner. Although testicular development initiates early in development, meiosis does not initiate until adult eclosion [32]. Our observation that SSL expression is also found in larval and pupal stages suggests that SSL's functions are not restricted to meiotic events and may also be involved in the ontogeny of testis development. Our interaction data and deletion analysis demonstrate that like CKII β , SSL is capable of complexing with CKII α forming an alternative CKII holoenzyme isoform, while differential abilities to rescue yeast would suggest functional differences. Future studies using β' /SSL transgenes for complementation of lethal/hypomorphic CKII β alleles and the global effort to isolate P-element insertions in all autosomal genes will provide insight into their functions.

Acknowledgments

We express our thanks to Madhavi Kalive and Rebecca Rummer for assistance. This work was supported by an American Cancer Society Grant RPG9918901 to A.P.B. and a Hurlbutt Undergraduate Honors Research Award to N.M.

References

- [1] L.A. Pinna, Protein kinase CK2: a challenge to canons, *J. Cell Sci.* 115 (2002) 3873–3878.
- [2] A. Saxena, R. Padmanabha, C.V.C. Glover, Isolation and sequencing of cDNA clones encoding α and β subunits of *Drosophila melanogaster* casein kinase II, *Mol. Cell. Biol.* 7 (1987) 3409–3417.
- [3] E. Hu, C.S. Rubin, Casein kinase II from *Caenorhabditis elegans*: properties and developmental regulation of the enzyme; cloning and sequence analysis of cDNA and the gene for the catalytic subunit, *J. Biol. Chem.* 265 (1990) 5072–5080.
- [4] I. Roussou, G. Dretta, The *Schizosaccharomyces pombe* casein kinase II α and β subunits: evolutionary conservation and positive role for the β subunits, *Mol. Cell. Biol.* 14 (1994) 576–586.
- [5] A.P. Bidwai, J.C. Reed, C.V.C. Glover, Casein kinase II of *Saccharomyces cerevisiae* contains two distinct regulatory subunits, β and β' , *Arch. Biochem. Biophys.* 309 (1994) 348–355.
- [6] M.A. Collinge, J.C. Walker, Isolation of an *Arabidopsis thaliana* casein kinase II β subunit by complementation in *Saccharomyces cerevisiae*, *Plant Mol. Biol.* 25 (1994) 649–658.
- [7] A.P. Bidwai, W.F. Zhao, C.V.C. Glover, A gene located at 56F1-2 in *Drosophila melanogaster* encodes a novel metazoan β -like subunit of casein kinase II, *Mol. Cell Biol. Res. Comm.* 1 (1999) 21–28.

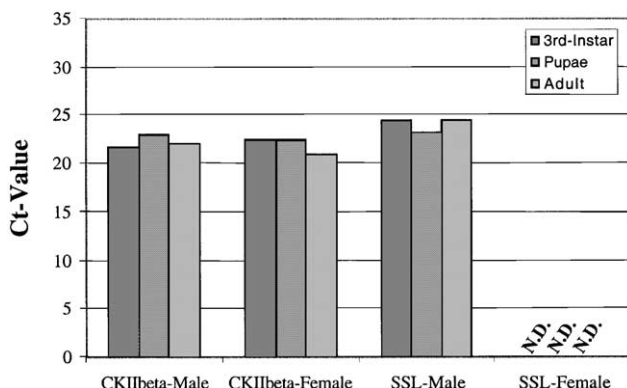


Fig. 4. Real-time RT-PCR analysis of CKII β and SSL. Primers specific to CKII β and to SSL were designed as described in Materials and methods, and used to screen RNA from single sex-selected third-instar-larva, pupa, and adult. Fluorescence output was captured and analyzed to calculate the threshold cycle (C_t). Higher C_t values correspond to a lower transcript copy number and C_t values greater than 32 represent levels that are non-detectable and are, therefore, indicated as ND.

- [8] E.A. Kuenzel, J.A. Mulligan, J. Sommercorn, E.G. Krebs, Substrate specificity determinants for casein kinase II as deduced from studies with synthetic peptides, *J. Biol. Chem.* 262 (1987) 9136–9140.
- [9] C.V.C. Glover, On the physiological role of casein kinase II in *Saccharomyces cerevisiae*, *Prog. Nucl. Acid Res. Mol. Biol.* 59 (1998) 95–133.
- [10] C. Cochet, E.M. Chambaz, Oligomeric structure and catalytic activity of G type casein kinase, *J. Biol. Chem.* 258 (1983) 1403–1406.
- [11] A.P. Bidwai, J.C. Reed, C.V.C. Glover, The phosphorylation of Calmodulin by the catalytic subunit of casein kinase II is inhibited by the regulatory subunit, *Arch. Biochem. Biophys.* 300 (1993) 265–270.
- [12] C.-K. Zhang, G. Vilk, D.A. Canton, D.W. Litchfield, Phosphorylation regulates the stability of the regulatory CK2 β subunit, *Oncogene* 21 (2002) 3754–3764.
- [13] A.P. Bidwai, J.C. Reed, C.V.C. Glover, Cloning and disruption of CKB1, the gene encoding the 38-kDa β subunit of *Saccharomyces cerevisiae* casein kinase II, *J. Biol. Chem.* 270 (1995) 10395–10404.
- [14] E. Jauch, J. Melzig, M. Brkulj, T. Raabe, In vivo functional analysis of *Drosophila* protein kinase casein kinase 2 (CK2) β -subunit, *Gene* (2002).
- [15] A.I. Kalmykova, Y.Y. Shevelyov, A.A. Dobritsa, V.A. Gvozdev, Acquisition and amplification of a testis-expressed autosomal gene, SSL, by the *Drosophila* Y chromosome, *Proc. Natl. Acad. Sci. USA* 94 (1997) 6297–6302.
- [16] K.J. Livak, Detailed structure of the *Drosophila melanogaster* *Stellate* genes and their transcripts, *Genetics* 124 (1990) 303–316.
- [17] M.D. Balakireva, Y.Y. Shevelyov, D.I. Nurminsky, K.J. Livak, V.A. Gvozdev, Structural organization and diversification of Y-linked sequences comprising su(ste) genes in *Drosophila melanogaster*, *Nucleic Acid Res.* 20 (1992) 3731–3736.
- [18] M.P. Bozzetti, S. Massari, P. Finelli, F. Meggio, L.A. Pinna, B. Boldyreff, O.G. Issinger, G. Palumbo, C. Ciriaco, S. Bonaccorsi, S. Pimpinelli, The Ste locus, a component of the parasitic cry-ste system of *Drosophila melanogaster* encodes a protein that forms crystals in primary spermatocytes and mimics properties of the β subunit of casein kinase II, *Proc. Natl. Acad. Sci. USA* 92 (1995) 6067–6071.
- [19] H.E. Feilotter, G.J. Hannon, C.J. Ruddell, D. Beach, Construction of an improved host strain for two hybrid screening, *Nucleic Acids Res.* 22 (1994) 1502–1503.
- [20] T. Durfee, K. Becherer, P.L. Chen, S.H. Yeh, Y. Yang, A.E. Kilburn, W.H. Lee, S.J. Elledge, The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit, *Genes Dev.* 7 (1993) 555–569.
- [21] J.H. Miller, in: *Experiments in Molecular Genetics*, Cold Spring Harbor, New York, 1972, pp. 352–355.
- [22] C. Guthrie, G.R. Fink, *Guide to yeast genetics and molecular biology*, *Methods Enzymol.* 194 (1991).
- [23] W. Zhao, A.P. Bidwai, C.V.C. Glover, Interaction of casein kinase II with ribosomal protein L22 of *Drosophila melanogaster*, *Biochem. Biophys. Res. Commun.* 298 (2002) 60–66.
- [24] R.L. Trott, M. Kalive, Z. Paroush, A.P. Bidwai, *Drosophila melanogaster* casein kinase II interacts with and phosphorylates the basic-helix-loop-helix (bHLH) proteins m5, m7, and m8 derived from the enhancer of split complex, *J. Biol. Chem.* 276 (2001) 2159–2167.
- [25] A.I. Kalmykova, Y.Y. Shevelyov, O.O. Poleskaya, A.A. Dobritsa, A.G. Evstafieva, B. Boldyreff, O.-G. Issinger, V.A. Gvozdev, CK2 β tes gene encodes a testis-specific isoform of the regulatory subunit of casein kinase 2 in *Drosophila melanogaster*, *Eur. J. Biochem.* 269 (2002) 1418–1427.
- [26] L. Chantalat, D. Leroy, O. Filhol, A. Nueda, M.J. Benitez, E.M. Chambaz, C. Cochet, O. Dideberg, Crystal structure of the human protein kinase CK2 regulatory subunit reveals its zinc finger-mediated dimerization, *EMBO J.* 18 (1999) 2930–2940.
- [27] A.P. Bidwai, D.E. Hanna, C.V.C. Glover, Purification and characterization of Casein Kinase II (CKII) from DCKA1 DCKA2 *S. cerevisiae* rescued by *Drosophila* CKII subunits: the free catalytic subunit of casein kinase II is not toxic in vivo, *J. Biol. Chem.* 267 (1992) 18790–18796.
- [28] K. Niefind, B. Guerra, I. Ermakowa, O.G. Issinger, Crystal structure of human protein kinase CK2: insights into basic properties of the CK2 holoenzyme, *EMBO J.* 20 (2001) 5320–5331.
- [29] K.A. Tenney, C.V.C. Glover, Transcriptional regulation of the *S. cerevisiae* ENA1 gene by casein kinase II, *Mol. Cell. Biochem.* 191 (1998) 161–167.
- [30] J.C. Reed, A.P. Bidwai, C.V.C. Glover, Cloning and disruption of CKB2, the gene encoding the 32 kDa regulatory β' subunit of *Saccharomyces cerevisiae* casein kinase II, *J. Biol. Chem.* 269 (1994) 18192–18200.
- [31] K. Niefind, B. Guerra, L.A. Pinna, O.G. Issinger, D. Schomburg, Crystal structure of the catalytic subunit of protein kinase CK2 from *Zea mays* at 2.1 Å resolution, *EMBO J.* 17 (1998) 2451–2462.
- [32] D.L. Lindsley, K.T. Tokuyasu, *Spermatogenesis*, in: *The Genetics and Biology of Drosophila*, Academic Press, New York, 1980, pp. 225–294.