Cyclin A1-deficient mice lack histone H3 serine 10 phosphorylation and exhibit altered aurora B dynamics in late prophase of male meiosis

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Abstract

Male mice lacking cyclin A1 protein are sterile. Their sterility results from an arrest in the meiotic cell cycle of spermatocytes, which we now identify as occurring at late diplotene, immediately before diakinesis. The stage of arrest in cyclin A1-deficient mice is distinct from the arrest seen in spermatocytes that are deficient in its putative catalytic partner Cdk2, which occurs much earlier in pachytene. The arrest in cyclin A1-deficient spermatocytes is also accompanied by an unusual clustering of centromeric heterochromatin. Consistent with a possible defect in the centromeric region, immunofluorescent staining of cyclin A1 protein shows localization in the region of the centromere. Phosphorylation of histone H3 at serine 10 in pericentromeric heterochromatin, which normally occurs in late diplotene, is reduced in spermatocytes from heterozygous Ccnal+/− testes and completely absent in spermatocytes with no cyclin A1 protein. Concomitantly, the levels of pericentromeric aurora B kinase, known to phosphorylate histone H3 during meiosis, are partially reduced in spermatocytes from testes of heterozygous mice and further reduced in homozygous null spermatocytes. These data suggest a critical and concentration-dependent function for cyclin A1 in the pericentromeric region in late diplotene of meiosis, perhaps in assembly or function of the passenger protein complex.

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Introduction

Mammalian meiosis is a tightly regulated process involving specialized cell cycle progression and morphogenetic changes. We previously identified a new cell cycle regulator of the A-type cyclin class, cyclin A1, that is expressed predominantly in the testis (Sweeney et al., 1996) and is essential for spermatogenesis (Liu et al., 1998). Cyclin A1-deficient mice were healthy and females were fully fertile, but homozygous males were infertile due to a block in spermatogenesis before the first meiotic division.

It was recently reported that males heterozygous for Ccnal show haplo-insufficiency, as evidenced by reduced male fertility, which was particularly striking in mice of a 129 genetic background (van der Meer et al., 2004). Cyclin A1-deficient spermatocytes were observed to undergo a robust apoptosis in a manner analogous to that observed in other targeted mutagenesis studies that resulted in an arrest in meiotic prophase (discussed in Salazar et al., 2003). We have demonstrated that the apoptotic response to the absence of cyclin A1 involved up-regulation of Bax and subsequent activation of caspase 3 (Salazar et al., 2005). Additionally, the apoptotic response was partially rescued in the absence of p53, although the triggers for this apoptotic response have not yet been determined.

Histone H3 becomes phosphorylated at serine 10 at the pericentromeric region in late diplotene of male meiosis and this phosphorylation persists through metaphase I (Cobb et al., 1999a, b). In mitosis, histone H3 has very low levels of phosphorylation at serine 10 at interphase but is highly phosphorylated at metaphase (Gurley et al., 1978). The precise function of this phosphorylation
is not known either for mitosis or meiosis, although some reports have suggested a function for this phosphorylation in proper chromosome segregation (Wei et al., 1999) and also for removal of the HP1 protein from chromatin (Fischle et al., 2005; Hirota et al., 2005). The kinase that performs this phosphorylation in both mitosis and meiosis is thought to be the aurora B kinase, a component of the passenger protein complex that also includes INCENP, survivin and borealin (Vagnarelli and Earnshaw, 2004). Aurora B kinase is believed to be critical for correct chromosome alignment at metaphase (Ditchfield et al., 2003). The passenger protein complex has been reported to have important roles in chromatin modification, kinetochore–microtubule interactions, chromosome bi-orientation, cell cycle checkpoint function, assembly of the central spindle, and cytokinesis (Vagnarelli and Earnshaw, 2004). The relationship between survivin and aurora B kinase is not well understood, but survivin appears to be required for correct localization of the other components of the passenger protein complex (Chen et al., 2003).

In the present study, we have used immunohistochemical staining of meiotic chromosome preparations to determine the precise time of meiotic arrest of cyclin A1-deficient spermatocytes, the chromosomal localization of cyclin A1 protein at the time when the cells arrest in cyclin A1-deficient cells, and the configuration of the chromosomes as the cells became apoptotic. We then closely examined events of late prophase spermatocytes that might be altered in the absence of cyclin A1 protein, including the levels and localization of components of the passenger protein complex and distribution of Ser10-phosphorylated histone H3. This study is the first demonstration of centromeric association of a meiotic cyclin, cyclin A1, and the first link between cyclin A1 activity and function of the passenger protein complex.

Materials and methods

Animals and tissues

A mouse line carrying a targeted disruption of the cyclin A1 gene (Liu et al., 1998) was maintained in a C57BL/6J×129/SV mixed background by mating heterozygous (Ccnal+/−) males and females. Genotyping was carried out by PCR analysis of genomic DNA as described previously (Liu et al., 1998). Dissected tissues for protein extraction were used immediately or frozen at −80 °C up to 1 month. Tissues for immunohistochemistry studies were fixed in 4% paraformaldehyde in PBS overnight at 4 °C.

Antibodies

Antisera specific for cyclin A1 were raised in a rabbit immunized with a GST fusion protein containing amino acids 3–204 of the murine cyclin A1. The antisera were affinity purified by incubation on nitrocellulose membrane blotted with the antigen fusion protein and used at 1/100 for immunoblotting and immunostaining. Rabbit and mouse anti-SCP1 and SCP3 antibodies were initially a gift from Peter Moens. Subsequently, identical results were obtained with antibodies purchased from Abcam (mouse monoclonal AB12452 and rabbit polyclonal AB15092) and were used at 1/1000 dilution. Anti-histone H3 (Upstate 06-570) was used at a 1/500 dilution, anti-phospho-Ser10 histone H3 (Upstate 07-145) was used at a 1/5000 dilution, anti-AUH1 (BD transduction laboratories 611082) was used at a 1/100 dilution, anti-γ-Ser139 phosphorylated H2AX (Trevigen 4411-PC-020) was used at a 1/500 dilution, anti-Cdc2 (Santa Cruz sc-6248) was used at 1/200 dilution, anti-survivin (Novus biologicals) was used at 1/500 dilution, and anti-Mih1 (BD Pharmingen 551091) was used at 1/100 dilution. Human CREST immune serum, which recognizes components of the centromere, was a gift from Dr. Peter Warburton and was used at a dilution of 1:500. Anti-GAPDH (BD Biosciences) was used at a dilution of 1:500 for immunoblotting.

Preparation and immunostaining of chromosome spreads from mouse testis

Meiotic chromosomal spreads were prepared using a protocol similar to that in Peters et al. (1997). Testes were freshly dissected from mice at day 17, 21 or 28, decapsulated, and the tunica albuginea and adherent extratubular tissue removed by rinsing the seminiferous tubules in PBS. Tubules were placed in hypotonic extraction buffer (30 mM Tris, 50 mM sucrose, 17 mM trisodium citrate dihydrate, 5 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, pH 8.2) for 30 min. One-inch lengths of tubules were placed in 20 μl of sucrose solution (100 mM sucrose pH 8.2, set with NaOH) and torn into small pieces with fine forceps. The volume was increased to 40 μl with sucrose solution and pipetted to give a cloudy suspension, which was then spread onto 2 slides dipped in paraformaldehyde solution (1% paraformaldehyde, 0.15% Triton X100 in water adjusted with sodium borate to pH 9.2). Slides were air-dried for 2 h and used immediately or stored at −20 to 80 °C.

For immunostaining, slides were rinsed for 5 min in PBS and then incubated for 30 min in wash buffer (0.3% BSA, 1% horse serum, 0.5% Triton X-100 in PBS). Antibodies were diluted in dilution buffer (3% BSA, 10% heat-inactivated horse serum, 0.5% Triton X100 in PBS) and incubated overnight at 4 °C. Following three 3-min washes in PBS, fluorescent conjugated secondary antibody, diluted 1/2000 in dilution buffer, was added and incubated for 45 min at RT in the dark. The slides were washed in PBS, stained with DAPI, and mounted using Glycergel.

Immunohistochemistry

Paraffin embedded sections (6 μM in thickness) from post-natal day 17, day 22, day 28, and adult mice were deparaffinized and antigen retrieval was performed by boiling the slides in 0.01 M citrate buffer, pH 6.0, in a microwave (Shi et al., 1991) for 10 min and washing extensively with H2O. The slides were treated with 0.03% H2O2 in methanol for 20 min, washed with PBST (1× PBS, 0.1% Triton X-100), and blocked for 1 h with 2.5% goat serum in PBST. The slides were incubated with the primary antibodies, as described for chromosome spreads in a humidified chamber overnight at 4 °C, washed three times with PBST and stained with Vectastain ABC kit (Vector Laboratories, Burlingame, CA). DAB-stained slides were counterstained with hematoxylin.

Immunoblot analysis

Mouse testis lysates were prepared as described in Liu et al. (2000), separated by SDS-PAGE, transferred to nitrocellulose membrane, and blocked in TBS/0.1% Tween 20 with 5% non-fat dried milk. Membranes were incubated overnight at 4 °C with primary antibody diluted in TBS/0.1% Tween 20 with 5% non-fat dried milk, washed briefly at room temperature in TBS/0.1% Tween 20, and incubated for 1 h with horseradish peroxidase-conjugated secondary antibody. After liberal washing in TBS/0.1% Tween 20, blots were incubated in Immobilon chemiluminescent reagent (Millipore) and visualized by exposure to ECL film (Kodak).

Results

Ccnal1−/− spermatocytes arrest in late diplotene following resolution of chiasmata and exhibit clustering of their centromeres

In order to precisely identify the meiotic stage of spermatocytes of wild-type and cyclin A1-deficient spermatocytes, we employed antibodies against the SCP3 protein, which forms part of the lateral element of the synaptonemal complex (Lammers et al., 1994). We also stained for a well-documented
marker of centromeres, the antigens recognized by human CREST autoimmune sera, which are believed to recognize at least in part the CENP-A, B and C proteins (Brenner et al., 1981; Earnshaw and Rothfield, 1985).

Staining and careful staging of chromosome spreads prepared from day 17, day 21, and day 28 wild-type and CcnA1−/− mice revealed normal meiotic progression until mid-diplotene (Fig. 1). Chiasmata appear to form and resolve normally. However in late diplotene, rather than undergoing diakinesis and proceeding to a metaphase I arrangement, spermatocytes in the CcnA1−/− mice arrested and no metaphase I chromosomes were observed. This arrest was distinct from and occurred later than the meiotic arrest observed in mice deficient in the putative cyclin A1 kinase partner, Cdk2. In Cdk2-deficient mice, arrest was observed in mid-pachytene spermatocytes and was accompanied by thin threads of SCP3 staining, perhaps indicating aberrant pairing (Ortega et al., 2003). Furthermore, unlike mice lacking Cdk2, pachytene chromosomes in cyclin A1-deficient mice were normal with respect to staining of SCP3 (Fig. 1).

Until the mid-diplotene stage, we noted no difference in centromere localization between wild-type and cyclin A1-deficient spermatocytes. However, the centromeres of late diplotene, cyclin A1-deficient spermatocytes appeared to cluster and there was a concomitant aggregation of centromeric heterochromatin into large clumps (Fig. 1F), phenomena that were never observed in the control CcnA1+/+ samples.

In order to identify any other abnormalities in chromosome pairing or structure, we next examined several other proteins with known functions and well documented staining patterns in meiotic prophase chromosomal pairing and dynamics by immunostaining. No differences were observed in the staining intensity and distribution of the synaptonemal complex proteins, SCP1 and STAG3 (Bayes et al., 2001), or proteins involved in sister chromatid cohesion, SMC1β and SMC3 (Cobbe and Heck, 2000) (data not shown). In addition there was no difference in the number of foci or qualitative intensity of the DNA repair protein, Mlh1, which localizes to chiasmata during meiosis and is required for completion of meiosis I (Baker et al., 1996) between cyclin A1-deficient and wild-type mice (data not shown). These data suggested that there were no overt defects in chromosome pairing or recombination in cyclin A1-deficient spermatocytes.

Another feature of meiotic prophase spermatocytes involves the formation of the XY chromosome bivalent, within which X and Y-linked genes are repressed. A phosphorylated form of H2AX, a histone variant implicated in DNA repair, has been shown to accumulate in the XY body in pachytene spermatocytes (Mahadevaiah et al., 2001) and to exhibit a characteristic staining pattern at each stage of meiosis. In our study, the Ser139-phosphorylated form of H2AX, or γH2AX, exhibited a staining pattern in meiotic prophase nuclei and in XY bodies of cyclin A1-deficient spermatocytes indistinguishable from that of the control CcnA1+/+ spermatocytes (Fig. 2, panels 1 and 2). However, at the point of meiotic arrest in diplotene in CcnA1−/− spermatocytes, γH2AX foci were observed first at the centromere and subsequently along the length of the chromosomal axes (Fig. 2, panels 3 and 4).

The appearance of γH2AX foci was concurrent with the observed aggregation of centromeric heterochromatin (Fig. 2, panels 4 and 5). In CcnA1+/+ spermatocytes throughout diplotene, each pair of chromosomes was associated at the centromere with a discrete pocket of dense heterochromatin, as visualized by DAPI staining (Fig. 2, panels 1 and 3). There was no difference in this pattern of heterochromatin in early diplotene CcnA1−/− spermatocytes (Fig. 2, panel 2). However, in late diplotene CcnA1−/− spermatocytes, centromeric heterochromatin from several chromosomes aggregated with a

Fig. 1. Spermatocyte spreads (day 28) stained with anti-SCP3 (red) and CREST antiserum (green). (A) Pachytene, (B) diplotene and (C) late diplotene stages of meiosis in CcnA1+/+ mice. (D) Pachytene, (E) diplotene and (F) arrest of spermatocytes in late diplotene in CcnA1−/− mice. Arrows denote chiasmata.
Fig. 2. Spermatocyte spreads (day 28) stained with anti-SCP3 (green), anti-Ser139γ phosphorylated H2AX (red) and DAPI (blue). Panel 1 depicts staining observed in Ccna1+/+ pachytene spermatocytes. Panel 2 depicts staining in Ccna1−/− pachytene spermatocytes. Panels 3 and 4 show staining in the late diplotene stage of meiosis in Ccna1+/+ and Ccna1−/− spermatocytes respectively. Panel 5 shows Ccna1−/− spermatocytes following meiotic arrest.
characteristic clustering appearance (Fig. 2, panels 4 and 5). It is not clear whether this aggregation occurred as part of the apoptotic response, but γH2AX foci accumulated after this aggregation.

**Pericentromeric localization of cyclin A1 protein in late diplotene and prometaphase**

In immunostained histological sections of testis, cyclin A1 showed a nuclear localization in spermatocytes from mid-pachytene through diplonete (Ravnik and Wolgemuth, 1996); however, whether there was any preferential localization within the nucleus could not be determined at this level of resolution. To assess the distribution of nuclear cyclin A1 in more detail we examined its localization in meiotic chromosome spreads using anti-cyclin A1 antibodies. This allowed us to assess localization of any cyclin A1 associated with the chromatin of the meiotic chromosomes. In Ccnal+/− spermatocytes, anti-cyclin A1 antibodies showed a diffuse staining of chromatin but also stained specific foci in the pericentromeric region from late diplotene onwards (Fig. 3A), coincident with the point of arrest in cyclin A1-deficient mice. This staining was lacking in the Ccnal−/− preparations, as a control for specificity (Fig. 3A, top panel).

There was partial co-localization of cyclin A1 with antigens recognized by CREST autoimmune sera (Fig. 3A, third panel from top). At late diplotene, there was little or no co-localization seen with cyclin A1 and SCP3 at centromeres, but at prometaphase there was some overlap with SCP3 staining (Fig. 3A, bottom panel). This pericentromeric localization is depicted in cartoon form in Fig. 3B.

We next examined the chromosomal distribution of Cdk2, a putative binding partner for cyclin A1 (Liu et al., 2000). The predicted distribution of Cdk2 protein was observed in the centromeric region, at telomeres, and at foci along chromosomes during pachytene to diplonete as reported by Ashley et al. (2001) and was not altered in cyclin A1-deficient mice (Fig. 4A). Interestingly, despite some overlap, cyclin A1 did not completely co-localize with its putative Cdk2 partner at the centromeres (Fig. 4B).

**Late diplotene cyclin A1-deficient spermatocytes lack histone H3 serine 10 phosphorylation**

The late diplotene stage of meiosis, when arrest of cyclin A1-deficient spermatocytes occurred, has been reported to be coincident with robust phosphorylation of histone H3 at serine 10 (Handel et al., 1999). We therefore examined the levels of phosphorylation of two histone modifications associated with the diplonete to metaphase transition, histone H3 serine 10 and serine 28. In Ccnal+/− spermatocytes, phosphorylation of H3 serine 28 was first observed at the prometaphase stage and persisted up to meiosis II (data not shown). Since this modification occurred after the point of arrest in Ccnal+/− spermatocytes, the serine 28 phosphorylation is unlikely to be important to the Ccnal−/− male meiotic arrest. In contrast, examination of histological sections of testes (Figs. 5A and B) and chromosome spreads (Fig. 5C) revealed that phosphorylation of H3 serine 10 was dramatically reduced in Ccnal+/− spermatocytes and undetectable in Ccnal−/− spermatocytes lacking cyclin A1 (Fig. 5C).

**Cyclin A1-deficient spermatocytes show reduced staining of aurora B kinase at the pericentromeric heterochromatin**

Histone H3 serine 10 is a known target of phosphorylation by the aurora B component of the passenger protein complex. The reduction of this phosphorylation in Ccnal+/− spermatocytes and its absence in Ccnal−/− spermatocytes prompted us to examine this complex in more detail. In addition, the point of meiotic arrest in cyclin A1 mice also overlapped with the assembly of the passenger protein complex. Reduction of levels of the passenger protein component survivin has been reported to result in mis-localization and reduced histone H3 kinase activity of aurora B (Chen et al., 2003). We therefore examined survivin dynamics in prophase and found its level and localization to be grossly indistinguishable between Ccnal+/− and Ccnal−/− spermatocytes when compared to Ccnal+/+ (Fig. 6). Survivin appeared at the centromeric heterochromatin in mid-pachytene and increased in amount until late diplonete. At prometaphase, survivin showed some, but not complete, overlap with SCP3 staining at the centromeres.

We next examined the levels and distribution of aurora B kinase. While immunoblot analysis of whole testicular lysates did not indicate a significant difference in levels of aurora B protein between Ccnal+/+ and Ccnal−/− testicular lysates (Fig. 7A), the amount of aurora B protein associated with meiotic chromosomes was clearly different (Fig. 7B). Interestingly, we found a reduced staining intensity of aurora B protein localizing to the pericentromeric heterochromatin in late diplonete Ccnal+/− spermatocytes (Fig. 7B, panels C and D). This reduced intensity was even more pronounced in the Ccnal−/− spermatocytes (Fig. 7B, panels E and F). It should be noted that great care was taken to expose the photomicrographs for identical periods of time so that relative intensity differences would be accurately portrayed.

**Discussion**

**Late prophase arrest of Ccnal−/− spermatocytes**

Consistent with our previous observations of histological sections and synaptonemal complex preparations of spermatocytes, examination of chromosome spreads from Ccnal−/− mice revealed no obvious defects in meiotic prophase prior to the diplonete stage. However, we have now been able to pinpoint the arrest to the late diplonete stage of meiosis, after resolution of chiasmata. This arrest was followed by a characteristic clustering of centromeric heterochromatin and the appearance of γH2AX foci, first at the centromere and then along the chromosomal axes. The clustering of centromeric heterochromatin may represent mis-localization or aberrant association of centromeres. The clustering was noted to precede immediate signs of apoptosis, such as phosphorylation of
Fig. 3. (A) Late diplotene spermatocytes spreads (day 28). Top panel: Ccna1−/− late diplotene spermatocyte spreads stained with anti-cyclin A1 (red), anti-SCP3 (green), and DAPI (blue). Second panel: Ccna1+/+ late diplotene spermatocyte spreads stained with anti-cyclin A (red) and anti-SCP3 (green) and DAPI (blue). Third panel: Ccna1+/+ late diplotene spermatocytes spreads stained with anti-cyclin A1 (red), CREST antiserum (green), and DAPI (blue). Bottom panel: Ccna1+/+ prometaphase spreads stained with anti-cyclin A1 (red), anti-SCP3 (green), and DAPI (blue). (B) Schematic of cyclin A1 protein pericentromeric localization.
Fig. 4. (A) Day 28 spermatocyte spreads from *Ccna1*^{+/+}* and *Ccna1^-/-* mice stained with anti-Cdk2 (green) and anti-SCP3 (red) and counterstained with DAPI (blue). (B) Day 28 spermatocyte spreads from *Ccna1*^{+/+}* mice stained with anti-Cdk2 (green) and anti-cyclin A1 (red) and counterstained with DAPI (blue).
Fig. 5. Top panel: paraformaldehyde fixed testis sections (day 28) from Ccn11+/+ (A) and Ccn11−/− mice (B) stained with anti-H3-Ser10 and counterstained with hematoxylin. The insert in A shows an enlarged image of H3-Ser10 nuclear staining in spermatocytes. (C) Spermatocyte spreads (day 28) from Ccn11+/+, Ccn11+/− and Ccn11−/− testes as indicated, stained with anti-SCP3 (green), anti-H3P-Ser10 (red), and DAPI (blue). Note that prometaphase spermatocytes are not observed in Ccn11−/− testes.
Ser139 γ-H2AX, but may simply be a feature of cells that are committed to apoptosis. It has been reported that, in mitotic cells, the condensation of chromatin thought to be characteristic of apoptosis is in fact caused by an aggregation of centromeric heterochromatin (Hendzel et al., 1998). Whether this occurs in meiotic cells is not known but this would be consistent with our data. To our knowledge this is the first report describing this kind of aggregation of centromeric heterochromatin upon meiotic arrest. It would be of great interest to see if this feature occurs in other mouse models exhibiting meiotic prophase arrest and apoptosis, such as the Spo11 (Baudat et al., 2000), Mlh1 (Edelmann et al., 1996), and Atm (Xu et al., 1996) knockout mice. It is also intriguing that γ-H2AX phosphorylation appears to begin at centromeres before spreading along the chromosomes. This may reflect a more rapid response at the location of the primary defect.

Distinct meiotic arrest in Ccna1−/− and Cdk2−/− spermatocytes

The clear difference in the phenotypes of cyclin A1- and Cdk2-deficient mice and the observation that Cdk2 protein localized normally in Ccna1−/− prophase spermatocytes raises exciting questions about the role of both proteins in meiosis. If Cdk2 partners with a cyclin for its critical functions in prophase of meiosis in spermatocytes, our localization data and the distinct points of meiotic arrest in Cdk2−/− and Ccna1−/− mice suggest that this partner is not cyclin A1. One possible partner for Cdk2 could be cyclin B3, which is expressed in leptotene and zygotene stages of meiosis (Nguyen et al., 2002). Cyclin B3 has been shown to interact with Cdk2, although it appears to be a poor activator of Cdk2 kinase activity. Alternatively, Cdk2 could be functioning during meiotic prophase in a cyclin-independent manner. There is increasing evidence for Cdk-independent functions for cyclins, that is, in addition to activating CDKs, cyclins also play kinase-independent functions. For example, D-type cyclins were shown to perform additional CDK-independent roles as co-activators or co-repressors of tissue-specific transcription factors (Zwijsen et al., 1997). Recent studies from Sicinski and colleagues indicate that E-type cyclins also have kinase-independent functions (Geng et al., 2007). This is of particular interest since the E-type cyclins, like the A-type cyclins, partner with Cdk1 and Cdk2.

As there was no complete co-localization of cyclin A1 and Cdk2 proteins in our studies, it is also probable that cyclin A1 partners with a protein other than Cdk2 for its role in late prophase. Cdk1 is an obvious candidate, as we have previously shown activity of cyclin A1/Cdk1 complexes immunoprecipitated from the testis (Liu et al., 2000; Sweeney et al., 1996). Cdk1 is localized to the nucleus throughout pachytene and diplotene of meiosis (Ravnik and Wolgemuth, 1999). In vitro comparison of the kinetic properties of human cyclin A1 and cyclin A2 in association with CDK1 suggests that cyclin A1 forms an efficient complex with CDK1 (Joshi, A., V. Jobanputra, K.M. Lele, and D.J. Wolgemuth, submitted). Additionally, cyclin A1/CDK1 complexes can phosphorylate pRb and p53 (possibly physiologically relevant in vivo substrates) more efficiently than cyclin A2/CDK1. It is possible therefore that Cdk1 is an in vivo partner for the centromere-associated function of cyclin A1. Unfortunately, we were unable to obtain staining of chromosome spreads with commercially available anti-Cdk1 antibodies to test possible co-localization.

Fig. 6. Chromosome spreads stained with anti-SCP3 (green) and anti-survivin (red) and counterstained with DAPI. Late pachytene spermatocytes from Ccna1+/+ (A) and Ccna1−/− (D) testes, late diplotene spermatocytes from Ccna1+/+ (B) and Ccna1−/− (E) testes. Ccna1−/− prometaphase staining (C) (this stage is not present in Ccna1−/− testis).
Requirement of cyclin A1 for histone H3 phosphorylation at serine 10

The role of histone H3 serine 10 phosphorylation in meiosis and even in mitosis is not well understood. However, the reduction of H3 Ser10 phosphorylation in Ccna1+/- spermatocytes provides an intriguing clue to the impaired fertility of these mice. Van der Meer and colleagues reported reduced numbers of haploid cells in heterozygous compared to wild-type mice (van der Meer et al., 2004). The phosphorylation levels of H3 serine 10 were lower than in wild-type in late diplotene spermatocytes, but identical to wild-type once prometaphase was reached. This may indicate a threshold level for H3 serine 10 phosphorylation that must be reached for meiosis to progress. Alternatively, this phosphorylation may simply be an indicator of low levels or activity of a kinase, possibly aurora B kinase, required for meiotic progression through phosphorylation of other targets. The apparent gradient effect observed in staining of aurora B kinase and phospho-H3-Ser10 lends weight to the hypothesis that absolute levels of cyclin A1 are critical to the progression of male meiosis. This may explain the cyclin A1 haplo-insufficiency observed by Van der Meer and colleagues.

Centromeric function of cyclin A1 protein

The localization of cyclin A1 at the centromeric region and the effects on other centromeric proteins in its absence suggest a critical role for cyclin A1 at this region. Although we have not identified the target(s) of cyclin A1/Cdk kinase activity that appear to be required for correct localization of aurora B kinase and subsequent phosphorylation of histone H3 at serine 10, we previously noted a reduction in cyclin B1/Cdk1 (MPF) activity in testis from Ccna1−/− mice (Liu et al., 2000). It has been reported that MPF can phosphorylate the passenger protein complex survivin and that this phosphorylation helps protect against apoptosis in mitotic cells. It is possible that reduced MPF activity observed in cyclin A1-deficient spermatocytes might result in reduced phosphorylation of key enzymes required for passenger protein complex localization and function. In contrast to the striking changes in the staining pattern of aurora B protein, preliminary observations did not reveal differences in the staining pattern of threonine 34-phosphorylated survivin prior to metaphase in Ccna1+/+ or Ccna1−/− spermatocytes (data not shown). Therefore, although this modification of survivin is important in sensing apoptosis in mitotic cells (Fortugno et al., 2002; O’Connor et al., 2000; Wall et al., 2003), we do not believe that it represents the primary response to apoptosis in Ccna1−/− spermatocytes. Future work should help to elucidate the potential targets and critical roles of cyclin A1-associated kinase activity in late diplotene of meiosis, in particular its role in regulating the activity of the passenger protein complex.

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