Two distinctive Tel2 pathways regulate protein maturation and refolding of ATM/ATR-related protein kinase Mec1 and Tel1 in budding yeast

Checkpoint signaling requires two evolutionarily conserved phosphatidylinositol 3-kinase (PI3K)-related protein kinases (PIKKs): ATM and ATR. In the budding yeast *Saccharomyces cerevisiae* ATM and ATR correspond to Tel1 and Mec1, respectively. Previous studies have uncovered the conserved activation mechanisms of ATM/Tel1 and ATR/Mec1, which are mediated by protein-protein interaction at sites of DNA damage. ATM- and ATR-mediated signaling is under several other layers of control. Recent studies of mammalian cells show that TTT is connected to the R2TP (Rvb1-Rvb2-Tah1-Pih1) complex via Tel2-Pih1 interaction. Whereas disruption of Tel2-Pih1 interaction nearly wipes out mTOR and SMG1 protein expression, it has an only minor impact on ATM and ATR protein levels.

The R2TP complex is well conserved from yeast to humans. Deletion of PIH1 did not alter expression levels of Mec1 and Tel1 at 30°C. Interestingly, however, Mec1 and Tel1 expression was lowered in *pih1Δ* mutants at 37°C. Correspondingly, *pih1Δ* mutation conferred checkpoint defects at 37°C. TEL2 is essential for cell proliferation whereas PIH1 is not. Previous studies have idenified that Asa1 is a TTT-interacting protein and a temperature *asa1* mutation (asa1-1) causes moderate Tel1 expression defects. We next characterized a role of Asa1 in protein stabilization of Mec1 and Tel1. Depletion of Asa1 comprimized Tel2-Mec1 or Tel2-Tel1 interaction and subsequently abolished Mec1 and Tel1 protein expression. Asa1 depletion caused significant checkpoint defects. Asa1-Pih1 interaction was undetected even at a higher temperature. Our findings indicate that two different Tel2 pathways contribute to protein maturation and refolding of Mec1 and Tel1 in budding yeast.

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