Aurora-A Kinase Interacting Protein (AIP), a Novel Negative Regulator of Human Aurora-A Kinase*

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Aurora kinases have evolved as a new family of mitotic centrosome- and microtubule-associated kinases that regulate the structure and function of centrosomes and spindle. One of its members, Aurora-A, is a potential oncogene. Overexpression of Aurora-A is also implicated in defective centrosome duplication and segregation, leading to aneuploidy and tumorigenesis in various cancer cell types. However, the regulatory pathways for mammalian Aurora-A are not well understood. Exploiting the lethal phenotype associated with the overexpression of Aurora-A in yeast, we performed a dosage suppressor screen in yeast and report here the identification of a novel negative regulator of Aurora-A, named AIP (Aurora-A kinase Interacting Protein). AIP is a ubiquitously expressed nuclear protein that interacts specifically with human Aurora-A in vivo. Ectopic expression of AIP with Aurora-A in NIH3T3 and COS cells results in the down-regulation of ectopically expressed Aurora-A protein levels, and this down-regulation is demonstrated to be the result of destabilization of Aurora-A through a proteasome-dependent protein degradation pathway. A noninteracting deletion mutant of AIP does not down-regulate Aurora-A protein, suggesting that the interaction is important for the protein degradation. AIP could therefore be a potential useful target gene for anti-tumor drugs.

Faithful chromosome segregation and cytokinesis are two essential steps in mitosis, which is responsible for the viability and genetic stability of daughter cells. This involves the concerted spatial and temporal interactions among various components such as the chromosomes, centrosomes, actin, and the microtubule cytoskeleton. Defined at the molecular level, the stability and the activation/inactivation of the proteins associated with these structures result in the regulation of the centrosome, centrosome, spindle microtubules, and actin dynamics. Reversible protein phosphorylation plays an important regulatory role in orchestrating the interactions among various proteins during mitosis (1–6). Many of the protein kinases and their opposing phosphatases, which are involved in these signaling cascades, have been identified (7). For example, the cyclin-dependent kinases are activated by cdc25 phosphatase (8) to regulate different stages of M phase, starting from triggering the mitotic entry, spindle formation, anaphase to cytokinesis (9–12). Similarly, polo-like kinases regulate several processes, which include centrosome maturation, cyclin-dependent kinase 1 activation and inactivation, and cytokinesis (9, 13–15). Other participating kinases include Bub1 kinase, which localizes to the kinetochore and regulates the anaphase checkpoint (16), and the centrosome-associated NimA-related kinase, which primarily regulates the centrosome cycles (17).

The stability of the mitotic regulators also plays a pivotal role in the progression and completion of mitosis. In mitotic cells, progression into anaphase depends on the activation of the anaphase-promoting complex/cyclosome (APC/C) by phosphorylation to degrade the mitotic regulators (18, 19).

Recently, the Aurora kinase family emerged as a new family of mitotic serine threonine kinases regulating the centrosomal and microtubule function, ensuring the accurate chromosome segregation and efficient completion of cytokinesis (20, 21). The Aurora kinase family was first identified in the budding yeast as Ipl1 (22), and subsequently various homologs of Ipl1 have been isolated from diverse organisms, ranging from Drosophila, Xenopus, Caenorhabditis elegans, mouse, rat, to human (20, 21). Ipl1 is the only representative of this family in yeast, two Aurora-related kinases are found in Drosophila and C. elegans, and three in mammals (20, 21). Their roles in chromosome segregation are implicated in the phenotypes of various mutants. S. cerevisiae ipl1 mutants showed abnormal chromosome segregation and ploidy (22–24). Drosophila Aurora mutants showed defective centrosome separation resulting in the formation of monopolar spindles (25). They share similarity in their kinase catalytic domain but no or little similarity in their N-terminal domain, which seems to be species- and memberspecific. Their expression and kinase activity are tightly cell cycle-regulated, peaking at M phase and disappearing rapidly upon mitotic exit (26–30). In mammals, three members of this family, designated Aurora-A, Aurora-B, and Aurora-C. Orthologs of Aurora-A kinase localize to the centrosome and mitotic spindle and function during the early part of the mitosis from prophase (27, 29, 31, 49). Aurora-B kinase localizes to the mid-body and postmitotic bridge, functioning during the late mitosis, and plays a role in cytokinesis (30, 32). Human Aurora-C kinase localizes to the anaphase centrosomes (33), and its function remains to be elucidated.

Increased attention has now been focused on Aurora-A kinase because of its suggestive role in tumorigenesis. Overexpression of Aurora-A kinase is observed in more than 50% of primary colorectal tumors and 6–18% of primary breast tumors (29, 31). Human Aurora-A kinase maps to chromosome 17q21-q23, and genetic instability at this site is associated with clinical outcome in colorectal and breast cancers (29, 31). Aurora-A kinase is an essential target for new anti-cancer agents (29, 31). Aurora-A kinase is a potential drug target for neoplastic disease. It is a new target for cancer therapy. Aurora-A kinase is an essential target for new anti-cancer agents. Aurora-A kinase is a potential drug target for neoplastic disease. It is a new target for cancer therapy.
20q-13.2 (30), which is frequently amplified in several human tumors (29, 34–38). Ectopic overexpression of Aurora-A in near diploid normal breast epithelial cells causes centrosome hyperamplification and aneuploidy (31). Also, overexpression of Aurora-A in rodent cells displayed cellular transforming activity, suggesting that when overexpressed, Aurora-A could function as a potential oncogene (29, 31). The extensive research done on the yeast homolog Ipl1, Xenopus homolog Eg2, and Drosophila aurora had shed some light into the functional role of Aurora-A in mitosis and meiosis. Kinesin-related protein, trimers of CIN8 (39), Pav (40) and Eg5 (41) were found to interact directly with the Aurora kinase homologs in yeast (Ipl1), Drosophila (aurora), and Xenopus (Eg2), respectively. Also, yeast Ipl1 is also found to interact with the kinetochore protein Ndc10, implying the possible role of Aurora kinase in the establishment of the mitotic checkpoint via monitoring the capture of the chromosome kinetochores by the spindle microtubule (24, 42, 43). Human Aurora-A kinase is shown to interact with Cdc20 (44), which is involved in the mitotic activation of anaphase-promoting complex APC/C (45).

Presently, only very limited knowledge is available on the function(s) of Aurora-A kinase in mammals. Understanding the functions of Aurora-A kinase and delineation of the Aurora-A kinase signaling pathway would definitely help us to have a clearer understanding of the role of the kinase in chromosome segregation and neoplastic transformation. Hence, in an effort to identify any interacting proteins as well as the negative regulators of Aurora-A kinase, a dosage suppressor screen in which HeLa cell cDNAs that can alleviate Aurora-A-mediated cytotoxicity in yeast has been carried out. In this paper, we report the identification of AIP, one such potential negative regulator of Aurora-A kinase.

EXPERIMENTAL PROCEDURES

Yeast Dosage Suppressor Screening—Yeast strain EGY188 (MATa trp1his3ura3leu2/2 LexAop-LEU2) was maintained in the rich YPD medium. Yeast transformation, plasmid isolation, and protein extracts were prepared as described (46). For cDNA library screening, EGY188 cells were grown to log phase in YPD and cotransformed with plasmids containing 150 μg of Aurora-A cDNA in pEG202 and 150 μg HeLa cell cDNAs in pMG4-5 using the LiOAc method (47). The resulting transformants were selected on galactose containing synthetic dropout media (SD–His–Trp). Yeast clones, which survived the Aurora-A-mediated cytotoxicity, were reconfirmed by sequencing, cells were grown to log phase in YPD and cotransformed with plasmids containing 150 μg of Aurora-A cDNA in pEG202 and 150 μg HeLa cell cDNAs in pMG4-5 using the LiOAc method (47). The resulting transformants were selected on galactose containing synthetic dropout media (SD–His–Trp). Yeast clones, which survived the Aurora-A-mediated cytotoxicity, were reconfirmed by sequencing.

Cloning of AIP and Plasmid Constructs—To clone a full-length AIP cDNA, a PCR-based approach was employed. Two primers, GSB (5′-CGC TGC CGA TCG GGG CCG ACT-3′) and GSG (5′-ACT ACG GAT CAC AGC AGC AAC-3′) were designed for PCR cloning of AIP from the HeLa cell cDNA library. All Aurora-A and AIP constructs were made in the mammalian expression vector pCDNA3 (Invitrogen). The cDNA expression plasmid pAPuro-CyclinB1 was a kind gift from Dr. Prochownik, Pittsburgh, PA. To trace the transfected AIP, a FLAG epitope was introduced at the N terminus of both truncated AIP constructs (87–600 bp) and full-length AIP (1–600 bp) constructs by PCR as described previously (28).

Northern Blot Analysis—Pre-made blots containing poly(A) RNA isolated from adult human tissues and a human cancer cell line panel were purchased from Clontech and used for hybridization with AIP-specific probe. Blots were hybridized according to Church and Gilbert (48) with a 477-bp AIP 3′-end fragment labeled using a random prime labeling kit. Blots were then stripped and reprobed with β-actin to quantitate RNA loading.

Cell Culture, Transfection, and Drug Treatment—NIH 3T3 and COS cells were maintained in Dulbecco’s modified Eagle’s medium and HeLa cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum. Transfections of cultured cell lines have been carried out using LipofectAMINE (Invitrogen) according to the manufacturer’s recommended protocol. Typically, 8 × 105 HeLa cells were seeded in a 60-mm dish 24 h prior to the transfection and transfected with different plasmids at a total concentration of 3 μg. For the in vivo interaction assay, equal amounts of either HA-Aurora-A or MmAurora-B plasmids were cotransfected with different combinations of control or AIP-expressing constructs using the LipofectAMINE PLUS reagent (15 μl of LipofectAMINE and 8 μl of PLUS reagent) for 5 h. Similarly, 7 × 105 NIH 3T3 cells or 1.8 × 106 COS cells were plated in a 60-mm dish 24 h prior to the transfection. For Aurora-A degradation study, COS cells were cotransfected with HA-Aurora-A and FLAG-tagged AIP at different ratios while maintaining the total amount of DNA transfected to 3 μg. The same optimized transfection conditions were used. For immunofluorescence staining, 3 × 105 cells were seeded on the coverslip placed in the 25-mm dish 1 day prior to the transfection. A total of 1 μg of plasmid DNA and 6 μl of LipofectAMINE/6 μl of PLUS reagent were used for transfection. To detect S phase and G2/M protein degradation, COS cells were treated with 20 μM N-Cbz-Leu-Leu-Leu-AL (MG132; Sigma), 25 μM ALLM (Calbiochem), 25 μM lactocystin β-lactone (Calbiochem), and 150 μM ALLN (Calbiochem) for 12 h.

Cell Lysis, Immunoprecipitation, and Immunoblotting—The cells were lysed for 15 min on ice in lysis buffer (1 × TBS, 10% glycerol, 1% Nonidet P-40) containing protease inhibitors mixture (Roche Molecular Biochemicals). The lysates were cleared by centrifugation at 13,000 rpm for 10 min at 4°C. The protein concentration of the lysates was measured by the Bio-Rad Protein Assay (Pierce). Prior to the immunoprecipitation, 1 mg of lysates was precleared by incubation with 80 μl of 50% slurry of protein G-agarose (Sigma) for 1 h at 4°C. For antibody coupling to the protein G-agarose, 20 μl of rabbit anti-HsAurora-A serum (44) or 6 μg of FLAG M2 mouse monoclonal antibody (Stratagene) was incubated with 80 μl of 50% slurry of protein G-agarose (Sigma) for 1 h at room temperature. For immunoprecipitation, the precleared lysate and antibody-coupled protein G-agarose were mixed and rotated for 2 h at 4°C. Immune complexes were washed twice with S buffer 1 (1 × TBS, 10% glycerol, 0.5% Nonidet P-40, 1% bovine serum albumin) and twice with wash buffer 2 (1 × TBS, 10% glycerol, 0.5% Nonidet P-40). The immune complexes were solubilized by boiling with SDS sample buffer and resolved by SDS-PAGE. The proteins were subsequently transferred to Hybond C+ nylon membrane (Amersham Biosciences). After blocking with 5% nonfat milk in TBS, the blots were incubated with rabbit anti-Aurora-A (1:5,000) or rabbit monoclonal anti IAK1 (Transduction Laboratories) at a dilution of 1:1,000 or FLAG M2 mouse monoclonal antibody (Stratagene) at 1:2,000 overnight at 4°C. The horseradish peroxidase-conjugated secondary antibodies were also diluted accordingly in blocking buffer (goat anti-rabbit horseradish peroxidase (Bio-Rad), 1:5,000; goat anti-mouse horseradish peroxidase (Pierce), 1:5,000) and incubated with the blot at 1 h at room temperature. The secondary antibodies were detected by enhanced chemiluminescence (ECL; Amersham Biosciences) and exposed to Kodak Biomax MR film.

Construction of AIP Deletion Mutants—Four AIP deletion mutants were created by PCR-based backward deletion. A 99-bp and a 198-bp deletion, each separately from the N and C terminus of AIP, were synthesized using four pairs of primers flanking the desired domain. The forward primers were designed to add the 8-amino acid FLAG tag to the N terminus of each mutant protein. The amplified fragments spanning different regions of AIP were cloned into pCDNA3 for expression purposes. The expected sequences of the deletion mutants were confirmed by sequencing.

In vivo interaction and degradation assays were carried out with these AIP mutants as described previously.

Immunofluorescence Staining—Cells grown on coverslips were fixed in −20°C methanol for 5 min at room temperature. After blocking for 1 h in 1% (v/v) blocking buffer (1:1 Tween 20:BSA, 1% Triton X-100, 100 mM NaCl, 0.02% sodium azide), cells were incubated with the primary antibody, mouse anti-FLAG (Stratagene; 1:800), for 1 h at room temperature. The cells were washed thoroughly in 1× TBS and incubated further with the respective secondary antibodies. Alexa Fluor 488-conjugated goat anti-mouse IgG (Molecular Probes) was used as the secondary antibody. Before immunofluorescence staining, cells were incubated with 0.05 μM/ml propidium iodide. Cells were analyzed by using a Leica epifluorescence microscope (Bio-Rad) equipped with a multiband filter set and/or confocal microscopy.

RESULTS

Molecular Cloning of AIP—Overexpression of Aurora-A kinase is lethal in yeast (29). By exploiting the lethal phenotype of Aurora-A kinase, we attempted to isolate mammalian proteins that can suppress the lethal phenotype when cotransfected and rescue the yeast from Aurora-A-mediated death.
For this purpose, a plasmid construct was made in yeast expression vector pEG202 where constitutive expression of the full-length Aurora-A kinase in yeast is achieved using the Gal1-induced alcohol dehydrogenase promoter. Yeast strain EGY188 was cotransformed with this Aurora-A plasmid and a HeLa cell cDNA library in pJG4-5 where cDNAs were expressed under a cotransformed with this Aurora-A plasmid and a HeLa cell alcohol dehydrogenase promoter. Yeast strain EGY188 was full-length Aurora-A kinase in yeast is achieved using the expression vector pEG202 where constitutive expression of the Aurora-A- mediated lethality by AIP led us to characterize AIP protein found a tandem bipartite nuclear localization signal, suggesting AIP could be a nuclear protein. Indeed, ectopically expressed FLAG epitope-tagged AIP was localized to the nuclear compartment of the cell (Fig. 2a). Computer-assisted search for the motifs presented in AIP protein can be coimmunoprecipitated independently of whether expressed the FLAG-tagged AIP cDNA into HeLa cells and acted with Aurora-A to activate the kinase. Preliminary information that AIP might interact directly with Aurora-A kinase came from the yeast two-hybrid in vivo interaction assay where the partial AIP cDNA interacted with Aurora-A to activate the LEU reporter in yeast (data not shown). To verify whether a similar interaction between AIP and Aurora-A occurs in mammalian cell context, we overexpressed the FLAG-tagged AIP cDNA into HeLa cells and attempted to coimmunoprecipitate the Aurora-A with the transfected AIP protein. The results presented in Fig. 3 indicate that AIP associates with Aurora-A in vivo and that AIP can be coimmunoprecipitated with Aurora-A, and conversely, Aurora-A can be coimmunoprecipitated with FLAG-tagged AIP using FLAG antibody. However, it is noted that the interaction of transfected AIP with the endogenous Aurora-A in vivo was difficult to demonstrate. We presumed that the difficulty in demonstrating the coimmunoprecipitation could be caused by the lower amounts of Aurora-A available in AIP-transfected cells. The result presented in Fig. 3a is the best that is achieved under the given experimental conditions. To explore the interaction further, HeLa cells that are otherwise contained in comparably lower levels of Aurora-A protein (data not shown) were transfected with HAurora-A in vivo was transferred with HAurora-A together with FLAG-tagged AIP, and coimmunoprecipitation followed by Western blot analysis were carried out. The results presented in Fig. 3b demonstrate that the transfected AIP protein and Aurora-A protein can be coimmunoprecipitated independently of whether...
AIP mRNA expression and nuclear localization. a, Northern blot analysis of AIP mRNA in adult human tissues and cancer cell lines was carried out with pre-made Northern blots purchased from Clontech. The blots were hybridized with the 477-bp AIP cDNA derived from the yeast dosage suppressor screen. The blot was stripped and reprobed with β-actin. b, HeLa cells were transiently transfected with a FLAG epitope-tagged AIP cDNA, and the subcellular localization of the transfected AIP protein (panel 1) was detected by staining with FLAG M2 monoclonal antibody (Stratagene) followed by confocal microscopy. Counterstaining of DNA was carried out with propidium iodide (panel 2). Panel 3 represents the merged image to show the nuclear localization of the transfected AIP protein.

FLAG M2- or Aurora-A-specific antibodies were used.

Overexpression of AIP Down-regulates Aurora-A Protein—Because AIP has been isolated as the negative regulator of Aurora-A kinase, we presumed that direct interaction of AIP with Aurora-A kinase should result in the down-regulation of either the stability and/or activity of Aurora-A kinase. In an attempt to study the impact of AIP-Aurora-A interaction on the Aurora-A protein, the levels of Aurora-A protein in AIP-transfected cells were investigated. Initial attempts to study the effect of AIP overexpression on the levels of Aurora-A protein were unsuccessful because of lower transfection efficiency, which was not sufficient to demonstrate the effect of AIP overexpression on endogenous Aurora-A protein. Hence, dividing NIH 3T3 or COS cells were cotransfected with FLAG-tagged AIP and HsAurora-A expression constructs at different ratios, and the levels of HsAurora-A protein were followed by Western blot analysis. A human Aurora-A-specific peptide antiserum was used to detect the transfected human Aurora-A in the background of the endogenous mouse and monkey Aurora-A protein. Ectopic expression of Aurora-A protein in human or monkey cell lines resulted in multiple Aurora-A-specific bands. These protein bands were verified to be Aurora-A-specific by Western blot analysis with two different Aurora-A-specific antibodies (data not shown). In COS cells, ectopic expression of Aurora-A results in two Aurora-A-specific bands of which the top band comigrated with the 46,000 endogenous Aurora-A protein from HeLa cells (data not shown). The nature of these other fragment(s), at present, is not clear. However, the results presented demonstrate that AIP, when overexpressed, could down-regulate the Aurora-A protein-specific bands in both a dose-dependent (Fig. 4a) and time-dependent manner (Fig. 4b). Both full-length AIP as well as the N-terminal truncated form of AIP (AIP-TR) were able to down-regulate Aurora-A protein (data not shown) although the truncated AIP was more efficient in that it could completely deplete the ectopic expressed Aurora-A protein in COS cells (Fig. 4, a and b).

AIP Interaction with Aurora-A Is Important for the Down-regulation of Aurora-A—To address the question of whether the interaction between AIP and Aurora-A is a necessary step for the down-regulation of Aurora-A, we attempted to isolate a deletion mutant of AIP protein, which does not interact with Aurora-A protein. A total of four deletion mutants lacking regions from either the N or C terminus of AIP protein were constructed and used for Aurora-A interaction studies as described previously. The size and location of these deletions in the different deletion mutants in relation to the wild type AIP protein are given in Fig. 5a. Expression of these deletion mutants in HeLa as well as COS cells showed that these mutant proteins have comparable stability (data not shown) except the
AIP Specifically Down-regulates Aurora-A

To verify the specificity of the effect of AIP overexpression on the down-regulation of Aurora-A protein, the effect of AIP overexpression on MmAurora-B, another member of the Aurora kinase family, as well as cyclin B1 was investigated. The rationale for selecting cyclin B1 is that, like Aurora-A protein, the proteasome-dependent pathway (50, 51) also degrades it. COS cells were transfected with FLAG-tagged AIP-TR together with either MmAurora-B or human cyclin B1 at ratio of 9:1, respectively, and the effects of AIP-TR overexpression on the levels of these proteins were analyzed. The data presented in Fig. 6, a and b, indicate that the overexpression of AIP-TR does not affect the down-regulation of either MmAurora-B or human cyclin B1 and support the notion that AIP down-regulates Aurora-A protein specifically. Also, the failure to down-regulate cyclin B1 suggests that the effect of AIP is not mediated by the generalized activation of the proteolytic machinery.

Proteasome Inhibitors Reverse the AIP-mediated Down-regulation of Aurora-A—It has been shown the proteasome plays a major role in the regulation of Aurora-A stability (50). Hence, it is possible that the effect of AIP overexpression on the down-regulation of Aurora-A could be mediated through the potentiation of proteasome-dependent degradation of Aurora-A. To address this question, COS cells were transfected with FLAG-tagged AIP-TR together with empty vector or HsAurora-A expression constructs, and the effect of AIP overexpression on the down-regulation of Aurora-A was followed in the presence and absence of proteasome inhibitors such as MG132, ALLN, and clasto-lactacystin β-lactone. As shown in Fig. 7, proteasome inhibitors could reverse the AIP-mediated down-regulation of Aurora-A protein to different levels depending on their potencies to inhibit the proteasome machinery. Calpain inhibitor ALLM could not reverse the AIP-mediated degradation of Aurora-A protein, suggesting that the cysteine protease calpain is unlikely to play a role in the AIP-mediated down-regulation of Aurora-A. Taken together, these results indicate that the proteasome plays a major role in AIP-mediated down-regulation of Aurora-A protein.

**DISCUSSION**

Aurora-A kinase is a member of a serine/threonine kinase family implicated in equal segregation of chromosomes between daughter cells. Aurora-A kinase is suggested to play a role also in tumorigenesis (29). Overexpression of Aurora-A kinase transforms cultured rodent cells and causes aneuploidy in near diploid mammary epithelial cells (31). Regulation of Aurora-A kinase expression and activity occurs at multiple levels such as gene amplification, transcription, phosphorylation, and degradation through the proteasome-dependent pathway (29, 31, 50). Currently, attempts are being made to understand the functions of Aurora-A kinase at the molecular level. In this paper, using a dosage suppressor screen in yeast, we have isolated and investigated AIP, a novel negative regulator of Aurora-A kinase. We have shown that AIP interacts specifically and down-regulates Aurora-A kinase by potentiating its degradation through the proteasome-dependent pathway. We demonstrated that both the full-length and N-terminal truncated AIP could interact with Aurora-A kinase, suggesting that the C-terminal portion of AIP alone is sufficient for the interaction. However, the interaction of endogenous Aurora-A kinase with AIP in cells overexpressing AIP was difficult to demonstrate probably because of the degradation of endogenous protein by AIP. This inference was supported by the observation that when Aurora-A protein levels were increased by the coexpression of Aurora-A and AIP, AIP and Aurora-A kinase can be communoprecipitated readily (Fig. 3, a and b). Similarly, both full-length and truncated AIP were effective in the down-regulation of Aurora-A kinase (data not shown).
However, the truncated AIP was more efficient in the down-regulation probably because of either the higher levels of the truncated protein accumulated inside the cell or the better binding to Aurora-A kinase. The increased level of truncated AIP was evident from all of our Western blot analysis that the cells accumulated more of the truncated AIP than the full-length AIP (Fig 3b). Analysis of the mutant AIP proteins for the interaction/degradation of Aurora-A protein demonstrated that the mutant ΔC198-AIP lacks the elements
essential for the AIP/Aurora-A interaction, and the interaction is important for the degradation of Aurora-A kinase (Fig. 5, a and b). Interestingly, this mutant lacks the nuclear localization signal, suggesting that the targeting of AIP to the nucleus may be necessary for the interaction and degradation of Aurora-A kinase.

We have shown that AIP down-regulates Aurora-A kinase possibly through proteasome-dependent degradation. AIP is not unique in that there are other examples of proteins involved in instigating the degradation of cell cycle-related interacting partners through the proteasome pathway. Jab1 has been shown to promote the degradation of the cell cycle regulator p27kip1 in a proteasome-dependent manner (52). However, the exact role of Jab1 in the degradation is still unclear. The WD repeat-containing protein cdc20 interacts with and targets the budding yeast anaphase regulator Pds1 (securin) for degradation through the APC/C (53). Similarly, it has been well documented that MDM2 can facilitate the degradation of p53 (54). In this case, it is evident now that MDM2 itself can act as the ubiquitin ligase facilitating the ubiquitination of p53 (55). The observation that AIP could also destabilize Aurora-A kinase specifically through 26 S proteasome raises an interesting question as to what proteasome-targeting mechanism is employed for AIP-mediated Aurora-A degradation. AIP sequence analyses do not reveal any similarity to either F box proteins (56) or U box proteins (57), which play crucial roles in targeting and ubiquitination, respectively. On the other hand, the failure of AIP-dependent cyclin B1 degradation in cells overexpressing AIP confirms the notion that AIP does not activate the 26 S proteasome machinery in a generic way. The specific interaction of AIP with Aurora-A kinase as well as the essential nature of the AIP/Aurora-A interaction for the degradation of Aurora-A supports interesting possibilities such as AIP directly modifying and/or targeting Aurora-A kinase for destabilization or AIP/Aurora-A kinase interaction being the key rate-limiting step in the Aurora-A kinase degradation pathway. Although it is known that Aurora-A kinase is polyubiquitinated before degradation by APC/cyclosome (50), it still remains to be shown that AIP plays a role in the ubiquitination of Aurora-A kinase. It has been shown that cdc20/tp53cdc, which is capable of activating APC, interacts with human Aurora-A (44). However, the question of whether cdc20 targets Aurora-A for degradation also remains to be answered.

Apart from its role in destabilization of Aurora-A kinase when overexpressed, the normal function of AIP is yet to be established. The results obtained so far point to the fact that AIP is a ubiquitously expressed nuclear protein. It will be interesting to investigate whether AIP is the normal physiological trigger for Aurora-A degradation. Other pertinent questions to be answered will include the subcellular location where interaction between AIP and Aurora-A occurs and at which stage of cell cycle AIP-mediated Aurora-A degradation occurs. Being a negative regulator of Aurora-A, a potential oncogene, there is a possibility that down-regulation of AIP could play a major role in tumorigenesis. Currently, experiments are being carried out to address these issues.

In summary, the findings reported in this paper identify a novel negative regulator of Aurora-A kinase. Understanding the normal function of AIP as well as the characterization of the molecular mechanisms involved in the AIP-mediated destabilization of Aurora-A will be the next chapter in this investigation. Moreover, the targeted degradation of Aurora-A by AIP provides us with the handle to manipulate the endogenous level of the oncogenic Aurora-A kinase. Hence, AIP could therefore be a potential target gene for anti-cancer drugs in the future.
Regulator of Aurora-A Kinase

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