

The Role of NEDD1 Phosphorylation by Aurora A in Chromosomal Microtubule Nucleation and Spindle Function

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Summary

Chromatin directs de novo microtubule (MT) nucleation in dividing cells by generating a gradient of GTP-bound Ran protein (RanGTP) that controls the activity of a number of spindle assembly factors (SAFs) [1]. It is now well established that these MTs are essential for the assembly of a functional bipolar spindle [2]. Although it has been shown that RanGTP-dependent MT nucleation requires γ -tubulin and a number of RanGTP-regulated proteins [3, 4], the mechanism involved is still poorly understood. We previously showed that the mitotic kinase Aurora A, which is activated in a RanGTP-dependent manner in mitotic cells, has a role in this pathway [5]. Here we show that Aurora A interacts with and phosphorylates the γ TURC adaptor protein NEDD1 at a single residue, Ser405. Ser405 phosphorylation is not required for centrosomal MT nucleation but is critical for MT nucleation in the vicinity of the chromosomes in mitotic cells. Moreover, it is essential for RanGTP aster formation and chromatin-driven MT assembly in *Xenopus* egg extracts. Our data suggest that one important function of Aurora A in mitotic cells is to promote MT nucleation around the chromatin by phosphorylating NEDD1, and thereby to promote functional spindle assembly.

Results

Aurora A Interacts with the γ TURC Adaptor Protein NEDD1

After nuclear envelope breakdown, chromosomes direct de novo microtubule (MT) assembly by generating a gradient of GTP-bound Ran protein (RanGTP) that promotes the release of various spindle assembly factors (SAFs) [1]. Very little is currently known about the mechanism that triggers MT nucleation through this pathway, but it involves basic components of the MT nucleation machinery like γ -tubulin [3] as well as the RanGTP-regulated protein TPX2 [4, 6] and the chromosomal passenger complex (CPC) [7, 8]. We previously showed that TPX2 activates the kinase Aurora A in a RanGTP-dependent manner [9] and in turn that Aurora A activity is required for RanGTP-dependent MT aster formation in *Xenopus* egg extracts [5]. Aurora A activity was also shown to be essential for kinetochore/chromatin-dependent MT assembly in human cells [7]. Recently, using a bioinformatics approach, we proposed that the γ TURC adaptor protein NEDD1 could be one of its substrates [10], suggesting a direct link between Aurora A and the MT nucleation machinery.

To investigate this possibility, we first examined whether Aurora A and NEDD1 interact in egg extract and mitotic HeLa

cells. Western blot analysis showed that Aurora A was coimmunoprecipitated by anti-NEDD1 antibodies both in cytosstatic factor (CSF)-arrested *Xenopus* egg extracts and in mitotic HeLa cell lysates (Figure 1A). Consistently, anti-Aurora A antibodies coimmunoprecipitated NEDD1 from egg extracts (Figure 1B). We conclude that Aurora A interacts with the γ TURC adaptor protein NEDD1 during M phase.

To confirm these data and map the domain (or domains) of NEDD1 involved in the interaction with Aurora A, we performed pull-down experiments in *Xenopus* egg extract using glutathione S-transferase (GST) fusion proteins corresponding to the N-terminal WD40 domain of NEDD1 (xNEDD1-NT) and the complementary C-terminal domain (xNEDD1-CT) (Figure 1D; see also Figure S1A available online). Western blot analysis showed that Aurora A was pulled down by GST-xNEDD1-CT, but not by GST-xNEDD1-NT or GST (Figure 1D). In vitro pull-down experiments using His-GFP-tagged xAurora A and the different GST-tagged NEDD1 fragments showed that there is a direct interaction between the C-terminal domain of NEDD1 and Aurora A (Figure S1B).

Double immunofluorescence analysis in mitotic HeLa cells showed that Aurora A and NEDD1 colocalized to the centrosomes and the spindle poles in metaphase (Figure 1C). Immunofluorescence studies in Aurora A- or NEDD1-silenced cells showed that they do not depend on each other for their localization (Figures S1C–S1E).

Altogether, we conclude that NEDD1 is a novel binding partner of Aurora A. It interacts directly with Aurora A through its C-terminal non-WD40 domain. Although the two proteins colocalize during mitosis, they are independently targeted to the centrosomes and spindle poles.

Aurora A Phosphorylates NEDD1 at Ser405

To determine whether Aurora A phosphorylates NEDD1 in vitro, we incubated Aurora A with each of the two NEDD1 fragments fused to GST (Figures S1F and S1G) in the presence of [³²P]ATP. Autoradiography showed that Aurora A efficiently phosphorylated GST-NEDD1-CT, but not GST-NEDD1-NT, for both the *Xenopus* and the human orthologs (Figures S1F and S1G). Mass spectrometry was then used to identify the Aurora A-dependent phosphorylated residue(s) in GST-xNEDD1-CT. A phosphopeptide containing Ser405 that is located in a consensus sequence for Aurora A phosphorylation [R/K]-X-[S/T]-[I/L/V] was identified [11] (Figure 1E). To confirm that NEDD1 Ser405 is the major site phosphorylated by Aurora A, we used site-directed mutagenesis to produce GST-NEDD1-CT S405A in which Ser405 was substituted by an alanine. In vitro kinase assays showed that this single amino acid substitution was sufficient to completely abolish the incorporation of ³²P upon incubation with xAurora A (Figure 1F). Altogether, this shows that Aurora A phosphorylates NEDD1 at Ser405 in vitro.

To determine whether Ser405 is phosphorylated during M phase in cells, we expressed Flag-hNEDD1 in HeLa cells and pulled down the protein from cells synchronized in mitosis. Mass spectrometry analysis of the hyperphosphorylated form of Flag-hNEDD1 identified a phosphorylated peptide containing Ser405 (Figure S2). Consistently, mass spectrometry analysis of xNEDD1 immunoprecipitated from

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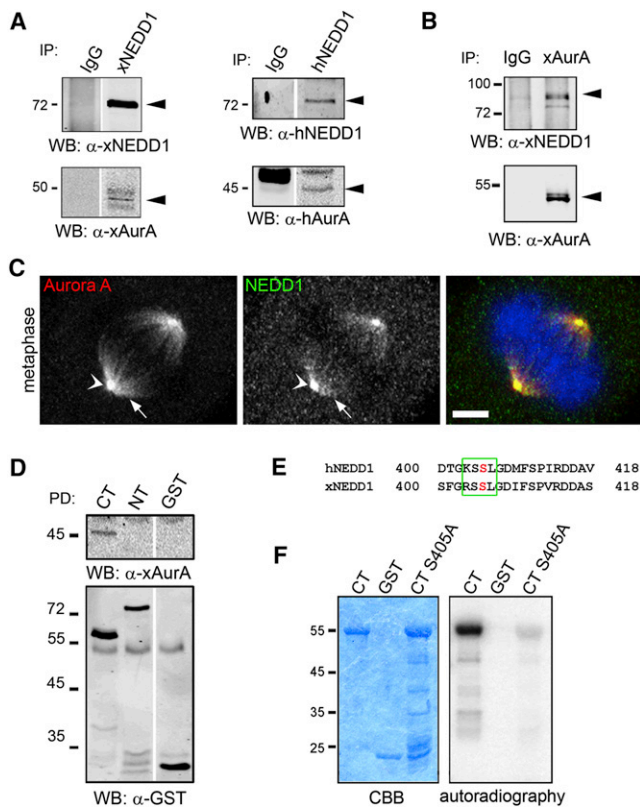


Figure 1. Aurora A Interacts With and Phosphorylates NEDD1 in Mitosis
(A) Western blot analysis of NEDD1 immunoprecipitations from CSF-arrested *Xenopus laevis* egg extracts (left) and mitotic HeLa cells lysates (right). Anti-xNEDD1 antibodies specifically immunoprecipitated xNEDD1 and xAurora A from egg extract. Mouse anti-hNEDD1 antibodies immunoprecipitated hNEDD1 and hAurora A from mitotic HeLa cells (the unspecific rabbit IgGs used as control are strongly recognized by the anti-rabbit secondary antibodies).
(B) Western blot analysis of xAurora A immunoprecipitation from CSF-arrested egg extracts. The anti-xAurora A antibodies specifically immunoprecipitate Aurora A and NEDD1.
(C) Confocal images of a HeLa cell in metaphase showing the localizations of Aurora A and NEDD1 by immunofluorescence. Aurora A and NEDD1 colocalize at the centrosome (arrowhead) and at the spindle MTs (arrow). A maximum projection is shown. In the merge image, Aurora A is in red, NEDD1 is in green, and DNA is in blue. Scale bar represents 5 μ m.
(D) Western blot analysis of a pull-down experiment using GST-fusion proteins of the C-terminal (CT) and N-terminal (NT) domains of xNEDD1. GST-NEDD1-CT (CT), GST-NEDD1-NT (NT), or GST alone were immobilized on anti-GST antibody-coated beads and incubated in CSF-arrested egg extract. The beads were retrieved, washed, and resuspended in loading buffer. The recombinant proteins are visualized with anti-GST antibodies in the lower panel. Aurora A was specifically recovered only with GST-xNEDD1-CT, as shown on the western blot with anti-Aurora A antibodies in the upper panel.
(E) Alignment of the human and frog NEDD1 sequences containing the conserved Ser405 found to be phosphorylated in vitro by Aurora A. The conserved motif for Aurora A phosphorylation [R/K]-X-[S/T]-[I/L/V] is framed in green, with Ser405 highlighted in red.
(F) Coomassie brilliant blue (CBB)-stained gel and autoradiography of an in vitro kinase assay with His-xAurora A and GST, GST-xNEDD1-CT, and GST-xNEDD1-CTS405A. xAurora A phosphorylates GST-xNEDD1-CT specifically, but not xNEDD1 CT S405A. This shows that Aurora A phosphorylates NEDD1 at Ser405.
 See also [Figures S1 and S2](#).

CSF-arrested egg extracts detected a phosphorylated peptide containing Ser405 (Figure S2). These results are consistent with previous reports describing the phosphorylation of NEDD1 on Ser405 in mouse and mitotic U2OS cells [12, 13]. We conclude that Aurora A phosphorylates NEDD1 on Ser405 during M phase.

Phosphorylation of NEDD1 by Aurora A at Ser405 Is Required for Functional Spindle Assembly and Chromosome Alignment

As a first approach to examine the role for NEDD1 Ser405 phosphorylation during cell division, we expressed Flag-NEDD1, Flag-NEDD1 S405A, or Flag-NEDD1 S405D in HeLa cells. Overexpression of any of these NEDD1 variants did not interfere with spindle formation and chromosome segregation (data not shown).

Reducing NEDD1 levels by small interfering RNA transfection in HeLa cells resulted in a range of spindle defects, including monopolar and unorganized spindles, as previously described [14, 15]. We then expressed RNAi-resistant Flag-NEDD1, Flag-NEDD1 S405A, or Flag-NEDD1 S405D in the silenced cells. We first looked at the localization of the different NEDD1 variants by immunofluorescence with an anti-Flag antibody (Figure 2A). All of the recombinant NEDD1 variants localized both to the centrosomes and to the spindle MTs like the endogenous protein (Figure 2A; Figure S3A). Consistently, γ -tubulin localized to both the spindle poles and the spindle MTs in all the cells (Figure 2B; Figure S3B). As a control, we performed the same analysis on NEDD1-silenced cells expressing Flag-NEDD1-S411A (Figures S3A and S3B). As described previously and in contrast to the S405 phospho variants, neither the S411A variant nor γ -tubulin localized to the spindle MTs [14]. These results suggested that phosphorylation of NEDD1 at Ser405 is not required for NEDD1 and γ -tubulin localization to the centrosome and spindle MTs.

Expression of Flag-NEDD1, Flag-NEDD1 S405A, or Flag-NEDD1 S405D in the silenced cells rescued the formation of bipolar spindles. However, cells expressing Flag-NEDD1 S405A showed a striking chromosome misalignment phenotype, with only $19.5\% \pm 2.9\%$ of the bipolar spindles showing fully aligned chromosomes, whereas $53.3\% \pm 5.4\%$ of bipolar spindles in cells expressing Flag-NEDD1 and $42.7\% \pm 2.3\%$ in cells expressing Flag-NEDD1 S405D had fully aligned chromosomes (Figure 2C). These data suggested that the phosphorylation of NEDD1 on Ser405 is important for the assembly of kinetochore fibers and their stable attachment to the chromosomes. To further test this idea, we monitored K-fiber stability using a cold-stable assay in NEDD1-silenced cells expressing the different NEDD1 variants (Figure 2D and 2E; Figure S3C). We found that silenced cells expressing Flag-NEDD1 S405A were more sensitive to cold-induced depolymerization than those expressing Flag-NEDD1 or Flag-NEDD1 S405D, indicating that their K-fibers were less stable (Figures 2D and 2E). Altogether, these results suggested that the phosphorylation of NEDD1 at S405 is important for the formation of functional K-fibers and chromosome alignment.

Phosphorylation of NEDD1 by Aurora A at Ser405 Is Essential for Chromatin-Dependent MT Assembly

The chromosome misalignment phenotype and K-fiber instability in silenced cells expressing Flag-NEDD1 S405A suggested that phosphorylation at this site could play a role in chromosomal MT nucleation [2, 7]. We therefore used a MT regrowth assay to clearly visualize the centrosomal and chromosomal

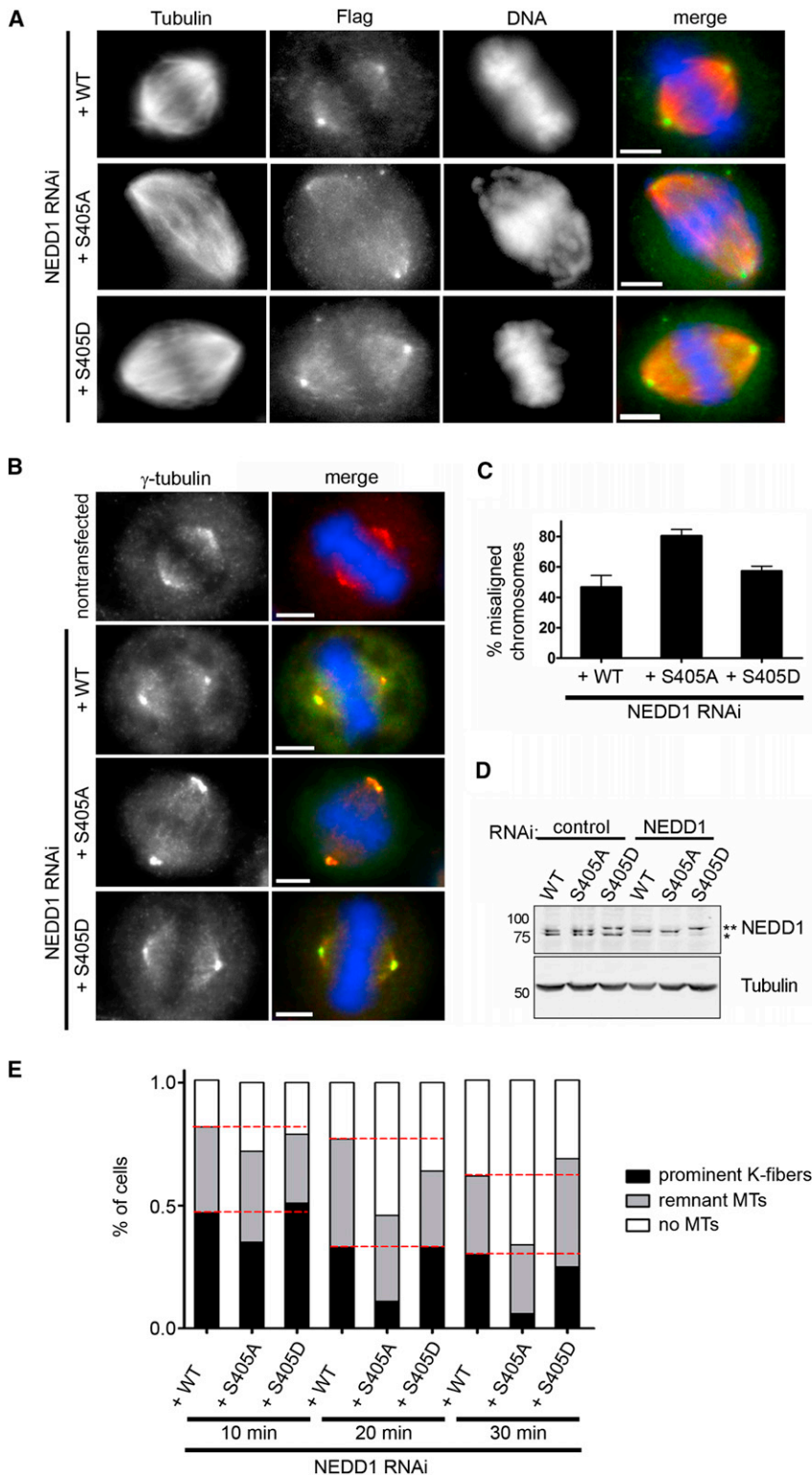


Figure 2. NEDD1 Phosphorylation Is Required for Chromosome Alignment, but Not for γ -Tubulin Targeting

(A) Immunofluorescence images of NEDD1-silenced cells transfected with RNAi-resistant Flag-NEDD1 wild-type (WT), Flag-NEDD1-S405A (S405A), or Flag-NEDD1-S405D (S405D). The exogenously expressed NEDD1 variants were detected with anti-Flag antibodies (in green). MTs are in red; DNA is in blue. Scale bar represents 5 μ m.

(B) Immunofluorescence images of NEDD1-silenced cells transfected with RNAi-resistant Flag-NEDD1-WT (WT), Flag-NEDD1-S405A (S405A), or Flag-NEDD1-S405D (S405D) and processed for immunofluorescence to visualize γ -tubulin (red), the Flag-tagged recombinant proteins (green), and DNA (blue). Scale bar represents 5 μ m.

(C) Quantification of the percentage of spindles with misaligned chromosomes in experiments similar to (B). Depicted is the average of two independent experiments and its SD.

(D) Western blot analysis of cell lysates from the different experimental conditions shown in (B). The anti-NEDD1 antibodies recognize both the endogenous and recombinant forms of NEDD1. In NEDD1-silenced cells, the endogenous protein (marked with one asterisk) is almost undetectable. The exogenously expressed recombinant proteins (marked with two asterisks) show levels comparable to endogenous NEDD1. Tubulin signal is shown as a loading control.

(E) Cold-stable assay to monitor K-fiber stability in NEDD1-silenced cells expressing Flag-NEDD1-WT (+WT), Flag-NEDD1-S405A (+S405A), or Flag-NEDD1-S405D (+S405D) as indicated. The quantification shows the percentage of metaphase-like cells with intact K-fibers (prominent K-fibers), K-fiber remnants (remnant MTs), or no MTs. The red dashed lines mark the percent of each category for the control condition (silenced cells expressing Flag-NEDD1-WT). The graph shows a representative experiment out of three independent experiments (each with $n \geq 30$). See also Figure S3.

MT asters on the chromatin [2, 6] (Figure 3). In agreement with previous reports, NEDD1-silenced cells displayed a significant reduction in the number of asters and in their intensity, suggesting that both the centrosomal and chromosomal pathways were strongly impaired [14] (Figures 3A and 3C). We then expressed the different Flag-tagged NEDD1 variants in the silenced cells to evaluate their capacity to rescue the formation of centrosomal and/or chromosomal MT asters.

MTs. Cells were incubated with nocodazole, released, and fixed. Immunofluorescence analysis showed that NEDD1 and Aurora A localized both to the centrosomal asters (as identified by the localization of the centrosomal marker Cep192) and to the chromosomal asters (Figure S4A) [14].

As described previously, control cells contained two prominent centrosome asters and a variable number of additional

Expression of Flag-NEDD1 or Flag-NEDD1 S405D in NEDD1-silenced cells rescued MT aster formation by both the centrosomes and the chromosomes in $54.3\% \pm 11.6\%$ and $53.5\% \pm 8.1\%$ of the cells, respectively (Figures 3B and 3C). Remarkably, the majority of the NEDD1-silenced cells expressing Flag-NEDD1 S405A contained only two prominent MT asters centered on the centrosomes as detected with GFP-centrin

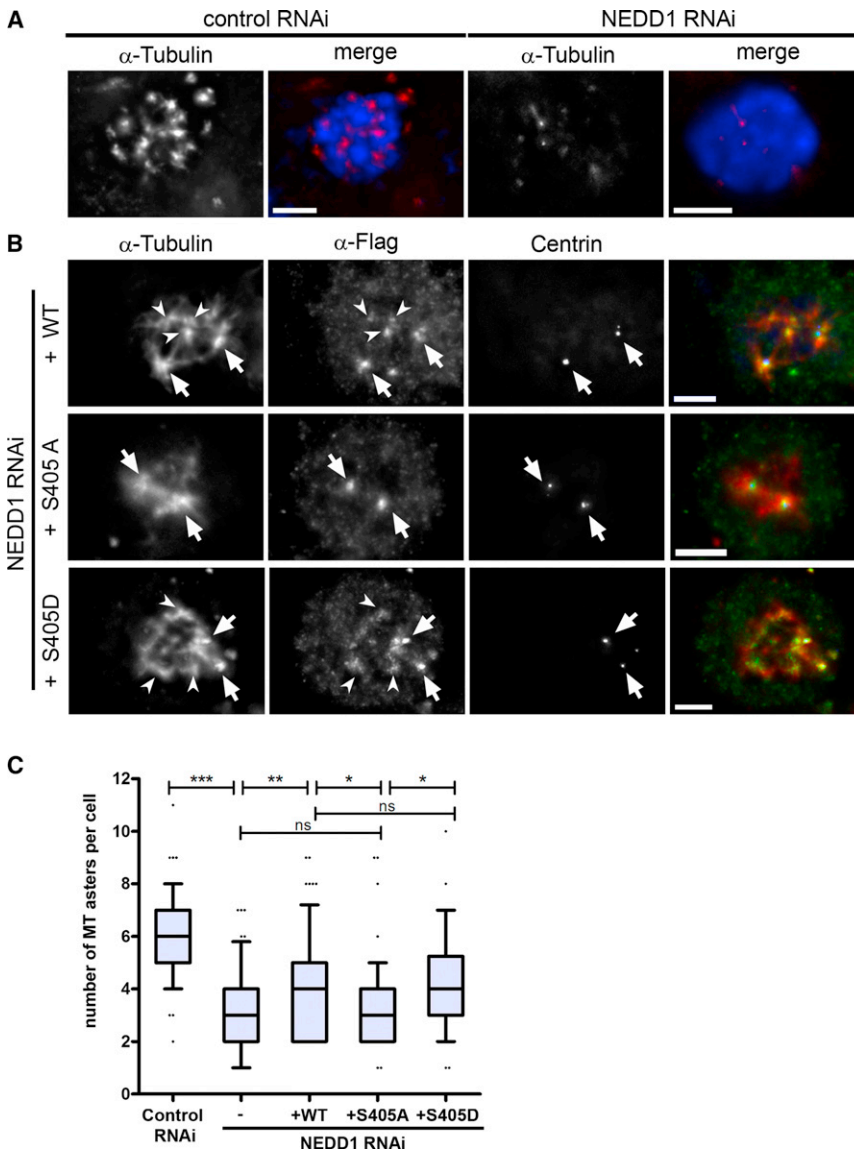


Figure 3. NEDD1 Phosphorylation at Ser405 Is Essential for Chromosome-Dependent MT Nucleation

(A) Immunofluorescence images of control and NEDD1-silenced HeLa cells incubated in nocodazole and fixed 5 min after washout. NEDD1-silenced cells are strongly impaired for MT regrowth. In the merge, MTs are in red and DNA is in blue. Scale bar represents 5 μ m.

(B) Immunofluorescence images of NEDD1-silenced HeLa cells expressing RNAi-resistant Flag-hNEDD1 (+WT), Flag-hNEDD1 S405A (+S405A), or Flag-hNEDD1 S405D (+S405D) incubated in nocodazole and fixed 5 min after washout. The centrosomes (indicated by arrows) are visualized by GFP-centrin (here shown in blue) stably expressed in this HeLa cell line. The recombinant proteins are detected with anti-Flag antibodies (displayed in green), and tubulin is shown in red. Chromosomal MTs marked with arrowheads are absent in cells expressing Flag-hNEDD1 S405A. Scale bar represents 5 μ m.

(C) Quantification of the number of MT asters per cell, 5 min after nocodazole washout. In the box-and-whiskers plot, boxes show the upper and lower quartiles (25%–75%) with a line at the median, whiskers extend from the 10th to the 90th percentile, and dots correspond to outliers. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.005$. The graph shows one representative experiment out of nine independent experiments (each with $n \geq 30$). See also Figure S4.

(Figure 3B). These results strongly suggested that NEDD1 phosphorylation on Ser405 by Aurora A is specifically required for the nucleation of chromosomal MTs.

We then examined cells at longer times after nocodazole washout to monitor spindle formation. Around 18% of control cells and Flag-NEDD1- or Flag-NEDD1 S405D-expressing cells had assembled a bipolar spindle with fully aligned chromosomes (Figure S4). By contrast, although Flag-NEDD1 S405A-expressing cells also rescued the spindle assembly defects due to NEDD1 silencing, only 5.2% formed a bipolar spindle with aligned chromosome (Figure S4). These data show that the phosphorylation of NEDD1 by Aurora A on Ser405 is essential for the nucleation of chromosomal MTs, and thereby for the assembly of a functional bipolar spindle.

Phosphorylation of NEDD1 by Aurora A at Ser405 Is Essential for RanGTP- and Chromatin-Dependent MT Assembly in *Xenopus* Egg Extracts

To gain further support for a role of phosphorylation of NEDD1 S405 by Aurora A in chromosome-dependent MT nucleation, we then turned to the *Xenopus* egg extract system. Depletion

of NEDD1 strongly impaired the formation of MT asters induced by addition of RanGTP to the extract. Moreover, the capacity of DNA-coated beads to promote MT assembly when incubated in depleted egg extract was also impaired (Figure 4). Both phenotypes were fully rescued upon addition of recombinant NEDD1 or NEDD1 S405E to the depleted extract. By contrast, addition of NEDD1 S405A was unable to rescue RanGTP aster formation and

MT assembly around DNA beads (Figure 4). These results provide strong support for an essential role of NEDD1 phosphorylation at Ser405 in the RanGTP-dependent MT assembly pathway during M phase.

Discussion

NEDD1 has recently emerged as a key targeting factor for the γ TURC required in all the known pathways leading to MT assembly [14–17]. It does indeed target the γ TURC to the centrosome, and to preexisting MTs through the augmin complex [17]. It is also required for chromosome-dependent MT assembly, although no mechanism for its targeting or activation in this pathway has been described so far. Interestingly, NEDD1 is hyperphosphorylated during M phase [14, 15]. Several studies have identified a number of sites phosphorylated by Cdk1 and Plk1 [12, 14, 16, 18, 19]. Phosphorylation at these sites plays important and complex regulatory functions at different levels, including the binding of NEDD1 to the γ TURC [12, 16, 20] and its targeting to the spindle MTs [14] and the centrosome [21].

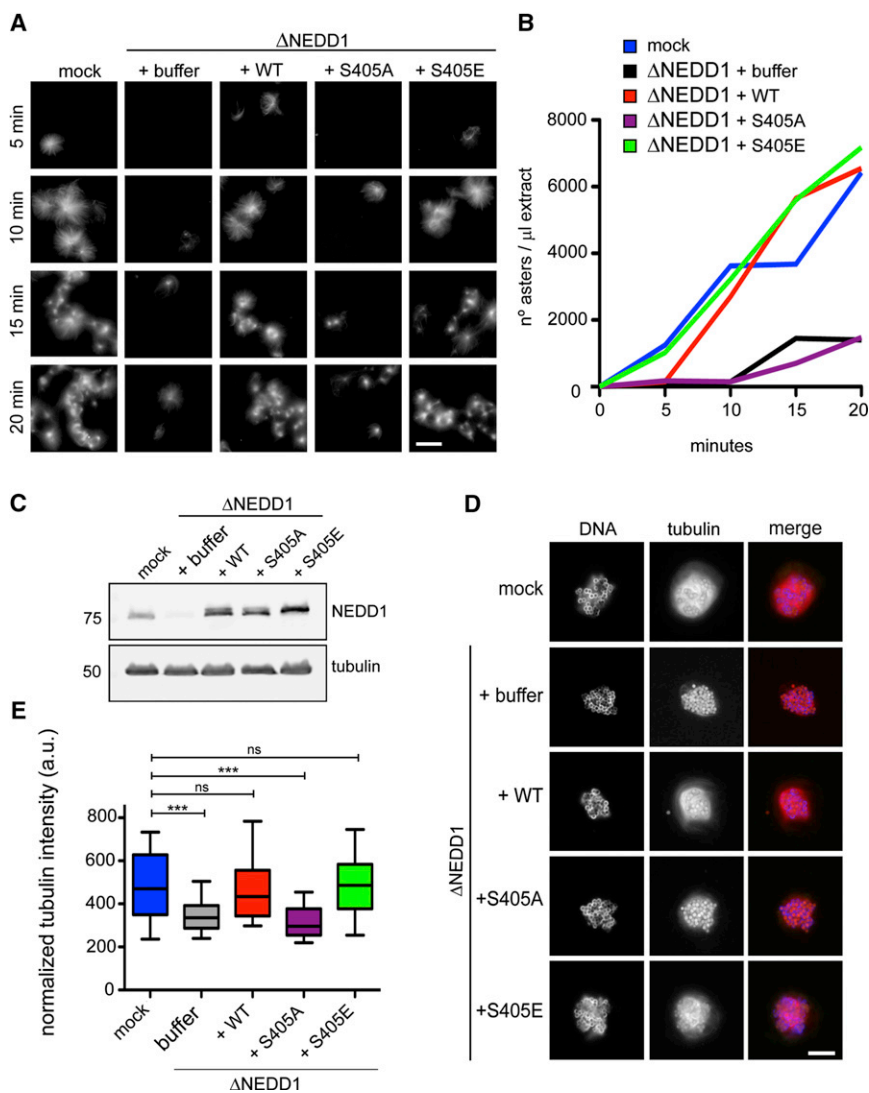


Figure 4. NEDD1 Ser405 Phosphorylation Is Essential for Chromatin- and RanGTP-Dependent MT Nucleation in *Xenopus laevis* Egg Extracts

(A) RanGTP asters formed in control mock-depleted egg extract (mock), NEDD1-depleted extract (+buffer), and depleted extract supplemented with human recombinant flag-NEDD1 (WT), Flag-NEDD1 S405A (+S405A), or Flag-NEDD1 S405E (+S405E). The images correspond to squashes taken at 5, 10, 15, and 20 min after RanGTP addition to a CSF extract. MTs are visualized by addition of rhodamine tubulin to the extract. Scale bar represents 25 μ m.

(B) Quantification of the number of RanGTP asters in each experimental condition. The graph shows one representative experiment out of four independent experiments.

(C) Western blot analysis of the extracts used for (A) and (B) showing that endogenous NEDD1 in mock extract was efficiently depleted, and showing the different recombinant proteins that were added to the depleted extract. The tubulin signal is shown as loading control.

(D) Images of DNA-coated beads incubated in cycled extract as indicated for 30 min. Rhodamine tubulin was added to the extract for visualization. DNA was stained with Hoechst. Note that the beads are autofluorescent. Scale bar represents 25 μ m.

(E) Quantification of the DNA bead-dependent MT nucleation shown in (D). The total tubulin intensity around each DNA bead cluster was measured and normalized by the cluster size. In the box-and-whiskers plot, boxes show the upper and lower quartiles (25%–75%) with a line at the median. *** $p < 0.005$; ns, no statistical difference using Student's *t* test. The graph shows one of two independent experiments.

tubulin to the centrosome during MT re-growth experiments [7].

We provide evidence in two experimental systems for an essential role of NEDD1 phosphorylation by Aurora A in

chromosomal MT assembly. The strong impairment of this pathway when NEDD1 cannot be phosphorylated on Ser405 is most likely due to defects at the level of MT nucleation. Although NEDD1 has also been implicated in another acentrosomal MT assembly pathway driving MT amplification through the augmin complex, our results do not indicate that this pathway is affected by NEDD1 S405 phosphorylation. Indeed, NEDD1 S405A localizes to the spindle MTs and does not interfere with the targeting of γ -tubulin to these spindle MTs, a process that has been shown to rely on NEDD1 and the augmin complex [17]. Moreover, we found that NEDD1 S405A pulled down FAM29A as efficiently as the wild-type protein in mitotic HeLa cell lysates (data not shown). In addition, in contrast to our results, it has been shown that the silencing of the augmin component FAM29A does not interfere with the early steps of chromosomal MT nucleation in cells released from nocodazole [17]. Our data therefore suggest that the phosphorylation of NEDD1 at Ser405 is specifically required for the direct nucleation of chromosomal MTs.

Although NEDD1 S405A strongly impairs chromosomal MT assembly, it supports the organization of the centrosomal MTs into a spindle-like configuration. Silencing the

Here we show that Aurora A phosphorylates NEDD1 at a single residue in mitotic cells and that this phosphorylation is essential for the assembly of chromosomal MTs. Extensive work supports a role for Aurora A in promoting MT assembly in M phase, both at the centrosome, where it accumulates in G2/M, and around the chromosomes through the RanGTP- and TPX2-dependent pathway [4, 5, 7, 22, 23]. The substrates of Aurora A identified so far support a main role for this kinase in MT stabilization and organization [5, 10, 24]. Our findings provide for the first time a direct link between Aurora A and the MT nucleation machinery, suggesting a novel role for this kinase in controlling MT nucleation.

The localization of Aurora A and NEDD1 at both the centrosome and spindle MTs suggests that NEDD1 phosphorylation by Aurora A could have a general regulatory function on the MT nucleation machinery. However, both NEDD1 S405A and NEDD1 S405D localize to the centrosomes and fully rescue their MT aster formation capacity in NEDD1-silenced cells. These data indicate that Ser405 phosphorylation is required neither for the targeting of the γ TURC to the centrosome nor for regulating its intrinsic MT nucleation activity. In line with these results, a previous report showed that Aurora A silencing does not interfere with the recruitment of γ -

RanGTP-regulated protein TPX2 also impairs chromosomal MT assembly, but it has more dramatic consequences on spindle formation [25, 26]. Because TPX2 has additional functions related to spindle organization and complex interactions in particular with motor proteins [27–29], our results here may more specifically reflect the consequences of the absence of chromosomal MTs on spindle assembly and functionality. We recently showed that interfering with chromosomal MT assembly by silencing MCRS1 leads to defects in K-fiber formation and dynamics [2]. Altogether, our results indicate that although centrosomal asters can organize a bipolar-like spindle, these structures are not functional and cannot align the chromosomes. Moreover, they suggest that an active chromosomal MT assembly pathway is essential for the assembly and functionality of the K-fibers.

In summary, our work strongly supports the key role of NEDD1 in the spatial and temporal control of MT nucleation in mitotic cells. Moreover, it provides a specific mechanism for the regulation of MT nucleation around the chromosomes. Under the influence of the RanGTP gradient, TPX2 activates Aurora A, which in turn specifically phosphorylates the γ TURC adaptor protein NEDD1 and triggers chromosomal-MT nucleation. This links for the first time the kinase Aurora A and the MT nucleation machinery and reveals a novel function for this kinase during mitosis.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2012.11.046>.

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References

1. Clarke, P.R., and Zhang, C. (2008). Spatial and temporal coordination of mitosis by Ran GTPase. *Nat. Rev. Mol. Cell Biol.* 9, 464–477.
2. Meunier, S., and Vernos, I. (2011). K-fibre minus ends are stabilized by a RanGTP-dependent mechanism essential for functional spindle assembly. *Nat. Cell Biol.* 13, 1406–1414.
3. Wilde, A., and Zheng, Y. (1999). Stimulation of microtubule aster formation and spindle assembly by the small GTPase Ran. *Science* 284, 1359–1362.
4. Gruss, O.J., and Vernos, I. (2004). The mechanism of spindle assembly: functions of Ran and its target TPX2. *J. Cell Biol.* 166, 949–955.
5. Sardon, T., Peset, I., Petrova, B., and Vernos, I. (2008). Dissecting the role of Aurora A during spindle assembly. *EMBO J.* 27, 2567–2579.
6. Tulu, U.S., Fagerstrom, C., Ferenz, N.P., and Wadsworth, P. (2006). Molecular requirements for kinetochore-associated microtubule formation in mammalian cells. *Curr. Biol.* 16, 536–541.
7. Katayama, H., Sasai, K., Kloc, M., Brinkley, B.R., and Sen, S. (2008). Aurora kinase-A regulates kinetochore/chromatin associated microtubule assembly in human cells. *Cell Cycle* 7, 2691–2704.
8. Sampath, S.C., Ohi, R., Leismann, O., Salic, A., Pozniakovski, A., and Funabiki, H. (2004). The chromosomal passenger complex is required for chromatin-induced microtubule stabilization and spindle assembly. *Cell* 118, 187–202.
9. Bayliss, R., Sardon, T., Vernos, I., and Conti, E. (2003). Structural basis of Aurora-A activation by TPX2 at the mitotic spindle. *Mol. Cell* 12, 851–862.
10. Sardon, T., Pache, R.A., Stein, A., Molina, H., Vernos, I., and Aloy, P. (2010). Uncovering new substrates for Aurora A kinase. *EMBO Rep.* 11, 977–984.
11. Ohashi, S., Sakashita, G., Ban, R., Nagasawa, M., Matsuzaki, H., Murata, Y., Taniguchi, H., Shima, H., Furukawa, K., and Urano, T. (2006). Phospho-regulation of human protein kinase Aurora-A: analysis using anti-phospho-Thr288 monoclonal antibodies. *Oncogene* 25, 7691–7702.
12. Gomez-Ferreria, M.A., Bashkurov, M., Helbig, A.O., Larsen, B., Pawson, T., Gingras, A.-C., and Pelletier, L. (2012). Novel NEDD1 phosphorylation sites regulate γ -tubulin binding and mitotic spindle assembly. *J. Cell Sci.* 125, 3745–3751.
13. Huttlin, E.L., Jedrychowski, M.P., Elias, J.E., Goswami, T., Rad, R., Beausoleil, S.A., Villén, J., Haas, W., Sowa, M.E., and Gygi, S.P. (2010). A tissue-specific atlas of mouse protein phosphorylation and expression. *Cell* 143, 1174–1189.
14. Lüders, J., Patel, U.K., and Stearns, T. (2006). GCP-WD is a gamma-tubulin targeting factor required for centrosomal and chromatin-mediated microtubule nucleation. *Nat. Cell Biol.* 8, 137–147.
15. Haren, L., Remy, M.H., Bazin, I., Callebaut, I., Wright, M., and Merdes, A. (2006). NEDD1-dependent recruitment of the gamma-tubulin ring complex to the centrosome is necessary for centriole duplication and spindle assembly. *J. Cell Biol.* 172, 505–515.
16. Zhang, X., Chen, Q., Feng, J., Hou, J., Yang, F., Liu, J., Jiang, Q., and Zhang, C. (2009). Sequential phosphorylation of Nedd1 by Cdk1 and Plk1 is required for targeting of the gammaTuRC to the centrosome. *J. Cell Sci.* 122, 2240–2251.
17. Zhu, H., Coppinger, J.A., Jang, C.Y., Yates, J.R., 3rd, and Fang, G. (2008). FAM29A promotes microtubule amplification via recruitment of the NEDD1-gamma-tubulin complex to the mitotic spindle. *J. Cell Biol.* 183, 835–848.
18. Johmura, Y., Soung, N.K., Park, J.E., Yu, L.R., Zhou, M., Bang, J.K., Kim, B.Y., Veenstra, T.D., Erikson, R.L., and Lee, K.S. (2011). Regulation of microtubule-based microtubule nucleation by mammalian polo-like kinase 1. *Proc. Natl. Acad. Sci. USA* 108, 11446–11451.
19. Haren, L., Stearns, T., and Lüders, J. (2009). Plk1-dependent recruitment of gamma-tubulin complexes to mitotic centrosomes involves multiple PCM components. *PLoS ONE* 4, e5976.
20. Manning, J.A., Shalini, S., Risk, J.M., Day, C.L., and Kumar, S. (2010). A direct interaction with NEDD1 regulates gamma-tubulin recruitment to the centrosome. *PLoS ONE* 5, e9618.
21. Sdelci, S., Schütz, M., Pinyol, R., Bertran, M.T., Regué, L., Caelles, C., Vernos, I., and Roig, J. (2012). Nek9 phosphorylation of NEDD1/GCP-WD contributes to Plk1 control of γ -tubulin recruitment to the mitotic centrosome. *Curr. Biol.* 22, 1516–1523.
22. Kufer, T.A., Silljé, H.H., Körner, R., Gruss, O.J., Meraldi, P., and Nigg, E.A. (2002). Human TPX2 is required for targeting Aurora-A kinase to the spindle. *J. Cell Biol.* 158, 617–623.
23. Brunet, S., Sardon, T., Zimmerman, T., Wittmann, T., Pepperkok, R., Karsenti, E., and Vernos, I. (2004). Characterization of the TPX2 domains involved in microtubule nucleation and spindle assembly in *Xenopus* egg extracts. *Mol. Biol. Cell* 15, 5318–5328.
24. Peset, I., Seiler, J., Sardon, T., Bejarano, L.A., Rybina, S., and Vernos, I. (2005). Function and regulation of Maskin, a TACC family protein, in microtubule growth during mitosis. *J. Cell Biol.* 170, 1057–1066.
25. Gruss, O.J., Wittmann, M., Yokoyama, H., Pepperkok, R., Kufer, T., Silljé, H., Karsenti, E., Mattaj, I.W., and Vernos, I. (2002). Chromosome-induced microtubule assembly mediated by TPX2 is required for spindle formation in HeLa cells. *Nat. Cell Biol.* 4, 871–879.
26. Garrett, S., Auer, K., Compton, D.A., and Kapoor, T.M. (2002). hTPX2 is required for normal spindle morphology and centrosome integrity during vertebrate cell division. *Curr. Biol.* 12, 2055–2059.
27. Ma, N., Tulu, U.S., Ferenz, N.P., Fagerstrom, C., Wilde, A., and Wadsworth, P. (2010). Poleward transport of TPX2 in the mammalian mitotic spindle requires dynein, Eg5, and microtubule flux. *Mol. Biol. Cell* 21, 979–988.

28. Gable, A., Qiu, M., Titus, J., Balchand, S., Ferenz, N.P., Ma, N., Collins, E.S., Fagerstrom, C., Ross, J.L., Yang, G., and Wadsworth, P. (2012). Dynamic reorganization of Eg5 in the mammalian spindle throughout mitosis requires dynein and TPX2. *Mol. Biol. Cell* 23, 1254–1266.
29. Vanneste, D., Takagi, M., Imamoto, N., and Vernos, I. (2009). The role of Hklp2 in the stabilization and maintenance of spindle bipolarity. *Curr. Biol.* 19, 1712–1717.