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# SACCHAROMYCES CEREVISIAE'DE ENOİL REDÜKTAZ-MİNUS MUTANTININ (FAS I -246) İŞARETLİ MUTASYONUNUN İZOLASYONU

# ISOLATION OF THE POINT MUTATION OF THE ENOYL REDUCTASE-MINUS MUTANT (FAS I-246), IN SACCHAROMYCES CEREVİSAE

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**SUMMARY**

The nuclaotide sequence of the fas I gene specifically coding for the enoyl reductase domain has been shown to be cloned into the plasmid YEp FAS I.The studies on the enoyl reductase minus-mutant (fas I-246) involved a yeast transformation technique using cloned plasmids, with yeast DNA fragments cleaved from the YEpFAS I clone. By cloning various overlapping yeast DNA fragments of YEpFAS I it was hoped to isolate the point mutation to a suitably sized fragment, which could be sequenced and the mechanism behind the mutation elucidated.

**ÖZET**

Enoil redüktaz bölgesi için spesifik olarak kodlanan yağ asidi sentetaz I geninin nukleotid dizisi YEpYAS I plazmidi içersine klonlanarak gösterilmiştir. Enoil redüktaz minus mutantı (FAS I-246) üzerine çalışmalar YEpYAS I klonundan ayrılan maya DNA fragmanları ile klonlanmış plazmidler kullanılan bir maya transformasyon tekniğini kapsar. Klonlama yoluyla YEpYAS I'in değişik, çakışan maya DNA fragmanları kullanılarak önceden dizilenmiş olan ve saptanan mutasyonunun arkasındaki mekanizmayı aydınlatan uygun ölçülere sahip bir işaretli mutasyonun izole edilebileceği planlandı.

Yeast genes introduced on plasmid vectors into E.coli are often active both in transcription and translation. The expression of cloned genes depends on the ability of the bacterial RNA polymerase to recognise eukaryotic promoters and the bacterial ribosomes to correctly translate the resulting messenger RNA's. Introns are apparently less common in fungi than in higher eukaryotes, thereby facilitating the expression of fungal genes in eukaryotes (1, 2). Hence it was probable that yeast fatty acid synthetase would be expressed in E.coli and the cloned yeast FAS genes could be isolated, if a suitable selection procedure were applied.

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The multi cistronic genes encoding the ∝ and ß subunits of yeast synthetase have been genetically mapped to the unlinked loci fas 2 and fas I, respectively (3, 4). Figure 1, shows restriction endonuclease maps for both loci. Using a yeast transformation technique (5), DNA clones of the genes coding for the ∝ and ß subunits of the synthetase have been isolated.. Fatty acid auxotrophs of S. cerevisiae were used to select clones from a bank of yeast DNA sequences which complemented mutations in the fas I and fas 2 loci (6). The bank of yeast DNA sequences was prepared from a partial Sau 3a digestion of yeast DNA and ligated into YEp13, a yeast-E.coli shuttle vector (7). The YEp13 plasmid contains the complete leu 2 gene of yeast and the entire pBR 322 genes (8). E.coli was transformed by the ligated plasmids, and ampicillin resistance- tetracyclin sensitive colonies were selected, pooled and amplified. Cells transformed with a plasmid containing yeast DNA sequences that complimented the fas I and fas 2 mutations were selected. The complimentary plasmid from each colony was isolated and named YEpFAS I and YEpFAS 2.

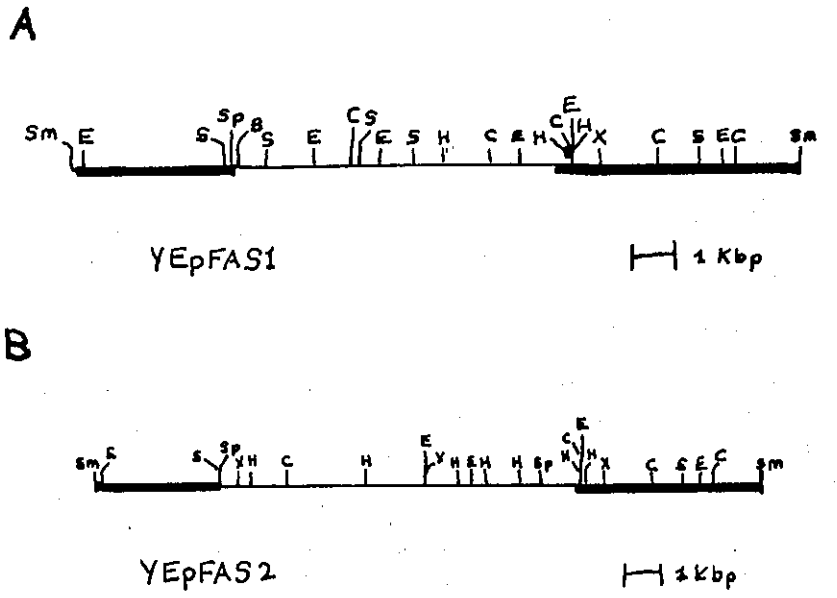



**Figure - 1:** Restriciton endonuclease maps of the loci, fas I and fas 2 cloned into the vector YEp13 (thick lines). Abbreviations are Sm, Sma I; E, EcoRI; H, Hind III; X, Xba I; S, SaI; C, Cla I; Sp, Sph I; and B, Bam HI.

Further sceening for sheared yeast DNA inserted into the Col EI vector, identified another fas I clone; 33FI. From Southern blot hybridization experiments homology between 33FI and YEpFAS I was demonstrated. This lead to further information that the plasmid proved to be a more accurate representation of the fas I locus. Fig.2 shows endonuclease restriction maps for the plasmids YEpFAS I and 33FI.

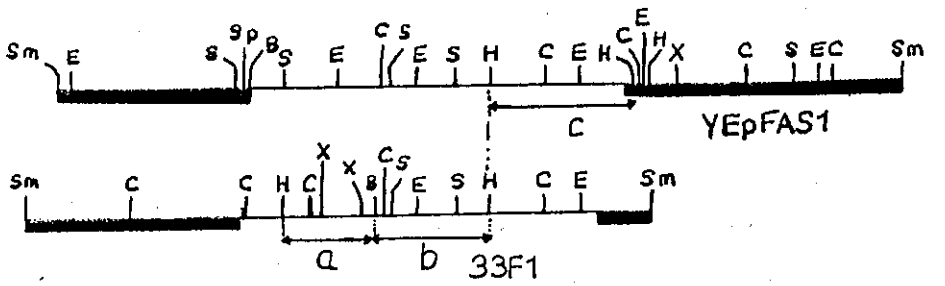



**Figure - 2:** Restriction endonuclease maps for YEpFAS I and YEp33FI. (Abbreviations as before). a = 2.I kb, b=2.8 kb, c= 3.5 kb.

The plasmid YEpFAS I has been shown to complement the fas I-246 mutation, associated with the enoyl reductase activity. The isolation of this mutation was to be explored further. Fig.3 gives a representation of the proposed site of the enoyl reductase transcription on the fas I gene. The three fragments labelled in connection with various activities of the enoyl reductase, have all been cloned from YEpFAS I and 33FI, into the vector pBR 322. These new clones were used in the initiation of a series of yeast transformations to elucidate the location and mechanism of the mutation fas I-246.

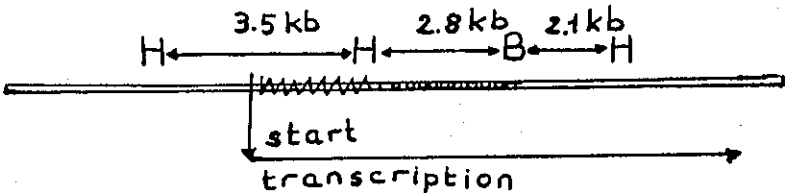



**Figure - 3:** The proposed sites of the enoyl reductase domain on the fas I gene (N-Terminus). H = Hind III, B=Bam HI, M=NADPH site, ooooooo = FMN site.

## EXPERIMENTAL PROCEDURES

### Strains and media

Yeast strain fas I-246a originally described by Schweizer (4), as a strain containing a mutation in the enoyl reductase activity was obtained from the American Type Culture Collection. This strain was crossed with GRF 18 (a, his 3-II, his 3-15, leu 2-3, leu 2-II2, can I-II, (9), random spores were isolated and the haploid strain fas I-246 leu 2 can R was selected and used in subsequent transformation experiment.

The fatty acid requiring yeast strain fas I-246 was grown in medium containing I% yeast extract, 2% bactopeptone, 2%: dextrose, supplemented with 0.5% Tween 40 and 2mM myristic acid (YPD + fatty acids). A minimal medium excluding the 0.5% Tween 40 and 2mM myristic acid, was employed in experiments involving transformed yeast cells. E.coli strains were grown on L-broth, and ampicillin was added to a final concentration of 80 ug/ml as required. Endonuclease restriction digest, vector dephosphorylations and ligation procedures, all followed manufacturers, suggested protocol.

### Transformation of bacteria

Purification of ligated plasmids for the transformation of yeats, was achieved initially through the transformation of the bacterium, E. coli (JMIOI), according to the method of Dagert and Ehrlich (10).

### DNA isolations

Large scale plasmid DNA preparations from E.coli (JMIOI) were performed using CsCl gradients. Mini plasmid preparations for screening potential plasmids of interest were carried out by the method of Birnboim and Doly (II).

### Yeast transformation with lithium treated cells

An overnght starter culture of the yeast strain to be transformed was grown in YPD + fatty acids. The next day, 500ml was innoculated with the starter culture to an A 600 of approximately 0.100. The cells were grown at 30°C to A 600 = 0.8, which corresponds to $7x10^6$ cells/ml,

and were harvested in 50 ml conical tubes in a swinging bucket rotor at 2500 rpm. The cells were washed once with sterile water and then resuspended in the fololwing solution; IOmM Tris-Cl, ImM EDTA and O.IM lithium acetate at pH 7.5, I/200 th (usually Iml) of the inital harvest volume (usually 20ml) povided the resuspension volume. The cells were then incubated at 30°C for I hour with agitation. The transformation was carried out in a microfuge tube - 0.Iml cells, 40ug sonicated carrier DNA and 20ug plasmid + insert DNA- this is then incubated for 30 minutes at 30°C. On addition of 0.7 ml (40% PEG 4000, O.I M LiAc, IOmM Tris - Cl and I mM EDTA) the cells were incubated for a further 30 minutes at 30°C, then heatshocked for 5 minutes at 42°C. The cells were centrifuged in a microfuge for 2 seconds, washed twice with 10 mM Tris - Cl and I mM EDTA solution (at pH 7.5) and then finally resuspended in I ml of this solution. The cells were plated on selected media; YPD agar (YPD media + 2% agar), tranformants appear after two days incubation at 30°C. On average the procedure yields 600 transformants per ug of yeast DNA, with a cell viability of about 40%.

## RESULTS AND DISCUSSION

The three DNA fragments 3.5, 2.8 and 2.1 kilobases were used in a yeast transformation of the mutant strain fas I-246. The result showed high number of mutants trasformants in the presence of the 2.8 kb. clone. This strongly suggests the region of the mutation to be located within the area of the enoyl reductase domain coding for the FMN bindig site. The next step in the study involved the isolation of a 3.8 kb fragment from the YEpFAS I plasmid (Fig.4), with the goal to clone the **105105fragment into a new plasmid PUC 18 (Fig.5).**

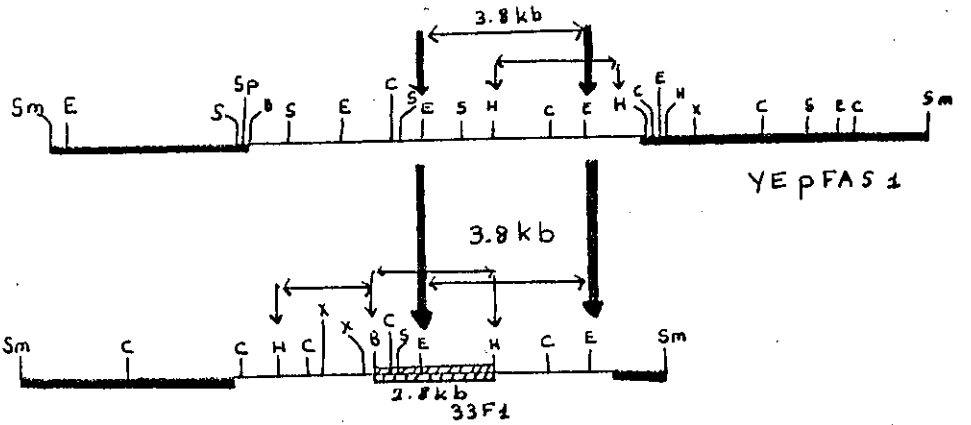



**Figure - 4:** The 3.8 kb fragment within the two clones YEpFAS I and 33FI. Abbreviatinos as before.

It was hoped that this would begin a series of yeast transformations, with different clones overlapping the 2.8 kb region (Fig.4) in an attempt to isolate the mutation down to an appropriate sized fragment (~ 1000 bases), suitable for sequencing. Using various hybridisation techniques with the aim to isolate the mutation to a single base. Once achieved further mutations around this area may well lead eventually to the mechanism behind the FMN - binding of the enoyl reductase activity in yeast fatty acid synthetase. In the future, the expression and mechanisms of all the patial activites of yeast FAS, may be determined by similar applications (12).

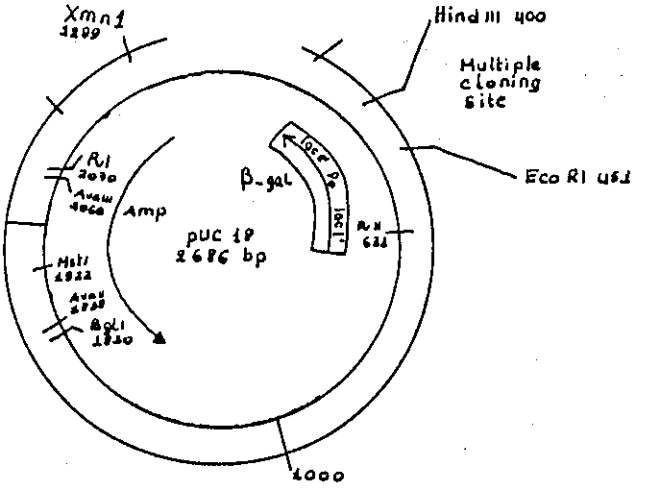
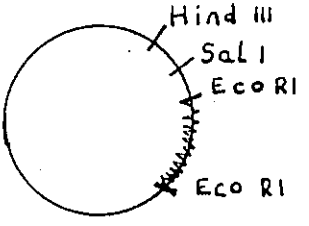
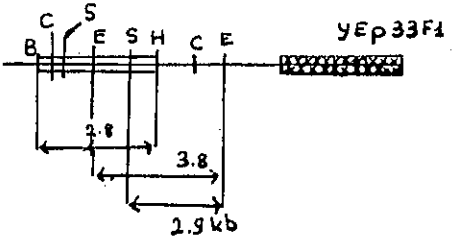



**Figure - 5:** The vector pUC 18 which contains the gene for ß-glactosidase and a multiple cloning site, is shown above (13). The two diagrams below show from left to right, the required insertion of the 3.8 kb fragment in the vector pUC 18, and the next proposed fragment to be cloned into pUC 18. Abbreviatinos as before.




ß - galactosidase activity

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## REFERENCES

1. Ng.R., Abelson, J.: *Proc.Natl. Acad. Sci. USA*, **77** 3912 - 3916 (1980).
2. Gallwitz, D., Sures,I.: *Proc.Natl. Acad. Sci. USA* **77**, 2546 - 2550 (1980).
3. Culbertson, M.R., Henry, S.A.: *Genetics*, **75**, 441 - 458 (1973).
4. Kuhn, L., Castorph, H., Schweizer, B.: *Eur. J. Biochem.*, **24**, 492 - 497 (1972).
5. Wieland, F., Siess, E., Renner, L., Verfurth, C., Lynen, F.: *Proc.Natl. Acad. Sci. USA*, **75**, 5792 (1978).
6. Kuziora, M.A., Douglas, M.G., Wakil, S.J.: *J.Biol. Chem.*, 258 11643 (1983).
7. Williamson, V., Bennetzen, V., Young, E.: *Nature*, **283**, 214 (1980).
8. Broach, J., Strathem, J., Hick, J.: *Gene.*, **8**, 121 (1979).
9. Sherman, F., Fink, G., Lawrence, C.: *Methods in Yeast Genetics*. Coldspring Harbor Labarotory, New York, 1975.
10. Dagert, M., Ehrlich, S.D.: *Gene.*, **6**, 23 - 28 (1979).
11. Birnboim and Doly.: *Nucl. Acids. Res.*, **7**, 1513, (1979).
12. Ulutin, T., Yardımcı, T.: *Acta. Pharmaceutica. Turcica*, (in Press), 1989.
13. Messing, J.: In the *"Third Cleveland Symposium on Macromolecules"* Recombinat DNA, ed. Walton A., Elsevier, Amsterdam, 143-153.

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