Diverse roles for CDK-associated activity during spermatogenesis

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The primary function of cyclin-dependent kinases (CDKs) in complex with their activating cyclin partners is to promote mitotic division in somatic cells. This canonical cell cycle-associated activity is also crucial for fertility as it allows the proliferation and differentiation of stem cells within the reproductive organs to generate meiotically competent cells. Intriguingly, several CDKs exhibit meiosis-specific functions and are essential for the completion of the two reductional meiotic divisions required to generate haploid gametes. These meiosis-specific functions are mediated by both known CDK/cyclin complexes and meiosis-specific CDK-regulators and are important for a variety of processes during meiotic prophase. The majority of meiotic defects observed upon deletion of these proteins occur during the extended prophase I of the first meiotic division. Importantly a lack of redundancy is seen within the meiotic arrest phenotypes described for many of these proteins, suggesting intricate layers of cell cycle control are required for normal meiotic progression. Using the process of male germ cell development (spermatogenesis) as a reference, this review seeks to highlight the diverse roles of selected CDKs their activators, and their regulators during gametogenesis.

Keywords: cyclin; cyclin-dependent kinase; meiosis; meiotic crossover; recombination; synopsis

Cyclin-dependent kinases: a diverse family with numerous activators and regulators

Cyclin-dependent kinases (CDKs) and their activating partner proteins, the cyclins, are often compared to the molecular engine and gearbox, which is utilized to drive a cell through the different phases of the cell cycle [1]. This simplistic analogy is representative of the cell cycle in eukaryotic organisms of lower complexity, such as yeast. These unicellular organisms possess a single ‘engine’ (CDK) and multiple cyclin ‘gears’. The sequential association of different cyclins with the CDK promotes the activation of distinct CDK/cyclin complexes to advance through each stage of the cell cycle. In more complex multicellular eukaryotes such as mammals, the CDK and cyclin gene families have become increasingly larger, as a result of multiple gene duplication events occurring throughout eukaryotic evolution. This has allowed some CDKs and cyclins to acquire specialized functions either in addition to, or independently of their roles in cell cycle progression [2,3]. There are currently 21 CDKs, and upwards of 30 cyclin proteins described in humans and

Abbreviations
CDKs, cyclin-dependent kinases; CKIs, CDK-inhibitor proteins; DSBs, double-strand breaks; FSH, follicle-stimulating hormone; GST, gono-cyte-spermatogonia transition; LINC, linker of nucleoskeleton and cytoskeleton; LRN, late recombination nodule-associated; RPM, rapid prophase movements; SSCs, spermatogonial stem cells.
mice. Only CDK1, 2, 3, 4, and 6 in complex with an A-, B-, C-, D-, or E-type cyclins, form canonical CDK/cyclin complexes with direct roles in driving mitotic cell cycle progression. The remaining CDKs and cyclins exhibit diverse functions and have been shown to facilitate many important cell cycle-independent roles including the modulation of transcription and RNA splicing [4]. Although much study has been devoted to the understanding of mammalian cyclins, it is clear that there are still many intricacies yet to be properly described. Recently, several ‘atypical’ cyclin family members have been identified. These proteins share limited homology with the cell cycle-associated cyclins. Many of these have yet-unknown functions, and their ability to bind and activate CDKs is mostly unexplored [4–10].

In addition to the cyclins, several mammalian CDKs can also be bound and activated by noncyclin CDK interactors from the Speedy/RINGO family of proteins. Noncanonical CDK/Speedy complexes are not subject to typical cell cycle regulation. Whereas the majority of CDK/cyclin complexes are reliant upon a critical activating phosphorylation to achieve full catalytic activity [11–13], CDK/Speedy complexes display activity in the absence of this modification [14–18]. Furthermore, CDK/Speedy complexes are also insensitive to inhibition by CDK-inhibitor proteins (CKIs), which physically bind and inactivate CDK/cyclin complexes [4, 19, 20]. These unique properties are thought to allow the formation of active CDK/Speedy complexes under circumstances, whereby CDK/cyclin complexes would usually be inactivated.

In regard to the role of CDK-associated activity in maintaining normal reproductive health, several mammalian CDKs, cyclins, and at least one Speedy protein (Speedy A) have been shown to be essential for the development and maturation of the germ cells within the male and female reproductive organs (gametogenesis). This is similarly true of members of the pRB-E2F signaling pathway, which represents a major downstream target of CDK activity and also specific CKI proteins. Many of these proteins show preferential or heightened expression in these tissues or, in some cases, exhibit differential expression of splice isoforms [21–24]. In accordance with these observations, knock-out mouse models of many of these proteins result in infertility due to arrested gametogenesis. Interestingly, the stage at which germ cell development is affected in these models is highly varied depending upon the protein which is knocked out. This highlights a complex network of often nonredundant interactions between CDKs, their activators, and their regulatory proteins during gametogenesis and offers a unique insight into the requirement of CDK-associated activity for normal fertility.

**Spermatogenesis as a model system to study the importance of CDK activity during germ cell development**

Much of the research performed to elucidate the roles of CDK-associated activity during gametogenesis has utilized the process of male germ cell development (spermatogenesis) as a developmental model. For such studies, female germ cell development (oogenesis) is disadvantaged by both the timing and frequency of the meiotic divisions observed in this system. In mice, the first meiotic division of female meiocytes (oocytes) is initiated in the embryonic ovary (~embryonic day E13.5 in the mouse). Meiosis is subsequently arrested prior to the onset of the second meiotic division and oocytes enter a long-term arrest state known as dictyate [25, 26]. As in humans, the generation of mouse oocytes occurs only once. Consequently, the analysis of early oocyte development and its first meiotic division must be performed during embryonic development. In contrast, once spermatogenesis is initiated in pubertal male mice (~postnatal day 14), the development of germ cells occurs in continuous waves throughout the lifetime of a mouse. This is facilitated by the continual production and maturation of male meiocytes (spermatocytes) from a self-renewing source of spermatogonial stem cells (SSCs) [27]. For normal spermatogenesis, a small pool of ‘fully undifferentiated’ or ‘primitive’ (A0) type SSCs must be maintained to supply a steady supply of cells competent to enter meiotic divisions. The division of these cells gives rise to either more A0 SSCs, which retain the capacity to self-renew, or early differentiating spermatogonia. Differentiating spermatogonia divide successively via mitosis without cytokinesis to form first pairs (A1), and then aligned chains (A4) of spermatogonia linked by cytoplasmic bridges. These chains can attain lengths of 16 or rarely 32 connected cells. It is generally considered that chains of at least 8 (A8) must be formed to enable further differentiation [28]. Committal of A4 spermatogonia cells to gametogenesis occurs in the absence of mitotic division and is characterized by morphological changes as well as changes in transcription [29, 30]. The resultant A1-type spermatogonia undergo sequential mitotic divisions to become A2, A3, and then A4-type spermatogonia. A4 spermatogonia undergo further divisions to become intermediate-type (In) and then B-type spermatogonia. At this stage, B-type spermatogonia are competent to differentiate into primary spermatocytes that can enter
meiosis. The stepwise nature of spermatogenesis as depicted in Fig. 1 is particularly suited to knockout studies. Here, the stage of germ cell development affected by the deletion of a single protein can be easily investigated to determine functionality.

**Meiotic prophase I: a hotbed for CDK-associated functions**

For the generation of haploid gametes, meiocytes must undergo two reductional meiotic divisions. The first meiotic division promotes the pairing and exchange of genetic material between maternal and paternal chromosomal pairs (homologs). This occurs during an extended prophase stage (prophase I) [31,32] and is followed by the segregation of homologs into two genetically heterogeneous daughter cells. This is followed by a second meiotic division, which is considered more comparable to mitotic divisions as it promotes the segregation of sister chromatids [33]. The prolonged timeframe of meiotic prophase I, reflects the complexity of this developmental process and can be broken down into five further substages: Leptotene, Zygotene, Pachytene, Diplotene, and Diakinesis. In regard to the knockout mouse models discussed within this review, the majority of meiotic defects observed typically arise during one or more of these substages. Therefore, this section will introduce the events of meiotic prophase I in detail, in order that the meiotic functions of CDK activity described later in this review can be better understood.

During leptonema, the earliest stage of prophase I, paternal and maternal bivalent chromosomes locate and pair (synapse) with their homologous counterpart. An integral part of this process is the self-imposed induction of double-strand breaks (DSBs). This is mediated primarily by the DNA topoisomerase, SPO11 [34,35]. SPO11-mediated DSB formation occurs preferentially within so-called ‘meiotic hotspots’ or ‘recombination hot regions’ of the genome. The biased nature of this process is enforced by epigenetic modifications placed proximal to the incipient break site [36–41]. These alterations are thought to create a preferred environment for the recombination machinery and are typically directed toward euchromatic regions depleted of nucleosomes [41–46]. Meiotic DSB formation results in the induction of approximately 150 and 200–300 DSBs during human and murine meiosis, respectively [34,47,48]. Although this is a phenomenon, which occurs in all organisms that are capable of meiotic division, the number of break sites is highly variable between organisms. In mice, meiotic DSB formation is essential for proper chromosomal pairing [49–51] and is tightly integrated with the assembly of a tripartite scaffolding structure, known as the synaptonemal complex [52]. The components comprising the
axial/lateral elements of this structure (SYCP2/3) localize primarily to chromatin decorated with markers of meiotic DSBs, in a manner that remains poorly understood. The subsequent self-assembly of axial filaments effectively anchors chromosome segments harboring meiotic break sites together within a single chromosomal core with the remaining chromatin organized in looped arrays tethered to these sites [53–56].

Zygonema marks the onset of homolog pairing (synapsis). Here, the transverse (SYCP1) and central elements (SYCE1, SYCE2, SYCE2, TEX12, SIX6OS1) bind the axial element scaffold surrounding each homolog and bring them together in a zipper-like fashion. This event brings meiotic DSBs close proximity with the near-perfect DNA template of its chromosomal homolog within the context of the synaptonemal complex scaffold. Upon nucleolytic processing of DSB sites, single-stranded 3′ DNA tails are generated and utilized to initiate homologous DNA damage repair in a process known as strand invasion [57]. This effectively creates a large number of initial recombination sites (also known as early recombination nodules) [58].

An essential precondition for normal synapsis during zygonema is the tethering of telomeres to the inner nuclear envelope. Nuclear envelope tethering is stabilized by the interaction of proteins at the inner (SUN1, SUN2) and outer nuclear envelope (KASH5), in addition to additional ancillary interactors (TERB1, TERB2, MAJIN). Together, these proteins comprise the ‘LINC’ (Linker of Nucleoskeleton and Cytoskeleton) complex ([59–65]; for review, see [66]). The LINC complex facilitates the creation of nucleocytoplasmic bridges connecting telomeres to microtubules within the cytoskeleton of the meiocyte allowing cytosomeal forces to be transmitted via the nuclear envelope to drive rapid telomeric movements [67–69]. These telomeric movements are crucial for establishing initial interactions between homologs and are thus essential for their eventual pairing. Much of the meiosis-specific functions of the proteins discussed in this review seems to revolve around the formation and stabilization of the LINC complex and will be discussed at length later in this review.

In pachynema, chromosomal homologs achieve a state of full synapsis. At this time, numerous DNA repair complexes are recruited to chromosomal axes to stabilize and repair DNA-intermediate structures created at recombination sites [70–72]. In the majority of cases, these sites are repaired without the exchange of genetic material from its homologs (non-crossovers). The remaining sites become stabilized in a manner which protects from this repair pathway. Instead, further processing results in the formation of crossover intermediate structures in so-called late recombination nodules. These late recombination nodules represent the latest stages of homologous recombination repair, which occur prior to the formation of meiotic crossover sites [73]. In mice, the formation meiotic crossovers resulting from the maturation of late recombination nodules occurs in a strictly regulated manner with 1–2 formed per homolog pair.

In diplonema, the synaptonemal complex lateral and central elements are disassembled, a process which is completed by diakinesis. Throughout these stages, sister chromatid arms remain closely connected via cohesive [74], but paired homologous chromosomes remain linked solely at meiotic crossover sites. These crossover sites act as anchor points, which allow bidirectional tension to be applied upon each homolog pair. This facilitates orderly alignment of homologs upon the meiotic spindle [75–79]. Subsequent recognition and cleavage of crossover sites by structure-specific endonucleases in anaphase I allows the release of tension on the spindle. This process is also known as crossover resolution [80–82]. Successful crossover resolution allows the segregation of homologs into two daughter cells and completes the reciprocal exchange of maternal and paternal DNA at the resolved crossover site. Achieving this genetic exchange of information during meiotic prophase I is a hallmark of meiotic division and is the major determinant of heterogeneity within the genome of developing germ cells [55,83–87].

**Aims**

Despite extensive research delineating the various roles of the CDKs, cyclins, and Speedy proteins during gametogenesis, pertinent questions remain even surrounding the best-characterized members of these protein families, particularly CDK2, CDK4, the E- and D-type cyclins, and Speedy A. This review aims to highlight the contribution of these selected CDKs in regard to their functions during gametogenesis. Specific effort is made in highlighting where gaps in the current literature can be addressed in future studies. Although also essential for gametogenesis, the A- and B-type cyclins in conjunction with CDK1 and/or CDK2 are not discussed at length in this review. For a comprehensive analysis of these proteins in relation to gametogenesis, the reader is directed to the following reviews by Chotiner *et al.* and Risal *et al.* [24,88].
Roles for canonical, cell cycle-associated CDK activity during germ cell development

One of the major requirements for CDK-associated activity during normal gametogenesis arises due to the need for mitotically dividing cells in the male and female reproductive organs. Typically, these mitotically active cell types consist of either precursor stem cells, which give rise to the meiocytes, or their respective supporting cell types, which promote meiocyte maturation. In this section, we will discuss the effects on fertility observed upon deletion of cell cycle-associated cyclins, their associated CDKs, and the CKI proteins, which regulate the activity of these complexes. For added context, this section will also explore the effects occurring upon perturbation of downstream CDK/cyclin signaling. For simplicity, the order of discussion will follow the typical order of expression of these proteins throughout the cell cycle starting with the G1 stage D-type cyclins and concluding with the S phase-associated CDK2.

Proposed roles for the D-type cyclins in regulating spermatogonial proliferation and differentiation

During mitotic cell division, D-type cyclins act primarily as the activating partners of CDK4 and CDK6. Here, CDK4- and CDK6-associated kinase activity serves an important role in driving entry into G1 from quiescence and also maintaining cellular proliferation in actively dividing cells [89–93]. The proliferative role of CDK/cyclin proteins, in general, is mediated through the phosphorylation of the major CDK-substrate, RB1 (Retinoblastoma) tumor suppressor, and its related proteins RBL1 (p107) and RBL2 (p130) [collectively referred to here as pRB]. In the absence of CDK-driven phosphorylation, these pRB proteins act to inhibit cellular proliferation by sequestering E2F transcription factor family members; effectively holding them in an inactive state. When released from pRB repression, E2F transcription factors promote cell cycle progression through the downstream transcription of genes that drive cell cycle progression [94].

Based on their cell-specific expression during spermatogenesis, cyclin D-associated kinase activity is thought to play a primarily proliferative role in driving the mitotic expansion of differentiating spermatogonia. During the first wave of spermatogenesis in which spermatogenic cells differentiate in a synchronous manner [95], each of the three D-type cyclin members (D1-D3) can be detected in A1 type spermatogonia as they enter a differentiating state. Later, during adult spermatogenesis, cyclins D1 and D3 can be detected in dividing spermatogonia during many stages of spermatogenesis, suggesting specific roles for these cyclins in driving cellular proliferation [96,97]. Although deletion of cyclin D1 [98,99] or cyclin D3 in isolation does not affect fertility [100], the deletion of both cyclins D1 and D3 in combination leads to severe developmental defects resulting in early lethality [101]. Since this early lethality precludes formal analysis of the relative requirement for cyclin D1 and/or D3 in spermatogenesis, it is likely that as in many other cell types, that at least one of these proteins is required to promote cellular division in spermatogonia.

Unlike cyclins D1 and D3, cyclin D2 expression is required for normal fertility in both male and female mice. During spermatogenesis, the expression of cyclin D2 remains specifically restricted to differentiating A1-type spermatogonia during adult spermatogenesis. It has been hypothesized that might reflect a role in the differentiation process of spermatogonia. Unfortunately, this is yet to be formally confirmed due to an incomplete analysis of the infertility phenotype in cyclin D2−/− testes.

In adult ovaries, which lack proliferating stem cells, cyclin D2 is expressed in the granulosa cells, which support the maturation of ovarian follicles. In this cell type, cyclin D2 expression is essential for cellular proliferation in response to the follicle-stimulating hormone (FSH) [102]. Interestingly, the proliferation of the corresponding testicular cell type, known as Sertoli cells, is similarly responsive to FSH signaling [103] and also seems to be influenced by cyclin D2 expression levels. This was best illustrated in studies of inhibitin α−/− mice, which are unable to properly regulate FSH production. In these mice, additional deletion of cyclin D2 was shown to slow the growth of gonadotropin-dependent gonadal tumors, which are comprised of the Sertoli or granulosa cell types in males and females, respectively [104]. Together, these data suggest that cyclin D2 is a FSH-responsive gene required for cellular proliferation in both testis and ovary. Future study is warranted to determine whether the spermatogenic defects observed in cyclin D2−/− mice arise from differentiation defects in spermatogonial stem cells or alternatively, the defective proliferation of Sertoli cells.

CDK4/CDK6

Mouse knockouts for the kinases partnering the D-type cyclins, CDK4 [105,106], and CDK6 [107] are viable. Expression of at least one of these proteins is
required for the early development of hematopoietic precursors and their combined knockout results in embryonic lethality due to the development of severe anemia [107]. This is also true of mice with the deletion of all D-type cyclins [108]. During spermatogenesis, the maximal expression of Cdk4 and Cdk6 is observed in immature testes at which time the testes consist primarily of spermatogonial stem cells [109–112]. Although Cdk6−/− mice show no overt defects in gametogenesis, Cdk4 deletion results in female infertility from birth and early-onset infertility in male mice. Interestingly in regard to female fertility, the phenotype upon deletion of Cdk4, however, seems to be distinct from that of the cyclin D2 knockout as normal follicular maturation could be observed in these mice with no defect seen in the proliferation of granulosa cells. Instead, postovulatory progesterone secretion was markedly impaired and fertility in these mice could be rescued by progesterone treatment [113]. In regard to male fertility, a low percentage (~20%) of Cdk4−/− males are initially fertile until around 2 months of age. The spermatogenic defects seen in Cdk4−/− testes increase in severity with age and fertility in these animals is invariably lost in older mice [105,106]. The importance of Cdk4 for fertility remains poorly understood. One proposal was that early-onset infertility in male Cdk4−/− mice might occur in a comorbid manner with the development of spontaneous nonobese diabetes mellitus [114], which is known to have a negative impact upon fertility [115,116]. Unfortunately, the analysis of the Cdk4−/− spermatogenic defect has not been extended further than the histological analysis of mutant testis sections. Potential spermatogonial stem cell proliferation/differentiation defects in this model are therefore yet to be investigated [114]. Additional unexplored roles for CDK4 in meiotically dividing spermatocytes have also been proposed by several groups and will be discussed later in the latter sections of this review.

Regulation of CDK4/CDK6 activity by the INK4 class of CKI proteins during spermatogenesis

During mitotic division, control of CDK4 and CDK6-associated kinase activity is exerted in part by the INK4 family of CKI proteins encoded by the genes of Cdkn2a, Cdkn2b, Cdkn2c, and Cdkn2d [117,118]. These proteins specifically inhibit CDK4 and CDK6 by inducing structural changes that prevent catalytic activation [119]. Each of the INK4 proteins show distinct expression patterns during spermatogenesis and can be observed in either spermatogonia (p16INK4A, p19ARF), spermatocytes (p19INK4D), or both (p15INK4B, p18INK4C) [112,120–123]. The Cdkn2a gene possesses distinct promoters upstream of alternate exons. This allows the expression of two distinct tumor suppressor proteins p16INK4A and p19ARF using different reading frames [124]. In its capacity as a CDK4/CDK6 inhibitor, p16INK4A prevents the phosphorylation of pRB proteins by directly binding CDK4 or CDK6, effectively repressing downstream E2F signaling. p19ARF will not be discussed further here as it cannot bind to CDK4/CDK6 and is therefore not considered a CDK-inhibitor [124]. Deletion of Cdkn2a leading to the loss of both p16INK4A and p19ARF yields viable and fertile animals [125], as does specific deletion of p16INK4A [126]. Similarly, singular, or combined deletion of either Cdkn2b (p15INK4B) or Cdkn2c (p18INK4C) does not result in overt defects in spermatogenesis suggesting nonessential roles for these proteins [127,128]. In contrast to the other INK4 family members, deletion of Cdkn2d (p19INK4D) results in the apoptosis of primary spermatocytes [129]. The severity of this phenotype is worsened by the additional deletion of Cdkn2d (Cdkn2c−/−, Cdkn2d−/−), suggesting that these proteins might have overlapping functions. Further analysis suggested that Cdkn2c−/−, Cdkn2d−/− spermatogonia show defects entering meiotic divisions. Resultant spermatocytes also fail to correctly undergo normal meiotic division [111]. The phenotypes seen in Cdkn2c−/−/− Cdkn2d−/− mice suggest that at least some inhibition of cyclin D-dependent kinase activity is essential for the normal progression of spermatogenesis. Somewhat contradictory to these findings are observations that in knockin mice, whereby CDK4 (Cdk4R24C) or CDK6 (Cdk6R31C) are refractory to inhibition by the INK4 inhibitors [130,131] are fertile, with no spermatogenic defects reported in either case [105,132]. Although compound Cdk4R24C Cdk6R31C mutant mice, which are fully insensitive to INK4 inhibitors have also been generated, it was not reported whether these mice exhibit any fertility defect. As such, it remains unknown to what extent the repression of CDK4/6/cyclin D activity might be required for normal spermatogenesis.

Regulation of the pRB/E2F pathway during spermatogenesis

During spermatogenesis, the prototypical CDK-substrate RB1 has been demonstrated to be expressed in both spermatogonia and spermatocytes. The inhibitory CDK-mediated phosphorylation at S795 has also been observed in subsets of spermatogonia. This indicates that active cellular proliferation is mediated by RB1 in these cells [121,133]. In contrast to a primarily
anti-proliferative role for RB1 during mitosis, RB1 in male germ cells seems to prevent differentiation. The conditional deletion of Rb1 expression in male germ cells promotes the differentiation of SSCs, preventing their continued self-renewal. Despite initial fertility in these conditional Rb1−/− animals, infertility occurs by 2 months of age due to a failure to replenish cells competent to enter meiosis [133]. Interestingly, no overt meiotic defects during the first waves of spermatogenesis were observed in these animals, suggesting that RB1 is not essential for either of the two meiotic divisions. Constitutive deletion of Rb1 results in early embryonic lethality [134–136]. In contrast, the RB-related proteins p107 and p130 are not required for viability or fertility [137,138], suggesting that these two proteins are not sufficient to compensate for the loss of Rb1 to ensure normal spermatogenesis. As RB1 is a repressor of E2F signaling, it is presumed that deletion of Rb1 results in unrestrained E2F transcriptional activity. Somewhat at odds with this interpretation is the overexpression of E2F1 in testes induces p53-independent apoptosis in the testes of transgenic mice and results in the apoptosis of both spermatogonia and early primary spermatocytes [139,140]. Such apoptotic cell death was not observed in Rb1 conditional knock-out germ cells.

Interestingly in the converse situation, mice with constitutive deletion of E2f1 exhibit testicular atrophy with aging. This results in the loss of germ cells and subfertility by 3 months of age. This phenotype was also described to progressively worsen with age suggesting that, E2F signaling, at least via E2F1 is required for normal spermatogenesis [141–143]. Similarly to the conditional Rb1 knockout, E2f1−/− testes exhibit a depletion of self-renewing SSCs [143]. Additionally, during the first waves of spermatogenesis, the apoptotic removal of spermatogonia is required for the correct establishment of germ/cell/Sertoli cell ratios [144–146] was noted to be perturbed and increased apoptosis was observed when these cells entered meiosis. E2F1 and several of the other E2F transcription factors have been described to exhibit specific localization during mouse spermatogenesis [143,147–150], both in spermatogonia and meiotically dividing spermatocytes. Despite this, the deletion of E2f2 [151], E2f3 [152], E2f4 [153], or E2f5 [154] does not cause overt spermatogenic defects when deleted in isolation. This, however, does not necessarily rule out the possibility of functional redundancy to allow compensation upon the deletion of certain E2F family members. The remaining E2F family members may modulate transcription independently of the pRB proteins as they lack a pocket protein binding domain and are, thus, less likely subject to CDK regulation [155,156].

Roles for CDK2 in the maintenance of SSC homeostasis

In addition to appropriate pRB-E2F signaling, the regulation of CDK2 activity is also important for normal SSC homeostasis. During spermatogenesis, CDK2 is primarily expressed at high levels in prophase I stage spermatocytes where it has essential functions in mediating homolog synapsis (for details, see below). Interestingly, CDK2 expression has also been noted in SSCs albeit at much lower levels [157,158]. The biological relevance of CDK2 activity in SSCs has only recently been addressed via the analysis of knockin mice with mutations which either partially (Cdk2Y15S/Y15S) or fully (Cdk2Y15A,Y15F/T14A,Y15F) ablate the inhibitory phosphorylatable ‘TY’ motif within the glycine-rich ‘inhibitory loop’ [159,160]. When phosphorylated, the conformation of the inhibitory loop renders the CDK unable to phosphorylate potential substrates due to multiple stable interactions occurring between the CDK catalytic site and bound ATP [161]. As such, CDK2 activity in these models is expected to become uncoupled from its regulatory phosphorylation potentially increasing its activity under circumstances when it should usually be inactivated. Both Cdk2Y15S/Y15S and Cdk2Y15A,Y15F/T14A,Y15F were reported as autosomal semidominant male infertility alleles, which cause male infertility when homozygous and less severe spermatogenic defects when heterozygous [159,160]. Although the spermatogenic defects observed in Cdk2Y15A,Y15F/T14A,Y15F mice have not been investigated in detail, male infertility in the case of Cdk2Y15S/Y15S arises due to a failure of SSC differentiation. Unlike spermatogenesis in adult animals, the first round of spermatogenesis is initiated by gonocytes [162]. Subsequent waves of spermatogenesis are initiated by SSCs following their differentiation from gonocytes during process known as gonocyte-spermatogonia transition (GST) [162,163]. Intriguingly, Cdk2Y15S/Y15S SSCs inappropriately exhibit properties of gonocytes including the cytoplasmic localization of FOXO1 [164,165], suggesting that GST is impaired in circumstances whereby CDK2 cannot be inhibited via its inhibitory loop phosphorylation. Although the initial wave of spermatogenesis is initiated as normal in Cdk2Y15S/Y15S mice and is able to generate spermatocytes proficient to enter meiosis I, subsequent rounds of spermatogenesis are not observed. Adult Cdk2Y15S/Y15S testes are severely atrophic and despite the presence of mitotically proficient SSC-like cells positive for the
self-renewal marker GFRA1 [166,167], these cells lack the ability to mature into differentiating spermatogonia. Furthermore, chains of spermatogonia in these mice were observed to inappropriately retain GFRA1 expression, which is downregulated in wild-type spermatogonia of the same stage. These results suggest that regulation of CDK2 activity is required to appropriately complete the developmental switch between gonocytes and spermatogonia, and the resultant differentiation status of abnormal SSCs is not sufficient to support their further differentiation [168]. Here, it is hypothesized that premature CDK2 activity in Cdk22155S/Y155S gonocytes results in the unscheduled phosphorylation of a factor required for GST. FOXO1 has previously been identified as a CDK2 substrate [169,170], making it an interesting candidate in mediating such a transitory role in the determination of germ cell fate [168]. It may be of interest for future studies to investigate whether the repression of CDK-associated kinase activity is a requirement for normal GST and whether this is only applicable to CDK2. Canonically, the ‘TY’ motif is phosphorylated and de-phosphorylated by the WEE1 kinase and CDC25 family of phosphatases, respectively [1,171–173]. As such, pharmacological inhibition or conditional knockout of these regulatory proteins might be useful in addressing such questions.

Regulation of CDK activity by the CIP/KIP class of CKI proteins during spermatogenesis

In addition to the INK4 protein family of CKI proteins, knockout mice have also been generated for the CIP/KIP family of CKI proteins comprised of p21CIP1 (Cdkn1a), p27KIP1 (Cdkn1b), and p57KIP2 (Cdkn2c). This family is less specific than INK4 and is, thus, able to interact with a broader range of CDK/cyclin complexes including CDK1/CDK2 in addition to CDK4/CDK6.

The deletion of Cdkn1a, Cdkn1b, or both in combination is not associated with spermatogenic defects in male mice [174–177]. Although female infertility is seen in Cdkn1b−/− female animals, this results from hyperplasia of the supporting granulosa cells of the ovary which negatively impact the maturation of ovarian follicles [174–176]. p21CIP1 and p27KIP1 also seem to play a role in restricting the proliferation of Sertoli cells in the testis in early development before these cells become mitotically quiescent. As such, testicular organomegaly is seen upon ablation of Cdkn1a, Cdkn1b, or both in combination [178], due to greater total numbers of Sertoli cells.

Interestingly, severe meiotic defects have been observed when endogenous levels of p27KIP1 are increased via deletion of the F-box protein SKP2. Under normal circumstances, SKP2 is required for the ubiquitination of p27KIP1, and thus, in this mutant, p27KIP1 is degraded at a lower rate. Minimal fertility resulting from widespread apoptotic loss of spermatogenic cell types is seen in Skp2−/− males, and severe ovarian degeneration was also noted in female animals. Although a detailed analysis of the arrest stage of gametogenesis in these mice was not performed, apoptosis was noted to affect cells in most stages of spermatogenesis. Deletion of Cdkn1b in Skp2−/− mice restores fertility, in both male and female mice confirming that abnormally high levels of p27KIP1 were indeed the cause of defective gametogenesis in these mice [179,180]. As p27KIP1 has the potential to inhibit both CDK2 and CDK4-associated complexes and both Cdk2−/− and Cdk4−/− mice display fertility defects, it is likely that these defects arise due to a repression of either one of these kinases. Unlike p21CIP1 or p27KIP1, specific expression of p57KIP2 has been noted during spermatogenesis in both spermatogonia and early spermatocytes [181,182]. The deletion of Cdkn2c leads to neonatal death in the majority of mutant mice [183,184]. The small number of Cdkn2c−/− mice that survive until adulthood exhibits immaturity of germinal tissues including testis and ovaries, which suggests that this gene might be necessary for the normal differentiation of germ cells [185]. However, this phenotype was not described in detail and is complicated by the many additional phenotypic defects caused by constitutive Cdkn2c deletion and would likely require the generation of a meiosis-specific conditional knockout model for any further investigation.

Meiosis-specific roles for CDK-associated activity

Aside from the well-characterized roles for the CDKs and their activating proteins during cell cycle progression, several of these proteins also exhibit meiosis-specific functions. The standout example of this is arguably CDK2. In addition to the aforementioned role in the differentiation and fate decision process of SSCs, this kinase seems to be involved in several aspects of meiotic division, particularly during meiotic prophase I. One of the most best-studied of these functions is promoting the normal synopsis of chromosomes homologs. In this context, CDK2 is reliant upon the expression of a non-cyclin-activating partner protein, Speedy A. To complicate matters, the canonical CDK2 activators, cyclin E1 and E2, are also essential...
for homolog synapsis. Accordingly, severe meiotic defects akin to that seen upon individual deletion of Cdk2 or Speedy A can also be observed upon the complete ablation of both E-type cyclins. The lack of functional redundancy between the Speedy A and the E-type cyclins, despite sharing CDK2 as their most likely kinase partner, is intriguing and requires further investigation to be properly understood. The following sections will primarily describe the known synaptic defects arising from deletion of CDK2 or its activating proteins. Additional topics discussed in this section are far more speculative and include poorly defined roles for the E-type cyclins in promoting telomere stability in addition to potential roles for CDK2 and CDK4 in regard to various stages of meiotic recombination.

**Potentiation of telomeric CDK2 binding by Speedy A during meiotic prophase I**

A telomeric function for CDK2 during meiosis was first investigated due to the localization of this kinase to telomeres throughout the duration of meiotic prophase I alongside components of the multiprotein shelterin complex [157]. In somatic cells, the shelterin complex forms a protective cap at telomeres, which prevents the inappropriate activation of the DNA damage response against telomeric DNA [186]. During meiotic prophase I, the shelterin complex fulfill a secondary role in ‘tethering’ telomeres to the LINC complex during zygonema (see introduction for prior discussion of the LINC complex). One initial step in the formation of the LINC complex is the interaction between the core shelterin complex member TRF1 and the noncanonical CDK2 activator: Speedy A. Here, TRF1 is thought to act as a scaffold to promote the interaction of Speedy A and CDK2 at the telomeres [65]. In accordance with this theory, telomeres of Trf1−/− spermatocytes show a complete failure to recruit both CDK2 and Speedy A [65]. Additional evidence suggests that the loading of Speedy A to shelterin is likely to occur at the nuclear envelope, as Speedy A can be detected as multiple foci at this structure prior to the initial tethering of telomeres to the nuclear envelope. Importantly, this interaction is also a prerequisite step for the telomeric binding of CDK2, as telomeric CDK2 foci cannot be observed in Speedy A−/− spermatocytes [187]. This CDK2-recruiting role for Speedy A is supported by the observation that mutagenesis of key Speedy A-interacting residues on CDK2 negatively impacts the ability of CDK2 to localize to telomeres [187]. One key factor driving the interaction between CDK2 and Speedy A seems to be the existence of a longer meiotic-specific splice isoform of CDK2 (p39) [22]. p39 CDK2 is a preferential partner of Speedy A in meiotic cells as opposed to the normal (<33 kDa) isoform, which forms canonical complexes with cyclins [22,187,188]. The relevance pertaining to the existence of two distinct isoforms of CDK2 during meiotic prophase is still not fully understood. However, this could potentially be utilized to compartmentalize the activities of both CDK2/Speedy A and CDK2/cyclin complexes for specific cellular processes.

**The roles of CDK2 and Speedy A in promoting stable LINC complex formation**

During the leptotene–zygotene transition, complex interactions occurring between components of the LINC complex and the cytoskeleton drive the polarization of membrane-bound proteins and the tethered telomeres. This forces telomeres into close proximity driving their clustering within a small area of the nuclear envelope, which is responsible for the characteristic ‘bouquet’ pattern of chromosomes observed at this stage [189,190]. Bouquet formation is absolutely dependent upon the prior establishment of telomere–LINC complex interactions in the leptotene stage, as this does not occur in mutants where LINC complex components or their interacting proteins have been deleted [59,61,62,65,67,187,191,192]. Following initial telomeric clustering, cytoskeletal forces promote rapid prophase movements (RPM), which allow chromosomes to sample their surroundings for homology in a controlled manner [193–195]. This is thought to be essential for homologs to locate each other and synapse without the occurrence of inappropriate pairing between nonhomologs (nonhomologous synopsis). The involvement of CDK2 and Speedy A during bouquet formation and synapsis is depicted in Fig. 2 with examples of meiotic defects observed upon a failure to establish normal telomere–nuclear envelope interactions.

Upon deletion of CDK2 or Speedy A, stable telomere–nuclear envelope interactions are lost and telomeres are observed to become detached from the inner nuclear envelope despite initial binding. In both models, it was noted that detached telomeres remain associated with vesicles containing proteins associated with the inner nuclear membrane including SUN1, suggesting a disconnect between the telomeres and the components of the LINC complex at the inner nuclear envelope [61,191]. As a consequence, extensive nonhomologous synopsis occurs between chromosomes.
addition, telomeres engage in inappropriate inter- and intrachromosomal interactions resulting in telomeric fusions and the observance of ring chromosomes [61,191]. Upon failure of synopsis in Cdk2<sup>−/−</sup> and Speedy A<sup>−/−</sup> spermatocytes, chromosomes are no longer able to access their homologous counterparts to initiate the repair of meiotic DSBs by homologous recombination. Meiotically arrested spermatocytes subsequently undergo apoptosis, causing a complete block of spermatogenesis and infertility [61,196,197]. The exact reason why CDK2 and Speedy A are required to maintain stable telomere–nuclear envelope interactions is uncertain. It is likely due to a failure to form proper interactions between the telomere and the inner nuclear envelope protein SUN1. In wild-type spermatocytes, SUN1 localizes to telomeres tethered to the nuclear envelope during leptonema and remains bound whilst chromosomes adopt a bouquet arrangement [59,62]. In Cdk2<sup>−/−</sup> and Speedy A<sup>−/−</sup> spermatocytes, SUN1 fails to form a polarized cap along the nuclear envelope leading to the formation of a characteristic bouquet pattern of chromosomes. Bouquet formation in pachynema brings chromosomal homologs into close proximity and facilitates homolog paring (synapsis), which is completed in pachynema. An example of meiotic defect occurring upon impaired formation and/or stability of the LINC complex is shown at the bottom left hand side. These defects are characteristic of Cdk2<sup>−/−</sup> and Speedy A<sup>−/−</sup> spermatocytes and also mutants whereby shelterin or LINC complex components have been deleted.
point linking the telomere and nuclear envelope, it is tempting to speculate that phosphorylation of SUN1 might stabilize its interaction with other LINC complex components, allowing telomeres to be grouped into their bouquet formation. The existence of a CDK2 substrate driving this process is supported by the fact that a knockin point mutant of Cdk2 where catalytic activity has been ablated (Cdk2<sup>D145N/D145N</sup>) shows essentially the same phenotype as both Cdk2<sup>+/−</sup> and Speedy A<sup>+/−</sup> spermatocytes [198]. This is despite normal telomeric localization of both mutant CDK2<sup>D145N</sup> and Speedy A (unpublished data from the Kaldis laboratory).

This observed requirement for CDK2 catalytic activity is somewhat at odds with the observation that a truncated Speedy A protein, which is competent to bind telomeres and CDK2 but unable to activate CDK2, is able to rescue impaired telomere–nuclear envelope interactions (when electroporated into Speedy A<sup>−/−</sup> testis). This suggested that CDK2/Speedy A catalytic activity (against SUN1 or otherwise) is not required for this process [187]. An alternate explanation is that catalytically inactive CDK2/Speedy A is able to maintain some degree of telomeric–nuclear envelope binding via noncatalytic interactions with SUN1 and/or its related protein SUN2. Relevant to this point is the observance that even in Sun1<sup>−/−</sup> mice, suboptimal levels of telomeric tethering and even bouquet formation can be observed although this is ultimately not sufficient for normal synopsis in pachynema [69]. Further investigation into the candidacy of SUN1 as a biologically relevant CDK2/Speedy A substrate will likely require the generation of a nonphosphorylatable Sun1 mouse mutant.

The importance of E-type cyclins for normal synopsis and telomere stability

Recent revelations obtained from knockout models of the LINC complex interactors TERB1, TERB2, and MAJIN have demonstrated the importance of a process termed telomere cap exchange for normal fertility. This event is thought to maintain the telomeric stability of membrane-bound chromosomes during pachynema [64,199]. During cap exchange, telomeric DNA is transferred to a complex containing TERB1, TERB2, and MAJIN. This requires the dissociation of the shelterin complex proteins from the telomeric ends and results in the formation of a structure known as the telomere attachment plate, which integrates telomeric DNA within the inner nuclear envelope [64,200–207]. The formation of telomere attachment plates can be determined via the appearance of conical thickenings at the ends of pachytene stage chromosomes. Interestingly, the typical formation of these structures is seemingly dependent upon the expression of the E-type cyclins. Upon deletion of cyclin E2, the conical thickening of telomeric ends is compromised in a manner that can be progressively worsened upon additional deletion of one, or both copies of cyclin E1. In such cyclin E mutant spermatocytes, the failure to develop normal telomere attachment plates was associated with the observance of telomeres within the nuclear space. Furthermore, such abnormal telomeres showed reduced localization of shelterin complex components and positivity for the DNA damage marker, γ-H2AX, suggesting inappropriate protection of telomeric DNA [208,209]. Despite such distinct telomeric defects, the E-type cyclins do not exhibit telomeric localization during meiotic prophase, making their interaction with telomeric CDK2 uncertain [209]. However, CDK2 has been shown to co-immunoprecipitate both cyclin E1 and cyclin E2 in protein extracts from wild-type pachytene spermatocytes. This suggests that a pool of likely nontelomeric CDK2/cyclin E is indeed present in meiotic cells [209]. A functional relationship between CDK2 and the E-type cyclins is also supported by the observation that the deletion of these proteins disrupts normal patterns of CDK2 localization during meiotic prophase. In cyclin E2<sup>−/−</sup> spermatocytes, for example, only 59% of telomeres were reported to show CDK2 binding at intensities similar to that of wild-type controls. The additional deletion of one or both copies cyclin E1 subsequently resulted in complete loss of CDK2 binding to the telomeres. As both the severity of telomere defects and loss of CDK2 localization were similarly affected by progressive cyclin E loss [208,209], it is likely that the action of these cyclins creates a stable environment at telomeres for CDK2 to bind.

In addition to the loss of shelterin integrity, another possibility could be that the cap exchange is defective in the absence of E-type cyclins. Since the process of telomeric cap exchange requires the dissociation of the shelterin complex from telomeric ends, it is possible that if this process is perturbed, uncapped telomeric ends dislodged from the nuclear envelope might be exposed within the nuclear interior. This would trigger a DNA damage response directed against the uncapped telomeres, similar to that seen upon ablation of the E-type cyclins. Fascinatingly, cap exchange has been shown to be influenced by CDK activity as the treatment of wild-type spermatocytes with the unspecific CDK2-inhibitor Roscovitine [210] leads to abolished cap exchange [64]. In regard to a potential CDK-substrate mediating this process, Thr647 of
TERB1 was shown to be phosphorylated during cap exchange. However, electroporation of a nonphosphorylatable TERB1 T647A protein into the testis of Terb1−/− mice was able to rescue otherwise defective cap exchange making the biological relevance of this phosphorylation uncertain [64].

**Potential CDK2 functions in the formation and/or designation of meiotic crossover sites**

In addition to promoting homolog synapsis via its function at meiotic telomeres, CDK2 is also known to localize to late recombination nodules during meiotic prophase. In this capacity, CDK2 foci can be transiently observed to localize to 1-2 interstitial sites associated with the chromosomal axes of each homolog pair during midpachytene stage of prophase I [157,159,191,209,211–214]. Here, CDK2 co-localizes with an E3 sumo-ligase, RNF212, and a proposed sumo-targeting ubiquitin ligase, CCNBIP1 (HEI10) [211,213]. These proteins, together with CDK2, have been described as pro-crossover factors. This is due to their role in the formation and maturation of late recombination nodules, which is associated with the subsequent recruitment of MutLγ (MLH1, MLH3). This is one of the final steps required for the eventual repair of recombination intermediates to form type I crossover sites [215–219]. Late recombination nodule-associated CDK2 foci appear independently of Speedy A, which is solely observed at telomeres during meiotic prophase I [187], suggesting that any activity of CDK2 at these sites is likely mediated by other activating proteins.

During early meiotic prophase, abundant RNF212 foci can be observed to localize to sites of recombination. During pachynema, a subset of RNF212 foci are able to achieve a ‘stable’ state. This is associated with the subsequent localization of HEI10. Both the increase in RNF212 foci size and the presence of HEI10 foci are considered indicators of sites selected for crossover designation. During mid-pachynema, a subset of RNF212 foci are able to achieve a ‘stable’ state. This is associated with the subsequent localization of HEI10. Both the increase in RNF212 foci size and the presence of HEI10 foci are considered indicators of sites selected for crossover designation. During mid-pachynema, a subset of RNF212 foci are able to achieve a ‘stable’ state. This is associated with the subsequent localization of HEI10. Both the increase in RNF212 foci size and the presence of HEI10 foci are considered indicators of sites selected for crossover designation.

**Fig. 3.** Schematic of the crossover designation process. Upper box: pattern of early and late recombination nodule formation during meiotic prophase I. During early pachynema, proteins associated with early recombination nodules (early RN) localize as many distinct foci along the chromosomal axes. Here, paired chromosomal axes are represented by a red line and early recombination nodules are represented by blue circles. By mid-pachynema, crossover designation leads to the selection of 1 or maximally 2 early recombination nodules to mature into late recombination nodules (late RN) as shown by green circles. At the same time, non-designated early recombination nodules are repaired and their associated proteins dissociate from the chromosomal axes. By late pachynema, only late RNs remain associated with the chromosomal axes. These mark the sites at which meiotic crossovers will form. Lower box: Events leading to crossover designation or crossover de-selection at early recombination nodules. During early pachynema, early RNs exhibit specific localization of many proteins including the MutS complex comprised of MSH4 (blue ring) and MSH5 (red ring). MutS is thought to be stabilized by SUMOylation mediated by RNF212 (yellow square). During mid-pachynema, one of two possibilities can occur at early RNs, crossover designation (left hand side) or crossover de-selection (right hand side). Crossover de-selection occurs when RNF212 becomes destabilized and dissociates from early RNs. This is thought to allow the ubiquitin ligase CCNBIP1 (HEI10) (red square) to target early RN proteins such as MutS for ubiquitination and degradation. These events are associated with the downstream repair of early RNs as non-crossovers. Crossover designation occurs when RNF212 remains stabilized at early RNs. This is associated with the localization of HEI10 and CDK2. These events are characteristic of late RN formation and leads to the downstream recruitment of the MutLγ complex comprised of MLH1 (green ring) and MLH3 (purple ring). These events are required for the downstream repair of late RNs as crossovers.
to become late recombination nodules. They are also termed as crossover designation [211]. Prior to crossover designation, RNF212-mediated SUMOylation is thought to stabilize the MutS\(_c\) (MSH5, MSH5) complex at recombination sites, promoting their progression toward crossover-associated repair. At smaller ‘undesignated’ RNF212 foci, RNF212-mediated SUMOylation also seems to act as a substrate for HEI10. This allows the ubiquitination and turnover of recombination proteins, including MutS\(_c\) and RNF212 itself, at recombination sites [220]. Sites depleted of MutS\(_c\) and RNF212, fail to mature, and are subsequently repaired as non-crossovers. Therefore, the action of HEI10 can be considered as a mechanism to deselect all but ‘designated’ RNF212 foci from maturing into late recombination nodules. This effectively prevents the formation of excess meiotic crossover sites. A model of crossover designation indicating the observance of CDK2 during this process is presented in Fig. 3. This is based upon the currently known functions of RNF212 and HEI10, as reported by the Hunter laboratory [211,213].

In addition to RNF212, HEI10, and CDK2, an atypical cyclin protein, ‘cyclin N-terminal domain-containing protein 1’ (CNTD1), has also been identified as a pro-crossover factor. This protein seems to be essential for the de-selection of undesignated crossover sites. In both Cntd1\(^{-/-}\) and Hei10\(^{-/-}\) spermatocytes, stable foci containing both MutS\(_c\) and RNF212 foci fail to undergo the de-selection process in pachynema and crossover designation does not occur [213,221]. The relationship between CNTD1 and HEI10 is still not understood, but one possibility could be that CNTD1 is important for the destabilization of RNF212 at ‘nondesignated’ sites. This destabilization would then allow HEI10 to better target RNF212-SUMOylated targets for degradation.

At present, the requirement for CDK2 localization at late recombination nodules remains unknown, but this is seemingly affected by the loss of other pro-crossover factors. Upon singular deletion of Rnf212, Hei10, or Cntd1, CDK2 localization to recombination nodules is severely depleted whilst telomeric CDK2 binding remains seemingly unaffected [211,214,221]. This indicates that these proteins are responsible or required for the localization of CDK2 to recombination nodules. As crossover designation fails to occur in each of these models, it is likely that the binding and/or action of CDK2 at these recombination nodules either occurs in parallel or directly after the observance of this process. In regard to the possible functions of CDK2 at these sites, it has previously been suggested that CDK2 might form a complex with CNTD1, which functions within the crossover designation process [221]. However, at present there is no cytological
Evidence that CNTD1 is able to localize to recombination nodules or indeed that this protein might interact with CDK2 to form an active kinase complex. It should be noted here that the homolog of CNTD1 in *C. elegans*, COSA-1, can localize to meiotic crossover sites [222–224]. However, the comparison of COSA-1 functions with mammalian CNTD1 is complicated by considerable differences in the process of crossover formation in *C. elegans*. For example, in this organism, MutS\(\gamma\) seems to function during the final stages of crossover formation and can be observed to localize at crossover sites in late meiotic prophase in a similar manner as described for mammalian MutL\(\gamma\). Furthermore, although MutS\(\gamma\) foci are stabilized upon deletion of *Cnd1*, MutS\(\gamma\) becomes destabilized in *Cosa-1* mutants [222]. One model addressing potential crossover-associated CDK2 functions suggested that HEI10 might promote the destruction of CDK2-bound cyclin allowing it to bind late recombination nodules, possibly in complex with another crossover-specific interactor [214]. As a matter of fact, the formal gene name for *Hei10* is cyclin B1-interacting protein 1 (*Cumb1ip1*), which was given due to the discovery that HEI10 can interact with cyclin B1 in a yeast two-hybrid screen [225]. In addition, HEI10 is also a known substrate of cyclin B-associated CDK activity in vitro [225] and contains several potential CDK consensus sites for phosphorylation within its C terminus. Of these sites, Ser242 (in mouse) is conserved in multiple eukaryotic species and harbors a putative cyclin binding motif upstream of this residue, making it a possible candidate substrate for CDK2 [225]. The importance of HEI10 for meiotic crossover formation was originally identified in an infertile, *N*-ethyl-*N*-nitrosourea-induced mutant mouse model (*Hei10*<sup>Mei4/Mei4</sup>) [214]. This model resulted in a partial deletion of the N terminus of HEI10 within another predicted cyclin binding motif, suggesting that loss of HEI10 functions, in this case, might have been caused by the loss of cyclin interaction. To unravel the mysterious functions of CDK2 and its associated proteins at late recombination nodules, it may be a requirement to determine the network of proteins localized specifically to these sites. This might be achievable via immunoprecipitation of the crossover-associated proteins HEI10 or RNF212 followed by mass spectrometry.

**Unexplored roles for cyclin D-dependent kinase activity during spermatogenesis**

In addition to being readily detectable in spermatogonia, CDK4 alongside its partner proteins cyclin D2 and cyclin D3 can also be detected in meiotically dividing spermatocytes [97,112,157]. In support of a functional role for CDK4 during meiotic division, at least two independent laboratories have indicated that CDK4 is able to localize to the chromosomal axes of spermatocytes in zygotene of meiotic prophase. Here, CDK4 appears as foci in areas where homologous chromosomes have synapsed. CDK4 foci persist until mid-pachynema at which time they can no longer be observed [157,221]. Such localization has not been reported for CDK6, suggesting that these roles during meiotic prophase might be unique to CDK4. The localization pattern of CDK4 suggests that this kinase might be required for homolog synopsis sometime after meiotic recombination has been initiated. Based on this localization pattern, it is possible that CDK4 might be required to stabilize interactions between the axial and lateral/central elements of the synaptonemal complex to promote their interaction. Many such proteins including SYCP1, SYCE1, and TEX12 have already been noted to be phosphorylated during meiotic prophase I [226,227]. Another possibility is that CDK4 might help to destabilize proteins associated with asynapsed axes to promote their dissociation upon completion of synopsis. For example, the HORMA domain-containing proteins, HORMAD1 and HORMAD2, are prevalent on asynapsed axes but are quickly removed upon synopsis [228,229]. One further possibility could be that CDK4 might interact with complexes such as MutS\(\gamma\) to stabilize early recombination nodules. In *Cnd1<sup>−/−</sup>* mice, CDK4 foci persist at high level throughout pachynema and can be observed in late pachytene spermatocytes. This was similar to the persistence of both RNF212 and the MutS\(\gamma\) complex, also observed in these mutant spermatocytes. This potentially suggests a link between stabilized recombination intermediates and CDK4. If such a stabilizing role is undertaken by CDK4, other possible CDK4 interactors could be the SPO16-SHOC2 complex and/or TEX11. These proteins are required for stable MutS\(\gamma\) foci formation and also appear to localize to chromosomal axes during similar stages of meiotic prophase, as described for CDK4 [230,231]. To date, interactions between CDK4 and meiotic interactors have not been investigated, and the role of this kinase is still uncertain. Elucidation of the possible function of axes associated CDK4 foci will likely require an in-depth analysis of the spermatogenic defect of *Cdk4<sup>−/−</sup>* mice. In regard to this question, the preparation of meiotic surface spreads from *Cdk4<sup>−/−</sup>* might be informative as to whether CDK4 contributes toward meiotic processes such as synopsis or meiotic crossover formation.
Potential CDK2 functions related to the regulation of transcription in male germ cells

In addition to the known and potential meiotic roles of CDK2 discussed above, the Kaldis laboratory has recently identified a novel interaction between CDK2 and chromatin [232]. This is mediated by interactions between CDK2 and the nuclear respiratory factor 1 (NRF1) in male germ cells. Remarkably, through ChIP-seq and ChIP-reChIP approaches, we detected interactions of CDK2 with chromatin-bound NRF1 at its target gene promoters. Despite the fact that this transcription factor is canonically associated with the regulation of mitochondrial respiration, many of the genes regulated by NRF1 in male germ cells were found to function within meiotic processes, a finding also noted by a prior study [232,233]. Although a thorough analysis of this relationship in meiotic cells was precluded by the early arrest stage of Cdk2<sup>−/−</sup> spermatocytes and the lethality associated with Nrf1 deletion [234], we were able to observe that CDK2 was a negative regulator of NRF1 transcriptional activity. In line with this theory, we were also able to demonstrate that the DNA binding domain of NRF1 is a substrate of CDK2 and that the phosphorylation by CDK2 decreased NRF1 binding activity in chromatin. In terms of the biological relevance of this interaction, we found that the expression of one of the many NRF1/CDK2 targets, Ehmt1, was elevated in germ cells upon conditional deletion of Cdk2. This was associated with the perturbation of EHMT1-dependent placement of H3K9me2, during the zygotene–pachytene transition. Together, these findings led us to hypothesize that the expression of CDK2 might be important to modulate NRF1 transcriptional activity during meiotic prophase, which affects the transcription of many meiotic genes including Ehmt1, Msh4, Asz1, Syce1, and Tex19.1. This hypothesis might be best tested in future studies via the conditional deletion of Nrf1 in spermatocytes, utilizing a previously described Nrf1<sup>fl/fl</sup> model [233]. This work further underlines that CDK2 has multiple functions in germ cell development in addition to the established function of tethering telomeres to the nuclear envelope. It will be interesting to determine the cyclin partners and the specific CDK2 substrates that regulate each of the diverse functions of CDK2.

Outstanding questions and outlook

Knockout mouse models have long been the method of choice when ascribing functions to the various cell cycle-associated genes. An almost exhaustive list of CDK and cyclin knockout mouse models have now been described, offering a great deal of information about the activity of CDKs and their regulatory proteins during gametogenesis. Although many of these roles have been described in the sections above, this is by no means an exhaustive list. Notable exclusions excluded from this review are CDK1, as well as the A-type and B-type cyclins. Currently, it is not possible to investigate the effects of cyclin A2 or cyclin B1 deletion due to their requirement for normal development [235,236]. Although conditional deletion of Cdk1 in spermatocytes or constitutive deletion of cyclin A1 has been shown to cause male infertility, these proteins primarily function to drive the exit from meiotic prophase I. Accordingly, these proteins are not required for meiotic recombination, synopsis of homologs, or meiotic crossover formation [237–240]. Similarly, cyclins B2 and B3 are not required during meiotic prophase. Although cyclin B3 deletion results in female infertility, this arises due to its role in triggering anaphase I exit in oocytes [236,241,242]. There is ample space for future investigations to determine the functions of these cell cycle regulators in germ cell development and meiosis.

In regard to future research, we perceive considerable scope to uncover novel information regarding CDK functions during germ cell development. For example, many of the so-called atypical cyclins are known to exhibit biased or heightened expression in meiotic tissues [243]. Although the function of these proteins is mostly unexplored, knockout models for several of these genes have been described to exhibit fertility defects [244,245]. In a similar vein, the Speedy/RINGO family members Speedy B1a, Speedy B1b, and Speedy B3 [16,246] also exhibit high levels of expression in meiotic tissues. At present, genetic knockouts for these proteins have not been described and it is, therefore, uncertain whether these proteins might be essential for meiotic division as has been described for Speedy A.

As illustrated by this review, normal reproductive health is dependent upon the proper expression and regulation of various CDK/complexes at distinct developmental stages of germ cell development. It is our view that important questions remain surrounding at least five major topics covered in this review:

1. What is the cause of spermatogenic arrest in Cdk4<sup>−/−</sup> mice? Does this arise from defects in spermatogonial proliferation/differentiation, or from a failure to complete meiotic division?
2 Why does CDK4 localize to early recombination nodules and what might its activating partner at such sites be?

3 Why do Speedy A and the E-type cyclins have nonredundant roles in promoting chromosomal synopsis? Is this due to their actions at different stages of meiotic prophase? Do the E-type cyclins form a complex with CDK2 during meiotic prophase or are their actions CDK2-independent?

4 How does CDK2/Speedy A stabilize the LINC complex to ensure proper bouquet formation and synopsis? Could this be via the phosphorylation of SUN1 or other related proteins?

5 What is the purpose of CDK2 localization to late recombination nodules? Does CDK2 partner with a canonical cyclin at these sites or alternatively, an atypical interactor such as CNTD1? Does CDK2 have a specific substrate at these sites, such as HEI10, which it must phosphorylate to promote crossover designation/maturation?

To elucidate the molecular mechanisms behind, the functions of CDKs in germ cell development will not only uncover more details about meiosis but will also help to understand the prevalent fertility issues that has been observed in the human population.

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References

1 Morgan DO (1997) Cyclin-dependent kinases: engines, clocks, and microprocessors. Annu Rev Cell Dev Biol 13, 261–291.

2 Cao L, Chen F, Yang X, Xu W, Xie J and Yu L (2014) Phylogenetic analysis of CDK and cyclin proteins in premetazoan lineages. BMC Evol Biol 14, 10.

3 Doonan JH and Kitsios G (2009) Functional evolution of cyclin-dependent kinases. Mol Biotechnol 42, 14–29.

4 Lim S and Kaldes P (2013) Cdks, cyclins and CKIs: roles beyond cell cycle regulation. Development 140, 3079–3093.

5 Loyer P, Trembley JH, Katona R, Kidd VJ and Lahti JM (2005) Role of CDK/cyclin complexes in transcription and RNA splicing. Cell Signal 17, 1033–1051.

6 Sherr CJ and Roberts JM (2004) Living with or without cyclins and cyclin-dependent kinases. Genes Dev 18, 2699–2711.

7 Malumbres M, Harlow E, Hunt T, Hunter T, Lahti JM, Manning G, Morgan DO, Tsai LH and Wolgemuth DJ (2009) Cyclin-dependent kinases: a family portrait. Nat Cell Biol 11, 1275–1276.

8 Malumbres M (2014) Cyclin-dependent kinases. Genome Biol 15, 122.

9 Murray AW and Marks D (2001) Can sequencing shed light on cell cycling? Nature 409, 844–846.

10 Hydbring P, Malumbres M and Sicinski P (2016) Non-canonical functions of cell cycle cyclins and cyclin-dependent kinases. Nat Rev Mol Cell Biol 17, 280–292.

11 Stevenson LM, Deal MS, Hagopian JC and Lew J (2002) Activation mechanism of CDK2: role of cyclin binding versus phosphorylation. Biochemistry 41, 8528–8534.

12 Morgan D (2012) The Cell Cycle Principles of Control. Oxford University Press, Oxford.

13 Solomon MJ (1993) Activation of the various cyclin/ cdc2 protein kinases. Curr Opin Cell Biol 5, 180–186.

14 Cheng A, Gerry S, Kaldes P and Solomon MJ (2005) Biochemical characterization of Cdk2-Speedy/Ringo A2. BMC Biochem 6, 19.

15 McGrath DA, Fifield BA, Marceau AH, Tripathi S, Porter LA and Rubin SM (2017) Structural basis of divergent cyclin-dependent kinase activation by Spy1/ RINGO proteins. EMBO J 36, 2251–2262.

16 Porter LA, DELLinger RW, Tynan JA, Barnes EA, Kong M, Lenormand JL and Donoghue DJ (2002) Human Speedy: a novel cell cycle regulator that enhances proliferation through activation of Cdk2. J Cell Biol 157, 357–366.

17 Karaiskou A, Perez LH, Ferby I, Ozon R, Jessus C and Nebreda AR (2001) Differential regulation of Cdc2 and Cdk2 by RINGO and cyclins. J Biol Chem 276, 36028–36034.

18 Ferby I, Blazquez M, Palmer A, Eritja R and Nebreda AR (1999) A novel p34cdc2 binding and activating protein that is necessary and sufficient to trigger G2/M
progression in Xenopus oocytes. Genes Dev 13, 2177–2189.
19 Cheng A, Xiong W, Ferrell JE Jr and Solomon MJ (2005) Identification and comparative analysis of multiple mammalian Speedy/Ringo proteins. Cell Cycle 4, 155–165.
20 Dinarina A, Perez LH, Davila A, Schwab M, Hunt T and Nebreda AR (2005) Characterization of a new family of cyclin-dependent kinase activators. Biochem J 386, 349–355.
21 Yang R, Morosetti R and Koellef HP (1997) Characterization of a second human cyclin A that is highly expressed in testis and in several leukemic cell lines. Cancer Res 57, 913–920.
22 Ellenrieder C, Bartosch B, Lee GY, Murphy M, Sweeney C, Hergersberg M, Carrington M, Jaussi R and Hunt T (2001) The long form of CDK2 arises via alternative splicing and forms an active protein kinase with cyclins A and E. DNA Cell Biol 20, 413–423.
23 Nguyen TB, Manova K, Capodieci P, Lindon C, Bottega S, Wang XY, Refik-Rogers J, Pines J, Wolgemuth DJ and Koff A (2002) Characterization and expression of mammalian cyclin B3, a prepauchyten meiotic cyclin. J Biol Chem 277, 41960–41969.
24 Chotiner JY, Wolgemuth DJ and Wang PJ (2019) Functions of cyclins and CDKs in mammalian gametogenesis. Biol Reprod, 101, 591–601.
25 Borum K (1961) Oogenesis in the mouse. A study of the meiotic prophase. Exp Cell Res 24, 495–507.
26 Speed RM (1982) Meiosis in the foetal mouse ovary. I. An analysis at the light microscope level using surface-spread. Chromosoma 85, 427–437.
27 Oakberg EF (1956) Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. Am J Anat 99, 507–516.
28 de Rooij DG and Russell LD (2000) All you wanted to know about spermatogonia but were afraid to ask. J Androl 21, 776–798.
29 Manova K, Nocka K, Besmer P and Bachvarova RF (1990) Gonadal expression of c-kit encoded at the W locus of the mouse. Development 110, 1057–1069.
30 Yoshinaga K, Nishikawa S, Ogawa M, Hayashi S, Kunisada T and Fujimoto T (1991) Role of c-kit in mouse spermatogenesis: identification of spermatogonia as a specific site of c-kit expression and function. Development 113, 689–699.
31 Liskay RM (1977) Absence of a measurable G2 phase in two Chinese hamster cell lines. Proc Natl Acad Sci USA 74, 1622–1625.
32 Leblond CP and El-Alfy M (1998) The eleven stages of the cell cycle, with emphasis on the changes in chromosomes and nucleoli during interphase and mitosis. Anat Rec 252, 426–443.
33 Bennett MD (1977) The time and duration of meiosis. Philos Trans R Soc Lond B Biol Sci 277, 201–226.
34 Keeney S, Giroux CN and Kleckner N (1997) Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell 88, 375–384.
35 Bergerat A, de Massy B, Godelle D, Varoutas PC, Nicolas A and Forster P (1997) An atypical topoisoasmease II from Archaea with implications for meiotic recombination. Nature 386, 414–417.
36 Baudat F, Imai Y and de Massy B (2013) Meiotic recombination in mammals: localization and regulation. Nat Rev Genet 14, 794–806.
37 Brick K, Smagulova F, Khil P, Camerini-Otero RD and Petukhova GV (2012) Genetic recombination is directed away from functional genomic elements in mice. Nature 485, 642–645.
38 Grey C, Barthes P, Chauveau-Le Friec G, Langa F, Baudat F and de Massy B (2011) Mouse PRDM9 DNA-binding specificity determines sites of histone H3 lysine 4 trimethylation for initiation of meiotic recombination. PLoS Biol 9, e1001176.
39 Baudat F, Buard J, Grey C, Fledel-Alon A, Ober C, Przeworski M, Coop G and de Massy B (2010) PRDM9 is a major determinant of meiotic recombination hotspots in humans and mice. Science 327, 836–840.
40 Parvanov ED, Petkov PM and Paigen K (2010) Prdm9 controls activation of mammalian recombination hotspots. Science 327, 835.
41 Walker M, Billings T, Baker CL, Powers N, Tian H, Saxl RL, Choi K, Hibbs MA, Carter GW, Handel MA et al. (2015) Affinity-seq detects genome-wide PRDM9 binding sites and reveals the impact of prior chromatin modifications on mammalian recombination hotspot usage. Epigenetics Chromatin 8, 31.
42 Pan J, Sasaki M, Kniewel R, Murokami H, Blitzblau HG, Tischfield SE, Zhu X, Neale MJ, Jasim M, Socci ND et al. (2011) A hierarchical combination of factors shapes the genome-wide topography of yeast meiotic recombination initiation. Cell 144, 719–731.
43 Baudat F and Nicolas A (1997) Clustering of meiotic double-strand breaks on yeast chromosome III. Proc Natl Acad Sci USA 94, 5213–5218.
44 Gerton JL, DeRisi J, Shroff R, Lichten M, Brown PO and Petes TD (2000) Global mapping of meiotic recombination hotspots and coldspots in the yeast Saccharomyces cerevisiae. Proc Natl Acad Sci USA 97, 11383–11390.
45 Buhler C, Borde V and Lichten M (2007) Mapping meiotic single-strand DNA reveals a new landscape of DNA double-strand breaks in Saccharomyces cerevisiae. PLoS Biol 5, e324.
46 Blitzblau HG, Bell GW, Rodriguez J, Bell SP and Hochwagen A (2007) Mapping of meiotic single-
stranded DNA reveals double-stranded-break hotspots near centromeres and telomeres. *Curr Biol* 17, 2003–2012.

47 Barlow AL, Benson FE, West SC and Hulten MA (1997) Distribution of the Rad51 recombinase in human and mouse spermatocytes. *EMBO J* 16, 5207–5215.

48 Cole F, Kauppi L, Lange J, Roig I, Wang R, Keeney S and Jasins M (2012) Homeostatic control of recombination is implemented progressively in mouse meiosis. *Nat Cell Biol* 14, 424–430.

49 Loidl J, Klein F and Scherthan H (1994) Homologous pairing is reduced but not abolished in asynaptic mutants of yeast. *J Cell Biol* 125, 1191–1200.

50 Baudat F, Manova K, Yuen JP, Jasins M and Keeney S (2000) Chromosome synopsis defects and sexually dimorphic meiotic progression in mice lacking Spo11. *Mol Cell* 6, 989–998.

51 Romanienko PJ and Camerini-Otero RD (2000) The mouse Spo11 gene is required for meiotic chromosome synopsis. *Mol Cell* 6, 975–987.

52 Page SL and Hawley RS (2004) The genetics and molecular biology of the synaptonemal complex. *Annu Rev Cell Dev Biol* 20, 525–558.

53 Kleckner N (2006) Chiasmata formation: chromatin/axis interplay and the role(s) of the synaptonemal complex. *Chromosoma* 115, 175–194.

54 Kleckner N (1996) Meiosis: how could it work? *Proc Natl Acad Sci USA* 93, 8167–8174.

55 Zickler D and Kleckner N (1999) Meiotic chromosomes: integrating structure and function. *Annu Rev Genet* 33, 603–754.

56 Moens PB and Pearlman RE (1990) Telomere and centromere DNA are associated with the cores of meiotic prophase chromosomes. *Chromosoma* 100, 8–14.

57 Szostak JW, Orr-Weaver TL, Rothstein RJ and Stahl FW (1983) The double-strand-break repair model for recombination. *Cell* 33, 25–35.

58 Carpenter AT (1975) Electron microscopy of meiosis in Drosophila melanogaster females: II. The recombination nodule—a recombination-associated structure at pachytene? *Proc Natl Acad Sci USA* 72, 3186–3189.

59 Ding X, Xu R, Yu J, Xu T, Zhuang Y and Han M (2007) SUN1 is required for telomere attachment to nuclear envelope and gametogenesis in mice. *Dev Cell* 12, 863–872.

60 Haque F, Mazzeo D, Patel JT, Smallwood DT, Ellis JA, Shanahan CM and Shackleton S (2010) Mammalian SUN protein interaction networks at the inner nuclear membrane and their role in laminopathy disease processes. *J Biol Chem* 285, 3487–3498.

61 Mikolcevic P, Isoda M, Shibuya H, del Barco Barrionuevo I, Igea A, Suja JA, Shackleton S, Watanabe Y and Nebreda AR (2016) Essential role of the Cdk2 activator RingoA in meiotic telomere tethering to the nuclear envelope. *Nat Commun* 7, 11084.

62 Viera A, Alsheimer M, Gomez R, Berenguer I, Ortega S, Symonds CE, Santamaria D, Benavente R and Suja JA (2015) CDK2 regulates nuclear envelope protein dynamics and telomere attachment in mouse meiotic prophase. *J Cell Sci* 128, 88–99.

63 Shibuya H, Ishiguro K and Watanabe Y (2014) The TRF1-binding protein TERB1 promotes chromosome movement and telomere rigidity in meiosis. *Nat Cell Biol* 16, 145–156.
74 Buonomo SB, Clyne RK, Fuchs J, Loidl J, Uhlmann F and Nasmyth K (2000) Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell 103, 387–398.

75 Hassold T and Hunt P (2001) To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2, 280–291.

76 Lacefield S and Murray AW (2007) The spindle checkpoint rescues the meiotic segregation of chromosomes whose crossovers are far from the centromere. Nat Genet 39, 1273-1277.

77 Sakuno T, Tanaka K, Hauf S and Watanabe Y (2011) Repositioning of aurora B promoted by chiasmata ensures sister chromatid mono-orientation in meiosis I. Dev Cell 21, 534–545.

78 Watanabe Y (2012) Geometry and force behind kinetochore orientation: lessons from meiosis. Nat Rev Mol Cell Biol 13, 370–382.

79 Klein F, Mahr P, Galova M, Buonomo SB, Michaelis C, Nairz K and Nasmyth K (1999) A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell 98, 91–103.

80 Allers T and Lichten M (2001) Differential timing and control of noncrossover and crossover recombination during meiosis. Cell 106, 47–57.

81 Wyatt HD and West SC (2014) Holliday junction resolvases. Cold Spring Harb Perspect Biol 6, a023192.

82 Osman F, Dixon J, Doe CL and Whitby MC (2003) Generating crossovers by resolution of nicked Holliday junctions: a role for Mus81-Eme1 in meiosis. Mol Cell 12, 761–774.

83 Moens PB (1973) Mechanisms of chromosome synapsis at meiotic prophase. Int Rev Cytol 35, 117–134.

84 Marcon E and Moens PB (2005) The evolution of meiosis: recruitment and modification of somatic DNA-repair proteins. BioEssays 27, 795–808.

85 Moens PB, Marcon E, Shore JS, Kochakpour N and Spyropoulos B (2007) Initiation and resolution of interhomolog connections: crossover and non-crossover sites along mouse synaptonemal complexes. J Cell Sci 120, 1017–1027.

86 Hunter N (2015) Meiotic recombination: the essence of heredity. Cold Spring Harb Perspect Biol 7, a016618.

87 Zickler D and Kleckner N (2015) Recombination, pairing, and synapsis of homologs during meiosis. Cold Spring Harb Perspect Biol 7, a016626.

88 Risal S, Adhikari D and Liu K (2016) Animal models for studying the in vivo functions of cell cycle CDKs. Methods Mol Biol 1336, 155–166.

89 Cheng M, Olivier P, Diehl JA, Fero M, Roussel MF, Roberts JM and Sherr CJ (1999) The p21Cip1 and p27Kip1 CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. EMBO J 18, 1571–1583.

90 Cobrinik D, Dowdy SF, Hinds PW, Mittnacht S and Weinberg RA (1992) The retinoblastoma protein and the regulation of cell cycling. Trends Biochem Sci 17, 312–315.

91 Dyson N (1998) The regulation of E2F by pRB-family proteins. Genes Dev 12, 2245–2262.

92 Nevins JR (1998) Toward an understanding of the functional complexity of the E2F and retinoblastoma families. Cell Growth Differ 9, 585–593.

93 Hinds PW, Mittnacht S, Dulic V, Arnold A, Reed SI and Weinberg RA (1992) Regulation of retinoblastoma protein functions by ectopic expression of human cyclins. Cell 70, 993–1006.

94 Dyson NJ (2016) RB1: a prototype tumor suppressor and an enigma. Genes Dev 30, 1492–1502.

95 Bellve AR, Cavicchia JC, Millette CF, O'Brien DA, Bhatnagar YM and Dym M (1977) Spermatogonial stem cells. The spindle checkpoint rescues the meiotic segregation of chromosomes. Cell 102, 57–63.

96 de Rooij DG (1998) Spermatogonial stem cells. Curr Opin Cell Biol 10, 694–701.

97 Beumer TL, Roepers-Gajadien HL, Gademann IS, Kal HB and de Rooij DG (2000) Involvement of the D-type cyclins in germ cell proliferation and differentiation in the mouse. Biol Reprod 63, 1893–1898.

98 Fantl V, Stamp G, Andrews A, Rosewell I and Dickson C (1995) Mice lacking cyclin D1 are small and show defects in eye and mammary gland development. Genes Dev 9, 2364–2372.

99 Sicinski P, Donaher JL, Parker SB, Li T, Fazeli A, Gardner H, Haslam SZ, Bronson RT, Elledge SJ and Weinberg RA (1995) Cyclin D1 provides a link between development and oncogenesis in the retina and breast. Cell 82, 621–630.

100 Sicinski E, Aifantis I, Le Cam L, Swat W, Borowski C, Yu Q, Ferrando AA, Levin SD, Geng Y, von Bohemmer H et al. (2003) Requirement for cyclin D3 in lymphocyte development and T cell leukemias. Cancer Cell 4, 451–461.

101 Ciemerych MA, Kenney AM, Sicinska E, Kalaszczyńska I, Bronson RT, Rowitch DH, Gardner H and Sicinski P (2002) Development of mice expressing a single D-type cyclin. Genes Dev 16, 3277–3289.

102 Sicinski P, Donaher JL, Geng Y, Parker SB, Gardner H, Park MY, Robker RL, Richards JS, McGinnis LK, Biggers JD et al. (1996) Cyclin D2 is an FSH-responsive gene involved in gonadai cell proliferation and oncogenesis. Nature 384, 470–474.

103 Walker WH and Cheng J (2005) FSH and testosterone signaling in Sertoli cells. Reproduction 130, 15–28.
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104 Burns KH, Agno JE, Sicinski P and Matzuk MM (2003) Cyclin D2 and p27 are tissue-specific regulators of tumorigenesis in inhibin alpha knockout mice. Mol Endocrinol 17, 2053–2069.

105 Rane SG, Dubus P, Mettus RV, Galbreath EJ, Boden G, Reddy EP and Barbacid M (1999) Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in beta-islet cell hyperplasia. Nat Genet 22, 44–52.

106 Tsutsui T, Hesabi B, Moons DS, Pandolfi PP, Hansel KS, Koff A and Kiyokawa H (1999) Targeted disruption of CDK4 delays cell cycle entry with enhanced p27kip1 activity. Mol Cell Biol 19, 7011–7019.

107 Malumbres M, Sotillo R, Santamaria D, Galan J, Cerezo A, Ortega S, Dubus P and Barbacid M (2004) Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6. Cell 118, 493–504.

108 Kozar K, Ciemerych MA, Rebel VI, Shigematsu H, Zagordzdon A, Sicinska E, Geng Y, Yu Q, Bhattacharya S, Bronson RT et al. (2004) Mouse development and cell proliferation in the absence of D-cyclins. Cell 118, 477–491.

109 Rhee K and Wolgemuth DJ (1995) Cdk family genes are expressed not only in dividing but also in terminally differentiated mouse germ cells, suggesting their possible function during both cell division and differentiation. Dev Dyn 204, 406–420.

110 Zhang Q, Wang X and Wolgemuth DJ (1999) Developmentally regulated expression of cyclin D3 and its potential in vivo interacting proteins during murine gametogenesis. Endocrinology 140, 2790–2800.

111 Zindy F, den Besten W, Chen B, Rehg JE, Latres E, Barbacid M, Pollard JW, Sherr CJ, Cohen PE and Roussel MF (2001) Control of spermatogenesis in mice by the cyclin D-dependent kinase inhibitors p16ink4c and p19ink4d. Mol Cell Biol 21, 3244–3255.

112 Bartkova J, Rajpert-De Meyts E, Skakkebaek NE, Lukas J and Bartek J (2000) Lack of p19 INK4d in human testicular germ-cell tumours contrasts with high expression during normal spermatogenesis. Oncogene 19, 4146–4150.

113 Gromley A, Churchman ML, Zindy F and Sherr CJ (2009) Transient expression of the Arf tumor suppressor during male germ cell and eye development in Arf-Cre reporter mice. Proc Natl Acad Sci USA 106, 6285–6290.

114 Quelle DE, Zindy F, Ashmun RA and Sherr CJ (1995) Alternative reading frames of the INK4a tumor suppressor gene encode two unrelated proteins capable of inducing cell cycle arrest. Cell 83, 993–1000.

115 Moons DS, Jirawatnotai S, Tsutsui T, Franks R, Parlow AF, Hales DB, Gibori G, Fazleabas AT and Kiyokawa H (2002) Intact follicular maturation and defective luteal function in mice deficient for cyclin-dependent kinase-4. Endocrinology 143, 647–654.

116 Mettus RV and Rane SG (2003) Characterization of the abnormal pancreatic development, reduced growth and infertility in Cdk4 mutant mice. Oncogene 22, 8413–8421.

117 Cameron DF, Murray FT and Drylie DD (1985) Interstitial compartment pathology and spermatogenic disruption in testes from impotent diabetic men. Anat Rec 213, 53–62.

118 Malumbres M and Barbacid M (2005) Mammalian cyclin-dependent kinases. Trends Biochem Sci 30, 630–641.

119 Sherr CJ and Roberts JM (1999) CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev 13, 1501–1512.

120 Malumbres M and Barbacid M (2005) Mammalian cyclin-dependent kinases. Trends Biochem Sci 30, 630–641.

121 Zindy F, Quelle DE, Roussel MF and Sherr CJ (1997) Expression of the p16INK4a tumor suppressor versus other INK4 family members during mouse development and aging. Oncogene 15, 203–211.

122 Bartkova J, Rajpert-De Meyts E, Skakkebaek NE, Lukas J and Bartek J (2003) Derepression of the G1/S-phase control in human testicular germ cell tumours. APMIS 111, 252–265.

123 Gromley A, Churchman ML, Zindy F and Sherr CJ (2009) Transient expression of the Arf tumor suppressor during male germ cell and eye development in Arf-Cre reporter mice. Proc Natl Acad Sci USA 106, 6285–6290.

124 Quelle DE, Zindy F, Ashmun RA and Sherr CJ (1995) Alternative reading frames of the INK4a tumor suppressor gene encode two unrelated proteins capable of inducing cell cycle arrest. Cell 83, 993–1000.

125 Serrano M, Lee H, Chin L, Cordon-Cardo C, Beach D and DePinho RA (1996) Role of the INK4a locus in tumor suppression and cell mortality. Cell 85, 27–37.

126 Churchman ML, Roig I, Jasmin S, Keeney S and Sherr CJ (2011) Expression of arf tumor suppressor in spermagonia facilitates meiotic progression in male germ cells. PLoS Genet 7, e1002157.

127 Franklin DS, Godfrey VL, Lee H, Kovaliev GI, Schoonhoven R, Chen-Kiang S, Su L and Xiong Y (1998) CDK inhibitors p16ink4c and p27kip1 mediate two separate pathways to collaboratively suppress pituitary tumorigenesis. Genes Dev 12, 2899–2911.

128 Latres E, Malumbres M, Sotillo R, Martin J, Ortega S, Martin-Caballero J, Flores JM, Cordon-Cardo C and Barbacid M (2000) Limited overlapping roles of p15ink4b and p16ink4a cell cycle inhibitors in proliferation and tumorigenesis. EMBO J 19, 3496–3506.
The text is a continuation of the previous one, discussing various studies and findings related to CDK/cyclin functions during spermatogenesis.
154 Lindeman GJ, Dagnino L, Gaubatz S, Xu Y, Bronson RT, Warren HB and Livingston DM (1998) A specific, nonproliferative role for E2F–5 in chorid plexus function revealed by gene targeting. Genes Dev 12, 1092–1098.

155 Chen HZ, Tsai SY and Leone G (2009) Emerging Morgunova E, Yin Y, Jolma A, Dave K, Schmierer B, Morgunova E, Yin Y, Jolma A, Dave K, Schmierer B, Ravnik SE and Wolgemuth DJ (1999) Regulation of Zhao H, Chen X, Gurian-West M and Roberts JM Singh P and Schimenti JC (2015) The genetics of Goertz MJ, Wu Z, Gallardo TD, Hamra FK and Pui HP and Saga Y (2017) Gonocytes-to- 159 Singh P and Schimenti JC (2015) The genetics of human infertility by functional interrogation of SNPs in mice. Proc Natl Acad Sci USA 112, 10431–10436.

160 Zhao H, Chen X, Guriand-West M and Roberts JM (2012) Loss of cyclin-dependent kinase 2 (CDK2) inhibitory phosphorylation in a CDK2AF knock-in mouse causes misregulation of DNA replication and centrosome duplication. Mol Cell Biol 32, 1421–1432.

161 Welburn JP, Tucker JA, Johnson T, Lindert L, Morgan M, Willis A, Noble ME and Endicott JA (2007) How tyrosine 15 phosphorylation inhibits the activity of cyclin-dependent kinase 2-cyclin A. J Biol Chem 282, 3173–3181.

162 Yoshida S, Sukeno M, Nakagawa T, Ohkó K, Nagamatsu G, Suda T and Nabeshima Y (2006) The first round of mouse spermatogenesis is a distinctive program that lacks the self-renewing spermatogonia stage. Development 133, 1495–1505.

163 de Rooij DG (1998) Stem cells in the testis. Int J Exp Pathol 79, 67–80.

164 Goertt MJ, Wu Z, Gallardo TD, Hamra FK and Castrillon DH (2011) Foxo1 is required in mouse spermatogonial stem cells for their maintenance and the initiation of spermatogenesis. J Clin Invest 121, 3456–3466.

165 Pui HP and Saga Y (2017) Gonocytes-to-spermatogonia transition initiates prior to birth in murine testes and it requires FGF signaling. Mech Dev 144, 125–139.

166 Meng X, Lindahl M, Hyvonen ME, Parvinen M, de Rooij DG, Hess MW, Raatikainen-Ahokas A, Sainio K, Rauvala H, Lakso M et al. (2000) Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. Science 287, 1489–1493.

167 Grasso M, Fusco A, Dovere L, de Rooij DG, Stefanini M, Boitani C and Vicini E (2012) Distribution of GFRA1-expressing spermatogonia in adult mouse testis. Reproduction 143, 325–332.

168 Singh P, Patel RK, Palmer N, Grenier JK, Paduch D, Kaldis P, Grimson A and Schimenti JC (2019) CDK2 kinase activity is a regulator of male germ cell fate. Development, https://doi.org/10.1242/dev.180273

169 Huang H, Regan KM, Lou Z, Chen J and Tindall DJ (2006) CDK2-dependent phosphorylation of FOXO1 as an apoptotic response to DNA damage. Science 314, 294–297.

170 Huang H and Tindall DJ (2007) CDK2 and FOXO1: a fork in the road for cell fate decisions. Cell Cycle 6, 902–906.

171 Den Haese G, Walworth N, Carr AM and Gould KL (1995) The Weel protein kinase regulates T14 phosphorylation of fission yeast Cdc2. Mol Biol Cell 6, 371–385.

172 Parker LL and Piwnica-Worms H (1992) Inactivation of the p34cdc2-cyclin B complex by the human WEE1 tyrosine kinase. Science 257, 1955–1957.

173 Johnson LN and Lewis RJ (2001) Structural basis for control by phosphorylation. Chem Rev 101, 2209–2242.

174 Nakayama K, Ishida N, Shirane M, Inomata A, Inoue T, Shishido N, Horii I, Loh DY and Nakayama K (1996) Mice lacking p27kip1 display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. Cell 85, 707–720.

175 Fero ML, Rivkin M, Tasch M, Porter P, Carow CE, Firpo E, Polyak K, Tsai LH, Broudy V, Perlmutter RM et al. (1996) A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27kip1-deficient mice. Cell 85, 733–744.

176 Kiyokawa H, Kineman RD, Manova-Todorova KO, Soares VC, Hoffman ES, Ono M, Khanam D, Hayday AC, Frohman LA and Koff A (1996) Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27kip1. Cell 85, 721–732.

177 Beumer TL, Kiyokawa H, Roepeps-Gajadien HL, van den Bos LA, Lock TM, Gademann IS, Rutgers DH, Koff A and de Rooij DG (1999) Regulatory role of p27kip1 in the mouse and human testis. Endocrinology 140, 1834–1840.

178 Holsberge DR, Buchold GM, Leal MC, Kiesewetter SE, O’Brien DA, Hess RA, Franca LR, Kiyokawa H and Cooke PS (2005) Cell-cycle inhibitors p27kip1 and p21cip1 regulate murine Sertoli cell proliferation. Biol Reprod 72, 1429–1436.
179 Fotovati A, Nakayama K and Nakayama KI (2006) Impaired germ cell development due to compromised cell cycle progression in Skp2-deficient mice. Cell Div 1, 4.
180 Fotovati A, Abu-Ali S, Nakayama K and Nakayama KI (2011) Impaired ovarian development and reduced fertility in female mice deficient in Skp2. J Anat 218, 668–677.
181 Kim ST, Park NC, Yi LS and Gye MC (2006) Expression of p57 in FEBS Letters 593.
182 Fotovati A, Abu-Ali S, Nakayama K and Nakayama KI (2006) Differentiation during mouse development. Dev Growth Differ 46, 495–502.
183 Yan Y, Frisen J, Lee MH, Massague J and Barbacid M (1997) Ablation of the CDK inhibitor p57KIP2 results in increased apoptosis and delayed differentiation during mouse development. Genes Dev 11, 973–983.
184 Zhang P, Wong C, DePinho RA, Harper JW and Elledge SJ (1998) Cooperation between the Cdk inhibitors p27KIP1 and p57KIP2 in the control of tissue growth and development. Genes Dev 12, 3162–3167.
185 Takahashi K, Nakayama K and Nakayama K (2000) Mice lacking a CDK inhibitor, p57KIP2, exhibit skeletal abnormalities and growth retardation. J Biochem 127, 73–83.
186 Palm W and de Lange T (2008) How shelterin protects mammalian telomeres. Annu Rev Genet 42, 301–334.
187 Tu Z, Bayazit MB, Liu H, Zhang J, Busayavalsa K, Risal S, Shao J, Satyanarayana A, Coppola V, Tesarollo L et al. (2017) Speedy A-Cdk2 binding mediates initial telomere-nuclear envelope attachment during meiotic prophase I independent of Cdk2 activation. Proc Natl Acad Sci USA 114, 592–597.
188 Tsai LH, Harlow E and Meyerson M (1991) Isolation of the human cdk2 gene that encodes the cyclin A- and adenovirus E1A-associated p33 kinase. Nature 353, 174–177.
189 Harper L, Golubovskaya I and Cande WZ (2003) Cyclin-dependent kinase 2 is essential for meiotic chromosome dynamics. Cell 114, 1715–1726.
190 Long J, Huang C, Chen Y, Zhang Y, Shi S, Wu L, Liu Y, Liu C, Wu J and Lei M (2017) Telomeric TERB1-TRF1 interaction is crucial for male meiosis. Nat Struct Mol Biol 24, 1073–1080.
191 Pendlebury DF, Fujiwara Y, Tesmer VM, Smith EM, Shibuya H, Watanabe Y and Nandakumar J (2017) Dissecting the telomere-inner nuclear membrane interface formed in meiosis. Nat Struct Mol Biol 24, 1064–1072.
192 Dunce JM, Milburn AE, Gurusaran M, da Cruz I, Sen LT, Benavente R and Davies OR (2018) Structural basis of meiotic telomere attachment to the nuclear envelope by MAJJIN-TERB2-TERB1. Nat Commun 9, 5355.
193 Link J and Jantsch V (2019) Meiotic chromosomes in motion: a perspective from Mus musculus and Caenorhabditis elegans. Chromosoma., [Epub ahead of print], https://doi.org/10.1007/s00412-019-00698-5.
194 Alsheimer M, von Glasenapp E, Hock R and Benavente R (1999) Architecture of the nuclear periphery of rat pachytene spermatocytes: distribution of nuclear envelope proteins in relation to synaptonemal complex attachment sites. Mol Biol Cell 10, 1235–1245.
195 Esponda P and Gimenez-Martín G (1972) The attachment of the synaptonemal complex to the nuclear envelope. An ultrastructural and cytochemical analysis. Chromosoma 38, 405–417.
206 Woollam DH, Millen JW and Ford EH (1967) Points of attachment of pachytene chromosomes to the nuclear membrane in mouse spermatocytes. Nature 213, 298–299.

207 Liebe B, Alsheimer M, Hoog C, Benavente R and Scherthan H (2004) Telomere attachment, meiotic chromosome condensation, pairing, and bouquet stage duration are modified in spermatocytes lacking axial elements. Mol Biol Cell 15, 827–837.

208 Manterola M, Sicinski P and Wolgemuth DJ (2016) E-type cyclins modulate telomere integrity in mammalian male meiosis. Chromosoma 125, 253–264.

209 Martinerie L, Manterola M, Chung SS, Panighrahi SK, Weisbach M, Vasileva A, Geng Y, Sicinski P and Wolgemuth DJ (2014) Mammalian E-type cyclins control chromosome pairing, telomere stability and CDK2 localization in male meiosis. PLoS Genet 10, e1004165.

210 De Azevedo WF, Leclerc S, Meijer L, Havlicek L, Reynolds A, Qiao H, Yang Y, Chen JK, Jackson N, Hunter N and Borts RH (1997) Mlh1 is unique among DNA mismatch repair and meiotic crossing over.

211 Liu W, Wang L, Zhao W, Song G, Wang G, Wang F, Li W, Lian J, Tian H et al. (2014) Phosphorylation of CDK2 at threonine 160 regulates meiotic pachytene and diplotype progression in mice. Dev Biol 392, 108–116.

212 Qiao H, Prasad Rao HB, Yang Y, Fong JH, Cloutier J, Deacon DC, Nagel KE, Swartz RK, Strong E, Holloway JK et al. (2014) Antagonistic roles of ubiquitin ligase HEI10 and SUMO ligase RNF212 regulate meiotic recombination. Nat Genet 46, 194–199.

213 Bose S, Kowalchuk M, Lespinasse F, Santucci-Darmanin S, Neyton S, Lepistinen S, Saunier A, Gaudry P and Paquis-Fluckinger V (2002) The DNA mismatch-repair MLH3 protein interacts with MSH4 in meiotic cells, supporting a role for this MutL homolog in mammalian meiotic recombination. Hum Mol Genet 11, 1697–706.

214 Liwicki SM, Wang V, Jacoby R, Banerjee-Basu S, Baxevanis AD, Lynch HT, Elliott RM and Collins FS (2000) MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. Nat Genet 24, 27–35.

215 Woods LM, Hodges CA, Baart E, Baker SM, Liskay M and Hunt PA (1999) Chromosomal influence on meiotic spindle assembly: abnormal meiosis I in female Mlh1 mutant mice. J Cell Biol 145, 395–406.

216 Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christie DM, Monell C, Arnheim N, Bradley A et al. (1996) Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. Nat Genet 13, 336–342.

217 Hunter N and Borts RH (1997) Mlh1 is unique among DNA mismatch repair and meiotic crossing over.

218 Lunar B, Alsheimer M, Hoog C, Benavente R and Scherthan H (2004) Telomere attachment, meiotic chromosome condensation, pairing, and bouquet stage duration are modified in spermatocytes lacking axial elements. Mol Biol Cell 15, 827–837.

219 Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christie DM, Monell C, Arnheim N, Bradley A et al. (1996) Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. Nat Genet 13, 336–342.
Keeney S et al. (2009) Mouse HORMAD1 and HORMAD2, two conserved meiotic chromosomal proteins, are depleted from synapsed chromosome axes with the help of TRIP13 AAA-ATPase. PLoS Genet 5, e1000702.

230 Zhang Q, Ji SY, Busayavalasa K and Yu C (2019) SPO16 binds SHOC1 to promote homologous recombination and crossing-over in meiotic prophase I. Sci Adv 5, eaau9780.

231 Yang F, Gell K, van der Heijden GW, Eckardt S, Leu NA, Page DC, Benavente R, Her C, Hoog C, McLaughlin KJ et al. (2008) Meiotic failure in male mice lacking an X-linked factor. Genes Dev 22, 682–691.

232 Palmer N, Talib SZA, Ratnacaram CK, Low D, Bisteau X, Lee JHS, Pfeiffenberger E, Wollmann H, Tan JHL, Wee S et al. (2019) CDK2 regulates the NRF1/Ehmt1 axis during meiotic prophase I. J Cell Biol 218, 2896–2918.

233 Wang J, Tang C, Wang Q, Su J, Ni T, Yang W, Wang Y, Chen W, Liu X, Wang S et al. (2017) NRF1 coordinates with DNA methylation to regulate spermatogenesis. FASEB J 31, 4959–4970.

234 Huo L and Scarpulla RC (2001) Mitochondrial DNA instability and peri-implantation lethality associated with targeted disruption of nuclear respiratory factor 1 in mice. Mol Cell Biol 21, 644–654.

235 Murphy M, Stinnakre MG, Senamaud-Beaufort C, Winston NJ, Sweeney C, Kubelka M, Carrington M, Brechot C and Sobczak-Thepot J (1997) Delayed early embryonic lethality following disruption of the murine cyclin A2 gene. Nat Genet 15, 83–86.

236 Brandeis M, Rosewell I, Carrington M, Crompton T, Jacobs MA, Kirk J, Gannon J and Hunt T (1998) Cyclin B2-null mice develop normally and are fertile whereas cyclin B1-null mice die in utero. Proc Natl Acad Sci USA 95, 4344–4349.

237 Gopinathan L, Szym R, Low D, Diril MK, Chang HY, Coppola V, Liu K, Tessarollo L, Guccione E, van Pelt AMM et al. (2017) Emi2 is essential for mouse spermatogenesis. Cell Rep 20, 697–708.

238 Clement TM, Inselman AL, Goulding EH, Willis WD and Eddy EM (2015) Disrupting cyclin dependent kinase 1 in spermatocytes causes late meiotic arrest and infertility in mice. Biol Reprod 93, 137.

239 Liu D, Matzuk MM, Sung WK, Guo Q, Wang P and Wolgemuth DJ (1998) Cyclin A1 is required for meiosis in the male mouse. Nat Genet 20, 377–380.

240 van der Meer T, Chan WY, Palazon LS, Nieduszynski C, Murphy M, Sobczak-Thepot J, Carrington M and Collode WH (2004) Cyclin A1 protein shows haplo-insufficiency for normal fertility in male mice. Reproduction 127, 503–511.

241 Li Y, Wang L, Zhang L, He Z, Feng G, Sun H, Wang J, Li Z, Liu C, Han J et al. (2019) Cyclin B3 is required for metaphase to anaphase transition in oocyte meiosis I. J Cell Biol 218, 1553–1563.

242 Karasu ME, Bouftas N, Keeney S and Wassmann K (2019) Cyclin B3 promotes anaphase I onset in oocyte meiosis. J Cell Biol 218, 1265–1281.

243 Diederichs S, Baumer N, Schultz N, Hamra FK, Schrader MG, Sandstede ML, Berdel WE, Serve H and Muller-Tidow C (2005) Expression patterns of mitotic and meiotic cell cycle regulators in testicular cancer and development. Int J Cancer 116, 207–217.

244 Terré B, Lewis M, Gil-Gómez G, Han Z, Lu H, Aguiler M, Prats N, Roy S, Zhao H and Stracker TH (2019) Defects in effenter duct multiciliogenesis underlie male infertility in GEMC1-, MCIDAS- or CCNO-deficient mice. Development 146, dev162628. https://doi.org/10.1242/dev.162628.

245 Nunez-Olle M, Jung C, Terre B, Balsiger NA, Plata C, Roset R, Pardo-Pastor C, Garrido M, Rojas S, Alameda F et al. (2017) Constitutive Cyclin O deficiency results in penetrant hydrocephalus, impaired growth and infertility. Oncotarget 8, 99261–99273.

246 Chauhan S, Zheng X, Tan YY, Tay BH, Lim S, Venkatesh B and Kaldis P (2012) Evolution of the Cdk-activator Speedy/RINGO in vertebrates. Cell Mol Life Sci 69, 3835–3850.