Abstract
Using a combination of database-mining and functional characterization, we have identified a component of the polyunsaturated fatty acid (PUFA) elongase. Co-expression of this elongating activity with fatty acid desaturases has allowed us to heterologously reconstitute the PUFA biosynthetic pathway. Both these enzymes (desaturases and elongase components) have undergone gene-duplication events which provide a paradigm for the diverged nature of PUFA biosynthetic activities.

Introduction
Unsaturated fatty acids are essential components required for normal cellular function, being involved in roles ranging from membrane fluidity to acting as signal molecules [1,2]. In particular, the class of fatty acids known as the polyunsaturated fatty acids (PUFAs) are of considerable interest on account of their potential pharmaceutical and nutraceutical roles [2,3]. PUFAs can be defined as fatty acids of 18 carbons or more in length, and containing two or more double bonds. These double bonds are inserted by specific enzymes (fatty acid desaturases), which have been the subject of much recent research [4,5]. PUFAs are also essential fatty acids, being required in the diet for normal development in mammals, which cannot synthesize the primary essential PUFA linoleic acid (18:2, n-6) [2]. More recently, interest has focused on the C_{20} fatty acid arachidonic acid (20:4, n-6), which has been shown to be involved in neonatal health, including retinal and brain development [6]. Arachidonic acid is synthesized by sequential desaturation, elongation and further desaturation of dietary 18:2, n-6 [2,5]. Although the two desaturases (Δ^6- and Δ^5- fatty acid desaturases) required for this pathway have recently been cloned from a number of different sources [7-14], no genes have been characterized for the C_{18} elongation of the C_{18} PUFA. Biochemical characterization of mammalian elongation systems (most notably from liver microsomes) has indicated that the 'elongase' actually consists of four enzyme activities, being made up of a condensing enzyme, a β-keto-reductase, a dehydrase and an enoyl reductase (reviewed in [15]).

Identification of a PUFA-elongating activity from Caenorhabditis elegans
To identify and functionally characterize potential components of a PUFA-specific elongase, we heterologously expressed a number of open reading frames (ORFs) of interest in yeast. These ORFs were identified from the PUFA-accumulating organism C. elegans [16], which was also the subject of a (now completed) genome-sequencing programme [17]. Candidate ORFs for functional characterization were selected on the basis of their potential to represent 'paralogues' of the yeast ELO1/2/3 gene family, which is involved in medium-chain fatty acid and sphingolipid elongation [18,19]. Expression of one C. elegans ORF (designated F56H11.4 by the Sanger Centre C. elegans genome project) was seen to direct the C_{18} elongation of γ-linolenic acid (18:3, n-6) to di-homo-γ-linolenic acid (20:3, n-6) [20]. Although this ORF showed a substrate preference for C_{18} Δ^5-desaturated fatty acids (either 18:3, n-6 or 18:4, n-3), other C_{18} fatty acids such as 18:2, n-6 and 18:3, n-3 were also elongated, though at a lower efficiency; no elongation of C_{20} substrates (saturated or unsaturated) was observed [20]. Interestingly, in the absence of any exogenously supplied substrates, the F56H11.4 ORF was also capable of directing the elongation of palmitoleic acid (16:1, n-7) to vaccenic acid (18:1, n-7) while not displaying any appreciable activity towards oleic acid (18:1, n-9). Thus it is apparent that this C. elegans ORF F56H11.4 displays a range of substrate specificities, although the resulting products are representative of the range of fatty acids found in this nematode worm [16]. In particular, the elongation

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Abbreviations used: PUFA, polyunsaturated fatty acid; ORF, open reading frame.

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of 16:1, n-7 to vaccenic acid may help to explain both the biosynthesis and the abundance of this unusual C\textsubscript{18} fatty acid [20].

**Reconstitution of PUFA biosynthetic pathway**

Since one of our long-term goals is the reconstitution of the C\textsubscript{20} PUFA pathway, we co-expressed the elongating activity of ORF F56H11.4 with either the \( \Delta^3\)-fatty acid desaturase [7] or the \( \Delta^2\)-fatty acid desaturase [12], as well as a combination of all three enzymes. These data clearly demonstrated that it was possible to heterologously reconstitute the PUFA biosynthetic pathway in yeast, obtaining both C\textsubscript{18} substrates [20]. This observation has important implications for the biotechnological production of C\textsubscript{20} PUFAs, though we also noted that the F56H11.4 elongating activity appeared to be unable to utilize C\textsubscript{20} substrates (as judged by a lack of any C\textsubscript{22} fatty acid products in all our experiments). Thus, the production of C\textsubscript{22} PUFAs (such as 22:6, n-3) may require an additional elongating activity.

**Genome organization of PUFA biosynthetic enzymes**

The deduced amino acid sequence of ORF F56H11.4 indicated that it is a member of a growing family of polypeptide sequences involved in lipid modification. Apart from the expected similarity to the yeast ELO1/2/3 ORFs, the mouse gene product Cig30 (and two related sequences described in [21]) are also related, though in the cases of both the yeast and mouse ORFs, they carry out different (i.e. non-PUFA) elongation reactions. Searching the entire *C. elegans* genome with F56H11.4 identifies a further seven related ORFs, which all show a number of conserved motifs (Figure 1). However, sequence analysis alone does not clarify the precise function.

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**Figure 1**

Sequence comparison of *C. elegans* ORF F56H11.4 with related sequences present in the *C. elegans* genome

Sequences are identified by their Sanger Centre notation. A number of conserved motifs are visible, including a 'histidine box' (H-X-X-H-H) and a 'triple tyrosine' motif (Y-X-Y-Y).
of ORF F56H11.4. Previous data for the FAE1 gene (which is required for the elongation of C\textsubscript{18:1} and C\textsubscript{20:1} monounsaturated fatty acids [22]) from Arabidopsis indicated that this sequence encoded a condensing enzyme, and that expression of this ORF in yeast could reconstitute the monounsaturated C\textsubscript{18:2} elongase [23]. FAE1 also shows some limited homology to other condensing enzymes, such as chalcone synthase and stilbene synthase. Unfortunately, F56H11.4 (and the related sequences found in yeast, C. elegans and mouse) shows no such homology and is unrelated to FAE1. Thus, although intuitively it is very likely that F56H11.4 encodes a condensing enzyme (based on the observed heterologous reconstitution in yeast of a PUFA elongase), this remains to be demonstrated biochemically.

Another intriguing observation is that F56H11.4 is present in the C. elegans genome as part of a tandem pair of related sequences; the ORF F56H11.3 is less than 2 kb downstream. We have observed previously that the \( \Delta^2 \) - and \( \Delta^4 \)-fatty acid desaturases from C. elegans are present as a tandem pair, separated by less than 1 kb. In both cases (desaturases and elongating activity) the genes in tandem contain conserved intron/exon junctions and are presumably the products of a gene-duplication event. In the case of the fatty acid desaturases, this duplication has resulted in the divergence of enzyme activity; it will therefore be of interest to determine the substrate(s) of the tandem ORF F56H11.3. We also noted two other C. elegans ORFs showing homology to the F56H11.4 elongating activity, present as a tandem pair (F41H10.7 and F41H10.8) less than 10 kb apart and also with conserved intron/exon junctions. Thus, four of the eight C. elegans ORFs that form a family of presumptive lipid-elongating activities have arisen as the result of gene-duplication events. It is tempting to speculate that these duplication events are not just re-iteration of a sequence generating functional redundancy, rather that they are responsible for the evolution of new enzyme activities (as seen in the case of the \( \Delta^3 \)- and \( \Delta^5 \)-fatty acid desaturases [12]).

**Conclusions**

We have identified a C. elegans component of the PUFA elongase via functional expression in yeast. Co-expression of this ORF with fatty acid desaturases allowed us to reconstitute the PUFA biosynthetic pathway. This elongating activity (F56H11.4) is a member of a family of C. elegans ORFs, several of which appear to have undergone gene-duplication events. We hypothesize that these gene duplications have driven the evolution of diversified enzyme activities while simultaneously allowing the proliferation of a number of conserved amino acid motifs.

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**References**


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