Abstract: Identification of two tyrosine kinase-like proteins as important modulators of the STATc-mediated response to hyperosmolarity

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In order to adapt to changes in environmental osmolarity cells initiate massive transcriptional changes. In *D. discoideum*, STATc (Signal Transducer and Activator of Transcription c) is a key regulator of the transcriptional response to hyperosmotic stress. Under this condition, STATc is phosphorylated on Tyr922, dimerises, and translocates to the nucleus, where it controls gene expression. In mammals, STATs are activated by JAKs (Janus kinases), however, *D. discoideum* does not encode bona fide tyrosine kinases and there is no JAK ortholog. Recent work revealed two direct STATc regulators, PTP3 (Protein Tyrosine Phosphatase 3) and Pyk2 (tyrosine-kinase like, TKL protein) (Araki *et al.*, 2008; Araki *et al.*, 2012). However, STATc was still phosphorylated in a pyk2⁻ strain, albeit with reduced efficiency.

Based on microarray studies, we chose three TKL proteins, Pyk3, Phg2, and MORN, as possible STATc protein kinase candidates. We analysed single knock-out mutants for these genes, a double knock-out mutant for Pyk3 and Phg2, as well as STATc-GFP overexpressing Ax2 wild-type and mutant cells with respect to transcriptional regulation, STATc phosphorylation, and nuclear translocation. Transcriptional regulation of STATc and STATc dependent genes was disturbed in pyk3, phg2, MORN, and pyk3/phg2 cells. The absence of Pyk3 resulted in significantly reduced and of Phg2 in completely abolished transcriptional activation of these genes in response to sorbitol and 8-Bromo cGMP. Phospho-STATc levels upon sorbitol and 8-Bromo cGMP treatment were significantly reduced in pyk3⁻ and phg2⁻ cells and further decreased in pyk3⁻/phg2⁻ cells. This result was reflected by delayed nuclear translocation of GFP-STATc in these strains. Furthermore, overexpression of Pyk3 led to constitutive STATc phosphorylation and Pyk3 directly phosphorylated bacterially expressed STATc in vitro. To further analyse Phg2 Myc-Phg2 overexpressing Ax2 wild-type cells and Myc-PTP3 overexpressing Ax2 wild-type and phg2⁻ cells were generated. By using PTP3 phosphoserine-specific antibodies we found that Phg2 is involved in the inhibition and phosphorylation of PTP3 on S747. Pull-down assays suggest a direct interaction between Phg2 and PTP3.

In response to hyperosmotic conditions, a bifurcated signaling pathway controls the activity of STATc. In the cGMP-dependent branch, Pyk3 directly phosphorylates STATc and Phg2 possibly PTP3 on S747. In the Ca²⁺-dependent branch, Pyk2 is the STATc kinase and we postulate a further protein kinase that phosphorylates PTP3 on S448. This complex regulation ascertains optimal STATc activation.