

# Response to Comments on “A Centrosome-Independent Role for $\gamma$ -TuRC Proteins in the Spindle Assembly Checkpoint”

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Weaver and Cleveland and Taylor *et al.* contend that our data on the involvement of the  $\gamma$ -tubulin ring complex ( $\gamma$ -TuRC) in the spindle assembly checkpoint (SAC) can be fully explained by kinetochore-derived checkpoint signaling. We maintain that (i) the interactions of  $\gamma$ -TuRC with Cdc20 and BubR1, and (ii) the activation of SAC by  $\gamma$ -TuRC depletion, in addition to the abrogation of kinetochore-microtubule interactions, argue for a more complex mechanism of SAC signaling.

$\gamma$ -tubulin ring complex ( $\gamma$ -TuRC) proteins constitute the microtubule nucleation complex involved in the building of bipolar spindles and are required for proper chromosome segregation in higher eukaryotic cells (1). The depletion of  $\gamma$ -TuRC components leads to abnormal spindle formation and chromosome segregation defects (2, 3). Their importance for microtubule nucleation as well as spindle formation and chromosome segregation suggests that  $\gamma$ -TuRC function is tightly interconnected to cell cycle regulatory mechanisms. We showed that  $\gamma$ -TuRC is integrated into spindle assembly checkpoint (SAC) signaling and interacts with the kinetochore checkpoint proteins BubR1 and Cdc20 in both *Drosophila* and human cells (4). Depletion of  $\gamma$ -TuRC components triggers checkpoint activation additional to the arrest caused by ablating kinetochore-microtubule binding, which demonstrates the presence of an additional trigger (4).

Weaver and Cleveland (5) state that our experiments are fully consistent with activation of the established pathway of checkpoint signaling in response to incomplete or unstable microtubule kinetochore attachment. We agree. We do not claim that incorrect microtubule kinetochore attachment, caused by depletion of  $\gamma$ -TuRC proteins, would not trigger the activation of the spindle assembly checkpoint. However, we demonstrate that depletion of  $\gamma$ -TuRC triggers a response that acts in addition to kinetochore-microtubule-dependent activation. We have not proposed that this is a new spindle assembly checkpoint. Indeed, we show that the classical spindle assembly components BubR1, Mad2, and Mps1 are required for the  $\gamma$ -TuRC protein depletion-mediated arrest. The suggestion by Weaver and Cleveland to demonstrate silencing of a kinetochore-derived checkpoint signaling

would be informative to clarify whether independent activation of SAC does exist. However, we have not suggested such a model and therefore did not see the need to demonstrate this in our study (4). Furthermore, we think it is unlikely that lack of kinetochore occupancy is a major trigger of checkpoint activation in SL2 cells depleted of  $\gamma$ -TuRC components through RNA interference (RNAi). Immunofluorescence labeling with an antibody to Bub1 demonstrated quantitatively that Bub1 is absent from the kinetochores in 69% of SL2 cells depleted for the  $\gamma$ -TuRC component Grip84, as it is in RNAi control cells (69%). The presence of Bub1 at the kinetochores is a marker for lack of kinetochore occupancy in the *Drosophila* SL2 system (6) used in our experiments.

Weaver and Cleveland (5) also note that we proposed that the SAC can be activated by errors in microtubule nucleation. The data we presented in (4) did not show this, and we regret that our statement that “the spindle checkpoint can be activated at the level of microtubule nucleation” was misleading in this respect. It was not intended to propose a novel checkpoint mechanism that detects aberrant microtubule nucleation at the microtubule minus end.

Taylor *et al.* (7) suggest that “the simplest explanation” for SAC activation is that  $\gamma$ -TuRC depletion causes spindle defects that lead to aberrant kinetochore microtubule attachment or tension. The statement that ablation of  $\gamma$ -TuRC might induce abnormal spindles, thus triggering SAC activation, is correct, and we indeed suggested this possibility (4). However, depletion of  $\gamma$ -TuRC components triggers mitotic arrest in addition to the arrest caused by ablating kinetochore-microtubule binding, thus suggesting an additional activation trigger. Hence, our study proposed that the depletion of  $\gamma$ -TuRC components contributes to SAC activation through BubR1 and Cdc20.

The spindle assembly checkpoint consists of two major parts: surveillance and signaling. The surveillance mechanism implements the cell cycle block (through proteins such as BubR1, Mad2, and Mps1) when necessary, that is, when

the signaling mechanism transmits that spindle assembly is compromised. The signaling mechanism may, therefore, through absence or mislocalization of a molecule or structure, trigger cell cycle block and induce cell cycle arrest (provided functional SAC components are present). It is evident that  $\gamma$ -TuRC is not a surveillance component of the SAC because, in its absence, the SAC is fully activated, resulting in cell cycle arrest. We have therefore proposed (4) that  $\gamma$ -TuRC proteins act as part of the signaling mechanism, because their absence or mislocalization trigger the checkpoint and induce arrest (again, provided that functional SAC components are present). We did not suggest that  $\gamma$ -TuRC itself is a component of the checkpoint surveillance mechanism.

Hence, the “simplest” explanation (6) does not sufficiently explain all of our data linking  $\gamma$ -TuRC to the spindle checkpoint machinery at the molecular level. There is evidence that SAC signaling is integrated into multiple cues from the kinetochore, cytoplasm, and centrosome. This view is supported by a series of recent publications (8–11) that suggest multiple roles of SAC components in cell cycle regulation, including inhibition of the anaphase-promoting complex (APC) in a kinetochore-independent manner. This is consistent with and supportive of our interpretation, showing that, in addition to a kinetochore-triggered signal derived from kinetochore ablation, depletion of  $\gamma$ -TuRC components triggers further checkpoint activation.

Our results are indeed consistent with the classical model of SAC activation. However, we maintain that the interactions of  $\gamma$ -TuRC with Cdc20 and BubR1 and the activation of SAC by  $\gamma$ -TuRC depletion, in addition to the abrogation of kinetochore-microtubule interactions, argue for a more complex mechanism of SAC signaling than previously thought. Future experiments are required to elucidate the precise molecular pathway involved.

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