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Survivin reads phosphorylated histone H3 threonine 3 to activate the mitotic kinase Aurora B

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SUMMARY

A hallmark of mitosis is the appearance of high levels of histone phosphorylation, yet the roles of these modifications remain largely unknown. Here we demonstrate that histone H3 phosphorylated at threonine 3 is directly recognized by an evolutionarily conserved binding pocket in the BIR domain of Survivin, a member of the chromosomal passenger complex (CPC). This binding mediates recruitment of the CPC to chromosomes and resulting activation of its kinase subunit Aurora B. Consistently, modulation of the kinase activity of Haspin, which phosphorylates H3T3, leads to defects in the Aurora B-dependent processes of spindle assembly and inhibition of nuclear reformation. These findings establish a direct cellular role for mitotic histone H3T3 phosphorylation, which is read and translated by the CPC to ensure accurate cell division.

The accurate segregation of chromosomes during mitosis depends on a complex signalling network, whose spatiotemporal coordination is achieved, in part, by restricting the activity of kinases to distinct subcellular structures (1). The kinase Aurora B, together with INCENP, Dasra (also known as Borealin) and Survivin, form the chromosomal passenger complex (CPC), which plays multiple roles during mitosis and meiosis by changing its localization (2). At the beginning of M phase, the CPC is localized to chromosomes, where it controls chromatin-dependent spindle assembly (3, 4) and processes at the centromere (2). During anaphase, the complex dissociates from chromosomes and relocates to the spindle midzone to stimulate cytokinesis (2). The mechanism by which the CPC is recruited to chromosomes in a cell cycle specific manner remains unknown.

In screening for mitotic histone-binding proteins, we found that the CPC interacts with histones H3/H4 in *Xenopus* cytosolic factor-arrested (CSF) egg extracts (Fig. S1). Given that Ser10 of histone H3 (H3S10) is an excellent substrate of Aurora B, we surmised that the

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Supporting Online Material

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Materials and Methods

Figs. S1 to S13

Table S1

References

GenBank accession number for *X. laevis* Haspin: HM559585.

CPC-H3/H4 interaction was mediated by the H3 tail (5, 6). In egg extracts the CPC did not show appreciable binding to a peptide corresponding to the N-terminal tail of H3 (H3₁₋₂₁) (Fig. 1A). However, all four CPC subunits bound to an H3 peptide phosphorylated on Thr3 (H3T3ph) more significantly than to H3₁₋₂₁, H3S10ph, scrambled H3 sequence peptide (Scr. H3) or a histone H4 N-terminal (H4₁₋₁₈) peptide (Fig. 1A).

The CPC can be separated into two functional modules (Fig. S2A): the kinase module consisting of Aurora B and the C-terminal domain of INCENP (IN box) (7, 8) and the chromosome-localization module consisting of the N-terminal region of INCENP, Survivin and Dasra, which form a ternary subcomplex through a tight helical bundle (4, 9). By introducing two mutations in INCENP (F21R and L33R) that disrupt this helical bundle (9) (Fig. S2B), we found that the chromosome-localization module is important for recruitment of the CPC to H3T3ph (Fig. 1B). A purified ternary complex of Survivin, Dasra A and INCENP₁₋₅₈-His₆, but not the kinase module of the CPC (Aurora B₆₀₋₃₆₁-INCENP₇₉₀₋₈₇₁) (8), specifically bound to H3T3ph peptide *in vitro* (Fig. 1C), demonstrating that the chromosome-localization module of the CPC directly recognizes H3T3ph.

Survivin was able to bind H3T3ph peptides in the absence of other CPC subunits (Fig. S2C), implying that it is the receptor for H3T3ph. We further defined this interaction using nuclear magnetic resonance (NMR) spectroscopy, taking advantage of published resonance assignments of human Survivin (10). Analysis of [¹⁵N, ¹H]-heteronuclear single-quantum coherence spectroscopy (HSQC) spectra of Survivin in the presence of increasing amounts of H3T3ph peptide revealed that several resonances were perturbed (Figs. 1D and S3). The migration pattern of these peaks indicated that the interaction was in slow chemical exchange and came to saturation (Fig. S3A), confirming a direct and strong interaction. When mapped onto the structure of Survivin (10), the chemical shift perturbations induced by H3T3ph peptide clustered to form a contiguous binding pocket in its BIR domain (Fig. 1E). Addition of unmodified H3 peptide to Survivin induced only a small subset of these chemical shift perturbations (Fig. S3B, C) demonstrating that in the absence of phosphorylation, H3 interacts with Survivin far less extensively (Fig. 1F). Among the residues within the H3T3ph-binding pocket is D71, which is critical for centromeric localization and function of the CPC *in vivo* (11, 12).

The binding interface of Survivin and H3T3ph is highly similar to that of the BIR3 domain of XIAP (X-linked inhibitor of apoptosis) and the N-terminus of processed SMAC (13, 14) or caspase-9 (15) (Fig. S4). In particular, E314 of XIAP (equivalent to Survivin D71) has been shown to coordinate the N-terminal alanine of its ligands and its mutation abrogates binding *in vitro* (13, 14), suggesting that recognition of the N-terminal alanine of histone H3 by Survivin is carried out in the same manner with D71 (Fig. S4D). By contrast, basic residues H80 and K62, which also shift upon H3T3ph binding, are unique to the Survivin family (Figs. S3C and S4) and may accommodate the negatively charged phosphate.

To further investigate the physiological significance of the CPC-H3T3ph interaction, we depleted the *Xenopus* homolog of the mitotic H3T3 kinase Haspin (16) from egg extracts (Figs. S5, S6). In control extracts, H3T3ph was found along entire chromosomes whereas Dasra A and INCENP localized to chromosome arms and were particularly enriched at centromeres (Figs. 2A and S7A). Haspin depletion abolished the majority of H3T3ph and, concomitantly, significantly reduced Dasra A and INCENP signals from chromosomes (Figs. 2A, S7, and S8). The kinase activity of Haspin is important for chromosomal recruitment of the CPC, since addition of wild-type Haspin, but not a kinase dead version (16), to Δ Haspin extracts rescued the ability of Dasra A and INCENP to bind chromosomes (Figs. 2A, S7 and S8).

We next biochemically tested the effect of Haspin depletion on binding of the CPC to chromatin. In egg extracts, DNA-beads assemble functional chromatin, recruiting the CPC and supporting bipolar spindle formation (3, 4). Consistent with Haspin's role in localizing the CPC to chromosomes, binding of the CPC to DNA-beads was reduced in Δ Haspin extracts and this defect was rescued by recombinant C-terminal kinase domain of Haspin (MBP-Haspin_c) (Figs. 2B and S9). However, Haspin does not appear to serve as a physical bridge to mediate the CPC-chromatin interaction, since the CPC bound H3T3ph peptides in Δ Haspin extracts (Fig. S10).

Chromatin assembled on DNA stimulated Aurora B autophosphorylation of its activation loop at threonine 248 (T248ph) (8) and hyperphosphorylation of the Aurora B substrate Op18 (4) in metaphase egg extracts (Fig. 3A). Chromatin failed to stimulate Aurora B activity in Δ Haspin extracts whereas excess MBP-Haspin_c accelerated the kinetics of chromatin-dependent Aurora B phosphorylation. Furthermore, MBP-Haspin_c rescued the inability of DNA and sperm chromosomes to activate Aurora B in Δ Haspin extracts (Fig. S11), establishing the role of Haspin in chromatin-dependent activation of Aurora B. However, it is unlikely that Haspin directly controls intrinsic Aurora B activity since it is not required for activation of Aurora B by stabilized microtubules, a chromatin-independent activator (4, 6) (Fig. S11).

Chromatin-induced Aurora B activation is required to drive spindle assembly around chromosomes by suppressing microtubule depolymerizing activities in *Xenopus* egg extracts (3, 4). In Δ Haspin extracts, this function was compromised, resulting in shorter spindle length (Fig. 3B and C, Table S1). Although spindles can still form, which may be attributable to residual CPC on chromosomes (Figs. 2 and S8), these results suggest that direct recruitment of the CPC by H3T3ph activates Aurora B on chromosomes to promote spindle assembly.

At the metaphase-to-anaphase transition, the CPC dissociates from chromosomes (2), a process critical to subsequent chromosome decondensation and nuclear reformation (17). Since H3T3ph is dephosphorylated upon exit from M phase (16, 18, 19) (Fig. S6B), we investigated whether its removal is essential to these processes in egg extracts. Metaphase chromosomes, preassembled in CSF control extracts, were decondensed 45 minutes after calcium addition, which induces transitioning to interphase (Fig. 4A). At this point, Dasra A and INCENP dissociated from chromosomes, H3T3ph and H3S10ph levels decreased (Fig. 4A), and functional nuclear formation was nearly complete (Fig. S12A, B). However, in extracts containing constitutively active MBP-Haspin_c, but not MBP-Haspin_c-KD, chromosomes remained condensed with robust staining of Dasra A, INCENP, H3T3ph and H3S10ph, and nuclear import was not initiated (Figs. 4A and S12A, B). These phenotypes were not due to a delay in cell cycle transition, since H1 kinase activity was not detectable fifteen minutes after calcium addition in all samples (Fig. 4B). The chromosome decondensation defect caused by MBP-Haspin_c was CPC-dependent, as expected if Haspin worked through CPC activation (Figs. 4C, D and S12C).

Our study demonstrates that M phase specific phosphorylation of histone H3 at Thr3 recruits the CPC to chromatin to activate Aurora B and that this interaction is mediated by Survivin. (Fig. S13). This interaction controls spindle assembly and nuclear re-formation in *Xenopus* egg extracts, and is important for spindle checkpoint signalling at the centromere in human cells (J. Higgins, accompanying paper). These results highlight a mechanism whereby the cell cycle control of Haspin activity restricts chromosomal Aurora B localization and activation to M phase through histone modification.

Supplementary Material

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Acknowledgments

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Fig. 1.

Survivin mediates the interaction between the CPC and H3T3ph. (A) Western blots (WB) of proteins copurified with indicated peptides from metaphase *Xenopus* egg extracts. Peptides were visualized by Coomassie brilliant blue (CBB). (B) Proteins copurified with indicated peptides from ΔCPC extracts supplemented with recombinant Aurora B and the indicated mRNAs. (C) Purified ternary complex consisting of INCENP₁₋₅₈-His₆, Dasra A and Survivin or the kinase complex consisting of Aurora B₆₀₋₃₆₁ and INCENP₇₉₀₋₈₇₁ was incubated with the indicated peptide-beads. Coomassie staining of input and bead fractions is shown. Asterisk: contaminating *E. coli* protein. (D) Superposition of ¹H, ¹⁵N-HSQC spectra with (red) and without (black) addition of 0.4 mM H3T3ph peptide (1-13) to a 0.2

mM ^{15}N -labeled human Survivin (1–120). Some of the NMR signals of the free protein that shift upon titration with peptide are shown. (E) H3T3ph (1–13) binding surface of Survivin (1–120). Magenta: residues that significantly shift upon addition of the peptide. (F) Unmodified H3 (1–20) binding surface of Survivin (1–120). Green: residues that significantly shift upon addition of the peptide.

Fig. 2.

The H3T3 kinase Haspin is required for recruitment of the CPC to metaphase chromosomes. **(A)** Immunofluorescence images of replicated sperm chromosomes in control or Δ Haspin metaphase extracts with nocodazole, supplemented with buffer, wild-type Haspin or Haspin-KD, stained with indicated antibodies. Scale bar, 5 μ m. **(B)** Western blots of proteins copurified with DNA-beads from control or Δ Haspin extracts supplemented with buffer or 2.5 μ M MBP-Haspin_c. Proteins were eluted from beads first with 0.6 M NaCl, and then with 2M NaCl. Ku70, a DNA-binding protein, serves as a loading control.

Fig. 3. Haspin controls chromatin-induced Aurora B activation to promote spindle assembly. **(A)** Plasmid DNA was incubated for the indicated times at 20°C with control egg extracts, Δ Haspin extracts or control extracts containing 2.5 μ M MBP-Haspin_c. Aurora B phosphorylation at T248 and hyperphosphorylation of Op18 (arrowhead) were monitored by Western blot. **(B)** Representative images of bipolar spindles formed on replicated sperm chromosomes in control or Δ Haspin extracts. Blue, DNA. Red, rhodamine-tubulin. Scale Bar, 10 μ m. **(C)** Histogram of metaphase spindle length in control and Δ Haspin extracts as measured in Table S1. Error bars represent ranges of three experiments.

Fig. 4.

Dephosphorylation of H3T3ph is required for chromosome decondensation and nuclear reformation. **(A, B)** Sperm nuclei incubated with CSF extracts for 45 min were subsequently treated with buffer, 2.5 μ M MBP-Haspin_c or MBP-Haspin_c-KD, together with calcium to induce exit from metaphase. **(A)** Indirect immunofluorescence 45 min after calcium addition. **(B)** Western blot analysis and histone H1 kinase assay (autoradiography). **(C, D)** Sperm nuclei and calcium were added together to CSF control or Δ CPC extracts containing buffer or MBP-Haspin_c. **(C)** Hoechst 33258 staining at 30 min after calcium addition. **(D)** Quantification of **(C)**. Mean and SEM of three independent experiments ($N > 30$). Scale bars, 10 μ m.