Saccharomyces cerevisiae

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Expression of genes encoding starch-degrading enzymes is regulated by glucose repression in the yeast *Saccharomyces cerevisiae.* We have identified a transcriptional repressor, Nrg1, in a genetic screen designed to reveal negative factors involved in the expression of *STA1*, which encodes a glucoamylase. The *NRG1* gene encoeds a 25-kDa C2H2 zinc finger protein which specifically binds to two regions in the upstream activation sequence of the STA1 gene, as judged by gel retardation and DNase I footprinting analyses. Disruption of the *NRG1* gene causes a fivefold increase in the level of the *STA1* transcript in the presence of glucose. The expression of *NRG1* itself is inhibited in the absence of glucose. DNA-bound LexA-Nrg1 represses transcription of a target gene 10.7-fold in a glucose-dependent manner, and this repression is ablished in both *ssn6* and *tup1* mutants. Two-hybrid and glutathione S-transferase pull-down experiments show an interaction of Nrg1 with Ssn6 both in vivo and in vitro. These findings indicate that Nrg1 acts as a DNA-binding repressor and mediates glucose repression of the STA1 gene expression by recruiting the Ssn6-Tup1 complex. Furthermore, in northern blot analysis, the mRNA level of *SUC2, PCK1, HXT2* in *nrg1* null mutant was relieved from glucose repression in repressed condition. Therefore it is thought that *NRG1* may be concerned in glucose repression of these genes.

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Development of Non-protoplast Transformation System in *Aspergillus oryzae*

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Aspergillus oryzae is a filamentous fungus classified in the group *Aspergillaceae Ascomycetes.* It is an important microorganism for industrial production of enzymes and fermented food productions. It secrets large quantities of proteins or enzymes into the culture medium which makes this organism appealing for the production of heterologous proteins. Recently electric field-mediated transformation method, electroporation, has been applied to fungal transformation. In this study, fungal transformation was carried out by bypassing the protoplast isolation step, decreasing the culturing time and non-protoplast transformation for the increment of transformation efficiency. Transformants were obtained with electroporation in optimal condition 2,500 voltage, 1,540 ohm and 0.50 capacitance. More than 1,000 transformants were obtained with 6-10 hrs cultured mycelia without enzyme treatment, called non-protoplast transformation.

SL312

The Biofuel Cell: Development of New Materials for Composing Electron Mediator-free and Electrochemical Active Acteria-free Biofuel Cell

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In this study biofuel cell is classified

into 5 generation fuel cell system based on structural and structural difference. I optionally named the biofuel cell with electron mediators prototype, that with electrochemical active bacterium 2nd generation, that with modified electrode with NR 3rd generation, that with catalytic active electrode 4th generation, and that using air as a catholyte instead of ion selective membrane and cathode, respectively. The electricity production was compared among 5 types biofuel cell and was confirmed to be 50-100% higher in 4th and 5th generation than in 1st to 3rd generation.

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Universal Conservation of Factors Promoting Protein Synthesis

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A universally conserved step in gene expression is the initiation of protein synthesis at an AUG codon using a specific initiator tRNAMet. The delivery of the Met-tRNAMet to the ribosome is catalyzed in prokaryotes by the translation factor IF2, a single polypeptide of ~90 kDa, and in eukaryotes by the heterotrimeric factor eIF2. Therefore, it was surprising when a bacterial IF2 homolog was identified in both yeast and archaea. *S. cerevisiae* strains lacking the *FUN12* gene encoding the yeast IF2 homolog had a severe slow-growth phenotype, and polyribosome profiles revealed a translation initiation defect. This translation defect was also apparent in extracts prepared from the *fun12* deletion strains and could be rescued by adding back recombinant yeast IF2 protein. These results indicate that yeast IF2 is a general translation factor. We identified a human IF2 homolog and found that the human protein, as well as archaeal IF2, could functionally substitute for yeast IF2 both in vivo and in vitro. Thus, IF2 is a universally conserved translation initiation factor. Recent biochemical analyses revealed that the eukaryotic IF2 homolog, now known as eIF5B, is required for the ribosomal subunit joining step of translation initiation and possesses 60S ribosomal subunit-dependent GTP hydrolysis activity. In addition, using yeast two-hybrid, co-immunoprecipitation, and in vitro binding assays, as well as genetic analyses, we recently found that eIF1A (the eukaryotic homolog of the bacterial translation factor IF1) physically and functionally interacts with eIF5B. We propose that eIF1A and eIF5B locate in A site of ribosome and stabilize proper binding of the initiator Met-tRNAMet in the ribosomal P site. Thus the bacterial translation factors IF2 and IF1 are conserved in eukaryotes and functionally cooperate to promote protein synthesis. Our studies reveal both similarities and differences in the functions of the eukaryotic and prokaryotic IF2 proteins, and we believe they offer important insights into the development of antibiotics that target specifically bacterial or fungal IF2 proteins.

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Production of Xylitol by Xylitol Dehydrogenase Defective Mutant of *Pichia stipitis*

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This study was carried out to develop