possible once myosin motors have redistributed over the apical surface. Another possibility is that a cell contracts as much as interactions with its neighboring cells allow. Contraction of a cell within an epithelium will inevitably lead to stretching of its neighbors and thus the extent by which the cell contracts might be limited by the elastic resistance of its neighboring cells. Studying the spatial and temporal correlation between contraction phases of neighboring cells might help to resolve this issue by providing insights into the correlation between contraction of one cell and stretching of its neighbors.

Importantly, cell surface area must be stabilized between contractions for pulses to result in a net decrease of tissue apical surface. Martin et al. propose that tension in the remaining acto-myosin cortex opposes stretching by neighboring cells during pauses between contractions. This implies that the cortical network displays a high elastic modulus that primarily depends on the level and nature of crosslinkers (Bausch and Kroy, 2006). It will thus be interesting to know whether twist is involved in the regulation of actin cortex crosslinking. Together with the observation of twist-dependent apical junction assembly, it also raises the question of whether actin crosslinking, cortical stiffening, and apical junction formation are interrelated processes. Mapping the tension distribution during ventral furrow formation by, for example, ablating single cells or the cortex within individual cells and correlating tension with the localization of junctional and cytoskeletal components would be helpful in resolving how pulsed apical cell contraction and stabilization are achieved.

Continuous tissue deformation driven by asynchronous shape changes of individual cells is likely to be a common feature in development. For example, convergent extension in *Xenopus* embryos proceeds at a constant rate, although it is driven by apparently uncoordinated cell movements (Keller et al., 2008). A similar mechanism at a different scale is also observed during muscle contraction, where sarcomere shortening is achieved by asynchronous steps of individual motors (Duke, 1999). Future studies will have to determine which developmental processes result from asynchronous behaviors and where (and how) synchrony is achieved (see also Duke, 1999 for a discussion of synchrony in sarcomere contraction). Theoretical modeling of cells interacting in a tissue (Farhadifar et al., 2007) may help reveal whether asynchronous pulsed contractions of individual cells provide a more efficient and/or robust tissue contraction mode than continuous synchronized contraction of all the cells together.

**References**


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**Relaying the Checkpoint Signal from Kinetochore to APC/C**

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The mitotic checkpoint delays chromosome segregation until the last chromosome has correctly attached to the spindle. Exactly how this unattached chromosome can generate a checkpoint signal and inhibit the anaphase promoting complex/cyclosome (APC/C) is unknown. Two Developmental Cell papers in this issue by Kulukian et al. and Malureanu et al. now provide insight into how checkpoint components Mad2 and BubR1 relay the checkpoint signal from kinetochores to APC/C.

A normal eukaryotic cell will not segregate its duplicated sister chromatids until the very last one of them properly attaches to the mitotic spindle (Rieder et al., 1994), because unattached kinetochores generate a checkpoint signal that prevents anaphase onset (Rieder et al., 1995). These studies suggested that unattached kinetochores serve as a platform...
for the generation of a diffusible anaphase inhibitor.

What is the nature of this diffusible inhibitor, and how does it inhibit anaphase onset? The answer to the latter part of this question is undisputed; the diffusible inhibitor selectively blocks Cdc20-dependent destruction of Cyclin B and securin by the APC/C, thus preventing the onset of anaphase (Peters, 2006). But the nature of the diffusible inhibitor has been controversial. Two checkpoint components, Mad2 and BubR1, have been prime suspects, as both fulfill the basic requirements: they rapidly cycle on and off unattached kinetochores in mitosis (reviewed in Musacchio and Hardwick, 2002) and both directly bind Cdc20 and inhibit Cdc20-dependent APC/C activity in vitro. The discovery that a mitotic checkpoint complex (MCC) can form in vivo containing Mad2, BubR1, Bub3, and Cdc20, suggested that Mad2 and BubR1 may act together. Consistently, the MCC was reported to be a more potent inhibitor of the APC/C than Mad2 alone (Sudakin et al., 2001). Meanwhile, structural studies demonstrated that Mad2 can adapt an open or closed conformation (Musacchio and Hardwick, 2002). In the closed conformation (C-Mad2), the C-terminal domain of Mad2 is wrapped around Mad1 (a checkpoint component stably associated to unattached kinetochores) or Cdc20, while in the open conformation (O-Mad2) this C-terminal “safety belt” is not engaged, preventing association with Mad1 or Cdc20. Importantly, C-Mad2 can dimerize with O-Mad2, which may prime O-Mad2 for capture of Cdc20. Indeed, dimerization-defective mutants of Mad2 fail to mount a proper checkpoint response (Musacchio and Hardwick, 2002). Thus, a fraction of Mad2 would lock onto Mad1 that is stably bound to the unattached kinetochore, and this complex can serve as a platform for the formation of a diffusible Mad2/Cdc20 complex. In agreement with this template model, two pools of Mad2 exist at kinetochores, one that cycles very rapidly and another that is more stably bound (Shah et al., 2004).

But where does BubR1 come in? Two new papers begin to address this question. To start, Kulukian et al. (2009) provide the first direct proof that unattached kinetochores can promote formation of a diffusible inhibitor. In an impressive tour de force, the authors use isolated chromosomes and a large array of purified checkpoint components to reconstitute an in vitro system of APC/C inhibition. In this system, purified checkpoint components can be preincubated with isolated chromosomes, which can subsequently be removed by centrifugation. Using this system, the authors demonstrate that isolated chromosomes act on Mad2, but not BubR1, to catalyze formation of a diffusible inhibitor (Figure 1). Catalysis requires binding of Mad2 to Mad1, present on the unattached kinetochores of the isolated chromosomes, and dimerization of Mad2, consistent with the template model. In addition, the authors find that unattached kinetochores merely accelerate, but are not required for, formation of the anaphase inhibitor. Indeed, earlier studies found that the MCC can form in the absence of a functional kinetochore (Fraschini et al., 2001; Sudakin et al., 2001). Furthermore, the authors find that the anaphase inhibitor produced by unattached kinetochores can block Cdc20 that is in complex with the APC/C, arguing against a model of simple sequestration. The authors fail to find evidence for catalysis of MCC formation by unattached kinetochores. In fact, most Cdc20 is bound to BubR1 and not to Mad2, following coincubation of all purified components with the isolated chromosomes. Importantly, the authors demonstrate that Mad2 that has been in contact with unattached kinetochores can convert BubR1 into a potent APC/C inhibitor in the absence of unattached kinetochores. These results suggest that Mad1/Mad2 complexes bound at unattached kinetochores facilitate binding of soluble Mad2 to Cdc20 and this latter complex acts as a (diffusible) transient precursor to the eventual BubR1/Cdc20 inhibitory complex.

A completely different approach by Malureanu et al. (2009) supports a similar model. Using BubR1 conditional knockout cells, the authors demonstrate that mutants of BubR1 that cannot bind to kinetochores are capable of activating the checkpoint in response to nocodazole. Instead, they find that the N-terminal Cdc20-binding domain of BubR1 is essential for checkpoint control, chromosome alignment and mitotic timing. These results are consistent with the above model, wherein BubR1 doesn’t need to contact kinetochores directly but instead forms a complex with Cdc20. Moreover, Malureanu et al. (2009) offer an attractive alternative for the sequestration model that was proven unlikely by Kulukian et al. (2009). They show that cells lacking BubR1 prematurely degrade Cyclin B in interphase. Inhibition of Cyclin B degradation does not require recruitment of BubR1 to kinetochores but depends on Cdc20 binding, indicating that BubR1 can function as a pseudosubstrate inhibitor of the APC/C, forming independently of unattached kinetochores. This may seem counterintuitive, as one might expect formation of the anaphase inhibitor to depend on the presence of unattached kinetochores. However, the experiments of Kulukian et al. (2009) also show that an APC/C inhibitor can form in the absence of kinetochores, it just happens more slowly. What’s more, Malureanu et al. (2009) find that premature Cyclin B degradation does not occur in cells lacking Mad2, suggesting that formation of the kinetochore-independent APC/C inhibitor does not require Mad2. All this points to a model in which an APC/C inhibitor, comprising BubR1 in complex with Cdc20, can form in the absence of unattached kinetochores and Mad2, but the latter two significantly accelerate its formation.

Nonetheless, several questions remain. For one, kinetochore-independent formation of the APC/C inhibitor has been proposed to control timing of mitosis (Meraldi et al., 2004). If the initial APC/C inhibitory entity can form in the absence of Mad2, as the data from Malureanu et al. (2009) seem to suggest, how does Mad2 control mitotic timing? Second, why does BubR1 cycle on and off kinetochores, and how can soluble BubR1 regulate chromosome alignment? Malureanu et al. (2009) argue that its role in chromosome alignment could be indirect, by preventing APC/C-dependent degradation of factors that control alignment. Since alignment defects are not seen after inactivation of Mad2, one would have to argue that these factors are primarily degraded in interphase, when Mad2 does not appear to be required for inhibition of APC/C activity. Possibly the most pressing question arising from these papers is: how transient is the Mad2/Cdc20 complex and how does it promote BubR1/Cdc20 complex formation? One
clue to the first part of the question comes from experiments by Kulukian et al. (2009) indicating that the Mad2/Cdc20 complex is sufficiently stable to survive the centrifugation steps required to remove the isolated chromosomes. How it promotes formation of a complex between BubR1 and Cdc20 will be more difficult to resolve and may require additional structural work. Despite these remaining issues, these two papers bring us several steps closer to understanding how the checkpoint signal is relayed from unattached kinetochores to APC/C.

Figure 1. The Checkpoint Relay from Unattached Kinetochore to APC/C
BubR1 functions as a diffusible APC/C inhibitor in G2 phase, when functional kinetochores have not yet assembled. This inhibition requires direct binding to Cdc20. Upon nuclear envelope breakdown, kinetochores assemble and recruit Mad1/Mad2 complexes that remain stably bound until the kinetochore attaches to the spindle. The Mad1/Mad2 complex promotes formation of a Mad2/Cdc20 intermediate (possibly already in complex with the APC/C) that can transfer Cdc20 (alone, or bound to the APC/C) into a complex with BubR1. This relay promotes rapid BubR1/Cdc20 complex formation, much more efficient than the spontaneous assembly of this complex that occurs in G2.

REFERENCES