

Characterization of *Saccharomyces cerevisiae* Transcription Factor Gcr1p Using 2-D Electrophoresis and Mass Spectrometry

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1) Background and Objective: In *Saccharomyces cerevisiae*, the transcription factor Gcr1p has been established as a phosphoprotein that is required for the transcription of glycolytic enzyme genes and ribosomal protein genes and may play a direct role in cell cycle progression. Using biochemical methods, knowledge of the phosphorylation of Gcr1 residues and any changes that may occur in those residues during the cell cycle will be explored. 2) Methods: KB3 cells containing the fusion protein Gcr1p-myc-his₆ and KB12 cells containing the fusion protein Gcr1p-GFP were grown. Gcr1p was partially purified from the cell lysates of each cell line using nuclear membrane purification scheme, 2-D electrophoresis and Western blotting techniques. In-gel trypsin digests were performed on identified bands from each cell prep and the samples were characterized using nanospray MS and MS/MS. 3) Results: With 2-D electrophoresis and Western blotting, Gcr1p was shown to be present in more than one species with pIs ranging from approximately 6.3-8.3. 4) Discussion and Conclusions: MS/MS analysis has determined peptide sequences that are exclusive to Gcr1p. This analysis has shown that Gcr1p exists in different states of phosphorylation *in vivo* and has provided information regarding the particular sites of phosphorylation of this important regulatory protein.