Quantitative analysis of single-cell MAPK signaling crosstalk in microfluidic concentration gradient device

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We investigate the yeast MAPK signaling pathways and their crosstalk, taking advantage of a new microfluidic device coupled to quantitative microscopy. We designed specific micropads to trap yeast cells in a single focal plane and modulate the magnitude of a given stress signal by microfluidic serial dilution while keeping other signaling inputs constant. This approach enabled us to quantify in single cells nuclear relocation of effectors responding to MAPK activation, like Yap1 for oxidative stress, and expression of stress-specific reporter expression, like pSTL1-qV and pFIG1-qV for high-osmolarity or mating pheromone signaling, respectively. Using this quantitative single-cell analysis, we confirmed bimodal behavior of gene expression in response to Hog1 activation, and quantified crosstalk between the pheromone- and cell wall integrity (CWI) signaling pathways. Importantly, we further observed that oxidative stress inhibits pheromone signaling. Mechanistically, this crosstalk is mediated by Pkc1-dependent phosphorylation of the scaffold protein Ste5 on serine 185, which prevents Ste5 recruitment to the plasma membrane.