Identification of MYB-Binding Protein 1A (MYBBP1A) as a Novel Substrate for Aurora B Kinase

Claudia Perrera1,2, Riccardo Colombo2, Barbara Valsasina, Patrizia Carpinelli, Sonia Troiani, Michele Modugno, Laura Gianellini, Paolo Cappella, Antonella Isacchi, Jurgen Moll, and Luisa Rusconi

From Nerviano Medical Sciences S.r.l.

Running title: MYBBP1A: a novel substrate for Aurora B kinase

Address correspondence to: Claudia Perrera, Ph.D, Nerviano Medical Sciences, viale L. Pasteur 10, 20014 Nerviano (MI), Italy. E-mail: Claudia.Perrera@nervianoms.com

2 These authors contributed equally to this work.

Aurora kinases are mitotic enzymes involved in centrosome maturation and separation, spindle assembly and stability, chromosome condensation, segregation and cytokinesis and represent well known targets for cancer therapy as their deregulation has been linked to tumorigenesis. The availability of suitable markers is of crucial importance to investigate Auroras’ functions and monitor kinase inhibition in in-vivo models and in clinical trials. Extending the knowledge on Aurora substrates could help to better understand their biology and could be a source for clinical biomarkers.

Using biochemical, mass spectrometry and cellular approaches we identified MYBBP1A as a novel Aurora B substrate and Serine 1303 as the major phosphorylation site. MYBBP1A is phosphorylated in nocodazole arrested cells and is dephosphorylated upon Aurora B silencing or by treatment with Danusertib, a small molecule inhibitor of Aurora kinases. Furthermore, we show that MYBBP1A depletion by RNAi causes mitotic progression delay and spindle assembly defects.

MYBBP1A has until now been described as a nucleolar protein, mainly involved in transcriptional regulation. The results presented herein show MYBBP1A as a novel Aurora B kinase substrate and reveal a not yet recognised link of this nucleolar protein to mitosis.

Aurora A, B and C are the three mammalian members of Aurora Ser/Thr kinase family. Aurora kinases are involved in multiple functions in mitosis, including centrosome separation and maturation, spindle assembly and stability, chromosome condensation, congression and segregation, and cytokinesis. They possess a conserved catalytic domain, while their N-terminal domain varies contributing to different localization of the kinase within the cell (1,2). While Aurora C is mainly expressed in meiotically dividing cells, both Aurora A and B are widely present in all proliferating tissues and their expression is cell cycle regulated, peaking at G2/M phase of the cell cycle. Expression of Aurora kinases has been found to be elevated in a number of diverse human cancers and their overexpression can cause chromosome number instability and cellular transformation (3,4). Thus, Aurora kinases represent attractive targets for anti-cancer drug development. Indeed, small molecule inhibitors have been developed and are currently being tested in clinical trials (5,6).

The identification of MOA-related biomarkers and the discovery of new substrates is of crucial importance not only to better characterize the cellular role of the Aurora kinases but also to provide a clear readout of the in vivo activity of compounds. Aurora kinases have already been shown to phosphorylate a number of different substrates. Specifically, Aurora A phosphorylates TPX2, LIM protein, CDC25B, p53, BRCA1, ASAP and others (7-13) whereas Aurora B phosphorylates histone H3, MCAK, histone H2A, topoisomerase II, INCENP, Survivin and CENP-A (14-20).
Together with INCENP, Survivin and Borealin, Aurora B is a component of the Chromosomal Passenger Complex (CPC) (21,22). In early mitosis, the CPC promotes correct chromosome alignment and is responsible for the displacement of HP-1 from mitotic chromosomes by modifying histone H3, whereas at the end of mitosis CPC regulates proper completion of cytokinesis (23,24). The non-enzymatic components of this complex control the targeting, enzymatic activity and stability of Aurora B kinase (25-28) and although the major role of CPC is in mitosis, its components are already expressed earlier in the cell cycle, when different complexes of chromosomal passenger proteins may exist (29). Recently, for example, Aurora B, INCENP and Borealin but not Survivin, have been reported to be components of the nucleolar proteome (30). Aurora B may also play a role in nucleolar cycle, as it regulates the RNA-methyltransferase activity of NSUN2, a protein involved in nucleolar architecture and nucleic acid metabolism during mitosis (31).

Here we report the identification of a novel in vivo Aurora B substrate, MYBBP1A (32). MYBBP1A was originally identified as a protein interacting with the leucine zipper of c-myb (33). This protein has been reported to bind to a number of transcription factors, including AhR, PGC-1alpha, NF-kB, Prep1, and to regulate their activity (34-37). MYBBP1A is ubiquitously expressed and is a nuclear protein mainly localized to the nucleolus. It shares some homology with a yeast protein called POL5, reported to be an essential DNA polymerase in S. cerevisiae (38). POL5 localises to the nucleolus where it binds near the enhancer region of rRNA-encoding DNA repeating units; indeed, pol5 mutants indicate that it is involved in rRNA synthesis in the nucleolus rather than in chromosomal DNA replication (39). Despite the homology with POL5, a role for MYBBP1A in the nucleolus has not yet been described.

Here we show a new role of MYBBP1A in mitosis. It is an in vitro and in vivo substrate for Aurora B and we have identified the Aurora B-dependent phosphorylation site on MYBBP1A as Ser1303, which falls in a consensus sequence for Aurora kinases. This phosphorylation event is modulated in cells both by RNAi against Aurora B and by treatment with Danusertib (previously known as PHA-739358), a small molecule inhibitor of Aurora kinases presently under clinical development. Furthermore we have shown that MYBBP1A depletion leads to prolongation of mitosis and mitotic spindle defects, suggesting an essential role of MYBBP1A in the normal progression of mitosis.

**Experimental Procedures**

*Sample preparation*- RIPA buffer (150 mM NaCl, 1%NP40, 0.5% Na deoxycholate, 0.1% SDS, 50 mM Hapes pH 7.9) was used to extract proteins from cytoplasmic and nuclear pellets of asynchronous HeLa cells purchased from CILBiotech. The cytoplasmic (CE) and nuclear (NE) pellets were resuspended in cold RIPA buffer, gently agitated at 4°C for 45 min, then centrifuged at 15000xg to separate cellular debris, and aliquoted for use in further experiments. The insoluble nuclear RIPA fraction (DNA-bound) was resuspended in buffer containing 50 mM Hapes pH 7.9, 2% SDS, 1 mM DTT, 10 U/ml benzonase. After resuspension, the solution was sonicated on ice 4x10 seconds to break DNA, then centrifuged at 25500xg for 10 minutes in a refrigerated centrifuge to remove debris and the supernatant was aliquoted and frozen in a bath of ethanol and dry-ice. In this way, the DNA-bound proteins, such as histones, were extracted.

*In vitro kinase assay*- To in vitro phosphorylate cytoplasmic or nuclear fractions, 1µg/ul of protein extract was used with 0.2 pmoles/ul of Aurora A kinase or nuclear fractions, 1µg/ul of protein extract was used with 0.2 pmoles/ul of Aurora A kinase in a buffer containing 50 mM Hapes pH 7, 60 mM NaCl, 4% glycerol, 1 mM ATP, 10 mM MgCl2, 1 mM DTT, protease and phosphatase inhibitors (Roche). The reaction took place at 30°C for 30 minutes and the samples were used for electrophoresis and Western blot analysis soon afterwards.
was used in a kinase buffer containing 50 mM Hepes pH 7.0, 10 mM MgCl₂, 0.2 µg/µl BSA, 1mM DTT, 25 nM [³²P]-ATP phosphatase and protease inhibitors; the reaction took place at 30°C for 30 minutes. In order to render the reaction specific, a low concentration of radiolabeled ATP was used, following the Kestrel approach (40).

**Ion-exchange fractionation** - The asynchronous HeLa nuclear extract was dialysed against 50 mM MES pH 6.5. A monoS column was pre-equilibrated in the presence of 50 mM MES pH 6.5 and the nuclear extract was loaded onto the resin using an AKTA Explorer. The unbound fraction (UB) was collected. After the loading, 4 elution steps at different NaCl concentrations (0.1 M, 0.3 M, 0.5 M and 1M) were performed and the eluting proteins were collected. The UB and the 0.1, 0.3, 0.5 and 1M fractions were then dialysed against the same buffer (kinase buffer, excluding ATP and MgCl₂) and concentrated for use in further experiments.

**Cloning and expression** - The cDNA of MYBBP1A was purchased from Geneservice and the coding sequence was cloned using the Gateway® system (Invitrogen) into pGEX2Tg. BL21 Codon Plus E.coli cells were used to express the protein (induction with 0.2 mM IPTG, 21°C overnight). The recombinant protein was then purified on GSH-Sepharose and on-column cleavage with PreScission protease was performed. For the over-expression in mammalian cells, MYBBP1A coding sequence was cloned into the mammalian expression vector PCMV-Tag2A FLAG vector (Stratagene) in EcoR1-Xho1 sites. Mutagenesis was performed with Stratagene’s QuikChange mutagenesis kit.

**Cellular lysis and Immunoblotting** - Cells were lysed in SDS-Buffer (125 mM Tris-HCl pH 6.8, 2% SDS) and after sonication and boiling, total extracts of the indicated samples were loaded on SDS-PAGE pre-cast gels (Invitrogen) or on 3-8% tris-acetate gels and immunoblotted. The following antibodies have been used: anti-MYBBP1A (Zymed), anti-BAF155 and anti-CDK2 (Santa Cruz Biotechnology), anti-FLAG (Sigma), anti-Phospho-Histone H3 Ser10 (Millipore), anti-Aurora A, anti-Aurora B, anti-CyclinB1 and anti-MCM2 (BD Biosciences), anti-Histone H3 (Abcam). Anti-Phospho-MYBBP1A, here called anti-P-p160, was produced by Zymed, immunizing rabbits using the peptide KENRESLVVN. Immunoreactive signals were detected with SuperSignal West Pico Chemiluminescent Substrate (Pierce).

**Flow cytometry** - For FACS analysis, cells were harvested, resuspended in cold PBS, fixed with cold (-20 °C) 70% ethanol in PBS and stored at 4°C for at least 1h. Cells were then stained with propidium iodide (25 µg/ml) in PBS containing 50 µg/ml RNase A.

**Cell culture and treatments** - HeLa and U2OS cells (ECACC) were grown in E-MEM medium. Medium was supplemented with 10% FBS and 2 mM L-Glutamine and cells were cultured at 37°C in a humidified incubator in the presence of 5% CO₂. U2OS-YFP alpha tubulin cells were transfected with a YFP-alpha tubulin over-expressing vector (Clontech) and selected in G418 (400 µg/ml).

For experiments involving over-expression of MYBBP1A or the S1303 mutants, HeLa cells were transiently transfected for 24h with 5 µg of the different constructs using Lipofectamine-2000 reagent (Invitrogen). Calyculin A (Sigma) was added 30 min before cellular lysis at 10 nM. For shake-off experiments, Nocodazole (75 ng/ml) or Taxol (0.5 µM) was added to the cells for 18h then mitotic cells were harvested by shake-off and treated in the presence of the mitotic inhibitors with DMSO or Danusertib (PHA-739358) (1 µM) together with the proteasome inhibitor MG132 (10 µM).

Lambda phosphatase (NEB) (400U) was used directly on cellular lysates (40 µg) for 1h at 30°C. RNAi experiments were performed with Oligofectamine (Invitrogen) for 72h. Different oligos were used: MYBBP1A (DHARMACON smart pool (M-020341-00-0010)), BAF155-oligo1 (ggaatgctcctaccaataaa), BAF155-oligo2 (ttacggatgagaagtcaaa), Aurora A (cgacaaagcttgtcctta), Aurora B (cggggcacttacaatgga), Aurora C
(gatgtgagtttccactat), MCM2 oligo-A (cctacggtagaatgtagtga) and MCM2 oligo-B (gggtgctcagatcaacatc). As a negative control an oligo targeting the luciferase gene was used: Luc (cctacgggaatatcga). All oligos were used at 80 nM.

**In-Gel Tryptic Digestion**- Protein digestion was performed with trypsin (Fluka) using the Digest Pro system (Intavis, Koeln, Germany) following the standard protocol. The elution mixture was then dried down in a speed vacuum and redissolved in 50% acetonitrile/0.1% TFA for MALDI-MS analysis or in deionized water for nanoLC-MS/MS analysis.

**MALDI-MS**-Samples for matrix-assisted laser desorption ionization-time of flight (MALDI-ToF) analysis were prepared by spotting 0.5µl of peptide mixture with 0.5µl of α-cyano-4-hydroxycinnamic acid (10 mg/ml in 50:50 acetonitrile:water containing 0.1% TFA) and analyzed on a Voyager DE-PRO (Applied Biosystems, Warrington, UK). All spectra were collected in reflector mode using four peptides of known mass as external calibration standards.

**Nano HPLC-ESI-MSMS**- NanoLC-MSMS was performed with a low flow rate reverse phase HPLC equipped with a nano-flow splitter (Ultimate, Dionex-LC Packings) coupled on-line to a hybrid quadrupole-time of flight instrument (Q-Tof2, Micromass) equipped with a Z-spray source. Samples were injected onto a C18 precolumn (PepMap 0.3 x 5mm, LC Packings) and pre-concentrated and desalted with mobile phase A (95:5 water:acetonitrile (v/v), 0.05% formic acid). The separation of the peptides was then performed on a 75 µm I.D. x 150mm PepMap column (LC Packings) with a gradient from 5% to 60% mobile phase B (5:95 water:acetonitrile (v/v), 0.06% formic acid) in 58 min followed by a washing step at 60% B in 15 min at a flow rate of 0.2 µl/min. The MS acquisition was performed in survey scan mode where the Q-Tof selected doubly, triply, quadruply charged ions above an intensity threshold of 10 counts/second (CPS) for MS/MS analysis from each survey scan (auto function switching). The instrument performed the MS/MS analysis under user-defined parameters of collision energy.

For the subsequent protein identification, the .raw data files were converted into .pkl files that were matched in MASCOT searches of the NCBI database for mammalian sequences. Protein identifications were confirmed by manual MS/MS data analysis for at least one peptide per protein.

**Time lapse**- U2OS cells stably over-expressing YFP-alpha tubulin were seeded in 12 well plate. Images of cells were taken every 30 minutes using a Time-Lapse microscope (Cell Observer Zeiss) with a 32X objective equipped with a motorized stage (Zeiss). Images were collected using the Axio Vision software (Zeiss).

**Immunofluorescence**- Cells were fixed 24h after transfection with 4% formaldehyde. After permeabilization in PBS1X containing 0.2% BSA, 0.1% triton X-100 for 10 minutes, staining was performed by incubating cells with an anti-Flag antibody (Sigma) diluted 1:2000 in PBS 1X containing 2% BSA. After 1 h, slides were rinsed in PBS 1X and exposed to CY3 secondary antibody 1:500 (Amersham). DNA was visualized with DAPI (1:1000). Images were acquired with an Axiovert 100 microscope (Zeiss) with a 60X objective.

**RESULTS**

A kinase substrate search identifies a 160 kDa protein as a new Aurora substrate - In order to identify new substrates of Aurora kinases suitable for biomarker discovery, an in vitro kinase substrate search was performed. Aurora A was tested against a panel of proteins containing the reported Aurora consensus phosphorylation site (41) and it was shown to phosphorylate Serine 220 of Mini-Chromosome Maintenance 2 (MCM2) (L. Rusconi, P. Carpinelli, personal communication), which in fact resides in a consensus motif for Aurora Kinases (KENR ESLV VN). MCM2 is required for replication origin activation along with other MCM partners and with members of the pre-replication complex, including origin recognition complex (ORC), cell division...
cycle 6 (Cdc6), and chromatin licensing and DNA replication factor 1 (Cdt1) (42), and has not previously been identified as an Aurora substrate either in vitro or in vivo. Therefore, we decided to raise a phospho-specific antibody against MCM2 Ser 220 to investigate the existence of this phosphorylation event in vivo. As shown in Fig. 1A, the phospho-Ser 220 MCM2 antibody indeed detects a single band specifically in extracts from nocodazole-treated mitotic HeLa cell and not from asynchronous cells. However, in contrast with the expected molecular weight of MCM2 (110-120 kDa), the detected band was at 160 kDa with no other bands visible even after prolonged exposure (data not shown). These results indicate that the anti-phospho-Ser220 MCM2 antibody indeed recognizes a band in cell lysates, although at a molecular weight different from the expected one and that this signal peaks upon nocodazole treatment. To explain this difference in molecular weight we hypothesized that: 1) some heavy post-translational modification was increasing the molecular weight of MCM2 or altering its electrophoretic mobility or 2) the antibody raised against the phospho-site Ser220 MCM2 was cross-reacting with some other unrecognized protein. To rule out the second hypothesis, we performed RNAi experiments against MCM2 followed by nocodazole treatment and Western blot analysis using total-MCM2 and phospho-MCM2 antibodies. As shown in Fig. 1B, despite the complete silencing of MCM2 obtained with two different siRNA oligonucleotides (A and B), the antibody against phospho-Ser220 MCM2 still detected a band at 160 kDa in lysates from nocodazole arrested cells. This indicates that the phospho-MCM2 antibody is cross-reacting in cellular protein extracts with a protein different from MCM2. Therefore, we tried to identify if this protein (that we denoted p160), was an in vivo substrate for Aurora.

**Identification of p160 as MYBBP1A**

The first step in the identification process was to check if p160 was also present in asynchronous cell extract and, if so, to verify if this could be phosphorylated in vitro using Aurora kinases, yielding a phospho-p160 signal. This would allow us to work with protein extracts from asynchronous cells which are more manageable than nocodazole-treated cell extracts and easier to obtain in large amounts. HeLa cytoplasmic (CE), nuclear (NE) and DNA-bound protein extracts were prepared from asynchronous cells, heated at 60 °C for 10 min to inactivate endogenous kinases and used in an in vitro kinase assay using Aurora A kinase (43). The presence of phospho-p160 was assessed by Western blot analysis. As a positive control, an extract from nocodazole arrested HeLa cells was included. As shown in Fig. 2A, phospho-p160 was detected only in the nuclear fraction after phosphorylation with Aurora A kinase, indicating that p160 is a predominantly nuclear protein. Both in the cytoplasmic and in the nuclear fraction, a band at 120 kDa was visible after in vitro phosphorylation. This band may correspond to MCM2 suggesting that under these in vitro conditions the antibody is also able to recognize phospho Ser220 MCM2. Given that it was possible to follow p160 presence by in vitro phosphorylation of asynchronous cell extracts and Western blot analysis with the phospho-Ser 220 MCM2 antibody, from now on denoted phospho-p160, we decided to fractionate the HeLa nuclear extract using monoS ion-exchange chromatography in order to enrich a fraction with p160 and try to isolate it. Binding of the nuclear extract to monoS resin was performed at pH 6.5 in MES buffer in the absence of salt. Elution was performed in a stepwise mode and 5 different fractions were collected corresponding to the Unbound (UB), 0.1, 0.3, 0.5, 1M NaCl elutions steps. All these fractions were dialysed against the same final buffer, concentrated as described in Experimental Procedures, and then used in an in vitro kinase assay. Following in vitro phosphorylation with Aurora kinase, the phospho-p160 signal was only detected in the 1M NaCl fraction (Fig. 2B).

The HeLa nuclear extract, cytoplasmic extract and 1 M fraction were then submitted to electrophoresis in a 3-8% tris-acetate gel in order to enhance resolution and achieve a clearer detection in the high-molecular weight range. Looking at the differential bands, we could detect a faintly Coomassie stained band.
at the expected molecular weight present only in the nuclear extract (NE) and in 1M fractions (Fig. 2C). We excised this band and subjected it to trypsin digestion for MS identification. MALDI-ToF analysis of tryptic digests from 1 M and NE fraction revealed the presence of a high-score candidate named MYBBP1A (Table S1). The tryptic digest was also analyzed by LC-MS/MS, which allowed us to confirm MYBBP1A identification and also revealed the presence in both samples of a second putative p160 candidate, the SNF complex 155 kDa component BAF155 (Table S2).

To discriminate between the candidates and confirm the identity of p160, RNAi experiments were performed against MYBBP1A or BAF155 (Fig. 2D). HeLa cells were transfected separately with two different RNAi oligonucleotides specific for BAF155 or with a pool of 4 oligos against MYBBP1A. Cells were then treated with nocodazole and only mitotic cells were harvested and analyzed to enrich the p160 mitosis-specific signal. As reported in Fig. 2D, MYBBP1A depletion abrogates the phospho-p160 signal, whereas depletion of BAF155, similarly to control cells (Luc), does not affect p160 phosphorylation, suggesting that indeed the p160 phosphorylation signal is related to MYBBP1A expression. No modulation was observed for MCM2, Aurora A or B levels upon MYBBP1A or BAF155 RNAi.

To rule out the possibility that p160 reduction could be simply due to a secondary event caused by MYBBP1A knock-down, MYBBP1A was cloned and over-expressed in HeLa cells as a Flag-tagged protein. As reported in Fig. 2E, Western blot analysis on asynchronous cells using the phospho-p160 antibody detected a p160 band only in cells transfected with MYBBP1A, confirming that MYBBP1A is recognised by this antibody. Furthermore, the MYBBP1A band was more intense when HeLa cells were treated with the phosphatase inhibitor calyculin A (Fig. 2F) and conversely, treatment of cellular lysates with lambda phosphatase completely abrogates the signal (Fig. 2G). This indicates that anti-phospho-p160 specifically recognises phosphorylated MYBBP1A.

Identification of the Aurora-dependent phosphorylation site on MYBBP1A - To verify if Aurora kinase can actually phosphorylate MYBBP1A generating a phospho-p160 signal in cellular lysates, MYBBP1A-depleted HeLa cell extracts were used in an in vitro Aurora kinase assay in comparison with control cell lysates. As shown in Fig. 3A, while phospho-p160 was clearly visible in luciferase (Luc) or BAF155 depleted extracts, it could hardly be detected in MYBBP1A-depleted samples, confirming in cells that p160 is MYBBP1A and that this protein can be phosphorylated by Aurora when present in cell extracts.

We then decided to map the Aurora-dependent phosphorylation site(s) of MYBBP1A. MYBBP1A recombinant protein was expressed and purified as a GST-fusion protein from E. coli, followed by on column tag removal with PreScission protease (Fig. 3B). The purified protein showed a number of degradation products below 160 kDa (eluted fractions E1 and E2), and the majority of these bands could be assigned by MALDI-ToF analysis to MYBBP1A fragments, deriving mainly from C-ter processing (data not shown). Recombinant MYBBP1A was then used in an in vitro kinase assay with Aurora A or Aurora B kinases in the presence of ^32P-ATP (Fig. 3C). The main labelled band corresponded to the full-length MYBBP1A protein indicating that the main phosphorylation site resides in the very C-terminal portion of the protein and both Aurora A and B kinases are capable of phosphorylating full length MYBBP1A in vitro (Fig. 3C). The bands visible in the lower part of the gel correspond to Aurora A and Aurora B auto-phosphorylated proteins, indicating that both kinases were active in these conditions. Sequence analysis of MYBBP1A showed the presence of an Aurora kinase phosphorylation consensus sequence in the C-terminal portion containing a single phosphorylatable residue, Ser 1303. Furthermore, Ser 1303 in MYBBP1A resides in a sequence (KARL\textsubscript{S}LVIRS) that resembles the MCM2 sequence used to raise the phospho-p160 antibody, explaining the observed cross-reactivity.
To confirm that Ser1303 is phosphorylated in vivo, two different MYBBP1A mutants were generated at this site, (MYBBP1A S1303D and MYBBP1A S1303A) and expressed in HeLa cells. Anti-phospho-p160 was not able to detect any band when the over-expressed protein harboured a mutation on Ser1303 to alanine or aspartic acid (Fig. 3D), even in the presence of calyculin A treatment, thus confirming that MYBBP1A S1303 is an in vivo phospho site.

**MYBBP1A is phosphorylated in vivo by Aurora B**- MYBBP1A is phosphorylated by Aurora kinases in vitro and is highly phosphorylated in nocodazole-arrested mitotic cells. Since Aurora kinases are known to be key mitotic regulators, we wanted to ascertain whether MYBBP1A was also regulated by Aurora kinases in vivo. To do this, we utilized a pan-Aurora inhibitor, Danusertib which has shown preclinical and clinical efficacy (5,6). Nocodazole or taxol-arrested mitotic HeLa cells were treated with Danusertib (1 µM) for 4 hours and MYBBP1A phosphorylation status was then investigated. Both, nocodazole or taxol-arrested cells show up-regulation of MYBBP1A phosphorylation which is completely abolished upon Danusertib treatment (Fig. 4A, lanes 2 and 6). This was shown to be a specific effect since it was maintained in the presence of MG132, which keeps cells arrested in M phase, even upon Aurora inhibition, by blocking the proteasomal mediated degradation of securin, cyclin B1, Aurora A and Aurora B (44,45) (Fig. 4A, compare lanes 2 and 6 with lanes 3 and 7). Thus, the enzymatic activity of the Aurora kinases is required to maintain MYBBP1A phosphorylation.

To understand which Aurora kinase regulates MYBBP1A phosphorylation in vivo, we separately depleted the three Aurora kinases (A, B or C) in HeLa cells and measured MYBBP1A phosphorylation in mitotic cells following nocodazole treatment. As reported in Fig. 4B, only Aurora B depletion decreased MYBBP1A phosphorylation, whereas Aurora A or Aurora C RNAi had no effect. In addition, under these experimental conditions, no effects were observed on the expression of total MYBBP1A or on the mitotic marker cyclin B1 following Aurora kinases RNAi, excluding RNAi related cell cycle effects. These results indicate that Aurora B is the main regulator of MYBBP1A S1303 phosphorylation in vivo.

**MYBBP1A has a role in the mitotic phase of the cell cycle**-MYBBP1A was shown to be phosphorylated by Aurora B, one of the main mitotic kinases required for proper mitotic progression (46), and MYBBP1A is phosphorylated in mitotic cells after nocodazole or taxol treatment. For these reasons, we decided to investigate the role of MYBBP1A in the mitotic phase of the cell cycle. MYBBP1A RNAi experiments were conducted in both HeLa and U2OS cells. As shown in Fig. 5, MYBBP1A is required for proper cellular proliferation: 72h after transfection, 75% MYBBP1A depletion (Fig. 5A) causes a 40% reduction of total cell number in both treated cell lines (Fig. 5B and D). Moreover, FACS profiles and phase contrast images indicate an enrichment in the G2/M phase of the cell cycle (Fig. 5C and D) and a detailed analysis by time-lapse videomicroscopy of U2OS cells over-expressing YFP-alpha tubulin shows that the absence of MYBBP1A causes a mitotic phase prolongation and defects in mitotic spindle assembly and stability (Fig. 5E), which ultimately leads to cell death.

Finally, immunofluorescence experiments performed in HeLa cells transfected with a Flag-tagged version of wild-type MYBBP1A confirmed its nucleolar localization in interphase cells and also showed a perichromosomal localization in mitotic cells from prophase to anaphase (Fig. 5F). This confirms a novel role of MYBBP1A in mitosis, which could be dependent on Aurora B-mediated phosphorylation.

**DISCUSSION**

Here we have identified and characterized human MYBBP1A (UniProt entry Q9BQG0) as a novel in vivo substrate of the Aurora B kinase. Orthologous genes for MYBBP1A sharing a high degree of similarity (approximately 80%) are present in rat and...
mouse (UniProt entries Q7TPV4 and O35821, respectively). Protein homologues have also been recognized in dog, bovine, and chicken and a MYBBP1A-like protein spanning 1269 residues and showing a 60% similarity to the human protein has been identified in zebrafish, suggesting that MYBBP1A is significantly conserved across vertebrate species (data not shown) (47). In addition, Ser1303, the residue that we have identified as an Aurora B dependent phospho-site, is conserved together with the surrounding Aurora consensus sequence, in rodents, dog and bovine (data not shown).

MYBBP1A was originally identified as a protein able to interact with the negative regulatory domain (NRD) of c-Myb, however, it was later shown to lack any significant effect in a Myb-dependent transcription reporter assay (32,33). MYBBP1A was shown to localize mainly in the nucleolus (32) where Myb is not detected (48). In addition, whereas c-Myb expression is tissue specific, being essential for hematopoietic cell proliferation and differentiation (49,50), MYBBP1A appears to be ubiquitously expressed (32). Taken together, these observations suggest that MYBBP1A has other functions independent from Myb. In fact, in studies from a number of different groups, MYBBP1A has been found to interact with and regulate several transcription factors: it binds and represses both Prep1-Pbx1, involved in development and organogenesis, and also PGC-1a, a key regulator of metabolic processes such as mitochondrial biogenesis and respiration and gluconeogenesis in liver (34,35). MYBBP1A acts as a co-repressor for RelA/p65, a member of the NFκB family, by competing with the co-activator p300 histone acetyltransferase for interaction with the transcription activation domain (TAD) of RelA/p65. It is also a co-repressor on the Period2 promoter, repressing the expression of Per2, an essential gene in the regulation of the circadian clock (37,51). Conversely, MYBBP1A is a positive regulator of the aromatic hydrocarbon receptor (AhR) which mediates transcriptional responses to certain hydrophobic ligands, such as dioxin, by enhancing the ability of AhR to activate transcription (36).

MYBBP1A has been confirmed as a resident protein of the nucleolus by three large-scale proteomic studies that have established a protein inventory of this sub-nuclear compartment (30,52,53). The nucleolus is a specialized nuclear domain and the site of ribosome biogenesis. It is a dynamic structure that disassembles at the beginning of mitosis when transcription shuts down and re-assembles at the end of mitosis/beginning of G1 around the ribosomal DNA (rDNA) genes, where the transcription and processing machineries responsible for ribosome biogenesis concentrate (54).

Although long dubbed “an organelle formed by the act of building a ribosome” (55), the nucleolus has now been shown to contain many proteins unrelated to ribosome biogenesis, including proteins involved in DNA replication and repair, telomere maintenance, protein degradation and cell-cycle regulation, supporting the hypothesis that this organelle fulfills several additional functions (54).

Lines of evidence for this hypothesis come from specific nucleolar proteins which make a connection between the nucleolus and the chromosomal periphery, such as Nucleolin and Rrs1. Human Nucleolin is one of the most extensively studied nucleolar proteins and has been shown to play essential roles in rDNA transcription and maturation, ribosome assembly and nucleo-cytoplasmic transport (56,57). During mitosis, Nucleolin is localised to the chromosome periphery, including the vicinity of the outer kinetochore of chromosomes, and Nucleolin depleted cells show disorganization of nucleoli in interphase and delayed cell cycle progression, mainly due to a delay at prometaphase with misaligned or non-aligned chromosomes and defects in chromosome biorientation (58). Rrs1 is a protein required for ribosome biogenesis in yeast (59). Its human orthologue localizes to nucleoli in interphase and at the chromosomal periphery from prometaphase to anaphase (60). Rrs1 depletion by RNAi results in defects in chromosome congression with
mitotic cells showing aberrant chromosome alignment at the metaphase plate (60). The chromosome periphery might then act as a means of transport for various proteins, including nucleolar proteins (61). Alternatively, co-localization and storage at the nucleolus might provide a regulatory mechanism for the assembly of specific nucleo-protein complexes and their timely release during mitosis, as suggested by Wong et al. (62). The kinetochore proteins CENPC1 and INCENP have indeed been shown to accumulate at the nucleolus in interphase in a centromeric α-satellite RNA-dependent manner. Aurora B has also been detected in purified nucleoli, together with its CPC partners INCENP and Borealin (30). Of additional relevance is the observation that Aurora B has been shown to regulate the nucleolar RNA methyltransferase NSUN2 in mitosis, through the inactivating phosphorylation of Ser139 (31). This phosphorylation event drives the dissociation of NSUN2 from its nucleolar partner NPM1, suggesting that Aurora B is a mitotic kinase that participates in regulating the assembly cycle of nucleolar RNA-processing machinery. Indeed, recent literature data show that NSUN2 is required for proper spindle assembly and chromosome segregation by regulating the localization of NuSAP, an essential spindle assembly factor; NSUN2 has been furthermore shown to be overexpressed in breast cancer (66,69).

Here we have shown that another nucleolar protein, MYBBP1A, is phosphorylated by the mitotic kinase Aurora B on a site that has been mapped to Serine 1303 (60). We have found that this phosphorylation event peaks in G2/M, upon treatment with mitotic poisons, and is completely abolished when cells are exposed to the Aurora inhibitor Danusertib. Moreover, only Aurora B silencing is effective in suppressing MYBBP1A Ser1303 phosphorylation, indicating that Aurora B, and not A or C, is responsible for this phosphorylation event in vivo.

MYBBP1A is reported to be a heavily phosphorylated protein in cells, according to several recent large-scale mass spectrometry-based phosphoproteomic studies (65-72). The majority of the phosphosites mapped in MYBBP1A in these studies (18 out of a total of 21) reside within the ~200 amino-acid long C-terminal portion of the protein, which has been shown to be relevant for its nuclear and nucleolar localization (73). Notably, MYBBP1A was also found to be also a component of the proteome as well as the phospho-proteome of the human mitotic spindle (71,74), pointing to an additional co-localization where Aurora B kinase may encounter and phosphorylate MYBBP1A. Indeed, the level of phosphorylation of a number of sites in MYBBP1A appear to increase massively in mitotic cells (66,69), including phosphorylation at Ser1303 (69) which is the site that we have shown to be Aurora B-dependent in cell lines, further confirming Ser1303 as an in vivo phospho-site for MYBBP1A. However, we have been unable to detect any difference in MYBBP1A cellular localization or transcriptional activity using GAL4-dependent or NFκB reporter assays in the presence of Danusertib or upon testing Ser1303 mutants (data not shown). Instead, MYBBP1A depletion by RNAi causes a delay in progression through mitosis and defects in mitotic spindle assembly and stability, indicating that, like other nucleolar proteins, MYBBP1A may have a role in insuring correct mitotic progression. This theory is supported by our immunofluorescence studies, which have shown a peri-chromosomal localization of MYBBP1A from prophase to metaphase. Taken together, our results indicate that MYBBP1A may be part of a nucleolar pool of proteins playing a role in mitotic progression. Further studies are needed to clarify the interplay between nucleolar and mitotic functions of MYBBP1A and also to understand the contribution of Aurora B to the regulation of MYBBP1A in mitosis and the relevance of Ser1303 phosphorylation.
REFERENCES

1. Kollareddy, M., Dzubak, P., Zheleva, D., and Hajduch, M. (2008) Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 152, 27-33
2. Vader, G., and Lens, S. M. (2008) Biochim Biophys Acta 1786, 60-72
3. Katayama, H., Brinkley, W. R., and Sen, S. (2003) Cancer Metastasis Rev 22, 451-464
4. Zhou, H., Kuang, J., Zhong, L., Kuo, W. L., Gray, J. W., Sahin, A., Brinkley, B. R., and Sen, S. (1998) Nat Genet 20, 189-193
5. Carpinelli, P., and Moll, J. (2009) Curr Opin Drug Discov Devel 12, 533-542
6. Pollard, J. R., and Mortimore, M. (2009) J Med Chem 52, 2629-2651
7. Dutertre, S., Cazales, M., Quaranta, M., Froment, C., Trabut, V., Dozier, C., Mirey, G., Bouche, J. P., Theis-Feuvre, N., Schmitt, E., Monsarrat, B., Prigent, C., and Ducommun, B. (2004) J Cell Sci 117, 2523-2531
8. Eyers, P. A., Erikson, E., Chen, L. G., and Maller, J. L. (2003) Curr Biol 13, 691-697
9. Giet, R., Uzbekov, R., Cubizolles, F., Le Guellec, K., and Prigent, C. (1999) J Biol Chem 274, 15005-15013
10. Hirota, T., Kunitoku, N., Sasayama, T., Zhang, D., Nitta, M., Hatakeyama, K., and Sen, S. (2003) Cell 114, 585-598
11. Liu, Q., Kaneko, S., Yang, L., Feldman, R. I., Nicosia, S. V., Chen, J., and Cheng, J. Q. (2004) J Biol Chem 279, 52175-52182
12. Ouchi, M., Fujiuchi, N., Sasai, K., Katayama, H., Minamishima, Y. A., Ongusaha, P. P., Deng, C., Sen, S., Lee, S. W., and Ouchi, T. (2004) J Biol Chem 279, 19643-19648
13. Venoux, M., Bambous, J., Berthenet, C., Prigent, C., Fernandez, A., Lamb, N. J., and Rouquier, S. (2008) Hum Mol Genet 17, 215-224
14. Bishop, J. D., and Schumacher, J. M. (2002) J Biol Chem 277, 27577-27580
15. Brittle, A. L., Nanba, Y., Ito, T., and Okhura, H. (2007) Exp Cell Res 313, 2780-2785
16. Crosio, C., Fimia, G. M., Loury, R., Kimura, M., Okano, Y., Zhou, H., Sen, S., Allis, C. D., and Sassone-Corsi, P. (2002) Mol Cell Biol 22, 874-885
17. Knowlton, A. L., Lan, W., and Stukenberg, P. T. (2006) Curr Biol 16, 1705-1710
18. Morrison, C., Henzing, A. J., Jensen, O. N., Osheroff, N., Dodson, H., Kandels-Lewis, S. E., Adams, R. R., and Earnshaw, W. C. (2002) Nucleic Acids Res 30, 5318-5327
19. Speliotes, E. K., Uren, A., Vaux, D., and Horvitz, H. R. (2000) Mol Cell 6, 211-223
20. Zeitlin, S. G., Shelby, R. D., and Sullivan, K. F. (2001) J Cell Biol 155, 1147-1157
21. Adams, R. R., Wheatley, S. P., Gouldsworth, A. M., Kandels-Lewis, S. E., Carmen, M., Smythe, C., Gerloff, D. L., and Earnshaw, W. C. (2000) Curr Biol 10, 1075-1078
22. Ruchaud, S., Carmen, M., and Earnshaw, W. C. (2007) Nat Rev Mol Cell Biol 8, 798-812
23. Adams, R. R., Wheatley, S. P., Gouldsworth, A. M., Kandels-Lewis, S. E., Carmen, M., Smythe, C., Gerloff, D. L., and Earnshaw, W. C. (2000) Curr Biol 10, 1075-1078
24. Ruchaud, S., Carmen, M., and Earnshaw, W. C. (2007) Nat Rev Mol Cell Biol 8, 798-812
25. Qatar, S., Kand, A., Suzuki, F., Sato, S., Takata, T., and Tatsuka, M. (2007) Mol Biol Cell 18, 1107-1117
26. Tavner, F. J., Simpson, R., Tashiro, S., Favier, D., Jenkins, N. A., Gilbert, D. J., Copeland, N. G., Macmillan, E. M., Lutwyche, J., Keough, R. A., Ishii, S., and Gonda, T. J. (1998) Mol Cell Biol 18, 989-1002
27. Favier, D., and Gonda, T. J. (1994) Oncogene 9, 305-311
28. Diaz, V. M., Mori, S., Longobardi, E., Menendez, G., Ferrai, C., Keough, R. A., Bach, A., and Blasi, F. (2007) Mol Cell Biol 27, 7981-7990
35. Fan, M., Rhee, J., St-Pierre, J., Handschin, C., Puigserver, P., Lin, J., Jaeger, S., Erdjument-Bromage, H., Tempst, P., and Spiegelman, B. M. (2004) *Genes Dev* **18**, 278-289
36. Jones, L. C., Okino, S. T., Gonda, T. J., and Whitlock, J. P., Jr. (2002) *J Biol Chem* **277**, 2215-22519
37. Owen, H. R., Elser, M., Cheung, E., Gersbach, M., Kraus, W. L., and Hottiger, M. O. (2007) *Proc Natl Acad Sci U S A* **99**, 9133-9138
38. Shimizu, K., Kawasaki, Y., Hiraga, S., Tawaramoto, M., Nakashima, N., and Sugino, A. (2002) *Proc Natl Acad Sci U S A* **99**, 9133-9138
39. Knebel, A., Morrice, N., and Cohen, P. (2001) *EMBO J* **20**, 4360-4369
40. Cheeseman, I. M., Anderson, S., Jwa, M., Green, E. M., Kang, J., Yates, J. R., 3rd, Chan, C. S., Drubin, D. G., and Cohen, P. (2002) *Cell* **111**, 163-172
41. Costa, A., and Onesti, S. (2008) *Biochem Soc Trans* **36**, 136-140
42. Boisvert, F. M., van Koningsbruggen, S., Navascues, J., and Lamond, A. I. (2007) *Nat Rev Mol Cell Biol* **8**, 574-585
43. Beausoleil, S. A., Jedrychowski, M., Schwartz, D., Elias, J. E., Villen, J., Li, J., Cohn, M. A., Cantley, L. C., and Gygi, S. P. (2004) *Proc Natl Acad Sci U S A* **101**, 12130-12135
44. Beausoleil, S. A., Villen, J., Gerber, S. A., Rush, J., and Gygi, S. P. (2006) *Nat Biotechnol* **24**, 1285-1292
45. Cantin, G. T., Yi, W., Lu, B., Park, S. K., Xu, T., Lee, J. D., and Yates, J. R., 3rd. (2008) *J Proteome Res* **7**, 1346-1351
We would like to thank Vanessa Marchesi, Francesco Sola, Rosario Baldi, Mauro Uggeri.

The abbreviations used here are: MYBBP1A, Myb-binding protein 1A; MOA, mechanism of action; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; RNAi, RNA interference.

**FIGURE LEGENDS**

**Fig. 1.** Anti-phospho MCM2 antibody detects a 160 KDa protein in nocodazole extracts independently from MCM2 presence. A, asynchronous and nocodazole-treated (75 ng/ml) HeLa cell lysates were loaded in a 4-12% SDS-PAGE gel and then immunoblotted (IB) with anti-phospho MCM2 antibody. A 160 KDa band was detected only in lysates from nocodazole-treated cells. Alpha tubulin antibody was used as a loading control. B, MCM2 RNAi does not abrogate p160 signal. HeLa cells were transfected with two different RNAi oligos specific for MCM2 (MCM2(A) and MCM2(B)) and, as negative controls cells were treated with an oligonucleotide specific for the luciferase gene (Luc) or with transfection reagent alone for 66 h. Nocodazole (75 ng/ml) was added for the last 14h and total lysates were probed with total MCM2, anti-phospho MCM2 (p160) and alpha tubulin antibodies.

**Fig. 2.** Identification of p160 as MYBBP1A. A, Aurora A phosphorylates p160 in nuclear extracts. Asynchronous HeLa cytoplasmic (CE), nuclear (NE) and DNA-bound cellular extracts were incubated with Aurora A in a kinase assay. As a control, a sample from nocodazole-treated HeLa cells was loaded. To verify the presence of Aurora A and MCM2, immunoblottting with anti-Aurora A and MCM2 antibodies was performed and also with anti-phospho-histone H3 Ser10 antibody to verify the quality of the DNA-bound extract. B, p160 signal appears only in 1M NaCl elution fraction of HeLa nuclear extracts. After fractionation and elution with different salt concentrations, unbound (UB) or 0.1, 0.3, 0.5, 1M NaCl elution fractions were incubated with Aurora A kinase and p160 signal was followed with anti-phospho-p160 antibody. As a control, unfractionated nuclear extract (NE) phosphorylated with Aurora A kinase was included. C, coomassie blu staining of cytoplasmic (CE), nuclear (NE) and 1M NaCl (1M) fraction. P160 protein is indicated. D, HeLa cells were transfected with two different RNAi oligos for BAF155 (1 and 2) or a pool of 4 different oligos for MYBBP1A. The luciferase gene RNAi-oligo (Luc) was used as a negative control. Cells were lysed after 72h. Nocodazole treatment was performed for the last 18 h and then cells were harvested and subjected to mitotic shake-off before total lysis. Immunoblot (IB) with anti-MYBBP1A, BAF155, MCM2, Aurora A, Aurora B, alpha tubulin (loading control) and anti-phospho p160 antibodies are shown. E, anti-phospho-p160 detects over-expressed MYBBP1A protein. HeLa cells were transfected with a Flag-tagged...
version of MYBBP1A or the empty vector and lysates were loaded and analyzed with anti-phospho p160, anti-Flag or anti-alpha tubulin (loading control) antibodies. F-G, the anti-phospho-p160 specifically detects phosphorylated MYBBP1A. Empty vector or Flag-MYBBP1A transfected HeLa cells were treated with 10 nM Calyculin A for 1h before lysis and samples were probed against anti-phospho p160, Flag, AuroraA, Aurora B or phospho-histone H3 Ser10 antibodies. F, Flag-MYBBP1A transfected HeLa cell lysates were treated with 400 Units of lambda phosphatase for 1h at 30°C and, following SDS-PAGE gel electrophoresis and western blottings, were probed with anti-phospho-p160 or anti-Flag antibodies. Ponceau S staining shows the presence of lambda phosphatase.

Fig. 3. **MYBBP1A is phosphorylated on Ser1303 by Aurora kinase.** A, Aurora A specifically phosphorylates MYBBP1A. HeLa cytoplasmic (CE) or nuclear extracts (NE) together with Luciferase (Luc), MYBBP1A or BAF155 (oligo2) RNAi oligo transfected samples, were incubated with Aurora A kinase in a kinase assay. Nocodazole treated HeLa cell lysates were loaded as a control for the phospho-p160 signal detected by immunoblot (IB). B, MYBBP1A recombinant protein was expressed and purified from E. coli. Pellet (P), Input (I), Flow-through (FT), Resin before elution (R), Wash (W), and two different elution steps after PreScission Protease cleavage (E1 and E2 fractions) are shown by Coomassie blue staining. In E1 and E2 eluates, full length MYBBP1A at 160 KDa (MW) and C-terminal degradation products are indicated. C, recombinant Aurora A and Aurora B kinases phosphorylate MYBBP1A full-length protein. Different amounts of MYBBP1A were added in a kinase assay experiment in the presence of 150 nM of Aurora A or Aurora B kinases. MYBBP1A Aurora A-dependent phosphorylation or Aurora kinases auto-phosphorylation were detected by autoradiography. D, Ser1303 phosphorylation was detected using the anti-phospho p160 antibody. HeLa cells were transfected with an empty vector (EV) or Flag-tagged versions of full-length wild type MYBBP1A or the two mutants S1303A or S1303D. 10 nM Calyculin A treatment was performed 1h before cellular lysis in the indicated samples. Lysates were loaded on a SDS-PAGE gel and immunoblotted (IB) with anti-phospho-p160, Flag or phospho-Histone H3 Ser10 antibodies to verify the phospho-p160 signal, transfection and Calyculin A treatment efficiency, respectively.

Fig. 4. **MYBBP1A is phosphorylated *in vivo* by Aurora B.** A, inhibition of Aurora kinases upon Danusertib treatment inhibits MYBBP1A Ser1303 phosphorylation. HeLa cells were treated with nocodazole (75 ng/ml) or taxol (0.5 µM) for 16h. Mitotic shake-off was performed and mitotic cells were replated in the presence of nocodazole or taxol together with Danusertib (1 µM) alone or in combination with MG132 (10 µM) for 4h before cellular lysis. Lysates were immunoblotted (IB) with anti-phospho p160, total MYBBP1A, phospho-histone H3 Ser10, Aurora A, Aurora B, histone H3 and Cyclin B1 antibodies, as indicated. B, Aurora B regulates MYBBP1A phosphorylation. RNAi experiments with Luciferase (Luc), Aurora A, Aurora B or Aurora C specific oligos in HeLa cells was performed. After 24h, nocodazole (75 ng/ml) was added for 15h. Mitotic cells were then harvested by mitotic shake-off and lysed. Anti-phospho p160, total MYBBP1A, Aurora A, Aurora B, Cyclin B1, alpha tubulin and histone H3 were analyzed by immunoblot (IB), as indicated. Aurora C mRNA knock-down efficiency was over 75% (data not shown).

Fig. 5. **MYBBP1A is required for correct mitotic progression and cellular proliferation.** A-D, HeLa and U2OS cells were transfected with MYBBP1A or Luciferase (Luc) specific RNAi oligos. After 72h, MYBBP1A protein levels (A), cellular count (B) and FACS profile analysis of G2/M population (C) were analyzed. In D, representative images of HeLa and U2OS cells are shown 72h after oligo transfection at 10X or 32X magnification. Scale bars equal to 50 µm are indicated. E, Asynchronous U2OS cells over-expressing a YFP-tagged version of alpha tubulin were followed by time lapse videomicroscopy 24h after MYBBP1A or Luc RNAi
experiments. Both bright-field and YFP-dependent fluorescent signals of a representative mitotic cell are shown. Images were taken every 30 minutes as indicated and scale bars equal to 10 µm are shown. The graph shows the average duration of mitosis on 25 luciferase (Luc) and 20 MYBBP1A-depleted cells. F, MYBBP1A has a peri-chromosomal localization in mitotic cells. Flag-MYBBP1A transfected Hela cells were analyzed by immunofluorescence 24 h after transfection. Representative images of cells in the different mitotic phases are shown. DNA was visualized by DAPI and MYBBP1A by anti-Flag antibody staining. Scale bars equal to 5 µm are shown.
Figure 1

A

IB: Anti-P-MCM2 (p160)  
IB: Anti-α-Tubulin

B

IB: Anti-P-MCM2 (p160)  
IB: Anti-MCM2  
IB: Anti-α-Tubulin
Figure 2

A

| Noc. | CE | NE | DNA Bound |
|------|----|----|-----------|
|      |    |    |           |

Phospho-p160
pS220-MCM2
IB: Anti-P-p160
IB: Anti-MCM2
IB: Anti-AurA
IB: Anti-AurA
IB: Anti-P-H3 S10

B

| +AurA |
|-------|
| UB    |
| 0.1   |
| 0.3   |
| 0.5   |
| 1M    |
| NE    |

IB: Anti-P-p160

C

| MW | CE | NE | 1M |
|----|----|----|----|
|    |    |    |    |

250kDa — 160kDa — 105kDa — 75kDa —
Coomassie

D

| RNAi |
|------|
| Luc  |
| Luc  |
| BAF155 (1) |
| BAF155 (2) |
| MYBBP1A |

IB: Anti-P-p160
IB: Anti-MYBBP1A
IB: Anti-BAF155
IB: Anti-MCM2
IB: Anti-AurA
IB: Anti- AurB
IB: Anti-αTubulin

E

| FL-MybBP1A |
| FL-MybBP1A |
| Empty Vector |
| Empty Vector |

IB: Anti-P-p160
IB: Anti-Flag
IB: Anti-αTubulin

F

| Calyc. A |
|         |
|         |

IB: Anti-Flag
IB: Anti-P-p160
IB: Anti-AurA
IB: Anti-AurB
IB: Anti-P-H3 S10

G

| λ-Phosphatase |
|               |
|               |

IB: Anti-P-p160
IB: Anti-Flag
Ponceau S
Figure 3

A

RNAi

CE NE Luc MYBBP1A BAF155 (2) Noc.

AurA + + + + -

250kDa —

160kDa —

105kDa —

IB: Anti-P-p160

Phospho-p160

B

MYBBP1A

C-ter degradation products

Coomassie

C

AurA 150 nM  AurB 150 nM

MYBBP1A µg

- 0.13 0.6 - 0.13 0.6

160kDa —

110kDa —

75kDa —

50kDa —

Autoradiography

D

MYBBP1A

Calyc. A

- + - + - + - +

IB: Anti-P-p160

IB. Anti-Flag

IB. Anti-P-H3 S10
**Figure 4**

### A

|       | + Nocodazole | + Taxol |
|-------|--------------|---------|
| DMSO  | +            | -       |
| Danusertib | -   | +       |
| MG132 | -            | +       |

![IB: Anti-P-p160](image)

![IB: Anti-MYBBP1A](image)

![IB: Anti-P-H3 S10](image)

![IB: Anti-H3](image)

![IB: Anti-AurA](image)

![IB: Anti-AurB](image)

![IB: Anti-CycB1](image)

### B

| RNAi  | + Noc. |
|-------|--------|
| Luc   |        |
| Aur-A |        |
| Aur-B |        |
| Aur-C |        |

![IB: Anti-P-p160](image)

![IB: Anti-MYBBP1A](image)

![IB: Anti-AurA](image)

![IB: Anti-AurB](image)

![IB: Anti-CycB1](image)

![IB: Anti-αTubulin](image)

![IB: Anti-H3](image)
Figure 5

A

Hela

U2OS

MYBBP1A protein levels %

B

Cells number %

C

Cells in G2/M %

RNAi 72h

Luc

MYBBP1A

Luc

MYBBP1A

D

HeLa RNAi 72h

U2OS RNAi 72h

Luc

MYBBP1A

Luc

MYBBP1A

10X

32X
Figure 5

E

RNAi Luc

RNAi MYBBP1A

Mitotic length

0 50 100 150 200 250 300 350 400

RNAi Luc MYBBP1A

00:00 00:30 01:00 01:30 02:00

00:00 00:30 01:00 01:30 02:00 02:30 03:00 03:30 04:00 04:30

F

DNA

MYBBP1A

Interphase Prophase Metaphase Anaphase Telophase
Identification of MYB-binding protein 1A (MYBBP1A) as a novel substrate for aurora B kinase
Claudia Perrera, Riccardo Colombo, Barbara Valsasina, Patrizia Carpinelli, Sonia Troiani, Michele Modugno, Laura Gianellini, Paolo Cappella, Antonella Isacchi, Jurgen Moll and Luisa Rusconi

*J. Biol. Chem.* published online February 22, 2010

Access the most updated version of this article at doi: 10.1074/jbc.M109.068312

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2010/02/22/M109.068312.DC1