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The pentafunctional *FAS1* genes of *Saccharomyces cerevisiae* and *Yarrowia lipolytica* are co-linear and considerably longer than previously estimated

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Summary. The fatty acid synthetase (FAS) gene *FAS1* of the alkane-utilizing yeast *Yarrowia lipolytica* was cloned and sequenced. The gene is represented by an intron-free reading frame of 6228 bp encoding a protein of 2076 amino acids and 229980 Da molecular weight. This protein exhibits a 58% sequence similarity to the corresponding *Saccharomyces cerevisiae* FAS β-subunit. The sequential order of the five *FAS1*-encoded enzyme domains, acetyl transferase, enoyl reductase, dehydratase and malonyl/palmityl-transferase, is co-linear in both organisms. This finding agrees with available evidence that the functional organization of FAS genes is similar in related organisms but differs considerably between unrelated species. In addition, previously reported conflicting data concerning the 3′ end of *S. cerevisiae FAS1* were re-examined by genomic and cDNA sequencing of the relevant portion of the gene. Thereby, the translational stop codon was shown to lie considerably downstream of both published termination sites. The *S. cerevisiae FAS1* gene thus has a corrected length of 6153 bp and encodes a protein of 2051 amino acids and 228667 Da molecular weight.

Key words: Yeast fatty acid synthetases – *Yarrowia lipolytica*/*Saccharomyces cerevisiae FAS1* sequence comparison – *S. cerevisiae FAS1* sequence correction

Fatty acid synthetases exist either as non-aggregated multicomponent enzymes (type II FAS) or as highly integrated multifunctional proteins (type I FAS). Characteristically, type I synthetases are found in the cytoplasm of eucaryotic cells while their occurrence in bacteria is rare (Lynen 1980; Kawaguchi and Okuda 1977). In yeast and other lower fungi, the enzyme is an $\alpha_6\beta_6$ heteromultimeric complex whose subunits are encoded by two multifunctional genes, *FAS1* (subunit β) and *FAS2* (subunit α) (E. Schweizer et al. 1978). The genetic and structural organisation of type I synthetases from other eucaryotic taxa differ from this (Wakil et al. 1983). To date, the multifunctional *FAS* genes of *Saccharomyces cerevisiae* (M. Schweizer et al. 1986; Chirala et al. 1987; E. Schweizer et al. 1987; Mohamed et al. 1988), *Penicillium patulum* (Wiesner et al. 1988), rat (M. Schweizer et al. 1989; Amy et al. 1989) and chicken (Yuan et al. 1988) have been cloned and sequenced. As is evident from these studies the sequences of isofunctional domains in different FAS enzymes are clearly similar, although the extent of sequence conservation is often very low (Beck et al. 1990). On the other hand, the sequential order of catalytic domains along the multifunctional FAS chains is comparable only among related species such as yeast and *Penicillium* (Wiesner et al. 1988) while the animal and fungal *FAS* genes have very different structures (M. Schweizer et al. 1989). The similarity of the *Yarrowia lipolytica* and *S. cerevisiae FAS1* sequences as reported in this study (Figs. 1 and 2) corroborates the above conclusions.

The complete nucleotide sequences of the *S. cerevisiae FAS1* and *FAS2* genes were first reported from our laboratory (M. Schweizer et al. 1986). Subsequent reports from Chirala et al. (1987) and from Mohamed et al. (1988) essentially confirmed our data but came to different conclusions regarding the *FAS1* translational termination site. While the *FAS1* open reading frame was originally proposed to end at position 7002 of the sequence shown in Fig. 1, Chirala et al. (1987) postulated that the stop codon is located at position 7409. Consequently, the corresponding gene products differed by 135 amino acids and 14947 Da molecular weight. This discrepancy prompted us to re-investigate the C-terminal sequence of the *S. cerevisiae FAS1* gene. These studies were complemented by *FAS1* sequence data of another yeast, *Y. lipolytica*, which were obtained in our laboratory at the same time.

Sequencing of the 3′-terminal portion of *S. cerevisiae FAS1* was performed using appropriate genomic and cDNA clones (Fig. 3). The latter allowed us to localize the transcriptional termination/polyadenylation site at

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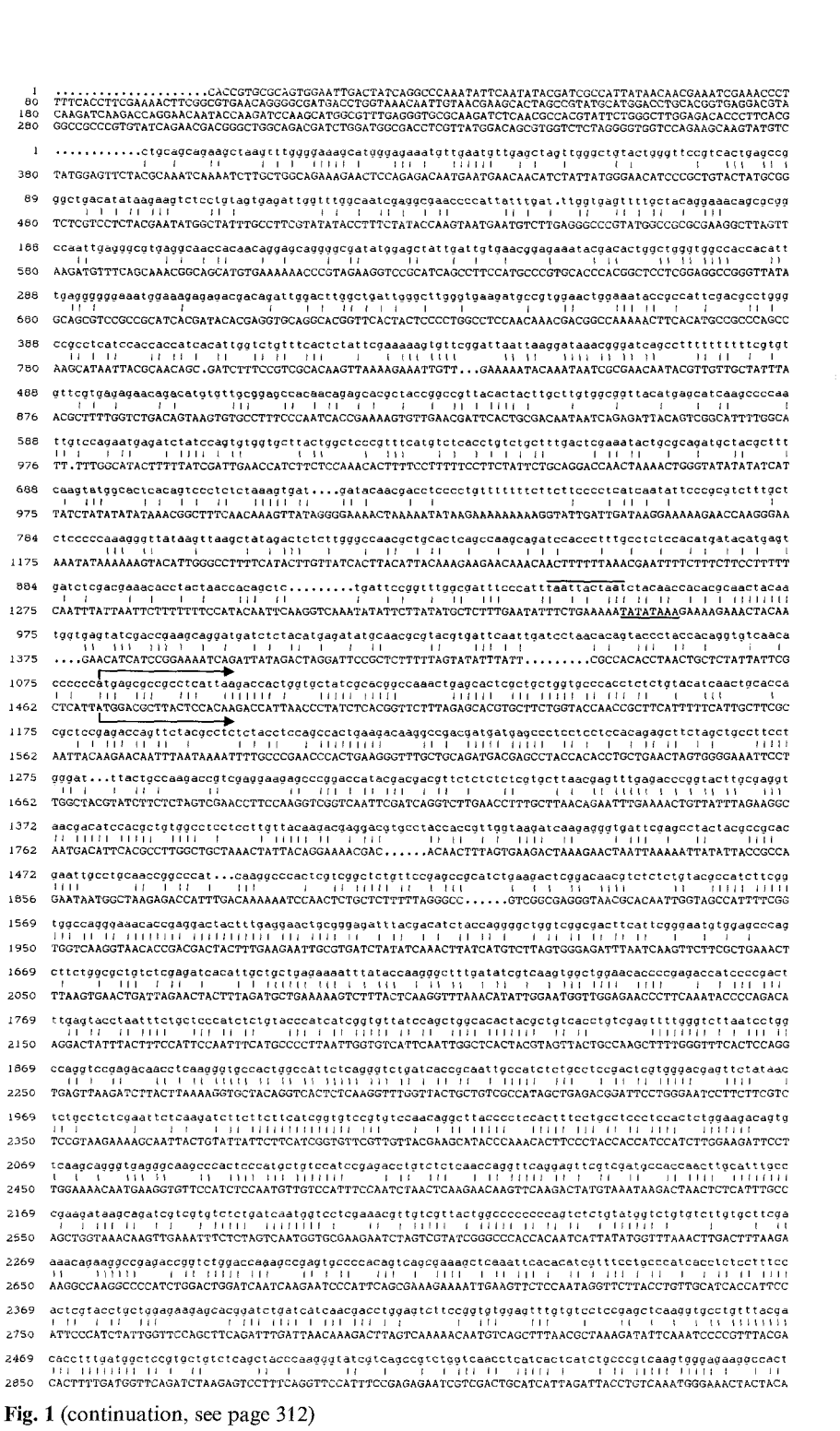

Fig. 1 (continuation, see page 312)