Current Biology, Vol. 15, 1308–1313, July 26, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.cub.2005.06.024

The Centrosomal Protein CP190 Regulates Myosin Function during Early *Drosophila* Development

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Summary

Centrosomes are the main microtubule (MT)-organizing centers in animal cells, but they also influence the actin/myosin cytoskeleton [1]. The *Drosophila* CP190 protein is nuclear in interphase, interacts with centrosomes during mitosis, and binds to MTs directly in vitro [2, 3]. CP190 has an essential function in the nucleus as a chromatin insulator [4], but centrosomes and MTs appear unperturbed in *Cp190* mutants [4, 5]. Thus, the centrosomal function of CP190, if any, is unclear. Here, we examine the function of CP190 in *Cp190* mutant germline clone embryos. Mitosis is not perturbed in these embryos, but they fail in axial expansion, an actin/myosin-dependent process that distributes the nuclei along the anterior-to-posterior axis of the embryo. Myosin organization is disrupted in these embryos, but actin appears unaffected. Moreover, a constitutively activated form of the myosin regulatory light chain can rescue the axial expansion defect in mutant embryos, suggesting that CP190 acts upstream of myosin activation. A CP190 mutant that cannot bind to MTs or centrosomes can rescue the lethality associated with *Cp190* mutations, presumably because it retains its nuclear functions, but it cannot rescue the defects in myosin organization in embryos. Thus, CP190 has distinct nuclear and centrosomal functions, and it provides a crucial link between the centrosome/MT and actin/myosin cytoskeletal systems in early embryos.

Results and Discussion

CP190 and CP60 are centrosomal microtubule-associated proteins (MAPs) that form a complex and shuttle between the nucleus in interphase and the centrosome in mitosis [2, 3]. Both proteins interact directly with MTs in vitro, but their concentration at centrosomes does not depend on MTs [2, 6]. We have shown that the *Cp190* gene is essential for viability, and homozygous mutant animals die during late stages of pupal development [7]. Surprisingly, these mutants had no detectable defects in mitosis, or in any aspect of centrosome or MT behavior. Moreover, a form of CP190 that cannot bind to centrosomes or MTs (CP190ΔM) could rescue the lethality associated with *Cp190* mutations, demonstrating that the ability of CP190 to interact with centrosomes and MTs is not essential for fly viability. Recently, CP190 has been shown to act in the nucleus as a chromatin-insulator element that sets up boundaries between different regions of chromatin [4]. Thus, CP190 appears to have essential functions in the nucleus, but its function at the centrosome, if any, remains unclear.

Several *Drosophila* centrosomal proteins are essential for the rapid rounds of mitosis that occur in the early embryo but are dispensable for mitosis at later stages of development [8–10]. We therefore wanted to test whether CP190 might have an essential role at the centrosome during early embryogenesis. This was not possible previously because CP190 mutant flies are inviable as a result of the nuclear requirements for CP190, and mutant flies rescued by CP190ΔM are generally unhealthy and are sterile [7]. We therefore recombined the $Cp190^{1}$ and $Cp190^{2}$ mutations onto an FRT chromosome so that we could generate germline clone (GLC) embryos (hereafter referred to as CP190GLCs—see Supplemental Experimental Procedures). These embryos develop from heterozygous females whose germline is homozygous for the *Cp190* mutation. CP190GLCs from either mutant contained essentially undetectable levels of the CP190 protein (Figure 1A), and we obtained similar results with both alleles in the experiments described here; all the data shown were obtained with $Cp190^{2}$. Although CP190 was no longer detectable at centrosomes, mitotic spindles appeared to function normally (Figure 1B), and the centrosomal localization of γ-tubulin, CNN (Figure 1C), D-TACC, and Msps (not shown) was largely unperturbed.

Although centrosomes and MTs appeared to behave normally in CP190GLCs, we noticed that these embryos had a defect in axial expansion. In syncytial *Drosophila* embryos, the first zygotic nucleus is usually positioned toward the anterior. During nuclear cycles 4–7, the process of axial expansion causes the nuclei to spread out along the anterior-to-posterior axis so that, by nuclear cycle 7–8, they are distributed evenly throughout the length of the embryo. In CP190GLCs, axial expansion failed, and the nuclei remained abnormally clustered at the anterior of the embryo (Figures 1D and 1E).

Axial expansion is a highly coordinated contractile process that requires both actin and cytoplasmic myosin II [5, 11–14]. A live analysis of myosin behavior, labeled by virtue of GFP-tagged myosin regulatory light chain (RLC, an obligatory subunit of functional myosin

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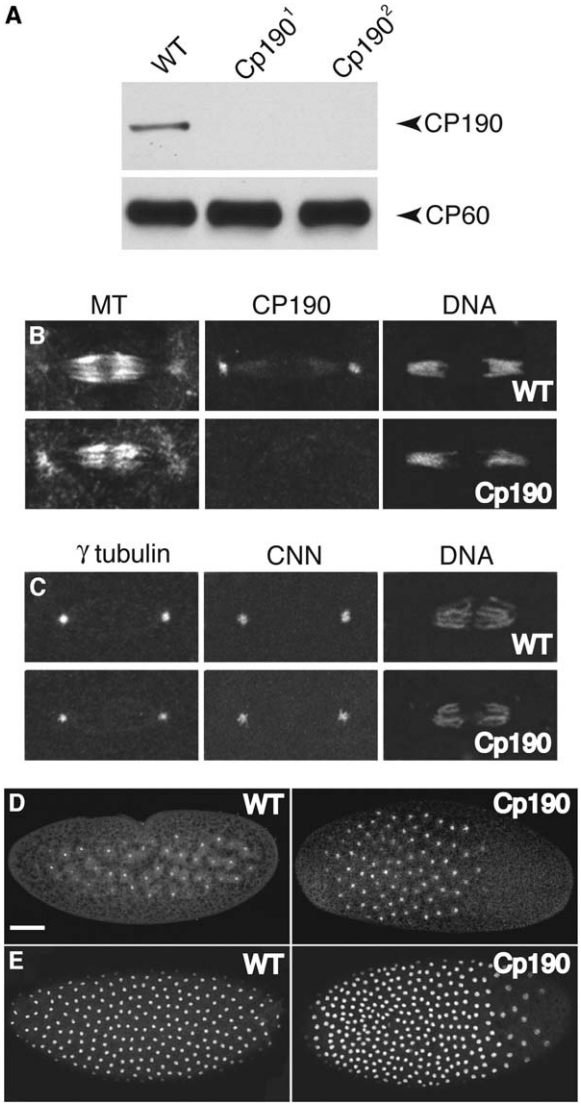

Figure 1. Mitosis Is Not Detectably Perturbed in CP190GLC Embryos, but Axial Expansion Is Defective

(A) A Western blot of wild-type (WT), *Cp190*[1], and *Cp190*[2] GLC embryos probed with anti-CP190 and anti-CP60 antibodies. The CP190 protein is not detectable in the GLC embryos.

(B) The distribution of MTs, CP190, and DNA in WT (top panels) and CP190[2] GLC (bottom panels) embryos during anaphase. CP190 is not detectable at centrosomes in GLC embryos.

(C) The distribution of γ-tubulin, CNN, and DNA in WT (top panels) and CP190[2] GLC (bottom panels) embryos during anaphase.

(D and E) The distribution of nuclei in WT (left panels) and CP190[2] GLC (bottom panels) embryos at approximately nuclear cycles 7 (D) and 10 (E). The GLC embryos have a severe axial-expansion defect, and the nuclei remain clustered toward the anterior of the embryo. Anterior is to the left in all embryos.

The scale bars represent 5 μm (B and C) and 50 μm (D).

II), has shown that during axial expansion myosin undergoes cycles of recruitment to and dispersion from the cortex, in coordination with the nuclear-division cycles of the internal nuclei [12]. Recruitment occurs during mitotic interphase and promotes a cortical contraction that is thought to drive axial expansion. This cyclical cortical recruitment of myosin requires the

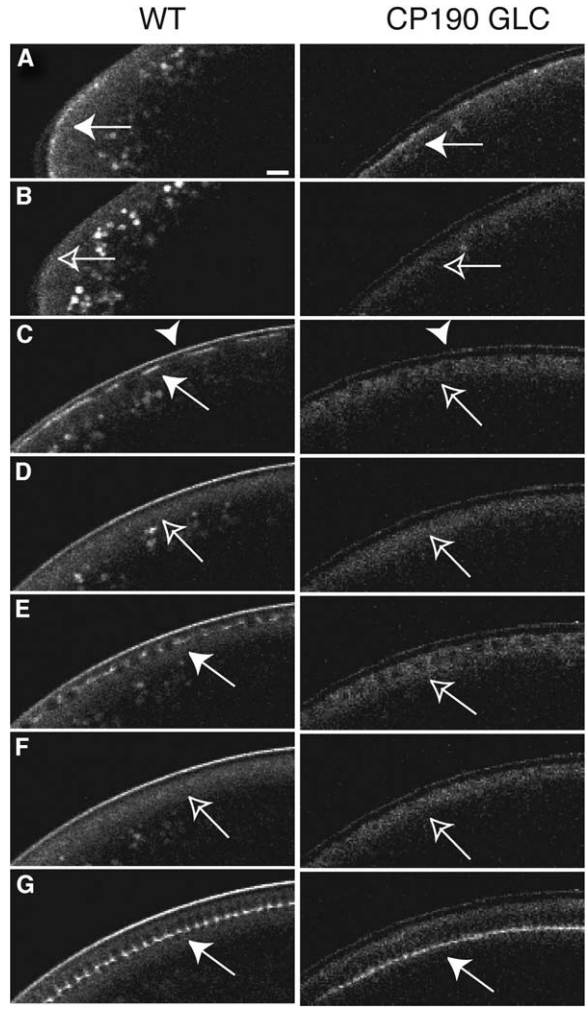

Figure 2. The Organization of the Myosin Regulatory Light Chain Is Disrupted in Living CP190GLCs

The behavior of GFP-RLC in WT (left panels) or *Cp190*[2] GLCs (right panels) was followed by time-lapse confocal microscopy. Confocal sections through the approximate middle of each embryo are shown, and different regions of the same embryo are shown in some panels because these regions most dramatically illustrate the differences or similarities in GFP-RLC recruitment. Solid arrows indicate high levels of cortical GFP-RLC (when the embryos are in interphase; [A, C, and E]), and hollow arrows indicate low levels (when the embryos are in mitosis; [B, D, and F]).

(A and B) In both WT and CP190 GLCs, cycles of cortical recruitment of GFP-RLC can be detected before the nuclei reach the cortex, but these are much weaker in the mutant embryo. In the images shown here, GFP-RLC is present at the cortex in both embryos, illustrating that this recruitment can occur in mutant embryos.

(C–F) In the blastoderm stage, when nuclei have reached the cortex, the WT embryo shows high levels of cortical GFP-RLC between the interphase nuclei (visible as dark circles), and low levels during mitosis. In contrast, the cortical accumulation of GFP-RLC during interphase is barely detectable in the mutant embryo.

(G) During cellularization, however, GFP-RLC is strongly concentrated at the leading edge of the cellularization furrow that invaginates around the nuclei in both WT and CP190GLC embryos. Note that the position of the vitelline membrane (which forms a solid line of autofluorescence around the embryo) is highlighted with an arrowhead. The brightness of the vitelline membrane varies somewhat between embryos, as well as in different images of the same embryo; we observe no consistent difference, however, in the brightness of the vitelline membrane between WT and CP190GLCs.

The scale bar represents 10 μm.

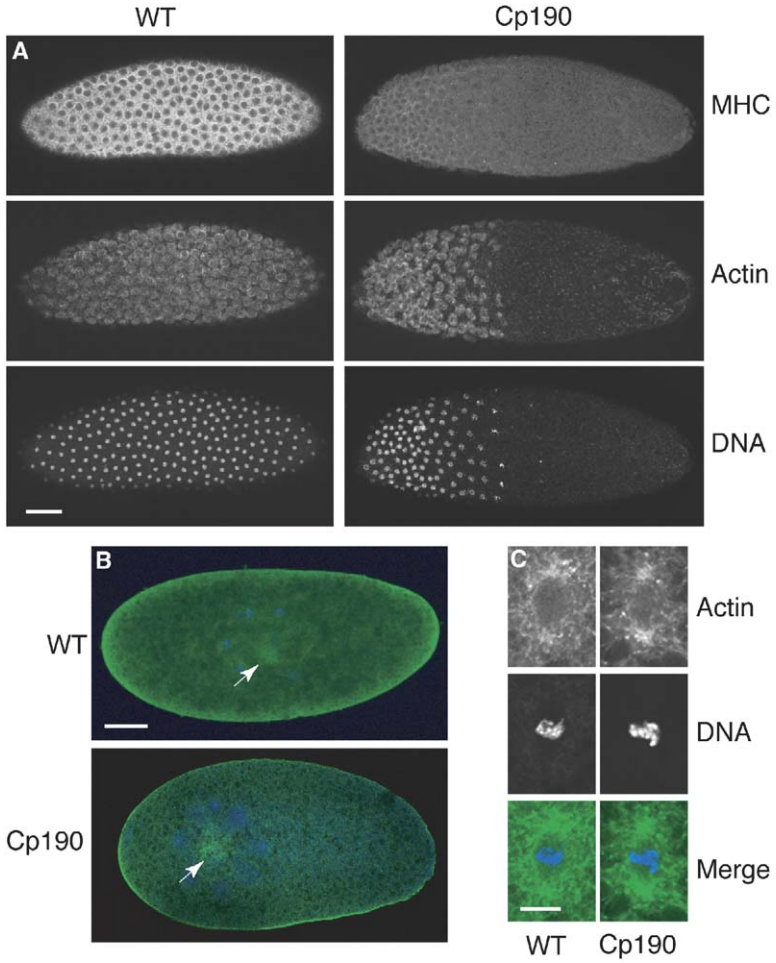

Figure 3. The Organization of Myosin Heavy Chain (MHC) Is Disrupted in CP190 GLCs, but the Organization of Actin Does Not Appear to Be Perturbed

(A) The distribution of MHC (top panels), actin (middle panels), and nuclei (bottom panels) in WT and *Cp190*^{2} GLC syncytial blastoderm embryos. In the mutant embryo, the organization of actin appears to be unperturbed, but the recruitment of MHC to the embryo cortex is very weak.

(B) The distribution of actin (green) and DNA (blue) in pre-blastoderm WT and *Cp190*^{2} GLCs. In both WT and mutant embryos, actin is concentrated at the cortex, and an internal "central domain" of actin associates with the nuclei (arrows).

(C) In early syncytial embryos, actin is also concentrated around the centrosomes in both WT and mutant embryos.

The scale bars represent 50 μm (A and B) and 5 μm (C).

phosphorylation of one of the activating residues of the RLC [12] (see below) but does not require either microtubules [12] or an intact actin network (it is not perturbed by cytochalasin or latrunculin injection; A.R. and R.K., unpublished data).

To test if these cycles of myosin accumulation occurred in CP190GLCs, we examined RLC-GFP behavior in CP190GLCs. In optical sections of wild-type (WT) embryos expressing one copy of RLC-GFP, cycles of myosin cortical accumulation and dispersion were observed prior to the arrival of the nuclei at the cortex, and these continued when the nuclei were at the cortex, with RLC-GFP being strongly recruited to the cortex in interphase and dispersing from the cortex during mitosis (Figure 2, left panels). By contrast, in CP190GLCs expressing one copy of RLC-GFP, only very weak cycles of myosin II accumulation at the cortex could be observed, and these were more uneven than those seen in WT embryos (Figure 2, right panels). Even after the nuclei had arrived at the cortex, the accumulation of RLC-GFP at the cortex in interphase was much weaker in CP190GLCs than in WT embryos. Surprisingly, however, the subsequent accumulation of RLC-GFP at the leading edge of the cellularization furrows was equally strong in CP190GLCs and WT embryos (Figure 2G). Moreover, in cellularized embryos, the accumulation of RLC-GFP in contractile rings during cytokinesis also appeared to occur normally in CP190GLCs (not shown). Thus, the organization of myosin appears to be disrupted in CP190GLCs specifically during the syncytial phase of embryogenesis.

We confirmed that myosin organization was disrupted in CP190GLCs by immunostaining fixed embryos with an anti-myosin heavy chain (MHC) antibody [14]. Although MHC staining was strong in the cortical regions surrounding the nuclei of WT embryos (Figure 3A) [12, 15] (average pixel intensity ± SD = 157 ± 9; see Supplemental Experimental Procedures), in CP190GLCs, MHC staining was much reduced and more irregular (101 ± 16) (Figure 3). As was the case with RLC-GFP, the localization of MHC to the leading edge of the cellularization furrow appeared to be normal in CP190GLCs (not shown).

Although myosin II behavior was profoundly disrupted in CP190GLCs, actin organization appeared to be unperturbed (Figure 3). In CP190GLC blastoderm embryos, cortical actin caps form over each nucleus (Figure 3A), just as in WT. In preblastoderm WT embryos, a network of actin fibers and granules lies below the actin-rich cortex, and an actin-rich "central domain" is associated with the internal nuclei during axial expansion [5] (arrow, Figure 3B); actin is also concen-

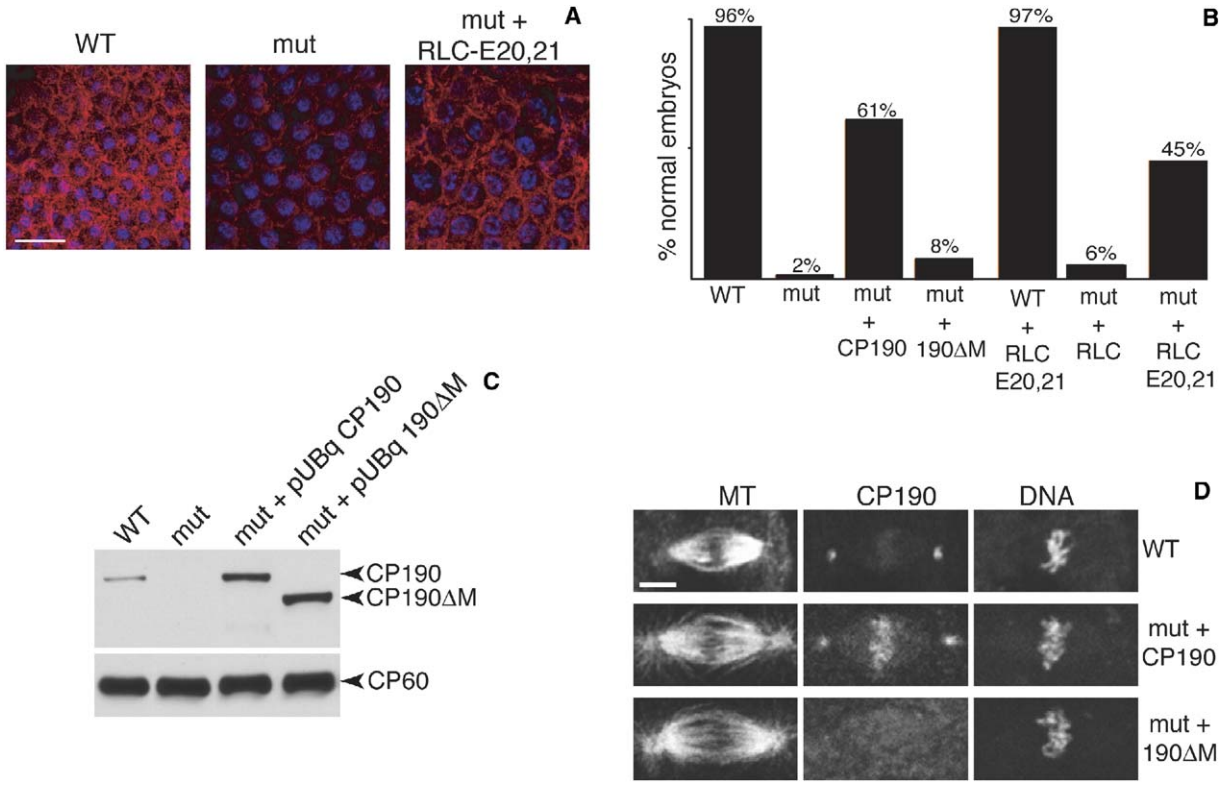

Figure 4. The Axial-Expansion Defects of CP190 GLCs Can Be Rescued by the Expression of an Activated RLC and WT CP190, but Not by the Expression of WT RLC and CP190ΔM

(A) The localization of MHC (red) and nuclei (blue) in WT, CP190GLCs, and CP190GLCs that express RLC-E20,21. The recruitment of MHC to the cortex is partially rescued by the expression of RLC-E20,21 (see also Figure S1).

(B) A graph showing the percentage of nuclear-cycle-7 to -14 embryos that do not have an axial-expansion defect. Embryos of the following genotypes were scored: WT (bar 1); *Cp190*^{2} GLCs (bar 2); *Cp190*^{2} GLCs expressing a pUbq-CP190 transgene (bar 3); *Cp190*^{2} GLCs expressing a pUbq-CP190ΔM transgene (bar 4); WT embryos expressing RLC-E20,21 driven from the RLC promoter (see text for details) (bar 5); *Cp190*^{2} GLCs expressing a WT version of RLC driven from the RLC promoter (bar 6); *Cp190*^{2} GLCs expressing RLC-E20,21 driven from the RLC promoter (bar 7). For each experiment, 50–80 embryos were scored.

(C) A Western blot showing the levels of CP190 and CP60 in embryos of the following genotypes: WT (lane 1); *Cp190*^{2} GLC (lane 2); *Cp190*^{2} GLC expressing a pUbq-CP190 transgene (lane 3); and *Cp190*^{2} GLC expressing a pUbq-CP190ΔM transgene (lane 4).

(D) The distribution of MTs, CP190, and DNA in *Cp190*^{2} GLCs (top panels) and in *Cp190*^{2} GLCs that also express pUbq-CP190 (middle panels) or pUbq-CP190ΔM (bottom panels). The scale bar represents 5 μm.

trated around the centrosomes during these early syncytial divisions (Figure 3C). All these features of actin organization were maintained in CP190GLCs.

These observations suggested that the failure in axial expansion in CP190GLCs is due to a failure to properly recruit cortical myosin. We confirmed by Western blotting that the levels of MHC were not altered in CP190GLCs (data not shown). To test whether CP190 might act upstream of myosin activation, we asked whether an "activated" RLC could rescue the axial expansion defect in CP190GLCs. The phosphorylation of the myosin RLC (on Ser-19 and, secondarily, on Thr-18 in vertebrates; these correspond to Ser-21 and Thr-20 in *Drosophila*) is required for myosin II motor activity [14, 16]. Blocking RLC phosphorylation, either by using mutant forms of the RLC in which these residues have been replaced by alanines (RLC-A20,A21) or by inhibiting Rho Kinase, whose activity is required for phosphorylating these residues, renders myosin II non-functional, eliminates its cortical localization [14, 17], and leads to a failure in axial expansion [14, 17] . In contrast, replacement of these sites by phospho-mimetic glutamates (RLC-E20,E21) restores activity, as defined genetically, and appears to render the myosin constitutively active [14, 17]. Thus, phosphorylation is essential for the function and localization of myosin [14, 17, 18].

We found that expression of one copy of a transgene encoding the activated form of RLC (RLC-E20,E21) partially rescued both the axial-expansion defects and myosin cortical recruitment in CP190GLCs (Figures 4A and 4B; also Figure S1 in the Supplemental Data available with this article online). Importantly, the expression of one copy of this transgene in WT flies had no effect on axial expansion, and the expression of one copy of a WT RLC-GFP transgene did not rescue the CP190GLC axial-expansion defect (Figure 4B). Thus, an "activated" form of RLC can recruit MHC to the cortex during interphase and can rescue the axial-expansion defect in CP190GLCs, strongly suggesting that CP190 normally acts upstream of myosin II activation to regulate axial expansion.

We have shown previously that a form of CP190 lack-

ing the centrosomal and MT binding domain of CP190 (CP190ΔM) can rescue the adult lethality associated with mutations in the CP190 gene [7], presumably because this form of the protein can still function as a chromatin insulator in the nucleus. We therefore tested whether the axial-expansion defects of the CP190GLCs could also be rescued by CP190ΔM. In CP190GLCs that expressed the full-length CP190 protein driven from the polyubiquitin promoter (Figure 4B), the axial-expansion defect was strongly suppressed (Figure 4B), and the transgenically supplied CP190 localized to centrosomes (Figure 4D). In CP190GLCs expressing CP190ΔM driven from the polyubiquitin promoter (Figure 4C), the axial-expansion defect was not significantly rescued (Figure 4B) and CP190ΔM did not localize to centrosomes (Figure 4D). Thus, it appears that CP190 requires its centrosome/MT binding domain to function properly in axial expansion.

How might CP190 influence myosin activity? The cycles of cortical myosin II recruitment that drive axial expansion are regulated by oscillations in the activity of Cdc2-Cyclin B, with levels of cortical myosin being high in interphase and low in mitosis [12]. This regulation is probably indirect; Cdc2-Cyclin B activity varies only locally around the nuclei during early embryo development [19], and cycles of myosin recruitment are initiated at the cortex long before the nuclei arrive there. Moreover, although Cdc2-Cyclin B can directly phosphorylate RLC in vitro, the removal of the potential Cdc2 phosphorylation sites in *Drosophila* RLC alters neither the myosin II recruitment cycles nor the ability of myosin to drive axial expansion [12]. How local fluctuations in Cdc2-Cyclin B activity around the nuclei direct cycles of myosin recruitment at the cortex is therefore unclear, but we speculate that CP190 plays a role in facilitating this process.

Cdc2-Cyclin B, for example, could regulate myosin by regulating the activity and/or localization of *Drosophila* rho kinase (Drok). This kinase is required for axial expansion [12], it regulates myosin II activity via phosphorylation of Thr-20 and Ser-21 [12, 17], and it is concentrated at centrosomes in at least some cell types [20]. Perhaps CP190 facilitates the activation of Drok at centrosomes or the targeting of Drok from centrosomes to the embryo cortex (either by diffusion or along MTs). We have shown previously that MTs are not essential for the cycling of myosin at the cortex [12], but these studies were performed when the nuclei had already reached the embryo cortex. Perhaps MTs are essential for the long-range signaling that must occur between the cortex and the nuclei/centrosomes during axial expansion. Because the interaction of CP190 with centrosomes and MTs is regulated during the cell cycle [2, 3], the involvement of CP190 in this process could ensure that the myosin-driven cortical contractions are coordinated with the cell-cycle state of the internal nuclei.

Our data suggest that, whatever its mechanism, CP190 serves as a crucial link between the centrosome/MT and actin/myosin cytoskeletal networks during the early stages of *Drosophila* embryonic development. This mechanism may be specific for organisms that have a syncytial phase of development and so require that centrosomes influence actin/myosin behavior over considerable distances. Indeed, no obvious orthologs of CP190 have been identified on the basis of sequence homology in species other than insects. On the other hand, the fertilized eggs of many species are very large, and special mechanisms that allow the long-range communication between the centrosomes and the cortical myosin network may be required in these systems.

Supplemental Data

Supplemental Experimental Procedures as well as a supplemental figure are available with this article online at http://www.current-biology.com/cgi/content/full/15/14/1308/DC1/.

Received: April 12, 2005
Revised: May 19, 2005
Accepted: June 8, 2005
Published: July 26, 2005

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