A Role for Cyclin J in the Rapid Nuclear Division Cycles of Early Drosophila Embryogenesis

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The nuclear division cycles of early Drosophila embryogenesis have a number of unique features that distinguish them from later cell cycles. These features include the lack of some checkpoints that operate in later cell cycles, the absence of gap phases, and very rapid DNA synthesis phases. The molecular mechanisms that control these rapid nuclear division cycles are poorly understood. Here we describe analysis of cyclin J, a previously uncharacterized cyclin which has an RNA expression pattern that suggests a possible role in early embryogenesis. We show that the cyclin J protein is present in early embryos where it forms active kinase complexes with cyclin-dependent kinase (Cdk) 2. To determine whether cyclin J plays a role in controlling the early nuclear cycles we isolated peptide aptamers that specifically bind to cyclin J and inhibit its ability to activate Cdns. We injected the inhibitory aptamers into syncytial Drosophila embryos and demonstrated that they caused defects in chromosome segregation and progression through mitosis. We obtained similar results by injecting cyclin J antibodies into embryos. Our results suggest that a cyclin J-associated kinase activity is required for the early embryonic division cycles. © 2000 Academic Press

Key Words: peptide aptamer; cell cycle; cyclin; Cdk; Drosophila; early embryogenesis; perturbagen.

INTRODUCTION

In Drosophila, as in other multicellular organisms, cell division is controlled by a highly conserved family of proteins called cyclin-dependent kinases (Cdns) and their regulatory subunits, the cyclins (reviewed in Morgan, 1995; Edgar and Lehner, 1996). Orderly progression through a typical somatic cell cycle requires the cyclic activity of several distinct Cdk/cyclin complexes. The transition from the G2 phase to mitosis (M) requires active complexes consisting of Cdk1 and one of the mitotic cyclins, the A- or B-type cyclins, which accumulate during interphase (Lehner and O'Farrell, 1990; Knoblich and Lehner, 1993; Jacobs et al., 1998). Exit from M phase requires the loss of Cdk1 activity that results from sequential proteolytic degradation of the mitotic cyclins (Dawson et al., 1995; Sigrist et al., 1995). Entry into the DNA synthesis (S) phase requires the activity of Cdk2, which forms complexes with cyclin E in late G1 phase (Knoblich et al., 1994). A number of other Drosophila Cdns and cyclins have been identified but their roles in regulation of the cell cycle have not been determined (Lahue et al., 1991; Leopold and O'Farrell, 1991; Finley et al., 1996; Sauer et al., 1996). Some of these uncharacterized Cdk/cyclin complexes may contribute to cell cycle regulation in specific developmental contexts. In most multicellular organisms the cell division cycles during early embryogenesis have unique properties that distinguish them from the cell cycles in later development. This is particularly evident in Drosophila, in which the initial 13 nuclear division cycles of embryogenesis take place in near synchrony within a common cytoplasm, or syncytium (reviewed in Foe et al., 1993). These cycles consist of alternating S and M phases, without the intervening gap phases that appear later in development. The nuclear cycles are also much faster than the typical somatic cell cycle; cycles 2–9 last as little as 8 min each and the entire genome is duplicated in as little as 5 min (Spradling and Orr-Weaver, 1987). Finally, the first 10 divisions appear to lack the checkpoint which in later cell cycles blocks entry into M phase in the presence of damaged or unreplicated DNA (Togashi and Okada, 1984; Raff and Glover, 1988; Sullivan et al., 1993). The molecular mechanisms that control the embryonic nuclear division cycles and that account for their unique features are largely unknown.

The syncytial embryo is supplied with maternal stores of the same Cdns and cyclins that are known to regulate the
typical cell cycles later in development. For example, Cdk1 and Cdk2 are both abundant in the early embryo, as are cyclins A, B, B3, and E (Lehner and O'Farrell, 1989; Whitfield et al., 1990; Richardson et al., 1993; Edgar et al., 1994; Sauer et al., 1995; Jacobs et al., 1998). Maternal Cdk1 and cyclin B have been shown to be required for the early embryonic cycles (Stern et al., 1993; Stiftler et al., 1999), whereas a requirement for the other Cdns and cyclins has not yet been demonstrated. At least some of the unique features of the nuclear division cycles may be mediated by novel regulation of these cell cycle proteins. For example, in contrast to later cycles, the overall levels of cyclin A and cyclin B do not oscillate during the early nuclear division cycles (Edgar et al., 1994). Instead, exit from mitosis coincides with localized degradation of mitotic cyclins and localized Cdk1 inactivation along the chromosomes (Su et al., 1998). Here we show that in addition to this embryo-specific regulation of known Cdns and cyclins, the early embryo contains a novel cyclin, cyclin J, which may contribute to some of the unique features of the nuclear division cycles.

Cyclin J was previously identified in a yeast two-hybrid screen for embryonic proteins that interact with Cdk2 (Finley et al., 1996). The amino acid sequence of cyclin J is most similar to that of the A-type cyclins, but lacks the destruction box and other consensus motifs found in all mitotic cyclins. Cyclin J mRNA is present in the embryo prior to cellularization but, in contrast with other cyclins, mitotic cyclins. Cyclin J mRNA is present in the embryo most similar to that of the A-type cyclins, but lacks the screen for embryonic proteins that interact with Cdk2.

Affinity Purification

cycJ cDNA was PCR-amplified from pJG4-5-Cdi5 (Finley et al., 1996) using primers 5′-GGAGAGATCTGACGACTGATTATTACC-3′ and BCO2 (Finley and Brent, 1995). The PCR product was cut with EcoRI and Xhol and inserted into pMLAL-c2 (NEB) for expression of MBP fusions, pGEX2 for GST fusions, or pET28a (Novagen) for His6 fusions. An EcoRI–Xhol Dacapo (Dap) fragment encoding amino acids 21 to 245 of Dap from pJG4-5-Cdi4 (Finley and Brent, 1994) was expressed from pMAL-c2 (NEB). EcoRI–SalI fragments encoding trxA-fused [1] J2, pepC, or DA2 were isolated from pJM-1 (Colas et al., 1996) and inserted into pET28a. MBP and GST fusions were expressed in Sure Escherichia coli (Strategane) and affinity-purified using amylase resin (NEB) or glutathione-Sepharose 4B (Pharmacia), respectively. His6 fusions were expressed in BL21/DE3 E. coli (Novagen) and affinity-purified using Ni-NTA agarose (Qiagen). Rabbit polyclonal cyclin J antibodies were affinity-purified using His6-CycJ immobilized on Ni-NTA agarose (Qiagen) or GST-CycJ immobilized on Affi-Gel 15 (Bio-Rad). Purified peptides and antibodies were concentrated and buffer-equilibrated using Centricon filters (Amicon).

ImmunobLOTS, Immunoprecipitations, and Histone H1 Kinase Assays

Cyclin J was immunodetected with mouse polyclonal or affinity-purified rabbit polyclonal antibodies raised against MBP-CycJc. Dechorionated embryos, larvae, or adult flies were Dounce-homogenized in lysis buffer (150 mM NaCl, 1% NP-40, 10% glycerol, 50 mM Tris–HCl, pH 8.0) containing 2.5 mM EDTA, 0.02% NaN3, 20 mM NaF, 0.3 mM Na2VO4, 1 mM PMSF, 1 μM/ml pepstatin A, 1 μM/ml aprotinin, 10 μM/ml chymostatin, 2 μM/ml leupeptin, 0.1 mg/ml TPCK, and 10 μM/ml soybean trypsin inhibitor. Extracts with approximately 20 mg/ml protein were cleared by centrifugation and frozen at −80°C until use. For each immunoprecipitation, 100 μl of protein A–Sepharose 6MB beads (Pharmacia) diluted 10-fold in lysis buffer was coated for 1 h at 4°C with 3 μg of affinity-purified cyclin J antibodies. For control experiments, protein A– or protein G–agarose (Bio-Rad) beads were coated with a similar amount of polyclonal cyclin B antibodies, Rb271 (gift from D. Glover) or polyclonal rabbit cyclin E antibodies (gift from H. Richardson), respectively. Beads were rocked for 1 h at 4°C with 5 mg of protein extract and then washed three times with 0.5 ml of lysis buffer. For competition experiments, immunoprecipitations were performed in the presence of 2.5 μM MBP-CycJc or MBP-PepC2. For immunoblots, beads were boiled for 5 min in 30 μl of SDS sample buffer, cleared by centrifugation, and resolved on a 10% SDS–PAGE gel. Immunoblotting was performed using Hybond-P membranes and ECL Plus detection kit (Amersham). All Cyc antibodies, as well as cyclin B antibodies Rb271, were used at 1:2000 dilution. Affinity-purified rabbit antibodies against Dro-
sophila Cdk1 and Cdk2 (gift from C. Lehner) were each used at 1:1000 dilution. For H1 kinase assays, beads with immunoprecipitates were each washed with 1 ml of 1× kinase buffer (50 mM Heps, pH 7.5, 10 mM MgCl2, 1 mM DTT) and then incubated for 30 min at 4°C in the presence or absence of 5 μl of a purified peptide, NBP-Dap, or buffer. The kinase reaction was initiated by addition of 10 μl of 1× kinase buffer containing 200 μg/ml histone H1 (Sigma), 60 μM ATP, and 0.2 mM [γ-32P]ATP. After 30 min of incubation at 25°C, 10 μl of SDS sample buffer was added; the samples were boiled for 5 min and run on 12% SDS-PAGE for subsequent autoradiography.

**Immunostaining**

Dechorionated embryos were fixed for 20 min in a two-phase mixture of equal volumes of heptane and PEMS (100 mM Pipes, pH 6.9, 1 mM MgCl2, 1 mM EGTA) + 3.7% formaldehyde. Devitellinization was performed by replacing the aqueous phase with methanol and shaking for 30 s. Devitellinized embryos were washed for 10 min with methanol and twice for 10 min with PBS + 0.2% saponin, blocked for 1 h at room temperature in PBS + 0.2% saponin + 10% normal goat serum, and incubated with primary antibodies in PBS + 0.2% saponin overnight at 4°C. Primary antibodies were washed off with PBS + 0.2% saponin three times for 10 min and secondary antibodies were applied for 1 h at 25°C. Finally, embryos were washed with PBS + 0.2% saponin three times for 10 min and stained for DNA in 1 μg/ml Hoechst 33258 for 10 min. Samples were mounted in Fluoromount G (immunofluorescence) or Aquamount (immunohistochemistry), and digital images were captured using a Nikon Optiphoto EFD-3 microscope and Photometrix Sensys digital camera setup. Cyclin J was detected using affinity-purified rabbit CycJ antibodies diluted 1:100. His6-fused peptides were detected with a monoclonal antibody against the T7 tag (Novagen) at 1:100 dilution. Secondary Texas red-conjugated goat anti-mouse, FITC-conjugated goat anti-rabbit (Jackson Laboratories), and HRP-conjugated goat anti-rabbit antibodies (Bio-Rad) were each used at 1:250 dilution.

**Yeast Manipulations**

Protein interaction assays and interactor hunts were performed using the version of the yeast two-hybrid system (Fields and Stern, 1994) developed by Brent and colleagues (Golemis et al., 2000). For additional details see http://cmmg.biosci.wayne.edu/rfinley/ lab.html. LexA-cyclin J (Finley and Brent, 1994) was used to screen a Drosophila ovario cDNA library RFLY3 (Finley et al., 1996). Yeast transformants (7.7 × 10^6) were screened and 40 galactose-dependent positives were isolated, 6 of which were Cdk2, 13 of which were Cdk1 lacking the N-terminal 17 amino acids, 1 of which was Scf, 2 of which were Arl2, 17 of which were S5a, and 1 of which was a novel protein named Dm5-306. To isolate cyclin J protein interactors, a random peptide library pPeAETRX (Colas et al., 1996) was screened by interaction mating as described (Kolonin and Finley, 1998). From 2.4 × 10^6 yeast transformants, 29 galactose-dependent positives were isolated, 7 of which activated the reporters better than any of the Drosophila cyclin J-interacting proteins. The specificity of all interactors was tested with the interaction mating assay (Finley and Brent, 1994). LexA-cyclin D and LexA-Cln3 bait plasmids have been described previously (Finley and Brent, 1994). LexA-cyclin C and LexA-cyclin A bait plasmids were constructed by inserting an EcoRI-XhoI fragment containing Drosophila cyclin C CDNA or an EcoRI-BamHI fragment containing Drosophila cyclin A CDNA into the corresponding sites of pEG202 or pG11da (Golemis et al., 2000), respectively. Complementation was performed as described (Finley et al., 1996). Drosophila cyclins were expressed from pRF80, a URA3, 2 μm vector containing a translational start signal flanked by an ADH1 promoter/terminator expression cassette (R.L.F., unpublished). TrxA-fused peptides were expressed from pJM-1 (Colas et al., 1996). Cyclin J aptamers had the following sequences: JA1, TLKHSSGWITAWLEMAVEGA; JA2, LVWRPLGSEGFLNLL-HRG; and JA3, CVRHIWVMGLCCRYLFAG.

**Embro Injection**

Dechorionated embryos were aligned on the edge of a glass slide coated with heptane-based glue for 10 min, covered with polycarbonate, and injected with peptides, affinity-purified antibodies, or preimmune serum in 5 mM NaCl, 0.1 mM NaHPO4, (pH 7.7) at a concentration of 2.5 mg/ml. The injected volume was estimated to be 1–5% of the total embryo volume. Injected embryos were aged at 18°C for 0–20 min (peptides) and 0–30 min (affinity-purified antibodies and serum). Embryos were then released in heptane and fixed with gentle rotation for 5 min at the interphase of equal volumes of heptane and 37% formaldehyde. Fixed embryos were spread on a Nitex mesh, washed with PBS, and attached to double-stick tape for manual devitellinization and subsequent immunostaining.

**RESULTS**

**Cyclin J Protein Is Present in Early Embryos**

To determine whether cyclin J protein is present during early embryogenesis we used rabbit antibodies raised against the unique C-terminal 100 amino acids of cyclin J (CycJc). Affinity-purified cyclin J antibodies recognized a single protein of the appropriate molecular weight (~50 kDa) in immunoblots from early embryos (Fig. 1a). The abundance of this protein diminished during embryogenesis, yet could be dramatically induced in older embryos that carried a heat-shock-inducible cyclin J transgene (hs > CycJ) (Fig. 1a). The cyclin J protein was abundant in unfertilized eggs and in embryos 0–1.5 h after egg deposition (AED), but diminished to a low level by 3 h AED (Fig. 1a). A similar pattern was observed by immunoprecipitation of proteins from embryo extracts using the affinity-purified rabbit cyclin J antibodies and probing immunoblots using a mouse antisera raised against CycJc; cyclin J protein was detected in unfertilized and 0- to 3-h-old embryos, but could not be detected in older embryos (Fig. 1b). Under the conditions used to stage these embryos, cellularization is completed between 2 and 3 h AED. Thus, cyclin J is most abundant in the syncytiotrophoblast embryo. This pattern is in contrast to the expression profile of cyclin B, which is abundant throughout embryogenesis (Fig. 1a), and of cyclins A, B3, and E, which are expressed in a temporal pattern similar to that of cyclin B (Lehner and O’Farrell, 1989; Whitfield et al., 1990; Richardson et al., 1993; Jacobs et al., 1998). Consistent with the immunoblots, the cyclin J antibodies strongly stained wild-type embryos undergoing the first 10
divisions (Fig. 2a). Starting at late syncytial stages embryo staining decreased to a background level (Figs. 2b–2d; data not shown). Cyclin J staining could be induced in later embryos that contained the hs > CycJ transgene (Fig. 2e) or in a striped pattern in embryos expressing CycJ under control of the prd promoter (data not shown). We observed no obvious subcellular localization in the early embryos or hs > CycJ embryos. In adult Drosophila cyclin J staining was strong in the cytoplasm of the ovarian nurse cells, but was not detected in testes in males (data not shown). Combined, our data suggest that cyclin J protein is deposited into eggs maternally and is degraded during early embryogenesis.

**FIG. 1.** Cyclin J protein is present in early embryos. (a) 10 μg of protein from the indicated stages was loaded per lane and immunoblotted with affinity-purified rabbit cyclin J antibodies or cyclin B antibodies. The cyclin J band migrating at an apparent molecular weight of 50 kDa (arrowhead) is detectable in wild-type females, unfertilized embryos, and embryos of the indicated ages and is induced in heat-shocked 3- to 20-h-old hsp70-GAL4/UAS-cycJ embryos (hs > J). Cyclin J is not detectable in wild-type males or in an equal mixture of L1–L3 stage larvae (L1–L3). (b) Protein complexes were immunoprecipitated with the affinity-purified rabbit cyclin J antibodies from embryos of the indicated ages and immunoblotted with mouse polyclonal cyclin J antibodies. The cross-reacting IgG band is indicated with an asterisk.

**FIG. 2.** Immunohistochemical detection of cyclin J in embryos. Staining with affinity-purified cyclin J antibodies in wild-type embryos (a–c) or in heat-shocked stage 12 hsp70-GAL4/UAS-lacZ (d) or hsp70-GAL4/UAS-cycJ (e) embryos. The wild-type embryos are at stage 2 during the first nuclear divisions (a), at stage 5 during cellularization (b), and at stage 11 during mitosis 16 in the epidermis (c). Staining (dark) was detected with HRP-conjugated secondary antibodies.
Cyclin J Interacts with Cdk2 in Early Embryos

To identify proteins that may form complexes with cyclin J, we used the two-hybrid system to screen two Drosophila cDNA libraries, one from ovaries and one from embryos (Fig. 3a). In addition to Cdk2, we isolated three other cyclin J-interacting proteins from the libraries. We isolated full-length versions of the DNA supercoiling factor Sce (Kobayashi et al., 1998) and of a putative GT Pase, Arl2 (Clark et al., 1993), and the carboxy-terminal 371 amino acids of SSA, the non-ATPase subunit of the 26S proteasome (Haracska and Udvardy, 1995). Each of these proteins, as well as the Cdk subunit Cks1, which previously had been shown to bind to cyclin J (Finley and Brent, 1994), interacted specifically with cyclin J but not with a panel of unrelated proteins in the two-hybrid assay (Fig. 3a; data not shown). We did not isolate any full-length Cdk5 other than Cdk2. Moreover, in direct tests, cyclin J failed to interact with other Cdns, including Cdk1 (Fig. 3a), Cdk4, and Cdk5 (data not shown). These results suggested that Cdk2 may be the kinase partner for cyclin J.

To determine whether Cdk2 interacts with cyclin J in Drosophila, we used affinity-purified cyclin J antibodies to precipitate cyclin J complexes from embryo extracts. We subjected the precipitates to immunoblotting with Cdk1 or Cdk2 antibodies (Fig. 3b). Consistent with the two-hybrid interactions, Cdk2 and cyclin J coimmunoprecipitated from unfertilized eggs and syncytial embryos. The cyclin J complexes contained both of the previously described phosphosforms (Sauer et al., 1995) of Cdk2 (Fig. 3b). We did not detect coimmunoprecipitation of cyclin J and Cdk2 from older embryos, unless the embryos ectopically expressed cyclin J from the hs > Cyp transgene (Fig. 3b). Cdk1 was not detected in complexes with cyclin J in early embryos. However, we did detect Cdk1 in cyclin J immunoprecipitates from unfertilized eggs and from older hs > Cyp embryos (Fig. 3b). Combined, these results suggest that cyclin J can bind both Cdk1 and Cdk2 in vivo and that Cdk2 is the principal kinase subunit for cyclin J in the syncytial embryo.

Early Embryos Contain a Cyclin J-Associated Kinase Activity

To determine whether cyclin J is associated with an active kinase in embryos we tested cyclin J immunoprecipitates for their ability to phosphorylate histone H1. We detected cyclin J-associated H1 kinase activity in newly laid unfertilized eggs and embryos 0–4 h AED (Fig. 4a). The activity diminished between 3 and 6 h and after 6 h reached the background level that can be immunoprecipitated with preimmune serum (Figs. 4a and 4b; data not shown). Precipitation of the cyclin J-associated kinase activity from both syncytial embryos and later embryos that ectopically expressed cyclin J was blocked by Cycjc peptide, but not by a control peptide, PepC2 (Fig. 4b). In contrast, Cycjc did not block immunoprecipitation of cyclin B-associated H1 kinase activity or the background activity (Fig. 4b). These data, combined with the results from coimmunoprecipitations and two-hybrid interaction assays, suggest that early embryos contain active Cdk2/cyclin J complexes which are inactivated around the time of cellularization by degradation of cyclin J.

Isolation of Peptide Aptamers to Specifically Inhibit Cyclin J in Vivo

A useful approach to studying the early embryonic division cycles has been to inject embryos with antibodies or small molecules that specifically inhibit maternally deposited proteins (Raff and Glover, 1988; Schubiger and Edgar, 1994; Su et al., 1997, 1998). To investigate the function of cyclin J in early development, we set out to isolate peptides that could be injected into living embryos to interfere with cyclin J function. First we identified peptide aptamers that bind specifically to cyclin J. We screened a yeast two-hybrid library that expresses random 20 amino acid peptides on the surface of a platform molecule, E. coli thioredoxin (trxA) (Colas et al., 1996), and isolated a number of trxA-fused peptides that interacted with cyclin J. Several of the cyclin J-interacting peptides (JA1, JA2, and JA3) interacted specifically with cyclin J but not with Drosophila Cdns A, D, or C (Fig. 3a) or cyclins E or H (data not shown). A control trxA-fused peptide, PepC2 (Colas et al., 1996), or a peptide aptamer specific for cyclin D, DA2, did not interact with cyclin J (Fig. 3a). The sequences of the cyclin J aptamers shared no strong similarity with each other or with previously identified proteins (see Materials and Methods).

To determine whether the cyclin J peptide aptamers could interfere with cyclin J function, we tested their ability to inhibit cyclin J-associated kinase activity. We immunoprecipitated cyclin complexes from embryo extracts with affinity-purified cyclin J antibodies and assayed the H1 kinase activity of the complexes in the presence or absence of purified trxA-fused peptides. In these experiments the peptides were incubated with the complexes after immunoprecipitation. As shown in Fig. 5, the cyclin J-associated kinase activity in the precipitates was inhibited by aptamer JA1 and JA2 at peptide concentrations of 1 to 10 μM, but not by the control peptide, pepC2. JA1 and JA2 inhibited the cyclin J kinase immunoprecipitated from syncytial embryos (Fig. 5a) and from later embryos that ectopically expressed cyclin J (Fig. 5b); the later extracts may contain cyclin J in complexes with Cdk1 in addition to Cdk2 (Fig. 3b). In contrast, the cyclin J aptamers did not inhibit cyclin B- or cyclin E-associated kinase activities (Fig. 5b). To compare the effects of JA1 and JA2 with those of known Cdk inhibitors, we tested cyclin J- and E-associated kinases for inhibition by a Cdk2 inhibitor Dap. As reported previously (de Nooij et al., 1996; Lane et al., 1996), Dap efficiently inhibited cyclin E-associated kinase activity (Fig. 5c). Dap also inhibited the cyclin J-associated kinase with half-maximal inhibition at 1–3 μM (Fig. 5c). These results indicate that the cyclin J aptamers inhibit
Cyclin J kinase with efficiencies comparable to that of a natural Cdk inhibitor.

We used a functional assay in yeast to test whether the cyclin J aptamers could inhibit cyclin J in living cells (Fig. 6). Like many other cyclins, Drosophila cyclin J is able to complement cln2 mutant yeast which lack the three yeast G1 cyclins, CLN1, CLN2, and CLN3 (Finley et al., 1996). We expressed trxA-fused JA1, JA2, or pepC2 in strains of cln2 yeast that depended on a Drosophila cyclin for growth. The cyclin J peptide aptamers dramatically inhibited the growth of cyclin J-dependent yeast (Fig. 6). In contrast, neither aptamer interfered with growth of strains dependent on Drosophila cyclin E (data not shown), indicating that the inhibition was specific for cyclin J kinase with efficiencies comparable to that of a natural Cdk inhibitor.

We used a functional assay in yeast to test whether the cyclin J aptamers could inhibit cyclin J in living cells (Fig. 6). Like many other cyclins, Drosophila cyclin J is able to complement cln− mutant yeast which lack the three yeast G1 cyclins, CLN1, CLN2, and CLN3 (Finley et al., 1996). We expressed trxA-fused JA1, JA2, or pepC2 in strains of cln− yeast that depended on a Drosophila cyclin for growth. The cyclin J peptide aptamers dramatically inhibited the growth of cyclin J-dependent yeast (Fig. 6). In contrast, neither aptamer interfered with growth of strains dependent on Drosophila cyclin E (Fig. 6) or cyclin D (data not shown), indicating that the inhibition was specific for
Cyclin J in Early Drosophila Embryogenesis

The observed functional inhibition of cyclin J by the aptamers may result from the aptamer blocking the cyclin J–Cdk interaction, as suggested by two-hybrid experiments for JA1 (data not shown). Alternatively, an aptamer may inhibit kinase activity by another mechanism, for example, by blocking access to the substrate. Combined, our results indicate that JA1 and JA2 are efficient and specific inhibitors of cyclin J function. Both aptamers bind to cyclin J and not to other cyclins, both specifically inhibit cyclin J-associated kinase activity from embryo extracts, and both are able to specifically inhibit cyclin J function in a yeast assay.

Cyclin J Aptamers and Antibodies Inhibit the Syncytial Cell Cycles

To determine the effects of inhibiting cyclin J function in vivo, we injected cyclin J aptamers or affinity-purified cyclin J antibodies into syncytial wild-type Drosophila embryos. We chose to inject purified peptides fused to the trxA platform because previous results had suggested that trxA peptides have higher affinity for their targets than the peptides alone (Cohen et al., 1998). We previously showed that expression of trxA-fused control peptides in Drosophila does not cause overt phenotypes during embryogenesis and later development (Kolonin and Finley, 1998). After injection with the aptamers or antibodies, embryos were incubated for variable times before fixation to allow nuclei to proceed through additional cycles. Localization of the injected peptides or antibodies was determined by immunofluorescence. For each injected agent we stained approximately 200 embryos with a DNA-staining reagent, Hoechst 33258, to look for cell cycle defects that colocalize with the injection site.
As shown for representative embryos in Fig. 7, injection of the cyclin J aptamers or antibodies resulted in profound effects on the syncytial cycles. Telophase chromatin bridges near the injection site were induced at high frequency by both the aptamers and the cyclin J antibodies (Figs. 7d, 7f, 7h, and 7j). In addition to the chromosome segregation defects, the cyclin J aptamers and antibodies caused an apparent delay in progression through mitosis. In many of the embryos that displayed localized staining for the aptamer or antibody, nuclei proximal to the injection site were at an early mitotic stage, whereas nuclei distal to the injection site had proceeded to progressively later stages (e.g., Fig. 7f). The gradient of mitotic progression and the higher frequency of chromatin bridges near the injection site suggest that the effect of the cyclin J inhibitors is dosage-dependent. Injection of two control trxA-fused peptides that do not interact with cyclin J (PepC2 and cyclin D aptamer, DA2), or of preimmune serum, did not significantly interfere with the nuclear cycles (Figs. 7a and 7b; Table 1).

Injected trxA-fused peptides rapidly diffused throughout the embryo and were no longer localized to the injection site within ~20 min after injection (data not shown). Consistent with this, we observed local cell cycle defects only when embryos were fixed within 10 min of peptide injection. To quantify the effects on cell cycle progression in embryos fixed later than 10 min after injection, we scored the cell cycle stages and the frequencies of chromatin bridges among all injected embryos (Table 1). Cyclin J aptamers and antibodies each caused chromatin bridges even in the embryos that did not display localized defects. For example, while only 3–7% of the embryos injected with the control peptides or preimmune serum had chromatin bridges; this frequency was increased to 24% (at least threefold) by JA1 and to 16% (two- to fivefold) by JA2 (Table 1). Forty-one percent of the embryos injected with cyclin J antibodies had chromatin bridges, sixfold over the control injections (Table 1). The cyclin J aptamers and antibodies also significantly increased the overall frequency of embryos with nuclei in mitosis (Table 1). For example, the frequency of embryos with nuclei undergoing mitosis increased from approximately 54% for the control peptides to 78% for each cyclin J aptamer. The injected cyclin J antibodies had a similar effect. In contrast, the frequencies of each cell cycle stage in embryos injected with the control peptides or serum were not significantly different from those observed for uninjected wild-type embryos (Table 1) (Edgar et al., 1994). These results suggest that a cyclin J-associated kinase activity is required for normal nuclear divisions during early embryonic development.

DISCUSSION

Peptide Aptamers to Study Protein Function
in Early Embryos

The mechanisms that regulate the earliest division cycles of Drosophila embryogenesis are poorly characterized. This is in part because of the technical challenges involved in studying the maternally encoded cell cycle regulators that drive these cycles. Loss-of-function mutations in the genes encoding these regulators are often difficult to study in the early embryo because they have essential functions at other stages of development. An effective alternative approach to study these cycles is to inject embryos with small molecules or antibodies that inactivate specific proteins (Schubiger and Edgar, 1994; Su et al., 1997). Here we applied a two-hybrid approach to isolate peptide aptamers that specifically inhibit cyclin J, a putative cell cycle regulator found in the early embryo. The phenotypes induced by injection of the cyclin J aptamers were specific: they were not observed with control peptides, and they could be phenocopied by injecting antibodies specific for cyclin J. The specificity was further supported by the fact that no abnormal phenotypes were detected upon expression of the cyclin J aptamers from heat-shock-inducible transgenes at later stages of development when cyclin J is not present (data not shown). In contrast, aptamers directed at Cdk1 or Cdk2 cause predicted developmental defects when expressed from transgenes later in development (Kolonin and Finley, 1998). The specificity of peptide aptamers suggests that they may be used to target proteins in vivo in much the same way antibodies have been used. For example, injection of inhibitory antibodies has been a useful approach to probe protein function, particularly in systems in which analysis of loss-of-function mutations is difficult or impractical, such as in cultured vertebrate cells and vertebrate and invertebrate embryos. Compared with antibodies, however, peptide aptamers are relatively easy to isolate and characterize. Moreover, the two-hybrid system also provides an assay to identify peptide aptamers that disrupt specific protein–protein interactions (Caponigro et al., 1998; Fabbrizio et al., 1999; Geyer et al., 1999; Norman et al., 1999; Kolonin et al., 2000). This strategy may be useful for thorough characterization of other proteins involved in multiple protein interactions in the early embryo.

Cyclin J Is Required for the Rapid Nuclear Cycles
in the Early Embryo

The division cycles of early Drosophila embryogenesis normally lack gap phases, have very rapid S phases, and lack some of the checkpoint controls found in later cell cycles (reviewed in Foe et al., 1993). In later cell cycles specific Cdk/cyclin complexes are known to control the length of gap phases, S phase, and various checkpoints. Thus, the unique features of the early cycles are likely to be mediated either by novel regulation of the Cdns and cyclins that also operate at other times during development or by embryo-specific Cdns or cyclins. Here we have shown that cyclin J and an associated kinase activity are present predominantly during early embryogenesis. This is in contrast to other known cyclins that are active throughout embryogenesis (Lehner and O’Farrell, 1989; Whitfield et al., 1990; Richardson et al., 1993; Jacobs et al., 1998). Thus, cyclin J is a
FIG. 7. Cyclin J aptamers and antibodies inhibit the syncytial cell cycles. Embryos were injected with control cyclin D aptamer DA2 (a, b, i), cyclin J aptamers JA1 (c, d, j) or JA2 (e, f), or affinity-purified cyclin J antibodies (g, h). After 10 min to allow progression of the nuclei through the cell cycle, the embryos were fixed and stained for trxA-fused peptides (a, c, e), IgGs (g), or DNA (b, d, f, h–j). The injection site is on the left in all embryos. Local concentration of the injected agent is visible (a, c, e, g). DNA staining reveals chromatin bridges (arrowheads) induced in embryos injected with JA1 (d) and, at higher magnification in (j), JA2 (f) and cyclin J antibodies (h).
TABLE 1
Cell Cycle Phenotypes of Injected Embryos

<table>
<thead>
<tr>
<th>Injection</th>
<th>No. scored</th>
<th>% bridges</th>
<th>% I</th>
<th>% P</th>
<th>% M</th>
<th>% A</th>
<th>% T</th>
<th>% mitosis</th>
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<tbody>
<tr>
<td>PepC2</td>
<td>175</td>
<td>6 ± 4</td>
<td>50 ± 3</td>
<td>3 ± 2</td>
<td>28 ± 8</td>
<td>5 ± 3</td>
<td>14 ± 3</td>
<td>50 ± 3</td>
</tr>
<tr>
<td>DA2</td>
<td>176</td>
<td>3 ± 1</td>
<td>43 ± 4</td>
<td>9 ± 3</td>
<td>31 ± 8</td>
<td>6 ± 2</td>
<td>11 ± 4</td>
<td>57 ± 4</td>
</tr>
<tr>
<td>Preimmune</td>
<td>220</td>
<td>7 ± 5</td>
<td>47 ± 15</td>
<td>8 ± 1</td>
<td>23 ± 10</td>
<td>4 ± 1</td>
<td>18 ± 4</td>
<td>53 ± 15</td>
</tr>
<tr>
<td>JA1</td>
<td>262</td>
<td>24 ± 11</td>
<td>22 ± 6</td>
<td>13 ± 6</td>
<td>41 ± 3</td>
<td>8 ± 2</td>
<td>16 ± 7</td>
<td>8 ± 7</td>
</tr>
<tr>
<td>JA2</td>
<td>212</td>
<td>16 ± 9</td>
<td>22 ± 6</td>
<td>10 ± 1</td>
<td>45 ± 3</td>
<td>5 ± 2</td>
<td>18 ± 7</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>Anti-CycJ</td>
<td>217</td>
<td>41 ± 12</td>
<td>19 ± 10</td>
<td>28 ± 13</td>
<td>22 ± 15</td>
<td>8 ± 1</td>
<td>23 ± 7</td>
<td>81 ± 10</td>
</tr>
</tbody>
</table>

Note. Embryos in cycle 5–11 were injected with control peptides PepC2 or DA2, aptamer JA1 or JA2, preimmune rabbit serum, or affinity-purified cyclin J antibodies (anti-CycJ). After 0–30 min embryos were fixed and analyzed. Embryos that stained positively for the J-associated kinase activity resulted in a delay in progression through mitosis and abnormal chromosome segregation. Nuclei entered mitosis with chromosomes remaining unresolved, leading to telophase daughter nuclei connected by chromatin bridges. A similar phenotype has been observed upon injection of agents that inhibit proteins required for DNA synthesis such as DNA polymerase α (Raff and Glover, 1988) and MCM proteins (Su et al., 1997), but not by inhibitors of transcription, protein synthesis, or microtubule dynamics (reviewed in Schubiger and Edgar, 1994). Telophase chromatin bridges arise presumably because Drosophila embryos lack the checkpoint that in later cycles prevents entry into mitosis in the presence of unreplicated or damaged DNA (Togashi and Okada, 1984; Raff and Glover, 1988; Sullivan et al., 1993). In addition to inducing chromatin bridges, DNA replication inhibitors, such as aphidicolin, can slow progression through mitosis in the early embryo (Raff and Glover, 1988). This can have the net effect of increasing the number of nuclei in various stages of mitosis, which we observed upon injection of cyclin J inhibitors. In contrast, inhibition of proteins required during mitosis causes an arrest at a specific mitotic stage, without chromatin bridges (Schubiger and Edgar, 1994; Ruden et al., 1997; Su et al., 1998). The possibility of cyclin J being an early embryo-specific S phase regulator seems particularly intriguing given the fact that genome duplication is proceeding at a uniquely rapid pace in the syncytium. The phenotypes resulting from cyclin J loss-of-function mutations may provide additional information on the precise cell cycle function of this unique cyclin.

Possible Roles for Cyclin J

The primary Cdk partner of cyclin J in the early embryo is Cdk2. A view supported by studies in a number of organisms is that the functional specificity of a Cdk/cyclin complex resides in the cyclin subunit (Nasmyth, 1993). However, the Cdk subunit can also determine the biological function of the complex. In vertebrates, for example, Cdk1/cyclin A complexes control mitotic events, whereas Cdk2/cyclin A complexes are required for S phase (reviewed in Pagano et al., 1992; Morgan, 1995). Moreover, although Cdk1 and Cdk2 each complex with more than one cyclin, all known Cdk1 complexes function in promoting M phase while all known Cdk2 complexes function in promoting S phase (Morgan, 1995). Thus, the association of cyclin J with Cdk2 in the Drosophila embryo raises the possibility that Cdk2/cyclin J complexes may function during S phase, similar to vertebrate Cdk2/cyclin A complexes. In this regard it may be significant that the closest homolog of cyclin J is cyclin A.

The phenotypic consequences of inhibiting cyclin J are consistent with a possible role for cyclin J in regulating the rapid S phases in the early embryo. Inhibition of cyclin J-associated kinase activity resulted in a delay in progression through mitosis and abnormal chromosome segregation. Nuclei entered mitosis with chromosomes remaining unresolved, leading to telophase daughter nuclei connected by chromatin bridges. A similar phenotype has been observed upon injection of agents that inhibit proteins required for DNA synthesis such as DNA polymerase α (Raff and Glover, 1988) and MCM proteins (Su et al., 1997), but not by inhibitors of transcription, protein synthesis, or microtubule dynamics (reviewed in Schubiger and Edgar, 1994). Telophase chromatin bridges arise presumably because Drosophila embryos lack the checkpoint that in later cycles prevents entry into mitosis in the presence of unreplicated or damaged DNA (Togashi and Okada, 1984; Raff and Glover, 1988; Sullivan et al., 1993). In addition to inducing chromatin bridges, DNA replication inhibitors, such as aphidicolin, can slow progression through mitosis in the early embryo (Raff and Glover, 1988). This can have the net effect of increasing the number of nuclei in various stages of mitosis, which we observed upon injection of cyclin J inhibitors. In contrast, inhibition of proteins required during mitosis causes an arrest at a specific mitotic stage, without chromatin bridges (Schubiger and Edgar, 1994; Ruden et al., 1997; Su et al., 1998). The possibility of cyclin J being an early embryo-specific S phase regulator seems particularly intriguing given the fact that genome duplication is proceeding at a uniquely rapid pace in the syncytium. The phenotypes resulting from cyclin J loss-of-function mutations may provide additional information on the precise cell cycle function of this unique cyclin.

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