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RESEARCH ARTICLE

SPECIAL ISSUE: CELL BIOLOGY OF MOTORS

RNA localization to the mitotic spindle is essential for early development and is regulated by kinesin-1 and dynein

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ABSTRACT

Mitosis is a fundamental and highly regulated process that acts to faithfully segregate chromosomes into two identical daughter cells. Localization of gene transcripts involved in mitosis to the mitotic spindle might be an evolutionarily conserved mechanism to ensure that mitosis occurs in a timely manner. We identified many RNA transcripts that encode proteins involved in mitosis localized at the mitotic spindles in dividing sea urchin embryos and mammalian cells. Disruption of microtubule polymerization, kinesin-1 or dynein results in lack of spindle localization of these transcripts in the sea urchin embryo. Furthermore, results indicate that the cytoplasmic polyadenylation element (CPE) within the 3′UTR of the *Aurora B* transcript, a recognition sequence for CPEB, is essential for RNA localization to the mitotic spindle in the sea urchin embryo. Blocking this sequence results in arrested development during early cleavage stages, suggesting that RNA localization to the mitotic spindle might be a regulatory mechanism of cell division that is important for early development.

KEY WORDS: Mitosis, RNA localization, Kinesin-1, Dynein, Embryonic development

INTRODUCTION

Mitosis is the fundamental cellular process in which a cell divides to become two identical daughter cells following replication of its DNA (Mcintosh, 2016). This process involves the division of its duplicated DNA in karyokinesis and separation of the cytoplasm in cytokinesis (Mcintosh, 2016). The mitotic spindle is the organelle that drives the segregation of chromosomes (Gadde and Heald, 2004). The spindle is comprised primarily of tubulin monomers that heterodimerize (Petry, 2016). These monomers polymerize through the action of enzymes such as XMAP215 (also known as CKAP5 in mammals), which is essential for the formation of mitotic spindles (Kronja et al., 2009). Actin also regulates mitosis by generating force within the dividing cell to orient the mitotic spindle, as well as separating chromosomes during anaphase (Anstrom, 1992; Kunda and Baum, 2009). Myosin II is the major motor protein that associates with actin and is indispensable for cytokinesis (Chaigne et al., 2016; Babkoff et al., 2021). Other actin regulators are essential for mitosis, such as cofilin family members, actin depolymerizers whose inactivation is necessary for proper spindle orientation during mitosis and are also responsible for importing actin into the nucleus (Pendleton et al., 2003; Kaji et al., 2008). Actin export from the nucleus is partially controlled through its interaction with profilin proteins, which are also required for cytokinesis in chondrocytes (Stüven et al., 2003; Böttcher et al., 2009).

The segregation of chromosomes is highly dynamic and microtubule motors, such as kinesin-5, are required to slide anti-parallel microtubule fibers polewards (Cochran et al., 2005; Mann and Wadsworth, 2019). CENP-E, centromeric protein E, is a plus-ended kinesin motor protein that assists in orienting chromosomes properly along the metaphase plate (Craske and Welburn, 2020). Conversely, dynein is a microtubule minus-end-directed motor protein that is known to regulate several aspects of mitosis, from centrosome separation to chromosome congression to spindle formation (Raaijmakers and Medema, 2014). Additionally, dynein and dynactin interact with NuMA (also known as NuMA1) to tether the astral microtubules to the cell cortex to orient the mitotic spindle (Hueschen et al., 2017; Okumura et al., 2018). NuMA also is essential for formation and maintenance of the spindle poles during mitosis (Zeng, 2000).

During early development, metazoan embryos undergo several rounds of rapid early cleavage divisions, where they cycle between mitosis (M) and synthesis (S) phases of the cell cycle, with minimal gap phases (Ikegami et al., 1994; Siefert et al., 2015). Diverse cells accomplish mitosis in a relatively constant time frame of between 30 to 60 min, indicating exquisite regulation of mitosis to ensure a timely completion of this process (Araujo et al., 2016). Prolonged mitosis has been shown to result in cell death, cell arrest or DNA damage (Rieder and Palazzo, 1992; Lanni and Jacks, 1998; Quignon et al., 2007; Uetake and Sluder, 2010; Orth et al., 2012). Thus, it is not surprising that mitosis is regulated by a plethora of mechanisms, from transcriptional regulation of cell cycle factors (Spellman et al., 1998; Whitfield et al., 2002) to post-translational regulation by phosphorylation and ubiquitylation (Stegmeier et al., 2007; Dephoure et al., 2008; Lindqvist et al., 2009). In general, transcription is globally inhibited during mitosis (Martínez-Balbás et al., 1995); however, transcription occurs at the centromeric regions. Centromere transcription is essential for CENP-A nucleosome assembly and centromere formation and maintenance (Perea-Resa and Blower, 2018). Transcribed centromeric RNAs ensure correct CENP-C (RNA-binding protein) levels and CENP-P (nucleosome) loading and accurate chromosome segregation. siRNAs and lncRNAs have also been found to be derived from centromeric regions and might play an important role in maintaining heterochromatin in the centromere domains (Hall et al., 2002; Volpe et al., 2002; Liu et al., 2015; Johnson et al., 2017; Perea-Resa and Blower, 2018).

As mitosis is under strict temporal control, it must be regulated in a rapid manner. As transcriptional regulation takes time, post-transcriptional and post-translational regulation play a key role during mitosis (Li and Zhang, 2017; Moura and Conde, 2019).

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Several critical steps in the cell cycle, and specifically mitosis, require post-translational regulation by kinases and phosphatases (Dephoure et al., 2008; Lindqvist et al., 2009; Combes et al., 2017; Gelens and Saurin, 2018; Moura and Conde, 2019). For example, entry into mitosis requires the mitotic kinase Cdk1–cyclin B1 and the phosphatase Cdc25 (Boutros et al., 2006; Lindqvist et al., 2009; Vigneron et al., 2018; Sun et al., 2019). Translation of cyclin B is required for sea urchin embryos to undergo mitosis (Chassé et al., 2016). Mitotic spindle elongation requires a perfect balance between kinase and phosphatase activities (Winey and Bloom, 2012; Nilsson, 2019). During metaphase, proper bipolar chromosome attachment is essential for progression through the spindle assembly checkpoint (SAC), which is passed partially through the inactivation of CDK1–cyclin B and the activation of the anaphase-promoting complex/cyclosome (APC/C) via phosphorylation (Castro et al., 2005). In addition, phosphatases regulate APC/C activity by directly dephosphorylating the Cdc20 and APC/C subunits, and indirectly through phosphatase-mediated silencing of checkpoint signaling from the kinetochores to promote mitotic exit (Labit et al., 2012; Craney et al., 2016; Hein et al., 2017; Lee et al., 2017). Aurora B kinase plays a role in sensing improperly attached chromosomes and phosphorylating kinetochore components Ndc80, KNL-1 and Dsn1 (also known as KNL-3) to reduce microtubule affinity and promote detachment from the kinetochore (Lens et al., 2003; Welburn et al., 2010). Aurora B kinase-mediated phosphorylation of Ndc80 also recruits Mps1 (also known as TTK in mammals), activating the SAC in the presence of chromosomes that are not attached to microtubules (Cheeseman et al., 2002, 2006; Combes et al., 2017). Thus, the coordinated regulation of hundreds of proteins by dynamic phosphorylation during mitosis is a key mechanism to ensure the timely and precise segregation of chromosomes.

Although post-translational regulation of mitosis by kinases and phosphatases has been well-studied (Dephoure et al., 2008; Lindqvist et al., 2009; Combes et al., 2017; Moura and Conde, 2019), post-transcriptional regulation through localization of important RNA transcripts is less well understood. Several different proteins have been identified to control localization of RNAs to the mitotic spindle. Aurora B protein is recruited to the different areas of the kinetochores by phosphorylated histones where it has been found to associate with hundreds of mRNAs that are enriched on mitotic spindles, many of which encode cytoskeletal proteins and transcription factors (Jambhekar et al., 2014). The binding of Aurora B to mRNA is essential for its localization to centromeres, as well as its ability to phosphorylate its substrates, including Polo kinase, p53, securin and APC/C, to initiate anaphase (Jambhekar et al., 2014). Staufen, an RNA-binding protein, is thought to mediate localization of subpopulations of RNAs to the spindles during mitosis (Hassine et al., 2020). Additionally, Staufen regulates localization of *prospero* in *Drosophila* neuroblasts, ensuring asymmetric division and correct cell fate after mitosis (Broadus et al., 1998). *Cyclin B* mRNA has previously been shown to have mitotic spindle localization in *Xenopus* and *Strongylocentrotus purpuratus*, as well as perinuclear localization in *Drosophila* embryos (Raff et al., 1990; Groisman et al., 2000; Yajima and Wessel, 2015). Interestingly, disruption of *Cyclin B* RNA localization results in defects in spindle architecture and ultimately abnormal cell division in *Xenopus* (Groisman et al., 2000). Many RNAs have been identified to localize to the mitotic spindle through biochemical assays (Blower et al., 2007; Sharp et al., 2011; Pascual et al., 2021) and several RNAs have been identified to localize to the centrosome, spindle midzone and microtubules in embryos (Kingsley et al., 2007; Lécuyer et al., 2007; Splinter et al., 2010). However, the impact of disruption of this localization has not been characterized in a developing embryo. In this study, we test the hypothesis that transcripts encoding proteins that regulate mitotic processes localize to the mitotic spindle, and that this localization is essential for early embryonic development.

We use the purple sea urchin embryo, *S. purpuratus*, to study RNA localization during mitosis. The sea urchin produces large and optically transparent blastomeres during cleavage stage, which enables easy visualization of perturbation phenotypes (McClay, 2011). Using the sea urchin embryo and mammalian cells, we identify several transcripts that encode proteins involved in mitosis located on the mitotic spindle, indicating that this RNA localization is evolutionarily conserved. Transcripts that we examined were selected for their known roles in regulating the progression of mitosis. Furthermore, this localization is dependent on microtubules and microtubule motor proteins kinesin-1 and dynein. Using reporter constructs, we also demonstrated that the 3′UTR of *Aurora B* is sufficient for spindle localization in the sea urchin embryo. Importantly, we identified a cytoplasmic polyadenylation element (CPE) sequence that is required for localization of *Aurora B* to the spindles and observed that blocking this CPE sequence results in developmental arrest. During cleavage stage development, the lack of gap phases during the relatively rapid cell divisions might mean spatial regulation of RNA transcripts of key players in mitosis is necessary to provide the cell another layer of control to an essential process. Our results reveal that RNA localization of the *Aurora B* transcript to the spindles is a novel mechanism in ensuring proper development.

RESULTS

RNA transcripts localize to the mitotic spindle

We examined the subcellular localization of the transcripts encoding select proteins involved in mitosis (Fig. 1A). The RNA transcripts of *Cyclin B*, *APC*, *Cdk*, *Aurora B*, *Polo kinase* and *CENP-E* were localized between the dividing chromosomes of sea urchin 16 to 32 cell cleavage stage embryos (Fig. 1A). At metaphase, *NuMA* was enriched at the spindle midzone (arrow) and at the presumed centrosome (arrowhead).

We also examined the subcellular localization of transcripts encoding cytoskeletal proteins, microtubule motor proteins, and regulators of other facets of mitosis (Fig. 1B). We observed that *β-actin*, *Myosin II*, *Tubulin α1*, *XMAP*, *Cofilin*, *Profilin*, *Dynein* and *Staufen* were localized between the dividing chromosomes of cleavage stage embryos (16 to 32 cells) undergoing mitosis (Fig. 1B). We also observe that the RNA transcripts of some of these genes (including *Aurora B*, *Polo kinase*, *β-actin*, *Tubulin α1*, *XMAP*, *Dynein* and *Staufen*) localized to the perinuclear region in blastomeres in interphase (Fig. 1 and data not shown). Transcript localization varied throughout the cell cycle, with *Aurora B*, *Polo kinase*, *Tubulin α1*, *Dynein* and *Staufen* localizing to the midzone of the mitotic spindle during metaphase and anaphase, then becoming more diffuse during telophase (Fig. S2 depicts this for *Aurora B*). These data indicate that the transcripts encoding proteins involved in mitosis are localized to the mitotic spindle.

To test the hypothesis that this localization of RNA transcripts to the mitotic spindle is selective and likely due to the function of these proteins in mitosis, we examined the subcellular localization of expressed transcripts that encode proteins of non-mitotic functions. Eve and Tbr are transcription factors that regulate endodermal and skeletal specification, respectively

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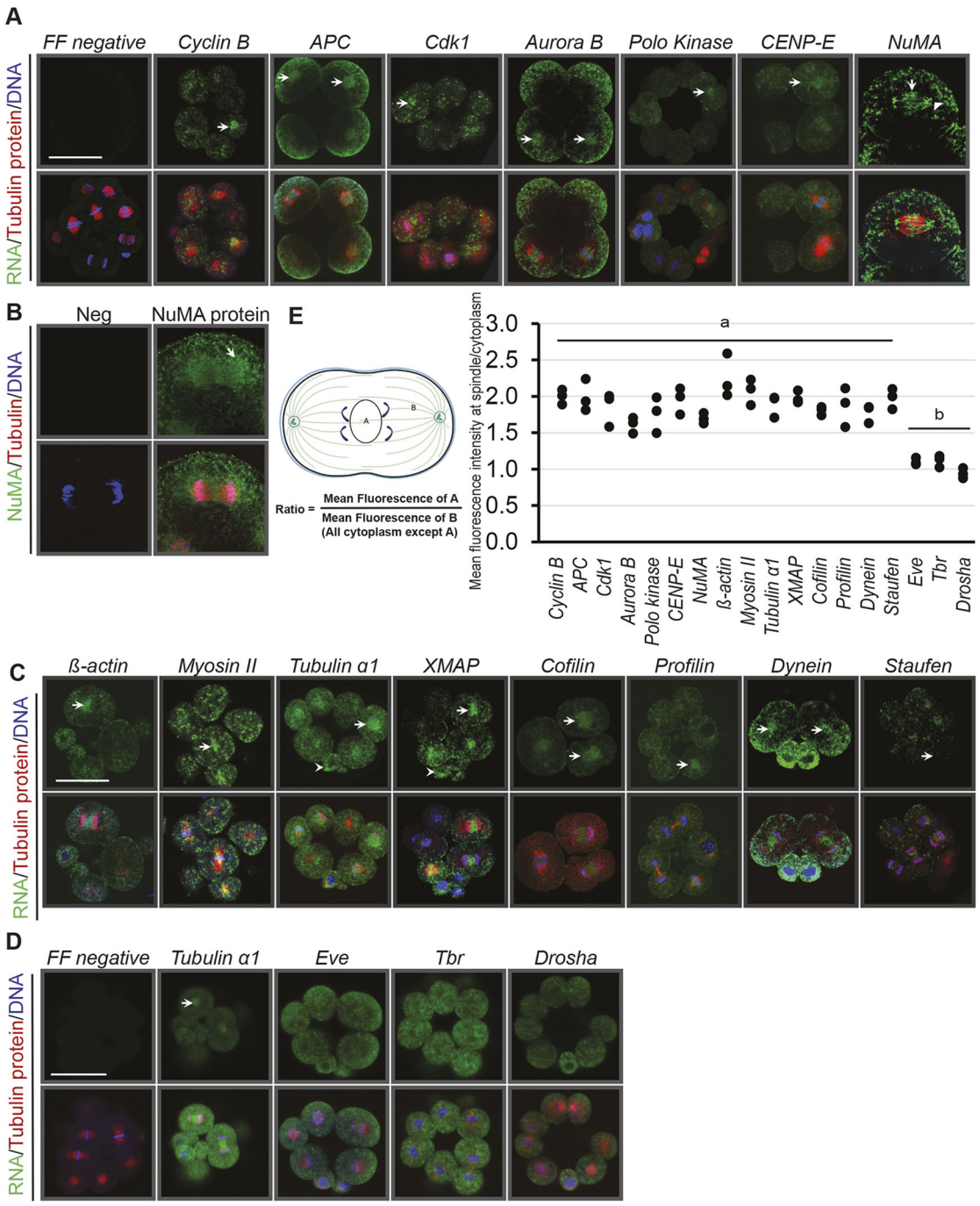

Fig. 1. RNA transcripts that encode proteins that regulate mitosis localize to the mitotic spindle in developing sea urchin embryos. (A) RNA transcripts that encode proteins that regulate mitosis localize to the mitotic spindle. Sea urchin embryos at the 16–32 cell stage were subjected to FISH, followed by immunolabeling with β-tubulin antibody, and then were counterstained with DAPI to detect DNA. Arrows indicate areas of RNA localization. Firefly probe (*FF negative*) is used as a negative control. Scale bar: 50 µm. (B) NuMA and α-tubulin protein localize to the mitotic spindle. The negative control (Neg) does not contain any primary antibody. Images shown are representative of three repeats. Scale bar: 10 µm. (C) RNA transcripts that encode cytoskeletal proteins, motor proteins or transport RNA localize to the mitotic spindle. Embryos at the 16–32 cell stage were subjected to FISH, followed by immunolabeling with β-tubulin antibody, and then were counterstained with DAPI to detect DNA. Arrows indicate areas of RNA localization. *Tubulin* α1 transcript is used as a positive control. Scale bar: 50 µm. (D) RNA transcripts that encode proteins that are not known to regulate mitosis and are expressed at the 16-32 cell stage do not localize to the mitotic spindle. Embryos were subjected to FISH using RNA probes, then immunolabeled with β-tubulin antibody, and counterstained with DAPI to detect DNA. Scale bar: 50 µm. *Tubulin α1* transcript is used as a positive control. Firefly probe (*FF negative*) is used as a negative control. Images shown are representative of three repeats. (E) The ratio of mean fluorescence intensity of RNA at the spindle to the cytoplasm was measured in wild-type embryos. A schematic of measured areas is shown, where area A represents a spindle region and area B represents the rest of the cytoplasmic region except for region A. The ratios of areas A and B represents the mean fluorescence intensity of RNA at the spindle to the cytoplasm. The ratio of mean fluorescence intensity of RNA at the spindle to the cytoplasm was measured in wild-type embryos for three blastomeres in anaphase. There is a significant increase in this ratio for *Cyclin B*, *APC*, *Cdk*, *Aurora B*, *Polo kinase*, *CENP-E* and *NuMA* compared to *Eve*, *Drosha* and *Tbr* using a one-way ANOVA with a post-hoc Tukey-Kramer test. Groups indicated with ‘a’ are significantly different from those with ‘b’. Grey-scale look-up tables for FISH images are available in Fig. S3.

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(Revilla-I-Domingo et al., 2007; Peter and Davidson, 2010). Drosha is a dsRNA-cleaving enzyme that processes microRNAs (Song et al., 2012). *Tbr* and *Drosha* are maternally present, whereas *Eve* is expressed by 6 hpf (Song and Wessel, 2007; Arshinoff et al., 2022). We observed that the RNA transcripts of *Eve*, *Tbr* and *Drosha* were present as diffuse signal throughout the cytoplasm of dividing cells (Fig. 1D). These data indicate that the localization of RNA transcripts to the mitotic spindle is not a general phenomenon that occurs with all RNA transcripts in the cell but is instead a regulated process.

To examine this enrichment quantitatively, we measured the mean fluorescence intensity at the midzone of an anaphase blastomere and in the cytoplasm, and took the ratio of these measurements (Fig. 1E). A ratio of greater than 1 indicates more enrichment at the mitotic spindle midzone, as this means the fluorescence intensity is higher at the midzone, whereas a ratio of 1 indicates that the transcript is evenly dispersed throughout the blastomere. We observed that transcripts of genes involved in mitosis (Fig. 1A,C) had significantly more enrichment at the mitotic spindle compared to transcripts of genes not involved in mitosis (Fig. 1D).

To test whether this RNA localization is evolutionarily conserved in other organisms and cells, we examined subcellular localization of a select set of these transcripts in pig epithelial kidney cells (LLC-PK1) (Hull et al., 1976). We observed *AURKB*, *PLK1* and *STAU1* transcripts to localize between dividing nuclei in LLC-PK1 cells (Fig. 2). These data indicate that the localization of RNA transcripts encoding proteins involved in mitosis is conserved from sea urchins to mammals.

NuMA and α-tubulin protein localize to the mitotic spindle in the region of their respective RNA transcripts

To determine whether the proteins encoded by *NuMA* and *Tubulin α1* RNA transcripts are localized in similar regions of the sea urchin blastomeres, we used immunolabeling to examine the subcellular localization of these proteins (Fig. 1B). Although we observed that *NuMA* RNA localized to the spindle midzone (arrows), as well as the region of the presumed centrosome (arrowhead), the NuMA protein localized to the region of the spindle midzone as well as the microtubules surrounding the presumed centrosome. For α-tubulin, both RNA and protein localized to the mitotic spindle (Fig. 1B,C). These data support the idea that the proteins encoded by *NuMA* and *Tubulin α1* RNA transcripts localize to a similar subcellular region to the transcripts.

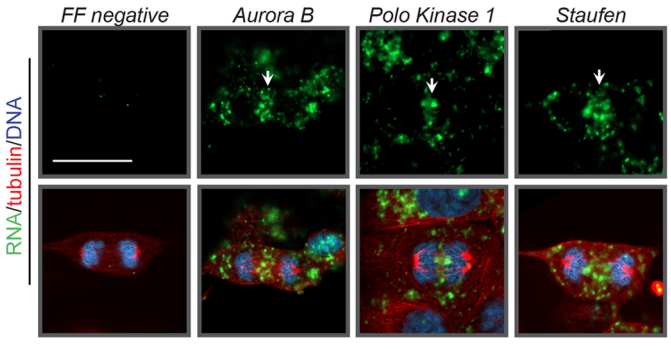

Fig. 2. Localization of RNA transcripts is evolutionarily conserved in mammalian cells. LLC-PK1 cells were subjected to FISH, followed by immunolabeling for α-tubulin (red), and then were counterstained with DAPI (blue). Arrows indicate RNA localization at the mitotic spindle. FF is used as a negative control. Scale bar: 10 µm. Images shown are representative of three repeats.

The localization of RNA transcripts to the mitotic spindle is not dependent upon actin dynamics

To understand how RNA transcripts are transported to the mitotic spindle, we used cytochalasin D (Lane et al., 1993) to disrupt actin dynamics within the sea urchin embryo, followed by examining the subcellular localization of specific transcripts. We found that the cytochalasin D resulted in an inability of the sea urchin embryos to undergo cell division in a dose-dependent manner (Fig. S1A), as well as a decrease in the amount of F-actin (Fig. S1B). However, cytochalasin D disruption of actin dynamics did not result in a change in localization of *Aurora B*, *Dynein*, *Staufen* or *Tubulin α1* transcripts (Fig. 3A). We found no significant difference in the ratio of fluorescence at the spindle to the cytoplasm between embryos treated with DMSO and embryos treated with cytochalasin D (Fig. 3B). This suggests that the transport of these RNA transcripts to the mitotic spindle is not dependent upon short-term (<30 min) disruption of actin dynamics. In order to prevent interference with cytokinesis, we did not treat embryos with cytochalasin D for longer than 30 min.

Additionally, we observed no significant difference in transcript level between embryos treated with DMSO and embryos treated with cytochalasin D, using real-time quantitative PCR (qPCR) (Fig. 3C).

Inhibition of microtubule polymerization disrupts localization of select RNA transcripts to the mitotic spindle

To test whether localization of RNA transcripts to the mitotic spindle is dependent upon intact microtubule fibers, we used colchicine to inhibit microtubule polymerization (Rieder and Palazzo, 1992), followed by detection of the subcellular localization of specific RNA transcripts. Colchicine disrupted formation of the mitotic spindle (as observed by the immunolabeling of tubulin), as well as resulting in a more diffuse distribution of *Aurora B*, *Dynein*, *Polo kinase* and *Tubulin α1* transcripts compared to that seen in embryos treated with DMSO (Fig. 4). Treatment with colchicine resulted in a significantly lower ratio of fluorescence at the spindle compared to the cytoplasm compared to that seen in control embryos (Fig. 4B), indicating that microtubule polymerization is required for localization of these RNA transcripts to the mitotic spindle. Although the subcellular localization of these transcripts was altered, we observed no significant difference in transcript level in control embryos compared to embryos treated with colchicine using qPCR (Fig. 4C).

Preventing kinesin-1 from interacting with its cargo results in reduced localization of RNA transcripts to the mitotic spindles

Given that our data suggest that RNAs are transported to the mitotic spindle along microtubule fibers, and typically RNAs are transported subcellularly by interacting with microtubule motors (Tekotte and Davis, 2002; Suter, 2018), we investigated the role of microtubule motors in localizing RNA transcripts to the mitotic spindle. Kinesin-1 is a conserved motor protein that is known to transport vesicles, organelles and ribonucleic proteins (RNP) complexes along microtubules (Hirokawa et al., 2009). Kinesin-1 has been identified to regulate the localization of RNA transcripts during *Drosophila* oogenesis, and in mammalian neurons and cardiomyocytes (Dimitrova-Paternoga et al., 2021; Fukuda et al., 2021; Scarborough et al., 2021). We used kinesore, a drug which binds to kinesin-1 at the cargo site and activates the ability of kinesin 1 to bind to microtubules (Randall et al., 2017). RNA localization to the mitotic spindles in embryos treated with kinesore was significantly reduced compared to sea urchin embryos treated with

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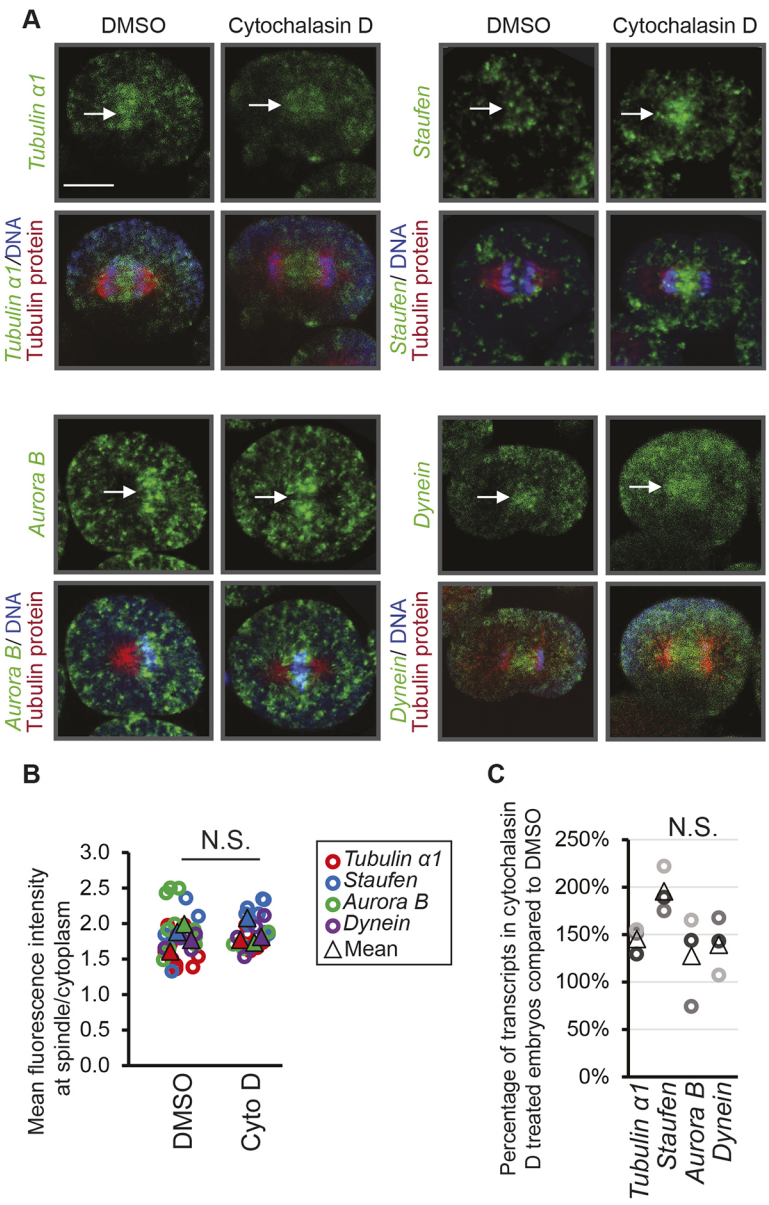

Fig. 3. Disruption of actin polymerization with cytochalasin does not change RNA localization to the mitotic spindle. (A) RNA localization to the sea urchin mitotic spindle is not affected by cytochalasin D treatment. Images are of single blastomeres of embryos at the 16–32 cell stage that were subjected to FISH with RNA probes that encode proteins known to regulate mitosis, then immunolabeled with β-tubulin antibody, and counterstained with DAPI. Arrows indicate areas of RNA localization. Scale bar: 10 µm. Images shown are representative of three repeats. (B) The ratio of the mean fluorescence intensity of the RNA at the mitotic spindle to the cytoplasm is unchanged in embryos treated with cytochalasin D compared to control embryos. An unpaired, two-tailed Student's *t*-test was performed using the means for each transcript. NS, not significant. Circles represent individual measurements; triangles represent the means for each transcript. *Tubulin α1* is in red, *Aurora B* is in green, *Staufen* is in blue, *Dynein* is in purple. (C) qPCR results indicate no difference in transcript level between control embryos and embryos treated with cytochalasin D using an unpaired, two-tailed Student's *t*-test; three biological replicates. The number of transcripts in cytochalasin D-treated embryos is expressed as a percentage of the number of transcripts in DMSO-treated embryos.

DMSO (Fig. 5A,B), as indicated by a statistically significant reduction of the ratio of mean fluorescence intensity at the mitotic spindle to the mean fluorescence intensity in the cytoplasm (Fig. 5B). This suggests that these RNAs are in part transported to the mitotic spindle by kinesin-1. We found that the total level of these transcripts was not significantly different in embryos treated with kinesore compared to that in embryos treated with DMSO (Fig. 5C).

Preventing dynein from transporting its cargo along microtubules alters localization of RNA transcripts at the mitotic spindle

As kinesin-1 is a plus-ended motor (Hirokawa et al., 2009), we also wanted to examine dynein, a minus-ended motor that has been identified to transport RNA transcripts in *Drosophila* oogenesis and neurons (Schnorrer et al., 2000; Xu et al., 2013; Herbert et al., 2017). We used ciliobrevin D to inhibit dynein from transporting its cargo along the microtubule filaments (Firestone et al., 2012). As has been reported previously, we observed smaller and more compact mitotic spindles in ciliobrevin-treated embryos compared to DMSO-treated sea urchin embryos (Fig. 6A, tubulin immunolabeling) (Firestone et al., 2012).

In control sea urchin embryos, RNA transcripts were enriched at the midzone of the mitotic spindle, whereas in ciliobrevin D-treated embryos, the RNA transcripts were enriched at the plus-ends of the astral microtubule filaments (Fig. 6A). The ratio of fluorescence at the spindle compared to the cytoplasm was significantly decreased in embryos treated with ciliobrevin D compared to that in embryos treated with DMSO, indicating that transport of the RNA transcripts to the spindle is dependent on dynein (Fig. 6B). Despite changes in the subcellular localization of *Staufen*, we observed no change in the level of *Staufen* in embryos treated with ciliobrevin D compared to control embryos, using qPCR (Fig. 6C).

The CPE within the 3′UTR of *Aurora B* is necessary for the localization of *Aurora B* RNA transcript to the mitotic spindle and is critical for early development

In order to understand how RNA transcripts are localized to the mitotic spindle, we investigated which region of the transcript is

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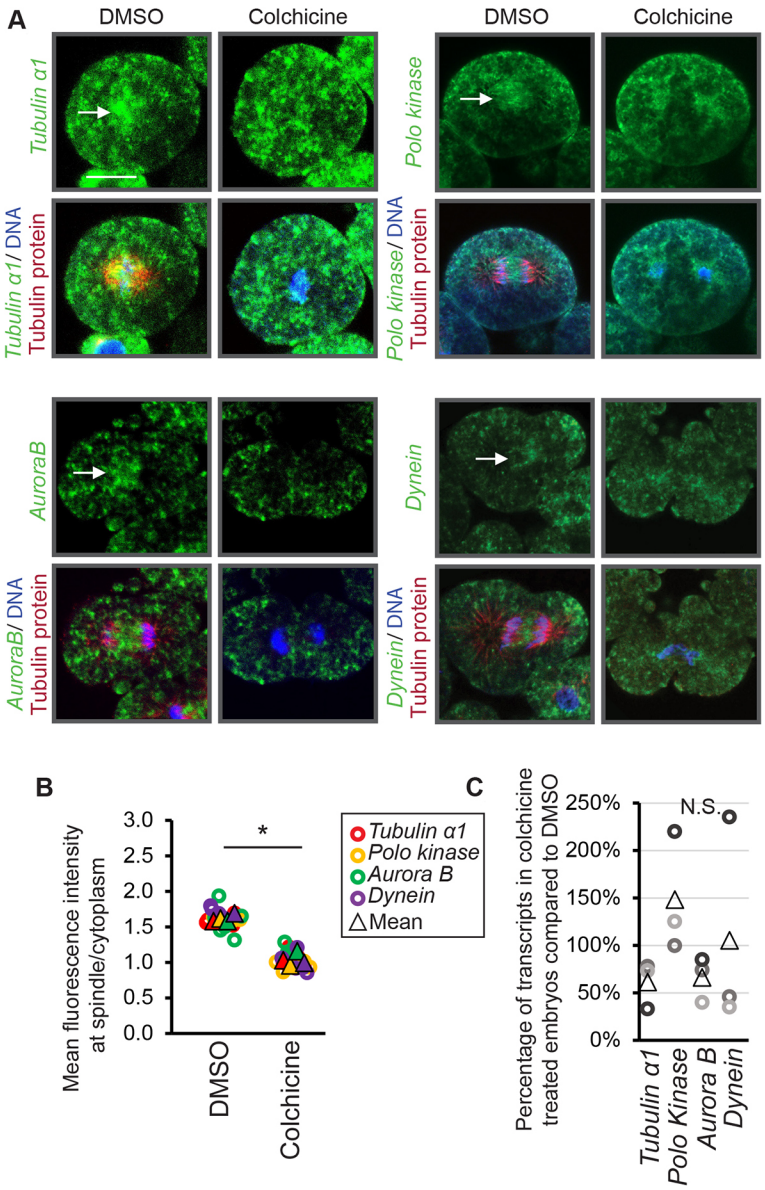

Fig. 4. Disruption of microtubule polymerization with colchicine abrogates RNA localization to the mitotic spindle. (A) RNA is no longer localized to the sea urchin mitotic spindle after embryos are treated with colchicine. Images are of single blastomeres of embryos at the 16-32 cell stage that were subjected to FISH with RNA probes of genes known to regulate mitosis, then immunolabeled with β-tubulin antibody, and counterstained with DAPI. Arrows indicate areas of RNA localization. Scale bar: 10 µm. Images shown are representative of three repeats. (B) The ratio of mean fluorescence intensity of the RNA at the mitotic spindle to the cytoplasm is significantly lower in embryos treated with colchicine compared to control embryos. An unpaired, two-tailed Student's *t*-test was performed using the means for each transcript. **P*<0.01. Circles represent individual measurements; triangles represent the means for each transcript. *Tubulin α1* is in red, *Polo Kinase* is in yellow, *Aurora B* is in green, *Dynein* is in purple. (C) qPCR results indicate no difference using an unpaired, two-tailed Student's *t*-test in transcript level between control embryos and embryos treated with colchicine; three biological replicates. The number of transcripts in colchicine-treated embryos is expressed as a percentage of the number of transcripts in DMSO-treated embryos.

necessary for localization. We cloned the 3′UTR of *Aurora B* downstream of a *Renilla* luciferase (*RLuc*) construct (*Aurora B-RLuc*) and tested its localization within the dividing sea urchin embryo (Fig. 7A,B). Results indicated that the 3′UTR is necessary and sufficient to localize *AuroraB-RLuc* to the mitotic spindles, whereas *RLuc* transcript by itself does not localize to the mitotic spindles (Fig. 7A,B). We bioinformatically identified a potential CPE within its 3′UTR. To test whether the CPE within *Aurora B* is critical for its localization to the mitotic spindles, we deleted the CPE in the *Aurora B* 3′UTR downstream of *RLuc* (Fig. 7A,B). Results indicate that deletion of the CPE abrogated localization of the *AuroraB-RLuc* transcript at the mitotic spindles (Fig. 7A).

To test whether localization of *Aurora B* RNA to the mitotic spindle has an impact on embryonic development, we designed a synthetic morpholino antisense oligonucleotide complementary to the CPE to block potential binding of CPEB to the endogenous CPE within the *Aurora B* 3′UTR (Fig. 7B). Results indicate that blocking the CPE significantly reduces localization of endogenous *Aurora B* RNA to the mitotic spindles (Fig. 7C), as the ratio of fluorescence at the spindle compared to the cytoplasm was significantly decreased in embryos in which the CPE is blocked compared to control embryos (Fig. 7C).

Importantly, blocking the *Aurora B* CPE resulted in early developmental defects. This is evident that as early as 2 hours post fertilization (hpf), when 61% of embryos injected with the control oligonucleotide had divided into two cells, whereas only 19% of embryos injected with CPE-blocking oligonucleotide had divided into two cells (Fig. 7D). This trend persisted throughout the early cleavage stages to 6 hpf, where 64.7% of embryos injected with the control oligonucleotide had reached the 16–32 cell stage, compared to 24.8% of the embryos injected with CPE-blocking oligonucleotide having reached the same developmental stage (Fig. 7D). There is no significant difference between embryos injected with the negative control oligonucleotide, which does not recognize specific sequences within the sea urchin genome, and the *Aurora B* control oligonucleotide, which is complementary to the *Aurora B* 3′UTR sequence upstream of the CPE. Of note, at 24 hpf, only 50% of the embryos injected with CPE-blocking oligonucleotide had developed into blastulae, compared to 85% of control injected embryos (Fig. S2). Taken together, as 50% of CPE-blocking

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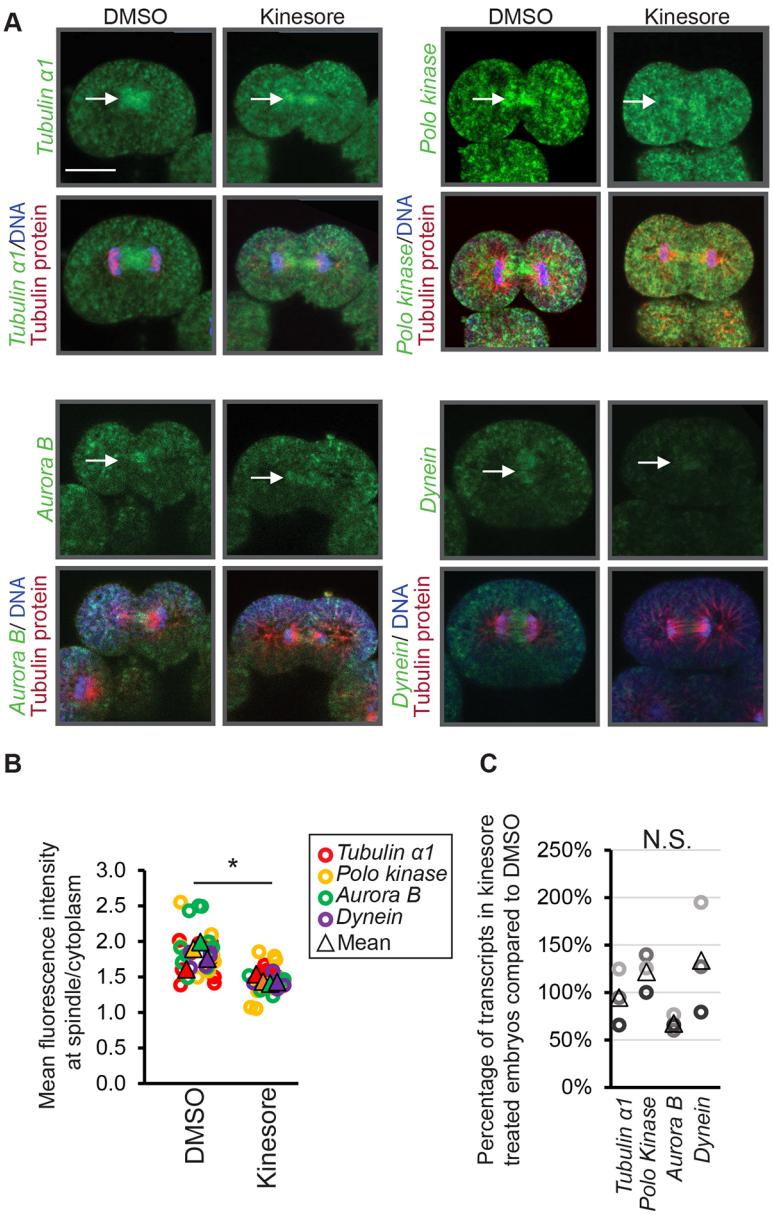

Fig. 5. Kinesore treatment diminishes RNA localization to the mitotic spindle. (A) RNA is less localized to the sea urchin mitotic spindle after embryos are treated with kinesore. Images are of single blastomeres of embryos at the 16–32 cell stage that were subjected to FISH with RNA probes of genes known to regulate mitosis, immunolabeled with β-tubulin antibody, and counterstained with DAPI. Arrows indicate areas of RNA localization. Scale bar: 10 µm. Images shown are representative of three repeats. (B) The ratio of mean fluorescence intensity of the RNA at the mitotic spindle to the cytoplasm is significantly lower in embryos treated with kinesore compared to control embryos. An unpaired, two-tailed Student's *t*-test was performed using the means for each transcript. **P*<0.01. Circles represent individual measurements; triangles represent the means for each transcript. *Tubulin α1* is in red, *Polo Kinase* is in yellow, *Aurora B* is in green, *Dynein* is in purple. (C) qPCR results indicate no difference using a unpaired, two-tailed Student's *t*-test in transcript level between control embryos and embryos treated with kinesore; three biological replicates. The number of transcripts in kinesore-treated embryos is expressed as a percentage of the number of transcripts in DMSO-treated embryos.

oligonucleotide-injected embryos did not survive to 24 hpf, mislocalization of *Aurora B* transcript likely leads to developmental arrest or lethality. Thus, these data indicate that localization of *Aurora B* transcript to the mitotic spindle is important for early development.

DISCUSSION

During early development, cells go through rapid cycles of mitosis, without intervening gap phases, making regulation of mitosis critical during this time (Siefert et al., 2015). We identified RNA localization as a potential regulatory mechanism that regulates the relatively fast cell divisions during the embryonic cleavage stages. Biochemical assays have identified transcripts that regulate cell cycle, cell division and chromosome function as being enriched in the subset of transcripts associated with mitotic spindles (Sharp et al., 2011). These assays were performed in *Xenopus* egg extract in which mitotic spindle formation was induced. To date, only a select few transcripts, such as *cyclin B* and *vasa*, have been visualized at the mitotic spindle in developing sea urchin, frog and zebrafish embryos (Groisman et al., 2000; Sharp et al., 2011; Yajima and Wessel, 2015; Takahashi et al., 2018; Waldron and Yajima, 2020; Fernandez-Nicolas et al., 2022).

We observed that transcripts encoding proteins involved in mitosis localize to the spindles (Fig. 1A,C), whereas transcripts encoding proteins that do not regulate mitosis do not show that localization (Fig. 1D). Additionally, we observed that the proteins encoded by two of these transcripts, NuMA and tubulin α1, also localize to a similar region (Fig. 1B). Prior research has indicated that localization of the RNA correlates with the site where the encoded protein functions (Mowry and Melton, 1992; Kloc and Etkin, 1994; Höfer et al., 1997; Joseph and Melton, 1998; Farina et al., 2003). For example, *Vg1* mRNA and protein localize to the vegetal pole of the *Xenopus* oocyte, where the localization of *Vg1* mRNA is known to be important for inducing endoderm and mesoderm in developing *Xenopus* embryos (Mowry and Melton, 1992; Kloc and Etkin, 1994; Joseph and Melton, 1998). Another example is that disruption of *β-actin* mRNA and protein localization to lamellipodia in chicken fibroblasts alters the polarization and migration of the cell (Höfer et al., 1997; Shestakova et al., 2001;

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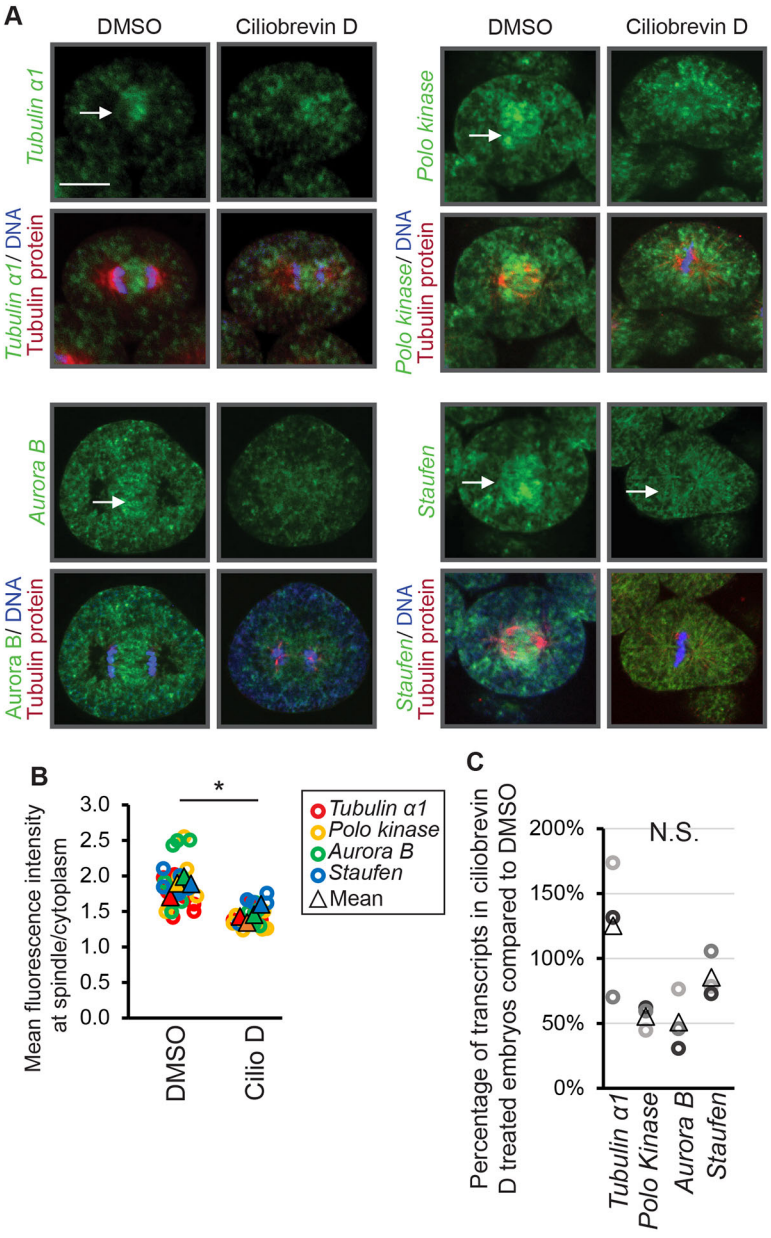

Fig. 6. Dynein inhibition alters RNA localization to the mitotic spindle. (A) RNA localization to the sea urchin mitotic spindle is altered after embryos are treated with ciliobrevin D. Images are of single blastomeres of embryos at the 16–32 cell stage that were subjected to FISH with RNA probes of genes known to regulate mitosis, immunolabeled with β-tubulin antibody, then counterstained with DAPI. Arrows indicate areas of RNA localization. Scale bar: 10 µm. Images shown are representative of three repeats. (B) The ratio of mean fluorescence intensity of the RNA at the mitotic spindle to the cytoplasm is significantly lower in embryos treated with ciliobrevin D compared to control embryos. An unpaired, two-tailed Student's *t*-test was performed using the means for each transcript. **P*<0.01. Circles represent individual measurements; triangles represent the means for each transcript. *Tubulin α1* is in red, *Polo Kinase* is in yellow, *Aurora B* is in green, *Staufen* is in blue. (C) qPCR results indicate no difference in transcript level using an unpaired, two-tailed Student's *t*-test between control embryos and embryos treated with ciliobrevin D; three biological replicates. The number of transcripts in ciliobrevin D-treated embryos is expressed as a percentage of the number of transcripts in DMSO-treated embryos.

Farina et al., 2003). In addition, intracellular RNA localization has been well studied in the context of neurons (Mayford et al., 1996; Huang et al., 2003; Dahm et al., 2007; Yoon et al., 2016, and many others). Neurons can have extremely long axons, and contain distinct intracellular regions, such as dendrites and synaptic boutons that have markedly different local environments (Li et al., 2021). The different local environments within different parts of a neuron are partially due to local translation of transcripts, such as *CaMKIIα* and *MAP2*, which is thought to regulate synaptic activity and neuronal plasticity (Huang et al., 2003; Dahm et al., 2007; Doyle and Kiebler, 2011). Additionally, there are some examples of RNAs that encode centrosomal proteins, such as *cen* and *PCNT*, localizing to the centrosome (Sepulveda et al., 2018; Bergalet et al., 2020). These examples highlight the functional importance between the localization of transcripts and the ultimate localization of their corresponding proteins.

Subcellular RNA localization has been identified to be mediated by a molecular motor transporting the RNA along a cytoskeletal element, such as an actin or microtubule filament (Tekotte and Davis, 2002). For example, RNAs known to be dependent upon actin for localization include *Ash1* in budding yeast (Takizawa et al., 1997; Beach and Bloom, 2001), and *β-actin* in embryonic fibroblasts (Latham et al., 2001), and *MAP2* in neurons (Balasanyan and Arnold, 2014). However, we found that whereas short-term disruption of actin dynamics by cytochalasin D disrupts development (Fig. S1A), it does not alter localization of RNA transcripts at the mitotic spindles (Fig. 3A,B). This result suggests that transport of the majority of these RNAs is not along actin filaments, or that the level of actin disruption was insufficient to disrupt RNA localization.

Of note is that in order to quantify changes in localization of RNA transcripts at the spindle, we utilized a ratio of the mean fluorescence of a region between the dividing nuclei at anaphase to the mean fluorescence of an identically sized region in the cytoplasm. Importantly, the ratio of fluorescence at the spindle compared to the cytoplasm is similar among the DMSO-treated

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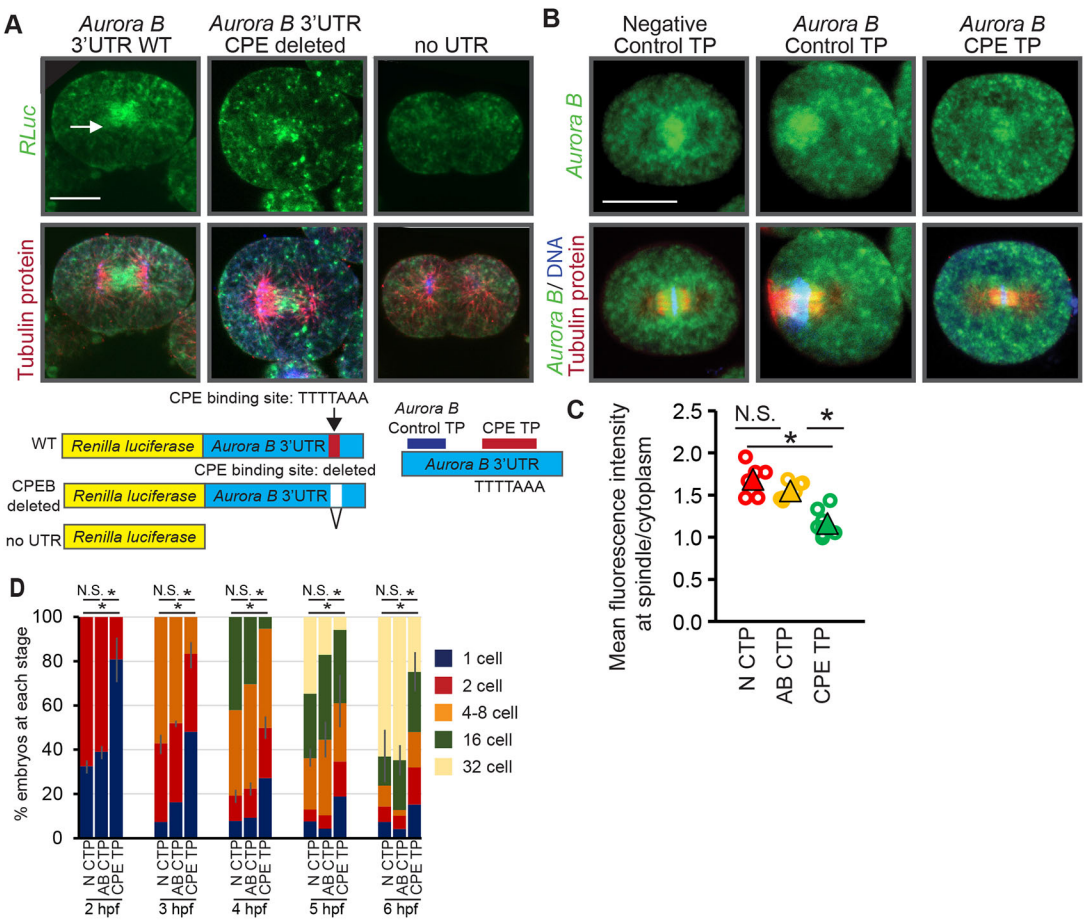

Fig. 7. The 3′UTR of *Aurora B*, and specifically the CPE sequence, is necessary for localization of RNA to the mitotic spindle. (A) Sea urchin embryos were injected with RNA constructs (shown underneath) containing *RLuc-Aurora B 3′UTR WT* RNA, *RLuc-Aurora B 3′UTR CPE deleted*, or *RLuc-no UTR*. Exogenously injected *RLuc-Aurora B 3′UTR WT* RNA is localized at the mitotic spindle, whereas *RLuc-Aurora B 3′UTR CPE* deleted is no longer localized, similar to *RLuc-no UTR* RNA. Images are of single blastomeres of embryos collected at the 16–32 cell stage then subjected to FISH with the *RLuc* probe, immunolabeled with β-tubulin antibody, then counterstained with DAPI. Arrows indicate areas of RNA localization. Scale bar: 10 µm. (B) Blocking the CPE within the 3′UTR of *Aurora B* results in less localization of endogenous *Aurora B* to the mitotic spindle compared to control embryos. Images are of single blastomeres of embryos collected at the 16–32 cell stage then subjected to FISH *Aurora B* RNA probe, immunolabeled with β-tubulin antibody, then counterstained with DAPI. Arrows indicate areas of RNA localization. Scale bar: 10 µm. Images shown in A and B are representative of three repeats. (C) The ratio of mean fluorescence intensity of the RNA at the mitotic spindle to the cytoplasm is significantly lower in embryos in which the CPE is blocked compared to control embryos. **P*<0.005; N.S., not significant (one-way ANOVA with a post-hoc Tukey–Kramer test). Circles represent individual measurements; triangles represent the means. (D) Embryos in which the CPE is blocked have significant developmental delay compared to control embryos. **P*<0.001; N.S., not significant (using a Cochran–Mantel–Haenszel test). *n*=210 negative control embryos, 255 *Aurora B 3′UTR* control embryos, 217 *Aurora B* CPE TP embryos; three biological replicates. Error bars indicate s.e.m.

controls for all the small-molecule inhibitors used (Figs 3B, 4B, 5B and 6B), as well as between the control-injected embryos (Fig. 7C). This indicates that this is a consistent way to objectively measure RNA localization to the spindle.

We observed that disruption of microtubule dynamics by colchicine abrogated the localization of these RNA transcripts at the mitotic spindle (Fig. 4A,B). This localization of transcripts could be due to RNA transcripts being actively transported along the microtubule filaments in a complex with a motor protein, as has been observed in neurons, muscles and fly embryos, among others (Lyons et al., 2009; Goldman and Gonsalvez, 2017; Denes et al., 2021). For example, the mammalian RNA-binding protein CPEB, which is known to localize *MAP2* to dendrites, has been found in granules with the motor proteins dynein and kinesin, suggesting that transport might occur along microtubule tracks (Huang et al., 2003). Alternatively, the disruption of localization of these RNA transcripts upon colchicine treatment could be due to the RNA transcripts being anchored at the spindle and that disruption of the microtubule filaments results in passive diffusion of the RNA transcripts. For example, apically localized transcripts in *Drosophila* blastoderm embryos, such as *run* and *ftz* transcripts, are transported to the apical end of the embryos by dynein, which then becomes anchored to sponge bodies, which are electron-dense particles related to P-bodies, in a microtubule-dependent manner (Delanoue and Davis, 2005). Disruption of microtubule dynamics altered localization of RNA to the mitotic spindle (Fig. 4A,B), indicating RNA localization to the spindle is dependent upon intact microtubules, but this experiment does not distinguish between transport of RNA along the microtubules and anchoring of RNA to the microtubules.

To identify whether RNAs are transported along microtubule filaments, we investigated the role of motor proteins. The main motors that have been implicated in RNA transport are myosin, which transports RNA along actin filaments, and kinesins and

dynein, which transport RNA along microtubule filaments (Takizawa et al., 1997; Januschke et al., 2002; Tekotte and Davis, 2002; Messitt et al., 2008; Xu et al., 2013). To identify the role of microtubule motors in localization of RNA transcripts to the mitotic spindle, we used kinesore, a small-molecule inhibitor which prevents kinesin-1 from binding to its cargo (Randall et al., 2017). We observed reduced localization of transcripts to the mitotic spindle in kinesore-treated embryos compared to control embryos (Fig. 5A,B). Our result is consistent with prior literature in which kinesin-1 has been identified to localize several RNA transcripts, such as *oskar* in *Drosophila* oocytes, *CaMKIIα* in oligodendrocytes (Kanai et al., 2004), and *cyclin B* in *Danio* oocytes (Takahashi et al., 2018). Both kinesin-1 and kinesin-2 are required to localize *Vg1* in *Xenopus* oocytes (Messitt et al., 2008). Our data indicate that kinesin-1 plays a role in the localization of RNA transcripts to the mitotic spindles in sea urchin embryos. We observe that kinesore has very little impact on the overall levels of transcripts, based on our qPCR data (Fig. 5C). Whereas RNA *in situ* using FISH suggests that there might be slightly less *Dynein* in kinesore-treated embryos compared to control, the qPCR shows a small decrease, with no statistical significance. One caveat is that although qPCR analysis is quantitative, the embryos collected for this analysis are not all undergoing mitosis. Thus, if *Dynein* or another transcript undergoes cell-cycle-specific changes in expression, this would not be detected with qPCR. However, the focus here is to examine the spatial localization of transcripts, and we found kinesin-1 to be important for RNA localization.

Inhibition of the AAA-ATPase of dynein with ciliobrevin D results in transcript accumulation to the plus-ends of the astral microtubules (Fig. 6A). This might be due to dynein directly transporting the RNA or through the ability of dynein to anchor RNA to microtubules (Delanoue and Davis, 2005). In addition, we observed that the mitotic spindle appears smaller (Fig. 6A), which has been observed previously in ciliobrevin D-treated cells (Firestone et al., 2012). The exact mechanism that mediates the smaller spindle is not known, but this might be due to the role of dynein in anchoring astral microtubules to the cortex of the cell (Hueschen et al., 2017), and its ability to mediate microtubule sliding in a cortical direction (Okumura et al., 2018). Given that motor proteins kinesin-1 and dynein, as well as intact microtubules are needed for the localization of RNA at the spindles, our overall results indicate that the transcripts are transported along microtubules to their final destination at the midzone of the mitotic spindle.

The transcripts are observed at the midzone of the sea urchin spindle throughout mitosis (Fig. 1A,C), and given that kinesin-1 is a plus-ended motor (Block et al., 1990), whereas dynein is typically a minus-ended motor (Raaijmakers and Medema, 2014), we can speculate that these motors are important for balancing the localization of RNA to the spindle midzone. It is known that kinesin-1 and dynein cooperate to ensure proper RNA localization in *Drosophila* embryos, where both kinesin-1 and dynein work together to properly localize *bcd* and *gurken* RNAs (Januschke et al., 2002). A caveat is that dynein is known to regulate mitotic spindle formation (Tanenbaum et al., 2008; Hueschen et al., 2017; Okumura et al., 2018) and kinesin-1 does regulate centrosomal positioning (Splinter et al., 2010) and microtubule sliding (Straube et al., 2006). Inhibiting these functions might result in spindle defects that alter RNA localization independently of RNA interactions with these motors. Further research will be needed to specifically separate these two functions.

We identified that the 3′UTR of *Aurora B* is necessary and sufficient for its localization to the mitotic spindles (Fig. 7B). In addition, deletion of the CPE or blockage of the CPE within the 3′UTR prevents localization of exogenous *Aurora B-Rluc* to the mitotic spindles (Fig. 7B,C). CPEB has been identified to be necessary for localization of *cyclin B* RNA of *Xenopus* embryos (Groisman et al., 2000), as well as for *BUB3* RNA, which encodes a mitotic checkpoint protein, to the mitotic spindle (Pascual et al., 2021). Preventing CPEB from binding to *cyclin B* RNA results in defects in mitosis (Groisman et al., 2000; Pascual et al., 2021). Similar to *cyclin B*, deletion of the CPE within the *Aurora B* 3′UTR completely abolished the localization of *Aurora B* (Fig. 7A). Furthermore, blocking the CPE site within the *Aurora B* 3′UTR also resulted in a significant reduction of localized transcript at the spindles (Fig. 7B,C). This reduction, rather than a complete abolishment of localization, might be due to the AT-rich sequence in the 3′UTR region that result in a weaker binding of the blocking oligo to the endogenous CPE within *Aurora B* transcripts. Importantly, we also observed that blocking the CPE in endogenous *Aurora B* transcript results in developmental arrest (Fig. 7D). Approximately 50% of the CPE blocking oligonucleotide-injected embryos do not live to the blastulae stage (24 hpf) (Fig. S2), indicating that the embryos experiencing developmental arrest do not survive. We do not know the exact mechanism of how mislocalization of *Aurora B* transcript away from the spindles causes developmental arrest and lethality. Potentially, disrupting localization of *Aurora B* transcript has a similar effect to blocking the localization of *cyclin B* transcript to the mitotic spindles (Groisman et al., 2000). In the case of mislocalization of *cyclin B*, these mitotic defects occur while cyclin B protein levels continue to display normal oscillations throughout the cell cycle, similar to in the control (Groisman et al., 2000). It has been suggested that the defects in mitosis are not due to a global deficit of cyclin B protein, but rather, the localization of the *cyclin B* transcript and its local translation at the mitotic spindle itself is important for progression through mitosis (Groisman et al., 2000). Given that ribosomal proteins and RNAs are present at the mitotic spindle and in early cleavage stage embryos (Hassine et al., 2020; Fernandez-Nicolas et al., 2022), an intriguing possibility is that local translation of these transcripts encoding proteins that regulate mitosis might be essential for mitotic progression.

Aurora B functions by sensing bi-polar attachment of chromosomes to the mitotic spindle (Krenn and Musacchio, 2015). This is essential to Aurora B-mediated regulation of the SAC through phosphorylation of its substrates leading to degradation of securin (Lens et al., 2003; Krenn and Musacchio, 2015). Interestingly, preventing Aurora B protein from localizing to the centrosomal region results in defects in mitosis, despite the fact that the protein retains its ability to act as a kinase (Scrittori et al., 2005). Aurora B also has a function in error correction during anaphase, where it rapidly corrects lagging chromosomes that, if left uncorrected, can result in micronuclei formation (Orr et al., 2021; Sen et al., 2021). Interestingly, we observe *Aurora B RNA* localized at the spindle midzone in both metaphase and anaphase (Fig. S2B), consistent with its roles in both phases of mitosis. Together with results from prior studies and our study, we propose that the function of Aurora B protein is tightly tied to its transcript localization which adds another layer of regulation of mitosis (Fig. 8). We propose that this regulation extends to other important mitotic regulators as well. Intriguingly, this RNA localization is a conserved phenomenon observed in mammalian cells as well (Fig. 2). Given that mitosis, especially during the early cleavage stages of development, must occur rapidly and be tightly controlled, localizing the RNA of key players of mitosis might be an evolutionarily conserved mechanism

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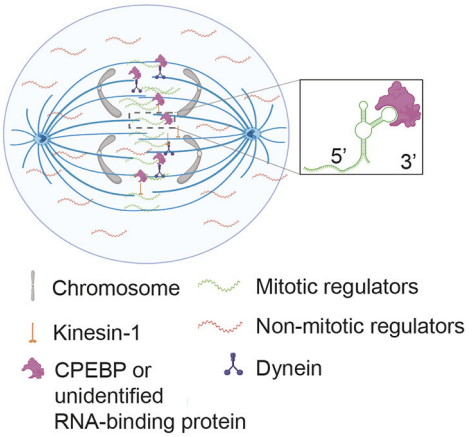

Fig. 8. Model of RNA transcript localization to the mitotic spindle. RNA transcripts that encode proteins that regulate mitosis are localized to the mitotic spindle. Microtubule motors kinesin-1 and dynein are involved in the transport of these RNA transcripts. Based on our results as well as previous research, we hypothesize that local translation of these transcripts (e.g. *cyclin B* and *Aurora B*) are essential for proper development (Groisman et al., 2000). The 3′UTR of the RNAs contain sequences responsible for binding of RNA-binding proteins, such as CPEB, which might be responsible for localization of the RNA to the mitotic spindle. RNA localization allows local translation at the spindles, which is a regulatory mechanism to ensure rapid cell divisions that occur during the early cleavage stage.

to facilitate rapid changes in the translation of these RNAs, allowing for proper cell division to occur.

MATERIALS AND METHODS

Animals

Adult *Strongylocentrotus purpuratus* were collected from Marinus Scientific, LLC (Lakewood, CA) or Point Loma Marine Invertebrate Labs (Lakeside, CA) and were maintained at 12°C in artificial sea water (ASW) made from distilled, deionized water and Instant Ocean©. Adults were induced to shed either through shaking or intracoelomic injection of 0.5 M KCl. Embryos were cultured at 12°C in filtered natural sea water (FSW) obtained from the Indian River Inlet (University of Delaware).

Cell culture

LLC-PK1 (Hull et al., 1976; LLC-PK1, ATCC No. CL-101) cells were maintained in DMEM/F12 (Thermo Fisher Scientific, Waltham, MA) medium supplemented with 10% fetal bovine serum (MilliporeSigma, Burlington, MA) at 37°C under 5% CO_2.

Fluorescence RNA *in situ* hybridization and immunolabeling

The steps performed for fluorescence RNA *in situ* hybridization (FISH) were as described previously with modifications (Sethi et al., 2014). RNA *in situ* hybridization probes were amplified using sea urchin cDNA for sea urchin-specific probes and porcine cDNA for mammalian probes. Primers were synthesized based on known sequences (IDTDNA, Coralville, Iowa) and amplicons were ligated into the ZeroBlunt vector (Thermo Fisher Scientific) (Table S1). Positive clones were sequenced (Genewiz Services, South Plainfield, NJ), digested (Thermo Fisher Scientific) and DIG-labeled using specific RNA polymerases (MilliporeSigma) as described in Table S1. Probe was used at 0.5 ng probe/µl to detect native transcript in embryos, according to previous protocols (Stepicheva et al., 2015). The embryos were incubated with anti-digoxigenin-POD antibody at 1:1000 (cat. no. 11207733910, MilliporeSigma) overnight at 4°C and amplified with Tyramide Amplification working solution (1:150 dilution of TSA stock with 1× Plus Amplification Diluent-fluorescence; Akoya Biociences, Marlborough, MA). The embryos were washed with MOPS buffer three times then with PBST (1× PBS with 0.1% Triton X-100) three times. After FISH, embryos were incubated for overnight at 4°C in E7 antibody against β-tubulin (Developmental Studies Hybridoma Bank, Iowa City, IA) diluted to 5 µg ml^{-1} in 4% sheep serum (MilliporeSigma) in PBST. Embryos were washed three times with PBST then incubated for 1 h at room temperature with secondary antibody (Alexa-Fluor-647 conjugated, Thermo Fisher Scientific) diluted at 1:300 in 4% sheep serum in PBST. Embryos were washed three times with PBST, then counterstained with DAPI (Thermo Fisher Scientific). Images were obtained with a Zeiss LSM 780 or 880 scanning confocal microscope (Carl Zeiss Incorporation, Thorwood, NY). Single digital image or the maximum intensity projections of the *Z*-stack of images were acquired with Zen software and exported into Adobe Photoshop and Illustrator (Adobe, San Jose, CA) for further processing.

LLC-PK cells were fixed (100 µM MOPS, 0.1% Tween 20, 4% paraformaldehyde in PBS) for 15 min at room temperature, then washed with PBS with 0.01% Tween 20. Cells were hybridized as described (Martín-Durán et al., 2017), and incubated with 0.5 ng $µl^{-1}$ probe at 50°C for 48 h. The cells were incubated with anti-digoxigenin-POD antibody at 1:1000 (MilliporeSigma) for 1 h at room temperature and amplified with Tyramide Amplification working solution (1:150 dilution of TSA stock with 1× Plus Amplification Diluent-fluorescence). Cells were then incubated at room temperature in anti-α-tubulin antibody (cat. no. 6603-1, Proteintech, Rosemont, IL) at 1:100 dilution in 4% sheep serum in PBST for 1 h at room temperature. Cells were washed three times with PBST then incubated for 1 h at room temperature with secondary antibody (Alexa Fluor 647, Thermo Fisher Scientific, Waltham, MA) diluted at 1:300 in 4% sheep serum in PBST. Cells were mounted in VectaShield Anti-Fade mounting medium with DAPI (Vector Laboratories, Newark, CA). Images were obtained with a Zeiss LSM 780 or 880 scanning confocal microscope. Single digital image or the maximum intensity projections of *Z*-stack of images were acquired with Zen software and exported into Adobe Photoshop and Illustrator (Adobe, San Jose, CA) for further processing. Excess DNA in the images is due to sperm and does not affect the interpretation of the results.

Microinjections and RNA constructs

Microinjections were performed as previously described with modifications (Cheers and Ettensohn, 2004; Song et al., 2012; Stepicheva and Song, 2015). Injection solutions contained 20% sterile glycerol, 2 mg ml^{-1} 10,000 MW FITC lysine charged dextran (Thermo Fisher Scientific) and 50 ng $µl^{-1}$ of *Renilla* Luciferase (RLuc) constructs. Injections were performed using the Pneumatic pump system (World Precision Instruments, Sarasota, FL) (Stepicheva and Song, 2015; Stepicheva et al., 2015). A vertical needle puller PL-10 (Narishige, Tokyo, Japan) was used to pull the injection needles (1 mm glass capillaries with filaments) (Narishige Tokyo, Japan).

The 3′UTR of *Aurora B* was amplified with PCR using sea urchin cDNA and cloned into the ZeroBlunt vector (Thermo Fisher Scientific) (Table S1, primers). Positive clones were sequenced (Genewiz Services, South Plainfield, NJ) and subcloned into *RLuc* as described previously (Stepicheva et al., 2015). The CPE element was identified bioinformatically and was deleted from the plasmid using site-directed mutagenesis (Lightning QuikChange Mutagenesis, Agilent, Santa Clara, CA). DNA sequencing of these plasmids indicated successful deletion (Genewiz, NJ). The plasmids were digested with EcoRI (Thermo Fisher Scientific) and RNA was *in vitro* transcribed using mMESSAGE mMACHINE Sp6 Transcription Kit (Thermo Fisher Scientific). mRNA was purified using NucleoSpin RNA clean up kit (Macherey-Nagel, Bethlehem, PA), and passed through a Millipore Ultrafree 0.22 µm centrifugal filter (MilliporeSigma) prior to microinjections. RNA constructs were injected at a final concentration of 50 ng $µl^{-1}$.

Block CPE element with antisense morpholino oligonucleotides (MASO)

To examine whether the CPE element was important for localization of *Aurora B* transcript to the mitotic spindles, we designed a target protector MASO (TP) blocking the cytoplasmic polyadenylation element (CPE): 5′-AGCTCGAATGATAAAGCTTACTTTAAAACA-3′, with the CPE sequence underlined (GeneTools, Philomath, OR). Owing to the high

A-T content of this CPE region, the TP sequence was designed to be a 30-mer to ensure sufficient affinity to the *Aurora B* transcript. For negative controls, we used 5′-CCTCTTACCTCAGTTACAATTTATA-3′, which targets a human β-globin intron mutation purchased from GeneTools (Philomath, OR). We also designed a negative control TP complementary to the 3′UTR of *Aurora B*, 5′-CTCAACATACGTTTTCATACAAAGT-3′, which is upstream of the CPE. Embryos were injected with a final concentration of 5 µM, 50 µM and 500 µM of the oligonucleotides, and observed at 24 hpf to determine which concentration resulted in 50% mortality. All experiments described were performed using a final concentration of 500 µM.

Embryos were injected with negative control, *Aurora B* TP control and *Aurora B* CPE TPs, then the embryos were assessed for stage of development every hour after fertilization until 6 hpf.

Drug studies

Wild-type embryos were fertilized and cultured to 16–32 cell stage (∼5 hpf) and were treated with either 50 µM kinesore, 100 µM ciliobrevin D, 10 mM colchicine, 20 µM cytochalasin D, or DMSO at equivalent concentrations in FSW for 30 min at 12°C. All drugs were obtained from MilliporeSigma and dissolved in DMSO. The embryos were then fixed immediately, and followed by FISH or collected for real-time quantitative PCR (qPCR).

qPCR

To examine the relative quantities of transcripts of *Aurora B*, *Polo kinase*, *Dynein*, *Staufen* and *Tubulin α1* after disruption of cytoskeletal dynamics, we used qPCR to examine their transcript levels. Two hundred eggs or embryos were collected immediately prior to treatment with kinesore, ciliobrevin D, colchicine, cytochalasin D or DMSO and immediately after treating for 30 min. Total RNA was extracted with NucleoSpin RNA XS kit (Macherey-Nagel, Bethlehem, PA). cDNA was synthesized using the iScript cDNA synthesis kit (BioRad, Hercules, CA). qPCR was performed using 7.5 embryo equivalents for each reaction using Fast SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, MA). Reactions were run on the QuantStudio 6 Real-Time PCR cycler system (Thermo Fisher Scientific, Waltham, MA), as previously described (Sampilo et al., 2018). Threshold cycle (Ct) values were normalized first to *ubiquitin* and are shown as the percentage of transcript compared with DMSO-treated embryos, using the $2^{-\Delta\Delta Ct}$ method (Stepicheva and Song, 2015) and converting fold-change to percentage. Primers were designed using Primer3 (Untergasser et al., 2012) (Table 1).

ImageJ analysis

To quantitatively analyze the enrichment of transcripts to the mitotic spindle, single plane images of embryos containing blastomeres in anaphase were exported from Zen as TIFFs. These images were opened in ImageJ (Schneider et al., 2012). A region spanning the area between the chromosomes was selected, and the mean fluorescence intensity (MFI) was measured and designated the spindle MFI. The spindle region was then masked from the entire blastomere area and the MFI of this region was measured and designated the cytoplasmic MFI. The ratio was then calculated by dividing the spindle MFI by the cytoplasmic MFI.

Immunolabeling and phalloidin staining

To examine the localization of NuMA and α-tubulin, embryos were immunolabeled with the respective antibodies, as previously described (Remsburg et al., 2021; Konrad and Song, 2022) with modifications. Embryos were fixed in 100% ice-cold methanol on ice for 10 min, then washed with PBST three times. They were then blocked in PBST with 4% sheep serum at room temperature for 1 h. Embryos were then incubated in anti-NuMA antibody (cat. no. 16607-1, Proteintech, Rosemont, IL) at 1:100 dilution and anti-α-tubulin antibody (cat. no. 6603-1, Proteintech, Rosemont, IL) at 1:100 dilution overnight at 4°C. Embryos were then washed with PBST three times and incubated sequentially in secondary antibody (anti-rabbit-IgG conjugated to Alexa Fluor 488 and anti-mouse-IgG conjugated to Alexa Fluor 647, Thermo Fisher Scientific) diluted to 1:300 in 4% sheep serum (MilliporeSigma) in PBST. Embryos were washed three times with PBST, then counterstained with DAPI (Thermo Fisher Scientific). Images were obtained with a Zeiss LSM 780 or 880 scanning confocal microscope (Carl Zeiss Incorporation, Thorwood, NY). Single digital image or the maximum intensity projections of *Z*-stacks of images were acquired with Zen software and exported into Adobe Photoshop and Illustrator (Adobe, San Jose, CA) for further processing.

To examine F-actin, embryos were labeled with fluorescently conjugated phalloidin as previously described (Konrad and Song, 2022) with minor modifications. Alexa Fluor-647-conjugated phalloidin was reconstituted in DMSO, then diluted to 10 U ml^{-1} in PBST. Embryos were washed three times with PBST, then counterstained with DAPI (Thermo Fisher Scientific). Images were obtained using Zen software and a Zeiss ObserverZ1 using an AxioCam MRm.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.M.R., K.D.K., J.L.S.; Methodology: C.M.R., K.D.K., J.L.S.; Validation: C.M.R., K.D.K.; Formal analysis: C.M.R., J.L.S.; Investigation: C.M.R., K.D.K., J.L.S.; Resources: J.L.S.; Data curation: C.M.R.; Writing - original draft: C.M.R.; Writing - review & editing: C.M.R., K.D.K., J.L.S.; Visualization: C.M.R., K.D.K., J.L.S.; Supervision: J.L.S.; Project administration: C.M.R., J.L.S.; Funding acquisition: J.L.S.

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Data availability

All relevant data can be found within the article and its supplementary information.

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