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Review

Cyclin A in cell cycle control and cancer

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Abstract. Cyclin A is particularly interesting among the cyclin family because it can activate two different cyclin-dependent kinases (CDKs) and functions in both S phase and mitosis. An embryonic form of cyclin A that is only essential for spermatogenesis is also present in some organisms. In S phase, phosphorylation of components of the DNA replication machinery such as CDC6 by cyclin A-CDK is believed to be important for initiation of DNA replication and to restrict the initiation to only once per cell cycle. In mitosis, the precise role of cyclin A is still obscure, but it may contribute to the control of cyclin B stability. Cyclin A starts to accumulate during S phase and is abruptly destroyed before metaphase. The synthesis of cyclin A is mainly controlled at the transcription level, involving E2F and other transcription factors. Removal of cyclin A is carried out by ubiquitin-mediated proteolysis, but whether the same anaphase-promoting complex/cyclosome targeting subunits are used as for cyclin B is debatable. Consistent with its role as a key cell cycle regulator, expression of cyclin A is found to be elevated in a variety of tumors.

Key words. Cell cycle control; cyclin; cyclin-dependent kinases; proteolysis; tumorigenesis.

Cyclin A and cell cycle control

Cyclins are defined as proteins that are related in sequence to the originally isolated A- and B-type mitotic cyclins [1–4]. The level of the mitotic cyclins oscillates in synchrony with the cell cycle, accumulates progressively throughout interphase and disappears abruptly at the end of mitosis. The region that shares the highest homology in the cyclin family is an ~100-residue region known as the cyclin box. The cyclin box assumes an α-helical fold composed of five helices, which is followed by a region that shares little sequence similarity with the cyclin box but nevertheless folds into the same three-dimensional structure to form a second cyclin box fold [5, 6]. Similar cyclin fold structures are also found in domains of several nuclear regulatory proteins such as the transcription factor (TF)IIB repeats and the pRB pocket region [7]. The N-terminal region of cyclin A contains several putative regulatory elements, including the destruction box and CDK phosphorylation site (fig. 1, see later).

Most cyclins are known to have a protein kinase partner called cyclin-dependent kinases (CDKs). The levels of most CDKs are relatively constant during the cell cycle, but their activities are highly regulated due to the fluctuation of the levels and activities of their cyclin partners and other regulators. In mammalian cells, cyclin B-CDC2 is the principal mitotic cyclin-CDK complex that regulates G_2-M transition. Cyclin D-CDK4/6 and cyclin E-CDK2 are important for G_1 progression and G_1-S transition, respectively. In cultured cells, cyclin A is synthesized and destroyed after cyclin E but slightly earlier than cyclin B during G_2 [8–10]. Cyclin A is especially interesting among the cyclins because it is associated with both CDC2 (also called CDK1) and CDK2, and has func-

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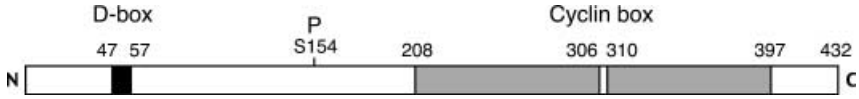

Figure 1. Schematic diagram of human cyclin A2. The positions of the conventional D-box, Ser154 phosphorylation site and the cyclin box (with the two cyclin box folds) are shown. Numbers represent the amino acid positions.

tions in both S phase and mitosis [11]. It is not very clear whether the same CDK subunit, when binding to different cyclins, has similar or distinct roles in vivo. It is likely that in addition to being an activating subunit, different cyclins also act as different targeting subunits for recognition of substrates. One example is the ZRXL motif (basic/Cys-Arg-basic-Leu) found in many proteins such as E2F-1, p107, p130, CDC25A and the p21$^{CIP1/WAF1}$ family of CDK inhibitors that binds to a conserved hydrophobic docking site on the surface of cyclin A [12].

Another interesting aspect about cyclin A is that there are both an embryonic and a somatic form of the protein. Organisms exemplified by *Drosophila* contain a single essential cyclin A gene [13, 14]. Other organisms such as *Xenopus* [15, 16], mice [17] and human [18] contain two A-type cyclins – an embryonic-specific cyclin A1 and a somatic cyclin A2. Cyclin A1 is only expressed in meiosis and very early embryos, whereas cyclin A2 is present in proliferating somatic cells. The only essential function of cyclin A1 in mice appears to be in spermatogenesis [19]. In contrast, cyclin A2 is essential, and disruption of its gene causes early embryonic lethality [20]. Based on sequence information alone, lower eukaryotes such as yeast do not contain A-type cyclin. The budding yeast Clb5 is probably the most similar in function to cyclin A.

Activation of CDC2 and CDK2 by cyclin A

The concentrations of cyclin A2 at its peak level in G_2/M are about 30-fold less than its partner CDC2 and about 8-fold less than CDK2 [21]. Activation of CDC2 and CDK2 requires binding to a cyclin subunit and phosphorylation on a threonine residue on a loop structure (T-loop) located near the mouth of the active site (Thr161 and Thr160 in CDC2 and CDK2, respectively) by CDK-activating kinase (CAK). In metazoans, the major CAK activity is composed of a cyclin-CDK pair, cyclin H-CDK7, with an assembly factor MAT1. Two residues on the activating loop region of CDK7 (Ser164 and Thr170 in human CDK7) are phosphorylated in the cell. Phosphorylation of Ser164 (a CDC2 consensus phosphorylation site) is dispensable for cyclin H-CDK7 activity, but phosphorylation of Thr170 (the equivalent site to Thr160 in CDK2) is required for cyclin H-CDK7 activity [9, 22]. Interestingly, both Ser164 and Thr170 in CDK7 can be phosphorylated by CAK targets, such as cyclin A-CDK2, in an autocatalytic loop [23, 24].

After binding to cyclin, CDC2 can be inactivated by phosphorylation on Thr14 and Tyr15 by WEE1 and MYT1. One possible difference between cyclin B-CDC2 and cyclin A-CDC2 is that while cyclin B-CDC2 is inactivated by Thr14/Tyr15 phosphorylation before entry into mitosis, no such phosphorylation is observed with cyclin A-CDC2 in *Xenopus* egg extracts [25].

Functions of cyclin A in mitosis and S phase

Cyclin B-CDC2 is the classic M phase-promoting factor (MPF) that drives G_2-M transition. Cyclin A can also bind CDC2, and microinjection of cyclin A into *Xenopus* oocytes or mammalian cells stimulates their entry into M phase [1, 3, 26–28]. Although cyclin A-CDK clearly exhibits MPF activity, the precise involvement of cyclin A in mitosis is obscure. Furthermore, these experiments did not reveal a distinct role for cyclin A in comparison to cyclin B during G_2-M. In support of a role of cyclin A in G_2-M, microinjection of antibodies against cyclin A into G_2 human cells leads to cell cycle arrest before mitosis [11]. Similarly, cells in *Drosophila* mutant lacking cyclin A are arrested in G_2 phase [13, 14]. One caveat is that *Drosophila* cyclin A only binds CDC2 but not CDK2 and is only important for G_2-M but not S phase.

One emerging hypothesis is that cyclin A may function before cyclin B and control the half-life of cyclin B. Once cyclin B-CDC2 is activated in mitosis, the activity of cyclin A-CDC2 is no longer required, and cyclin B-CDC2 triggers the cyclin destruction pathway and drives mitosis exit. In accordance with this idea, it has been reported that while cyclin B can trigger ubiquitin-mediated cyclin degradation, cyclin A prevents the degradation of cyclins [26, 29, 30]. A possible mechanism is that phosphorylation of CDH1 by cyclin A-associated kinase prevents the formation of APC^{CDH1} and in turn delays cyclin B ubiquitination and degradation [29] (see below).

Cyclin A is implicated in the control of DNA replication because ectopic expression of cyclin A in mammalian cells accelerates the entry of G_1 cells into S phase [31, 32]. Likewise, cyclin A can promote DNA replication in cell-free extracts from *Xenopus* eggs [33] or human cells [34], and is sufficient to initiate SV40 DNA replication in G_1 cell extracts [35]. Microinjection of antisense cyclin A or anti-cyclin A antibodies blocks progression through S phase [11, 36, 37], and immunodepletion of cyclin A from S cell extracts partially inhibits SV40 origin-driven

DNA replication [38]. Consistent with its role in the control of DNA replication, cyclin A is synthesized at the onset of S phase and localizes to the sites of DNA replication [39, 40]. The current model of DNA replication implicates roles for cyclin A-CDK both in the initiation of DNA replication and in the restriction of initiation to only once per cell cycle.

Many known substrates of cyclin A are components of the DNA replication machinery. RPA, a cellular single-stranded DNA binding complex that is essential for the initiation and elongation of simian virus 40 (SV40) DNA replication, is phosphorylated by cyclin A-CDK [41, 42]. The functional significance of phosphorylation of the 34-kDa subunit of RPA remains to be established, since both phosphorylated and unphosphorylated forms of RPA are equally active in SV40 DNA replication [43, 44]. Several proteins, including origin recognition complex (ORC), CDC6, and the MCM protein complex, need to be assembled on the chromatin before the initiation of DNA replication. Cyclin A-CDK2, but not cyclin B- or cyclin E-CDK complexes, binds CDC6 through the N-terminal ZRXL motif and phosphorylates CDC6 [45, 46]. After phosphorylation by cyclin A-CDK2, CDC6 is relocalized from the nucleus to the cytoplasm and is destroyed. One possibility is that phosphorylation by cyclin A-CDK2 results in the destruction of free CDC6 that are not assembled into replication complexes, and prevents the re-replication of DNA after G_1. Cyclin A-CDK2 can phosphorylate MCM4 in the MCM4-MCM6-MCM7 DNA helicase complex, resulting in the inactivation of its DNA helicase activity [47]. These results raise the possibility that the inactivation of CDC6 and MCM4-associated helicase activity by cyclin A-CDK is part of the system for preventing DNA re-replication. In agreement with this, the priming activity of DNA polymerase α-primase is inhibited by cyclin A-CDK2 phosphorylation [48]. Elongation by DNA polymerase δ, on the other hand, is shown to require the activity of cyclin A [49].

Synthesis of cyclin A

The messenger RNA (mRNA) of cyclin A2 starts to accumulate during S phase and diminishes at mitosis, slightly ahead of cyclin B mRNA [8]. The role of E2F in the regulation of cyclin A2 transcription is particularly well characterized. E2F is inhibited by binding to hypophosphorylated pRb family proteins during G_1, but their phosphorylation by cyclin D/E-CDK complexes releases E2F, which is then able to activate the transcription of genes involved in S phase progression (including that of cyclin A2). Cyclin A2 promoter is repressed during the G_0/G_1 and is activated at S phase entry [50]. This repression of cyclin A2 transcription during G_0/G_1 is attributed to the occupation of a repressor element termed cell-cycle-responsive element (CCRE) or cell-cycle-dependent element (CDE) located in the cyclin A2 promoter [51, 52]. Mutation of the CCRE/CDE resulted in a complete loss of cell cycle regulation of cyclin A2 transcription. The CCRE/CDE element in fact contains an E2F binding site, and binding of p107 (but not pRb) to E2F represses the promoter [53, 54]. Adenovirus E1A can activate the cyclin A2 promoter through interaction with p107 [55].

Cyclins synthesized in G_1 can stimulate the transcription of cyclin A2 in S phase. Accordingly, cyclin E-CDK2 can directly bind to E2F/p107 complexes formed on the cyclin A2 promoter and activate the transcription of cyclin A2 [56]. Similarly, cyclin D can activate cyclin A2 transcription [54] and restore the transcription of cyclin A2 due to the loss of cell adhesion to substratum [57]. A considerable body of evidence suggests that E2F can also interact with cyclin A2 via a small domain near its amino terminus, and the transcriptional activity of E2F is turned off by cyclin A2-CDK2 phosphorylation [58–60], completing a negative feedback loop that limit the transcription of cyclin A2.

In addition to CCRE/CDE, another element termed the cell cycle genes homology region (CHR) six nucleotides 3′ to the CCRE/CDE is also important for the repression of cyclin A2 transcription in G_0/G_1 [51]. Only putative CHR-binding activities, which are unrelated to E2F, have been identified so far [61, 62].

Apart from E2F, other transcription factors are also known to regulate cyclin A2 transcription. TAFII250, a subunit of TFIID, can stimulate cyclin A2 transcription through the TSRE enhancer element [63], and MDM2 can bind to TAFII250 and potentiate cyclin A2 transcription [64]. Cyclin A2 transcription can be negatively regulated by p53 [65], but probably not through direct interaction of p53 to its cognate consensus sequence [66]. Finally, cyclin A2 transcription can be stimulated by cyclic AMP (cAMP) [67, 68] or repressed by transforming growth factor-β (TGF-β) through an ATF/CREB site in the promoter [69, 70].

Apart from transcriptional regulation, the stability of the cyclin A mRNA also appears to be cell cycle regulated. Cyclin A2 mRNA has a longer half-life from G_1-S transition to G_2-M, and a shorter half-life in early G_1 [71]. The stabilization of cyclin A mRNA is at least in part attributed to the binding of HuR to the 3′-untranslated region [72].

Degradation of cyclin A

Live cell imaging using cyclin A2-green fluorescent protein fusion proteins shows that human cyclin A2 begins to be degraded in early prometaphase and is completed at metaphase [73, 74]. Degradation of the mitotic cyclins requires a short sequence near their N-terminus called the

destruction box (D-box), which acts as a signal for ubiquitin-dependent proteolysis [75, 76]. The major ubiquitin ligase in mitosis is the anaphase-promoting complex/cyclosome (APC/C) [77, 78]. The APC/C core complex is under complex control via phosphorylation and is activated by binding to targeting subunits including CDC20 and CDH1. CDC20 accumulates during late S phase and mitosis as a result of transcriptional activation [79]. Destruction of CDC20 at the end of mitosis is in part mediated by its own D-box and APC/C. Formation of APC/C^{CDC20} complexes alone is probably insufficient to trigger degradation of their substrates, and may require the phosphorylation of APC/C by cyclin B-CDC2 and PLK [80, 81]. After APC/C^{CDC20} is inactivated, APC/C activity is maintained from the end of mitosis to late G_1 by binding to CDH1 [79]. Interaction between CDH1 and APC/C is inhibited from S phase until the end of mitosis by CDK phosphorylation [82], most likely through cyclin A-CDK2 [29].

It is clear that the major mitotic cyclin, cyclin B1, is targeted for ubiquitin-mediated proteolysis by APC/C^{CDC20}. However, the protein(s) that targets cyclin A for proteolysis is still a mystery. Evidence that the same destruction mechanism is used to destroy cyclin A and cyclin B1 certainly abounds. Cyclin A does contain a similar D-box to cyclin B1 near the N-terminus of the protein, and the integrity of the D-box is required for the proper destruction of cyclin A in M-phase extracts [83–86]. A dominant-negative mutant of Ubc10, a ubiquitin carrier, arrests cells in mitosis with high levels of cyclin A and cyclin B1 [87, 88]. In *Drosophila*, the CDC20 homologue Fizzy is required for the degradation of both cyclins A and B [89, 90]. Adding anti-Fizzy antibodies to *Xenopus* egg extracts inhibits degradation of both cyclin A1 and cyclin B1 [91]. Similarly, injection of antibodies against the APC/C component CDC27 or CDC20 inhibits the degradation of cyclin A2 in mammalian cells [74]. Furthermore, APC/C^{CDC20} or APC/C^{CDH1} can promote ubiquitination of cyclin A2 in vitro [74, 78]. In this connection, it was reported that human CDC20 can interact with cyclin A2 and can be phosphorylated by cyclin A2-associated kinase [92]. This interaction is mediated through the WD40 repeats of CDC20 and the region of cyclin A2 between the D-box and the cyclin box.

Despite the evidence that cyclin A is degraded by the same mechanisms as cyclin B1, there are clear differences between their destruction behavior that suggest distinct mechanisms may be involved. Most important, cyclin A disappears before cyclin B1 in the cell cycle. Moreover, activation of the spindle assembly checkpoint, which delays metaphase-anaphase transition until all chromosomes are attached to the mitotic spindles, inhibits cyclin B1 but not cyclin A degradation [73, 74, 88, 93, 94]. The current consensus is that the spindle assembly checkpoint exerts its effects through the inhibition of APC/C by MAD2. This implie that cyclin A degradation is not mediated through the same APC/C as cyclin B1, or cyclin A and cyclin B1 have different susceptibility to APC/C.

Apart from the differences in timing of proteolysis, the D-box of cyclin A also behaves differently to that of cyclin B1. Unlike that of cyclin B1, the D-box of cyclin A1 cannot act as an independent destruction module when grafted onto heterologous proteins. It was shown that substituting the D-box of *Xenopus* cyclin B1 with that of cyclin A1 renders cyclin B1 nondegradable, whereas the D-box of cyclin B1 supports the proteolysis of cyclin A1 [95, 96]. In human cells, the D-box of cyclin A2 is not sufficient for targeting cyclin A2 for destruction, and an additional short sequence following the D-box is also required for its proteolysis [73, 74]. In accord with this idea, a similar extended D-box is present in another APC/C substrate, NEK2A, which is a NIMA-related protein implicated in regulating centrosome structure [97]. Interestingly, *Drosophila* cyclin A, which has a rather different N-terminal region from other metazoan cyclin A, requires the two D-boxes and a KEN box in that region for proper destruction [98]. Taken together, these suggest that cyclin A requires sequence elements in addition to the D-box for efficient destruction.

On the dependence of the CDK partner for cyclin degradation, it was found that degradation of *Xenopus* cyclin A1 but not cyclin B1 requires binding to the CDK partner [83]. However, mutants of human cyclin A2 that cannot bind CDK are still destroyed in vivo, albeit after a delay [73]. Phosphorylation of human cyclin E at Thr380 by its partner CDK2 is important for their ubiquitin-dependent degradation [99, 100]. Similarly, cyclin A2 can be phosphorylated by its partners CDC2 or CDK2 on Ser154 [101]. However, unlike cyclin E, phosphorylation of Ser154 does not affect the degradation of cyclin A2.

Cyclin A and tumorigenesis

Conceptually, deregulation of cell cycle regulators such as cyclin A2 is likely to contribute to tumorigenesis. Increased expression of cyclin A has been detected in many types of cancers (table 1). The majority of these studies rely on immunohistochemical detection of cyclin A2 in cancer cells in comparison to the surrounding noncancer cells. However, the important question of whether elevation of cyclin A2 is a contributing factor to tumorigenesis or a mere consequence of increased cell proliferation is not easily addressed. Not surprisingly, cyclin A2 is typically coexpressed with proliferation markers such as PCNA (proliferative cell nuclear antigen) and Ki67. Despite these limitations, expression of cyclin A2 in many types of cancers appears to be of prognostic values such as prediction of survival or early relapse.

Table 1. Overexpression of cyclin A in cancer.

Tumor	References	Methods	Correlations
Astrocytoma	122, 123	IH	tumor stage, proliferation
Breast cancer	124, 125	IH/flow cytometry	aneuploid, proliferation, poor prognosis
Cervical cancer	126	IH	proliferation
Colorectal cancer	127	IH	poor prognosis
Gastric cancer	128	IH	
Leukemia and lymphoma	129–131	IB/mRNA	proliferation, poor prognosis
Liver cancer	102, 103, 107, 108	IB/Southern/PCR/mRNA	proliferation, poor prognosis
Lung cancer	132–136	IH	proliferation, poor prognosis, better chemotherapy response
Melanoma	137–140	IB/IH	tumor thickness, tumor stage, poor prognosis
Esophageal cancer	141, 142	IH	tumor stage, poor prognosis
Oral cancer	143–145	IH	proliferation
Osteosarcoma	146	IH	poor prognosis
Ovarian cancer	124, 147, 148	IH	tumor stage, poor prognosis, better chemotherapy response
Prostate cancer	149, 150	IH	tumor stage
Renal cancer	151–153	IH	tumor stage, proliferation, poor prognosis
Smooth muscle cancer	154, 155	IB/IH	poor prognosis
Soft tissue sarcoma	156–158	IH	tumor stage, poor prognosis, better chemotherapy response
Testicular cancer	159	IH	proliferation

PCR, polymerase chain reaction. Several common human tumors, selected references, detection methods for cyclin A (IB, immunoblotting; IH, immunohistochemical staining), and correlations with several clinical factors are summarized.

A good illustration of the deregulation of cyclin A2 is in hepatocellular carcinoma (HCC). Increased expression of cyclin A2 in HCC ranges from about 40% in one study [102] to about 80% in another [103]. Increased expression of cyclin A2 is due to a combination of gene amplification, posttranscription, and posttranslational mechanisms [102]. Increase in cyclin A2 at the mRNA level has been demonstrated by expressed sequence tag sequencing and complementary DNA microarray analysis [104], and at the protein level by immunohistochemical analysis [105] and immunoblotting [103, 106]. The kinase activities associated with the CDK partners of cyclin A2 are activated in HCC [106]. Although allelic loss or rearrangement of cyclin A gene in HCC is rare [107, 108], a case of HCC in which the hepatitis B virus (HBV) is found to integrate into the cyclin A2 gene has been discovered. This produces a stable hybrid HBV-cyclin A2 fusion protein that lacks the N-terminus of cyclin A2, including the D-box, of which transcription is driven by the strong viral promoter [109, 110].

What are the consequences of having too much cyclin A? Clues can be obtained from experiments that overexpress cyclin A in different systems. Transgenic mice overexpressing the wild type of nondegradable cyclin A2 in the mammary glands exhibit hyperplasia and nuclear abnormalities suggestive of preneoplastic alterations [111]. In mammalian cells, increasing the levels of cyclin A2 delays metaphase and anaphase onset [73, 74]. Interestingly, failure to destroy cyclin A2 does not arrest cells in metaphase, but at later stages of mitosis. Similarly, expression of nondegradable cyclin A in *Drosophila* arrests cells transiently at metaphase, whereas expression of stable cyclin B arrests cells at later stages of mitosis [90, 112]. In this connection, *Drosophila* cells at the gastrula stage delay in metaphase after DNA damage, and this delay correlates with the stabilization of cyclin A. Furthermore, mutant cells lacking cyclin A are unable to delay in mitosis and enter anaphase with an increased number of lagging chromosomes [113]. This implicates cyclin A2 in the DNA damage checkpoint, and suggests that a decrease rather than an increase in cyclin A2 may play a role in tumorigenesis. Increased expression of cyclin A2 may simply reflect a high rate of cell proliferation once the tumor has developed.

Apart from potentially deregulating the cell cycle and checkpoints directly, it is possible that cyclin A-CDK also contributes to tumorigenesis by phosphorylating other oncoproteins and tumor suppressors. For example, phosphorylation of p53 on Ser315 by cyclin A2-CDK stimulates sequence-specific DNA binding by p53 [114], and phosphorylation of mouse MDM2 by cyclin A2-CDK2 weakens its interaction with p53 [115]. Hence increased expression of cyclin A2 appears to enhance the activity of p53.

Expression of cyclin A1 in cancer is arguably more interesting than cyclin A2 because somatic cells do not usually contain any cyclin A1. Human cyclin A1 is highly expressed in certain myeloid leukemia cells [18, 116, 117]. Transgenic mice with overexpression of cyclin A1 in the myeloid lineage exhibit abnormal myelopoiesis [118], but cyclin A1 alone is not sufficient to induce myeloid leukemia. Cyclin A1 promoter activity is highest during late S and G_2-M phase, and is dependent on the binding of members of the Sp1 family to the four GC boxes [119]. Silencing of the cyclin A1 promoter in cancer cell lines is associated with CpG methylation, but tissue-specific repression of the cyclin A1 promoter occurs independently of CpG methylation [120]. Binding of c-MYB to the cyclin A1 promoter may also contribute to the preferential activation of cyclin A1 promoter in acute myeloid leukemia [121].

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