Report

The Inositol 5-Phosphatase dOCRL Controls PI(4,5)P2 Homeostasis and Is Necessary for Cytokinesis

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Summary

During cytokinesis, constriction of an equatorial actomyosin ring physically separates the two daughter cells. At the cleavage furrow, the phosphoinositide PI(4,5)P2 plays an important role by recruiting and regulating essential proteins of the cytokinesis machinery [1]. Accordingly, perturbation of PI(4,5)P2 regulation leads to abortive furrowing and binucleation [2–4]. To determine how PI(4,5)P2 is regulated during cytokinesis, we individually knocked down each of the enzymes controlling the phosphoinositide (PIP) cycle in *Drosophila*. We show that depletion of the *Drosophila* ortholog of human oculocerebrorenal syndrome of *Lowe 1* (OCRL1), an inositol 5-phosphatase mutated in the X-linked disorder oculocerebrorenal Lowe syndrome, triggers a high rate of cytokinesis failure. In absence of dOCRL, several essential components of the cleavage furrow were found to be incorrectly localized on giant cytoplasmic vacuoles rich in PI(4,5)P2 and in endocytic markers. We demonstrate that dOCRL is associated with endosomes and that it dephosphorylates PI(4,5)P2 on internal membranes to restrict this phosphoinositide at the plasma membrane and thereby regulates cleavage furrow formation and ingression. Identification of dOCRL as essential for cell division may be important to understand the molecular basis of the phenotypic manifestations of Lowe syndrome.

Results and Discussion

dOCRL, the *Drosophila* Ortholog of OCRL1, Is Required for Cell Division

To identify which of the enzymes controlling the PI(4,5)P2 cycle are essential for cytokinesis, we individually depleted the 34 *Drosophila* putative kinases (13), phosphatases (16), and phospholipases (5) that directly or indirectly regulate PI(4,5)P2 levels, and we recorded cell divisions in an α-tubulin-GFP *Drosophila* S2 cell line [5]. Among these enzymes, inactivation of CG9115 and CG3573 led to a significant increase in cytokinesis failure (see Figures S1A and S1C available online). Whereas the myotubularin CG9115 has already been implicated in cell-cycle progression [6], we report here the first evidence that the inositol 5-phosphatase CG3573 is important for cytokinesis. The cytokinesis defects observed in the absence of CG3573 function correlated with the appearance of giant multinucleated cells, with cells that can reach ten times the size of control cells (Figure 1A).

The CG3573 gene encodes a protein of 850 amino acids that we named dOCRL after one of its two human orthologs, OCRL1 (oculocerebrorenal syndrome of Lowe 1) (Figure S1B). dOCRL comprises an inositol polyphosphate 5-phosphatase catalytic domain (IPP) and a C-terminal RhoGAP-like domain that is catalytically inactive in both dOCRL human orthologs [7]. Mutations in OCRL1 are responsible for Lowe syndrome, a rare X-linked disorder seen in ∼1 in 200,000 births and for some cases of Dent disease [8, 9]. The cellular function of OCRL1 is poorly characterized; it has been proposed to modulate membrane traffic [10], actin dynamics [11], and/or Pi(4,5)P2 homeostasis [12]. Because investigation of OCRL1 functions is hampered by functional redundancy between OCRL1 and INPP5B in mammals [13], we further analyzed the function of its *Drosophila* ortholog in cultured cells. The polyploidy phenotype was specific to dOCRL knockdown because two additional independent double-stranded RNAs (dsRNAs) (dOCRL dsRNA2 and dOCRL dsRNA 5′UTR) triggered cytokinesis failure in S2 cells (Figure 1B). We then performed a rescue experiment by stably expressing an mCherry-dOCRL, a GFP-dOCRL, or a GFP-dOCRL phosphatase-dead cDNA that are not targeted by the dOCRL 5′UTR dsRNA. Although dOCRL constructs restored efficiency of cytokinesis to levels seen in nontreated cells, the phosphatase-dead mutant was not able to rescue cytokinesis defects (Figure 1B; Figure S1D). These results demonstrate that dOCRL is necessary for cytokinesis via its inositol 5-phosphatase enzymatic activity.

dOCRL Is Associated with Endocytic Compartments

We took advantage of the fact that dOCRL fused to GFP or mCherry was functional (Figure 1B) to investigate the subcellular localization of the enzyme. Besides a diffuse staining in the cytoplasm, dOCRL was associated with cytoplasmic vesicles, visualized as dot-like or ring-like structures (Figure 2A). We further found that GFP-dOCRL partially colocalized with the small GTPase Rab5, a marker of early endosomes (Figure 2A; 17.5% of dOCRL vesicles were positive for Rab5). Because dOCRL was also present on vesicles that were not positive for Rab5, we explored the potential association of dOCRL with endosomes of other origin. We cotransfected mCherry-dOCRL with GFP-Rab7 or YFP-Rab11 [14] and found that dOCRL was also associated with these markers of late and recycling endosomes (Figure 2A; 62.3% and 53.5% of dOCRL vesicles were positive for Rab7 and Rab11, respectively), indicating that similarly to its human orthologs, dOCRL is associated with endosomes of diverse origins [15–17]. Remarkably, when dOCRL was depleted, Rab5, Rab7, and Rab11 were present on giant abnormal vacuoles rarely seen in control cells (Figure 2B), showing that in the absence of dOCRL, the endocytic compartments are disorganized.

dOCRL Controls PI(4,5)P2 Homeostasis

Because the preferential substrate of the inositol 5-phosphatase OCRL1 is the phosphate at the 5′ position of PI(4,5)P2 [18], we analyzed the distribution of PI(4,5)P2 upon dOCRL depletion using GFP-Tubby or mCherry-PH$^{PLC\delta}$ as PI(4,5)P2 biosensors [19]. In control and in dOCRL knockdown cells, PI(4,5)P2 was associated with the plasma membrane
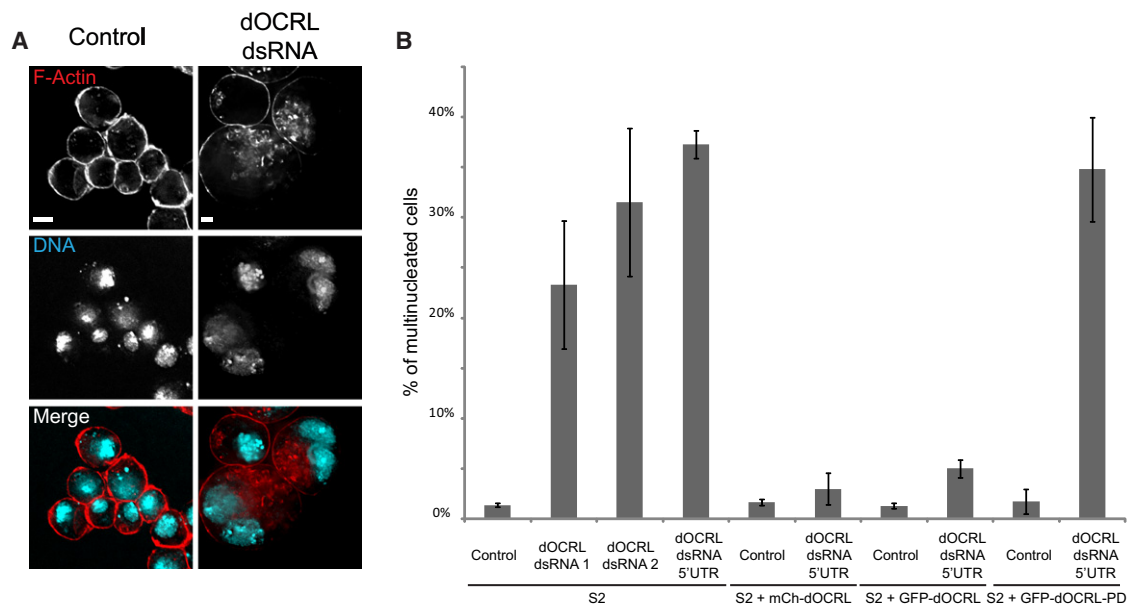
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Figure 1. dOCRL Is Required for Cytokinesis in *Drosophila* S2 Cells

(A) Control or dOCRL-depleted cells were labeled for F-actin (red) and DNA (blue). dOCRL-depleted cells are multinucleated and significantly larger than controls. 27.8% of the dOCRL-depleted cell population was more than twice as large as the mean area of control cells (vs. 0.47% of the control population); n = 1522 dOCRL-depleted cells, n = 633 controls.

(B) S2 cells or S2 cells stably expressing the indicated dOCRL constructs were treated by the indicated dsRNA and scored for polyploidy (minimum of three independent experiments, error bar represents standard deviation (SD), a minimum of 1500 cells per condition were scored). Scale bar represents 5 μm.

(Figure 2C; Figure S2A). Strikingly, we found that PI(4,5)P2 accumulated on the abnormal vacuoles observed in dOCRL knockdown cells. To test whether dOCRL depletion modifies PI(4,5)P2 homeostasis, we monitored GFP-Tubby or mCherry-PH$^{PLC\delta}$ fluorescence and quantified the ratio of signal associated with endomembranes to that associated with the plasma membrane. We measured a significant increase of the ratio of PI(4,5)P2 localized on endomembranes to that associated with the plasma membrane when dOCRL was depleted when compared to control cells (Figure 2D; Figure S2B). This defect of PI(4,5)P2 homeostasis was rescued when mCherry-dOCRL was expressed in absence of endogenous dOCRL (Figure 2D). These results indicate that dOCRL controls PI(4,5)P2 enrichment on the plasma membrane by dephosphorylating PI(4,5)P2 on endomembranes.

dOCRL Is Required for Proper Furrowing of the Contractile Ring

During cytokinesis, PI(4,5)P2 accumulates at the contractile ring of mammalian cells while it furrows [2, 4]. Furthermore, a tight control of PI(4,5)P2 levels at the cortex of dividing cells is important to maintain cleavage furrow stability and cytokinesis efficiency [2, 3, 20]. We therefore investigated whether dOCRL depletion can impinge on PI(4,5)P2 mitotic distribution and cleavage furrow stability (Figure 3A; Movie S1; Movie S2). Whereas in control cells PI(4,5)P2 was enriched at the cleavage furrow, this accumulation was deficient upon dOCRL depletion (Figure 3A; Figure S3A). We measured that in control conditions, enrichment of PI(4,5)P2 at the equatorial cleavage furrow was progressive and occurred when cells advanced from metaphase to telophase. Interestingly, we found a similar progressive increase of PI(4,5)P2 associated with endomembranes when dOCRL-depleted cells went from metaphase to telophase (Figure 3A; Figure S3B). This shows

that the PI(4,5)P2-rich endomembranes observed in dOCRL-depleted cells share similarity with the plasma membrane of the cleavage furrow.

Because PI(4,5)P2 plays a key role in controlling actin dynamics [21], we then asked whether PI(4,5)P2 deregulation could impinge on filamentous actin (F-actin) organization. In control telophase cells, F-actin was mainly cortical and was enriched at the cleavage furrow. Although F-actin was enriched at the cleavage furrow of dOCRL-depleted cells, it also abnormally accumulated on the PI(4,5)P2-rich endomembranes (Figure S3C). We then assessed the dynamics of F-actin in mitotic cells stably expressing GFP-utrophin as a F-actin reporter [22] (Figure 3B). We used the equatorial enrichment of GFP-utrophin fluorescence to mark the longitudinal width of the contractile ring. In control cells, contractile rings occupied an average of 12.5% of the cell length, whereas in dOCRL-depleted cells, the contractile rings were twice as broad (Figure S3D). This indicates that dOCRL function is necessary to spatially constrain the contractile ring. Similarly to fixed conditions, we measured a significant defect in F-actin homeostasis during cell division with a high level of F-actin fluorescence at the surface of the endomembranes (Figure S3E). These results establish that dOCRL is necessary for stabilization of the contractile ring and suggest that the furrowing deficiency observed upon dOCRL depletion occurs through defective homeostasis of PI(4,5)P2 and F-actin.

dOCRL Is Required for the Proper Assembly of Components of the Cytokinetic Machinery

We then asked whether, similarly to F-actin, other components of the cytokinetic machinery were also mistargeted to the endomembrane surface instead of accumulating solely at the cytokinetic ring. During furrowing, signals sent at the equator by the mitotic spindle trigger actomyosin contractions by
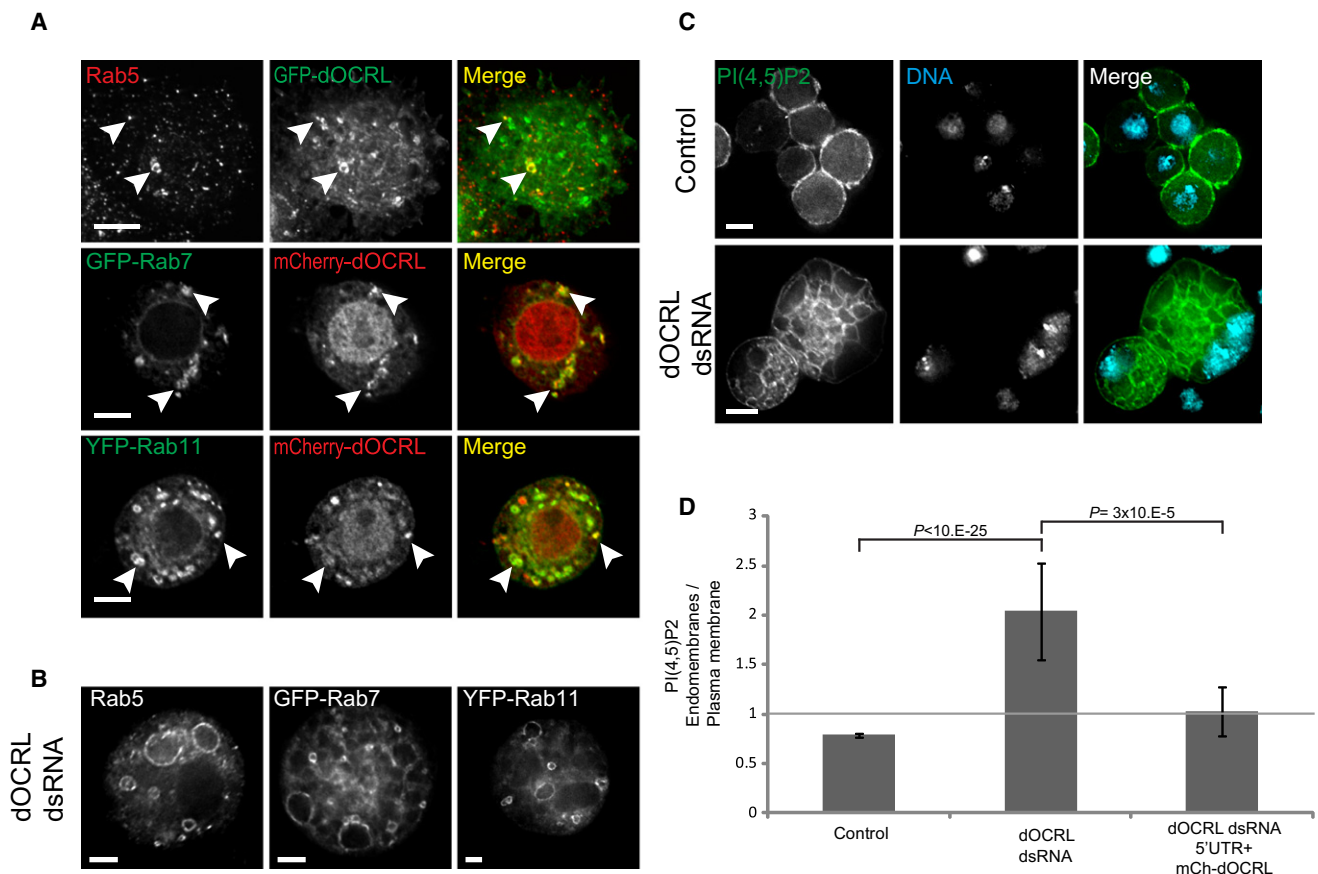
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Figure 2. dOCRL Is Localized on Endocytic Compartments and Controls PI(4,5)P2 Homeostasis

(A) S2 cells expressing GFP-dOCRL (green) were immunostained for Rab5 (red) and labeled for DNA (blue). Cells coexpressing mCherry-dOCRL (red) with GFP-Rab7 or YFP-Rab11 (green) were labeled for DNA (blue). Arrows show colocalization of the indicated proteins on endosomes.

(B) dOCRL-depleted cells were either immunostained for Rab5 or transiently transfected by GFP-Rab7 or YFP-Rab11.

(C) Control or dOCRL-depleted cells stably expressing GFP-Tubby (green) as a marker of PI(4,5)P2 were labeled for DNA (blue). 56.6% ± 13.7% of dOCRL-depleted cells presented PI(4,5)P2 intracellular vacuoles versus 5.6% ± 1.9% of control cells; n = 350.

(D) The ratio of GFP-Tubby fluorescence associated with endomembranes to that associated with the plasma membrane was measured using ImageJ on fixed cells (minimum of two independent experiments, error bar represents SD). Scale bar represents 5 μm.

locally activating the small GTPase RhoA [23, 24]. In turn, RhoA drives polymerization of F-actin and activation of myosin II to generate the forces necessary for ingression of the cleavage furrow. While in control cells, RhoA accumulated at the cytokinetic ring; when dOCRL was depleted, RhoA was mistargeted to endomembranes (Figure 4A). This shows that dOCRL is necessary to correctly localize RhoA during cytokinesis. Interestingly, PI(4,5)P2 was shown to play an important role in concentrating RhoA at the division site of budding yeast [25].

We then monitored cell division in S2 cells stably expressing mCherry-anillin and the regulatory light chain of myosin II, Sqh-GFP (Figure 4B; Movie S3; Movie S4) [26]. These two proteins are essential components of the cytokinetic machinery and their individual depletion promotes a high rate of cytokinesis failure [27–29]. Whereas myosin II is essential for actomyosin contractions, anillin is a multidomain protein capable of scaffolding different components of the cytokinetic ring at the plasma membrane [30, 31]. As previously reported [26, 32], anillin was nuclear in control interphase cells, became uniformly cortical in metaphase, and accumulated at the cytokinetic ring in anaphase (Figure 4B; Figure S4A). myosin II was mostly cytoplasmic in interphase and was evenly distributed at the metaphase cortex and

enriched at the cleavage furrow (Figure 4B; Figure S4B). In dOCRL-depleted cells, we found that myosin II and anillin were mistargeted to the abnormal giant vacuoles during cell division. Similarly to PI(4,5)P2, we measured that association of anillin and myosin II with endomembranes increased when dOCRL-depleted cells progressed from metaphase to telophase (Figures S4A and S4B). Together, these results show that contractile ring components are aberrantly recruited to the PI(4,5)P2-enriched endomembranes when function of dOCRL is impaired. This further implies that dOCRL and PI(4,5)P2 are required to specify the molecular distinction between the cytokinetic ring membrane and endomembranes.

The spatial restriction of specific phosphoinositides on different cellular membranes contributes to membrane structural heterogeneity. The majority of PI(4,5)P2 is concentrated at the plasma membrane; however, the mechanisms controlling PI(4,5)P2 homeostasis remain mysterious. Our studies identify the *Drosophila* ortholog of the human inositol 5-phosphatases OCRL1 and INPP5B as essential for PI(4,5)P2 homeostasis.

We propose that in wild-type conditions, dOCRL dephosphorylates PI(4,5)P2 on membranes of the endocytic compartment to reduce PI(4,5)P2 intracellular levels. Depletion of
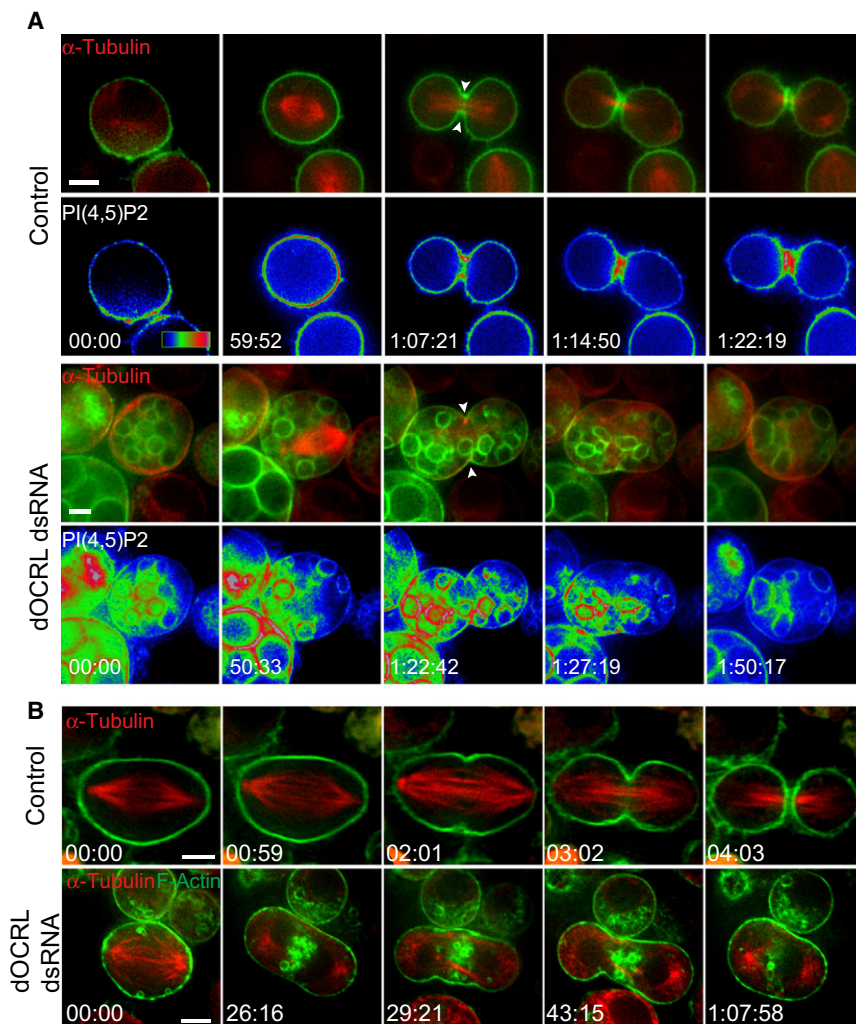
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Figure 3. dOCRL Is Required for Cleavage Furrow Positioning and Stabilization

(A) Selected frames of dividing control or dOCRL-depleted cells expressing the PI(4,5)P2 biosensor GFP-Tubby (green) and α-tubulin-mCherry (red). GFP-Tubby was pseudocolored in a rainbow heatmap to underline variations in its levels (lower rows).

(B) Selected frames of dividing control or dOCRL-depleted cells stably expressing utrophin-GFP (green) and α-tubulin-mCherry (red). Scale bar represents 5 μm.

dOCRL leads to a defect in PI(4,5)P2 homeostasis with a net increase of this phosphoinositide on endomembranes. During cell division, enrichment of PI(4,5)P2 at the plasma membrane of the cytokinesis ring is critical for the stability of the furrow. In dOCRL-depleted cells, defects in cytokinesis could arise through at least two distinct mechanisms. One possibility is that accumulation of F-actin, RhoA, myosin II, and anillin on vacuoles of endocytic origin reflects a natural trafficking process that transports cytokinetic machinery elements to the furrow [33]. In that model, PI(4,5)P2 homeostasis and dOCRL function are necessary to regulate the trafficking and fusion of endosomes carrying cytokinetic machinery elements to the cleavage furrow. Alternatively, defects in PI(4,5)P2 homeostasis directly trigger mislocalization of key components of the cytokinetic machinery to intracellular membranes at the expense of the cortical cleavage furrow. In that model, progressive enrichment of PI(4,5)P2 at the cleavage furrow represents a spatial cue that helps specify recruitment and localization of the cytokinetic machinery components. In accordance with this model, we found a progressive enrichment of PI(4,5)P2, anillin, and myosin II at the surface of the abnormal endomembranes while dOCRL-depleted cells progressed from metaphase to telophase. These two models are not mutually exclusive and could operate in concert to control cytokinesis. The importance of dOCRL for PI(4,5)P2 homeostasis, endocytic compartment specification, and cytokinesis may also be relevant to understand how mutations in OCRL1 produce the phenotypic manifestation of the Lowe syndrome.
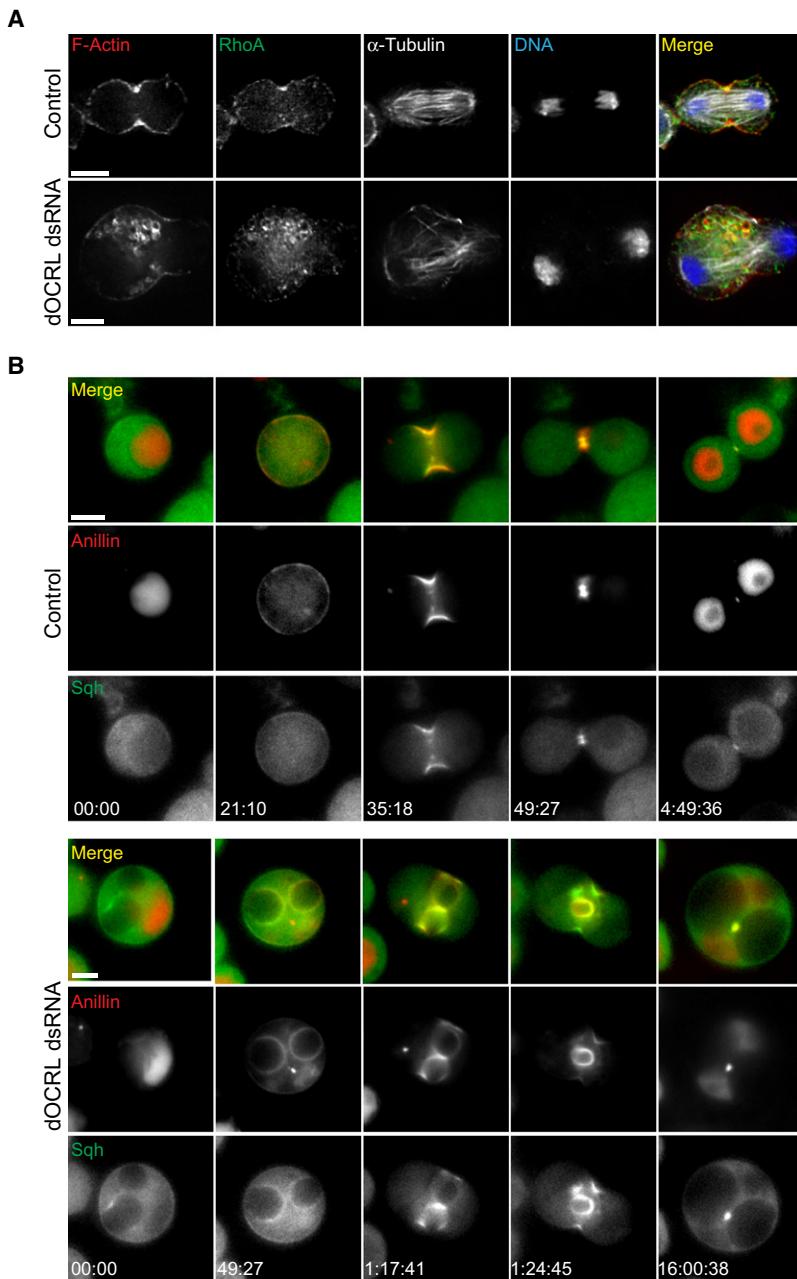
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Figure 4. dOCRL Function Is Required to Localize the Cytokinetic Ring at the Equatorial Cortex

(A) Control or dOCRL-depleted cells were labeled for F-actin (red), RhoA (green), α-tubulin (white in merge channel), and DNA (blue).

(B) Selected frames of dividing control or dOCRL-depleted cells stably expressing anillin-mCherry (red) and Sqh-GFP (green). Scale bar represents 5 μm.

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