SL307 Characterization of yeast transcription factor Gcn4p and its regulation of ADE3 gene

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The Gcn4p, the yeast transcriptional activator protein, binds to the specific sequence in the promoters of many amino acids biosynthetic genes for the general control. We developed a screening system which can isolate Gcn4p derivatives with mutations in the DNA binding domain. In order to identify amino acids in Gcn4p that involve in protein-protein interaction, we performed saturation mutagenesis in the DNA binding domain of Gcn4p using oligonucleotides containing randomized codon bases. These mutations were assayed for their ability to support transcriptional activation, binding activity by checking sensitivity of 3-aminotriazole(3-AT), and *in vitro* binding assay. The two mutations (R241L and S242L), which have decreased transcriptional activation ability, can bind to the AP-1 sequence. The residues identified in these mutants may play a role in the interaction with other protein(s) for Gcn4p activation. To find protein(s) which interact with Gcn4p functionally *in vivo*, the suppression study with these mutants are in progress. Previous studies showed that Gcn4p also effeiciently activates transcription of purine biosynthetic genes in response to purine limitation. The protein product of the *Saccharomyces cerevisiae*, ADE3 gene C_1-tetrahydrofolate synthase, is a trifunctional enzyme, which possesses formyl-THF synthetase, methenyl-THF cyclohydrolase, methylene-THF dehydrogenase activities. This trifunctional enzyme involves in one-carbon metabolism, THF interconversion, which is dependent on the expression of C_1-THF synthase. 10-formyl-THF generated by C_1-THF synthase is required in purine biosynthesis. The ADE3 promoter contains putative binding sites for the yeast regulatory factor, Gcn4p, Pho4p, Abf1p and Tuf1p. Through deletional analysis of the ADE3 promoter, we identified 3 GCREs(Gcn4p responsive elements) as the cis-regulatory element required for transcriptional expression. The first Gcn4p binding site was required for high levels of transcription *in vivo*. The second Gcn4p binding site also affected on transcriptional expression. The wild type ADE3 promoter containing both Gcn4p sites is bound Gcn4p *in vitro*. The ADE3-LacZ fusion gene was not expressed in GCN4 deletional strain. Also deletional analysis showed Abf1p binding sites prior to Gcn4p binding sites might be required for maximal levels of ADE3 transcription. These results suggest that the transcriptional factor Gcn4p induce transcriptional expression of ADE3 gene and Abf1p may involve the ADE3 promoter function.