REPRODUCTION
REVIEW

The APC/C in female mammalian meiosis I

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Abstract

The anaphase-promoting complex or cyclosome (APC/C) orchestrates a meticulously controlled sequence of proteolytic events critical for proper cell cycle progression, the details of which have been most extensively elucidated during mitosis. It has become apparent, however, that the APC/C, particularly when acting in concert with its Cdh1 co-activator (APC/C^{Cdh1}), executes a staggeringly diverse repertoire of functions that extend its remit well outside the bounds of mitosis. Findings over the past decade have not only earmarked mammalian oocyte maturation as one such case in point but have also begun to reveal a complex pattern of APC/C regulation that underpins many of the oocyte's unique developmental attributes. This review will encompass the latest findings pertinent to the APC/C, especially APC/C^{Cdh1}, in mammalian oocytes and how its activity and substrates shape the stop–start tempo of female mammalian first meiotic division and the challenging requirement for assembling spindles in the absence of centrosomes.

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Introduction

Meiosis is the unique cell division that halves the chromosome compliment by coupling two successive nuclear divisions with a single round of DNA replication. In mammalian oocytes, this process is further complicated by being drawn out over unusually protracted periods that are punctuated by two arrest stages.

Following recombination between homologous chromosomes during fetal life, oocytes arrest at the dictyate stage of prophase of the first meiotic division (meiosis I or MI) until postnatal life. Following an extended growth phase, hormonal cues that lead to ovulation induce oocytes to resume and complete MI. Resumption of MI is marked by breakdown of the membrane of the oocyte's large nucleus, referred to as germinal vesicle breakdown (GVBD), following which recombined homologous chromosomes (termed bivalents) are segregated (so-called homologue disjunction) between the secondary oocyte and the first polar body (PB). Notably, MI – the interval commencing with homologous chromosome recombination and ending with first PB extrusion (PBE) – in mammalian oocytes incorporates one of the longest M-phases of any known cell type (lasting 7–11 h in mice and 24–36 h in humans vs tens of minutes for mitosis). Following PBE, oocytes progress uninterruptedly to metaphase of the second meiotic division (meiosis II or MII) and become arrested for a second time under the influence of cytostatic factor (CSF). It is the CSF-arrested egg that is released from its associated somatic follicular compartment at the time of ovulation. Significantly, although primordial germ cells in the ovary commit to meiosis during fetal life, it is not until postnatal adulthood that mature oocytes (or eggs) with the capacity to support fertilisation and embryogenesis are produced, a period lasting up to four to five decades in women. Intriguingly, the ovulated egg has not yet halved its chromosome compliment as the second meiotic chromosome segregation will ordinarily only occur following fertilisation-induced breakage of CSF arrest.

A striking feature of human reproduction is the exponential deterioration in fertility and pregnancy success associated with female ageing (Homer 2007), which in turn has been linked with declining oocyte quality. While the oocyte's developmental program provides a biologically plausible explanation for this phenomenon – oocytes ovulated later in life would have been subjected to greater 'ageing effects' than oocytes ovulated in earlier years – exactly what the key molecular players might be that succumb to the ageing process remain to be elucidated. Before being able to identify any such pivotal regulatory nodes, it is first important to develop a detailed understanding of how the oocyte, in conjunction with its follicular investment, orchestrates maturation at the molecular level.

Over the past decade, increasing insight has been gained into the role that the master regulator known as the anaphase-promoting complex or cyclosome (APC/C)

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plays during meiotic maturation in mammalian oocytes, most notably the mouse model. The APC/C has been found to be involved in virtually every stage of mammalian oocyte development including G2/prophase arrest, the G2-M transition, prometaphase I progression, the metaphase I-anaphase I transition, the transition between MI and MII, CSF-mediated MII arrest and calcium-related events at fertilisation. Significantly, disordered control of either the APC/C or its substrates is severely detrimental, in keeping with which mice bearing an oocyte-specific deletion of *Apc2* (encoding an APC/C subunit) exhibit major fertility defects (McGuinness *et al*. 2009). The APC/C therefore qualifies as a key molecular determinant of oocyte quality of direct relevance to reproductive performance.

The intention here is to review current understanding of how the APC/C and its substrates take the mammalian oocyte through the gears of MI with a focus on recent developments, most of which relate to the Cdh1-activated species of APC/C (APC/C^{Cdh1}) and its control of the G2-M transition and acentrosomal spindle assembly during prometaphase I. I also refer to the Cdc20-activated APC/C (APC/C^{Cdc20}) but do not delve into great depth regarding its regulation by the spindle assembly checkpoint (SAC) as this has been the subject of recent excellent reviews (Homer 2011, Eichenlaub-Ritter 2012, Nagaoka *et al*. 2012, Sun & Kim 2012).

Overview of the APC/C during mammalian oocyte maturation

The APC/C is a cullin-RING finger E3 ubiquitin ligase comprising 15–17 subunits (depending on the organism) which, through ubiquitylation, targets key substrates for destruction by the 26S proteasome (Pines 2011). Structurally, the APC/C is broadly composed of two sub-complexes, one that is catalytic and the other that mediates binding with pivotal WD40-containing co-activators, the two best characterised ones being Cdc20 and Cdh1 (Cdc20 homologue 1; also known as Fizzy-related 1 of Fzr1). APC/C in conjunction with one of its co-activators recognises substrates bearing specific motifs or degrons, most of which can be divided into two classes, D-boxes and KEN boxes, although there are rarer motifs including the O-, G-, A- and CRY boxes. Although the APC/C has been most extensively studied for its roles in regulating the mitotic cell cycle, important non-mitotic functions have emerged; one such function involves the unique transitions required for transforming immature mammalian oocytes into fertilisable eggs.

A striking feature of MI in mammalian oocytes is the sequence by which the APC/C becomes activated by Cdc20 and Cdh1. During mitosis, APC/C is activated first by Cdc20 in prometaphase followed by Cdh1 during mitotic exit: APC/C^{Cdc20} brings about anaphase by directing securin and cyclin B1 destruction before handing over the baton to APC/C^{Cdh1} to complete proteolysis in the lead up to exit from M-phase. In mammalian oocytes, APC/C^{Cdh1} is active at an earlier stage than in mitosis, during prophase I arrest (equivalent to late G2-phase in mitosis, hereafter G2/prophase) and prometaphase I, before APC/C^{Cdc20} rises during late prometaphase I to initiate homologue disjunction (Homer 2011). Thus, APC/C^{Cdh1} activity coincides with two unique aspects of mammalian oocyte development: a protracted G2/prophase arrest and an unusually extended prometaphase I.

APC/C^{Cdh1} at the G2-M boundary: a delicate balancing act

Maintenance of G2/prophase arrest requires suppression of the major cell cycle kinase, cyclin-dependent kinase 1 (Cdk1), a heterodimer composed of a catalytic Cdk1 subunit and an activating accessory cyclin subunit. In contrast to the relatively low Cdk1 activity state of G2/prophase arrest, the cellular processes involved in entry into M-phase are mediated by Cdk1 activation. Thus, the mammalian oocyte is tasked with suppressing Cdk1 for prolonged periods while at the same time maintaining the receptivity to activate Cdk1 in response to ovulatory hormonal cues.

Cdk1 activity is determined by Cdk1's phosphorylation profile as well as the levels of its cyclin activating partner. Inhibitory Cdk1 phosphorylation important for G2/prophase arrest is induced by cAMP-dependent protein kinase A (PKA) activity, which enhances the embryonic Cdk1 inhibitory kinase, Wee1B (or Wee2), and suppresses the activating Cdc25B phosphatase (Han *et al*. 2005, Pirino *et al*. 2009, Oh *et al*. 2010, Solc *et al*. 2010).

A novel aspect of APC/C regulation is the involvement of APC/C^{Cdh1} in regulating G2/prophase arrest in mammalian oocytes (Reis *et al*. 2006). Important inputs have since been shown to provide opposing influences on APC/C^{Cdh1}, altogether furnishing highly sophisticated fine-tuning of cyclin levels and hence of Cdk1 activity that maintains GV arrest on the one hand while facilitating re-entry into MI on the other hand.

Maintaining G2/prophase arrest through APC/C^{Cdh1}

The importance of APC/C^{Cdh1} for restraining Cdk1 through low-level cyclin B1 degradation was initially demonstrated *in vitro* using morpholino antisense-mediated depletion of Cdh1 in fully grown oocytes denuded of their surrounding follicular cells (Reis *et al*. 2006). It was found that such Cdh1-depleted oocytes exhibited an increased susceptibility to undergoing GVBD even under conditions in which cAMP levels were chemically sustained using the phosphodiesterase inhibitor, milrinone. More recently, this mechanism has

been shown to be physiologically relevant for sustaining the reservoir of G2/prophase-arrested oocytes using an *in vivo* mouse model in which *Fzr1* was specifically deleted from growing and mature oocytes using Cre-LoxP technology ($Fzr1^{\Delta/\Delta}$ mice; Holt *et al*. 2011). Significantly, even within the inhibitory cAMP-rich follicular environment within the ovary, $Fzr1^{\Delta/\Delta}$ oocytes exhibited an increased tendency to undergo GVBD that was related to elevated cyclin B1 levels. Importantly, in $Fzr1^{\Delta/\Delta}$ oocytes, there was no change in the levels of Cdc25B, a potential APC/C^{Cdh1} substrate (Holt *et al*. 2011), altogether underlining the importance of the APC/C-cyclin B1 pathway for regulating G2/prophase arrest independent of Cdk1 phosphorylation status. Interestingly, even though many aspects of follicular physiology remained intact in $Fzr1^{\Delta/\Delta}$ mutant mice, there was nevertheless a 35–40% reduction in the numbers of cumulus-enclosed oocytes (Holt *et al*. 2011), suggesting that defects in oocyte-centred control brought about through APC/C de-regulation were detrimental to overall follicular health and integrity.

Unexpectedly, one input required for sustaining APC/C^{Cdh1} activity and hence the G2/prophase arrest state is the SAC protein, BubR1(encoded by *BUB1B*) (Homer *et al*. 2009), most extensively characterised for its role in regulating APC/C^{Cdc20} at the metaphase-to-anaphase transition (Foley & Kapoor 2013). Depletion of BubR1 using morpholino antisense in mouse oocytes led to escape from G2/prophase arrest during culture in medium treated with the phosphodiesterase inhibitor, 1-isobutyl 3-methylxanthine (IBMX). This was a consequence of reduced APC/C^{Cdh1} activity secondary to Cdh1 instability in turn resulting in marked securin stabilisation, which, as discussed below, is an important determinant of cyclin B1 stability in GV-stage oocytes.

In addition to the modulation of APC/C^{Cdh1} activity through control of Cdh1 levels, there is evidence that APC/C^{Cdh1} activity is also determined by phosphorylation status in G2/prophase oocytes. Phosphorylation of Cdh1 by Cdk prevents it from binding to the APC/C holoenzyme (Kramer *et al*. 2000) and can be reversed by phosphatases. Cell division cycle homologue 14 (Cdc14) is a dual-specificity phosphatase that counteracts Cdk-mediated phosphorylation with two homologues, Cdc14A and Cdc14B, being expressed in mammals. Recently, Cdc14B depletion using double-stranded RNA-mediated RNA interference was found to promote GVBD during culture in milrinone and was associated with elevated histone H1 kinase activity (Schindler & Schultz 2009*b*). Conversely, over-expression of Cdc14B from exogenous cRNA inhibited GVBD along with which H1 kinase activity and cyclin B1 levels were reduced (Schindler & Schultz 2009*b*). Notably, the effects of Cdc14B over-expression could be attenuated by depleting Cdh1, altogether indicating that Cdc14B probably positively regulates APC/C^{Cdh1}, thereby contributing to the G2/prophase arrest state. In contrast to Cdc14B, Cdc14A does not appear to play a significant role at the G2-M boundary (Schindler & Schultz 2009*a*).

Modulating APC/C^{Cdh1}-mediated cyclin B1 proteolysis to avoid refractoriness

BubR1 and Cdc14B represent two mechanisms that are important for sustaining active APC/C^{Cdh1} and hence for maintaining the G2/prophase arrest state. Oocytes must nevertheless retain the capacity to respond to ovulatory cues and therefore employ counter mechanisms that help to limit cyclin proteolysis.

One counter-APC/C^{Cdh1} mechanism involves the APC/C inhibitor, early mitotic inhibitor 1 (Emi1; also known as FBXO5 for F-BoX Only 5), which binds to APC/C^{Cdh1} but resists degradation, thereby acting as a pseudo-substrate inhibitor (Miller *et al*. 2006), although other mechanisms may contribute (Pines 2011). In mouse oocytes, over-expression of *Emi1* promotes entry into MI whereas antisense-mediated depletion induces the opposite effect (Marangos *et al*. 2007). The latter effect following Emi1 depletion was correlated with blunted Cdk1 activation as measured using histone H1 kinase assays and with increased cyclin B1 instability as determined by fluorescence monitoring of exogenous cyclin B1-GFP. Significantly, after co-depleting Cdh1, Emi1-depleted oocytes exhibited a high propensity for undergoing GVBD akin to Cdh1 single-depleted oocytes. Altogether, these data support a model in which Emi1 inhibits APC/C^{Cdh1}-dependent cyclin destruction to enable entry into MI (Fig. 1). Emi1's activity must presumably be restricted to a relatively narrow window when the fully grown oocyte responds to ovulatory cues as otherwise, prematurely increased cyclin levels would trigger unscheduled GVBD, potentially compromising the ovarian oocyte reservoir. Exactly how such temporal control is achieved, for instance through regulation of protein levels during the growth phase and/or through spatial redistribution around the time of GVBD, and how this is integrated with signalling mechanisms associated with ovulation are important future questions.

In contrast to the pseudo-substrate activity of Emi1, a surprising finding was that securin, a *bona fide* APC/C^{Cdh1} substrate, is also important for shielding cyclin B1 from APC/C^{Cdh1} (Marangos & Carroll 2008; Fig. 1). It was found that morpholino-induced depletion of securin led to reduced cyclin B1 levels culminating in a roughly fourfold reduction in the ability of oocytes to undergo GVBD by 3 h after release from IBMX. This effect of securin depletion could be fully reversed by co-depleting Cdh1. Furthermore, while GVBD could be rescued in securin-depleted oocytes by co-expressing full-length securin, this was not the case for a mutant form of securin lacking both D and KEN boxes (which could therefore not engage APC/C^{Cdh1}). Overall, therefore, these data underscored a model of competitive

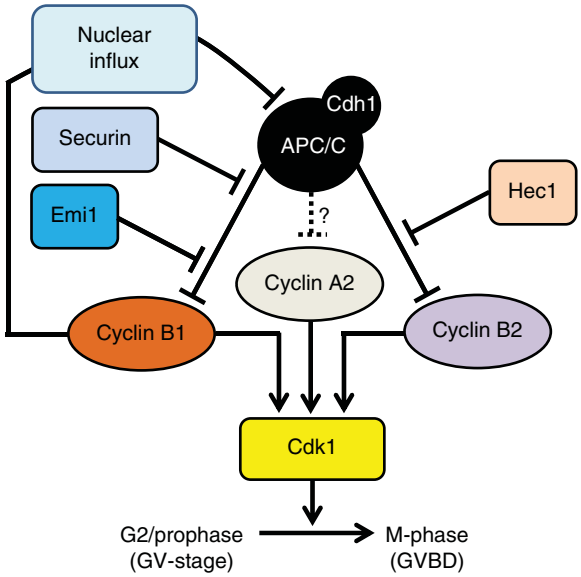

Figure 1 Regulating APC/C^{Cdh1} at the G2-M transition. APC/C^{Cdh1}-mediated proteolysis suppresses cyclin B1 levels to sustain G2/prophase arrest (Reis *et al.* 2006). Conversely, Emi1 and securin serve to limit such proteolysis, thereby allowing cyclin B1 accumulation and Cdk1 activation required for GVBD (Marangos *et al.* 2007, Marangos & Carroll 2008). Influx of cyclin B1 into the nuclear interior around the time of GVBD is thought to overwhelm nuclear-concentrated APC/C^{Cdh1}, thereby promoting further cyclin B1 accumulation (Holt *et al.* 2010). Recent data also indicate that cyclin B2 is also important for Cdk1 activation at GVBD and that Hec1 sustains cyclin B2 levels by sequestering it away from APC/C^{Cdh1} (Gui & Homer 2013). It also appears that cyclin A2 makes a contribution to Cdk1 activation (Touati *et al.* 2012). Note that although the effect of exogenous cyclin A2 cRNA constructs bearing D-box mutations on GVBD in mouse oocytes is indicative of regulation by APC/C^{Cdh1} (Touati *et al.* 2012), this remains to be formally proven.

substrate inhibition in which the ability of endogenous levels of securin to bind APC/C^{Cdh1} as a substrate prevented cyclin B1 from dropping to levels that would compromise entry into MI. Interestingly, the property of securin over-expression to promote GVBD did not extend to Aurora kinase B (Marangos & Carroll 2008), another known APC/C^{Cdh1} substrate, indicating that being an APC/C substrate does not in itself necessarily confer the ability to modulate the G2-M transition.

Cyclin B2 and cyclin A2: new dimensions to MPF in oocytes

Maturation-promoting factor (MPF) was initially identified in starfish and frog oocytes as the physiological entity responsible for promoting entry into M-phase (Doree & Hunt 2002). MPF was first purified from starfish oocytes as a complex of Cdk1 (previously Cdc2; see Doree & Hunt (2002)) and cyclin B (Labbe *et al.* 1989). Although the overwhelming majority of insight into cyclin-related regulation in mouse oocytes has centred on cyclin B1 (Polanski *et al.* 2012), recent data (discussed next) suggest that non-cyclin B1 cyclins – cyclin A2 and cyclin B2 – also participate in activating Cdk1 at the G2-M boundary and may therefore make an important contribution to MPF.

Mammals express three B-type cyclins and two of these, cyclin B1 and cyclin B2, encoded by *CCNB1* and *CCNB2* respectively are known to activate Cdk1 (Satyanarayana & Kaldis 2009). The indispensability of cyclin B1 is underlined by the embryonic lethality of cyclin B1 knockout mice (Brandeis *et al.* 1998). In contrast, cyclin B2 knockout mice are morphologically normal (Brandeis *et al.* 1998), suggesting that, at least in somatic cells, cyclin B2 function is non-essential and redundant with other cyclins such as cyclin B1. Notably, however, although mice nullizygous for cyclin B2 are viable, they have been reported to produce smaller litters (Brandeis *et al.* 1998). Altogether, these data suggested an important fertility- and meiosis-specific role for cyclin B2 in mammals.

In line with this notion, and while exploring the function of Ndc80/Hec1 (hereafter Hec1 for highly expressed in cancer 1), a critically important kinetochore component (Tooley & Stukenberg 2011, Foley & Kapoor 2013), we unexpectedly uncovered a key role for cyclin B2 in mouse oocytes (Gui & Homer 2013). Kinetochores are macro-molecular protein complexes that assemble at centromeres and form the interface between chromosomes and microtubules of the spindle apparatus. Hec1 along with Spc24, Spc25 and Nuf2 are the four subunits making up the Ndc80 complex, one of the most pivotal kinetochore components for interacting directly with microtubules and an important platform for SAC signalling (Tooley & Stukenberg 2011, Foley & Kapoor 2013). Through its capacity to mediate binding with microtubules, Hec1 has been characterised most extensively for its role in bringing about chromosomal bi-orientation during M-phase in a wide variety of systems. Surprisingly, however, depletion of Hec1 from mouse oocytes using morpholino antisense was associated with reduced ability to undergo GVBD pointing for the first time to a role for Hec1 at the G2-M transition (Gui & Homer 2013). Furthermore, although compromised GVBD after Hec1 depletion was associated with reduced Cdk1 activity, cyclin B1 levels nevertheless remained similar to those in wild-type oocytes. This prompted examination of the other potential Cdk1-activating B-type cyclin, cyclin B2, which was known to be expressed (at least at mRNA level) in oocytes (Chapman & Wolgemuth 1993, Ledan *et al.* 2001). Remarkably, and in stark contrast with cyclin B1, cyclin B2 levels were roughly halved following Hec1 depletion (Gui & Homer 2013). Cyclin B2 instability in Hec1-depleted oocytes turned out to be responsible for compromised M-phase entry as co-expressing cyclin B2 from exogenous cRNA restored GVBD. Furthermore, independently depleting cyclin B2 with a *CCNB2*-targeting morpholino compromised GVBD

while over-expressing cyclin B2 had the opposite effect, the latter consistent with previous findings (Ledan *et al.* 2001). Subsequent investigation revealed that cyclin B2 was subject to APC/C^{Cdh1}-mediated proteolysis in oocytes and that Hec1 served to counter this, thereby contributing to cyclin B2 stability (Fig. 1). Thus, cyclin B2 is independently required for Cdk1 activation at the G2-M transition and requires Hec1 for stability.

An important finding was that wild-type levels of cyclin B1 were not sufficient for M-phase entry when cyclin B2 was lacking (Gui & Homer 2013). In keeping with this, cyclin B1 over-expression was far less effective in promoting GVBD on a cyclin B2 knockdown background than in cyclin B2-replete oocytes. This indicates that endogenous levels of cyclin B1 are not sufficient on their own to fully activate Cdk1, which instead requires a contribution from cyclin B2 (Fig. 1). One possibility is that by restraining cyclin B1 accumulation, APC/C^{Cdh1} underlies cyclin B1's comparative impotence in oocytes. In contrast, in mitosis in which APC/C^{Cdh1} is not a prominent feature of G2, near-complete depletion of cyclin B2 has virtually no effect on cell cycle progress unless cyclin B1 is co-depleted (Bellanger *et al.* 2007) consistent with the notion that although cyclin B2 can confer Cdk1 activity, the lack of APC/C^{Cdh1} allows somatic cells to accumulate sufficient cyclin B1 to cover cyclin B2's loss. Analogously, APC/C^{Cdh1}-mediated Cdc20 proteolysis during prometaphase I (Reis *et al.* 2006) could also account for the increased vulnerability of meiosis in mouse oocytes to partial Cdc20 reduction compared with mitosis (Jin *et al.* 2010).

One possible interpretation of the foregoing is that cyclin B2- and cyclin B1-dependent Cdk1 activities might simply be additive for generating sufficient MPF activity for entry into M-phase. It is notable in this regard that early reports of MPF purified from *Xenopus* eggs found it to contain roughly equivalent amounts of Cdk1 complexed with either cyclin B1 or cyclin B2 (Gautier *et al.* 1990). This requires further investigation as other potential mechanisms exist. For instance, cyclin B2-Cdk1 could instigate phosphorylation-related pathways that activate cyclin B1-Cdk1 akin to that proposed for cyclin A2-Cdk1 in mitosis (Fung *et al.* 2007). While it remains to be determined whether cyclin B2-Cdk1 is a component of MPF in mammalian oocytes or contributes to its activation, these data nevertheless identify an important role for cyclin B2 in sustaining basal Cdk1 tone and for facilitating Cdk1 activation during re-entry into MI (Fig. 1).

The cyclin-Cdk1 saga relevant to the APC/C does not end there as recent data also implicate cyclin A2, another known APC/C substrate, as being important for the G2-M transition in mouse oocytes (Touati *et al.* 2012). Mammals express two A-type cyclins capable of binding and activating Cdk1, cyclin A1 and A2 (Satyanarayana & Kaldis 2009). Cyclin A1 knockout mice are developmentally normal but exhibit a defect specifically related to male meiosis (Liu *et al.* 1998). In contrast, cyclin A2 is an essential cyclin, the loss of which is embryonic lethal (Murphy *et al.* 1997) in keeping with which somatic cell culture experiments report severe defects at the G2-M transition after cyclin A2-depletion (Fung *et al.* 2007, Gong *et al.* 2007).

The role of cyclin A2 in mouse oocytes was recently examined using two commercially available anti-cyclin A antibodies, one raised in mouse (Abcam, Cambridge, UK) and the other in rabbit (Santa Cruz Biotechnology), along with a variety of exogenous cyclin A2 cRNA constructs (Touati *et al.* 2012). Microinjection of both the mouse and rabbit antibodies into GV-stage oocytes inhibited GVBD, an effect that could be negated using epitope-blocked antibody or by expressing exogenous cyclin A2, indicating a role for cyclin A2 at the G2-M transition (Fig. 1). In keeping with this and with APC/C involvement, over-expression of two exogenous constructs stabilised against proteolysis by virtue of lacking their D-box degrons induced spontaneous GVBD in dbcAMP-treated medium to an even greater extent than that produced by a cyclin B1 mutant lacking its D-box. Moreover, stabilised exogenous cyclin A2 could no longer promote GVBD when additional mutations were introduced that prohibited binding to the catalytic Cdk subunit, altogether suggesting that cyclin A2's positive influence on M-phase entry was conferred by affecting Cdk1 activity. In support of this, using a heterologous assay system involving mouse somatic cells, it was found that cyclin A2 immunoprecipitated by the mouse antibody lacked any associated histone H1 kinase activity. Assuming that the mouse antibody exerts the same effect in mouse oocytes as in the somatic cell assay, one can interpret these data as indicating that cyclin A2-asscoiated Cdk1 activity is important for promoting entry into M-phase (Fig. 1). As discussed for cyclin B2 above, however, how this cyclin A2-associated Cdk1 activity fits into the pathway of MPF activation remains to be determined. Interestingly, there may be non-Cdk1-related mechanisms by which cyclin A2 feeds into G2-M regulation as the rabbit antibody impaired GVBD to the same extent as the mouse antibody but in stark contrast to the latter, marked histone H1 kinase activity remained associated with cyclin A2 that was immunoprecipitated by the rabbit antibody in the heterologous assay.

Spatial regulation of cyclin B localisation and G2-M control

Cellular compartmentalisation of cyclins between the nucleus and cytoplasm plays an important role in G2-M regulation in mammalian oocytes. Recent evidence indicates that unlike mitosis, G2 arrest in oocytes is very sensitive to intra-nuclear cyclin B1 levels. Thus, a nuclear-targeting cyclin B1 construct markedly increases GVBD in oocytes (Holt *et al.* 2010) whereas a similar construct has little effect on the timing of entry

into mitosis (Hagting *et al*. 1998). Consequently, it is especially critical to maintain low cyclin B1 levels within the interior of the GV. It turns out that in order to accomplish this, one strategy is to spatially enrich components of the APC/C proteolytic pathway within the GV including the exclusive expression of the α splice variant of Cdh1, which is targeted to the nuclear interior, and not the cytoplasmic β variant (widely expressed in other cell types) (Holt *et al*. 2010). It has been proposed that the primary function of spatially enriched proteolysis within the GV is to maintain the G2/prophase arrest state by maintaining nuclear cyclin at low levels (Holt *et al*. 2010). It is also possible that compartmentalised proteolysis, by helping to preserve a large cytoplasmic reservoir of cyclin B, might assist in averting refractoriness.

Interestingly, similar to cyclin B1, immunostaining studies reveal that cyclin B2 and Hec1 are also enriched externally to the GV (Gui & Homer 2013). Hec1 has itself recently been shown to be an APC/C^{Cdh1} substrate (Li *et al*. 2011), and as discussed above, cyclin B2 is subject to APC/C^{Cdh1}-mediated proteolysis in GV-stage oocytes (Gui & Homer 2013). Although it is not currently known how Hec1 affords protection to cyclin B2, one possibility is that Hec1 sequesters cyclin B2 away from the GV interior where proteolytic activity is maximal. This could occur through direct binding in keeping with which cyclin B2 but not cyclin B1 was found to co-immunoprecipitate with Hec1 from mouse oocyte lysates (Gui & Homer 2013). Another possibility is that Hec1's influence on cyclin B2 stability might also reflect competitive substrate inhibition akin to the securin-cyclin B1 paradigm discussed above (Marangos & Carroll 2008). Arguing against this, however, over-expression of Hec1 did not stabilise cyclin B2 (Gui & Homer 2013). This regulatory mechanism deserves further attention, however, as over-expressed Hec1 alone might not replicate the function of native Hec1, which is ordinarily tightly complexed with Nuf2 (Campbell & Desai 2013). It is noteworthy that the subcellular compartmentalisation of other non-cyclin Cdk1 regulators, such as Cdc25B and Wee1B, is also important for control at the G2-M boundary (Oh *et al*. 2010).

APC/C-mediated control of M-phase progression

Following entry into M-phase, a singularly important objective for all cell types including oocytes is to establish properly configured attachments between kinetochores and spindle microtubules, a process referred to as chromosomal bi-orientation, and an indispensable pre-requisite for ensuring chromosome segregation fidelity (Foley & Kapoor 2013). Sequential APC/C^{Cdh1} and APC/C^{Cdc20} activities are important for the chain of events leading up to and following bi-orientation, including the ensuing anaphase I and transition into MII.

Prometaphase APC/C^{Cdh1} activity and acentrosomal spindle assembly

Following GVBD, APC/C^{Cdh1} continues to be active during early prometaphase I when slow-rising Cdk1 activity is thought to provide a permissive environment that is not inhibitory to APC/C^{Cdh1} (Reis *et al*. 2007). With increasing Cdk1 activation, APC/C^{Cdh1} activity is extinguished, allowing Cdc20 to accumulate and APC/C^{Cdc20} activity to rise – Cdc20 being subject to degradation by APC/C^{Cdh1} before this (Reis *et al*. 2007, Homer 2011). In addition to delaying Cdc20 accumulation, recent data also highlight an important role for APC/C^{Cdh1} during prometaphase I spindle assembly in mouse oocytes.

In oocytes of many species, spindle assembly proceeds in the absence of two pre-defined centrosomes as in mitosis, so-called acentrosomal spindle assembly (Dumont & Desai 2012). In mouse oocytes, the spindle is nucleated from ~80 microtubule organising centres (MTOC's; Schuh & Ellenberg 2007). The early acentrosomal spindle assumes a spherical form which, during an hours-long process during prometaphase I, is subsequently remodelled into a characteristic barrel-shaped structure as MTOCs become sorted into two distinct poles (Breuer *et al*. 2010, Gui & Homer 2012; Fig. 2). Two proteins that are APC/C^{Cdh1} substrates, targeting protein for the *Xenopus* kinesin xklp2 (TPX2) and hepatoma up-regulated protein (HURP), have been shown to be important for stable acentrosomal bipolar spindle formation (Brunet *et al*. 2008, Breuer *et al*. 2010). In mouse oocytes, spindle assembly and spindle pole integrity are dependent on TPX2, probably through N-terminal region-dependent transforming acidic coiled-coil 3 (TACC3) phosphorylation acting via Aurora A kinase (Brunet *et al*. 2008). HURP is important for bipolarisation in mouse oocytes by stabilising a central microtubule array that acts as a scaffold for sorting MTOCs into two distinct poles (Breuer *et al*. 2010).

Recent findings on *Fzr1*$^{\Delta/\Delta}$ oocytes provide surprising new insights into how APC/C^{Cdh1} affects MI progression through acentrosomal spindle assembly (Holt *et al*. 2012). Consistent with APC/C^{Cdc20} being the target of inhibitory regulation by the SAC (Foley & Kapoor 2013), Cdc20 over-expression can over-ride the SAC in other systems (Hwang *et al*. 1998, Mondal *et al*. 2007). Unexpectedly, however, although Cdc20 levels were nearly 25-fold higher in *Fzr1*$^{\Delta/\Delta}$ oocytes, Cdc20 stabilisation *per se* was not the main driver for earlier anaphase I-onset observed in such oocytes (Holt *et al*. 2012). If it were, then one would predict that APC/C^{Cdc20} would become active even in the face of ongoing SAC signalling. This is not the case; however, as in *Fzr1*$^{\Delta/\Delta}$ oocytes, anaphase I is only initiated after mitotic arrest-deficient 2 (Mad2) is displaced from kinetochores (Mad2 enrichment at kinetochores is indicative of ongoing SAC-mediated inhibition). Furthermore, low doses of

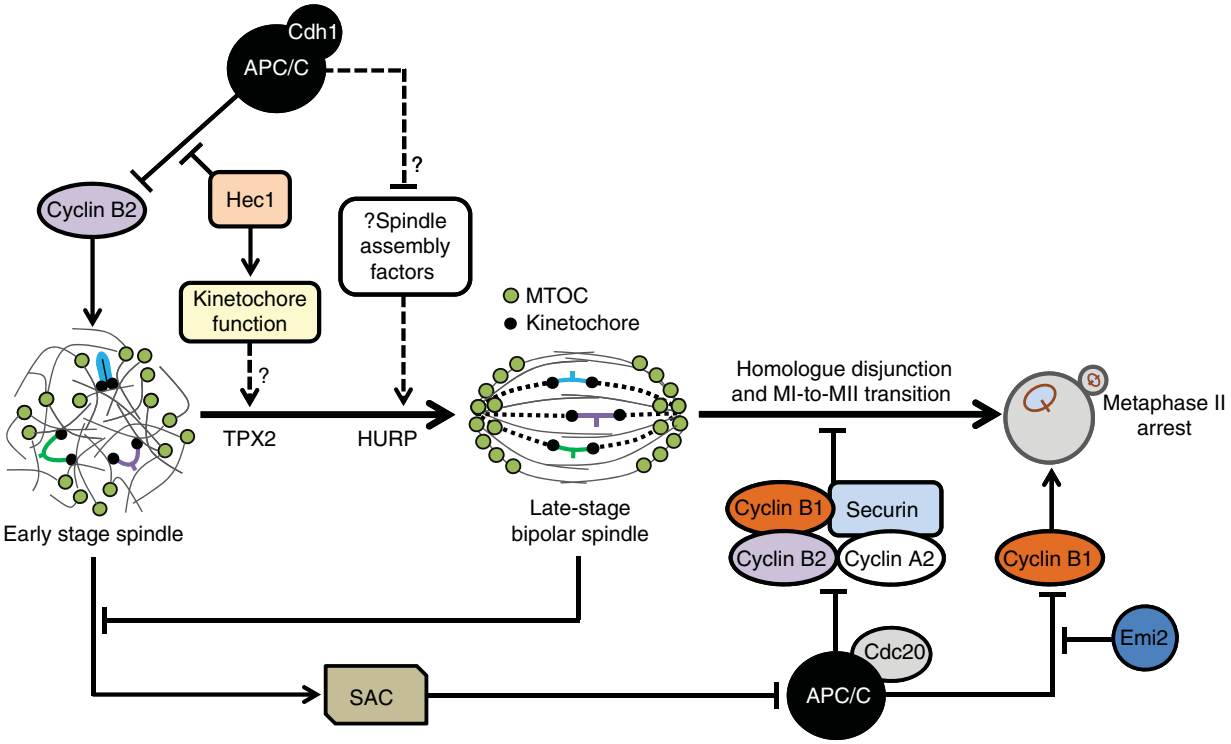

Figure 2 The APC/C in acentrosomal spindle assembly during MI and progression to MII. The schematic diagram depicts two stages in acentrosomal spindle assembly in mouse oocytes, an early stage when spindles are roughly spherical and the late stage when spindles have acquired a bipolar phenotype (Schuh & Ellenberg 2007, Gui & Homer 2012, 2013). Another characteristic feature of mouse oocytes (not explored in this review) is the configuration of homologous kinetochore pairs, which are juxtaposed at the early-spindle stage before re-orienting to face in opposite directions by the late stage (see Kitajima *et al.* 2011, Gui & Homer 2013). Such re-orientation is illustrated by the three bivalents (blue, lilac and green), which undergo a concomitant change in morphology from 'compact' to 'extended' (see Gui & Homer 2012, 2013). Hec1-dependent cyclin B2 stabilisation is important for early-stage spindle assembly after which preliminary evidence suggests that kinetochore re-orientation, in part mediated by Hec1- and CENP-E-dependent kinetochore functions (Gui & Homer 2012, 2013), supports late-stage bipolarisation, a model that will require further investigation. It is hypothetically possible that Hec1 stabilisation in $Fzr1^{\Delta/\Delta}$ oocytes helps to promote the accelerated spindle assembly and in particular, faster kMt attachment formation, that are observed (Holt *et al.* 2012), but this remains to be formally tested. The schematic therefore incorporates APC/C^{Cdh1} targets that act as 'spindle assembly factors', which may or may not turn out to include Hec1/cyclin B2. Although TPX2 and HURP are known to promote acentrosomal spindle assembly (Brunet *et al.* 2008, Breuer *et al.* 2010) and are potential APC/C^{Cdh1} substrates, in mouse oocytes, they may not be subject to direct APC/C^{Cdh1} regulation (Holt *et al.* 2012). Following the establishment of kMt attachments, SAC-mediated inhibition dissipates, thereby allowing activation of APC/C^{Cdc20}, which could then be tasked with degrading not only cyclin B1 and securin but also cyclin A2 and cyclin B2 (Touati *et al.* 2012, Gui & Homer 2013). It should be noted that APC/C^{Cdh1}-mediated regulation of APC/C^{Cdc20} through Cdc20 proteolysis (Reis *et al.* 2007) is not depicted here. Following exit from MI, Emi2 limits APC/C^{Cdc20} activity allowing for cyclin B1 accumulation and spindle stabilisation in MII (Madgwick *et al.* 2006).

nocodazole that disrupt microtubule attachments and lead to Mad2 recruitment to kinetochores induce an equally strong MI arrest whether or not Cdh1 is lacking, indicating that the capacity of $Fzr1^{\Delta/\Delta}$ oocytes to mount an SAC response to kinetochore-microtubule (kMt) attachment defects remains intact. Further investigation of $Fzr1^{\Delta/\Delta}$ oocytes revealed that earlier onset of anaphase I was in fact related to earlier satisfaction of the SAC brought about by a faster rate of spindle assembly that was accompanied by earlier establishment of stable kMt attachments as determined by earlier redistribution of Mad2 from kinetochores to spindle poles. The authors examined the possibility that accelerated Cdk1 activation in $Fzr1^{\Delta/\Delta}$ oocytes secondary to cyclin B1 stabilisation might account for their findings but observed similarly accelerated spindle assembly with or without the Cdk1-specific inhibitor, flavopiridol (Holt *et al.* 2012).

Altogether, the foregoing data suggested that one or more APC/C^{Cdh1} substrates important for spindle assembly as well as for mediating kMt attachments became de-regulated in $Fzr1^{\Delta/\Delta}$ oocytes. Notably, however, sixfold over-expression of TPX2 did not augment and indeed disrupted, spindle assembly leading to multipolar spindles (Brunet *et al.* 2008) arguing against the notion that TPX2 stabilisation in $Fzr1^{\Delta/\Delta}$ oocytes accounted for accelerated bipolar spindle formation. In keeping with this, TPX2 levels remained unaltered in GV-stage $Fzr1^{\Delta/\Delta}$ oocytes (Holt *et al.* 2012), albeit it is not known what the levels were during M-phase while spindle assembly is underway. As with TPX2, HURP over-expression also had a disruptive effect

on spindle assembly (Breuer *et al*. 2010) and no changes in HURP expression were observed in GV-stage *Fzr1*$^{\Delta/\Delta}$ oocytes (Holt *et al*. 2012). Additionally, although BubR1 has been to shown to contribute to stable kMt attachment formation in mitosis and in mouse oocytes (Lampson & Kapoor 2005, Homer *et al*. 2009, Wei *et al*. 2010), BubR1 levels were not increased in *Fzr1*$^{\Delta/\Delta}$ oocytes and in fact were reduced by about one-half (Holt *et al*. 2012). Overall, therefore, although TPX2 and HURP are known APC/C substrates and are important for acentrosomal spindle assembly in oocytes, it appears that they may not be subject to APC/C^{Cdh1} control in oocytes leaving it unknown how Cdh1 loss culminates in accelerated spindle assembly and kMt attachment dynamics.

Independent recent findings regarding Hec1 are of particular interest regarding this issue since, as mentioned above, Hec1 is an APC/C^{Cdh1} substrate (Li *et al*. 2011) and is known to be central to kMt attachment formation (Tooley & Stukenberg 2011). Significantly, Hec1 depletion was found to impair acentrosomal spindle assembly in mouse oocytes on two levels (Gui & Homer 2013). Firstly, through its role in stabilising cyclin B2 (another APC/C^{Cdh1} substrate), it was found to be important for early-stage spindle assembly (Fig. 2). Secondly, through a more canonical kinetochore-involved function, it was required for later stages of bipolarisation (Fig. 2). Given that reduced levels of Hec1/cyclin B2 severely impair spindle assembly, one might speculate that increased levels of this pairing could have some part to play in the accelerated dynamics of spindle assembly and kMt attachment formation observed in *Fzr1*$^{\Delta/\Delta}$ oocytes.

A challenging environment for APC/C^{Cdc20} during late MI in oocytes

In order to safeguard against chromosome mis-segregation, the surveillance system known as the SAC is intended to couple APC/C^{Cdc20} activation with the completion of proper bi-orientation (Foley & Kapoor 2013). A series of papers have demonstrated that SAC components including Mps1 (Hached *et al*. 2011) and members of the Mad and budding uninhibited by benzimidazole (Bub) family of proteins such as Mad2 (Wassmann *et al*. 2003, Homer *et al*. 2005*b*, 2005*c*, Niault *et al*. 2007), Bub1 (Yin *et al*. 2006, Leland *et al*. 2009, McGuinness *et al*. 2009), Bub3 (Li *et al*. 2009) and BubR1 (Homer *et al*. 2009, Wei *et al*. 2010) are capable of delaying anaphase I onset and are important for averting chromosome mis-segregation in oocytes. Significantly, however, it has become apparent that the oocyte's SAC does not incorporate the same level of stringency in its ability to reliably monitor small numbers of misaligned chromosomes as is characteristic of mitosis (Nagaoka *et al*. 2011, Gui & Homer 2012, Kolano *et al*. 2012, Lane *et al*. 2012). For further details relating to SAC signal transduction in mammalian oocytes, the reader is referred to recent in-depth reviews (Homer 2011, Eichenlaub-Ritter 2012, Nagaoka *et al*. 2012, Sun & Kim 2012).

Although cyclin B1 is the canonical cyclin that is degraded following SAC satisfaction, APC/C^{Cdc20} may have a broader cyclin substrate load in oocytes. In mitosis, cyclin A2 undergoes destruction shortly after nuclear envelope breakdown (den Elzen & Pines 2001, Geley *et al*. 2001) through preferential Cdc20 binding in concert with a Cdk kinase subunit (Cks)-dependent mechanism that enables APC/C^{Cdc20}-mediated destruction even in the face of an active SAC (Wolthuis *et al*. 2008, Di Fiore & Pines 2010). In contrast, analysis of fluorescently labelled constructs in mouse oocytes showed that degradation of cyclin A2 did not occur any earlier than that of securin in late MI (McGuinness *et al*. 2009, Jin *et al*. 2010). This would be consistent with APC/C^{Cdh1}-mediated proteolysis restricting Cdc20 accumulation to late MI (Reis *et al*. 2007), thereby preventing earlier prometaphase cyclin A2 degradation. It is noteworthy, however, that levels of endogenous cyclin A2 detected by immunoblotting in a recent paper appear to decline ahead of securin (see Fig. 1B in Touati *et al*. (2012)). As the focus of this paper was not to quantitatively analyse such changes, further clarification is required regarding the timing of endogenous cyclin A2 destruction. New findings indicate that cyclin B2 is also subject to APC/C^{Cdc20}-mediated proteolysis in late MI which, like securin and cyclin B1 degradation, is under the control of the SAC (Gui & Homer 2013).

Thus, following SAC satisfaction in oocytes, APC/C^{Cdc20} could be tasked with degrading securin, cyclin B1, cyclin A2 and cyclin B2 (Fig. 2). This constitutes a considerable substrate load for the metaphase-APC/C, which, along with restricted Cdc20 accumulation, could make for an unfavourable APC/C^{Cdc20}:substrate ratio perhaps explaining, at least in part, why proteolysis occurs over such a protracted period of around 2–3 h in oocytes (Herbert *et al*. 2003, Homer *et al*. 2005*a*, 2005*c*, Lane *et al*. 2012).

It has been proposed that these slow degradation kinetics furnish a buffer period during which bivalents have the opportunity to complete bi-orientation in advance of anaphase I-onset (Lane *et al*. 2012), a facility predicted to be especially important in the presence of a porous SAC. Although this remains to be formally tested, the finding that anaphase I-onset is advanced by chemically mediated Cdk1 inhibition induced after proteolysis is underway (Lane *et al*. 2012) provides one experimental means with the necessary temporal control for doing so.

A slow Cdk1 inactivation profile could also be relevant to the need to rapidly re-establish Cdk1 activity following exit from MI so as to promote entry into MII while avoiding DNA replication. At this stage, yet another APC/C regulator, Emi2 (for early mitotic inhibitor

2/Emi-related protein 1), is important for establishing the MII/CSF arrest state by restraining APC/C^{Cdc20} to enable cyclin B re-accumulation (Madgwick *et al*. 2006; Fig. 2).

Conclusion

It is roughly a decade since the APC/C's role in mammalian oocytes was first identified when the focus was on the Cdc20-activated species and its importance for triggering anaphase I (Herbert *et al*. 2003). More recently, interest has extended to APC/C^{Cdh1} and how this form of the APC/C engineers unique features at the G2-M boundary and prometaphase I progression characteristic of mammalian oocytes.

As new discoveries emerge so too, inevitably, do new questions. For instance, is Emi1-mediated inhibition of APC/C restricted to a narrow window around the time of GVBD? What is the relative importance of Emi1 inhibition vs the spatial redistribution of cyclin B? How do cyclin B2 and cyclin A2 contribute to MPF? How does Hec1 regulate cyclin B2 at the G2-M boundary and is this also dependent upon an element of spatial regulation? During M-phase, what are the important APC/C^{Cdh1} substrates that affect spindle assembly? What is the relationship between APC/C^{Cdc20} capacity and substrate load in late MI?

The coming decade will undoubtedly yield exciting new findings that will provide invaluable insights into the molecular dimensions of oocyte quality, critically important for advancing clinically relevant issues such as assisted reproductive treatments, *in vitro* maturation and female fertility preservation.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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