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MPS1-mediated activation of AURORA-A is required for efficient correction of erroneous kinetochore-microtubule attachments

Master's Dissertation

Master in Molecular Genetics

Work supervised by

Bjorn Fredrik Johansson

Carlos Alberto da Silva Conde

October, 2023

DEDICATION

I wholeheartedly dedicate my Master's thesis to the most extraordinary person in my life, my beloved mother, Mónica Barbosa. Her importance in this academic journey cannot be overstated. She is the foundation upon which my quest for knowledge and achievement has been built.

First and foremost, my mother has given me the gift of life and has dedicated herself to giving me every opportunity for a better future. She was my unwavering source of support, ensuring that nothing essential was ever lacking. Through her selfless sacrifices, she not only nurtured my education but also cultivated my character.

Her commitment knows no bounds. Even on weekends, she would tirelessly drive me to the institute and patiently wait for me as I delved into the intricacies of my research in the lab. She encouraged and lifted me up during the most challenging and demanding moments of this academic journey. Her resilience and boundless love have been my constant motivation.

Despite the financial hardships she faced, she spared no effort to provide me with the best possible opportunities and experiences, all in the pursuit of my happiness and success. Her sacrifices and unwavering belief in me have been the pillars of my educational journey.

This thesis is a testament to the love and devotion of my mother, Mónica. It is a humble offering, a tangible symbol of my gratitude for her unwavering support and for shaping me into the person I am today. I am extremely fortunate to have her as my mother and this work is dedicated to her with deep love and appreciation.

ACKNOWLEDGEMENTS

As I reach the culmination of this important chapter in my academic journey, I am filled with a profound sense of achievement and gratitude. I must begin by expressing my deep affection for the thematic underpinnings of the project I undertook, which truly captivated me from the start.

I would like to thank my supervisor, Bjorn, for sharing his knowledge during my MSc in Molecular Genetics, in the subjects of Genetics and Molecular Biotechnology, and Metabolic Engineering.

I would like to thank all the members of my laboratory for their insightful comments at lab meetings: Cell Division & Genomic Stability and Epithelial Polarity & Cell Division – i3S research groups.

I am deeply grateful to my i3S supervisor, Carlos, whose unwavering support and mentorship has played a paramount role in my academic journey. His belief in my abilities, along with the invaluable opportunity to work in his laboratory, has had a transformative impact on both the content of this thesis and my personal and academic growth. Under Carlos' guidance, I have not only refined my research skills but also gained a deeper understanding of the cell division process. His mentorship was an anchor in times of uncertainty and fostered an environment of intellectual curiosity. In essence, Carlos' pivotal role as a supervisor has been instrumental in shaping the trajectory of my academic journey, and I am immensely grateful for the privilege of having him by my side.

Nelson holds a special place in my academic journey and I owe him a debt of gratitude that words can hardly express. His unwavering support and mentorship have been instrumental in my research endeavours. Nelson went above and beyond to ensure that I had all the resources and guidance I needed in the lab. He consistently demonstrated remarkable patience and dedication, always taking the time to explain complex techniques and experiments. His willingness to share his knowledge and expertise was invaluable and I owe many of my research skills and insights to his guidance. Nelson's contributions to my academic and personal growth are immeasurable and I am deeply grateful for the profound impact he has had on my research journey.

Margarida, during Nelson's 4-month absence, admirably assumed his function. Her tireless efforts and willingness to assist me during this period were truly commendable. Margarida's contributions were critical in ensuring the continuity of my research, and her ability to step in and provide the necessary guidance when it was most needed is proof of her commitment to our shared goals.

I would like to express my sincere gratitude to Tanguy, my best friend, whose invaluable help in reviewing my work and troubleshooting played a pivotal role in this thesis.

Last but not least, I have to thank my parents. Without them, I wouldn't be here to tell this story.

STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

RESUMO

A segregação precisa dos cromossomas na mitose requer que os cinetocoros-irmãos se liguem a microtúbulos provenientes de polos opostos do fuso (ligações anfitélicas). Devido ao facto de ligações impróprias ocorrerem frequentemente durante a mitose, resultantes da natureza estocástica das interações entre os cinetocoros e os microtúbulos, as células recorrem a mecanismos coordenados pelas cinases AURORA para detetar e corrigir ligações incorretas. A atividade da AURORA-A é particularmente importante para desestabilizar as ligações sintélicas de cromossomas que se encontram próximos do polo do fuso.

Neste estudo, relatamos que a proteína MPS1 recrutada para os cinetocoros potencia a ativação da AURORA-A nos centríolos de células de *Drosophila* S2. Apresentamos provas bioquímicas e celulares de que a MPS1 fosforila a AURORA-A na sua posição T360, o que subsequentemente promove a auto-fosforilação ativadora da AURORA-A na alça T (T311). Em concordância com isto, demonstramos que a ativação da AURORA-A nos centríolos aumenta significativamente quando se encontra na proximidade dos cinetocoros e diminui à medida que estes se afastam do polo do fuso em direção ao equador da célula. Notavelmente, a potenciação localizada da atividade da AURORA-A é suprimida em células onde a acumulação da MPS1 nos cinetocoros é experimentalmente impedida.

De forma surpreendente, a depleção da MPS1 aumenta dramaticamente a frequência de ligações sintélicas e, simultaneamente, atrasa a congressão dos cromossomas. Estes defeitos são, no entanto, resgatados quando a AURORA-A é tornada constitutivamente ativa (T311D) ou quando a posição T360 é convertida em aspartato mimético de fosforilação (T360D), ou mesmo quando uma versão da MPS1 ligada ao centríolo é expressa.

Estes resultados revelam um papel *sui generis* da MPS1 em auxiliar a AURORA-A na correção de ligações sintélicas de cromossomas polares, que, se não forem resolvidas, podem levar à aneuploidia, um *hallmark* do cancro.

Palavras-chave: MPS1; AURORA-A; Correção de Erros.

ABSTRACT

Faithful chromosome segregation in mitosis requires sister-kinetochores to attach to microtubules from opposite spindle poles (amphitelic attachments). Because improper attachments often occur during mitosis as a result of the stochastic nature of interactions between kinetochores and microtubules, cells use mechanisms orchestrated by AURORA kinases that sense and correct inaccurate attachments. The activity of AURORA-A is particularly important to destabilize syntelic attachments of chromosomes that are near the spindle pole.

Here, we report that MPS1 recruited to kinetochores, potentiates the activation of AURORA-A at centrosomes of *Drosophila* S2 cells. We provide biochemical and cellular evidence that MPS1 phosphorylates AURORA-A in its T360, which subsequently promotes the activating auto-phosphorylation of AURORA-A T-loop (T311). In line with this, we demonstrate that activation of AURORA A at centrosomes increases significantly when at the vicinity of kinetochores and decreases as kinetochores move away from the spindle pole towards the cell equator. Notably, the localized potentiation of AURORA-A activity is supressed in cells where kinetochore accumulation of MPS1 is experimentally impaired.

Strikingly, depletion of MPS1 increases dramatically the frequency of syntelic attachments and concomitantly delays chromosome congression. These defects are however rescued when AURORA-A is rendered constitutively active (T311D) or T360 is converted to phospho-mimicking aspartate (T360D), or even when a centrosome-tethered version of MPS1 is expressed.

These results unveil a novel role for MPS1 in assisting AURORA-A in the correction of syntelic attachments of polar chromosomes, which, if left unresolved, can lead to aneuploidy, a hallmark of cancer.

Keywords: MPS1; AURORA-A; Error Correction

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LIST OF ABBREVIATIONS AND ACRONYMS

A	Amino acid Alanine
A box/DAD	D-box activating domain
APC/C	Anaphase promoting complex / cyclosome
AURORA-A WT	AURORA-A wild-type
CCAN	Constitutive centromere-associated network
Cdc20	Cell division cycle 20
CDK1	Cyclin-dependent kinase 1
CENP	Centromere protein
D	Amino acid Aspartate
D-boxes	Degradation boxes
DNA	Deoxyribonucleicacid
dsRNA	Double-stranded RNA
E	Amino acid Glutamic acid
EGFP	Enhanced green fluorescent protein
FHA2	Forkhead-associated 2
FRET	Fluorescence resonance energy transfer
K-fibre	Kinetochore fibre
MCC	Mitotic checkpoint complex
MDS	MPS1 degradation signal
MPS1	Monopolar spindle 1
MPS1 WT	MPS1 wild-type
MR	Middle region
MTOCs	Microtubule-organising centres
nt	Nucleotides
NTE	N-terminal extension
OD	Optic density
PBS	Phosphate-buffered saline
PBSTF	PBS with 0.05% Tween20 and 10% fetal bovine serum
PBT	Pbs 0.05% tween20
PCR	Polymerase chain reaction
PFA	4% Paraformaldehyde in PBS
SAC	Mitotic spindle assembly checkpoint
TPR	Tetratricopeptide repeat
γ-TuRC	γ-tubulin ring complex

Units of measurement

rpm	revolutions per minute
µg	micrograms
µL	microliters
µM	micromolar
M	molar
mL	milliliters
mM	millimolar
°C	degrees Celsius

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CHAPTER 1 - INTRODUCTION

1.1 The cell cycle

In unicellular and multicellular eukaryotes, the cell cycle is tightly regulated to ensure that each cell undergoes division at the right time and properly (Barbosa et al., 2020; Conde & Gassmann, 2020). Disruptions in this regulation can lead to severe health conditions, including cancer (Leal-Esteban & Fajas, 2020; Matthews et al., 2022). Understanding the different phases of the cell cycle is crucial for studying cell biology and developing treatments for various diseases (Wenzel & Singh, 2018).

The cell cycle refers to the series of events that take place in a cell from the time it is formed until it divides into two daughter cells (Barbosa et al., 2020; Choi et al., 2017). It can be broadly divided into two main phases: the interphase and the mitotic phase (**Figure 1**) (Cheeseman & Desai, 2008).

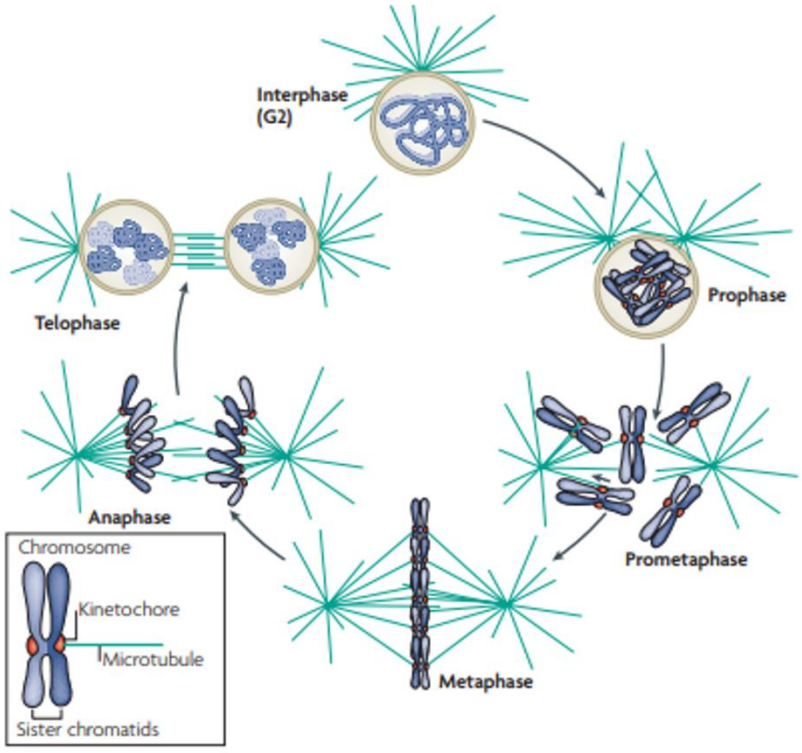


Figure 1. Schematic representation of G2 (interphase) and the mitotic stages (Figure from Cheeseman & Desai, 2008). Cells progress from interphase (G2) through prophase, prometaphase, metaphase, anaphase, and telophase.

Interphase is the longest phase of the cell cycle, and it can be further divided into three subphases: G1 (pre-replicative phase), S (synthesis phase), and G2 (post-replicative phase). The G1 phase is the gap between the end of cytokinesis of the previous division and the beginning of the S phase and it is the phase in which the decision of whether a cell will stay in a non-proliferative/quiescent state also known as G0 is taken. During G1, the cell grows and synthesizes RNA and proteins in preparation for DNA replication. At the end of G1, a series of regulatory pathways examines internal and external cues to assess whether or not the cell should move forward to the S phase of the cell cycle (G1 checkpoint). Some of the main cues analysed in this checkpoint are the cell size, the available nutrients in the medium,

the presence of growth factors, and DNA integrity. In the S phase, DNA replication occurs and each chromosome is duplicated, becoming two sister chromatids that are kept together until anaphase by the cohesin complex. Centrosome duplication also takes place in this phase. The G2 phase is the gap between the end of the S phase and the start of mitosis (M phase). During G2, the cell synthesizes RNA and proteins needed for mitosis. Furthermore, before the M phase, the cell goes through another checkpoint where DNA integrity and duplication are analysed to make sure the cell is ready for cycle division (Wenzel & Singh, 2018; Matthews et al., 2022; Carlton et al., 2020).

The M phase is the shortest phase of the cell cycle, but it is crucial for the cell division process (**Figure 1**). It can be divided into five subphases: prophase, prometaphase, metaphase, anaphase, and telophase (Cheeseman & Desai, 2008). During prophase, centrosomes separate and migrate to opposite sides of the cell, the chromatin condenses into discrete and highly compact chromosomes, the mitotic spindle begins to form with microtubules growing from each of the centrosomes and the nuclear envelope disassembles. Prometaphase is the phase in which the attachments of spindle fibres to the kinetochores of the chromosomes start forming. These interactions are mediated through large disc-shaped protein networks called kinetochores that assemble around the centromere (Matthews et al., 2022). The attachment of kinetochores to spindle microtubules is crucial for the proper alignment of the chromosomes in the cell equator, chromosome biorientation, and sister chromatids separation during subsequent phases of mitosis (Tanaka, 2008). In metaphase, the chromosomes align along the cell equator, and each pair of sister chromatids has each kinetochore bound to microtubules eradiating from opposite centrosomes. The spindle assembly checkpoint (also known as mitotic checkpoint), ensures that the metaphase-anaphase transition only occurs once all kinetochores are attached to spindle microtubules (Karess et al., 2013). During anaphase, the spindle fibres pull the sister chromatids apart and move them to opposite poles of the cell. Finally, in telophase, the chromosomes reach the opposite poles of the cell, the mitotic spindle disassembles, the chromatin decondenses, and the two new nuclear envelopes are reformed. The cell then undergoes cytokinesis, during which the cytoplasm is divided, giving rise to two daughter cells (Carlton et al., 2020; Wenzel & Singh, 2018).

1.2 Mitotic spindle

The spindle is a highly dynamic structure composed of tubulin, molecular motors, and other regulatory and structural molecules. In animals, centrosomes usually localize to the spindle poles and have three major functions: 1) organising spindle microtubules, 2) serving as the locus towards which chromosomes move in anaphase, and 3) specifying the axis that determines the orientation and position of the plane of

cytoplasmic division (Carlton et al., 2020). Each centrosome consists of two centrioles surrounded by pre-centriole material (PCM) (Jamasbi et al., 2022). Acting as microtubule-organising centres (MTOCs), centrosomes are responsible for microtubule nucleation, orientation, and anchoring. Individual microtubules are nucleated from ~25 nm ring-shaped structures (γ-TuRC: γ-tubulin ring complex) composed of a ubiquitous centrosome protein, γ-tubulin, and additional minor components. The microtubule nucleation sites are held together and associated with coiled-coil proteins, which are abundant in the centrosome and are thought to maintain the overall shape of the pericentriolar material by acting as a centrosome scaffold. To anchor the microtubule minus ends, molecules with affinity for microtubules end are embedded in the pericentriolar material. It has become clear that the centrosome is not only involved in microtubule organisation, but also in the regulation of a wide range of cellular activities, such as signal transduction and cell cycle control (Kuriyama, 2006; Popova et al., 2022).

1.2.1 Mitotic spindle assembly

The mitotic spindle is transient as it is assembled at the onset of the M phase and disappears entirely after the completion of mitosis (Carlton et al., 2020). Because it is composed of dynamic microtubules, the mitotic spindle is also dynamic, undergoing continuous morphological alterations in mitosis (Popova et al., 2022). These movements lead to their positioning at the spindle equator, before finally migrating to the poles following sister chromatid separation. This stochastic movement towards the equator corresponds to the onset of prometaphase and is referred to as chromosome congression. Chromosome congression truly represents the initial challenge of mitosis, culminating in the formation of a metaphase plate. It occurs in precise spatiotemporal coordination with the assembly of the mitotic spindle, which facilitates the microtubule-chromosome interactions essential for chromosome movement (Maiato et al., 2017). The assembly of a bipolar spindle is essential for the accurate segregation of chromosomes during mitosis (Barbosa et al., 2020; Conde et al., 2013b; Prosser & Pelletier, 2017).

Microtubules are assembled from dimers of α- and β-tubulin, a process that is initiated from γTuRCs that serve as a template for the assembly of the protofilaments that make up a growing microtubule and as a cap for the microtubule minus end (Prosser & Pelletier, 2017). Microtubules continuously polymerize and depolymerize, undergoing rapid cycles of growth and shrinkage before ultimately disassembling (Saurin, 2018).

Kinetochore microtubules (K-MTs) attach to the kinetochores (Tanaka, 2008), non-kinetochore microtubules (nK-MTs) extend towards the equator, where microtubules from each pole overlap, resulting in an antiparallel array that connects both spindle poles and astral microtubules (A-MTs) anchor spindle

poles to the cell cortex (**Figure 2**). The K-MTs play a crucial role in separating the sister chromatids during anaphase, segregating them towards the spindle poles. Attachment of a number of K-MTs to kinetochore results in their stabilization into a kinetochore fibre (K-fibre) (**Figure 2**). A-MTs extend from each spindle pole of the cell and are critical for spindle positioning because of the interaction with the cell cortex (Popova et al., 2022). nK-MTs originate from the opposing poles and help to separate them, providing stability to the spindle (Prosser & Pelletier, 2017).

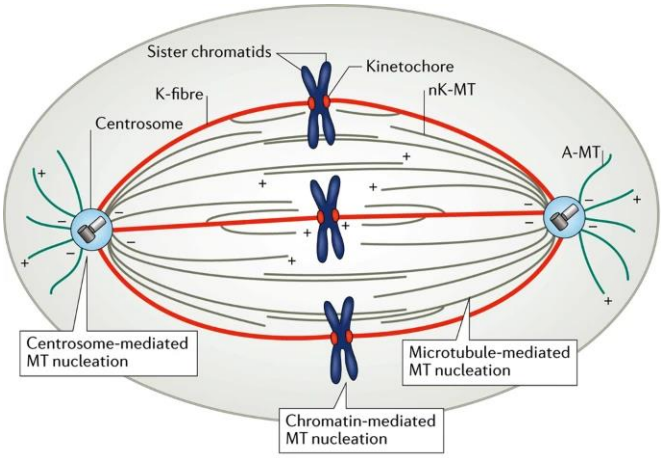


Figure 2. Overview of the mitotic spindle (Figure from Prosser & Pelletier, 2017). The spindle is a self-organized bipolar array of microtubules. Three pathways drive the nucleation of microtubules to form a common mitotic spindle: the centrosome, chromatin and microtubule-mediated microtubule nucleation pathways. The resulting spindle comprises three different types of microtubules: kinetochore microtubules (K-MTs), non-kinetochore microtubules (nK-MTs), and astral microtubules (A-MTs).

1.2.2 Centromere and kinetochore

A centromere is a chromatin structure that specifies the site for the assembly of a kinetochore (Jamasbi et al., 2022). The centromere is a critical component of the chromosome that plays a crucial role in the proper segregation of genetic material during mitosis (McKinley & Cheeseman, 2016). The centromere DNA is typically composed of repetitive sequences, and it is epigenetically marked by the presence of specific chromatin modifications, such as the histone H3 variant centromere protein A (CENP-A/ CENH3) (McKinley & Cheeseman, 2016; Prosser & Pelletier, 2017).

The kinetochore is a multiprotein complex assembled on the centromere (Nicholson & Cimini, 2011) and consists of three discrete regions: the inner kinetochore, the outer kinetochore, and the fibrous corona (Kops and Gassmann, 2020). The kinetochore has three main functions: 1) serve as the site where spindle microtubules attach to the chromosomes, 2) transduce the microtubules forces to chromosomes that are required for chromosomes to align at the spindle midzone (congression) during prometaphase and move toward the pole (segregation) in anaphase, and finally 3) determine the timing of anaphase

onset (McKinley & Cheeseman, 2016; Saurin, 2018). Because a kinetochore assembles in each chromatid, two kinetochores are arranged back-to-back and the microtubule attachment sites face opposite directions in a single chromosome (Krenn & Musacchio, 2015).

The stochastic nature of the interaction between microtubules and kinetochores throughout mitosis, particularly in prometaphase, often leads to different kinetochore-microtubule attachments: (1) amphitelic, (2) monotelic, (3) merotelic and (4) syntelic (Tanaka, 2008) (**Figure 3**). In an amphitelic attachment, each kinetochore of a chromosome is attached to spindle fibres from opposite spindle poles. This is the proper attachment conformation that ensures faithful chromosome segregation in anaphase (Barbosa et al., 2020; Manic et al., 2017; Moutinho-Santos et al., 2012). In a monotelic attachment, only one kinetochore of a chromosome is attached to spindle fibres, while the other is unattached. In a merotelic attachment, a single kinetochore is attached to spindle fibres from both spindle poles of the cell. Finally, in a syntelic attachment, both kinetochores of a chromosome are attached to spindle fibres from the same pole of the cell (**Figure 3**) (Barbosa et al., 2019; Barbosa et al., 2022; Krenn & Musacchio, 2015; Prosser & Pelletier, 2017). These last three attachment conformations, if not repaired before anaphase onset, can lead to chromosome segregation errors and result in aneuploidy and/or micronuclei formation, a hallmark of cancer (Tanaka, 2008; McKinley & Cheeseman, 2016; Audett & Maresca, 2020).

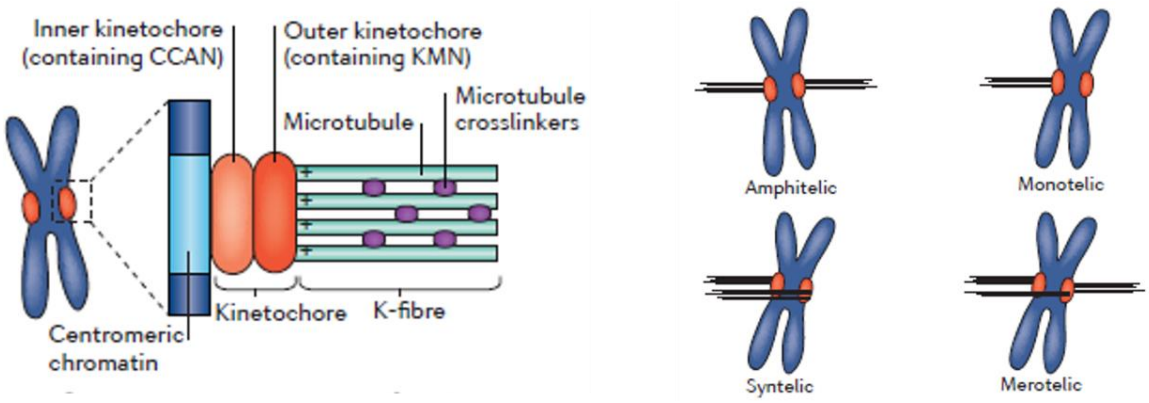


Figure 3. Representation of the spindle fibre attachments (Figure from Prosser & Pelletier, 2017). In order for chromosomes to attach to the spindle, they must be recognised by the spindle apparatus. A constitutive centromere-associated network (CCAN) localises to the centromere throughout the cell cycle, and members of this complex bind directly to CENPA/CENH3. The CCAN provides a link between the centromere and the inner kinetochore, while the outer kinetochore provides a physical link that allows microtubules to recognise and form stable attachments to each chromosome. Within the outer kinetochore, the KNL1-MIS12-NDC80 (KMN) network forms the core microtubule-binding site, while other kinetochore proteins act to modulate and amplify microtubule interactions. The attachment of 20-30 microtubules to the kinetochore results in the formation of a kinetochore fibre (K-fibre), which mediates chromosome movement.

1.3 The spindle assembly checkpoint

The spindle assembly checkpoint (SAC) is a regulatory mechanism that ensures the accurate segregation of chromosomes during cell division (Dou et al., 2015; Karess et al., 2013; London & Biggins, 2014). The SAC functions to prevent premature separation of sister chromatids by arresting mitosis until all kinetochores are properly attached to microtubules. Defects in the SAC can lead to chromosomal instability, aneuploidy, and cancer (Audett & Maresca, 2020; Conde et al., 2013b; Conde et al., 2013a; Jamasbi et al., 2022; Moura et al., 2017).

The SAC is activated by a complex network of regulatory proteins that monitor the attachment of kinetochores to microtubules (Lara-Gonzalez et al., 2021). SAC is imposed by the assembly of the mitotic checkpoint complex (MCC) at unattached or tensionless kinetochores (London & Biggins, 2014). This is a tetrameric complex composed of MAD2 in its closed conformation (c-MAD2), CDC20, BUBR1, and BUB3 proteins (Audett & Maresca, 2020; Cunha-Silva & Conde, 2020; Conde & Gassmann, 2020; Moura et al., 2017). MCC prevents chromosome segregation and mitotic exit by inhibiting an E3 ubiquitin ligase known as the anaphase promoting complex/cyclosome (APC/C). When microtubules bind to kinetochores, SAC signalling from those kinetochores is switched off (Barnum & O'Connell, 2014). Complete SAC silencing takes place when all kinetochores achieve stable attachments and are aligned at the metaphase plate (London & Biggins, 2014). At this point the MCC production ceases, the APC/C becomes active and anaphase takes place. The APC/C activation results in the ubiquitination and consequent degradation of SECURIN and CYCLIN B (Conde & Gassmann, 2020; Jamasbi et al., 2022). SECURIN degradation renders the protease SEPARASE able to cleave the cohesin rings holding the sister chromatids together, thus allowing chromatid separation and subsequent segregation. The degradation of CYCLIN B results in mitotic exit (Karess et al., 2013; Wenzel & Singh, 2018; Nicholson & Cimini, 2011; Tanaka, 2008). The presence of a single unattached kinetochore is sufficient to produce enough MCC to prevent APC/C activation. This ensures that anaphase only takes place once all kinetochores are bound to microtubules, thus safeguarding faithful chromosome segregation (Conde et al., 2013a; Chen et al., 2022; Lara-Gonzalez et al., 2021; Moura et al., 2017).

As the cell goes from prophase to prometaphase, microtubules grow from each spindle pole and undergo a highly dynamic "search and capture" routine in attempts to attach to kinetochores. Due to the stochastic nature of the interaction between microtubules and kinetochores, this is unpredictable and may result in both correct and erroneous attachments (Dou et al., 2015; Jamasbi et al., 2022). Amphitelic attachments silence the SAC because in this conformation sister kinetochores are bioriented and under tension (London & Biggins, 2014).

Sister chromatids align at the metaphase plate as a result of these stable attachments, and in anaphase, they segregate toward the opposing spindle poles (**Figure 4A**) (Barbosa et al., 2019; Barbosa et al., 2020; Manic et al., 2017). On the other hand, the previously mentioned incorrect attachments (monotelic, syntelic, and merotelic) are perceived as a threat since they might result in chromosomal mis-segregation if not corrected before anaphase onset (**Figure 4B**) (Barbosa et al., 2022). Both unattached kinetochores and kinetochores interacting with microtubules but lacking tension like monotelic and syntelic are reported to trigger SAC signalling (Nezi & Musacchio, 2009). This results in the generation of the aforementioned "wait anaphase signal" that halts the metaphase to anaphase transition as long as these attachments persist and until the bi-orientation (amphitelic attachment) of the entire set of sister chromatids is achieved (**Figure 4B**) (Krenn & Musacchio, 2015; Manic et al., 2017).

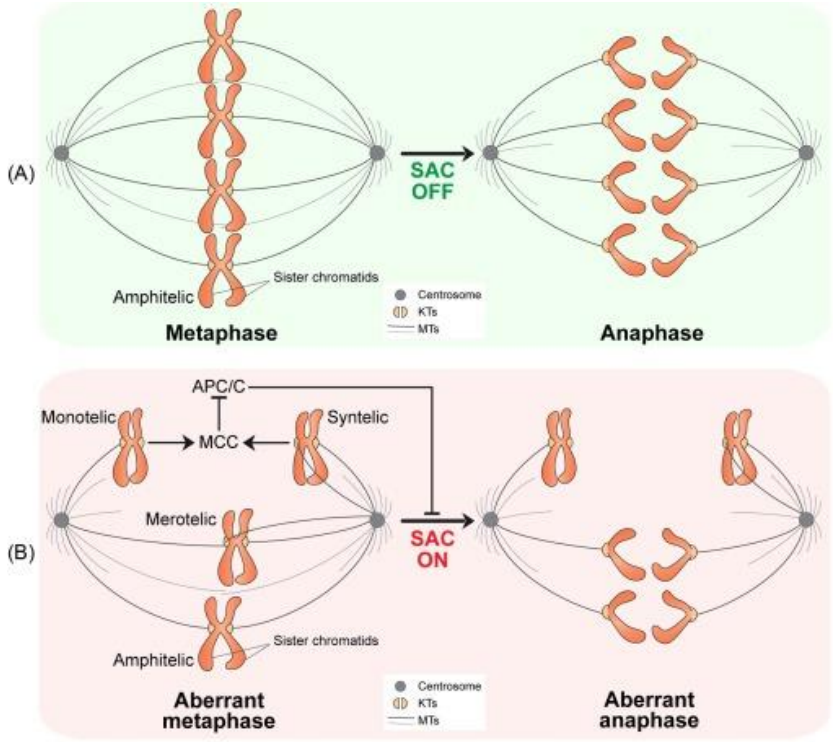


Figure 4. Kinetochore orientations and anaphase onset (Figure from Manic et al., 2017). (A) Bioriented sister chromatids align at the metaphase plate during standard mitoses before being distributed in the two daughter cells during anaphase. (B) In early or abnormal mitoses, monotelic and syntelic attachments trigger the SAC by releasing the MCC from tensionless or unattached kinetochores, inhibiting the APC/C. By doing so, the transition to anaphase is delayed until all kinetochores are bioriented, avoiding chromosome mis-segregation.

It is therefore imperative for the cell, to correct erroneous kinetochore-microtubule attachments in order to allow cell cycle progression and prevent genome mis-segregation.

The SAC and error correction pathway (discussed in the next section) are closely intertwined. For instance, any incorrectly attached chromosome will detach from microtubules, reactivating SAC signalling and both processes share numerous molecular players (Krenn & Musacchio, 2015; Pachis & Kops, 2018).

1.4 Error correction mechanism

Error correction is the process by which improper kinetochore-microtubule attachments are destabilised so that the chromosome can reach an amphitelic configuration. Each replicated chromosome must be bioriented prior to anaphase onset to ensure the equitable transmission of the genome. However, biorientation is not inherent, as erroneous kinetochore-microtubule attachments frequently form and must be resolved to avoid aneuploidy. The canonical error correction pathway described to destabilise syntelic attachments is thought to be mainly mediated by the centromere-enriched AURORA-B kinase (Salemi et al.,2013). However, more recent studies have shown that the centrosome-associated AURORA-A kinase also has an important role in this regulation (Ye et al., 2015; DeLuca et al. 2018). In addition, the spindle checkpoint kinase MPS1 has been proposed to be directly involved in error correction. MPS1 coordinates error correction of kinetochore-microtubule attachments with spindle checkpoint initiation by transiently localising to end-on attached kinetochores. Ultimately, it promotes microtubule release and removal of erroneous microtubule attachments (Hayward et al., 2022).

1.5 Protein kinases in mitotic regulation

Protein kinases are essential for controlling mitosis and guaranteeing the proper and efficient execution of the intricate procedures involved in cell division (Bayliss et al., 2012). Several protein kinases are involved in mitotic regulation, such as CDK1 (cyclin-dependent kinase 1), AURORA kinases, POLO/PLK1 (polo-like kinase 1), and MPS1 (monopolar spindle 1) (Colón-Marrero et al., 2021; Moura & Conde, 2019). This report focuses on the biochemical and cellular mechanisms involving MPS1 and AURORA-A kinases.

1.5.1 MPS1 kinase

MPS1 is a dual-specificity kinase that is mainly expressed in proliferating cells (Liu & Winey, 2012; Manic et al., 2017). It phosphorylates a wide range of substrates, including microtubule-associated proteins, kinetochore proteins, and checkpoint proteins, to ensure that cell division proceeds accurately and efficiently (Koch et al., 2019; Hayward et al., 2022).

MPS1 has a highly dynamic intracellular location. During interphase, MPS1 is largely found in the cytosol, but in late G2 it starts to accumulate at the nuclear envelope. During prometaphase, MPS1 accumulates

rapidly at unattached kinetochores. Some studies have suggested that MPS1 removal from the kinetochore coincides with the attachment to the spindle microtubules, but more recent studies show that MPS1 is still present at attached kinetochores (Althoff et al., 2012; Dou et al., 2015; Pachis & Kops 2018; Moura et al., 2017; Hayward et al., 2022).

1.5.1.1 Structure of MPS1

Members of the MPS1 kinase family are 85–95 kDa proteins and have conserved C-terminal kinase domains (Liu & Winey, 2012). The N-terminal extension (NTE), the tetratricopeptide repeat (TPR), and the middle region (MR) (**Figure 5**) are three N-terminal functional modules that allow MPS1 kinase to localise to kinetochores (Lara-Gonzalez et al., 2021). For MPS1 to function properly, homodimer formation is promoted by the TPR repeat (Dou et al., 2015). However, the absence of a TPR domain in *Drosophila* MPS1 suggests a different mechanism underlying MPS1 kinetochore localisation in flies (Conde et al., 2013a). The control of cellular MPS1 levels is mediated by the MPS1 degradation signal (MDS) (Bayliss et al., 2012). Structurally, the MPS1 kinase domain adopts the canonical kinase architecture, with an N-terminal region connected by a hinge loop to the C-terminal region containing the catalytic loop, the activation loop (T-loop), and the P+1 loop (Pachis & Kops, 2018).

Figure 5. Schematic representation of human MPS1 motifs (Figure adapted from Roorda et al., 2018). Structurally, MPS1 consists of an N-terminal region containing 3 functional regions – NTE, TPR, and MR – and a C-terminal region which is the kinase domain. These domains are connected by the MDS region.

1.5.1.2 MPS1 function

MPS1 is an upstream component of the SAC signalling cascade and has been proposed to directly contribute to sister kinetochore bi-orientation (Dou et al., 2015; Koch et al., 2019; Roorda et al., 2019). There, MPS1 is responsible for the initiation and transduction of the SAC cascade by (1) promoting the hierarchical recruitment of most SAC proteins to the kinetochore, (2) promoting the assembly of the MCC complex and its inhibitory function on APC/C, (3) helping to maintain SAC signalling, and (4) helping to coordinate the AURORA B-mediated error correction mechanism with the SAC (**Figure 6**) (Audett & Maresca, 2020; Jamasbi et al., 2022; Manic et al., 2017; Saurin, 2018). Importantly, more recent studies

have suggested a direct role for MPS1 in the destabilisation of kinetochore-microtubules as an error correction mechanism (Hayward et al. 2022).

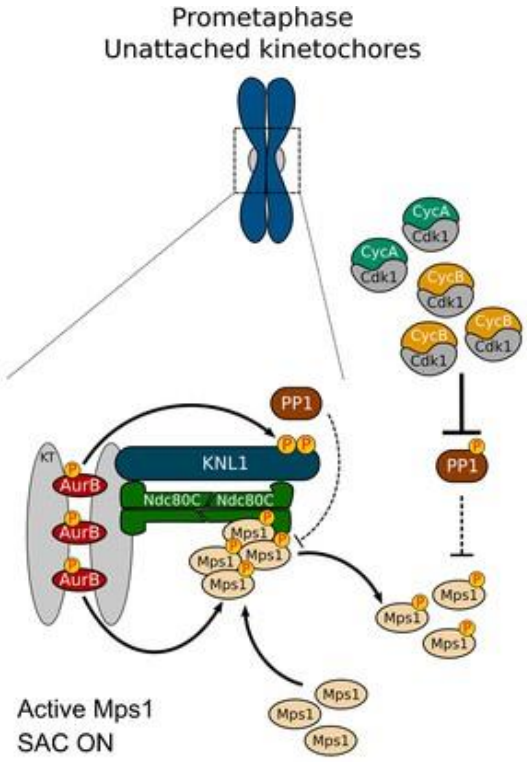


Figure 6. Schematic representation of the regulation of Mps1 activation in mitosis (Figure from Moura et al., 2017). During prometaphase, AURORA-B potentiates the recruitment of MPS1 to unattached kinetochores and phosphorylates KNL1/Spc105 to limit PP1 kinetochore association. This allows the accumulation of active MPS1 at unattached kinetochores to instate efficient MCC assembly.

1.5.2 AURORA kinases

The AURORA kinase family is a group of serine/threonine protein kinases that play a crucial role in the regulation of the cell cycle and mitotic processes (Colón-Marrero et al., 2021). For this reason, to ensure faithful mitosis, AURORA kinases activity has to be fine-tuned in both spatial and temporal manners (Baldini et al., 2016; Marumoto et al., 2005).

AURORA kinases are essential components of all eukaryotic mitotic machinery. AURORA A, B, and C are the only known members of the AURORA kinase family in mammals (Yan et al., 2016). In the *Drosophila* genera, the AURORA C type is missing (Bayliss et al., 2012). During mitosis, AURORA-A is predominantly found at centrosomes, whereas AURORA B kinase localizes to the inner centromere (Baldini et al., 2016; Mou et al., 2021).

Briefly, AURORA B regulates spindle assembly and proper kinetochore-microtubule attachments (Colón-Marrero et al., 2021; Jamasbi et al., 2022; Mou et al., 2021). Its activity is critical to resolve syntelic

attachments (Karess et al., 2013; Funabiki, 2019) (**Figure 7**). Moreover, two recent studies have shown that AURORA-A is also important for the correction of these attachments (Ye et al. 2015; DeLuca et al. 2018).

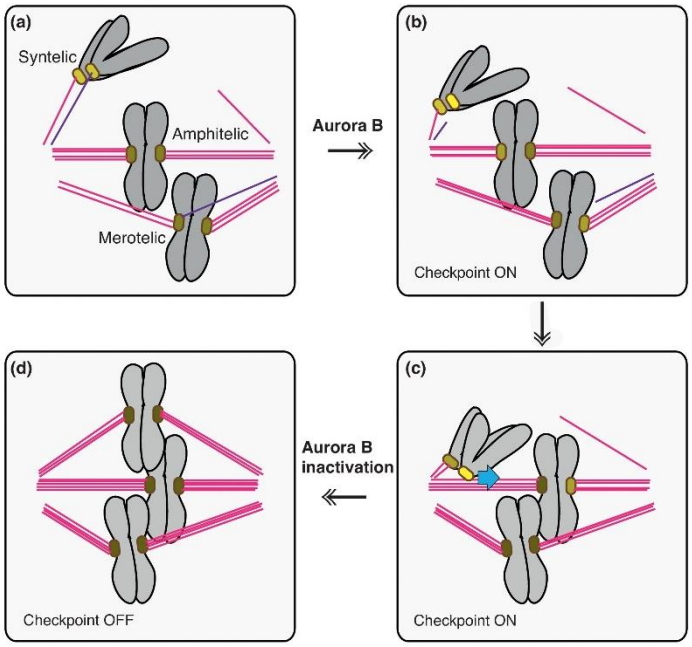


Figure 7. Simplified schematic representation of AURORA-B-dependent error corrections of aberrant attachments (Figure from Funabiki, 2019). (a) Normal (amphitelic) and aberrant (syntelic and merotelic) attachments. (b) AURORA-B-mediated microtubule dissociation from aberrant attachments. The formation of an unattached kinetochore (yellow) activates the spindle checkpoint. (c) Kinetochores attached to the lateral side of microtubules can slide toward the spindle equator via the CENP-E motor. Lateral attachment does not silence the checkpoint (yellow). (d) Conversion of lateral attachment to end-on attachment and stabilisation of amphitelic attachments, which silences the spindle checkpoint. The brightness of each kinetochore indicates the level of AURORA-B-dependent phosphorylation or checkpoint strength.

1.5.2.1 Structure of AURORA-A

AURORA-A kinases consist of a β-stranded N-terminal lobe and an α-helical C-terminal lobe connected by a hinge region that controls the active conformation. AURORA-A is structurally divided into three domains: N-terminal, catalytic, and C-terminal (**Figure 8**) (Baldini et al., 2016). Different intracellular localisations, substrate selectivity, and activities depend on the N-terminal domain. Autophosphorylation of the AURORA-A activation loop (T-loop) occurs at Threonine311 in *Drosophila* (Threonine288 in mammals). The T-loop is located in the large and conserved catalytic domain and is vital for the kinase activity of the protein (Willems et al., 2018).

This protein features several degradation boxes (D-boxes) (Mou et al., 2021; Willems et al., 2018; Yan et al., 2016). Adaptors of the APC/C recognize the D-box activating domain (A box/DAD), which designates

these proteins for destruction via the ubiquitin/proteasome-dependent pathway (Baldini et al., 2016; Marumoto et al., 2005). Other regulatory motifs found in AURORA are the KEN-motifs which also serve for degradation signalling (de Almeida Magalhães et al., 2020).

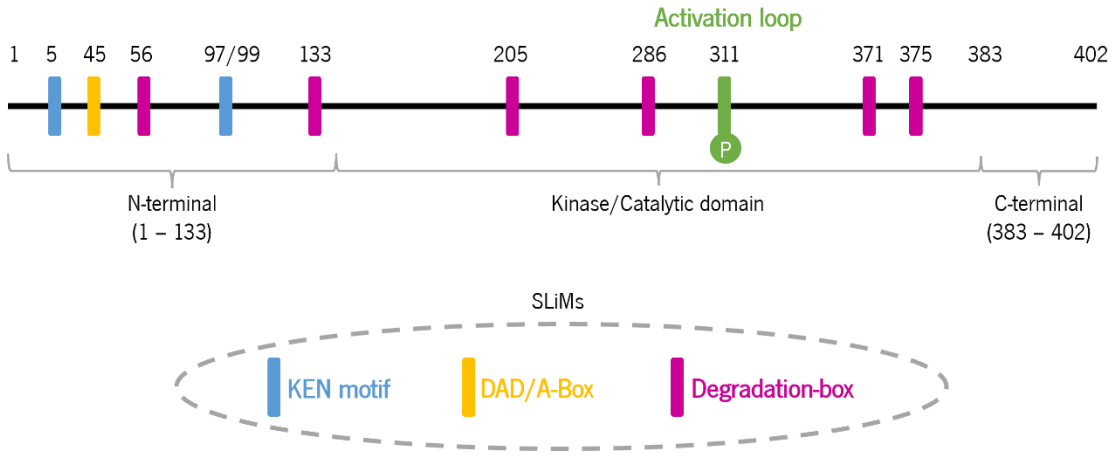


Figure 8. Schematic representation of *Drosophila* AURORA-A motifs (Figure adapted from Willems et al., 2018). Structurally, AURORA-A consists of an N-terminal and a C-terminal region, both belonging to the regulatory domain. The N-terminal region contains KEN motifs and A-box residues. Both the N- and C-terminal regions contain D-box residues. All domains belong to the group of SLiMs (Short Linear Motifs).

1.5.2.2 AURORA-A functions

AURORA-A is a regulator of mitotic progression, where it plays a crucial role in mitotic entry, centrosome separation and maturation, chromosome alignment, mitotic spindle assembly, and cytokinesis (de Almeida Magalhães et al., 2020; Colón-Marrero et al., 2021; Yan et al., 2016).

In G1 phase, the level of AURORA-A is barely detectable. During S phase, a small amount of AURORA-A is first detected at centrosomes. In late G2 phase, AURORA-A accumulates at the centrosomes and undergoes auto-activation. During prometaphase and metaphase, active AURORA-A localises to bipolar spindles and spindle poles following nuclear envelope breakdown (Marumoto, 2005). At the metaphase-anaphase transition, the majority of AURORA-A is inactivated and degraded. However, a small fraction of AURORA-A remains on centrosomes and spindles at the onset of anaphase and localises to the spindle midzone and centrosomes during late anaphase and telophase/cytokinesis (Yan et al., 2016).

In the current scientific consensus, it is understood that AURORA-A kinase plays a pivotal role in the regulation of kinetochore-microtubule interactions during cell division. It opposes polar ejection forces and facilitates the phosphorylation of kinetochore substrates near spindle poles, thereby destabilizing kinetochore-microtubule attachments in this region. This spindle pole-centred AURORA-A activity gradient

contributes to the correction of misoriented kinetochore-microtubule attachments, ensuring efficient error correction in both *Drosophila* and mammalian cells (DeLuca et al., 2018; Ye et al., 2015).

1.6 Objectives of the present study

Previous findings in the host laboratory have shown through *in vitro* kinase assays that in the presence of MPS1 activity, AURORA-A activation status is significantly increased (**Figure 9A, B**). The activation status was probed using a phospho-specific antibody for AURORA-A T-loop autophosphorylation (T288Ph). Importantly, AURORA-A chemical inhibition in the presence of active MPS1 prevented the previously observed increase (**Figure 9A, B**). This observation suggests that MPS1 is phosphorylating AURORA-A in a residue to potentiate its activating auto-phosphorylating in the T-loop. Mass spectrometry analysis led to the identification of Threonine 360 (T360) as an MPS1-phosphorylated residue. This residue is conserved in both human (T337) and *Drosophila* AURORA-A sequences and is preceded by two negatively charged amino acids – aspartate (D) and glutamic acid (E) – which resembles the described consensus phosphorylation sequence associated with the MPS1 kinase (**Figure 9C**). Figure 9D displays the differences in T-loop accessibility of *Drosophila* AURORA-A WT (blue) and AURORA-A T360D (red) structures.

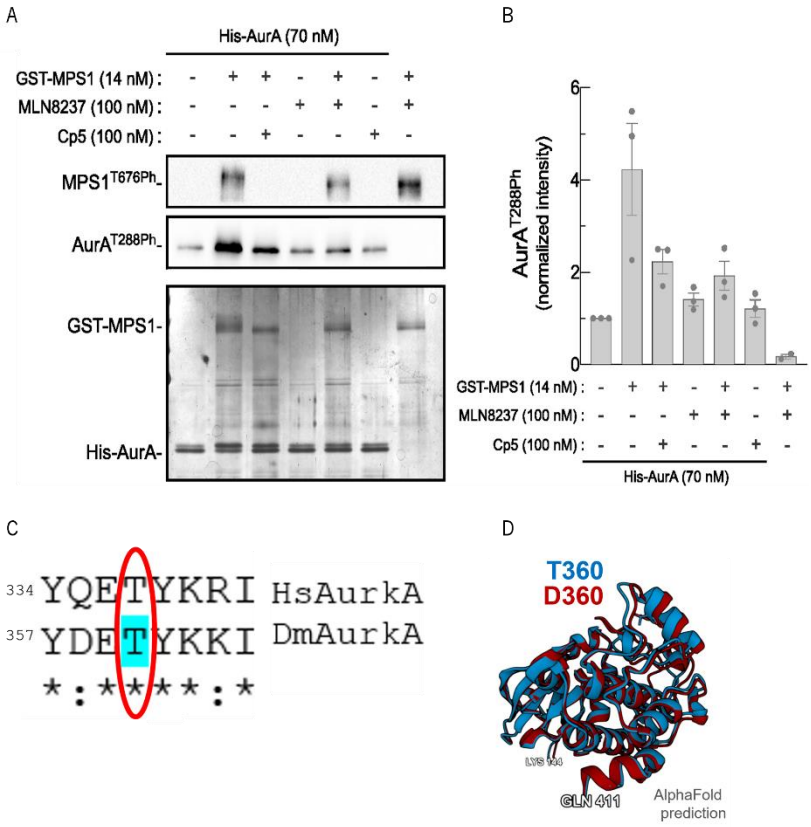
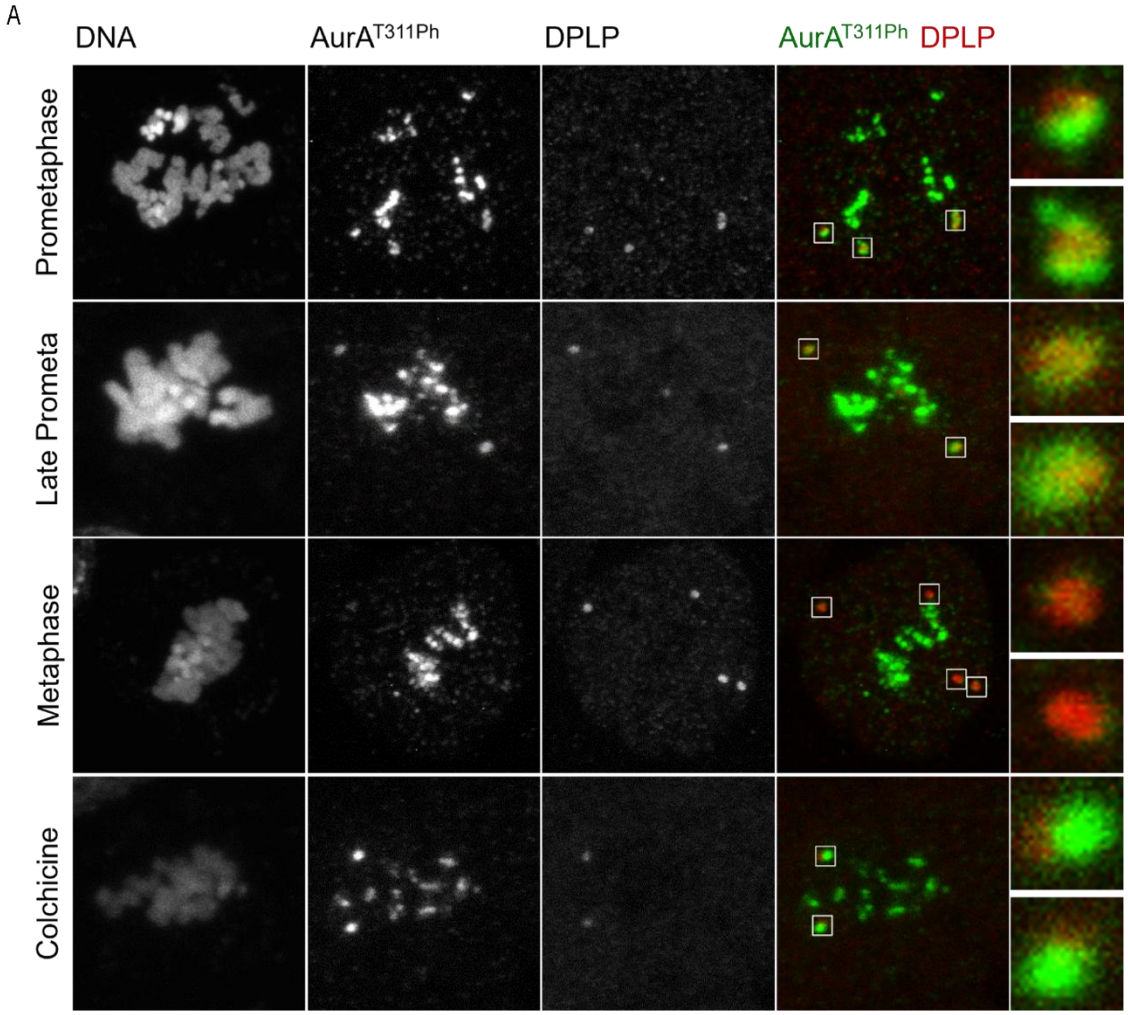


Figure 9. MPS1 potentiates AURORA-A T-loop autophosphorylation *in vitro* (Figure from Leça et al., unpublished)**.** (A) *In vitro* kinase assay of recombinant human AURORA-A and MPS1 in the indicated conditions. The assay was performed at 30 °C for

30 minutes. Phosphorylation levels were assessed through western blot using phosphospecific antibodies for MPS1 T-loop autophosphorylation (MPS1 T676Ph) and AURORA-A T-loop autophosphorylation (AURORA-A T288Ph). Total levels of proteins were determined by silver staining of the gel. (B) Quantification of the normalized intensity of the AURORA-A T-loop autophosphorylation bands observed in (A). (C) Clustal omega alignments of amino acid residues of Human (HsAurkA) and *Drosophila* (DsAurkA) AURORA-A. Highlighted in red is the putative MPS1-phosphorylation site identified by Mass Spectrometry. (D) AlphaFold prediction of *Drosophila* AURORA-A WT (blue) and AURORA-A T360D (red) structures. Superimposition of structures shows differences in T-loop accessibility.

To add to this, previous data in the host laboratory, using a phosphospecific antibody for AURORA-A activating T-loop autophosphorylation, has shown that centrosomal activation of AURORA-A is higher in prometaphase, in either asynchronous or MG132-treated *Drosophila* S2 cells (**Figure 10A, B**). Accordingly, a correlation between AURORA-A activation status and the kinetochore-centrosome distance was found. The further away the kinetochore is from a centrosome, the lower the activation status of AURORA-A (**Figure 10A, C**). Strikingly, preventing MPS1 expression or recruitment to kinetochores significantly decreases the levels of AURORA-A activation in prometaphase cells (**Figure 10D**).



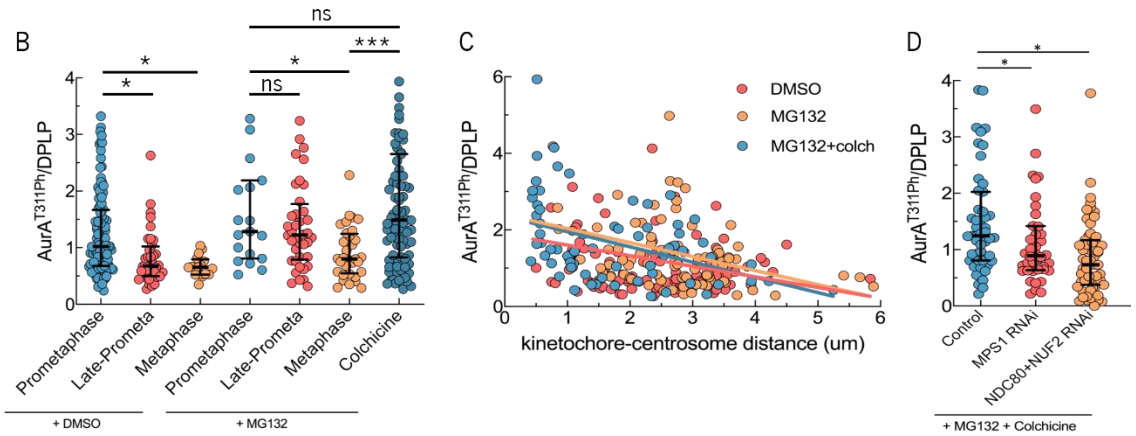


Figure 10. AURORA-A activation status decreases with kinetochore-microtubule attachments (Figure from Leça et al., unpublished). (A) Representative images of confocal immunofluorescence analysis of dividing *Drosophila* S2 cells in the indicated mitotic stages. (B & D) Quantification of the normalized intensity of AURORA-A T-loop autophosphorylation in the indicated conditions. (C) Correlation between the normalized intensity of AURORA-A T-loop autophosphorylation and the kinetochore-centrosome distance. Statistical analysis was calculated using a non-parametric Kruskal-Wallis 1-way ANOVA with Dunn's multiple comparison test. P values: ns, not significant; *< 0.05; **< 0.01; ***< 0.001.

To assess AURORA-A activity, a previously described FRET (fluorescence resonance energy transfer) sensor known to be a substrate of AURORA kinases (Ye et al. 2015) was redesigned to include a CM2 domain to ensure its centrosomal localisation (**Figure 11A, B**). Briefly, this FRET sensor is a fusion protein composed of 4 domains: a fluorescent donor (blue molecule, mTurquoise2), an FHA2 domain, an AURORA-A substrate sequence, and a fluorescent acceptor (yellow molecule, mVenus). The donor is excited with blue light and emits green light due to fluorescence. However, a resonance effect (known as FRET) may happen between the donor and the acceptor leading to an emission of lower energy than the usual emission by the donor. In this case, when the AURORA substrate sequence is phosphorylated, the FHA2 domain interacts strongly with it, resulting in a conformational change that decreases the levels of FRET (**Figure 11A**). Hence, the levels of FRET from the sensor can be used to assess the levels of centrosomal AURORA-A substrate phosphorylation/AURORA-A activity: the higher the FRET levels, the lower AURORA-A activity (**Figure 11B**). In agreement with AURORA-A activation status described in the previous results, the FRET sensor shows higher levels of AURORA-A activity in prometaphase. However, when MPS1 is depleted, prometaphase cells exhibit metaphase levels of AURORA activity even in prometaphase. Moreover, when MPS1 is overexpressed and present across the whole cytoplasm (including near the centrosomes) even metaphase cells exhibit high levels of AURORA-A activity. To put it succinctly, these results suggest that MPS1 present at kinetochores near the centrosome are able to directly phosphorylate AURORA-A, potentiating its autoactivation and activity.

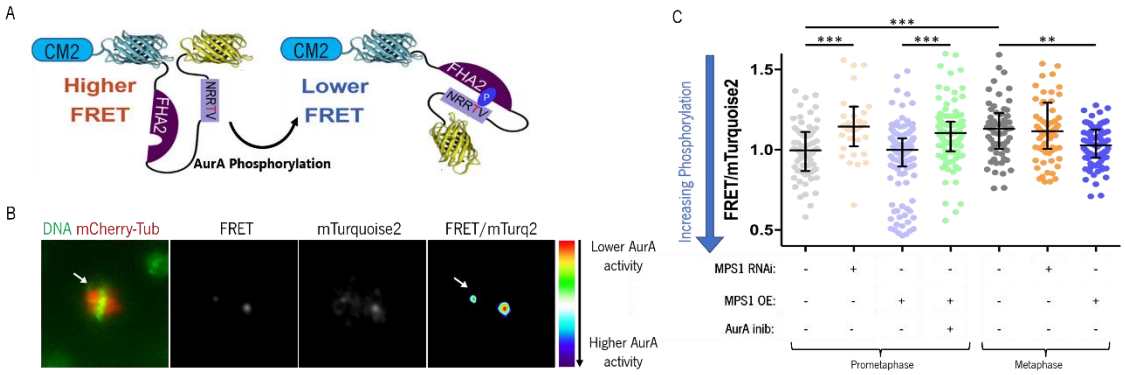


Figure 11. High centrosome AURORA-A activity in prometaphase is caused by the presence of MPS1 near the poles (Figure from Leça et al., unpublished). (A) Schematic of the centrosome-targeted CM2-Aurora FRET sensor used in this study. (B) Representative images of the FRET reporter of a late prometaphase with one chromosome near the left centrosome. The FRET emission ratio image ''FRET/mTurq2'' is presented with the "Thermal" LUT from ImageJ and its respective color wedge. The white arrow indicates the position of a chromosome near the left centrosome. (C) Quantification of the normalized FRET levels in the indicated conditions. Statistical analysis was calculated using a non-parametric Kruskal-Wallis 1-way ANOVA with Dunn's multiple comparison test. P values: ns, not significant; *< 0.05; **< 0.01; ***< 0.001.

Besides, data from the host laboratory has also shown that MPS1 overexpression in S2 cells causes an abnormal spindle rotation during mitosis. Importantly, this phenotype is also observed when a constitutively active version of AURORA-A (T311D) is expressed, and AURORA-A inhibition or depletion in S2 cells overexpressing MPS1 resulted in a rescue of the rotation. It was then tested whether phosphorylation of the T360 residue promotes AURORA-A hyperactivation – like the one in AURORA-A T311D. To do that, a phosphomimetic mutation was performed in the T360 (T360D) and the spindle rotation was analysed by live imaging. In parallel, a phosphodefective mutant was created in which the residue becomes unphosphorylatable because it is mutated to an alanine (T360A). Live imaging of *Drosophila* S2 cells expressing EGFP-AURORA-A and αTubulin-mCherry allowed the evaluation of the spindle rotation phenotype in each of the AURORA-A mutants. Expression of the AURORA-A T360D mutant in S2 cells shows a rotation phenotype, suggesting that in this condition, AURORA-A is hyperactive as in AURORA-A T311D and when MPS1 is overexpressed, in contrast to the phosphodefective mutant (T360A) which does not show a significant spindle rotation (**Figure 12**).

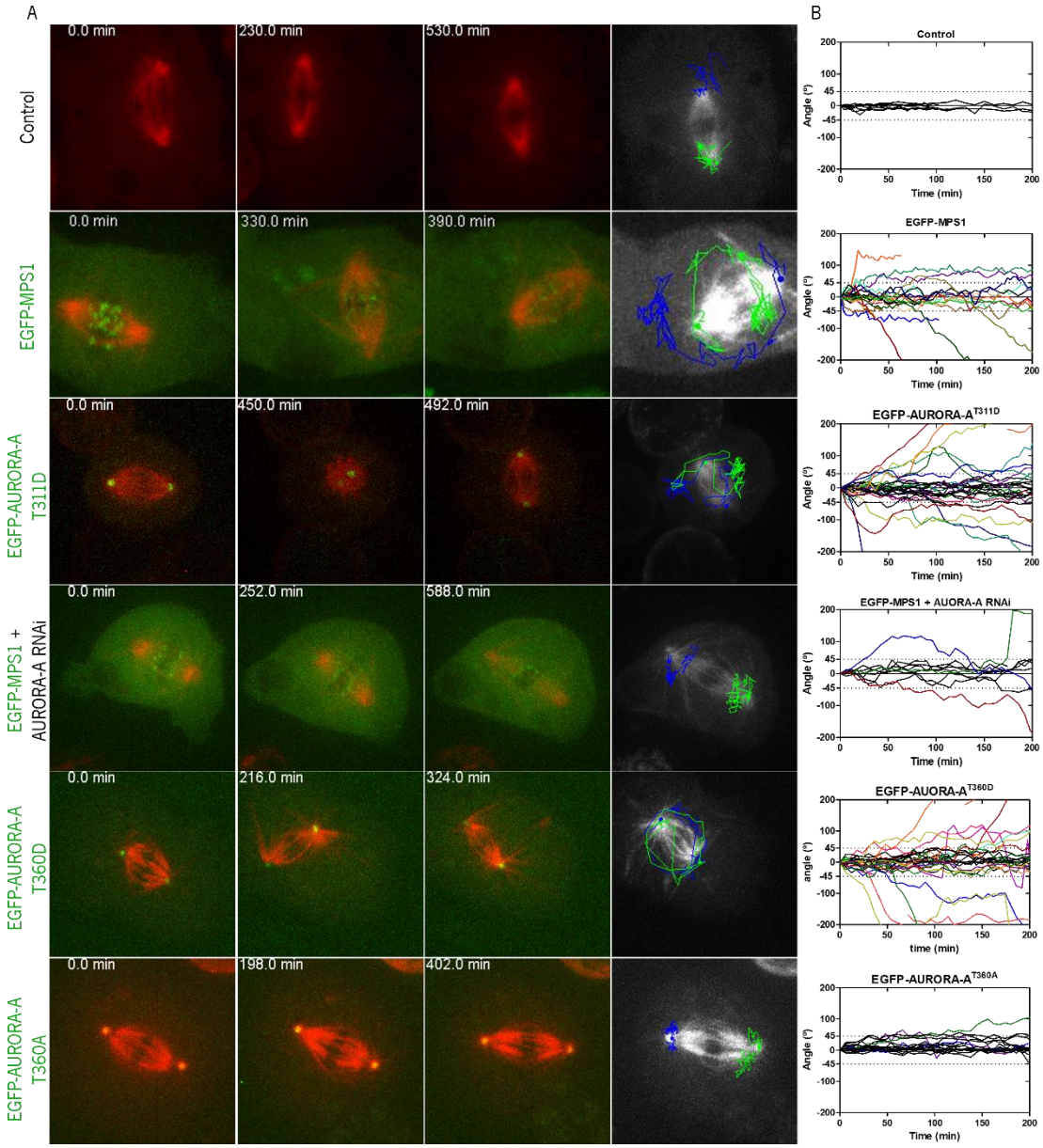


Figure 12. Phosphorylation of AURORA-A T360 by MPS1 increases AURORA-A activity (Figure from Leça et al., unpublished)**.** (A) Representative spindle positioning in *Drosophila* S2 cells expressing EGFP-AURORA-A and αTubulin-mCherry in the indicated conditions monitored by time-lapse microscopy. Selected stills of live-cell imaging are depicted and the respective time is shown in min. Spindle pole positions (centrosomes) are represented by the green and blue spots. The green and blue lines represent all the positions tracked along the movie, respectively. (B) Time-course quantification of the angle between the pole-to-pole axis in each time frame and the initial position for the indicated conditions. Black lines represent cells that never exceeded the 45-degree threshold relative to their initial position.

These findings further suggest that AURORA-A T360 phosphorylation by MPS1 potentiates AURORA-A activation.

As previously discussed, MPS1 and AURORA-A have recently been described as having a role in the correction of erroneous kinetochore-microtubule attachments (Ye et al. 2015; DeLuca et al. 2018; Hayward et al. 2022). However, it is unclear if they work through different mechanisms (**Figure 13, Model 1**), or if they are part of the same signalling cascade. Moreover, if they do work through the same pathway, it is then critical to clarify which one of them is the upstream regulator of the other (**Figure 13, Model 2 or 3**), in order to better understand the mechanisms by which syntelic attachments are corrected in mitosis. The results discussed in this section suggest that there is a direct interaction between both kinases that happens mainly in early mitosis when erroneous attachments are frequently formed. Therefore, the aims of this study are to uncover a potential physiological interaction between MPS1 and AURORA-A in dividing cells and to understand if it has any relevance in the correction of erroneous attachments and proper chromosome congression to the metaphase plate.

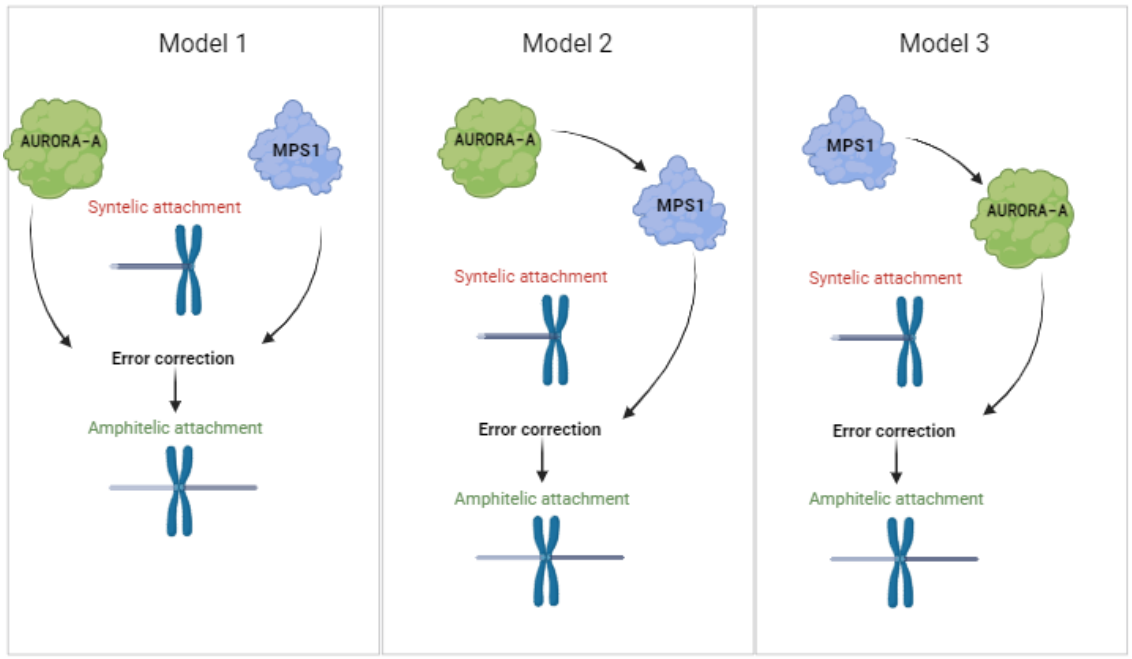


Figure 13. Schematic representation of hypothetical error-correction models involving MPS1 and AURORA-A (Figure created with Biorender.com)**.** In Model 1 AURORA-A and MPS1 work independently of each other in correcting syntelic attachments. In Model 2 AURORA-A acts upstream of MPS1 which in turn directly destabilises erroneous attachments. Model 3 illustrates the hypothesis in which MPS1 acts upstream of AURORA-A in an AURORA-A-mediated error correction signalling.

CHAPTER 2 - MATERIALS AND METHODS

2.1 Cloning and mutagenesis

PCR reactions to generate the desired constructs were conducted using Phusion polymerase (New England Biolabs, Ipswich, MA). The resulting PCR products were then incubated with DpnI restriction enzyme (New England Biolabs) for 1 hour to remove the template plasmid from the mix. Transformation of competent bacteria was then carried out to select positive clones.

To generate AURORA-A T360A and AURORA-A T360D mutants, by site-directed mutagenesis, the following primers harbouring the desired mutation and a MboI restriction site were employed:

Fw dAURORA-A T360 MboI: 5' – CTACAAGAAGATCCTCAAGGTGG – 3' (23nt)

Rev dAURORA-A T360A: 5' – ATCTTCTTGTAGGCCTCGTCATAG – 3' (24nt)

Rev dAURORA-A T360D: 5' – ATCTTCTTGTAGTCCTCGTCATAG – 3' (24nt)

Sanger sequencing reactions were performed on each of the plasmids generated to confirm their sequences.

2.2 Rubidium Chloride competent cells generation and transformation

To produce competent *Escherichia coli* DH5α cells (Invitrogen), 5 mL of a starting culture (grown overnight, at 37 °C) was used to inoculate 500 mL of LB medium, which were grown at 37 °C until they reached an OD595 of 0.5. At this time, cells were cooled down on ice, for 15 minutes. Afterward, the culture was centrifuged for 10 minutes at 4500 rpm and 4 °C to collect the cell pellet, which was resuspended in 30 mL of Tfb I (100 mM RbCl, 50 mM MnCl2 · 4H2O, 30 mM Potassium Acetate, 10 mM CaCl2 · 2 H2O and 15% Glycerol, prepared in deionized H2O) and incubated for another 15 minutes on ice. Then, cells were centrifuged for 5 minutes at 4000 rpm and 4 °C, and the cell pellet was resuspended in 6 mL Tfb II (0.2 M MOPS, 10 mM RbCl, CaCl2 · 2 H2O and 15% Glycerol). Aliquots were frozen in dry ice and stored at -80 °C.

For each transformation, one 50 μL aliquot of DH5α competent cells was defrosted on ice for a short period of time. About 5 μL of a recombination reaction were then added to the cells and the mixture was incubated on ice for 20 minutes.

The transformation was induced through heat-shock at 42 °C for 45-60 seconds. After 2 minutes on ice, 200 μL of LB medium was added and the culture was incubated at 37 °C for 1 hour. To select transformed bacteria, cells were plated on LB agar supplemented with specific antibiotics (ampicillin or kanamycin were used at the final concentrations of 100 μg · mL-1 and 50 μg · mL-1, respectively).

Plasmid extractions were done with Fast-n-Easy Plasmid Mini-Prep Kit (Jena Bioscience GmbH) according to the manufacturer's instructions.

2.3 *Drosophila* S2 cell transfection

The transfection of recombinant plasmids into *Drosophila* S2 cells was carried out using the Effectene Transfection Reagent (Qiagen, Hilden, Germany). To initiate each transfection, 10^6 *Drosophila* S2 cells were initially seeded in an 800 μL volume of Schneider's medium (Sigma) supplemented with 10% fetal bovine serum (FBS) within a twelve-well plate. After an h, the medium was exchanged with 500 μL of fresh Schneider's medium containing 10% FBS, along with a plasmid mix. The plasmid mix, composed of 0.5 μg of the plasmid of interest and either 0.5 μg of pAC-Tub-mCherry or 0.5 μg of pCoBlast, was prepared using 75 μL of EC Buffer, 8 μL of Enhancer, 10 μL of Effectene, and 1 mL of Schneider's medium with 10% FBS, following the manufacturer's instructions. For stable transfections, a three-day incubation period was allowed, after which the selection process commenced using blasticidine at a final concentration of 25 mg/mL.

MPS1 overexpression was done using a pMT-EGFP vector (Invitrogen, Carlsbad, CA) where either the coding sequences of MPS1 WT or the catalytic domain of MPS1 fused with CM2 (a domain of centrosomin which is responsible for its recruitment to the centrosome) were cloned in-frame with N-terminal EGFP or mCherry under the control of a metallothionein promoter. The various versions of AURORA-A were expressed using a pHGW (Gateway Vectors) where AURORA-A genomic sequences were cloned in-frame with N-terminal EGFP under the control of the Hsp70 promoter.

To induce MPS1-EGFP expression, S2 cells were incubated with 200 μM $CuSO_4$ at 25 °C overnight. For co-expression of EGFP-AURORA-A WT/T360A and mCherry-MPS1, cells were heat shocked at 37 °C for 30 minutes prior to incubation with $CuSO_4$. To induce expression of any mutant of EGFP-AURORA-A, cells were heat shocked at 37 °C for 30 minutes and allowed to rest for at least 6-hour before processing for immunofluorescence or live cell analysis.

2.4 RNA interference (RNAi) synthesis and depletion

Double-stranded (ds)RNA targeting specified regions (**Table 1**) was synthesized according to the manufacturer's instructions using the TranscriptAid T7 High Yield Transcription Kit (Thermo Scientific TM). An *in vitro* transcription reaction was performed, maintaining the temperature at 37 °C for 16 h. Subsequently, the resulting single-stranded RNA was denatured at 96 °C for 5 minutes to eliminate secondary structures, and then gradually cooled down at a rate of 2 °C per minute to facilitate the

formation of dsRNA duplexes. The synthesized dsRNA duplexes' integrity, concentration, and purity were assessed using 1% agarose gel electrophoresis.

Table 1. List of primers used in dsRNA synthesis.

Target region	Primer sequence		
CDC27	FW	TAATACGACTCACTATAGGGAACAATAGC	29nt
	REV	TAATACGACTCACTATAGGGTCTTCATGTAGAATTGCATGGC	42nt
3'UTR MPS1	FW	TAATACGACTCACTATAGGGTCATTCCGCTGCAGAA	36nt
	REV	TAATACGACTCACTATAGGGAAGGATTTATGATGT	35nt
5'UTR MPS1	FW	TAATACGACTCACTATAGGGGGTCACACTTGAATA	35nt
	REV	TAATACGACTCACTATAGGGAGTCCCAGTGCCATCATG	38nt
CDS MPS1	FW	TAATACGACTCACTATAGGGTCTTCCAAACACCTATGACCG	41nt
	REV	TAATACGACTCACTATAGGGCGTTTAGATATCCCTGCACCA	41nt
3'UTR AURORA-A	FW	TAATACGACTCACTATAGGGAGAACACATTCTTGTTTAATTTTC	44nt
	REV	TAATACGACTCACTATAGGGAGAGAAAACACACACAAACTTT	42nt
5'UTR AURORA-A	FW	TAATACGACTCACTATAGGGAGAACTTGCCATTCGCCTCATC	42nt
	REV	TAATACGACTCACTATAGGGAGAGACGGCACAGGCACTCG	40nt

FW: Forward sequence 5' to 3'
REV: Reverse sequence 3' to 5'
nt: number of nucleotides in the primer sequence

For each depletion, 0.5 x 10^6 *Drosophila* S2 cells were seeded in 0.5 mL of Schneider's medium in twelve-well plates, and 15 μg of the corresponding dsRNA were added. Following a 1-hour incubation at 25 °C, the cells were supplemented with 1 mL of Schneider's medium containing 10% FBS. The incubation period was 120 hours for AURORA-A depletion and 98 hours for MPS1 depletion.

2.5 Antibodies

Immunofluorescence of S2 *Drosophila* cells used several fluorescent antibodies. The primary antibodies used for immunofluorescence are listed in Table 2. Secondary antibodies conjugated to fluorescent dyes were from the Alexa series (Invitrogen) and were used according to the manufacturer's instructions at a dilution of 1:1000, except for anti-guinea pig 568 and anti-chicken 488, which were used at 1:2000.

Table 2. Primary antibodies used for immunofluorescence analysis.

Primary Antibodies	Manufacturer	Dilution
chicken anti-GFP ab13970	Abcam, Cambridge, UK	1:2000
chicken anti-dPLP	Gift from Mónica Bettencourt-Dias, Gulbenkian, Oeiras, Portugal	1:2000
guinea pig IB anti-MPS1 Gp15	Gift from Scott Hawley, Stowers Institute for Medical Research, Kansas City, EUA	1:250
mouse anti-αtubulin B512	Sigma- Aldrich	1:3000
mouse anti-GFP	Gift from Carla Lopes, i3S, Porto, Portugal	1:100
rabbit anti-CENP-C Rb1	Heeger et al., 2005	1:3000
rabbit anti-phosphorylated AURORA-A T311D	Cell Signaling Technology, Massachusetts, EUA	1:500

2.6 Immunofluorescence analysis

To evaluate the rescue of syntelic kinetochore-microtubule attachments by AURORA-A when potentiated by MPS1, a calcium treatment was performed. This treatment enables the destabilization of microtubules that were not connected with any kinetochore (Kapoor et al., 2000). In a six-well plate, 1 to 5 x 10^5 *Drosophila* S2 cells were seeded onto glass coverslips that had been pre-treated with concanavalin A for 30 minutes. After 1 hour at 25 °C, the cells were supplemented with 1 mL of Schneider's medium containing 10% FBS. Following at least a 4-hour incubation at 25 °C, the medium was removed, and 1.5 mL of the permeabilization solution was added for a duration of 2 minutes. Subsequently, 1.5 mL of a 4% paraformaldehyde (PFA) solution was added for 10 minutes. The slides were then washed three times with 1.5 mL of 0.1% Triton X-100 for 5 minutes each.

To assess the amount of phosphorylated AURORA-A in MPS1-overexpressing cells, cells were treated with 20 μM of the proteasome inhibitor MG132 (Calbiochem) for about 2 hours prior to collection to inhibit mitotic exit. Cells were then collected by Cytospin and fixed with PFA.

Fixed cells were blocked for 1 hour in PBS with 0.05% Tween20 and 10% fetal bovine serum (PBSTF) at room temperature and then incubated overnight at 4 °C with primary antibodies (prepared in blocking solution). After three five-minute washes in PBS with 0.05% Tween 20 (PBT), cells were incubated with fluorescent-labelled secondary antibodies diluted in PBSTF for 1 hour at room temperature. Finally, cells were washed thrice with PBT for 5 minutes and slides were mounted with 1 mg/mL of Vectashield mounting medium containing fluorescence DAPI (Vector Laboratories, UK).

Images were collected in a Leica TCS SP8 laser scanning confocal microscope (Leica Microsystems, Germany). Data stacks were analysed using ImageJ 1.49k software (http://rsb.info.nih.gov/ij/). The mean pixel intensity was obtained from maximum projected raw images acquired with fixed exposure acquisition settings for immunofluorescence quantification of proteins.

2.7 Live cell imaging

Live analysis of mitotic *Drosophila* S2 cells was performed using various cell lines expressing specific constructs. After the desired treatment, 7.5 x 10^5 cells were plated in MatTek glass bottom dishes (MatTek Corporation) that had been pre-treated with Concanavalin A at a concentration of 0.25 mg/mL (Sigma). Prior to the visualisation under the microscope, 20 μM MG132 (Calbiochem) was added to the cells when no CDC27 depletion had been done. Six-dimensional datasets were collected at 25 °C using a spinning disc confocal system (Revolution; Andor) equipped with an electron-multiplying charge-coupled device camera (iXonEM+; Andor) and a CSU-22 unit (Yokogawa), all integrated with an inverted microscope (IX81; Olympus). Near-simultaneous excitation of EGFP and mCherry-tubulin was achieved using two laser lines (488 nm and 561 nm) respectively. A UPLSAPO 100x/NA 1.40 Oil objective was utilized. The imaging system was controlled by iQ software (Andor). Time-lapse imaging of z stacks with 0.8 μm steps covering the entire volume of the cell was acquired at 30-second intervals, and subsequent image sequence analysis and video assembly were performed using ImageJ and iQ software.

2.8 Statistical analysis

All statistical analysis were conducted using GraphPad Prism V5.0f (GraphPad Software, Inc.). To determine statistical significance, a non-parametric Kruskal-Wallis 1-way ANOVA with Dunn's multiple comparison test was used. A significance level of $P < 0.05$ was considered statistically significant.

CHAPTER 3 - RESULTS AND DISCUSSION

3.1 AURORA-A T360D rescues the delay observed in the congression of polar chromosomes in MPS1-depleted S2 cells

AURORA-A has been previously described as having an important role in the correction of erroneous kinetochore-microtubule attachments (like syntelic) when chromosomes are near the centrosome (polar chromosomes). This is achieved through the destabilization of these attachments by the phosphorylation of the kinetochore protein NCD80. This phosphorylation is key in promoting the establishment of correct kinetochore-microtubule attachments (bioriented), timely chromosome congression to the metaphase plate, and proper segregation of the sister chromatids in anaphase (Ye et al., 2015; Medina et al., 2021). To assess the importance of AURORA-A phosphorylation by MPS1 in the efficiency of AURORA-A-mediated error correction, we resorted to live cell imaging of mitotic *Drosophila* S2 cells expressing EGFP-tagged Histone2B (H2B), mCherry-tagged αTubulin. AURORA-A, which is always localized at the centrosomes due to CM2 fusion, is also associated with EGFP-fluorophores (**Figure 14A**). We measured the time cells depleted of MPS1 took from Nuclear Envelop Breakdown (NEB) to full chromosome congression (metaphase) (**Figure 14B**) and the time each individual polar chromosome took to be brought to the metaphase plate (**Figure 14C**). It is already understood that cells depleted of MPS1 are known to have premature mitotic exit due to impaired mitotic checkpoint signaling. MPS1 enables the cell to enter anaphase even if the chromosomes aren't properly aligned at the metaphase plate. To tackle this, CDC27, an APC subunit, was also depleted in all conditions analyzed to prevent the aforementioned premature mitotic exit and allow us to understand the real chromosome congression time and specifically monitor the polar chromosomes behavior.

Our data show that CDC27-depleted cells (control condition) fully align all chromosomes in the metaphase plate in an average time of 17 minutes, with each chromosome taking on average 6.5 minutes. When endogenous MPS1 is depleted, cells take longer to solve or align polar chromosomes, with an average time of 35 minutes for full congression, with each polar chromosome taking about 30 minutes. These results demonstrate that MPS1 is important for chromosome congression. Notably, in cells depleted of MPS1, most polar chromosomes appear to exhibit syntelic attachments (**Figure 14A** white arrow). In fact, MPS1 has been described as playing an important role in error correction (Santaguida et al., 2010; Hayward et al., 2022). On the other hand, when the phosphomimetic mutant for AURORA-A T360 (T360D) is expressed in cells depleted of CDC27 and MPS1, the congression time is rescued to values similar to the control situation. These results indicate that the phosphorylation of this residue is indeed important for the role of AURORA-A in error correction. Moreover, the fact that this mutant is able to rescue MPS1 depletion suggests that MPS1 acts on error correction through this phosphorylation.

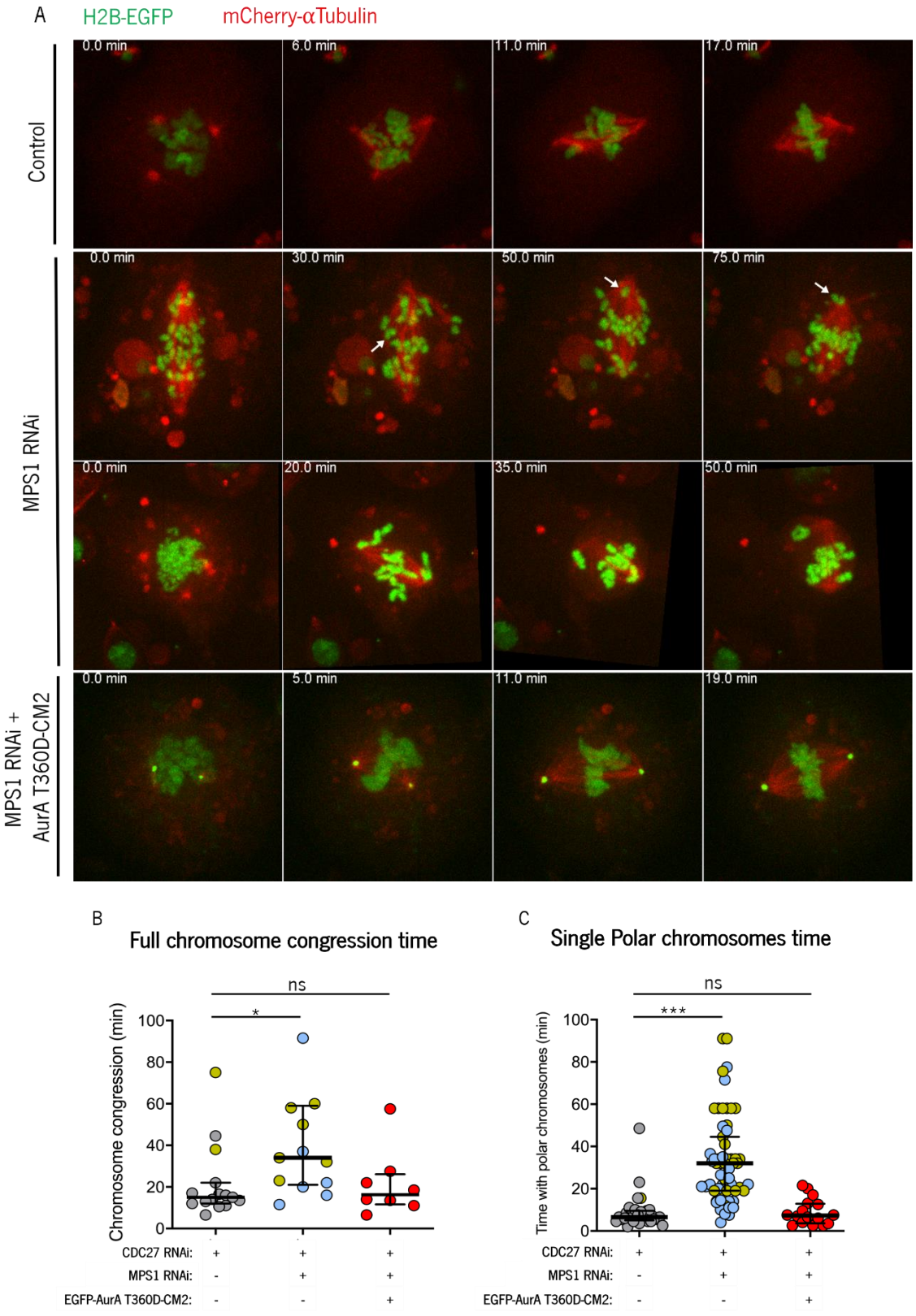


Figure 14. AURORA-A T360D rescues the delay observed in the congression of polar chromosomes in MPS1-depleted S2 cells.

(A) Representative stills from time-lapse movies of dividing *Drosophila* S2 cells expressing H2B-EGFP and mCherry-α-Tubulin in the indicated conditions. To prevent premature mitotic exit, caused by the absence of MPS1, CDC27, an APC subunit was also depleted in all analyzed conditions. (B) Quantification of the time to full chromosome congression (NEB to metaphase)

for each cell in the indicated conditions. (C) Quantification of the time that each polar chromosome takes to be removed from the centrosome region back to the metaphase plate in the indicated conditions. Statistical analysis was calculated using a non-parametric Kruskal-Wallis 1-way ANOVA with Dunn's multiple comparison test. P values: ns, not significant; *< 0.05; **< 0.01; ***< 0.001.

3.2 Centrosome-tethered MPS1 or AURORA-A T360D can promote the correction of syntelic kinetochore-microtubule attachments in MPS1-depleted S2 cells

Live cell imaging analysis revealed the importance of AURORA-A phosphorylation in its T360 by MPS1 for timely chromosome congression to the metaphase plate, suggesting this phosphorylation is key in the correction of erroneous kinetochore-microtubule attachments. To better assess this hypothesis, immunofluorescence analysis was used to examine the attachment status of kinetochores in *Drosophila* S2 cells depleted of MPS1 (**Figure 15**). Cells were fixed and stained for EGFP, α-Tubulin, one of the main microtubule monomers, and CENP-C, a kinetochore protein, and the configuration of the kinetochore-microtubule attachment was analysed. To make sure only stable kinetochore-microtubule attachments were analysed, unstable microtubules were depolymerized by a calcium treatment before fixing cells (Kapoor et al., 2000). Once again, all cells were depleted of CDC27 to prevent premature mitotic exit due to the absence of MPS1.

Expectedly, in control cells, that were only depleted of CDC27, the median percentage of syntelic chromosomes per cell was 0% (**Figure 15 A,B**). This result is easily explained by the fact that CDC27 depletion results in the accumulation of cells in metaphase where, in cells with proper error correction mechanisms, most erroneous attachments were already destabilized to promote the establishment of bioriented chromosomes. Accordingly, the bioriented chromosomes percentage is maximum (**Figure 15 C**). However, when the endogenous MPS1 was depleted, 45% (median value) of the chromosomes in each cell were syntelic (**Figure 15 A-C**). Moreover, whereas control cells exhibit few polar chromosomes (1.8% of chromosomes/cell), depletion of MPS1 resulted in a 4-fold increase in the frequency of polar chromosomes (**Figure 15 A, B)**. Hence, in agreement with the live cell imaging data, the increase in the percentage of syntelic and polar chromosomes shows that MPS1 is important for destabilizing syntelic attachments and promoting proper attachments required for chromosome congression.

Because the host lab has shown that MPS1 recruited to kinetochores of polar chromosomes potentiates AURORA A activation, we tested whether this function was required for error correction. To do so, we expressed the MPS1 catalytic domain fused with the centrosome targeting domain of centrosomin (CM2 motif) in cells depleted of endogenous MPS1. As expected, the EGFP-MPS1-CM2 fusion accumulated specifically at spindle poles, where AURORA-A is localized. Strikingly, when this centrosome-tethered

version of MPS1 is expressed on top of the CDC27 and MPS1 depletions a significant rescue of syntelic attachments is observed (median of 18% syntelic chromosomes/cell; **Figure 15B**). In addition, the same very low percentage of polar chromosomes was obtained (median of 2% polar chromosomes/cell; **Figure 15C**) as in the control condition. This result suggests that MPS1-mediated potentiation of AURORA A activation contributes heavily to the error correction mechanism. It should be noted however, that a full rescue of biorientation was not achieved (median of 83% bioriented chromosomes/cell; **Figure 15D**), which might reflect the need for kinetochore-specific functions of MPS1 for complete correction of syntelic into amphitelic attachments.

Interestingly, the expression of a wild-type AURORA-A failed to rescue the defects of MPS1 depletion in the configuration of the kinetochore-microtubule attachments. Specifically, cells expressing AURORA-A wild-type presented relatively high percentages of syntelic (median of 33% of syntelic chromosomes/cell; **Figure 15B**) and polar chromosomes (median of 6.8% of polar chromosomes/cell; **Figure 15C**). On the other hand, the expression of a constitutively active version of AURORA-A (T311D) – where the activating T-loop autophosphorylation residue is mutated to an aspartate – was able to efficiently restore chromosome biorientation and congression. In fact, the expression of AURORA-A T311D after MPS1 and CDC27 depletion resulted in the highest percentage of bioriented chromosomes of all non-control conditions (median of 92% of bioriented chromosomes/cell; **Figure 15D**) and only 2.3% of polar chromosomes/cell (**Figure 15C**). Altogether these findings reveal that in the absence of MPS1, merely overexpressing AURORA-A is not sufficient to correct the syntelic attachments, whereas the expression of constitutively activated AURORA-A does. Hence, AURORA-A accumulation at the centrosome by itself is not sufficient for the error correction of these attachments. MPS1 near the pole is still required, unless AURORA-A is in its active form. This further indicates that a role of MPS1 in error correction revolves around potentiating AURORA-A activity.

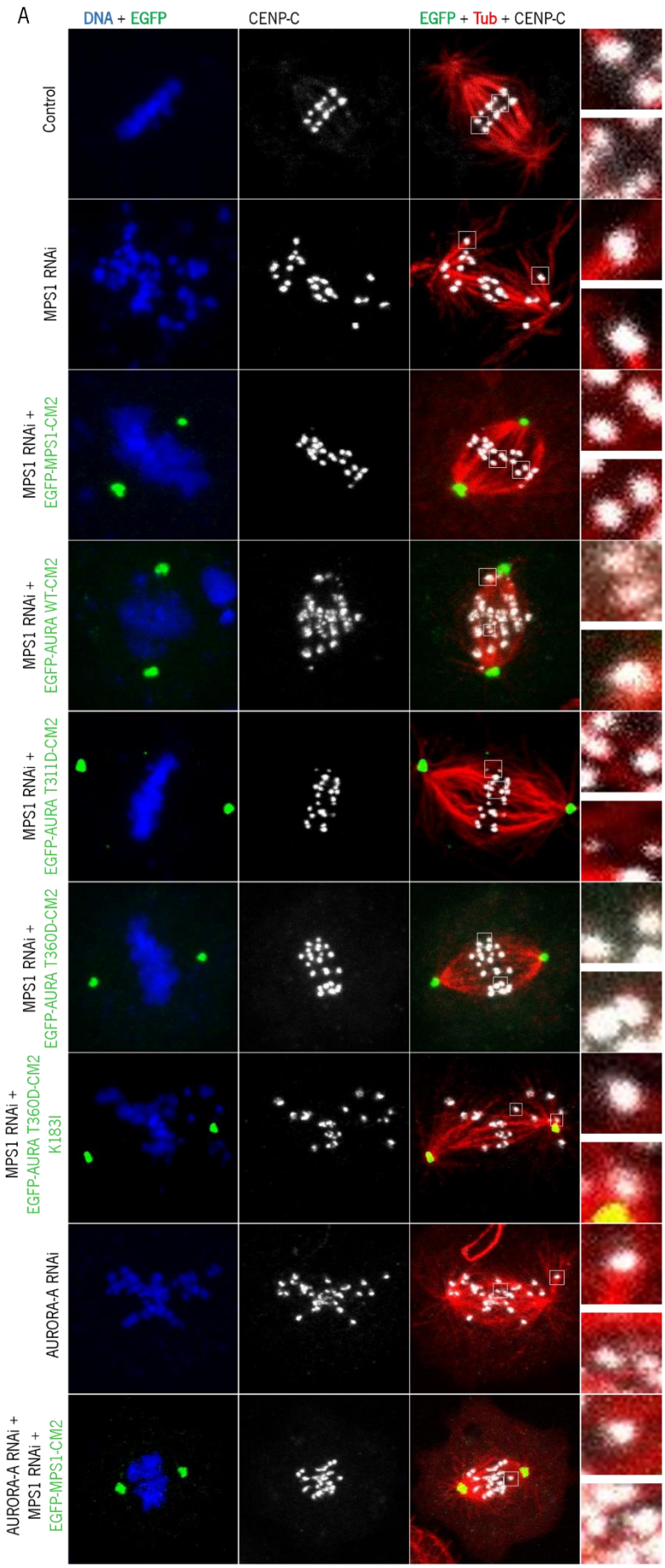
Previous data in the host lab suggests that AURORA-A phosphorylation in its T360 by MPS1 results in more AURORA-A activation and efficiency in correcting incorrect attachments. Therefore, to understand if the defects observed in cells depleted of MPS1 are a direct consequence of the lack of AURORA-A T360 phosphorylation in these cells, the expression of a phosphomimetic mutant of AURORA-A (T360D) was done in cells depleted of CDC27 and MPS1.

Importantly, the expression of phosphomimetic mutant version of AURORA-A for the MPS1 phosphorylation site (T360D) resulted in a significant rescue of the attachment configuration. Cells expressing AURORA-A T360D presented a median of 17% of syntelic chromosomes/cell (**Figure 15D**) and a very reduced percentage of polar (3.1% chromosomes/cell; **Figure 15C**). The fact that the expression

of a centrosomal MPS1 or the AURORA-A T360D phosphomutant were able to significantly restore biorientation in cells depleted of endogenous MPS1 supports even further the hypothesis that MPS1, when near the centrosome, phosphorylates AURORA-A on its T360 to promote AURORA-A-mediated correction of syntelic chromosomes and efficient chromosome congression (**Figure 15A-D)**.

To assess whether the phosphomimetic mutant AURORA-A T360D ability to rescue MPS1 depletion is due to increased AURORA-A activity, we expressed a catalytic-inactive version of the T360D mutant (K183I T360D). This version of AURORA-A completely failed to rescue syntelic attachments (median of 40% of syntelic chromosomes/cell; **Figure 15B**) and the accumulation of polar chromosomes observed in MPS1 depletion because AURORA-A K183 T360D achieved a median of 4.8% of polar chromosomes/cell (**Figure 15C**). This suggests that indeed MPS1 near the centrosome phosphorylates AURORA-A in its T360 to increase its activity towards syntelic attachments.

Depletion of AURORA-A resulted in a similar frequency of syntelic attachments (median of 33% of syntelic chromosomes/cell; **Figure 15B**) observed in cells depleted of MPS1 whereas when both AURORA-A and MPS1 were depleted, even the expression of centrosome-tethered MPS1 was insufficient to rescue the defects observed in MPS1 depletion to the same extent, achieving only 71% of bioriented chromosomes (**Figure 15D**). This further implies that centrosomal-MPS1 works mainly through AURORA-A in promoting error correction.


A
DNA + EGFP
CENP-C
EGFP + Tub + CENP-C
Control
MPS1 RNAi
MPS1 RNAi + EGFP-MPS1-CM2
MPS1 RNAi + EGFP-AURA WT-CM2
MPS1 RNAi + EGFP-AURA T311D-CM2
MPS1 RNAi + EGFP-AURA T360D-CM2
MPS1 RNAi + EGFP-AURA T360D-CM2 K183I
AURORA-A RNAi
AURORA-A RNAi + MPS1 RNAi + EGFP-MPS1-CM2

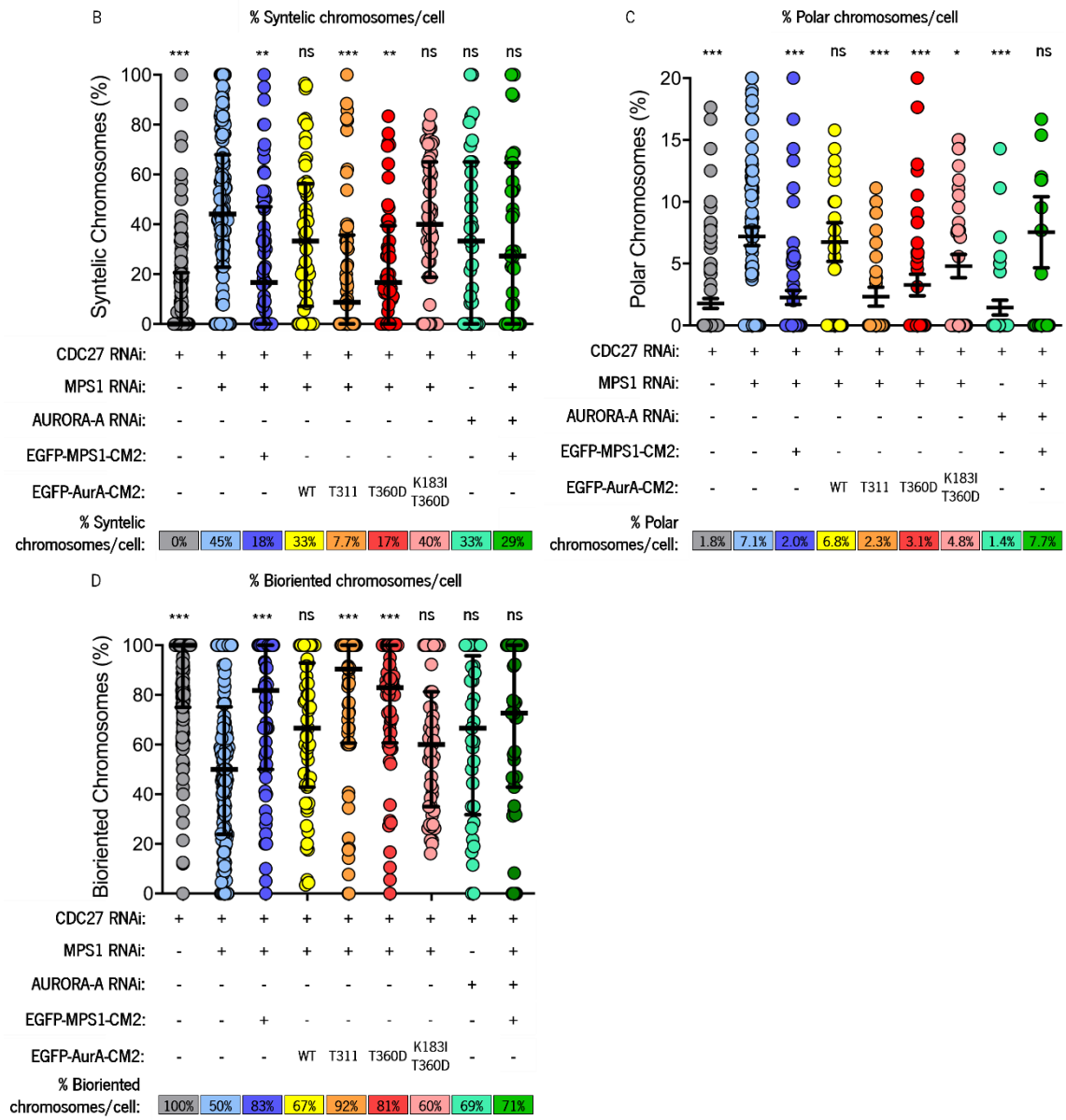


Figure 15. Phosphorylation of AURORA-A T360 by MPS1 promotes the correction of syntelic kinetochore-microtubule attachments in MPS1-depleted S2 cells. (A) Representative images of confocal immunofluorescence analysis of dividing *Drosophila* S2 cells in the indicated conditions. (B-D) Quantification of the percentages of syntelic (B), polar (C), and bioriented (D) chromosomes per cell, in the indicated conditions. Statistical analysis was calculated using a non-parametric Kruskal-Wallis 1-way ANOVA with Dunn's multiple comparison test relative to the MPS1 RNAi condition. P values: ns, not significant; *< 0.05; **< 0.01; ***< 0.001.

It is therefore clear that AURORA-A has an essential role in the correction of errors in the kinetochore-microtubule attachments, but this role is potentiated by MPS1.

CHAPTER 4 - CONCLUSIONS AND FUTURE PERSPECTIVES

4.1 Conclusions

In this study, we sought to elucidate the previously described roles of MPS1 and AURORA-A in the correction of erroneous kinetochore-microtubule attachments (Hayward et al., 2022; Ye et al., 2015; DeLuca et al., 2018; London & Biggins, 2014; Maure et al., 2007). In summary, the results presented in this thesis show that kinetochore MPS1 phosphorylates AURORA-A T360 to potentiate AURORA-A activation and consequently, increase its activity to ensure efficient and timely AURORA-A-driven error correction of syntelic attachments and chromosome congression. Therefore, from the three hypothetical models previously presented in the introduction, the model that better fits the results obtained is Model 3 (**Figure 16**), in which MPS1 acts upstream of AURORA-A to promote an AURORA-A-mediated error correction mechanism that leads to the formation of amphitelic attachments.

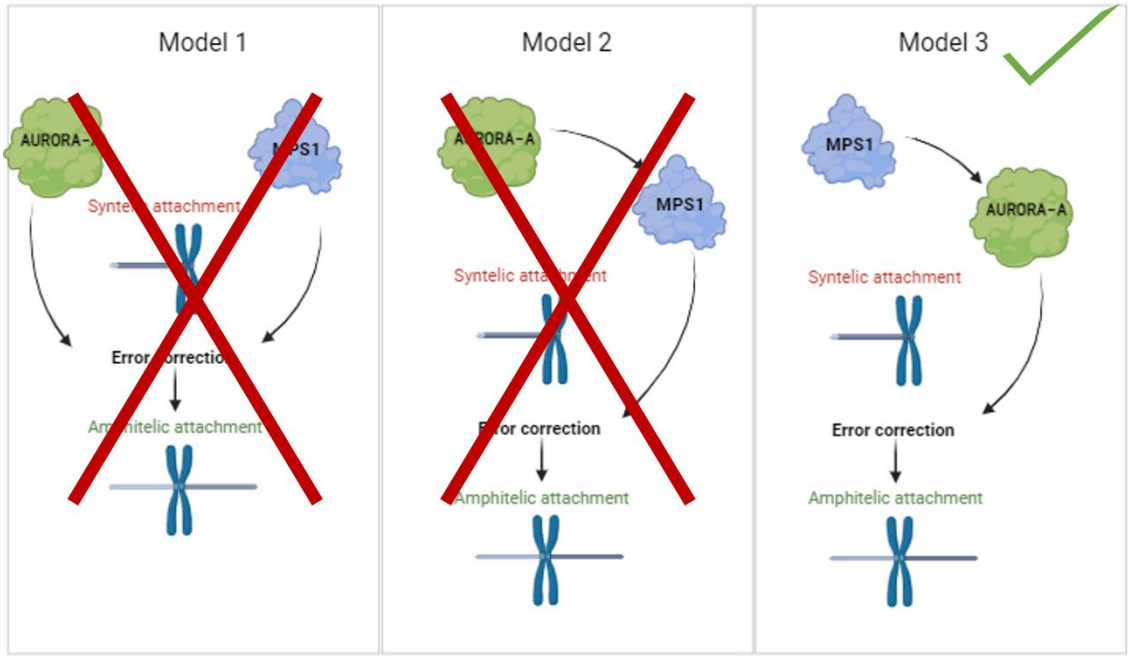


Figure 16. Schematic representation of the most feasible error-correction model involving MPS1 and AURORA-A (Figure created with Biorender.com)**.** In Model 1 AURORA-A and MPS1 work independently of each other in correcting syntelic attachments. In Model 2 AURORA-A acts upstream of MPS1 which in turn directly destabilises erroneous attachments. Model 3 illustrates the hypothesis in which MPS1 acts upstream of AURORA-A to promote an AURORA-A driven error correction. Our results strongly support Model 3.

In our model, after nuclear envelope breakdown the microtubules of the mitotic spindle start a "search and capture" process where they randomly interact with kinetochores that assembled on the centromeres of the condensed chromosomes (Cheeseman & Desai, 2008). Due to the stochastic nature of this process, the formation of incorrect attachments, like syntelic attachments, often occurs. If left uncorrected, these attachments will result in an uneven distribution of the genetic material to the daughter

cells (Maure et al., 2007). It is, therefore important that the cell corrects these incorrect attachments, before anaphase onset, to ensure the proper segregation of the duplicated genome. Syntelic attachments are known to be corrected by AURORA kinases by the phosphorylation of kinetochore proteins that lead to the destabilisation of kinetochore-microtubule attachments (DeLuca et al. 2018). Specifically, AURORA-A, present at the centrosomes, was described as being important for the correction of syntelic attachments when chromosomes are near the spindle poles (polar chromosomes) (Ye et al., 2015). However, whether this mechanism is always ON or whether it needs to sense the presence of polar chromosomes to be turned ON is still unclear. This study uncovers a novel function for MPS1 acting upstream of AURORA-A in this mechanism. We hypothesize that in prometaphase when a chromosome is near the centrosome, kinetochore MPS1 and centrosomal AURORA-A get close enough to be within each other's zone of influence. In this setting, MPS1 phosphorylates AURORA-A on its threonine 360, potentiating AURORA-A T-loop autophosphorylation and rendering the kinase more efficient at correcting syntelic attachments (**Figure 17**). After one (or both) of the attachments are destabilized, the forces keeping the chromosome near the poles are more easily counterbalanced and the chromosome can then be brought to the metaphase plate by motor protein-assisted congression (Maiato et al., 2017). Once near the metaphase plate new kinetochore microtubule attachments are formed. If the new attachments are amphitelic, the chromosome is now bioriented and ready for anaphase onset. This mechanism is crucial for proper chromosome segregation and maintenance of genomic integrity in dividing cells. Importantly, the uncovered phosphorylation site is conserved in *Drosophila* and in humans as well, which suggests a conservation of this mechanism across species. Moreover, consistently with this, work in frame of a collaboration with Marin Barisic's lab shows that in human cells, AURORA-A activity is also increased as chromosomes are near the centrosome and that this is dependent on MPS1 activity (data not shown).

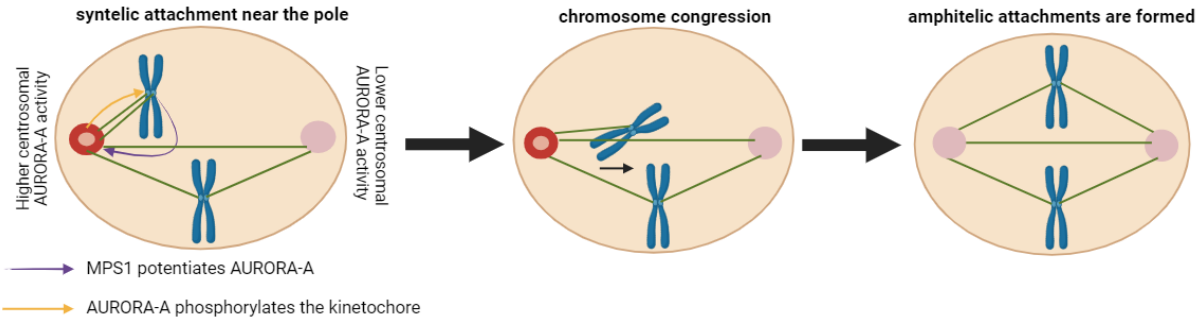


Figure 17. **A kinetochore-centrosome cross-talk ensures efficient correction of erroneous kinetochore-microtubule attachments and timely chromosome congression** (Figure created with Biorender.com). Kinetochore-associated MPS1 on polar chromosomes reaches centrosomal AURORA-A and phosphorylates its T360. This phosphorylation promotes the activating auto-phosphorylation of AURORA A T-loop through a yet elusive mechanism. Robust activation of AURORA-A driven by MPS1 ensures efficient phosphorylation of kinetochore proteins to promote destabilization of syntelic attachments. Unattached

kinetochores are exposed to spindle microtubules to attempt the formation of amphitelic attachments and congress to the metaphase plate.

In this work, we present a novel role for kinetochore MPS1 as a potentiator of AURORA-A activity in the presence of polar chromosomes and unravel a new cross-talk between polar chromosomes and centrosomes that increases the efficiency of AURORA-A-mediated correction of syntelic chromosomes and promotes accurate genome distribution in mitosis.

4.2 Future perspectives

Aiming to dissect the molecular underpinnings of MPS1-mediated activation of AURORA-A and the physiological relevance of this interaction on the correction of inaccurate kinetochore-microtubule attachments further biochemical and cellular tests should be done. Specifically, live cell imaging analysis of the same conditions used in the calcium treatment experiments would be ideal to follow chromosome congression through time. This will allow measuring the timely efficiency of the error correction of each syntelic attachment in these conditions. Additionally, to further confirm that indeed AURORA-A phosphorylation in its T360 by MPS1 results in a higher AURORA-A activity, AURORA-A activity and activation status should be assessed in the AURORA-A T360A and T360D both in the presence and the absence of MPS1. This could be done both in *in vitro* kinase assays and in the cellular context. To delve deeper into the effects of the uncovered phosphorylation in potentiating AURORA-A activity, X-ray crystallography analysis of AURORA-A T360A and T360D mutants could be used to understand whether this phosphorylation results in conformational changes that could explain increased AURORA-A activity.

In this work, we present a series of results that point towards a critical role for MPS1 in potentiating AURORA-A in the correction of syntelic attachments. However, AURORA-B has been described in many studies as the main kinase for error correction (Krenn & Musacchio, 2015; Funabiki, 2019). It becomes then important to address AURORA-B role in light of our results as well as understand whether MPS1 may also have a direct role in AURORA-B-driven error correction. Importantly, AURORA-A and AURORA-B share very similar catalytic domains, including the uncovered MPS1 phosphorylation site. Hence, new questions can then be raised: 1) Is the AURORA-B pathway completely independent of the uncovered mechanism? 2) Is AURORA-B phosphorylated by MPS1 in this site as well? 3) Does MPS1 also act upstream of AURORA-B in its well-established error correction pathway? Assessing whether MPS1 phosphorylates AURORA-B in this site and whether that is important for error correction and proper chromosome congression will generate critical knowledge to better describe the role of MPS1 in the correction of syntelic attachments.

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