FEBS Letters 356 (1994) 317–321

FEBS 14911

Cloning and characterization of the *Drosophila melanogaster* CDK5 homolog

Mark R. Hellmich[a,*], James A. Kennison[b], Lori L. Hampton[a], James F. Battey[a]

[a]*Laboratory of Biological Chemistry, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Building 37, Room 5D02, 9000 Rockville Pike, Bethesda, MD 20892, USA*
[b]*Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA*

Received 13 October 1994

Abstract The *D. melanogaster* homolog of mammalian CDK5 has been cloned and its chromosomal location determined. The gene for *Cdk5* consists of 4 exons separated by 3 short introns ranging in size from 61–160 bp. Northern blot analysis revealed a single mRNA of approximately 1.6 kb that is expressed at highest levels in the adult fly. The putative amino acid sequence for *Drosophila Cdk5* predicts a protein with a mass of approximately 32 kDa that is 77% identical to its mammalian counter-parts. *Drosophila Cdk5* gene is located in polytene chromosomal region 52BC of the right arm of chromosome 2. This study provides the framework for a molecular genetic analysis of CDK5 function.

Key words: CDK5; cDNA; Cyclin-dependent kinase; Development; *Drosophila*

1. Introduction

The coordinated progression of a eukaryotic cell through DNA replication and cell division is dependent on the activity of a family of protein kinases structurally and functionally related to the yeast cell division control kinases, cdc2 and CDC28 [1]. This family of proteins is known as the cyclin dependent kinases (CDKs), because the active enzyme is a heterodimeric protein complex consisting of a catalyic subunit and a regulatory subunit called a cyclin. The regulatory subunit derives its name from the oscillatory nature of its expression [2]. Activation of the catalytic subunit requires association with a specific cyclin as well as selective phosphorylation and dephosphorylation events. The index members of this enzyme complex are $p34^{cdc2}$ kinase (CDK1) and cyclin B. Cyclin B protein expression increases during S phase, peaks as the cell enters mitosis and decreases rapidly as the cell exits mitosis [3]. CDK1 activation at the G2-M boundary requires association with cyclin B, phosphorylation of threonine residue 161 by cdc2 activating kinase (CAK) [4] and dephosphorylation of tyrosine 15 and threonine 14 within the putative ATP binding site [5].

In yeast, progression from G1 to S phase and G2 to mitotis is controlled by the activity of a single kinase, either cdc2 (*Schizosaccharomyces pombe*) or CDC28 (*Saccharomyces cerevisiae*) [6]. In contrast, cell division in multicellular organisms requires the activity of multiple CDKs. Currently, clones for twelve catalytic subunits [7–12] and eight cyclins (A–H) have been identified [13–18]. Six catalytic subunits are known to associate with specific cyclins: CDK1 with cyclins A and B, CDK2 with A and E, and CDK4–6 with the D-type cyclins. In addition, CDK1–4 and CDK6 have been shown by either yeast complementation assays, dominant-negative mutant transfection assays [19] or immunoprecipitation studies [20] to be active during the cell cycle. In contrast, CDK5, although structurally similar to these other kinases, failed to complement budding yeast containing temperature sensitive mutants of CDK1 [9].

Previously, we have described the cloning and characterization of CDK5, which was isolated from a rat brain cDNA library by low stringency screening with a ^{32}P-labelled mouse CDK1 cDNA probe [10]. We originally called this clone 'neuronal-cdc2-like kinase' or nclk because its structure is closely related to both CDK1 and CDK2, sharing approximately 60% amino acid sequence identity. However, unlike CDK1 and CDK2, CDK5 mRNA was shown to be expressed predominantly in terminally differentiated (post-mitotic) neurons of the central and peripheral nervous system in both E20 rat embryos and adults. In contrast to the selective expression pattern observed in normal tissues, CDK5 mRNA is expressed at high levels in all cultured cells investigated to date, including both primary and immortalized cell-lines. These observations have been corroborated and extended by other investigators. Recently, Tsai et al. [21] have shown that CDK5 protein was specifically expressed in post-mitotic neurons and that enzymatically active kinase could be immunoprecipitated from mouse brain tissue extracts, but not other normal mouse tissues. Furthermore, they observed that various cultured cell lines contained high levels of CDK5 protein, but active kinase could not be immunoprecipitated from extracts of those cells. Similarly, Xiong and co-workers [12] have cloned CDK5 from a human HeLa cell expression library and demonstrated that CDK5 could be co-immunoprecipitated with anti-D-type cyclin antibodies from cell lysates of WI38 human diploid fibroblasts, however, the enyzme from these cultured cells was inactive. Finally, Lew et al. [22] purified active CDK5 from bovine brain based on its ability to phosphorylate a synthetic peptide derived from the sequence of $p60^{c\text{-}src}$. The active kinase in their preparation consisted of two proteins, a 33 kDa catalytic subunit (CDK5) and a non-cyclin 25 kDa protein.

These data clearly demonstrate that CDK5 expression alone is not sufficient for its activity and suggest that CDK5 may serve some unique function in neurons. Perhaps CDK5 is a specific regulator of neuronal development and/or differentiation, two processes which like the cell division cycle require the coordinated progression of a series of precisely timed and regulated events. One method of addressing this possibility is to

*Corresponding author. Fax: (1) (301) 480 2514.

SSDI 0014-5793(94)01298-9

develop a model for studing CDK5 function in a genetically tractable organism like *Drosophila melanogaster*. *Drosophila* provides the combination of identified cells of known lineage, the ability to trace growth cones and axons, and the potential for molecular genetic analysis making it an excellent organism to ask questions concerning neuronal development and differentiation.

This report describes the cloning, structural characterization, and chromosome mapping of the *D. melanogaster* homolog of vertebrate CDK5. It represents the initial framework necessary to develop this organism into a genetic model for the investigation of CDK5 function.

2. Materials and methods

2.1. Drosophila melanogaster *stocks*

Wild-type stain was *Oregon R* stock. The deficiencies were obtained from the stock center at Indiana University, Bloomington, ID.

2.2. Southern blot analysis

40 μg of *D. melangaster* genomic DNA and 10 μg of human genomic DNA were cut with either *Bam*HI or *Eco*RI for 1 h at 37°C, resolved on a 0.8% agarose gel, and blotted onto nitrocellulose, as described previously [23]. The hybridization probe was a rat CDK5 cDNA fragment (*Eco*RI fragment), isolated from a low-melting point agarose gel using the Wizard PCR Preps DNA purification system (Promega, Madison WI) and labelled by nick-translation using the method described previously [23]. The blot was hybridized overnight at 37°C in 10% dextran sulfate, 40% formamide, 4 × SSC (1 × SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0), 20 mM Tris pH 7.4, 1 × Denhardt's (0.02% Ficoll, 0.02% polyvinlypyrrolidone, 0.02% bovine serum albumin), and 20 μg/ml herring sperm DNA. Filters were washed twice at room temperature for 15 min in 2 × SSC, 0.1% SDS followed by two additional 15 min washes at 37°C in 0.1 × SSC, 0.1% SDS. Hybridization was visualized by autoradiography using Kodak XAR5 film.

2.3. Library screening

The *D. melanogaster* CDK5 genomic clone was isolated from a lambda GEM11 library (Stratagene, La Jolla CA). Approximately 5×10^5 plaques were screened using a ^{32}P-labelled rat CDK5 cDNA fragment labelled by nick-translation [23]. Filters were hybridized and washed as described in section 2.2. After autoradiography for 1 to 2 days, positive clones were plaque purified, subcloned into pGEM4 plasmid vector (Promega, Madison WI), and both stands sequenced using the f-mol sequencing kit (Promega, Madison WI).

2.4. Preparation of RNA

Adult *Oregon R* flies (approximately 2 ml dry volume) were placed into a glass-on-glass Dounce homogenizer and first homogenized in 5 ml of GIT buffer (4 M guanidine isothiocyanate, 25 mM sodium citrate (pH 7.0), 0.7% β-mercaptoethanol), then 0.55 ml of 20% sarcosyl was added and the sample was homogenized a second time. GIT/fly solution (5 ml) was layered onto a 5 ml cesium chloride cushion (5.8 M CsCl, 10 mM EDTA) and the RNA pelleted by spinning overnight at 32,000 × *g* in a swinging bucket rotor (Beckman, SW41TI). The pellet was washed with 70% EtOH, dried, and resuspended in 0.5 ml of sterile H_2O. Poly(A)$^+$ RNA was isolated using a poly (A) Quik Kit (Stratagene, La Jolla CA). The typical yield using this procedure is 10 to 15 μg of poly(A)$^+$ RNA.

Two to three thousand embryo or several hundred fly larvae were collected on grape-juice agar plates, washed 3 times with 10 mM Tris-Cl (pH 7.4), 1 mM EDTA, and homogenized (Dounce homogenizer) in 1–2 ml of 4 M guanidine isothiocyanate, 0.5% sarcosyl, and 25 mM sodium citrate (pH 7.0). After homogenization, an equal volume of H_2O-saturated phenol was added plus 0.1–0.2 ml of 2 M sodium acetate and 0.2–0.4 ml of chloroform. The samples were mixed and incubated on ice for 15 min, spun for 20 min (10,000 × *g*) at 4°C, the upper aqueous layer removed, and precipitated at −20°C for 1 h in an equal volume of isopropanol. The pellets were resuspended in 1 ml of the guanidine isothiocyanate buffer and re-precipitated in an equal volume of isopropanol. The pellets were then washed with 70% EtOH, resuspended in DEPC-treated H_2O, and precipitated a third time in 3 M LiCl for 2 h at −20°C.

2.5. cDNA cloning: RT/PCR

D. melanogaster CDK5 cDNA was amplified from mRNA by the RT/PCR method. Briefly, 1 μg poly(A)$^+$ RNA (isolated from 12–24 h embryos) was primed with either random hexamers (0.1 μg) or a gene specific anti-sense oligo-nucleotide (5′-GCTGGACAAAGTCTCGCATT-3′) (0.1 μg) and reverse transcribed with the Moloney murine leukemia virus (MMLV) reverse transcriptase (RT) (200 U) in a reaction buffer containing the following: 50 mM Tris-Cl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 40U RNasin, and 0.5 mM each of dGTP, dATP, dCTP, and dTTP. After incubating for 1 h at 37°C, the RT was inactivated by heating to 70°C for 5 min. For amplification of CDK5, 2 μl of the RT reaction was added to a PCR reaction containing the following: 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 0.1 μg each of an anti-sense (5′-CGGCTTAAGTCTAATCAAAG-3′) and sense (5′-CCCCGGAGTTTTCCATCTGA-3′) oligo nucleotide primer, 0.5 mM each dNTP, and 5 U *Taq* DNA polymerase. This reaction mixture was heated to 95°C for 5 min, cooled to 72°C for 2 min, and cycled 35 times at 94°C for 15 s, 55°C for 15 s, and 72°C for 30 s. Products of both the hexamer primed and gene specific primed RT/PCR reactions were resolved on a low melt agarose gel, purified, subcloned into pGEM4 (Promega, Madison, WI), and sequenced using the f-mol sequencing kit (Promega, Madison, WI).

2.6. 3′-RACE (rapid amplification of cDNA ends)

A reverse transcription assay was performed as described above in section 2.5 using 1 μg of poly(A)$^+$ RNA from adult flies (*Oregon R*) as template and an adaptor plus -dT17 (5′-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3′) oligo nucleotide as a primer. PCR was performed using a 5′ sense gene specific primer (5′-GACGCGACCTGCTGCAAAAG-3′) and a 3′ anti-sense primer containing the adaptor sequence as described previously [24].

2.7. Northern blot analysis

Total or poly(A)$^+$ RNA was prepared from embryos, larve, and adult flies as described above, resolved by electrophoresis on 1% agarose-formaldehyde gels, and blotted to nitrocellulose membranes using standard methodology [23]. After baking at 80°C, membranes were hybridized with a ^{32}P-labelled *D. melanogaster* cDNA fragment, washed at high stringency (65°C in 0.1 × SSC, 0.1% SDS, 2 × 15 min), and subjected to autoradiography for 1 to 2 days.

2.8. In situ hybridization to salivary gland chromosomes

Preparation of slides and hybridization followed standard methods [25]. Slides were probed with either digoxigenin labelled cDNA fragments or DNA fragments generated by PCR from genomic clones 3a and 3b. To generate PCR fragments the following primers were used: Sense primers, 5′-GGATGAGCAGGAAGTGATTC-3′, 5′-GCCATAATGTCCTGCATCGC-3′, and 5′-CCCCGGAGTTTCCATCTGA-3′. Anti-sense primer, 5′-GCTGGACAAAGTCTCGCATT-3′. DNA fragments were labelled with digoxigenin-11-dUTP using the random primers method described in the Genius 2 DNA labelling Kit (Boehringer Mannheim, Indianapolis, IN). Specific hybridization was visualized by immunostaining the slides using an alkaline phosphatase conjugated antidigoxigenin antibody (Boehringer Mannheim, Indianapolis, IN). Chromosomes were counter-stained in freshly prepared and filtered 5% Giemsa, washed with distilled H_2O, air dried, and mounted.

3. Results

Southern blot analysis was performed to determine whether *D. melanogaster* contained a gene homologous to rat CDK5. Fly DNA was digested with either *Bam*HI or *Eco*RI, hybridized with a rat CDK5 cDNA probe labelled by nick-translation and washed under low-stringency conditions. The rat probe hybridized to a single fragment in both the *Bam*HI and *Eco*RI cut fly DNA (Fig. 1). The *Bam*HI fragment is approximately 4.5 kb and the *Eco*RI fragment is about 3.5 kb in size.

A *Drosophila* genomic library constructed in lambdaGEM-

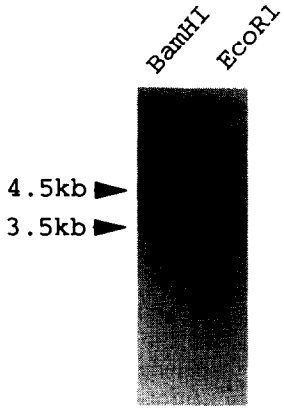


Fig. 1. Southern blot analysis of *D. melanogaster* genomic DNA probed with a ^{32}P-labelled rat CDK5 cDNA probe. A single band is revealed in each digest: a 4.5 kb *Bam*HI fragment and 3.5 kb *Eco*RI fragment.

11 (Stratagene, La Jolla, CA), was screened using the rat CDK5 cDNA probe. Three clones were identified in the initial screen of 5×10^5 plaques. Two clones (3a and 3b) were plaque purified and subcloned into pGEM4. Each clone contained an *Sst*I insert of approximately 9–10 kb. The *Sst*I fragments were digested further with various restriction endonucleases (Fig. 2A) and after Southern blot analysis, the fragments that hybridized to a rat CDK5 cDNA were subcloned into pGEM4 and sequenced. Digestion of the two clones gave dissimilar banding patterns depending on the enzyme used. These differences appear to be the result of polymorphism.

A restriction map of clone 3b is shown diagramatically (Fig. 2A). The entire coding region of the fly *Cdk5* is contained on four exons separated by 3 short introns ranging in size from 61 to 160 base pairs (Fig. 2B). The intron/exon splice sites, 5′-GT donor and 3′-AG acceptor sequences of the introns, as well as the consensus recognition sequence for poly-adenylation are underlined. To verify the intron-exon structure of the genomic clone, a cDNA clone was isolated from 12–24 h fly embryo mRNA by RT/PCR as described in section 2. By RT/PCR we isolated the *Cdk5* coding region that is approximately 900 base pairs in length, and verified the location of intron boundaries.

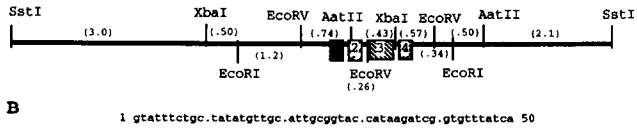
Northern blot analysis was performed using a hexamer primed ^{32}P-labelled fly *Cdk5* cDNA fragment as a probe. In 1–12 h embryos, larvae, and adult flies a single band approximately 1.6 kb in size hybridized with the probe (Fig. 4). Although 12–24 h embryos appear to contain very low levels of *Cdk5* message, we were able to clone *Cdk5* cDNA from this RNA preparation by RT/PCR. In situ hybridization of whole mount embryos revealed uniform, specific staining at the blastoderm stage, suggesting that *Cdk5* message at this stage is of maternal origin (data not shown). All other embryonic stages showed no detectable *Cdk5* message over background.

To identify the remaining 500–600 base pairs predicted from the message size observed on Northern blots, a 3′-RACE (Rapid Amplification of cDNA Ends) reaction was performed. Southern blot analysis of the 3′-RACE reaction revealed 3 predominant products, ranging in size from 0.2 to 0.6 kb. These three fragments were subcloned into pGEM4 and sequenced. Sequence analysis showed that the three fragments overlapped, and contained genomic sequences contiguous to the 3′ end of the coding region without additional interruptions by introns. Isolation of these RACE products allowed us to account for the entire size of the mRNA observed by Northern blot. The identification of three fragments of different size appears to be the result of mis-priming of the dT17 oligo during the reverse transcription reaction, since the 3′UT domain contains numerous A-rich sequence motifs.

Comparison of putative amino acid sequence of *D. melanogaster Cdk5* to the known vertebrate CDK5 clones is shown in Fig. 3. *D. melanogaster Cdk5* is approximately 77% identical to human, rat, and bovine sequences. By comparison the vertebrate sequences share greater than 99% identity to each other.

To initiate a genetic study of *Cdk5* function we mapped the chromosomal location of the *Cdk5* gene. To do this we performed in situ hybridization on polytene chromosomes from 3rd instar larval salivary glands (Fig. 5A,B). The cytological location of the *Cdk5* gene was first determined in wild-type (Oregon R) flies (Fig. 5B). The *Cdk5* gene maps to the right arm of chromosome two in region 52. To confirm and more precisely define this location, overlapping deletions in this region were analyzed by in situ hybridization. These deficient chromosomes are maintained in the heterozygous state, i.e. one normal copy of the gene(s) in the deleted region. Therefore if the gene

A



B

```
   1 gtatttctgc.tatatgttgc.attgcggtac.cataagatcg.gtgtttatca  50
  51 ttgtcggtct.atattcgtat.gtttagctaa.tatcgagtcc.aaataaaaca 100
 101 ataaaattat.aacaaaagtt.ttatcaacat.tttttaaatg.atatctgtta 150
 151 attttgagta.tttttggtga.cagcagactg.tcagctgcga.tgttggttag 200
 201 tcaccgcgtg.ctgtaagcga.ataacagtgt.gctgttaggc.gcacgaatcc 250
 251 ctggccagca.aaaacaaact.gcaaaaacaa.taatccccgg.agtttttccat 300
 301 ctgaacgcaa.aataatacac.accaaaacac.agcaaaATGC.AGAAGTACGA 350
 351 TAAAATGGAG.AAGATCGGGG.AGGGCACCTA.CGGCACGGTG.TTCAAGGGTC 400
 401 GCAACCGCGC.AACCATGGAA.ATAGTGGCCC.TGAAACGGGT.GCGACTGGAC 450
 451 GAGGACGACG.AGGGTGTGCC.TAGTTCTGCC.CTCCGGGAGA.TCTGCCTGCT 500
 501 GAAGGgtcag.tcggatgagc.aggaagtgat.tccagtttat.aaactaaatc 550
 551 ggcacattac.catccgcagA.GCTGAAGCAC.AAGAACATTG.TGCGCCTCAT 600
 601 AGACGTCCTG.CACTCGGACA.AGAAACTCAC.CCTGGTCTTC.GAGCACTGCG 650
 651 ACCAGGACCT.CAAGAAGTAC.TTTGACAGCC.TCAACGGGGA.GATCGACATG 700
 701 GCCGTCTGCA.GGAGCTTTAT.GCTCCAACTG.CTCCGCGGAC.TGGCCTTCTG 750
 751 CCACAGgtat.acctatatat.gtatcagcac.tggattaggg.tttatcttgt 800
 801 atcggtgata.gttagaatca.agtgttattt.ggagtaactg.catctgtggc 850
 851 acatttccga.atgatatcca.ttttatagca.ctgagactta.acttacgcta 900
 901 cattatccta.ttttagCCAT.AATGTCCTGC.ATCGCGATCT.GAAACCACAG 950
 951 AACCTGCTGA.TCAACAAAAA.CGGCGAGCTA.AAGCTGGCTG.ACTTTGGTCT 1000
1001 GGCTAGAGCC.TTTGGCATTC.CCGTGAAGTG.CTACTCCGCA.GAAGTGGTGA 1050
1051 CCCTGTGGTA.CCGACCACCG.GATGTACTGT.TTGGAGCCAA.GCTCTATACG 1100
1101 ACCAGCATAG.ACATGTGGTC.CGCTGGATGC.ATTTTGGCCG.AACTTGCGGA 1150
1151 CGCTGGACGA.CCGCTGTTTC.CTGGCTCTGA.CGTGCTCGAT.CAGCTGATGA 1200
1201 AGATCTTCCG.TGTGCTGGGC.ACCCCCAACG.AGGACTCCTG.GCCAGGAGTT 1250
1251 TCGCACCTCT.CCGACTACGT.GGCGCTTCCA.TGtAgggatt.ctagacggat 1300
1301 tttatttatc.gataccaata.ttaaaagcct.atttatatta.tagCTTTTCC 1350
1351 GGCCATCACC.TCGTGGAGTC.AGCTGGTACC.TAGGCTAAAC.TCCAAGGGAC 1400
1401 GCGACCTGCT.GCAAAAGCTG.CTCATCTGCC.GGCCAAATCA.GCGAATTAGC 1450
1451 GCGGAGGCGG.CGATGCAGCA.TCCCTACTTT.ACGGACAGCA.GCTCTTCGGG 1500
1501 ACACTGAggt.cgaaaggttc.tttccaagcc.actgttttca.cgacctttga 1550
1551 ttagacttaa.gccgaaatgc.gagactttgt.ccagcaaaga.tcttttaatt 1600
1601 atatatgtat.gtccttgcag.tagagttcga.ttatagtata.agataaaaaa 1650
1651 ttctttataa.tttcattagt.ttgatttacc.aaagcaaaag.gtttgattta 1700
1701 tgacaatccc.taccaatgtg.acacttaata.tagccatttg.agctgccaaa 1750
1751 ttaacattta.tcgataaaat.agttattgat.tcagtaaaat.agttttattc 1800
1801 ttaaaaattt.ttatgttgtg.tattgttaag.atccgaaaat.tgttcaaatt 1850
1851 cctagacgat.atctactgtt.tgtgtgaatg.tggaaaaata.tttgaaatcc 1900
1901 tagttttctgt.tttcaaaaac.taaaaactag.gcaatacatc.tggttttcaa 1950
1951 atttaatgag.tgtcgacgat.tttcttatta.catttattgt.gaatttttaa 2000
2001 gaatttcgat.ctttctgaat.gatatgagtc.attaagtaaa.ttagaatcaa 2050
2051 aaaaattgag.atgtaaccta.gatttttaaa.ccaataaaga.cacttaccgc 2100
2101 agcactaagt.cgtcgcagca.tgatgaatat.tttgaagtgg.tcgcctcaac 2150
2151 ccccggcact.caataggggt.taaagtcgtt.ctagcgcact.cgcgagtt   2198
```

Fig. 2. (A) Restriction endonuclease map of *D. melanogaster Cdk5* genomic clone 3b. The locations of various endonuclease sites were determined by Southern blot analysis and/or nucleotide sequencing. The shaded boxes (1–4) represent the putative *Cdk5* coding sequence. The numbers in parentheses indicate the distance between endonuclease sites in kilobases. (B) Nucleotide sequence of *D. melanogaster Cdk5* gene. Putative coding region is indicated in upper case letters. 5′- and 3′-untranslated regions and intron sequences are in lower case. The dinucleotide donor (gt) and acceptor (ag) sequences which define intron/exon splice sites as well as the polyadenylation recognition site (aataaa) are underlined.

```
        1                                                   50
Human   ----E-L--- ---------- AK--E-H--- --------D- ----------
Rat     ----E-L--- ---------- AK--E-H--- --------D- ----------
Bovine  ----E-L--- ---------- AK--E-H--- --------D- ----------
Fly     MQKYDKMEKI GEGTYGTVFK GRNRATMEIV ALKRVRLDED DEGVPSSALR

        51                                                  100
Human   ---------- ------H--- ---------- -F-------- ---C--DL-P
Rat     ---------- ------H--- ---------- -F-------- ---C--DL-P
Bovine  ---------- ------H--- ---------- -F-------- ---C--DL-P
Fly     EICLLKELKH KNIVRLIDVL HSDKKLTLVF EHCDQDLKKY FDSLNGEIDM

        101                                                 150
Human   EIVK--LF-- -K--GF---R ---------- -----R---- ----------
Rat     EIVK-LLF-- -K--GF---R ---------- -----R---- ----------
Bovine  EIVK--LF-- -K--GF---R ---------- -----R---- ----------
Fly     AVCRSFMLQL LRGLAFCHSH NVLHRDLKPQ NLLINKNGEL KLADFGLARA

        151                                                 200
Human   -----R---- ---------- ---------S ---------- -F----N---
Rat     -----R---- ---------- ---------S ---------- -F----N---
Bovine  -----R---- --------S- ---------S ---------- -F----N---
Fly     PGIPVKCYSA EVVTLWYRPP DVLFGAKLYT TSIDMWSAGC ILAELADAGR

        201                                                 250
Human   -----N--D- --KR---L-- --T-EQ--SM TK-P--KPY- MY--T--LVN
Rat     -----N--D- --KR---L-- --T-EQ--AM TK-P--KPY- MY--T--LVN
Bovine  -----N--D- --KR---L-- --T-EQ--AM TK-P--KPY- MY--T--LVN
Fly     PLFPGSDVLD QLMKIFRVLG TPNEDSWPGV SHLSDYVALP SFPAITSWSQ

        251                                                 294
Human   V--K--AT-- ----N--K-N -V------E- L-----S-FC PP.
Rat     V--K--AT-- ----N--K-N -V------E- L-----S-FC PP.
Bovine  V--K--AT-- ----N--K-N -V------E- L-----S-FC PP.
Fly     LVPRLNSKGR DLLQKLLICR PNQRISAEAA MQHPYFTDSS SSGH.
```

Fig. 3. Comparison of the putative amino acid sequence of *D. melanogaster Cdk5* to the known mammalian CDK5 gene products. The fly sequence has an overall identity of 77% with the human (Accession No. X66364), rat (Accession No. 102121) and bovine (Accession No. 104798) proteins.

of interest maps to the region covered by the deletion only one-half the width of the polytene chromosome will hybridize with the probe. To facilitate analysis, we also used heterozygous chromosomal inversions to asynapse this region of the chromosome. By comparing the hybridization of these chromosomes to the wild-type, we determined that the *Cdk5* gene is located between 52 A14 and C9.

4. Discussion

This paper describes the identification, primary structure, and gene localization of the *D. melanogaster* homolog of CDK5. CDK5 is a serine/threonine protein kinase that is structurally related to the family of cyclin-dependent protein kinases (CDKs) that play a pivitol role in controlling cell cycle progression. However, unlike other members of this family of kinases, mammalian CDK5 is selectively expressed as an active enzyme only in terminally differentiated (post-mitotic) neurons. This observation suggests that CDK5 may have a function that is unique to neurons and not related to the regulation of cell division.

Homologs of CDK1, mitotic cyclins, and other cell cycle related proteins such as cdc25 protein phosphatase (string) have been cloned in *Drosophila*. These proteins have been shown to share similar functions with regard to cell cycle progression within the fly [26]. We hypothesize that *Drosophila Cdk5* will also share a similar function with its mammalian counterpart. Presently, very little is known about the biological role of mammalian CDK5. In vitro phosphorylation experiments using CDK5 purified from bovine brain have demonstrated that it will phosphorylate the lysine-serine-proline repeats contained within the tail domain of the high and middle molecular weight neurofilament proteins [11]. Phosphorylation of this class of intermediated filaments is thought to be important to axon stabilization and possibly axonal-transport. Others have identified the neuronal microtubule-associated protein *tau* as a possible CDK5 substrate [27]. Hyper-phosphorylated *tau* is the main component of the pathological paired helical filaments observed in post-mortem biopsies of Alzheimer patients. Homologs of these substrates have not been identified in *Drosophila*. However, other members of the intermediate filament family may serve as substrates for *Cdk5* mediated phosphorylation.

Development of both the vertebrate and invertebrate nervous systems can be divided into a series of overlapping stages [28]: (i) induction and patterning of the neurogenic regions; (ii) birth and migration of neurons and glia; (iii) generation of specific cell fates; (iv) guidance of neuronal growth cones; (v) formation of synaptic connections; (vi) role of trophic factors in regulating cell death; (vii) competitive rearrangement of functional synapses, and (viii) continued synaptic plasticity throughout the life of the organism. *Drosophila* has been an important model for the elucidation of the molecular mechanisms regulating the progression of these stages. Particularly with regard to the early stages of neurogenesis and growth cone guidance, *Drosophila* and vertebrates use related molecules and mechanisms to construct the basic framework of the nervous system.

The identification, characterization, and mapping of *D. melangaster Cdk5* represent the foundation on which a more

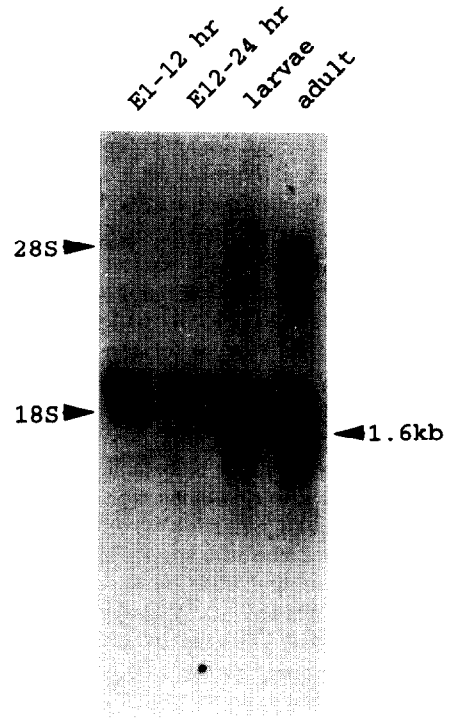


Fig. 4. The developmental expression of *D. melanogaster* CDK5 mRNA as revealed by Northern blot analysis. Autoradiography reveals a single band of hybridization of approximate 1.6 kb, in 1–12 h embryos (E1–12), larvae, and adult flies. 12–24 h embryos (E12–24) appear to express very little CDK5 message as detected by this method. Equal loading of RNA/lane was confirmed by ethidium bromide staining. The size of the hybridizing band was determined by comparison of its mobility to the mobility of the 28S (5 kb) and 18S (1.9 kb) ribosomal RNAs.

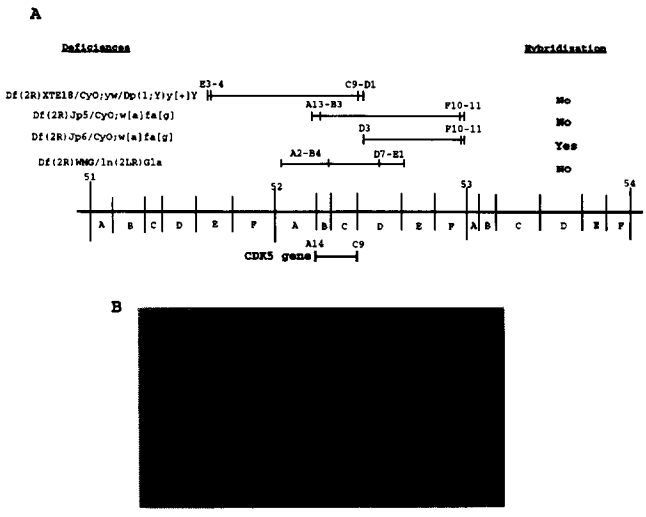


Fig. 5. (A) Diagram showing results of in situ hybridizations and the location of deficiences (Df) used to map the chromosomal location of *D. melanogaster Cdk5* gene. Df(2R)XTE18/CyO;yw/Dp(1;Y)y[+]Y, Df(2R)Jp5/CyO;w[a]fa[g], and Df(2R)WMG/ln(2LR0Gla) showed hybridization of a digoxigenin labelled *Cdk5* genomic DNA probe to only one-half the polytene chromosome indicating that *Cdk5* gene is located within the region covered by the deficiency. In contrast, Df(2R)Jp6/Cyo;w[a]fa[g] showed a single band of hybridization across the entire width of the polytene chromosome. The *Cdk5* gene was localized to region 52 between bands A14 and C9 on the right arm of chromosome 2. (B) In situ hybridization of the salivary gland polytene chromosomes from an *Ore-R* (wild-type) fly probed with a Digoxygenin labelled genomic DNA fragment.

comprehensive genetic investigation into the biological function of CDK5 can be built and its possible role in neuronal development or differentiation determined.

Acknowledgements: We wish to thank Drs. Peter Worland, Glenn Kroog and Richard Kahn for reviewing this manuscript, and Dr. Ravi Kamkadur for performing in situ hybridization experiments on whole mount embryos.

References

[1] Pines, J. (1993) Trends Biochem. Sci. 18,195–197.
[2] Murray, A.W., Solomon, M.J. and Kirschner, M.W. (1989) Nature 339, 280–286.
[3] Pines, J. and Hunter, T. (1989) Cell 58, 833–846.
[4] Solomon, M.J., Lee, T. and Kirschner, M.W. (1992) Mol. Biol. Cell 3, 13–27.
[5] Atherton-Fessler, S., Parker, L.L., Geahlen, R.L. and Piwnica-Worms, H. (1993) Mol. Cell. Biol. 13, 1675–1685.
[6] Pines, J. and Hunter, T. (1990) New Biol. 2, 389–401.
[7] Hanks, S.K., Quinn, A.M. and Hunter, T. (1988) Science 241, 42–52.
[8] Tsai, L.-H., Harlow, E. and Meyerson, M. (1991) Nature 353, 174–177.
[9] Meyerson, M., Enders, G.H., Wu, C.-L., Su, L.-K., Gorka, C., Nelson, C., Harlow, E. and Tsai, L.-H. (1992) EMBO J. 11, 2909–2917.
[10] Hellmich, M.R., Pant, H.C., Wada, E. and Battey, J.F. (1992) Proc. Natl. Acad. Sci. USA 89, 10867–10871.
[11] Lew, J., Winkfein, R.J., Paudel, H.K. and Wang, J.H. (1992) JJ. Biol. Chem. 267, 25922–25926.
[12] Xiong, Y., Zhang, H. and Beach, D. (1992) Cell 71, 505–514.
[13] Wang, J., Chevenisse, X., Henglein, B. and Brechot, C. (1990) Nature 343, 555–557.
[14] Swenson, K.I., Farrell, K.M. and Ruderman, J.V. (1986) Cell 47, 861–870.
[15] Koff, A., Cross, F., Fisher, A., Schumacher, J., Leguellec, K., Philippe, M. and Roberts, J.M. (1991) Cell 66, 1217–1228.
[16] Lew, D.J., Dulic', V. and Reed, S.I. (1991) Cell 66, 1197–1206.
[17] Tamura, K., Kanaoka, Y., Jinno, S., Nagata, A., Ogiso, Y., Shimizu, K., Hayakawa, T., Nojima, H. and Okayama, H. (1993) Oncogene 8, 2113–2118.
[18] Fisher, R.P. and Morgan, D.O. (1994) Cell 78, 713–724.
[19] van den Heuvel, S. and Harlow, E. (1993) Science 262, 2050–2054.
[20] Meyerson, M. and Harlow, E. (1994) Mol. Cell. Biol. 14, 2077–2086.
[21] Tsai, L.-H., Takahashi, T., Caviness Jr., V.S. and Harlow, E. (1993) Development 119, 1029–1040.
[22] Lew, J., Beaudette, K., Litwin, C.M.E. and Wang, J.H. (1992) J. Biol. Chem. 267, 13383–13390.
[23] Davis, L., Kuehl, M. and Battey, J. (1994) Basic Methods in Molecular Biology, 2nd Edn., Appleton & Lange, Norwalk, Connecticut.
[24] Frohman, M.A., Dush, M.K. and Martin, G.R. (1988) Proc. Natl. Acad. Sci. USA 85, 8998–9002.
[25] Engels, W.R., Preston, C.R., Thompson, P. and Eggleston, W.B. (1986) Focus 8, 6–8.
[26] Edgar, B.A., Sprenger, F., Duronio, R.J., Leopold, P. and O'Farrell, P.H. (1994) Genes Dev. 8, 440–452.
[27] Baumann, K., Mandelkow, E.-M., Biernat, J., Piwnica-Worms, H. and Mandelkow, E. (1993) FEBS Lett. 336, 417–424.
[28] Goodman, C.S. and Doe, C.Q. (1993) in: The Development of *Drosophila melanogaster*, pp. 1133–1205, Cold Spring Harbor Press, Cold Spring Harbor, NY.