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Oliferenko and Balasubramanian reply:

We have read the correspondence submitted by Toda and colleagues with great interest. The authors have performed additional experiments using the *mia1/alp7* deletion mutant. In our paper, we have shown that *mia1/alp7* mutants are predominantly devoid of astral microtubules, are delayed in metaphase with short spindles, and that this block is relieved by *atf1*, a proposed downstream effector of the spindle orientation checkpoint. Toda and colleagues also observe the lack of astral microtubules in *mia1/alp7* mutants and suggest that *mia1/alp7* might activate the spindle orientation checkpoint, as we have previously proposed. However, Toda and colleagues reach two conclusions on the basis of synthetic lethality between the *mia1/alp7* deletion mutant and various mutants defective in SAC function. Thus, these authors make two statements: first, that it is SAC, not SOC, that keeps *mia1/alp7* cells alive; second, that the *mia1/alp7* mutant is not well suited for study of the SOC.

We will address these two points: first, we have independently identified genetic interactions between *mia1/alp7* and *mad2* mutants (S.O. and M.K.B, unpublished observations). We agree that the double-mutant data generated by Toda and colleagues and ourselves establish that *mia1/alp7* has multiple functions and activates at least two different checkpoint mechanisms. However, our manuscript did not claim that the spindle orientation checkpoint keeps *mia1/alp7* cells alive, and furthermore, we have proposed that this checkpoint is not essential for cell survival[1]. Thus, a major difference between our interpretation and that of Toda and colleagues pertains to the essential nature of the checkpoint[2]. We believe that the SOC is non-essential and therefore that *mia1/alp7 atf1* double mutants are capable of colony formation, albeit with a higher proportion of cells containing chromosome segregation defects. Consistent with the idea that the SOC is non-essential, we have identified two mutants, *kin88* (A. Bimbo, S.O. and M.K.B., unpublished observations) and *sps1* (S.O., unpublished observations), that seem to activate the spindle orientation checkpoint. In both cases, introducing the *atf1* deletion into these backgrounds is not lethal. We think a thorough analysis of several mutants that activate the SOC is required to firmly establish whether this checkpoint is essential, even though our own data from analysis of three mutants suggests that it is not.

Second, the *mad2 mia1/alp7* double-mutant experiment suggests that the *mia1/alp7* mutant is deficient in some aspect of spindle function (but not for assembly or anaphase elongation, as spindle assembly and spindle elongation proceed with wild-type kinetics in these mutants). The fact that Mad2–GFP is present on kinetochores in an increased number of *mia1/alp7* cells suggests that these cells might have an imperfect kinetochore attachment mechanism. Thus, the question that had to be addressed was whether there is a Mad2p-independent metaphase delay mechanism in *mia1/alp7* cells. A direct approach to answer this question is to study spindle and Mad2–GFP dynamics in *mia1/alp7* mutants, given that the other approach of studying the metaphase delay under *mia1/alp7 mad2* mutants was not feasible because of the lethality of this strain. In our hands, approximately 35% of *mia1/alp7* cells with short spindles exhibit Mad2–EGFP on kinetochores (10–11% total cell population). We have performed time lapse-analysis of Mad2–EGFP behaviour in wild-type and *mia1/alp7*cells, where we could also observe spindle dynamics using a spindle pole body marker Sid2–EGFP. We did observe that in general, Mad2–EGFP persisted longer on the kinetochores of *mia1/alp7* cells. However, after attachment (as indicated by disappearance of Mad2-GFP from kinetochores), there was an additional metaphase delay in these cells (S.O. and M.K.B., unpublished observations). Our observations suggest that in general, wild-type cells remained in metaphase for approximately 5 min after kinetochore attachment has occurred, whereas the *mia1/alp7* cells were delayed for approximately 15 min. These time-lapse experiments further establish that *mia1/alp7* cells activate a mechanism to delay onset of anaphase that is not related to attachment of kinetochores. Given the lack of astral microtubules in *mia1/alp7* cells, the presence of mis-oriented spindles and the rescue of the elongation defect by *atf1*, we conclude that *mia1/alp7* cells, as we proposed earlier, activate the spindle orientation checkpoint. Although activation of both SAC and SOC complicates the situation, *mia1/alp7* is one of the few mutants we are aware of that is devoid of astral microtubules. Therefore, this mutant will continue to be a valuable tool to understand both SAC and SOC. We are currently trying to isolate alleles of *mia1/alp7* that specifically activate SAC or the SOC.

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