Mechanosensor-Mediated Hsp70 Phosphorylation Orchestrates the Landscape of the Heat Shock Response

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Cells must be able to respond rapidly to changes in their surrounding temperature, accomplished by activation of the core heat shock response (HSR). The HSR promotes the expression of molecular chaperones such as Hsp70 and Hsp90 that refold denatured proteins restoring proteostasis. Recent studies have identified numerous post-translational modifications (PTMs) on Hsp70 (the "chaperone code)" that fine-tunes the activity of Hsp70 through alteration of co-chaperone and client interactions. To understand how heat shock impacts Hsp70 PTMs, we performed quantitative mass spectrometry analysis on yeast Hsp70 purified from untreated and heat-shocked cells. We identified a single site on the substrate binding domain of Hsp70 that was uniquely upregulated in response to heat. Interestingly, activation of this site was dependent on the cell integrity MAP kinase pathway, another pathway activated by heat. Phosphorylation at this unique site was activated rapidly (<5mins) by heat and by other cell-wall damaging agents and was dependent on proteins in the cell-wall integrity pathway. Mutating the site to an alanine (phospho-mutant) resulted in yeast being unable to survive exposure to heat stress or cell wall damaging agents. The phospho-mutant was unable to activate the heat shock response (HSR) confirming the importance of this unique phosphorylation for survival at high temperatures. Taken together we believe that this phosphorylation of Hsp70 is an initiating event in the heat shock response, a process that may be universally conserved in all organisms.

Keywords- Hsp70, phosphorylation, heat shock, yeast