J Biosci (2019) 44:147
DOI: 10.1007/s12038-019-9963-6

Haploinsufficient tumor suppressor Tip60 negatively regulates oncogenic Aurora B kinase

Arnab Bose[1], Surabhi Sudevan[1], Vinay J Rao[1], Hiroki Shima[2], Arun Kumar Trivedi[3], Kazuhiko Igarashi[2] and Tapas K Kundu[1]*

[1]*Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, Karnataka, India*

[2]*Department of Biochemistry, Tohoku University Graduate School of Medicine, Seiryo-machi 2-1, Aoba-ku, Sendai 980-8575, Japan*

[3]*Division of Cancer Biology, CSIR-Central Drug Research Institute (CSIR-CDRI), Sector-10, Jankipuram Extension, Lucknow, UP 226031, India*

**Corresponding author (Email, tapas@jncasr.ac.in)*

MS received 28 March 2019; accepted 25 September 2019; published online 6 November 2019

The Aurora kinases represent a group of serine/threonine kinases which are crucial regulators of mitosis. Dysregulated Aurora kinase B (AurkB) expression, stemming from genomic amplification, increased gene transcription or overexpression of its allosteric activators, is capable of initiating and sustaining malignant phenotypes. Although AurkB level in cells is well-orchestrated, studies that relate to its stability or activity, independent of mitosis, are lacking. We report that AurkB undergoes acetylation *in vitro* by lysine acetyltransferases (KATs) belonging to different families, namely by p300 and Tip60. The haploinsufficient tumor suppressor Tip60 acetylates two highly conserved lysine residues within the kinase domain of AurkB which not only impinges the protein stability but also its kinase activity. These results signify a probable outcome on the increase in "overall activity" of AurkB upon Tip60 downregulation, as observed under cancerous conditions. The present work, therefore, uncovers an important functional interplay between AurkB and Tip60, frailty of which may be an initial event in carcinogenesis.

Keywords. AurkB; acetylation; kinase activity; stability; Tip60

Abbreviations: AurkB, aurora kinase B; Tip60, HIV-1 Tat interacting protein, 60 kDa; KAT, lysine acetyltransferase

1. Introduction

AurkB is one of the three human paralogs of Aurora kinases and is a member of the chromosome passenger complex. It plays pivotal roles in chromosome congression, spindle bi-orientation and cytokinesis (Ruchaud *et al.* 2007) and undergoes a multitude of post translational modifications which modulate its spatio-temporal behaviour necessary for proper mitotic progression (Monaco *et al.* 2005; Ban *et al.* 2011; Fernández-Miranda *et al.* 2010; Catherine *et al.* 2016). Although *AurkB* transcription or protein stability is tightly regulated prior to mitotic entry or following mitotic exit respectively (Kimura *et al.* 2004; Park and Song 2018), this regulation is lost in cancer cells, leading to elevated steady state AurkB protein levels. It is frequently overexpressed in both solid and haematological malignancies, and is therefore a potential target of anti-neoplastic therapies (Borisa and Bhatt 2017).

Proteome-wide high throughput studies have unveiled the "lysine acetylome" of a cell (Choudhary *et al.* 2009). Interestingly, many cell cycle regulators were found to be acetylated. Congruently, histone deacetylase (HDAC) inhibitors were found to affect kinetochore assembly through decreased pericentromeric targeting of AurkB (Robbins *et al.* 2005). HDAC3 was observed to not only assist in histone H3 deacetylation during mitosis to provide a hypoacetylated H3-tail for phosphorylation by AurkB (Li *et al.* 2006) but also deacetylate AurkB and enhance its kinase activity (Fadri-Moskwik *et al.* 2012). Additionally, AurkB interacts with class IIa histone deacetylases in determining their nuclear localization during mitosis (Guise *et al.* 2012).

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s12038-019-9963-6) contains supplementary material, which is available to authorized users.

We observe that p300 and Tip60 acetylate AurkB *in vitro*. Tip60-mediated acetylation of AurkB at two highly conserved K85 and K87 residues negatively influence its protein stability and kinase activity. We propose that under normal physiology Tip60-mediated acetylation serves to contain AurkB oncogenicity. However, upon Tip60 downregulation, as observed in different cancers (Gorrini *et al*. 2007; Mattera *et al.* 2009), AurkB is not only stabilized but also exhibits increase in its kinase activity, causing an overall increase in activity of the oncogenic kinase.

2. Materials and methods

2.1 *Cell culture, antibodies and reagents*

HEK293 and HEK293T (purchased from ATCC), MDA-MB-231 and MCF7 were grown at 37°C in DMEM (Invitrogen, Thermo Fisher Scientific) containing 10% fetal bovine serum. To generate Flag-AurkB overexpressing stable cell line, pCDH adenoviral vector containing N-terminal Flag-AurkB fusion was co-transfected with lentiviral gene containing plasmids (psPAX2, pRSV-Rev and pCMV-VSV-G) into HEK293T cells to package the mammalian clone into the viral particles. Forty-eight hours post-transfection, the generated viral particles were used to infect HEK293 cells, and subsequently sorted for the GFP-positive cell population. Constitutive Tip60 knockdown cell line and the corresponding control cell line were similarly established in HEK293 using Tip60 directed shRNA or a non-silencing shRNA cloned in pGIPZ vector (Dharmacon). Transient transfection of 2N3T-Tip60 or the corresponding control empty vector was carried out for 48 hours into HEK293 cells using Lipofectamine 2000 transfection reagent (Invitrogen); UV irradiation was carried out at a dose of 15 mJ/cm^2 using Stratagene Stratalinker 1800; HEK293 cells overexpressing Flag-AurkB was treated with 10 μM of trichostatin A (TSA) (Sigma-Aldrich) or DMSO for 12 hours. Cells for the various treatments or transfection conditions were harvested by scraping and lysed in lysis buffer containing 50 mM tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% NP40, and 1 X protease inhibitory cocktail (Sigma-Aldrich). Anti-Aurora kinase B (Abcam; ab45145), anti-Flag (Sigma; F-1804), anti-β-actin (Sigma; A3854), anti-HA (Sigma; H9658), and anti-α tubulin (EMD Millipore; DM1A) antibodies were purchased from the defined vendors.

2.2 *Cycloheximide chase assay*

3 X Flag pCMV10-AurkB WT or the corresponding acetylation defective (K85R-K87R) and mimetic (K85Q-K87Q) mutants were transiently transfected into HEK293 cells in 60 mm dish format using Lipofectamine 2000. Twenty-four hours post transfection, the cells were trypsinized and re-seeded into 6-well-plate and allowed to grow for 24–36 h more to grow till 60–70% confluence, after which they were treated with 150 μg/ml of cycloheximide (Sigma-Aldrich) for the indicated time periods. The cells were harvested by scraping and lysed in Laemmli buffer.

2.3 *In vitro lysine acetyltransferase (KAT) assay*

KAT assay was performed using highly purified, baculovirus-expressed, recombinant full-length lysine acetyltransferases-His_6-Tip60, Flag-PCAF or His_6-p300 from Sf21 insect cells as enzymes and His_6-AurkB, as substrates, in a 30 μl final reaction volume. Briefly, the KAT enzyme activities were normalized for the individual enzymes toward purified histones (histone H3 for p300 and PCAF; histone H4 for Tip60). Equal activity (30,000 counts per minute, as determined by filter binding assay) for each of the enzymes was used for final reaction mixture consisting of 50 mM tris-HCl, pH 8.0, 10% (v/v) glycerol, 1 mM dithiothreitol (DTT), 1 mM phenyl methyl sulfonyl fluoride (PMSF), 0.1 mM EDTA, pH 8.0, 10 mM sodium butyrate, and 1 μl of 3.3 Ci/mmol of H^3-acetyl Coenzyme A (acetyl-CoA) and the indicated proteins were incubated at 30°C for 30 min. Mass acetylation was carried for 2.5 hours at 30°C (with replenishment of indicated enzymes and acetyl-CoA every 30 min, thrice). To visualize the radiolabelled acetylated protein, the reaction products were resolved on 12% SDS- polyacrylamide gel upon electrophoresis and stained by coomassie brilliant blue (CBB) to ascertain the presence of proteins. The gel was then dehydrated in DMSO for 1 h and incubated with scintillation fluid (22.5% w/v PPO solution in DMSO) for 30 min and finally equilibrated in water for two hours. The rehydrated gel was dried using a gel drier and exposed to X-ray film for 10-12 days.

2.4 *In vitro kinase assay*

Recombinant histone H3, expressed and purified from *E. coli*, was incubated with either mass acetylated (Tip60-mediated) or mock acetylated His_6-Aurora kinase B, in a 30 μl reaction mixture containing 50 mM tris-HCl, 100 mM NaCl, 0.1 mM EGTA, 10 mM MgCl2, 0.2% β-mercaptoethanol and [γP32] ATP (Specific Activity 3.5 Ci/mmol). The reaction mixture was incubated at 30°C for 15 min. The reaction was inhibited over ice for 5 min, constituent proteins were denatured by the addition of gel loading dye containing SDS, heated at 90°C for 5 min and resolved using 12% denaturing PAGE, stained by CBB, and followed by autoradiography using X-ray films.

3. Results and discussion

3.1 *Tip60 acetylates AurkB at two highly conserved lysine residues*

In order to identify the putative KAT(s) responsible for the lysine acetyltransferase activity toward AurkB, we adopted an

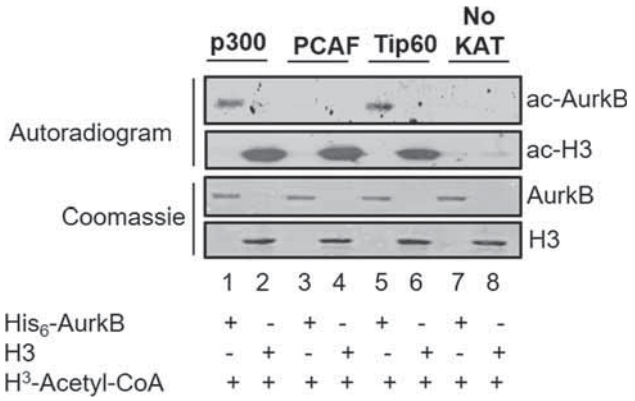

Figure 1. Tip60 and p300 acetylate AurkB. 500 ng each of recombinant His_6-AurkB and histone H3, purified from *E. coli*, were subjected to *in vitro* lysine acetyltransferase (KAT) assay by using His_6-p300, Flag-PCAF and His_6-Tip60 purified from Sf21 cells using suitable baculoviral constructs and tritiated acetyl-CoA (H^3-acetyl-CoA). No KAT (lanes 7 and 8) served as the negative control in which none of the KATs were added.

unbiased approach and considered the screening of KATs belonging to three different families of lysine acetyltransferases, namely p300, PCAF and Tip60. Each of these enzymes were purified from Sf21 insect ovarian cells using suitable baculoviral constructs containing the human KAT homolog. Recombinant His_6-AurkB, expressed and purified from *E. coli* was used for an *in vitro* lysine acetyltransferase (KAT) assay. It was intriguing to find that p300 and Tip60 were capable of acetylating AurkB, while PCAF could not (figure 1).

The haploinsufficient tumor suppressor, Tip60 is known to oppose oncogenesis. Factual downregulation of Tip60 expression in multiple cancer types and its direct involvement in acetylation dependent inhibition of oncogenic pathways (Du *et al.* 2010; Kim *et al.* 2007) led us to study the further possibility of a Tip60 dependent regulation of AurkB in the context of acetylation. In order to identify the acetylation sites, we performed *in vitro* mass acetylation of recombinant full length His_6-AurkB using His_6-Tip60. The acetylated protein was subjected to liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS) and nine probable acetylated lysine residues were

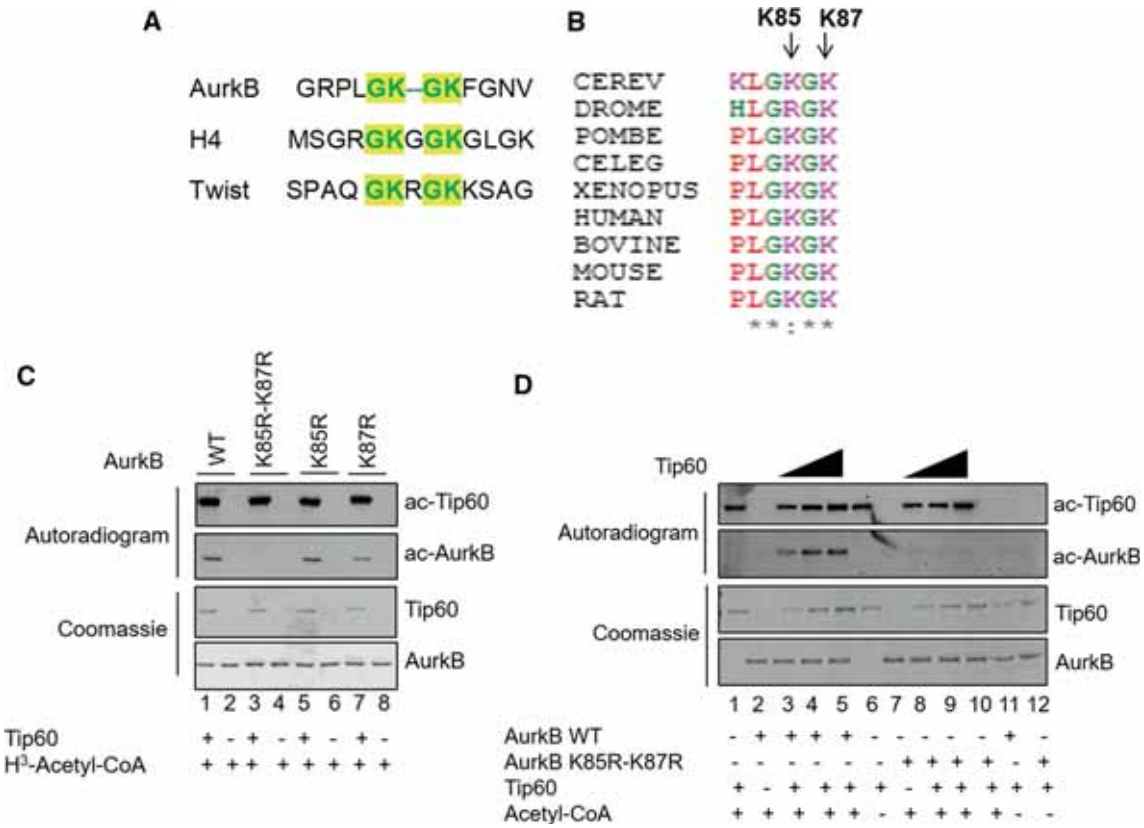

Figure 2. Tip60 acetylates AurkB at K85 and K87. (**A**) Local alignment of oligopeptide sequence displaying the similarities of putative Tip60-mediated acetylation motif on AurkB, histone H4 and Twist. (**B**) Local alignment exhibiting the extent of conservation of K85 and K87 residues of AurkB in different organisms- CEREV, Saccharomyces cerevisiae; DROME, Drosophila melanogaster; POMBE, Schizosaccharomyces pombe; CELEG- Caenorhabditis elegans; XENOPUS, Xenopus laevis; HUMAN, Homo sapiens; Bovine, Bos Taurus; Mouse, Mus musculus; RAT, Rattus rattus. (**C**) 500 ng each of His_6- AurkB WT or the indicated mutant His_6- AurkB proteins were used to carry out an *in vitro* KAT assay using His_6-Tip60 purified from Sf21 cells and H^3-acetyl coenzyme A. (**D**) 500 ng each of His_6-AurkB WT or His_6- AurkB K85R-K87R double point mutant proteins were used to carry out an in vitro KAT assay using increasing concentrations of His_6-Tip60 purified from Sf21 cells and H^3-acetyl coenzyme A. The coomassie staining for each of the lanes is shown below the autoradiogram profiles for comparing the loading levels across each lane.

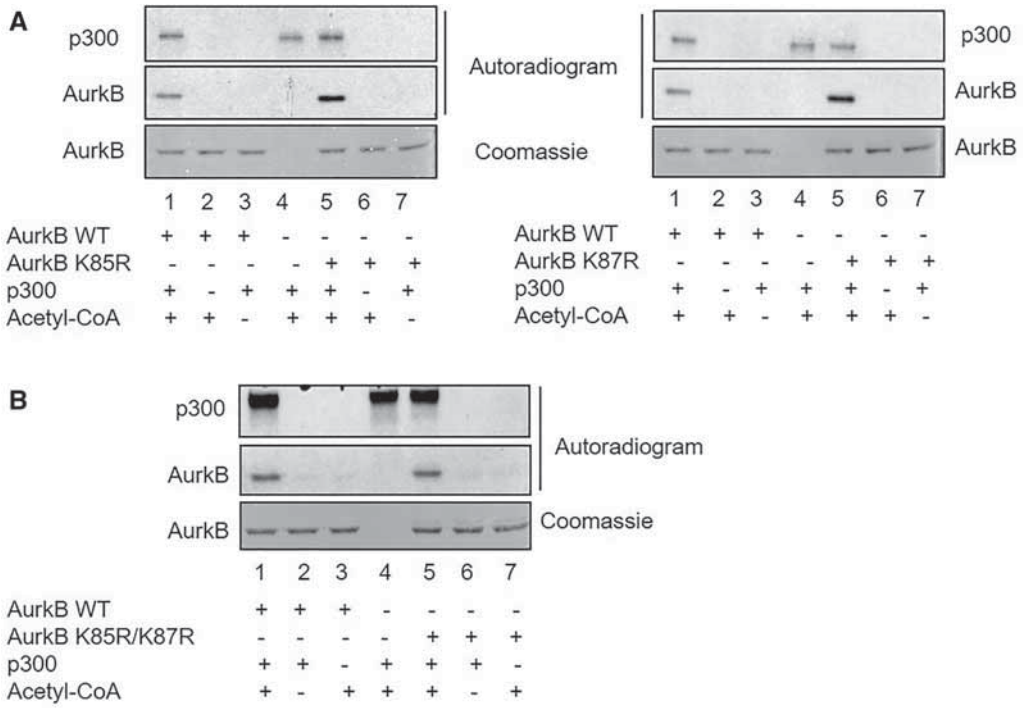

Figure 3. Validation of p300-mediated acetylation of AurkB for Tip60 targeted lysine residues. 500 ng each of recombinant His_6-AurkB WT or the His_6-AurkB mutant proteins (single point mutants-K85R and K87R, as in panel (**A**) or the double point mutant (His_6- AurkB K85R/ K87R, as in panel (**B**) were used to carry out an *in vitro* KAT assay using His_6-Tip60 purified from Sf21 cells and H^3-acetyl coenzyme A. The coomassie staining for each of the lanes is shown below the autoradiogram profiles for comparing the loading levels across each lane.

identified- K4, K31, K56, K85 (supplementary figure 1, panel I), K87 (supplementary figure 1, panel II), K115, K168, K195, and K202. Additionally, we observed that K85 and K87 residues reside in a glycine-rich microenvironment and is part of a G-K-G-K motif, which is similar to G-K-X-G-K motif found in the well-studied Tip60 substrates- histone H4 and Twist (figure 2A). Noting the high extent of conservation of these two lysine residues (figure 2B), we mutated them, either singly or in combination, to arginine and the *E. coli* purified recombinant WT and mutant AurkB proteins were subjected to *in vitro* KAT assay. While K85R point mutant exhibited minimal alteration in acetylation levels, the K85R-K87R double point mutant led to a complete abrogation of Tip60-mediated acetylation (figure 2C). Furthermore, the K85R-K87R mutant did not show any acetylation even for higher Tip60 concentrations (figure 2D), emphasizing both of the residues to be the major Tip60-mediated acetylation sites. However, the fact that K87R showed drastic, yet not complete, reduction in acetylation might indicate the possibility of preferential acetylation site amongst the two lysine residues.

3.2 *Tip60 and p300 may not possess overlapping acetylation sites on AurkB*

The screening of different KATs had highlighted that p300, apart from Tip60, is also capable of acetylating AurkB *in vitro*. We subsequently studied the acetylation of K85 and K87 residues of AurkB by p300 and observed that p300 is capable of acetylating either of the individual point mutants-K85R and K87R (figure 3A) or the K85R-K87R double mutant (figure 3B). These results emphasize either the exclusivity of K85 and K87 residues toward Tip60-mediated acetylation or portrays the possibility of numerous p300-mediated acetylation sites on AurkB, and mutation of two (K85 and K87) amongst many, is incapable of altering the overall p300 dependent acetylation status of the AurkB protein.

3.3 *Tip60 interacts with and acetylates AurkB in cells*

We studied if AurkB and Tip60 interact with each other in a cellular context. Reciprocal co-immunoprecipitation of ectopically overexpressed 2N3T-Tip60 (henceforth referred to as HA-Tip60) in HEK293 cells constitutively expressing Flag-AurkB confirmed their physical interaction (figure 4A). These results demonstrate that Tip60 interacts with AurkB in cells and acetylate it at K85 and K87 residues. We next asked whether Tip60 is capable of acetylating AurkB in cells. To this end, we co-transfected Flag-AurkB and HA-Tip60 in HEK293T cells and observed that Tip60 overexpression enhanced the acetylation levels of AurkB in cells despite reduced overall Flag-AurkB levels (figure 4B). This result

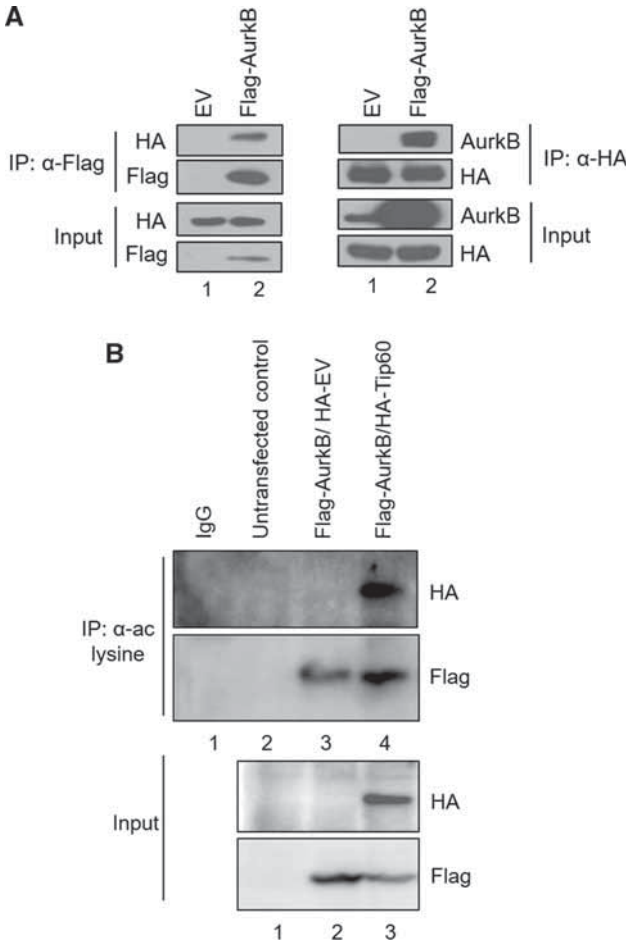

Figure 4. Tip60 acetylates AurkB in cells. (**A**) Western blot analysis for reciprocal co-immunoprecipitation showing the interaction between HA-Tip60 (transiently overexpressed for 48 hours) and Flag-AurkB in HEK293 cells constitutively overexpressing Flag-AurkB protein. Whole cell lysates were incubated with the desired antibodies (either anti-Flag or anti-HA) conjugated to agarose beads and probed with the indicated antibodies. IP represents the immunoprecipitated fraction from the whole cell lysate and input represent 2% of the whole cell lysate. (**B**) Western blot representation for Flag-AurkB acetylation by HA-Tip60 (transiently co-overexpressed for 48 hours). IP represents the immunoprecipitated fraction from whole cell lysate and input represent 2% of the whole cell lysate.

signifies that the physical interaction observed between Tip60 and AurkB culminates into AurkB acetylation in cells.

3.4 *Tip60 regulate protein levels of AurkB in cells*

Histone deacetylases play important roles in mitosis. Broad and non-selective HDAC inhibitors like trichostatin A (TSA) inhibits class I HDACs (HDAC1, 3, 4, 6, and 10) and has been shown to induce G2/M arrest by targeting HDAC3 (Li *et al.* 2006). Moreover, HDAC3 was also reported to deacetylate AurkB (Fadri-Moskwik *et al.* 2012). Since HDAC inhibition results in a gross increase in acetylation levels of proteins in cells, we were prompted to study the effect of TSA treatment on the levels of AurkB. Strikingly, treatment of HEK293 cells stably expressing Flag-AurkB with TSA led to a marked reduction of the AurkB protein pool (figure 5A). Combining this result with the outcome of a decrement in Flag-AurkB levels upon HA-Tip60 overexpression (figure 4B; input), we wondered about the likelihood of acetylation of AurkB as a trigger for its downregulation. Concordantly, we overexpressed HA-Tip60 in HEK293 cells and found that the endogenous AurkB levels exhibited a marked reduction, which could be rescued by simultaneous Tip60 knockdown (figure 5B, panel-I; compare lane 1 v/s 2 and 2 v/s 3). Similar rescue was also observed when Flag-AurkB was transiently overexpressed in constitutive Tip60 knockdown HEK293 cells (figure 5B, panel II; supplementary figure 2). We could faithfully reproduce these results in two other breast cancer cell lines, MDA-MB-231 and MCF7 (figure 5C, panel I and II, respectively). We also generated point mutants corresponding to acetylation defective (K85R-K87R) or mimetic (K85Q-K87Q) conditions, on Flag-AurkB mammalian expression vector, and observed that the later exhibited reduced stability under cycloheximide chase assay conditions (figure 5D). These observations collectively confirm that Tip60-mediated acetylation of AurkB at K85 and K87 residues result into its destabilization in cells.

We next asked if AurkB modulation agrees with a physiologically relevant scenario where Tip60 exhibits differential expression levels in cells. An earlier observation had demonstrated enhanced stabilization of Tip60 protein in cells upon UV exposure (Legube *et al.* 2002), owing to an inhibition of its degradation by MDM2. In agreement with this study, we observed that UV treatment caused enhancement in Tip60 levels in HEK293 cells, peaking at 6 hours post irradiation. Interestingly, AurkB was concomitantly observed to be downregulated under such conditions (figure 5E).

3.5 *Tip60-mediated acetylation inhibits kinase activity of AurkB*

K85 and K87 residues lie within the kinase domain of AurkB. This propelled us to study if the kinase activity is affected upon acetylation by Tip60. Recombinant His_6-AurkB was acetylated in vitro with Tip60 and the acetylated kinase was used in an in vitro kinase assay with histone H3 as a substrate. We found that acetylation of AurkB lowered its kinase activity, when compared to the mock acetylated control (supplementary figure 3; figure 6A). We extended our study in assessing the point mutants of AurkB and observed that the acetylation mimetic K85Q-K87Q mutant exhibited reduced kinase activity as compared to either the WT or the acetylation defective K85R-K87R mutant (figure 6B). The acetylation defective mutant, however, showed

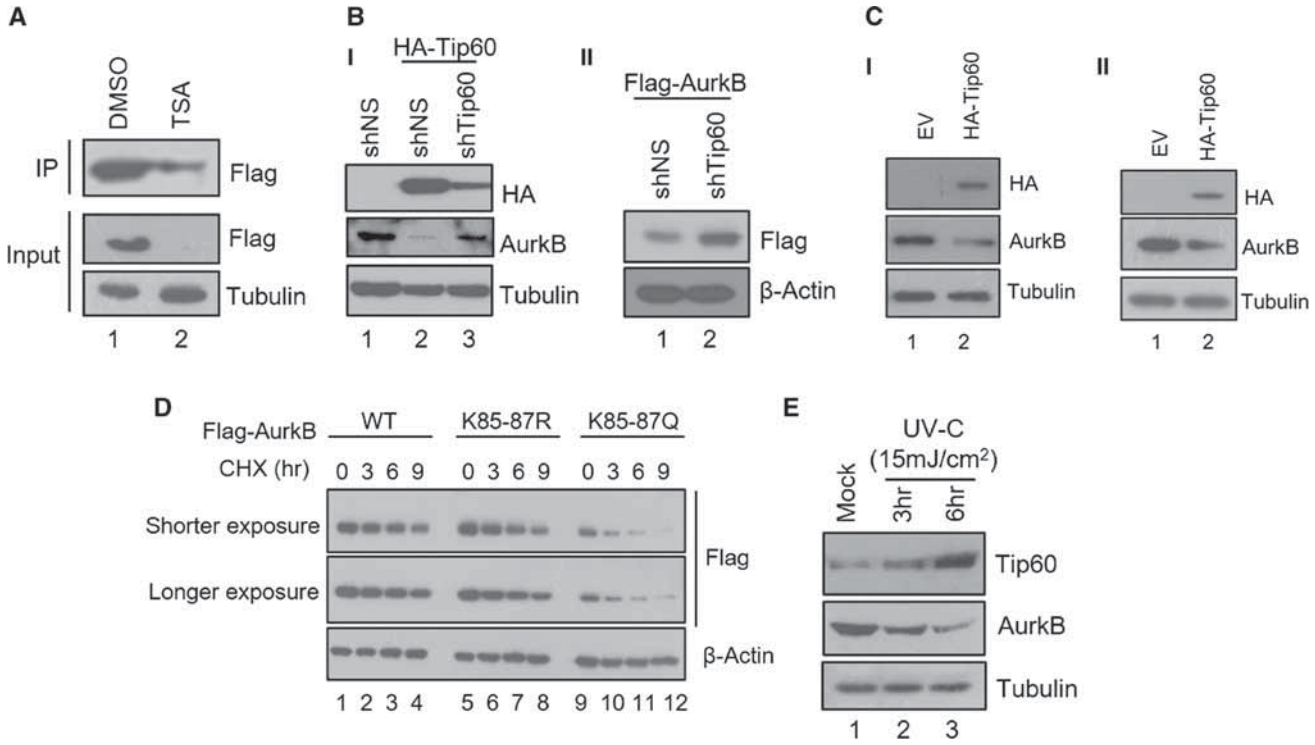

Figure 5. Tip60 regulates AurkB levels in cells. (**A**) Western blot representation of the indicated proteins upon treatment of stably overexpressing Flag-AurkB HEK293 cells with TSA or the corresponding DMSO control. IP represent the immunoprecipitated fraction from 1 mg of whole cell lysate; input represent the whole cell lysate fraction. (**B**) Western blot analysis showing that Tip60 overexpression reduces, while simultaneous Tip60 knockdown rescues AurkB levels in HEK293 cells (**panel I**) [shNS represent non-silencing shRNA control]. (**panel II**) Transient overexpression of Flag-AurkB in stable shNS and shTip60 cell lines showing the higher levels of Flag-AurkB in the Tip60 knockdown cell line as compared to the control shNS cells. (**C**) Western blot representation for overexpression of Tip60 in MDA-MB-231 cells (**panel I**) or MCF7 cells (**panel II**) and study of the proteins with the indicated antibodies. (**D**) Cycloheximide chase assay for WT, acetylation defective (K85R-K87R) or acetylation mimetic (K85Q-K87Q) mutants of AurkB. (**E**) Western blot representation of HEK293 cells exposed to 15 mJ/cm^2 UV-C (254 nm) and harvested at the indicated time points after irradiation for the indicated proteins.

higher kinase activity, even as compared to the WT kinase, the cause of which is presently unclear. These results confirm that Tip60 dependent acetylation inhibit the kinase activity of AurkB.

4. Discussion

AurkB is the catalytic component of the chromosomal passenger complex (CPC) and ensures proper chromosome alignment and segregation (Adams *et al*. 2001). It also assists in cleavage furrow formation, and cytokinesis and hence a reduction in the kinase activity by either chemical inhibitors or RNA interference gives rise to chromosome segregation defects and aneuploidy (Ditchfield *et al.* 2003; Hauf *et al.* 2003). Paradoxically, AurkB overexpression in cultured mammalian cells and mouse model is reported to cause an increased tumor incidence by tetraploidization or inhibiting p53 and p21 levels and functions (Gully *et al.* 2012; González-Loyola *et al.* 2015; Nguyen *et al.* 2009) or by modulation of Myc dependent tumors (den Hollander *et al.* 2010; Yang *et al.* 2010). These evidences indicate the necessity for fine tuning of kinase activity of AurkB in cells for error-free mitotic progression. Moreover, the paralogous kinase- AurkA, has been reported to participate in varying signalling events which culminate into tumorigenesis and cancer progression (Katayama *et al.* 2004; Briassouli *et al.* 2007; Dar *et al.* 2009; Otto *et al.* 2009).

Here, we report a previously unanticipated link between AurkB and Tip60 whereby Tip60 contributes to its tumor suppressive effects by not only inhibiting the kinase activity but also impinging the destabilization of AurkB protein, through acetylation of two highly conserved lysine residues present within the kinase domain of AurkB. This phenomenon may have roles to play in normal and altered physiology. We observed that UV induced stabilization of Tip60 concomitantly reduces AurkB levels. Since AurkB is necessary for cell cycle progression, the present set of results indicate the molecular roles Tip60 may play to ensure genomic stability by ensuring sufficient time for UV induced DNA damage repair before cell division commences. Furthermore, Tip60 expression has been shown to be perturbed

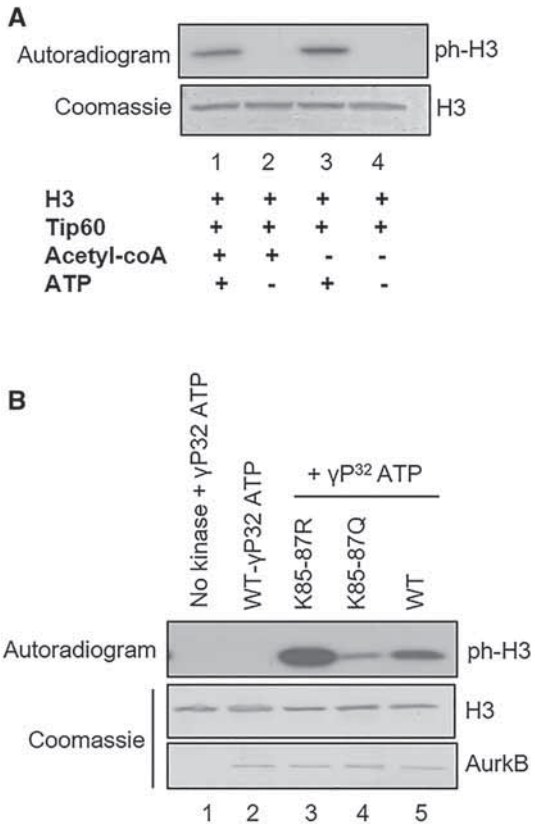

Figure 6. Tip60-mediated acetylation inhibits AurkB activity. (**A**) 500 ng of His_6-AurkB was either acetylated with Tip60 or mock acetylated (control) in vitro. One-tenth of the acetylation reaction was used to carry out in vitro kinase assay with 500 ng of recombinant H3. The coomassie staining for each of the lanes is shown below the autoradiogram profiles for comparing the loading levels across each lane. (**B**) *In vitro* kinase assay was carried out using 100 ng each of WT, acetylation defective mutant (K85R-K87R), or acetylation mimetic mutant (K85Q-K87Q) of AurkB using histone H3 as a substrate. Lesser extent of kinase activity was observed for the K85Q-K87Q mutant as compared to either the WT or the K85R-K87R mutant.

in a various solid and haematological cancers (Gorrini *et al.* 2007; Mattera *et al*. 2009). We propose that the widespread Tip60 downregulation in cancers may favour the deregulated increase in the overall activity of AurkB in tumor cells which in turn may act as a causative factor for the associated alteration of p53 or c-Myc pathways. Furthermore, as earlier studies elucidate the direct links between Tip60 and genomic aberrations (Bassi *et al.* 2016), it is worthwhile to study if any of such effects are mediated by the altered activities of AurkB.

Acknowledgements

The authors thank Prof. Didier Trouche (LBCMCP, Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, UPS, Toulouse, France) for 2N3T empty vector and 2N3T-Tip60 plasmids and Dr. Amit Dutt (Integrated Cancer Genomics Lab, ACTREC, Mumbai, India) for sharing MDA-MB-231 and MCF7 breast cancer cells. AB is a recipient of Senior Research Fellowship from the Council of Scientific and Industrial Research (CSIR), Government of India; SS and VJR were supported by Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR). This study was supported in part by JNCASR and Sir JC Bose National Fellowship, Department of Science and Technology, India, to TKK. This work was also supported by Indo-Japan bilateral joint research program by Department of Science and Technology, India, and Japan Society for the Promotion of Science (JSPS), Japan. Studies at Tohoku University was supported in part by grants-in-aid from JSPS (17K07278 and 25670156).

References

Adams RR, Maiato H, Earnshaw WC and Carmena M 2001 Essential roles of Drosophila inner centromere protein (INCENP) and aurora B in histone H3 phosphorylation, metaphase chromosome alignment, kinetochore disjunction, and chromosome segregation. *J. Cell Biol.* **153** 865–880

Ban R, Nishida T and Urano T 2011 Mitotic kinase Aurora-B is regulated by SUMO-2/3 conjugation/deconjugation during mitosis. *Genes Cells* **16** 652–669

Bassi C, Li YT, Khu K, Mateo F, Baniasadi PS, Elia A, Mason J, Stambolic V, Pujana MA, Mak TW and Gorrini C 2016 The acetyltransferase Tip60 contributes to mammary tumorigenesis by modulating DNA repair. *Cell Death Differ.* **23** 1198–1208

Borisa AC and Bhatt HG 2017 A comprehensive review on Aurora kinase: Small molecule inhibitors and clinical trial studies. *Eur. J. Med. Chem.* **140** 1–19

Briassouli P, Chan F, Savage K, Reis-Filho JS and Linardopoulos S 2007 Aurora-A regulation of nuclear factor-kappaB signaling by phosphorylation of Ikappa B alpha. *Cancer Res.* **67** 1689–1695

Catherine L, Grant R, and Min M 2016 Ubiquitin-mediated degradation of Aurora kinases. *Front. Oncol.* **5** 307

Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, Olsen JV and Mann M 2009 Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* **325** 834–840

Dar AA, Belkhiri A and El-Rifai W 2009 The aurora kinase A regulates GSK-3beta in gastric cancer cells. *Oncogene* **28** 866–875

den Hollander J, Rimpi S, Doherty JR, Rudelius M, Buck A, Hoellein A, Kremer M, Graf N, *et al.* 2010 Aurora kinases A and B are up-regulated by Myc and are essential for maintenance of the malignant state. *Blood* **116** 1498–1505

Ditchfield C, Johnson VL, Tighe A, Ellston R, Haworth C, Johnson T, Mortlock A, Keen N and Taylor SS 2003 Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. *J. Cell Biol.* **161** 267–280

Du Z, Song J, Wang Y, Zhao Y, Guda K, Yang S, Kao HY, Xu Y, Willis J, Markowitz SD, Sedwick D, Ewing RM and Wang Z 2010 DNMT1 stability is regulated by proteins coordinating deubiquitination and acetylation-driven ubiquitination. *Sci. Signal* **3** ra80

Fadri-Moskwik M, Weiderhold KN, Deeraksa A, Chuang C, Pan J, Lin SH and Yu-Lee LY 2012 Aurora B is regulated by acetylation/deacetylation during mitosis in prostate cancer cells. *FASEB J.* **26** 4057–4067

Fernández-Miranda G, Pérez de Castro I, Carmena M, Aguirre-Portolés C, Ruchaud S, Fant X, Montoya G, Earnshaw WC and Malumbres M 2010 SUMOylation modulates the function of Aurora-B kinase. *J. Cell Sci.* **123** 2823–2833

González-Loyola A, Fernández-Miranda G, Trakala M, Partida D, Samejima K, Ogawa H, Cañamero M, de Martino A, *et al*. 2015 Aurora B overexpression causes aneuploidy and p21Cip1 repression during tumor development. *Mol. Cell Biol.* **35** 3566–3578

Gorrini C, Squatrito M, Luise C, Syed N, Perna D, Wark L, Martinato F, Sardella D, *et al*. 2007 Tip60 is a haplo-insufficient tumour suppressor required for an oncogene-induced DNA damage response. *Nature* **448** 1063–1067

Guise AJ, Greco TM, Zhang IY, Yu F and Cristea IM 2012 Aurora B-dependent regulation of class IIa histone deacetylases by mitotic nuclear localization signal phosphorylation. *Mol. Cell Proteom.* **11** 1220–1229

Gully CP, Velazquez-Torres G, Shin JH, Fuentes-Mattei E, Wang E, Carlock C, Chen J, Rothenberg D, *et al*. 2012 Aurora B kinase phosphorylates and instigates degradation of p53. *Proc. Natl. Acad. Sci. USA* **109** E1513–E1522

Hauf S, Cole RW, LaTerra S, Zimmer C, Schnapp G, Walter R, Heckel A, van Meel J, Rieder CL and Peters JM 2003 The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. *J. Cell Biol.* **161** 281–294

Katayama H, Sasai K, Kawai H, Yuan ZM, Bondaruk J, Suzuki F, Fujii S, Arlinghaus RB, Czerniak BA and Sen S 2004 Phosphorylation by aurora kinase A induces Mdm2-mediated destabilization and inhibition of p53. *Nat. Genet*. **36** 55–62

Kim MY, Ann EJ, Kim JY, Mo JS, Park JH, Kim SY, Seo MS and Park HS 2007 Tip60 histone acetyltransferase acts as a negative regulator of Notch1 signaling by means of acetylation. *Mol. Cell Biol.* **27** 6506–6519

Kimura M, Uchida C, Takano Y, Kitagawa M and Okano Y 2004 Cell cycle-dependent regulation of the human aurora B promoter. *Biochem. Biophys. Res. Commun*. **316** 930–936

Legube G, Linares LK, Lemercier C, Scheffner M, Khochbin S and Trouche D 2002 Tip60 is targeted to proteasome-mediated degradation by Mdm2 and accumulates after UV irradiation. *EMBO J.* **21** 1704–1712

Li Y, Kao GD, Garcia BA, Shabanowitz J, Hunt DF, Qin J, Phelan C and Lazar MA 2006 A novel histone deacetylase pathway regulates mitosis by modulating Aurora B kinase activity. *Genes Dev.* **20** 2566–2579

Mattera L, Escaffit F, Pillaire MJ, Selves J, Tyteca S, Hoffmann JS, Gourraud PA, Chevillard-Briet M, Cazaux C and Trouche D 2009 The p400/Tip60 ratio is critical for colorectal cancer cell proliferation through DNA damage response pathways. *Oncogene* **28** 1506–1517

Monaco L, Kolthur-Seetharam U, Loury R, Murcia JM, de Murcia G and Sassone-Corsi P 2005 Inhibition of Aurora-B kinase activity by poly(ADP-ribosyl)ation in response to DNA damage. *Proc. Natl. Acad. Sci. USA* **102** 14244–14248

Nguyen HG, Makitalo M, Yang D, Chinnappan D, St Hilaire C and Ravid K 2009 Deregulated Aurora-B induced tetraploidy promotes tumorigenesis. *FASEB J.* **23** 2741–2748

Otto T, Horn S, Brockmann M, Eilers U, Schüttrumpf L, Popov N, Kenney AM, Schulte JH, Beijersbergen R, Christiansen H, Berwanger B and Eilers M 2009 Stabilization of N-Myc is a critical function of Aurora A in human neuroblastoma. *Cancer Cell* **15** 67–78

Park J and Song EJ 2018 Deubiquitinase USP35 as a novel mitotic regulator via maintenance of Aurora B stability. *BMB Rep*. **51** 261–262

Robbins AR, Jablonski SA, Yen TJ, Yoda K, Robey R, Bates SE and Sackett DL 2005 Inhibitors of histone deacetylases alter kinetochore assembly by disrupting pericentromeric heterochromatin. *Cell Cycle* **4** 717–726

Ruchaud S, Carmena M and Earnshaw WC 2007 Chromosomal passengers: conducting cell division. *Nat. Rev. Mol. Cell Biol*. **8** 798–812

Yang D, Liu H, Goga A, Kim S, Yuneva M and Bishop JM 2010 Therapeutic potential of a synthetic lethal interaction between the MYC proto-oncogene and inhibition of aurora-B kinase. *Proc. Natl. Acad. Sci. USA* **107** 13836–13841

Yun Li, Kao GD, Garcia BA, Shabanowitz J, Hunt DF, Qin J, Phelan C and Lazar MA 2006 A novel histone deacetylase pathway regulates mitosis by modulating Aurora B kinase activity. *Genes Dev.* **20** 2566–2579

Corresponding editor: Sorab Dalal