Change of serine$^{309}$ into proline causes temperature sensitivity of the nuclear NAM1/MTF2 gene product for yeast mitochondria

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Recent independent isolation and sequencing of the nuclear genes NAM1 (1) and MTF2 (2) from Saccharomyces cerevisiae demonstrated their identity. The respective gene product is important for the biogenesis of functional mitochondria and affects mitochondrial splicing, transcription and protein synthesis (1, 3).

In order to get further insights into functional aspects of that factor we turned our attention to mutant pet-ts3504. This mutant represents a temperature sensitive allele of NAM1/MTF2 (3). Using the wild-type gene as a probe a chromosomal DNA fragment carrying the reading frame was isolated from that mutant. Sequencing of the complete reading frame identified a single base substitution in comparison to the wild-type. The respective region is shown in Figure 1.

The nucleotide change from T to C does not interrupt the reading frame of 440 amino acids but results in a single amino acid substitution at position 309 in the protein. In that way the serine of the wild-type is replaced by a proline in the mutant. To further verify that this change is responsible for the temperature sensitive phenotype of the mutant a small DNA fragment from the wild-type reading frame of this region was used to directly transform the mutant. Homologous recombination of the fragment into the mutated reading frame results in restoration of temperature sensitivity proving that this single point mutation is responsible for the phenotype of pet-ts3504.

In that way a functionally important domain of the protein has been localized and it is possible to explain the defect of the altered gene product on the molecular level. The change of serine at position 309 into proline probably results in a heat-labile form of the protein due to the fact that the amino acid proline cannot take part in the formation of alpha-helical structures.

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REFERENCES

wild-type $5'\ldots$ AAGTACACATTGATCTCCTATGTTTATAATGAA$\ldots3'$
304-K Y T L I S Y V Y N E-315
K Y T L I P Y V Y N E
pet-ts3504 $5'\ldots$ AAGTACACATTGATCCCTATGTTTATAATGAA$\ldots3'$

Figure 1. A single base substitution (*) distinguishes the wild-type from the mutant gene.