



Effects of aluminum exposure and involvement of Tor1 signaling on cell cycle regulation in *Schizosaccharomyces pombe*

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Abstract: This study was conducted to investigate the effects of AlCl_3 toxicity on wild-type and Target of Rapamycin knockout (ΔTor1) strains of *Schizosaccharomyces pombe* growth and cell cycle to explore the likely roles of the Tor1 signaling pathway in mediating Al toxicity. The growth of yeast cells was assessed through measuring cell density in media containing increasing concentrations of AlCl_3 in 3 h intervals. Yeast cells were cultured in liquid media containing 0, 750, and 1500 μM of AlCl_3 for 3 and 6 h. Increasing concentrations of AlCl_3 reduced the growth rate of both wild type and ΔTor1 strains in the same pattern; however, ΔTor1 strain grew better in solid media at high AlCl_3 concentration. The expression of the *cdc2* gene measured after 3 h of incubation was higher in ΔTor1 compared to wild type strains. The results of the current work demonstrate the likely involvement of the Tor1 signaling pathway in Al-induced effects on *S. pombe* cell cycle regulation.

• Introduction

Aluminum (Al) exposure can cause various dysfunctions in the organism. Human overexposure to Al causes organ-specific disorders, including bone diseases, neurological disorders, and breast cancer possibly as a consequence of toxic effects on the genetic materials of cells from various organs. Target of rapamycin (TOR) is a serine/threonine protein kinase belonging to the phosphatidylinositol-3 kinase-related kinases (PIKKs) family. The TOR signaling pathway is involved in modulating cell growth in response to varying environmental conditions.

The fission yeast *Schizosaccharomyces pombe* is a model organism with major contribution in investigating biological processes due to its similarity to humans, including gene structures, chromatin dynamics, and the control of gene expression. Hence, the objective of the current work was to investigate the effects of Al toxicity on *S. pombe* growth and expression of genes involved in cell cycle regulation in wild-type and Tor1-deficient strains to explore the likely role of Tor1 in Al-induced cell cycle regulation alterations.

• Material and method

The wild type and ΔTor1 strains were cultured in increasing AlCl_3 concentrations, including 0, 10, 20, 40, 80, 160, 320, 640, 1280, 2460, 4920 and 10000 μM . The cell growth was measured as the difference in light absorbance at 600 nm (OD_{600}) using Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader at 3, 6, and 9 h.

Spot test on solid YES media supplemented by 0, 300, 500, 1000, 2500, 5000, and 10000 μM of AlCl_3 was used to evaluate the capability of yeast cells to grow under Al-induced stress.

The expression of genes involved in cell cycle regulation, including *cdc2*, *cdc13*, *cdc15*, and *cdc25*, was measured by qRT-PCR.

• Results and discussions

The increasing concentration of AlCl_3 reduced growth rates of wild-type and Tor1 mutant strains in the same pattern; however, there were some differences between strains in some AlCl_3 concentrations, especially after 9 h of incubation, in which wild-type strains showed higher growth rates. The spot test experiment showed the same growth rates at low AlCl_3 concentrations. The

expression of *cdc2* was significantly higher in Tor1-deficient compared to the wild-type strain. Increasing AlCl_3 supplementation level in *S. pombe* growth media caused an enhancement in expression of *cdc13* and *cdc15*. In *S. pombe*, *cdc2* is a protein coding gene with a central role in regulating the mitotic cell cycle and the capability to phosphorylate *cdc15*, *cdc23*, and *cdc25* in various mitotic phases.

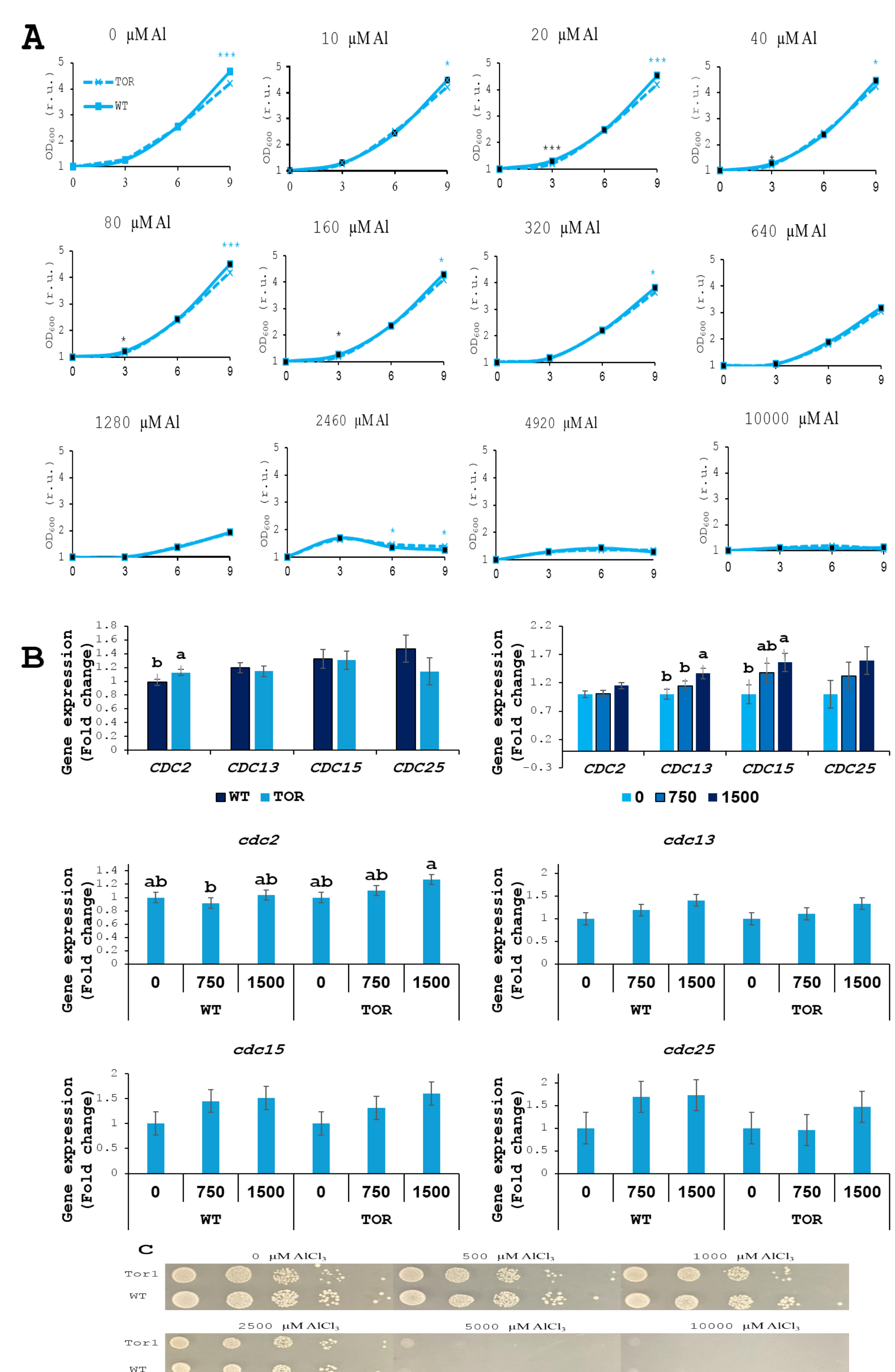


Figure 1. A. Growth rates of wild-type and Tor1-deficient *S. pombe* cells exposed to increasing concentrations of AlCl_3 incubated for 3, 6, and 9 h (relative units, r.u. represent relative increase in OD_{600} compared to time point 0 h), B. The growth ability of serially diluted wild type and Tor1-deficient *S. pombe* on plates containing solid YES media supplemented with indicated AlCl_3 concentrations for 2-3 days, C. The fold change in expression of genes involved in cell cycle regulation of *S. pombe*, comparing to the control group affected by the type of strains, AlCl_3 concentrations, and their interaction.

In recent experiments in our lab, the ΔTor1 strain of *S. pombe* showed higher tolerance and growth rates compared to the wild-type strain when exposed to toxic concentrations of cadmium and nickel and this capability was related to the involvement of Tor1 in ion trafficking and metabolism. The enhanced expression of Cdc13 and Cdc15 in response to AlCl_3 supplementation in the current study can be considered as a strategy acquired by the yeast cells to overcome the likely cell death induced by AlCl_3 toxicity.

• Conclusions

The results of the current study demonstrate that AlCl_3 supplementation decreases *S. pombe* wild-type and ΔTor1 strains in a dose-dependent manner; however, there were some slight differences. Higher expression of *cdc2* in ΔTor1 compared to the wild-type strain might be an indicator of the Tor1 pathway in cell cycle regulation.

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