From the nuclear pore to the kinetochore: a MAD journey to preserve genome stability

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Mad1-Mad2: how to go from nuclear pores to kinetochores?

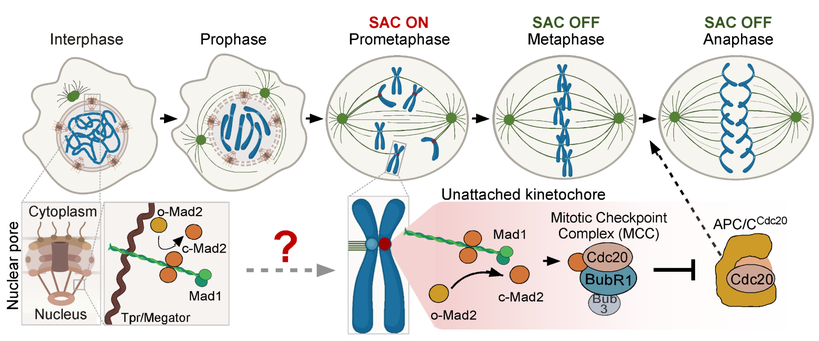

Figure 1: Schematic representation of Mad1-Mad2 localization throughtout cell cycle. The Spindle Assembly Checkpoint (SAC) prevents chromosome mis-segregation by restraining anaphase onset until all the kinetochores are correctly attached to microtubules from opposite poles. During prometaphase, Mad1-Mad2 complexes localize at unattached or tensionless kinetochores and catalyze the conversion of o-Mad2 to c-Mad2 which together with BubR1, Bub3 and Cdc20 forms the Mitotic Checkpoint Complex (MCC). The MCC inhibits APC/C^{Cdc20} thereby preventing the transition to anaphase. In interphase, Mad1-Mad2 is docked at nuclear pore complexes (NPCs) through Mad1 binding to Megator/Tpr. However, the events controlling Mad1-Mad2 redistribution as cells enter mitosis remain unclear.

1. Mps1 excludes Mad1 from nuclear pore complexes during prophase

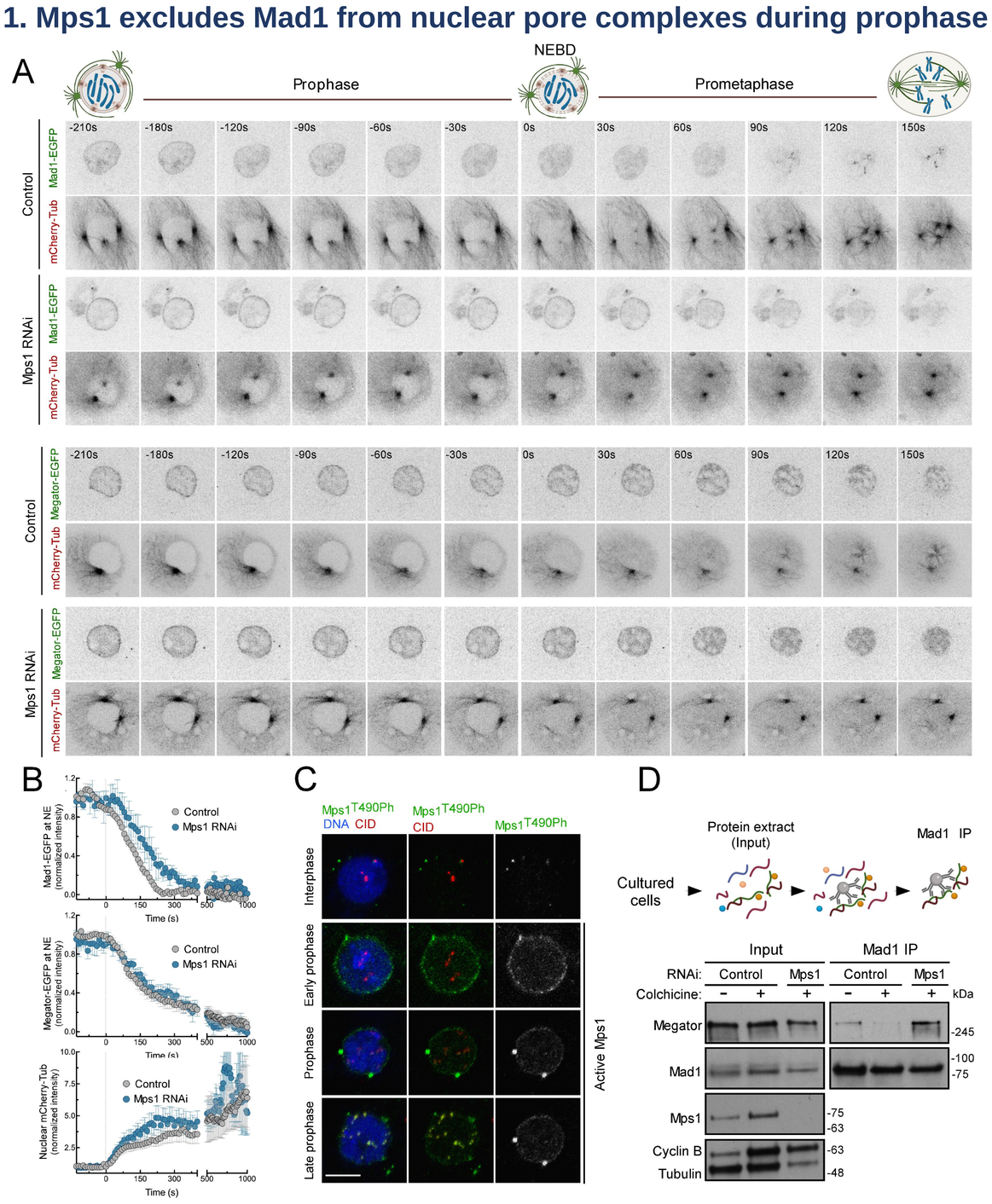

Figure 2: Mps1 promotes Mad1 dissociation from nuclear pores during prophase. (A-B) Depletion of Mps1 delays Mad1 release from the Nuclear Envelope (NE). (A) Stills from time lapse-movies of control or Mps1 depleted *Drosophila* S2 cells expressing EGFP-Mad1 or EGFP-Megator and mCherry-tubulin. (B) Quantification of EGFP-Mad1 and EGFP-Megator levels at NE and nuclear mCherry-tubulin over time. (C) Mps1 is active at the nuclear envelope during mitotic entry. Immunolocalization of Mps1^{T490Ph} (green), CID (red) and DNA (blue) in S2 cells. (D) Co-immunoprecipitation (Co-IP) of Mad1 from lysates of control and Mps1-depleted S2 cells.

2. Mps1 phosphorylates Megator to disrupt its interaction with Mad1

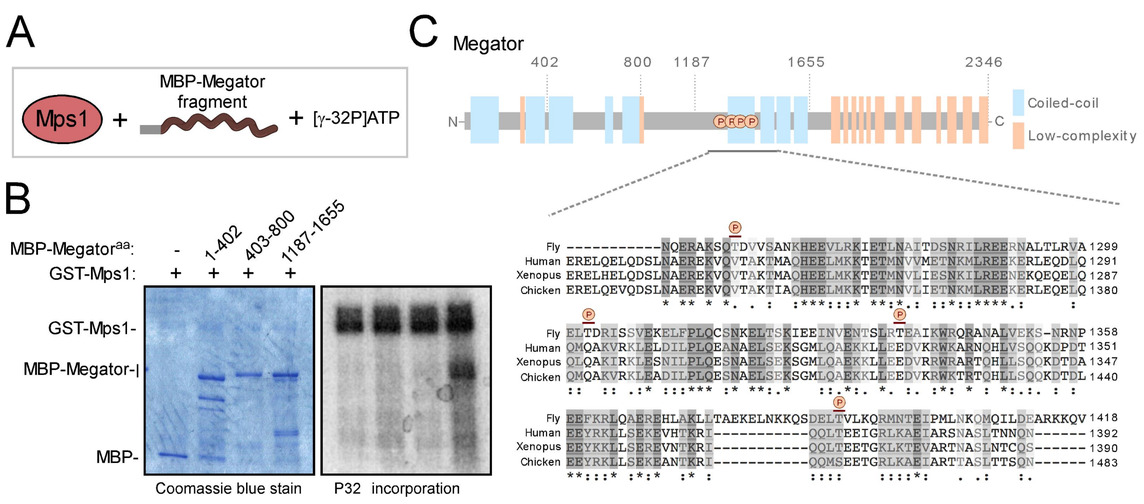

Figure 3: Mps1-mediated phosphorylation of Megator disrupts its interaction with Mad1. (A,B) Mps1 phosphorylates Megator *in vitro*. Recombinant fragments of MBP-Megator were used as substrates for GST-Mps1 in the presence of ATP [P^{32}]. (C) Schematic representation of *Drosophila* Megator and local alignment of protein orthologues. Megator$^{1187-1655}$ phosphorylated residues were identified by MS/MS analysis (D) Phosphorylation of Megator by Mps1 decreases its interaction with Mad1 *in vitro*. Pull-downs of recombinant Megator fragments by bead-immobilized 6xHis-Mad1$^{1-493}$ and corresponding quantifications from two independent experiments. B-beads; FT- flowthrough.

3. Mps1-mediated phosphorylation of Megator is required for Mad1 kinetochore recruitment and sustained SAC response

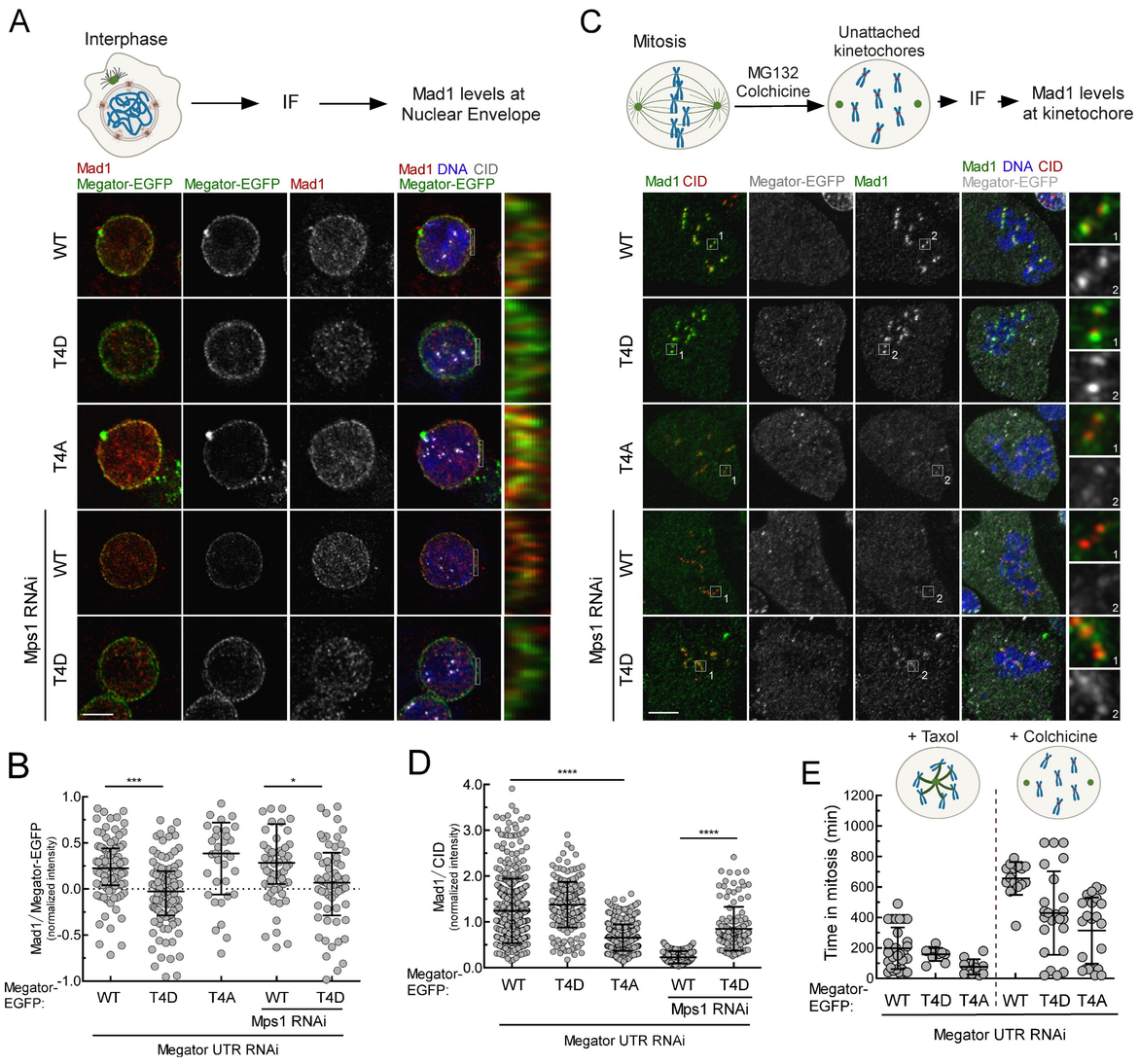

Figure 4: Mps1-mediated phosphorylation of Megator promotes Mad1 kinetochore recruitment and sustained SAC response. (A,B) Phosphomimetic version of EGFP-Megator excludes Mad1 from nuclear envelope of interphase cells. (A) Immunolocalization of the indicated proteins (B) Quantification of Mad1/EGFP-Megator levels at nuclear envelope; **P<0.01, *** P<0.001, **** P<0.0001 Kruskal-Wallis, Dunn´s multiple comparison test. (C,D) Mps1-mediated phosphorylation of Megator is required for proper recruitment of Mad1 to unattached kinetochores. (C) Immunolocalization of the indicated proteins in S2 cells treated with colchicine and MG132 for 30 min. (D) Quantification of Mad1 levels at kinetochores; **** P<0.0001 Kruskal-Wallis, Dunn´s multiple comparison test (E) Mps1 phosphorylation of Megator is required for a sustained SAC response. S2 cells co-expressing EGFP-Megator phosphomutants and mCherry-tubulin or H2B-mCherry were treated with Taxol or Colchicine and mitotic progression monitored through time-lapse microscopy. Graph represents mean+SD of the time that cells spent in mitosis.

4. Depletion of Megator in *mps1-null* mutant neuroblasts rescues mitotic fidelity

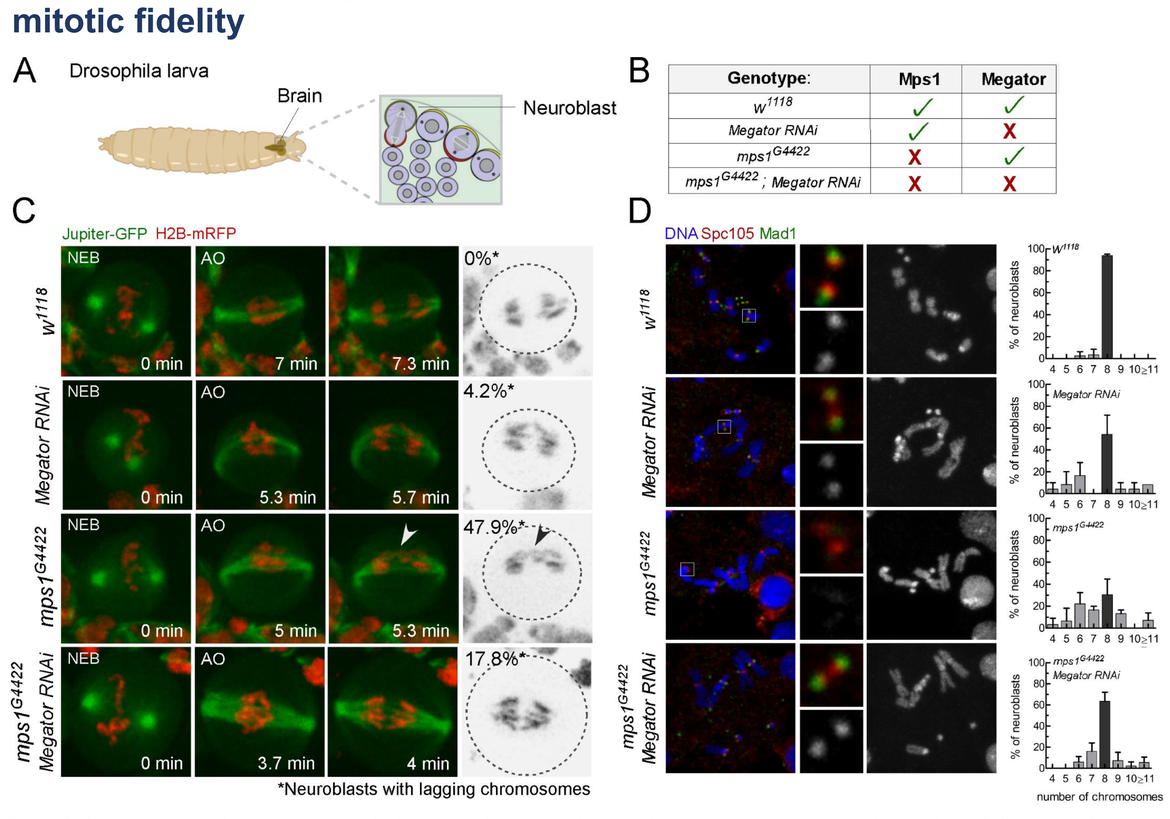

Figure 5: Depletion of Megator in *mps1-null* mutant neuroblasts rescues Mad1 kinetochore recruitment and mitotic fidelity. (A;B) Scheme of *Drosophila* neuroblasts from 3rd instar larva used to perform live cell imaging and immunofluorescence assays (A) and list of the genotypes used (B). (C) Stills from time-lapse movies of with the indicated genotypes and co-expressing Jupiter-EGFP and H2B-RFP. NEB - nuclear envelope breakdown; AO - anaphase onset. The percentage of neuroblasts exhibiting anaphases with lagging chromosomes is indicated. (C) Immunolocalization of Mad1 at kinetochores of neuroblasts with the indicated genotypes and corresponding ploidy histograms.

Mps1 controls timely dissociation of Mad1 from Megator to ensure a functional mitotic checkpoint signalling and genome stability

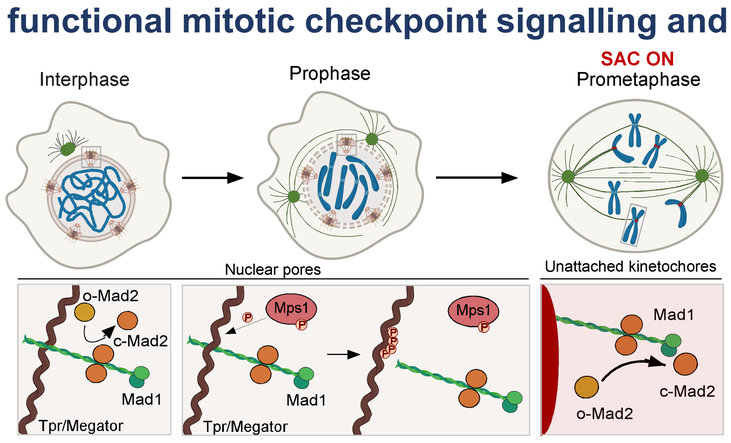

Figure 6: Phosphorylation of Megator by Mps1 licences Mad1 for kinetochores accumulation and robust checkpoint signalling. During interphase, Mad1-Mad2 is docked at nuclear pore complexes (NPCs) through Mad1 binding to Megator/Tpr. This is required for Mad1-Mad2 proteostasis and assembly of pre-mitotic MCC. During prophase, Mps1 phosphorylates Megator disrupting its interaction with Mad1. This is required for proper accumulation of Mad1 at mitotic kinetochores to levels that ensure a robust SAC signalling.

Our findings unveil a kinetochore-extrinsic mechanism regulated by Mps1 that licenses Mad1 to instate efficient SAC signalling at kinetochores and accurate chromosome segregation. Thus, a key function of non-kinetochore Mps1 is to coordinate Mad1 subcellular localization with cell cycle progression, so that both nuclear pores in interphase and kinetochores in mitosis generate anaphase inhibitors that preserve genomic stability.

Acknowledgements

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