PROTEOMIC AND PHOSPHOPROTEOMIC STUDY OF THE MACROPHAGE RESPONSE TO CANDIDA ALBICANS USING SILAC AND SIMAC

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Host-pathogen interaction studies open interesting opportunities in the search of new virulence determinants and new targets for antimicrobial therapies. Protein phosphorylation is a complex network of signalling and regulatory events that affects virtually every cellular process. In the light of the importance of macrophages for optimal host protection against *Candida albicans* invasive infections, we have chosen the murine macrophage cell line RAW 264.7 to study the host response to *C. albicans*. Previous studies from our group showed that macrophages respond to the interaction with *C. albicans* with a proinflammatory reaction, anti-apoptotic signals, cytoskeletal rearrangement and other cellular processes (Martínez-Solano *et al*., 2006, 2009 and unpublished results). In the present work, we have employed SILAC (Stable Isotope Labelling by Amino acids in cell Culture) to study changes in macrophage proteins and phosphopeptides expression in response to the yeast. For the phosphoproteomics approach we have used SIMAC (IMAC and $Ti0_2$) and CPP (Calcium Phosphate Precipitation and $Ti0_2$) enrichment. From a total of 2833 proteins identified, 71 macrophage proteins showed differential expression during the interaction. These included proteins involved in the immune response, phagocytosis, anti-apoptosis and cytoskeletal rearrangement. Using SIMAC and CPP, we report the identification of 841 nonredundant phosphorylation sites from 522 proteins. The response of the macrophage phosphoproteome to *C. albicans* reflects changes in 191 phosphopeptides related with innate and adaptive immunity, protein kinases, cytoskeletal rearrangement as well as other cellular processes.