Poster

Bub1 as a recruitment platform for Spindle Assembly Checkpoint components

Velasco-Gomariz M., Iglesias-Romero AB., Flor-Parra I. and Rodríguez Daga R.(1)

(1) Área de Genética, Departamento de Biología Molecular e Ingenieria Bioquímica, Centro Andaluz de Biología del Desarrollo (CABD), Universidad Pablo de Olavide, Carretera Utrera km. 1, 41013, Sevilla, Spain

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ABSTRACT

Motivation: The Spindle Assembly Checkpoint (SAC) is a safeguard mechanism conserved in all the eukaryotes that ensures the correct chromosome segregation in mitosis by preventing the premature mitotic exit in condition of unattached kinetochores. SAC defects lead to chromosome mis-segregation provoking aneuploidies that has been widely associated with cancer. Kinase Bub1 is a key player in SAC function because it maintains a proper centromeric cohesion and serves as a platform for other SAC components such as Mad1, Mad2 and Mad3. In this study, we are characterizing a *bub1* mutant allele which exhibits an impaired SAC function. The phenotype shown by this mutant has not been previously described. This mutant can provide new insights about the Bub1-dependent recruitment of SAC components to the kinetochores and the mechanism of mitosis arrest.

Methods: We made use of live imaging techniques to study SAC function by using GFP-tagged alleles of the main SAC components (like Mad1, Mad2 and Mad3), comparing the wild type background versus the *bub1* mutant allele. Furthermore, we have used a cold sensitive tubulin mutant that allows us to test SAC activity; in response to microtubules damage induced by this mutant, cells activate the SAC and arrest in metaphase. We have also tested Bub1 protein levels in a wild-type and in the Bub1-mutant by Western Blot. Finally, we are performing a two-hybrid screening using a *S. pombe* library-strain to detect differences in the interactome of the Bub1-mutant compared with the wild-type.

Results: We have demonstrated that in the Bub1-mutant background, Mad1 correctly localizes at the kinetochores meanwhile Mad2 does not. Bub1 protein levels turned out to be quite similar in both strain. Additionally, we have observed that the SAC defects notice in the Bub1-mutant in the cold sensitive background partially phenocopies the one seen in the Bub1-deleted cells.

Conclusions: Our Bub1-mutant is unable to maintain a proper metaphase block in the cold-sensitive tubulin background and exhibit a SAC failure. We are working in a model where Bub1 could be regulating SAC activity by promoting Mad2 recruitment to kinetochores.

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