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Dipen S Mehta, Augusta University
Hala Zein-Sabatto, Emory University
Pearl Ryder, Emory University
Jina Lee, Emory University
Dorothy Lerit, Emory University

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**Drosophila centrocortin is dispensable for centriole duplication but contributes to centrosome separation**

Dipen S. Mehta,1,† Hala Zein-Sabatto 2,† Pearl V. Ryder 2,3,† Jina Lee 3, Dorothy A. Lerit 2,4

1College of Science and Mathematics, Augusta University, Augusta, GA 30912, USA,
2Department of Cell Biology, Emory University School of Medicine, Atlanta, GA 30322, USA,
3Wandr, Atlanta, GA 30340, USA,
4Emory College of Arts and Sciences, Emory University, Atlanta, GA 30322, USA

*Corresponding author: Department of Cell Biology, Emory University School of Medicine, 615 Michael Street, Room 444 Whitehead Building, Atlanta, GA 30322, USA. Email: dlerit@emory.edu
†These authors contributed equally to this work.

**Abstract**

Centrosomes are microtubule-organizing centers that duplicate exactly once to organize the bipolar mitotic spindle required for error-free mitosis. Prior work indicated that *Drosophila centrocortin* (cen) is required for normal centrosome separation, although a role in centriole duplication was not closely examined. Through time-lapse recordings of rapid syncytial divisions, we monitored centriole duplication and the kinetics of centrosome separation in control vs cen null embryos. Our data suggest that although cen is dispensable for centriole duplication, it contributes to centrosome separation.

**Keywords:** centrosome; mitosis; RNA localization; centrocortin

**Introduction**

Centrosomes are the microtubule-organizing centers (MTOCs) of most eukaryotic cells and promote error-free mitosis through organization of the bipolar mitotic spindle. Centrosome function as a MTOC is instructed by the pericentriolar material (PCM), a matrix of proteins that encircles the central pair of centrioles (Nigg and Raff 2009).

Centrosomes are essential for early *Drosophila* embryogenesis, where they coordinate rapid S-M abridged nuclear division cycles and cortical migration of the nuclei to give rise to the syncytial blastoderm embryo (Rothwell and Sullivan 2000; Stevens et al. 2007; Gonzalez 2008). As conserved in many organisms, *Drosophila* embryogenesis requires a maternal supply of mRNAs and proteins to support early development until zygotic genome activation; thus, syncytial-stage embryos are largely transcriptionally quiescent (Tadros and Lipshitz 2009). The early embryonic mitotic divisions, therefore, are predominantly regulated by maternally endowed supplies.

Among the resident components of centrosomes are mRNAs (Marshall and Rosenbaum 2000; Alliegro 2011). A genome-wide screen within early *Drosophila* embryos identified a subset of transcripts enriched near spindle poles and encoding proteins key to centrosome function and mitotic progression (Lécuyer et al. 2007). The localization of mRNAs to centrosomes is evolutionarily conserved and observed across taxa (Groisman et al. 2000; Lambert and Nagy 2002; Alliegro et al. 2006; Blower et al. 2007; Lécuyer et al. 2007; Sepulveda et al. 2018; Zein-Sabatto and Lerit 2021). Despite this high level of conservation, functions of centrosome-localized mRNAs remain underexplored.

Systematic functional analysis of a few individual mRNAs suggests local RNAs support spindle morphogenesis and mitotic progression, as demonstrated for localized cyclin B mRNA in *Xenopus* oocytes (Groisman et al. 2000). In *Drosophila*, recent work indicates centrocortin (cen) mRNA localized to centrosomes is similarly required for error-free mitosis. Here, the cen coding sequence is necessary and sufficient for cen mRNA localization to pericentrosomal RNA granules, which likely function as sites of translational regulation (Bergalet et al. 2020; Ryder et al. 2020). Indeed, high-throughput analyses support an emerging view that co-translational transport supports the localization of several conserved centrosome mRNAs (Sepulveda et al. 2018; Chouaib et al. 2020; Kwon et al. 2021; Safieddine et al. 2021).

Historically, several hypotheses were suggested to account for why mRNAs reside at centrosomes, including the postulation that mRNA may instruct centrosome duplication (Alliegro et al. 2006). In this view, mRNA may serve as the genetic material required to support the semi-conservative replication of centrioles, where an older “mother” centriole serves as the template for the younger “daughter” centriole (Pelletier and Yamashita 2012). Alternatively, mRNA may support the structural integrity of the centrosome (Woodruff et al. 2018). Other models of centrosomal mRNA functions are also possible (Ryder and Lerit 2018). Importantly, these models remain to be tested.
In this study, we investigated whether cen contributes to centriole duplication. Normally, centrioles duplicate only once per cell cycle, and this regulation is key to prevent multipolar spindles and chromosomal instability (Wong and Stearns 2003; Tsou and Stearns 2006). In Drosophila, cen mutant embryos show mitotic defects, including errant centrosome separation and multipolar spindles, despite normal microtubule assembly (Kao and Megraw 2009). However, centriole duplication was not previously examined in cen mutants. Given the requirement for local cen mRNA for the integrity of centrosome functions, we assessed the requirement of cen in centriole duplication and centrosome separation in live embryos.

**Materials and methods**

**Fly stocks**

Drosophila strains used in this study are summarized in Table 1.

Flies were raised on Bloomington formula “Fly Food B” (LabExpress, Ann Arbor, MI, USA), and crosses were maintained at 25°C in a light and temperature-controlled incubator chamber. To examine maternal effects, cen mutant embryos are progeny derived from homozygous cen<sup>504787</sup> mutant mothers.

**Generation of RFP-PACT**

P[Ubi-RFP-PACT] was a gift from Nasser Rusan (NIH) and was generated by introducing amino acids 2,479–3,555 from PLP-PF (Fragment 5), into the vector pURW (plasmid 1282, Drosophila Genomics Resource Center) to generate an RFP fusion, as described for the GFP fusions in Galletta et al. (2014). Transgenic animals were generated by BestGene, Inc. (Chino Hills, CA, USA).

**Microscopy**

For live imaging, dechorionated 1–2 h embryos were adhered to a 22 × 30 mm #1.5 coverslip using glue extracted from double-sided tape (3M), covered with Halocarbon oil 700 (H8898, Millipore-Sigma), and inverted onto a 50-mm gas permeable dish (Sarstedt) with broken #1 coverslips used as spacers. Images were captured on a Nikon Ti-E inverted microscope fitted with a Nikon T-E immersion objective, using a Nikon 100x, 1.49 NA Apo TIRF oil immersion objective. Images were acquired at 300 ms exposure times over 0.5 s/m of tissue at 20 s time intervals for one or more complete embryonic nuclear cycles (NCs).

**Image analysis**

Images were randomized, and the experimenter was blinded to genotype during analysis. We measured the time elapsed from when centrioles were duplicated, visible as 2 proximal RFP-PACT signals, until they were fully separated. We define full separation as the moment when centrioles remain at distal sides of the nucleus, approximately late prophase. Time-lapse recordings from age-matched nuclear division cycle 13 samples were used. Once centrosomes were fully separated, the distance between centrosomes was measured using the line tool. The time between successive centriole duplication events was monitored between NCs 12 and 13. Image analysis was completed in FIJI (NIH; Schindelin et al. 2012). Images were assembled using Adobe Photoshop and Illustrator software.

**Statistical analysis**

Data were unblinded, plotted, and statistical analysis was performed using Microsoft Excel and GraphPad Prism software. Normal data distribution was tested using a D’Agostino and Pearson normality test, followed by a 2-tailed nonparametric Mann–Whitney test, 2-tailed t-test with Welch’s correction, or a chi-square test, as indicated in the figure legend. Data shown are representative results from at least 2 independent experiments.

**Results and discussion**

To address whether cen contributes to centriole duplication, we examined the kinetics of centrosome duplication and separation in live control vs cen mutant Drosophila embryos. For these studies, we used embryos collected from homozygous cen<sup>504787</sup> mothers. The cen<sup>504787</sup> allele is a PBac transposon insertion disrupting the cen locus (Bellen et al. 2011). Prior work confirms cen<sup>504787</sup> animals are null for Cen protein (Kao and Megraw 2009) and mRNA (Berghalet et al. 2020).

NC 13 control and cen mutant embryos co-expressing GFP-Cnn to label the PCM and RFP-PACT to mark the centrioles were monitored by video microscopy (Fig. 1, a and b, Supplementary Videos 1 and 2). Through blinded image analysis, we calculated the time elapsed from when centrosomes are duplicated, visible as 2 proximal RFP-PACT signals, until they were fully separated (Fig. 1c). Similar to controls, centrosomes from cen null embryos duplicated and initiated centriole separation. However, about 20% of cen centrosomes required more time to fully separate (12/52 centrosome pairs; open dots, Fig. 1c) or failed to separate (arrow), consistent with prior work (Kao and Megraw 2009). On average, centrosomes from NC 13 cen mutant embryos required about 30% more time than controls to separate (time to separate, expressed as mean ± SD, was 396.1 ± 350.1 s for cen mutants vs 306.3 ± 65.8 s for controls; F = 0.0071 by Mann–Whitney test). Examination of the frequency distribution of centrosome separation velocities confirmed that the centrosomes in cen mutant embryos require more time than controls (>400s) to separate (Fig. 1d; *P = 0.01081 by chi-square test). These data indicate cen is required to complete efficient centrosome separation.

Our data show Cen supports the kinetics of centrosome separation. We next measured the distance between centrosomes once fully separated (Fig. 1, a and b; 4:20) to further investigate the extent of centrosome separation in cen mutant embryos. On average, control centrosomes were separated 4.4 ± 0.7 μm in NC 13 late prophase embryos (Fig. 1e). In contrast, a subset of cen mutant centrosomes failed to separate or separated less than 3 μm (7.5%, N = 4/53; Fig. 1e, open symbols). Most cen mutant centrosomes, however, did successfully separate. Unexpectedly, many cen mutant centrosomes separated greater distances than controls, perhaps due to centrosome detachment errors.

### Table 1 Drosophila strains used.

<table>
<thead>
<tr>
<th>Drosophila strain</th>
<th>Source</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>P[Ubi-RFP-PACT]</td>
<td>This paper</td>
<td>N-terminal fusion of RFP to a C-terminal fragment of PLP containing the PACT domain</td>
</tr>
<tr>
<td>cen&lt;sup&gt;504787&lt;/sup&gt;</td>
<td>Kao and Megraw (2009)</td>
<td>Protein and RNA null allele; Bloomington stock #18805</td>
</tr>
</tbody>
</table>
To determine if cen is required for centriole duplication, we quantified the time between successive centriole duplication events, spanning NC 12 to 13. All examined centrioles completed duplication, indicating that cen is dispensable for centriole duplication (Fig. 1f). A small proportion of cen mutant centrioles duplicated at faster rates than controls (Fig. 1f). While the underlying mechanism of this defect remains unknown, these more rapidly dividing centrioles likely correspond to anucleated centrosomes following the ejection of damaged nuclei from the syncytial blastoderm cortex. Taken together, our analysis indicates cen contributes to normal centrosome separation kinetics but is not required for centriole duplication. Further work is required to unearth precisely how cen promotes centrosome separation.

Although several mRNAs localize to centrosomes, the functions of most centrosome proximal mRNAs remain unstudied (Zein-Sabatto and Lerit 2021). Drosophila cen mRNA is a notable exception, as mislocalization of cen mRNA to the anterior cortex via the bicoid (bcd) 3′-UTR demonstrated that local cen mRNA is required to recruit Cen protein to distal centrosomes and support spindle morphogenesis, genome stability, and embryonic viability (Ryder et al. 2020). Among the phenotypes noted in that study were mispositioned centrosomes, which could be an indirect effect of the large-scale nuclear fallout DNA damage response observed in cen-bcd-3′UTR embryos. To circumvent this technical challenge, we examined centrosome separation in cen null embryos, which lack mRNA and protein. From this study, we conclude that local cen activity is dispensable for normal centriole duplication but supports centrosome separation. Whether local cen mRNA or protein is sufficient to support centrosome separation remains to be determined.

**Data availability**

Strains are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables. Supplemental data are available on Figshare: time-lapse recordings Supplementary Video 1 (control) and Supplementary Video 2 (cen mutant) show embryos expressing RFP-PACT and GFP-Cnn. Supplemental Material included at figshare: https://doi.org/10.25387/g3.17096684.

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DSM: formal analysis, writing—review editing. HZ-S: investigation, formal analysis, writing—review editing. PVR: investigation, conceptualization. JL: investigation. DAL: conceptualization, formal analysis, supervision, visualization, writing—original draft, writing—review editing.

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**Conflicts of interest statement**

None declared.

**Literature cited**


