Talk

Phosphorylation-mediated regulation of the gamma tubulin complex

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ABSTRACT

Motivation: Microtubules perform vital functions such as chromosome segregation, nuclear positioning, vesicular transport and determination of cell polarity and morphogenesis. The gamma tubulin complex is an essential element responsible for microtubule nucleation and is highly conserved from yeast to human and the core of the complex is basically composed of three esential proteins (Alp4/GCP2, Alp6/GCP3 and Gtb1/GTB). Its function is subject to a multiple regulation processes both temporally and spatially, and phosphorilation is one of the main postranslational modifications to regulate protein function. The fission yeast Schizosaccharomyces pombe is a potent model for studying microtubules (MT) nucleation because of its simple MT cytoskeleton, genetic tractability, limited number of regulators, and amenability to assays of individual nucleation events. In this work, we use S. pombe to determine the role of different putative phosphorylation sites to understand the regulation of this complex in different stages of the cell cycle.

Methods: In our laboratory, a collection of mutants of Alp4 and Alp6 genes was generated. These mutants consisted of the modification of the phosphorylation sites described in massive phosphoproteomes. Usind directed mutagenesis we generated non-phosphorylatable and phosphomimetic alleles (changins Serines to Alanine or Aspartic acid residues respectively. In this project, a phenotypic analysis of the different mutants from this collection has been performed using different approaches such as spot tests, western blots, fluorescence microscopy with live cells, and genetic interaction analysis techniques.

Results: We found that different mutants display phenotypic differences under the conditions analysed. The most representative phenotypes are related to delayed and aberrant mitotic spindle formation, no mitotic spindle formation and the appearance of stress granules at high temperatures. This establishes a connection between the phosphorylation state of Alp4 and Alp6 with microtubule formation, and its implication in the biology of microtubules in the cell.

Conclusions: Our data suggest that phosphorylation of components of the gamma tubulin complex is involved in the regulation of microtubule nucleation in different stages of the cell cycle and cell phisiology. This shed light in new molecular mechanisms not been previously described.

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