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Biology of the Cell 96 (2003) 215–229

Biology of the Cell

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Review

# Aurora A, Meiosis and Mitosis

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Received 2 September 2003; accepted 5 September 2003

**Abstract**

The Aurora family kinases are pivotal to the successful execution of cell division. Together they ensure the formation of a bipolar mitotic spindle, accurate segregation of chromosomes and the completion of cytokinesis. They are also attractive drug targets, being frequently deregulated in cancer and able to transform cells in vitro. In this review, we summarize current knowledge about the three family members, Aur-A, Aur-B and Aur-C. We then focus on Aur-A, its roles in mitotic progression, and its emerging roles in checkpoint control pathways. Aur-A activity can be controlled at several levels, including phosphorylation, ubiquitin-dependent proteolysis and interaction with both positive regulators, such as TPX2, and negative ones, like the tumor suppressor protein p53. In addition, work in Xenopus oocytes and early embryos has revealed a second role for Aur-A, directing the polyadenylation-dependent translation of specific mRNAs important for cell cycle progression. This function extends to post-mitotic neurons, and perhaps even to cycling somatic cells.
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*Keywords:* Aurora; Mitosis; Meiosis; Xenopus; Oocyte

## 1. Introduction

Investigations over the past 20 years have resulted in the discovery of numerous proteins that are required to ensure the correct timing, order, dependency and accuracy of DNA replication, chromosome segregation and cytokinesis. Work from several groups led to the identification of MPF (M phase promoting factor) as a complex of cyclin B and cdc2, the kinase that drive cells from G2 into M phase (Dunphy et al., 1988; Gautier et al., 1988; Labbe et al., 1988; Draetta et al., 1989; Labbé et al., 1989; Meijer et al., 1989; Westendorf et al., 1989), and the identification of the multisubunit ubiquitin ligase, the APC/C, that is responsible for the destruction of mitotic cyclins and other mitotic regulatory proteins during mitotic exit (Irniger et al., 1995; King et al., 1995; Sudakin et al., 1995). Much of this work was done using the frog *Xenopus*, the clam *Spisula* and several species of starfish, all of which provide large numbers of oocytes that are naturally arrested at the G2/M border of meiosis I. When stimulated by the appropriate mitogen, their oocytes proceed rapidly and synchronously into M phase, meiosis I and meiosis II. *Xenopus* oocytes have the further advantage that following the meiotic cycles, they undergo a second natural cell cycle arrest, this time at the metaphase/anaphase transition of meiosis II. Fertilization releases this arrest and initiates the rapid embryonic cell division cycles. This article is dedicated to Marcel Doree, whose creative and insightful work has contributed much to our understanding of the fundamental mechanisms that control the cell division cycle.

There is now a deep molecular understanding of the regulatory networks that prevent activation of mitotic cyclin A/cdc2 and cyclin B/cdc2 complexes, and thus the G2/M transition, until DNA replication is completed and DNA damage has been repaired. We also understand several of the checkpoint mechanisms that prevent APC/C-mediated destruction of mitotic cyclins and other proteins, and thus exit from M phase, when the mitotic spindle is damaged or the chromosomes are incompletely aligned on the metaphase plate (Morgan, 1997; Harper et al., 2002; Nasmyth, 2002; Peters, 2002). Imaging studies have provided additional information about the spatial regulation of the mitotic cdc2 complexes. In mammalian somatic cells, studies using phospho-specific antibodies that primarily detect the activated form of cyclin B/cdc2 complexes suggest that cdc2 is preferentially activated and localized at centrosomes (Jackman et al., 2003). In *Drosophila* embryos, GFP-tagged cyclin B accumulates at centrosomes in interphase, in the nucleus in

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doi:10.1016/j.biolcel.2003.09.008

prophase, on the mitotic spindle in prometaphase, and then on the microtubules that overlap in the middle of the spindle in metaphase. Spindle-associated cyclin B disappears in a wave that starts at the spindle poles and spreads to the equator When the cyclin B-GFP on the spindle is almost undetectable, the chromosomes enter anaphase (Huang and Raff, 1999; Wakefield et al., 2000).

In contrast to cdc2, much less is understood about the roles of the Aurora family of kinases (Aur-A, Aur-B and Aur-C), which are required for the accuracy of progression through mitosis. Even less is known about when and how the Aurora kinases are turned on and off, and how they function in checkpoint signaling pathways. We first briefly summarize current views of how Aur-A, Aur-B and Aur-C contribute to mitotic progression. Next, we discuss the roles of Aur-A in chromosome segregation and mitotic exit during the normal cell cycle. We describe how overexpression of Aur-A results in spindle defects and the appearance of aneuploid cells containing multiple centrosomes, and promotes tumor formation. We then summarize known molecular mechanisms that regulate Aur-A activation, inactivation and destruction during the mitotic cell division cycle. Finally, we return to the *Xenopus* oocyte, where our interest in Aur-A first began, and examine the role of Aur-A in the translational activation of an important meiotic regulator, Mos, and the roles of Aur-A in the two meiotic cell division cycles.

## 2. The Aurora family

The Aurora kinases were first identified in cell cycle studies as *Xenopus* Eg2 (Paris and Philippe, 1990; Andresson and Ruderman, 1998; Roghi et al., 1998), yeast Ipl1 (Chan and Botstein, 1993) and *Drosophila* aurora (Glover et al., 1995). We now know that there are three types of Aurora kinases in vertebrates mammals (Aur-A, Aur-B and Aur-C), two in frog, *Drosophila* and C. elegans (the A- and B-types), and a single one in budding yeast (Ipl1) and fission yeast (Ark1) which, so far, seem mostly B-like in their functions. The initial diversity of names of these kinases stemmed from the diversity of routes leading to their discoveries. For example, within the group now known to be A-type Auroras, *Xenopus* Aur-A (Eg2), was first identified in a screen for *Xenopus* egg cDNAs aimed at finding mRNAs that became deadenylated after fertilization (Paris and Philippe, 1990). Eg2 was subsequently shown to be a kinase with important roles in the signal transduction pathway that controls the G2/meiosis I transition in *Xenopus* oocytes (Andresson and Ruderman, 1998) and mitotic spindle function in *Xenopus* eggs (Roghi et al., 1998). Budding yeast Ipl1 was found in a screen for mutants that affected chromosome segregation and ploidy. *Drosophila* aurora was identified as a maternal effect mutant in which late stage embryonic cells arrested with condensed mitotic chromosomes associated with defective spindles or circular, monopolar spindles. Additional Aur-A orthologs have since been identified in many species, including rodents and humans (Katayama et al., 2003). For example, BTAK was identified as a gene amplified in human breast tumors. STK15 was the 15$^{th}$ kinase identified in a screen for kinases in human breast cancer cells. Others, like *C. elegans* Air-1, were named on the basis of their sequence similarity to the original *Drosophila* and yeast genes, such as *C. elegans* Air-1 (Aurora, Ipl1-related), the mouse ortholog IAK (Ipl1, aurora-like kinase) and human Aurora-2. Others, now known to be B- or C-type Auroras, received similar names, such as human Aurora-1, human HsAIRK, rat AIM-1 and mouse STK-1 (Aur-B orthologs), and human STK-13, human Aurora-3, and mouse AIE1 (Aur-C orthologs) (Nigg, 2001; Katayama et al., 2003).

While we now routinely refer to the individual kinases as being an Aur-A, Aur-B or Aur-C, the recognition that there are three types was not immediate. In somatic cells, the protein levels and activities of all the Aurora-like kinases peaked in mitosis and declined rapidly during mitotic exit. Their genes each mapped to chromosomal loci that are amplified, overexpressed or both in many cancer cell lines, as reviewed elsewhere recently (Goepfert and Brinkley, 2000; Katayama et al., 2003). Overexpression studies found that all of Aurora family kinases examined interfered with mitotic progression. Sequence alignments suggested the existence of three and possibly more classes within vertebrates, but were not definitive. Sorting by mutant phenotypes was not straightforward. For example, the original *Drosophila* Aur-A mutant phenotype suggested that Aur-A was required for centrosome separation and formation of a bipolar spindle, whereas in the original *C. elegans* Aur-A mutant, bipolar spindles were able to form but then collapsed. Furthermore, we now know that certain point mutants and truncations in Aur-A have the potential to act as dominant gain-of-function mutants, as discussed later. Whether differences in the original *Drosophila* and C. elegans Aur-A mutant phenotypes are due to variations in type of mutation, timing of expression, extent of penetrance, or stage-specific differences in roles of other proteins, is only beginning to be sorted out (Giet et al., 2002). Comparisons using RNAi may be more informative in certain cases, but the actual design and interpretation of RNAi-mediated ablations of cell cycle proteins are challenging. This is true both for early embryos, where maternal stockpiles of proteins exist in addition to mRNA, and in somatic cells, where the addition of a particular RNAi to an asynchronous culture could lead to a mix of two or more arrests types in the population (as would be predicted for cyclin A, for example).

The most informative property for sorting out the A, B and C classes turned out to be their subcellular localization figure 1. The Aur-As localize to centrosomes from early S phase onward; during mitosis, a fraction associates with spindle microtubules most proximal to the spindle poles. The Aur-Bs associate with chromosomes in early mitosis and relocalize to the spindle midzone at anaphase, typical of a small number of other well-characterized chromosomal passenger proteins. The Aur-Cs, which localize to centrosomes, have not yet

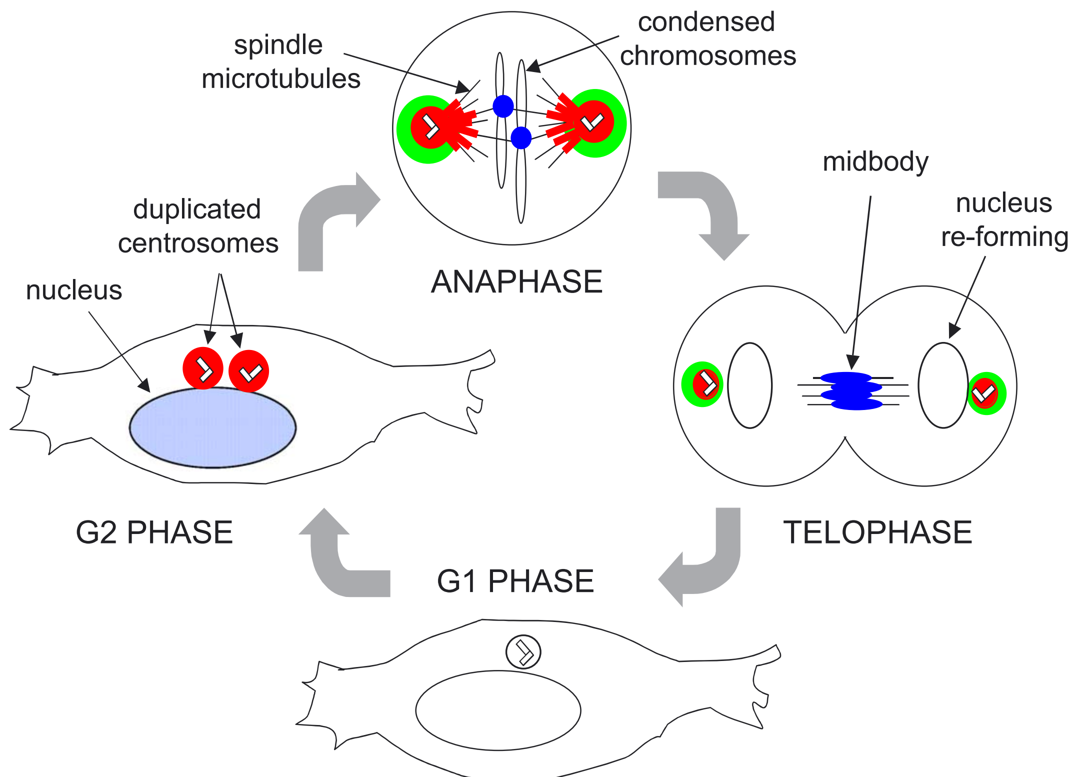


Fig. 1. Localizations of Aur-A (red), Aur-B (blue) and Aur-C (green) during the cell cycle.

been well-studied. Taken with sequence differences, and emerging differences in the types of proteins found to be associated with each kinase, these properties allowed sorting family members into three classes with the simplified and more consistent nomenclature of Aur-A, Aur-B and Aur-C (Nigg, 2001).

The catalytic domains of the three Aurora types show only minor differences in sequence, and there is no good understanding yet of how those variations may be important functionally. By contrast, there are important differences in their non-catalytic N-terminal sequences, their interacting proteins, their subcellular localizations, and, in some cases, their substrates. In this article, we first briefly summarize the properties of Aur-C and Aur-B, and then focus on the regulation of Aur-A and its roles in meiosis and mitosis.

## 3. Aurora-C

Aur-C was first identified in a screen for kinases expressed in mouse sperm and eggs (Tseng et al., 1998). While it is found prominently in testis (Tseng et al., 1998, Hu et al., 2000, Bernard et al., 1998; Kimura et al., 1999), Aur-C is also detected in other cell types and is overexpressed in certain cancer cell lines (Kimura et al., 1999 and Crosio et al., 2002). The Aur-C gene maps to 19q13.43, a region often translocated or deleted in certain cancer tissues (Kimura et al., 1999 and Bernard et al., 1998). Aur-C protein levels are low during interphase and peak at G2 and/or mitosis (Kimura et al., 1999and Crosio et al., 2002). Aur-C first appears at centrosomes in anaphase and persists there until cytokinesis, suggesting it may have a role during the later stages of M phase (Kimura et al., 1999). Little is known about the regulation of Aur-C protein and kinase activity levels. Protein kinase A (PKA) can phosphorylate mouse Aur-C at T171 in the presumed activation loop, and mutation of this residue to alanine enhances its activity *in vitro*, suggesting that this phosphorylation may negatively regulate its activity (Chen and Tang, 2002). Mutation of T174 to ala also enhances activity. However, it remains to be shown whether Aur-C is a substrate for PKA *in vivo* and whether T171 phosphorylation is indeed regulated in cells. The region containing these two threonine residues is conserved in almost all Aurora kinases. T171 in Aur-C corresponds to T295 in the *Xenopus* Aur-A activation loop (T288 in human Aur-A), which can also be phosphorylated *in vitro* by PKA *in vitro* (see below). *Xenopus* Aur-A has recently been shown to be capable of autoactivation through auto-phosphorylation at T295 (Eyers et al., 2003; Tsai et al., 2003), further emphasizing the regulatory potential of residues in this region of Aur-C.

Initial reports about the sequences of non-catalytic N-terminal region of human Aur-C varied. A short protein with a five residue N-terminal region was described by two groups for STK13/AIE2 (Bernard et al., 1998; Tseng et al., 1998), while a third group described a longer, 39 residue region for AIK3 (Kimura et al., 1999). Antibodies raised against the C-terminus of Aur-C recognize a protein of 36 kDa (Kimura et al., 1999) which is most consistent with the predicted size of the longer protein. Nevertheless, analysis of the genomic sequence reveals that the longer protein

derives from a translation initiation site within an intron that had not been spliced from the AIK3 cDNA. Thus it seems most likely that the non-catalytic N-terminal region of Aur-C is a mere five residues, (M)RRLTV, and does not contain any obvious destruction signals (Crane and Ruderman, in preparation).

## 4. Aurora-B

Aur-B, which has been widely studied in recent years, is required for, or coordinates, several important processes in chromosomes segregation and cytokinesis. For example, in *C. elegans*, disruption of Aur-B expression by RNAi produces single cell embryos that undergo multiple rounds of DNA replication and normal microtubule rearrangements in the absence of cytokinesis, leading to extreme polyploidy (Schumacher et al., 1998). Histone H3, an excellent substrate for Aur-B, fails to be phosphorylated during mitosis, and both chromosome condensation and segregation are defective. Some of these defects are similar to those seen in *Drosophila* S2 cells in which Aur-B has been depleted using RNAi (Adams et al., 2001c; Giet and Glover, 2001). Chromosome alignment in those cells is highly abnormal, and mitotic exit and cytokinesis are defective. Kinetochores bind to spindle microtubules, but sister chromatid separation does not seem to occur, and paired sister kinetochores still persist in anaphase (Adams et al., 2001c). By analogy with yeast Ipl1, Aur-B may function to destabilize kinetochore attachments to spindle microtubules until correct bi-orientation is achieved and the sister kinetochores are under tension (Biggins and Murray, 2001). Work with pharmacological inhibitors of Aurora kinases in mammalian cells is also consistent with a role for Aur-B in the spindle checkpoint (Ditchfield et al., 2003; Hauf et al., 2003).

Recent work has shed particular light on Aur-B's role in cytokinesis. RNAi experiments in *C. elegans* and *Drosophila* found that the cleavage furrow in Aur-B depleted cells form as in wild-type cells, but then regresses, indicating that Aur-B is also required for completion of cytokinesis (Schumacher et al., 1998; Adams et al., 2001b). While some cleavage furrow proteins, such as actin and NMY-2 localize normally to the forming furrow in *C. elegans* embryos lacking Aur-B, midbody-associated proteins such as ZEN-4 and polo-like kinase cannot be recruited to the midbody microtubules (Schumacher et al., 1998; Severson et al., 2000; Speliotes et al., 2000). These results further suggest that some events during cytokinesis occur normally in Aur-B deficient cells. The absence of midbody-associated proteins in these embryos seems to be due to an extremely disorganized spindle midzone (Speliotes et al., 2000). However, in *Drosophila* and mammalian cells, Aur-B does not seem to be required for localizing the orthologs of ZEN-4 (pavarotti/MKLP-1), suggesting that Aur-B has other targets during late cytokinesis (Adams et al., 2001b; Minoshima et al., 2003). One such target appears to be MgcRacGAP, a GTPase activating protein that associates with midbody microtubules and is required for late cytokinesis events. In HeLa cells, Aur-B binds and phosphorylates MgcRacGAP at a residue whose phosphorylation activates its GAP activity toward RhoA, a protein required for the completion of cytokinesis. This residue is phosphorylated *in vivo* and seems to target MgcRacGAP to the midzone; mutation to alanine prevents the completion of cytokinesis (Minoshima et al., 2003).

Aur-B is first found along the length of chromosomes during prophase, at the inner centromere regions near the kinetochores during prometaphase and metaphase, and with the spindle midzone during anaphase, eventually ending up in the midbody at the end of cytokinesis (reviewed by (Adams et al., 2001a; Descamps and Prigent, 2001; Shannon and Salmon, 2002). Aur-B associates with two other passenger proteins, Survivin and INCENP; reduction of any one of these three proteins interferes with the localization of the other two, indicating that their accurate targeting and function during mitosis require each other. Beyond the scope of this article, but well-reviewed (Earnshaw, 2001; Katayama et al., 2003) is a considerable body of evidence that the molecular roles for the Aur-B/INCENP complex include localizing Aur-B to centromeres at metaphase, where it phosphorylates histone H3 to regulate chromosome segregation, enhancing Aur-B kinase activity at particular times during mitosis, and translocation of the complex from centromeres to the spindle midzone during cytokinesis. Intriguingly, Survivin (Ambrosini et al., 1997), INCENP (Adams et al., 2001b) and Aur-B (Tatsuka et al., 1998) are overexpressed in many transformed and cancer cell lines, leading to the idea that the coordinated increase in this complex and, possibly enhanced Aur-B activity through increased expression with Survivin, is important for in some aspect of transformation or tumor formation (Katayama et al., 2003).

## 5. Aurora-A

Aur-A is an important regulator of spindle formation, and therefore essential for accurate chromosome segregation. Depending on the type of cell cycle and organism, Aur-A mutations interfere with recruitment of γ-tubulin ring complexes and other proteins during centrosome maturation, formation of a bipolar spindle, maintenance of the bipolar spindle, and chromosome segregation. This topic has been discussed in detail recently (Goepfert and Brinkley, 2000; Dutertre et al., 2002; Blagden and Glover, 2003). Accurate chromosome segregation depends on the accurate duplication, maturation, separation, positioning and, usually, the microtubule-nucleating activity of centrosomes. The centrosomes serve as microtubule organizing centers in interphase and in mitosis, and can also serve as organizers of cell polarity (see reviews by Brinkley and Goepfert, 1998; Brinkley, 2001; Doxsey, 2001; Hansen et al., 2002; Nigg, 2002). In most vertebrate somatic cells, the centrosome consists of a pair of centrioles surrounded by a pericentriolar matrix that

contains numerous components, including many copies of the γ tubulin ring complex, which is responsible for nucleating microtubules. Centrosome duplication begins during late G1 or S, depending on the cell type. At the onset of mitosis, replicated centrosomes separate, migrate to opposite poles of the cell, and organize the bipolar mitotic spindle. At the same time, the kinetochores of the replicated sister chromatids capture and/or nucleate microtubules. Once all kinetochores are associated with microtubules, and the sisters are aligned on the metaphase plate with the kinetochores under equal and opposite tension, anaphase begins. Checkpoint pathways that sense spindle damage, incomplete kinetochore occupancy, or lack of tension usually delay or block anaphase onset.

## 6. Localization of Aur-A

As seen in several studies, Aur-A staining is detected in the pericentriolar material (PCM) of centrosomes beginning in late G1 or early S phase and increases as the cell cycle proceeds. Aur-A remains associated with centrosomes at the mitotic poles and, later, is found in adjacent spindle microtubules as well (reviewed by Donaldson et al., 2001; Dutertre et al., 2002). Photobleaching studies show that there are rapidly exchanging pools of centrosome-associated and cytoplasmic Aur-A (Berdnik and Knoblich, 2002; Stenoien et al., 2003). Live imaging studies using GFP-tagged Aur-A also show that, during late G2, the Aur-A-staining centrosomes move to opposite sides of the nucleus and appear to push inward, with the nucleus taking on a dumbbell shape (Sugimoto et al., 2002). As the nuclear envelope dissociates and the bipolar spindle starts to form, the Aur-A signal increases at the centrosome, appears on spindles, and remains associated with them through telophase. The signal then quickly fades, presumably through destruction, delocalization or both. From these studies, it appears that Aur-A destruction probably occurs late in mitotic exit.

The N-terminal non-catalytic domain of Aur-A can localize to the centrosome in *Xenopus* egg extracts, while GFP fusions of either the N-terminal or catalytic domains are targeted to the centrosome in *Xenopus* XL2 cells (Giet and Prigent, 2001). Localization of the N-terminus-GFP fusion to the centrosome is microtubule dependent, and overexpression of the N-terminus inhibits bipolar spindle assembly in *Xenopus* egg extracts. This suggests the N-terminus of Aur-A may interact directly with the spindle microtubules or microtubule-associated proteins. Interestingly, localization of both full length and C-terminus-GFP fusion Aur-A proteins is microtubule-independent.

## 7. Regulation of Aur-A by phosphorylation and dephosphorylation

In somatic cells, both the amount of Aur-A protein and its kinase activity peak in mitosis (Bischoff et al.; 1998 ). During

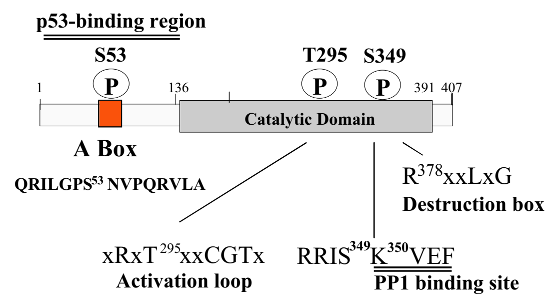


Fig. 2. Functional domains of *Xenopus* Aur-A.

the early embryonic cell cycles, Aur-A protein is stable whereas its kinase activity oscillates (Littlepage and Ruderman, 2002), providing an excellent opportunity to investigate regulation of Aur-A kinase activity, free from the complicating effects of synthesis and degradation. Previous work on the role of Aur-A during the G2/M transition in *Xenopus* oocyte maturation (discussed later) had shown that Aur-A activation requires one or more phosphorylations (Andresson and Ruderman, 1998). When the major metaphase phosphorylation sites of Aur-A were mapped by mass spectroscopy, three were identified (Figure 2): S53, T295 and S349 (equivalent to S51, T288 and S342 in human Aur-A) (Littlepage and Ruderman, 2002). To investigate how phosphorylation might affect activity, each of the sites were mutated to alanine or aspartic acid, expressed in Sf9 cells, and assayed for kinase activity. Neither of the S53 mutations had any significant effect on Aur-A activity. However, mutation of S53 to aspartic acid, an acidic residue that can sometimes mimic the effect of phosphorylation, did block the *in vitro* destruction of Aur-A, suggesting that phosphorylation of S53 might negatively regulate destruction until the time when it is normally degraded during mitotic exit (Littlepage and Ruderman, 2002).

Both T295A and T295D mutations completely block activity (Littlepage et al., 2002). An equivalent mutation in human Aur-A (T288A) also blocks activity (Bischoff et al., 1998; Walter et al., 2000). Furthermore, inactivating mutations at this site completely block the ability of Aur-A to transform tissue culture cells and promote tumor formation (Bischoff et al., 1998; Walter et al., 2000; Littlepage et al., 2002). Crystal structures of the Aur-A catalytic domain expressed in Sf9 cells, which can contain several different phosphorylated forms of Aur-A with differing activities (Andresson and Ruderman, 1998; Littlepage et al., 2002), reveal that it has a typical bilobed organization (Cheetham et al., 2002; Nowakowski et al., 2002). The structure of the activation loop, which contains the T295 residue whose phosphorylation is required for activity, is partially disordered. In one of the structures (Nowakowski et al., 2002), free phosphate ion bound near T295 can be seen, suggesting that the phosphate-bound Aur-A structure mimics the catalytically active conformation. The activation loop, which contains residues specific to the aurora family of kinases, displays several features that could be exploited in the design of specific inhibitors. Given the increasing evidence that over-

expression of Aur-A generates aneuploidy and is associated with tumor formation, Aur-A has become an attractive target for drug development. One such inhibitor has been described recently, ZM447439, which inhibits both Aur-A and Aur-B *in vitro*, and seems to preferentially inhibit Aur-B *in vivo* (Ditchfield et al., 2003). Another small molecular inhibitor of mitotic progression, hesperadin, may also affect Aurora kinase-dependent processes (Hauf et al., 2003).

In *Xenopus* Aur-A, the S349A mutation does not seem to affect kinase activity significantly, whereas the S349D mutation abolishes activity. Although open to other interpretations, these results suggested to us that phosphorylation of the activation loop residue T295 is required for activity, while phosphorylation of S349 might negatively regulate its activity (Littlepage and Ruderman, 2002). The corresponding residue in human Aur-A, T288, is also required for activity (Bischoff et al., 1998; Walter et al., 2000). However, in a recent study where quantitative mass spectroscopy analysis was used to map the auto-phosphorylation sites on bacterially expressed, active *Xenopus* Aur-A, Maller, Ahn and colleagues found that 12 sites, including S349, were phosphorylated, and that 100% of the S349 sites were phosphorylated (Eyers et al., 2003) indicating that phosphorylation of S349 does not inhibit activity of the purified protein *in vitro*. Intriguingly S349 is immediately adjacent to $K^{350}$VEF, which binds PP1, a negative regulator of Aur-A activity, as discussed next.

## 8. Inhibition of Aur-A by the phosphatase PP1

There is now good evidence that Aur-A and PP1, both of which are enriched on centrosomes, can associate and that, *in vitro*, each can negatively regulate the other (Katayama et al., 2001). Aur-A has two PP1-binding motifs, one that includes the catalytic lysine residue ($K^{169}$VLF) and the other immediately adjacent to S349 ($K^{350}$VEF). A portion of endogenous Aur-A and PP1 was co-immunoprecipitated during mitosis. Aur-A mutants that cannot bind PP1 were hyperphosphorylated suggesting that PP1 negatively regulates Aur-A by dephosphorylation of one or more critical residues. Conversely, Aur-A was able to phosphorylate and inhibit PP1 *in vitro*. There are now several examples where PP1 targeting subunits are phosphorylated within or adjacent to the K/RVxF PP1-binding motif in response to incoming signals, and this phosphorylation leads to decreased PP1 binding (Beullens et al., 1999; McAvoy et al., 1999; Walker et al., 2000). One possibility is that, *in vivo*, phosphorylation of S349 decreases binding of PP1 to $K^{350}$VEF, thus preventing dephosphorylation of T295 and/or other sites required for Aur-A activity.

## 9. Activation of Aur-A by TPX2

TPX2 (Target Protein for *Xenopus* kinesin-like protein 2) is a microtubule-associated protein required both for stability of the mitotic spindle, and for localizing Aur-A to the spindle poles (Wittmann et al., 2000; Gruss et al., 2002; Kufer et al., 2002). Recent work shows that TPX2 stimulates Aur-A activity by binding and stimulating autophosphorylation of Aur-A on T295 (Eyers et al., 2003; Tsai et al., 2003). It is believed that TPX2 and another spindle assembly factor, NuMA (Nuclear Mitotic Apparatus protein) are inhibited in interphase by the binding of importin-α and -β (better known as cargo receptors for nuclear transport). TPX2 and NuMA appear to become activated when the importins are displaced by the small GTPase, Ran (also known as a regulator of nuclear transport). Consistent with this, spindle assembly depends in part on regulation by Ran, and the activated form of Ran, Ran-GTP, can induce mitotic spindle assembly in *Xenopus* egg extracts and *in vivo* (Guarguaglini et al., 2000; Moore et al., 2002). Spindles nucleated at centrosomes are stabilized by attachment to chromosomes at their kinetochores, and/or by the chromosomes themselves which, in the absence of centrosomes, can generate spindle-like arrays in a process promoted by microtubule-associated motors (Kirschner and Mitchison, 1986; Heald et al., 1996; Walczak et al., 1998; Karsenti and Vernos, 2001). The current view is that during interphase, Ran-GTP causes dissociation of cargo from importins in the nucleus, so that Ran-GTP is responsible for the directionality of cargo transport. At the onset of mitosis, Ran-GTP becomes more concentrated around chromosomes through association with its chromatin-bound exchange factor, RCC1. It is thought that Ran-GTP dissociates spindle assembly factors from the importins in a small area around the chromatin, initiating microtubule assembly at the centrosomes (reviewed by Zhang and Clarke, 2001; Dasso, 2002). Separated centrosomes also approach the nuclear envelope closely during early mitosis, where they would be in a good position to interact with importins (Sugimoto et al., 2002). Understanding how TPX2 stimulates Aur-A autophosphorylation and Aur-A activity, and how this is negatively regulated by PP1, are important questions.

## 10. Inhibition of Aur-A by p53

The p53 tumor suppressor protein plays many important roles in maintaining genomic stability, through its ability to respond to numerous events including DNA damage, spindle damage and inappropriate activation of oncoproteins (Morgan and Kastan, 1997; Taylor et al., 1999). One of its best understood roles is the transcriptional activation of several target genes that can induce checkpoint arrests in G1 and/or G2, depending on the cell type and context. Several studies show that p53 on its own can cause G2 arrest in the absence of DNA damage, and can also extend the G2 arrest that occurs in response to DNA damage (e.g. Agarwal et al., 1995; Taylor et al., 1999; Taylor and Stark, 2001). In addition, p53 appears to play important checkpoint role(s) during mitosis (Fukasawa et al., 1996; Duensing et al., 2000; Duensing et al., 2001; Tarapore and Fukasawa, 2002). A portion of

p53 is found at the centrosomes during mitosis, and reduction or loss of p53 results in the formation of cells with multiple centrosomes and spindle defects. Significantly, these defects resemble those induced by overexpression of Aur-A, defects which are especially marked in cells lacking p53.

Human p53 protein directly binds and inhibits the activity of human Aur-A (Chen et al., 2002). Using a transactivation-defective p53 as bait in a two-hybrid screen, Aur-A was isolated as a p53-interacting protein. When purified recombinant p53 and Aur-A proteins are co-incubated, p53 binds directly to the N-terminal non-catalytic domain of Aur-A and inhibits its kinase activity *in vitro*. The same transcriptionally inactive p53 can suppress Aur-A-induced centrosome amplification *and* cellular transformation, further supporting the idea that p53 negatively regulates Aur-A by a direct protein-protein interaction. By contrast, p53 does not suppress transformation induced by Aur-A lacking the N-terminus.

*Xenopus* eggs contain large, cytoplasmic stores of p53 protein. The early cell cycles proceed without any detectable transcription, and the effects of p53 (injection of additional p53 slows the cell cycle for example) all result from non-transcriptional effects (Tchang et al., 1993; Amariglio et al., 1997; Wallingford et al., 1997; Tchang and Mechali, 1999). Recent work from our lab confirms that *Xenopus* p53 and Aur-A also interact (Gadea and Ruderman, in preparation). Defining the precise regions of interactions, and the molecular mechanism by which p53 inhibits Aur-A's kinase activity are important future questions.

## 11. Targets of Aur-A

The past five years have revealed an increasing number of proteins that are phosphorylated by Aur-A *in vitro*, some of which probably represent real *in vivo* targets. A subpopulation of Aur-A associates with TACC, a microtubule-associated protein that also localizes to centrosomes and is involved in stabilizing centrosomal microtubules (reviewed by Gergely, 2002). In *Drosophila*, RNAi-mediated reduction and specific point mutants of Aur-A show that Aur-A is required to localize TACC and its binding partner MSPS to the centrosomes both in embryos and neuroblasts (Giet et al., 2002). Aur-A can bind and phosphorylate Eg5, a kinesin-like motor protein whose localization pattern resembles that of Aur-A and which is essential for the formation and maintenance of a normal bipolar spindle (Giet et al., 1999; Mayer et al., 1999). As discussed above, Aur-A can phosphorylate and inhibit the phosphatase PP1, which is itself a negative regulator of Aur-A, suggesting that the two participate in a regulatory feedback loop (Katayama et al., 2001). As discussed later, Aur-A phosphorylates the translational regulatory protein CPEB during oocyte maturation, leading to the adenylation and translation of stored Mos mRNA TPX2 (Kuffer et al., 2002; Eyers et al., 2003; Tsai et al., 2002), but the physiological role of this phosphorylation needs further investigation. Finally, we know that Aur-A can activate itself through autophosphorylation (Eyers et al., 2003; Tsai et al., 2003; Wu, Littlepage, and Ruderman in prep.).

## 12. Ubiquitin-mediated destruction of Aur-A

Aur-A activity is regulated by phosphorylation, ubiquitin-dependent proteolysis or both, depending on the cell type. In somatic cells, two studies have suggested that during mitotic exit Aur-C protein disappears first, followed by the loss of Aur-A and then Aur-B (Bischoff et al., 1998; Taguchi et al., 2002). It is notable that Aur-B protein levels, which decline during late mitotic exit, never seem to disappear completely. The pathways responsible for these regulated, selective destructions are not well understood; most information at this point has come from work with Aur-A.

Destruction of Aur-A is dependent on a specialized ubiquitin ligase, APC/C-Cdh1, which recognizes at least two sequences on Aur-A. Early work from our lab and others found that the selective ubiquitin-dependent proteolysis of several mitotic regulatory proteins at specific times during mitotic progression is catalyzed by the APC/C, a multisubunit ubiquitin ligase (E3), in conjunction with a small ubiquitin carrier protein (E2) called here by its human name, ubcH10 (Hershko et al., 1994; Irniger et al., 1995; King et al., 1995; Sudakin et al., 1995; Aristarkhov et al., 1996; Yu et al., 1996; Townsley et al., 1997). Work in yeast and *Drosophila* identified two APC/C activator subunits, cdc20 (fzy) and cdh1 (fzr) that are responsible for APC/C activation, for substrate recognition and binding, and for temporally selective ubiquitination of those substrates (reviewed in Amon, 1999; Fang et al., 1999; Farr and Cohen-Fix, 1999; Hershko, 1999; Morgan, 1999). The cdh1-activated form of the APC/C is required for ubiquitin-dependent destruction of both *Xenopus* Aur-A (Castro et al., 2002; Littlepage and Ruderman, 2002) and human Aur-A (Crane et al., in preparation). Cdh1, which is also required for the destruction of several mitotic regulatory proteins during late mitotic exit, recognizes two well-characterized APC/C recognition signals: the destruction box and the three residue KEN sequence. Both signals are highly conserved in Aur-A and Aur-B. In *Xenopus* eggs and early embryos, cdh1 protein is absent and consequently Aur-A protein levels are constant across the early mitotic cell cycles (Littlepage and Ruderman, 2002). Addition of cdh1 protein to *Xenopus* egg extracts undergoing mitotic exit restores destruction of both endogenous Aur-A and radiolabeled Aur-A *in vitro* translation product. Furthermore, in COS cells, overexpression of cdh1 leads to a decrease in the steady state level of a co-expressed tagged Aur-A protein and requires an intact destruction box sequence (Taguchi et al., 2002). Cdh1-dependent destruction of Aur-A does not require the KEN sequence but does require the C-terminal destruction box and a sequence in the non-catalytic N-terminal domain, the A box (Littlepage and Ruderman, 2002). The A box is highly conserved in Aur-A orthologs; it is not found in Aur-B or Aur-C. Intriguingly, the A box also

contains the residue S53, which is phosphorylated during mitosis and whose phosphorylation may block destruction until late in mitotic exit (Littlepage et al., 2002). A fusion protein consisting of an N-terminal fragment containing the A box plus the catalytic C-terminal domain of Aur-B can be destroyed in Cdh1-supplemented egg extracts, suggesting that the A box and C-terminal destruction box may act cooperatively to signal destruction during M phase exit (Castro et al., 2002). Similar assays using lysates prepared from HeLa cells shortly after mitotic exit and human Aur-A show that these sequence requirements for mitotic destruction are highly conserved between *Xenopus* and human Aur-A (Crane et al., in preparation).

## 13. Aur-A and checkpoint control in somatic cells

A preliminary but intriguing report found that, in HeLa cells, where DNA damage delays the G2/M transition, forced overexpression of Aur-A overcame this delay (Marumoto et al., 2002). This result suggests that Aur-A may be involved in G2/M checkpoint control. Furthermore, when cells were treated with nocodazole to disrupt microtubules, Aur-A appeared to activate properly but then stayed active. In another study (Anand et al., 2003), Aur-A overexpression in HeLa cells led to spindle abnormalities and defects in chromosome alignment (as previously seen), plus failures in the attachment of some kinetochores to microtubules. Kinetochores showed persistent mad2 staining during anaphase, suggesting that the spindle checkpoint had been activated and that overexpression of Aur-A bypassed that checkpoint. Through transfection, dominant negative bub1 protein was able to suppress the Aur-A –induced accumulation of cells in G2 and/or M and the formation of multinucleate (probably tetraploid) cells. Furthermore, overproduction of Aur-A induced a strong increase in resistance to taxol-induced apoptosis, raising the possibility that Aur-A gene amplification could contribute to drug-resistance in tumors.

## 14. Aur-A and Cancer

There is strong evidence that Aur-A is a clinically important oncogene. Human Aur-A localizes to chromosome 20q13, a region that is frequently amplified and overexpressed in breast cancers and correlated with poor prognosis (Kimura et al., 1997; Sen et al., 1997). In a range of primary colorectal cancers tested, over 50% had amplified Aur-A DNA and overexpressed mRNA (Bischoff et al., 1998), while among colorectal cancers, a correlation was found between Aur-A expression, invasiveness and high p53 levels (Takahashi et al., 2000). Aur-A was overexpressed in 12-62% of primary breast tumors and multiple cancer cell lines; in some cases high mRNA levels were observed in the absence of DNA amplification (Zhou et al., 1998; Miyoshi et al., 2001). Aur-A was also found to be overexpressed in 94% of invasive ductal adenocarcinomas of the breast, but not in the corresponding normal tissues (Tanaka et al., 2002), in all 9 of 9 pancreatic tumors examined in a recent study (Li et al., 2003).

Forced overexpression of either human or *Xenopus* Aur-A is sufficient to transform mammalian cells and promote formation of tumors in nude mice (Bischoff et al., 1998; Littlepage et al., 2002). At the cellular level, overexpression of Aur-A in HeLa cells, which lack p53 function, generates aneuploid, often 4N, cells containing multiple centrosomes (Littlepage et al., 2002; Meraldi et al., 2002). Reintroduction of p53, which can bind and inactivate Aur-A as discussed earlier (Chen et al., 2002), suppresses the ability of Aur-A to generate tetraploid cells with multiple spindle poles (Meraldi et al., 2002). Forced expression of Aur-A in near diploid human breast epithelial cells also leads to centrosome amplification and aneuploidy (Zhou et al., 1998). These findings suggest an important and perhaps delicate balance *in vivo* between p53, a tumor suppressor protein, and Aur-A, an oncoprotein. Overexpression of Aur-A can also cause defects in spindle morphology, a low frequency of metaphase chromosome misalignment, and failure of kinetochores to capture free microtubule ends (Anand et al., 2003). In that work, mad2 staining remained on the chromatin during anaphase, suggesting that a spindle checkpoint was being bypassed as discussed in the previous section. It will be interesting to learn whether Aur-A's oncogenic activity is due to these checkpoint effects, or to other as yet unknown mechanisms.

## 15. The roles of Aur-A during the meiotic cell cycles of *Xenopus* oocytes

Studies of *Xenopus* oocyte maturation have revealed an interesting function of Aur-A that appears to have little to do with spindle formation, but instead to do with mRNA adenylation and translation (Figure 3). *Xenopus* oocytes arrest naturally during the G2/M transition at prophase of meiosis I. Gonadotropins from the pituitary stimulate the surrounding follicle cells to secrete steroid hormones, including progesterone, that break the G2/M arrest, induce progression through the two meiotic cycles and lead to differentiation of the immature oocyte into a mature, fertilizable oocyte, now called the egg (Smith, 1989). The egg then arrests at metaphase of meiosis II until fertilization breaks the arrest. Progesterone applied directly to oocytes lacking follicle cells stimulates all these events, which are collectively referred to as oocyte maturation. Early work also showed that progesterone activates the G2-arrested oocyte through a non-transcriptional pathway. After physical enucleation of the oocyte or blockade of transcription by Actinomycin D, progesterone was still able to able to induce MPF activation (later identified as cyclin B/cdc2), nuclear envelope breakdown (germinal vesicle breakdown, GVBD), chromosome condensation, progression through the meiotic cycles, and formation of the meiosis II-arrested egg.

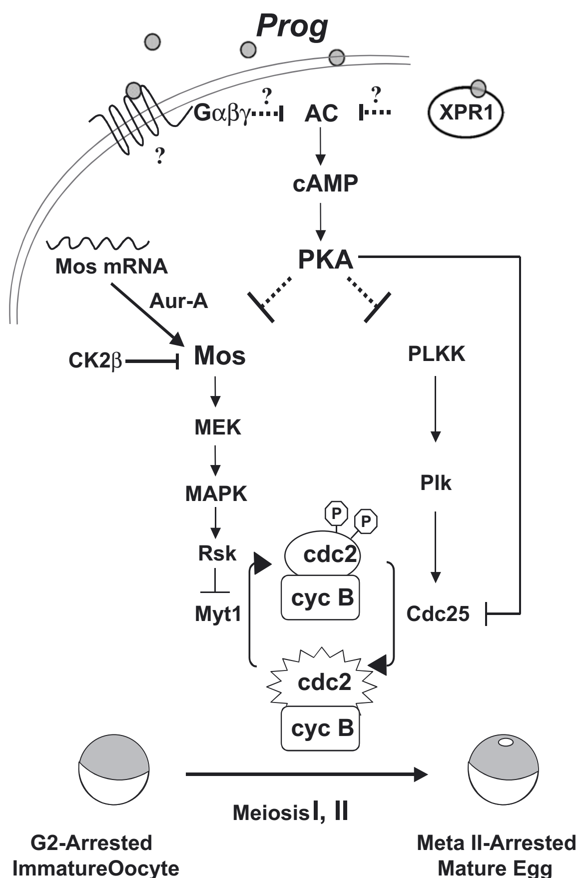


Fig. 3. Progesterone activates two parallel signaling pathways that converge to activate cdc2/cyclin B.

The ability of progesterone to induce amphibian oocyte maturation through a non-transcriptional pathway, first demonstrated in the late 1960s, remains one of the most compelling examples of the rapid, non-genomic effects of steroid hormones. Androgens such as testosterone are potent inducers of *Xenopus* oocyte maturation in the absence of transcription, and there is now very good evidence that testosterone provides the main stimulus for oocyte maturation *in vivo* (Fortune, 1983; Lutz et al., 2001; Lutz et al., 2003; Yang et al., 2003). So far, the signaling pathways used by androgens appear quite similar to those described previously for progesterone, suggesting that, with the exception of the receptors themselves, most of what we have learned from more than 30 years of investigating how progesterone activates the frog oocyte probably holds for androgen-stimulated oocyte maturation as well.

Within minutes of exposure to progesterone, there is a rapid decrease in adenylyl cyclase activity and cAMP levels, both in whole oocytes and in isolated surface vesicle preparations (Maller and Krebs, 1977; Maller et al., 1979; Finidori-Lepicard et al., 1981; Sadler and Maller, 1981; Cicirelli and Smith, 1985). We now know that progesterone functions through one, and possibly two, receptors: XPR-1/xPR, a conventional transcriptional receptor that can also initiate cytoplasmic signaling (Bayaa et al., 2000; Tian et al., 2000), and a recently identified membrane-associated protein that may mediate G protein down regulation of adenylyl cyclase (Zhu et al., 2003a; Zhu et al., 2003b). While the earliest progesterone-dependent steps have not yet been worked out, many of the downstream responses are now well understood. Progesterone induces activation of what appear to be two parallel signaling pathways that converge on the activation of cyclin B/cdc2 (see Stanford et al., 2003 and refs therein). One pathway inhibits an inhibitor of cdc2 (the kinase Myt1); the other activates an activator of cdc2 (the phosphatase cdc25). In the first pathway, activation of Aur-A induces the polyadenylation and polyadenylation-dependent translation of maternal mRNA encoding Mos, a MAP kinase kinase kinase, as discussed below. Increasing evidence suggests that newly made Mos protein is bound and inhibited by a maternal store of CK2β, the noncatalytic subunit of the kinase CK2 (Chen and Cooper, 1997; Chen et al., 1997), Lieberman and Ruderman, in prep.). Once Mos accumulates beyond this threshold, it is able to induce activation of MAPK. MAPK then binds and activates the kinase RSK; RSK in turn binds and inactivates Myt1, overcoming its ability to keep MPF repressed through inhibitory phosphorylations on cdc2 (Palmer et al., 1998). Mos also appears to be able to directly inactivate Myt1 (Peter et al., 2002). In the second pathway, progesterone leads to activation of the kinase Plk and cdc25, the phosphatase that removes the inhibitory phosphorylations from cdc2 (Hoffmann et al., 1993; Izumi and Maller, 1993; Qian et al., 1998; Qian et al., 2001). Both pathways can be blocked by high PKA activity as found in oocytes before progesterone stimulation. In the first pathway, PKA blocks synthesis and/or accumulation of Mos, the activation of Mos, and the ability of Mos to induce GVBD (see (Stanford et al., 2003) and refs therein). PKA inhibits the second pathway through an inhibitory phosphorylation of cdc25 (Duckworth et al., 2002). PKA may also have a noncatalytic role in blocking the G2/Meiosis I transition (Schmitt and Nebreda, 2002).

By contrast, less is known about the pathways that connect the earliest progesterone-induced events to the activation of the two cytoplasmic signaling pathways. Several years ago we undertook a screen designed to identify proteins that became phosphorylated, or otherwise modified, soon after progesterone stimulation, using a variant of the ″small pools cDNA″ protocol (Stukenberg et al., 1997). One of the first hits in this screen was the kinase Eg2, now recognized as *Xenopus* Aur-A (Andresson and Ruderman, 1998). We found that Aur-A was phosphorylated soon after progesterone stimulation of the oocyte, that overexpression of Aur-A accelerated both the appearance of newly synthesized Mos protein and GVBD, and that overexpression of Aur-A lowered the concentration of progesterone needed to induce Mos synthesis and GVBD. These results together suggested that Aur-A played a role early in the pathway, somewhere between the receptor and the translational activation of Mos. These results were significant because (i) newly made Mos protein is critical for GVBD and the completion of meiosis, (ii) Mos is one of the earliest proteins whose synthesis is

activated by progesterone, and (iii) injection of Mos mRNA into G2-arrested oocytes induces GVBD and the resumption of the meiotic divisions. Crucially, ablation of Mos mRNA blocks GVBD and formation of a meiosis I spindle (reviewed by (Nebreda and Ferby, 2000). In addition to its role in entry into Meiosis I, Mos is required for accurate progression through meiosis I and II, and to establish the metaphase II-arrest of the unfertilized egg. Subsequent work found that the early activation of Aur-A has a direct, positive effect on the polyadenylation and translational activation of Mos mRNA, as described below.

The oocytes and early embryos of all species examined rely extensively on translationally-regulated switches in the synthesis of individual proteins. In almost all cases, the cell cycle stage-specific, selective translation of these proteins is controlled in turn by the selective adenylation of their mRNAs. The first and arguably still the most dramatic example of this regulatory mechanism was the rapid switch in the adenylation and translation of the mitotic cyclins in clam oocytes (Rosenthal et al., 1980; Rosenthal et al., 1983). Work from numerous groups established that Mos mRNA has a short 3' oligo(A) tail and is translationally inactive in the oocyte. In response to progesterone, Mos mRNA is adenylated by a cytoplasmic poly(A) polymerase, an event that is required for, and directly promotes, its translation (reviewed by (Richter, 2000; Wickens et al., 2000). Adenylation of Mos mRNA requires the presence of two sequences in its 3'UTR, the conventional AAUAAA polyadenylation signal and a short cytoplasmic polyadenlyation element (CPE) upstream of that. In the oocyte, the CPE is bound by CPEB (cytoplasmic polyadenylation element binding protein). Given that Aur-A overexpression accelerated the appearance of Mos protein in progesterone-stimulated oocytes, we asked if CPEB might be a direct target of Aur-A.

Several studies now strongly support the idea that Aur-A phosphorylates CPEB soon after progesterone stimulation, converting CPEB from a repressor to an activator of Mos mRNA translation. In response to progesterone, CPEB is phosphorylated on S174, and this phosphorylation is required for Mos mRNA adenylation, Mos translation and GVBD. *In vitro*, CPEB is phosphorylated at a single site, S174, in a sequence context that we now know is an Aur-A phosphorylation motif, and a CPEB peptide spanning S174 delays GVBD. Egg extracts, in which Aur-A kinase activity is high, phosphorylate CPEB at this and several other sites; extracts from which Aur-A was depleted did not phosphorylate CPEB on ser-174 (Mendez et al., 2000). Further evidence for an early, translational role of Aur-A comes from the work of (Ma et al., 2003). For use as bait in a yeast two-hybrid screen, they constructed a myristylated version of Aur-A (Myr-Aur-A) which is, for reasons not yet understood, constitutively active. In the absence of progesterone, injection of Myr-Aur-A into G2-arrested oocytes induces CPEB phosphorylation, Mos synthesis, activation of MAP kinase, activation of MPF and GVBD. These results add further support to the idea that Aur-A functions early in the G2/MI transition, upstream of Mos translation and MPF activation.

The mechanism by which Mos mRNA polyadenylation leads to its translational activation is fairly well understood. In unstimulated oocytes, CPEB interacts with the protein maskin and the mRNA cap-binding factor eIF4E in a way that prevents binding of eIF4E to the scaffold protein eIF4G. Thus, formation of the translation initiation complex is prevented. Upon phosphorylation of CPEB by Aur-A, CPSF (cleavage and polyadenylation specificity factor) is recruited to the AAUAAA sequence of Mos mRNA. CPSF, in turn, recruits cytoplasmic poly(A) polymerase and initiates polyadenylation. The newly elongated poly(A) tail then binds PABP (poly(A) binding protein). The PABP-eIF4G complex is believed to displace maskin from eIF4E. This allows the assembly of eIF4E, the 40S ribosomal subunit and other proteins, on the eIF4G scaffold, leading to translational activation (see Cao and Richter, 2002) and refs therein). Consequently, early activation of Aur-A appears to be critical for triggering Mos translation and, thus, the Mos-dependent inhibition of the cdc2 inhibitor Myt1. This is not unique to *Xenopus*: Aur-A plays a similar role in positively regulating the polyadenylation of mRNAs in mouse oocytes (Hodgman et al., 2001).

The role of Aur-A in translational activation in oocytes may extend to the early embryonic cell cycles. CPEB, maskin and Cyclin B1 mRNA have all been found on mitotic spindles in early embryos (Groisman et al., 2000). It has also been reported that the polyadenylation of cyclin B mRNA is low in interphase and high in M phase in somatic cells. Addition of a CPEB antibody to cycling *Xenopus* extracts (going through alternating interphase and M phases) was able to block polyadenylation and cyclin B1 protein accumulation, causing an interphase arrest. Phosphorylation of CPEB on S174 occurs during M phase, suggesting that Aur-A contributes to this regulated translation (Groisman et al., 2001). These data don't quite mesh with a large body of evidence demonstrating that mitotic entry requires cyclin B synthesis during the preceding interphase rather than in mitosis itself, (see, for example, (Murray and Kirschner, 1989; Hunt et al., 1992)). One possible explanation is that the extracts used to measure polyadenylation rates in those experiments were taken during interphase or M phase but actually proceeded into the next cell cycle phase while the polyadenylation assays were being carried out. If so, then phosphorylation of CPEB occurring in mid-interphase would promote adenylation, translation, and accumulation of cyclin B protein during interphase, as is known to occur.

The translational roles of Aur-A are not limited solely to the G2 /M cell cycle transition. Aur-A also appears to be used in the selective polyadenylation of spatially regulated mRNAs in neurons, where localized translation is involved in modulating synaptic plasticity. In mammalian hippocampal neurons, for example, activation of synapses results in the polyadenylation and translation of the CPE-containing mRNA encoding α-CaMKII, but not in mRNAs lacking CPEs (Wu et al., 1998). Several of the proteins that control cytoplasmic polyadenylation in *Xenopus* oocytes are also

found localized at synaptic sites in these neurons, including CPEB, maskin, CPSF and Aur-A. Glutamate stimulation of the NMDA receptor in synaptosome preparations induces an Aur-A-like kinase activity that stimulates CPEB phosphorylation and α-CaMKII polyadenylation (Huang et al., 2002). Similar conclusions about the roles of CPEB and polyadenylation of serotonin-induced changes in the polyadenylation of neuronal actin mRNA have been reached using neurons of the invertebrate *Aplysia* (Liu and Schwartz, 2003).

Not surprisingly, Aur-A also functions during the two meiotic divisions. During normal, progesterone-induced meiotic maturation, Aur-A activity appears to drop during the completion of meiosis I and rise again as cells enter meiosis II, as judged by changes in its phosphorylation (Ma et al., 2003). In oocytes injected with muristylated, which constitutively localizes to the oocyte membrane, cells proceeded Aur-A, cells proceeded past GVBD and then arrested with highly condensed metaphase-like chromosomes that lacked detectable associated spindle structures, failed to migrate to the oocyte cortex in preparation for the completion of meiosis I and formation of the first polar body, and failed to show the partial destruction of cyclin B that normally occurs between meiosis I and II. While the molecular mechanisms underlying these effects are unknown, one obvious possibility is that membrane-associated Myr-Aur-A is prevented from associating with centrosomal material required to organize the poles of the first meiotic spindle (Ma et al., 2003). An important role for Aur-A in the completion of meiosis I is also indicated by a second study in which oocytes were injected with Aur-A antibodies (which did not interfere with its kinase activity *in vitro)* and then stimulated with progesterone (Castro et al., 2003). In those cells, cdc2 activation and GVBD occurred on schedule, and condensed chromosomes aligned on the metaphase I plate parallel to the surface of the oocyte, as in controls. However, the metaphase plate then failed to rotate 90° and, in most cases, failed to undergo anaphase II. While the mechanisms by which injected Aur-A antibodies blocked the completion of meiosis I are not yet known, it is possible that the antibodies interfere with targeting Aur-A to important spindle sites or accessibility to certain substrates such the spindle motor protein Eg5 (Roghi et al., 1998; Giet et al., 1999) or components of the dynactin-dynein complex that may be needed for spindle rotation (Castro et al., 2003).

## 16. Conclusions

The discoveries of the last few years have begun to illuminate the many functions of the Aurora kinase family, and many questions remain unanswered. The substrates and precise mechanisms that underlie the Aurora kinases' roles in spindle formation, chromosome segregation and cytokinesis are still being worked out. In addition, Aur-A seems to have a fascinating double life as a regulator of spindle formation and protein translation. Not only are the Aurora kinases interesting subjects for the cell biologist, but they are also important targets for pharmacological intervention. The unraveling of the mechanisms by which they promote tumor formation will be a particularly interesting story to follow in the next few years.

## Acknowledgements

We thank all the members of the Ruderman lab for useful discussions. This work was supported by NIH Grant HD37636 to J.V.R.

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