Activity and mRNA abundance of Δ-5 and Δ-6 fatty acid desaturases in two human cell lines

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Abstract We analyzed fatty acid biosynthesis in Chang and ZR-75-1 cells. Both cell lines could desaturate and further elongate substrates for Δ-5 desaturase. ZR-75-1 but not Chang cells showed Δ-6 desaturation of 18:2*n*-6, 18:3*n*-3, 24:4*n*-6 and 24:5*n*-3. In both cell lines, the mRNA abundance can be related to Δ-5 or Δ-6 fatty acid desaturase activities. These results suggest that desaturase genes could have, at least in part, independent control mechanisms and that Δ-6 desaturase impairment is not specific to any particular step of the fatty acid metabolic pathways, which may diminish the rationale for the existence of at least two distinct enzymes.

Key words: Desaturase; Elongation; Gene expression; Polyunsaturated fatty acid; Chang; ZR-75-1; Essential fatty acid metabolism

1. Introduction

In mammalian cells, polyunsaturated fatty acids (PUFAs) are synthesized from essential fatty acid precursors, 18:2*n*-6, linoleic acid, and 18:3*n*-3, α-linolenic acid, through a microsomal Δ-6 desaturation that yields 18:3*n*-6 and 18:4*n*-3, and a subsequent elongation to 20:3*n*-6 and 20:4*n*-3, respectively. These fatty acids are then substrates of a Δ-5 desaturase that generates 20:4*n*-6 and 20:5*n*-3 which are further elongated twice to 24:4*n*-6 and 24:5*n*-3, respectively. In recent years, Sprecher and co-workers have shown that a second microsomal Δ-6 desaturation takes place in the PUFA synthesis on 24:4*n*-6 and 24:5*n*-3 [1]. The products of this desaturation, 24:5*n*-6 and 24:6*n*-3, are converted to 22:5*n*-6 and 22:6*n*-3, respectively, by a peroxisomal β-oxidation [1,2]. Most of the enzymology of these metabolic pathways has been studied, although the genes involved are only beginning to come to light. Recently, the cloning and expression of mammalian Δ-6 and Δ-5 desaturases have been described [3–5]. In fact, three genes encoding membrane-anchored proteins from the human fatty acid desaturase family have been characterized within human chromosomal band 11q12–q13.1. Their expression profile has also been assessed in various human tissues [6]. The regulation of these desaturases, in particular Δ-6 desaturase, has been explored from different angles and found to be associated with hormones, diet, and age [7]. Recent reports have also proposed that certain activators (i.e. Wy14643) of the transcription factor peroxisome proliferator-activated receptor isoform α coordinately induce the transcription and mRNA abundance of both Δ-5 and Δ-6 desaturases [8].

Results from Cho et al. [4,5] have shown that enzyme activity does not always consistently correlate with the quantity of Δ-6 desaturase mRNA in different human tissues, i.e. brain and liver. Therefore, in order to explore further the relationship between enzyme activity and gene expression in human cells, this study was designed to assess and compare the ability of two human cell lines to metabolize *n*-3 and *n*-6 PUFAs and to correlate enzymatic activity with mRNA presence for Δ-5 and Δ-6 desaturases.

2. Material and methods

2.1. Cell culture

ZR-75-1 (human mammary carcinoma) and Chang (human adult liver) cell lines (American Type Culture Collection, Rockville, MD, USA) were maintained in Dulbecco's modification of Eagle's medium (DMEM) containing 10% fetal bovine serum in a humidified atmosphere of 95% air and 5% carbon dioxide at 37°C. Cell culture media and supplements were obtained from Gibco BRL (Grand Island, NY, USA).

2.2. Incubation with radiolabelled fatty acids

Both human cell line cultures were supplemented with fatty acids as previously described [9]. These conditions were selected based on our previous experiments in which optimum fatty acid uptake, desaturation, and elongation were detected in mammalian cells in culture [10]. Briefly, 3×10^5 cells were plated in 35 mm Petri dishes and allowed to grow for 24 h. At approximately 90% confluence, the medium was removed and the cell monolayers were rinsed twice with fresh serum-free DMEM medium. Cells were then incubated in DMEM medium containing 0.27% fatty acid-free bovine serum albumin (BSA, Sigma Chemical Company, St. Louis, MO, USA) for 18 h prior to the addition of fatty acids. [1-^{14}C]Linoleic acid (18:2*n*-6; specific activity (s.a.) 53 mCi/mmol), [1-^{14}C]α-linolenic acid (18:3*n*-3; s.a. 52 mCi/mmol) and [1-^{14}C]dihomo-γ-linolenic acid (20:3*n*-6; s.a. 52 mCi/mmol) were purchased from NEN Research Products (Du Pont, Boston, MA, USA). The radiolabelled and unlabelled fatty acids (Sigma), pre-dissolved in ethanol, were diluted in DMEM medium containing 0.27% BSA and immediately added to the Petri dishes to give a fatty acid concentration of 60 μM. The incubation volume was 1.5 ml with a final ethanol concentration of 0.1% and total radioactivity of 0.25 μCi. At different time intervals, between 0.5 and 24 h, the cell monolayer was washed three times with phosphate-buffered saline and the

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Abbreviations: s.a., specific activity; PUFA, polyunsaturated fatty acid; fatty acids are designated with a number that refers to the number of carbons in the chain; the number after the colon indicates the number of double bonds and the *n* refers to the location of the nearest double bond from the methyl end

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cells were harvested using a rubber policeman. In separate experiments, [1-^{14}C]24:4*n*-6 and [1-^{14}C]24:5*n*-3 (s.a. 55 mCi/mmol for both fatty acids; ARC, St. Louis, MO, USA) were incubated for 12 h at a final concentration of 60 μM or 2.8 μM with a total radioactivity of 0.25 μCi.

Separate dishes of cells were treated under the same conditions with 60 μM unlabelled fatty acid and used for protein determination [11].

2.3. Extraction and lipid analysis

Lipids were extracted from harvested cells and the pooled media and washings, according to the method of Folch et al. [12]. Aliquots of the total lipid extracts were taken for radioactivity determination, whereas other aliquots were methylated using BF_3–methanol. The radiolabelled fatty acid methyl esters (FAME) were analyzed as previously described [13] using a Hewlett Packard (1090, series II) chromatograph equipped with a diode array detector set at 205 nm, a radioisotope detector (model 171, Beckman, Fullerton, CA, USA) with a solid scintillation cartridge (97% efficiency for ^{14}C detection) and a reverse-phase ODS (C-18) Beckman column (250 mm×4.6 mm i.d.; 5 μm particle size) attached to a pre-column with a μBondapak C-18 (Beckman) insert. FAME were separated isocratically with acetonitrile/water (95:5 v/v) at a flow rate of 1 ml/min and were identified by comparison with authentic standards.

2.4. RNA extraction and Northern blot analysis

RNA was isolated from both human cell lines using Trizol® (Gibco BRL, Gaithersburg, MD, USA) based on the method of Chomczynski and Sacchi [14]. Total RNA (5 μg) from each cell line was size-fractionated in a 1.0% formaldehyde agarose gel. The gel was treated with 50 mM NaOH and 100 mM Tris–HCl, pH 7.0 each for 20 min before equilibration in 20×SSC for 30 min. The Zeta-Probe® blotting membrane was prepared according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA) and the RNA was transferred overnight at room temperature with 10×SSC. Both UV cross-linking and baking at 80°C for 60 min fixed the RNA to the membrane. The abundance of each transcript of interest was determined by sequential hybridization using specific cDNA probes corresponding to nucleotide positions 722–1173 and 262–1006 downstream of the ATG for human Δ-5 and Δ-6 desaturase respectively [4,5], and from nucleotide positions 71–1053 for the human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe (Clontech, Palo Alto, CA, USA). The probes were radiolabelled by random priming with [α-^{32}P]dCTP (NEN Life Science Products, Boston, MA, USA) using DNA Labeling Beads (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and the unincorporated nucleotides removed by exclusion chromatography using MicroSpin™ Sephadex G-25 columns according to the supplier's protocol (Amersham Pharmacia Biotech). Prehybridization and hybridization were conducted at 42°C and the membrane was washed under high stringency conditions (0.1×SSC at 65°C). The membrane was then exposed to Kodak BioMAX MS film (Kodak, Rochester, NY, USA) for an autoradiographic analysis.

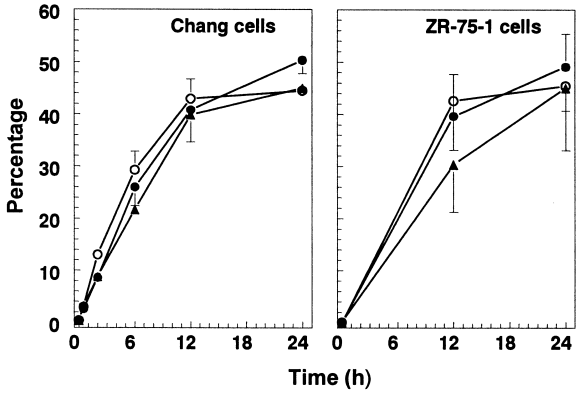

Fig. 1. Percent of radioactivity recovered in Chang and ZR-75-1 cells during a 24 h incubation with 60 μM of radiolabelled polyunsaturated fatty acids: [1-^{14}C]18:2*n*-6 (●), [1-^{14}C]20:3*n*-6 (○) and [1-^{14}C]18:3*n*-3 (▲). Data are the mean ± S.D. of at least four determinations.

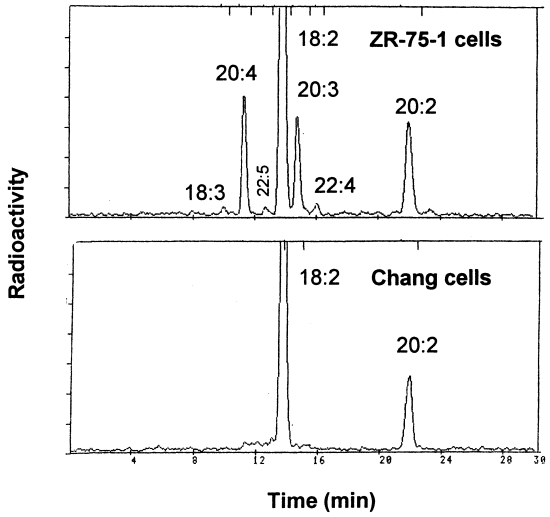

Fig. 2. HPLC radiochromatograms of FAME obtained from total lipids of Chang and ZR-75-1 cells incubated with [1-^{14}C]18:2*n*-6 acid for 24 h.

3. Results

Fig. 1 shows that the uptake of 18:2*n*-6, 20:3*n*-6, or 18:3*n*-3 was monophasic and the maximum labelling, achieved within 12 h of incubation with all of these PUFAs, was quantitatively similar in either Chang or ZR-75-1 cells. The detection of desaturation and elongation to higher homologues of radiolabelled 18:2*n*-6, 20:3*n*-6, or 18:3*n*-3 increased linearly with time in both cell lines (data not shown). Two selected radiochromatograms (Fig. 2) of *n*-6 metabolites obtained after a 24 h incubation with [1-^{14}C]18:2*n*-6 show the capability of ZR-75-1 cells to produce 18:3*n*-6 through a Δ-6 desaturation system. This fatty acid was elongated to 20:3*n*-6 which was further desaturated to 20:4*n*-6 by Δ-5 desaturase and then elongated to 22:4*n*-6. Two of the higher homologues of this fatty acid (i.e. 24:4*n*-6 and 24:5*n*-6) and the final product in the pathway, 22:5*n*-6, were not detected. The direct elongation metabolite of 18:2*n*-6 (i.e. 20:2*n*-6) was also produced. Conversely, Chang cells were only able to elongate 18:2*n*-6 without any Δ-6 desaturation. Furthermore, no desaturation products were detected in the supernatant at this or at any other time point (data not shown).

A comparative percent distribution of radioactivity among *n*-6 and *n*-3 metabolites after 12 h of incubation with either [1-^{14}C]18:2*n*-6, [1-^{14}C]18:3*n*-3, or [1-^{14}C]20:3*n*-6 expands these findings (Tables 1, 2 and 5). In Chang cells, the percent of radioactivity recovered in 18:2*n*-6 was 1.5-fold higher than in ZR-75-1 cells (Table 1). The direct elongation product, 20:2*n*-6, with a 1.2-fold higher percentage of radioactivity to that detected in ZR-75-1 cells, made up the balance. [1-^{14}C]18:3*n*-6 was not detected at this time point nor in the 0.5 or 6 h incubations. In ZR-75-1 cells, the highest percentage of radioactivity in *n*-6 metabolites was found in 20:4*n*-6.

Similar results were observed when both cell lines were incubated with [1-^{14}C]18:3*n*-3 (Table 2). In Chang cells, most of the radioactivity remained in the fatty acid precursor, however, the balance was made up by elongation products (i.e. 20:3*n*-3 and 22:3*n*-3) and a 20:4*n*-3 isomer at a slightly higher percentage than that observed with 18:2*n*-6. In ZR-75-1 cells,

Table 1
Percent of radioactivity recovered in *n*-6 desaturation and elongation metabolites of [1-^{14}C]18:2*n*-6 incubated with Chang and ZR-75-1 cells for 12 h

Cells	Distribution of radioactivity (%)					
	18:2	18:3	20:2	20:3	20:4	22:4
Chang	88.2 ± 0.2	ND	11.8 ± 0.2	ND	ND	ND
ZR-75-1	61.1 ± 1.4	2.1 ± 0.2	9.2 ± 0.5	10.1 ± 0.4	13.7 ± 0.6	2.8 ± 0.5

Values are the mean ± S.D. of at least three determinations. ND: not detected.

Table 2
Percent of radioactivity recovered in *n*-3 desaturation and elongation metabolites of [1-^{14}C]18:3*n*-3 incubated with Chang and ZR-75-1 cells for 12 h as described in Section 2

Cells	Distribution of radioactivity (%)						
	18:3	18:4	20:3	20:4*	20:5	22:3	22:5
Chang	83.9 ± 1.9	ND	7.2 ± 0.5	3.0 ± 0.2	ND	2.8 ± 0.3	ND
ZR-75-1	55.3 ± 4.1	2.4 ± 0.4	9.1 ± 0.6	11.4 ± 0.5	13.9 ± 3.9	ND	4.6 ± 0.5

Values are the mean ± S.D. of at least three determinations. ND: not detected. * indicates $\Delta^{5,11,14,17}$20:4 and/or $\Delta^{8,11,14,17}$20:4.

the distribution of radioactivity was almost identical to that detected when these cells were incubated with [1-^{14}C]18:2*n*-6. The reverse-phase HPLC analysis of PUFAs did not allow us to distinguish between the 20:4*n*-3 isomers (i.e. $\Delta^{5,11,14,17}$20:4 and $\Delta^{8,11,14,17}$20:4). Thus, the ratio of these fatty acids produced in ZR-75-1 cells remains unknown.

The Δ-6 desaturase activity in the 'Sprecher pathway' [2] was also analyzed in both cell lines using custom-made radiolabelled substrates. Chang cells were unable to desaturate 24:4*n*-6 or 24:5*n*-3 to 24:5*n*-6 and 24:6*n*-3, respectively, at either of the test concentrations (Tables 3 and 4). Conversely, in ZR-75-1 cells, 18.1% of 24:5*n*-6 and 15.8% of 24:6*n*-3 were produced from 24:4*n*-6 and 24:5*n*-3, respectively, within 12 h incubation (Tables 3 and 4). In spite of maximizing the detection of the radiolabelled products by increasing the specific activity of the substrate by 20 times (when the total concentration was 2.8 μM), no major differences were observed in the radiolabelled profiles in these two cell lines. The radiochromatograms also showed peaks that correspond to 14:0, 16:0, and 18:1 which may be de novo synthesis products of endogenous fatty acids and radiolabelled acetate released by partial β-oxidation of 24:4*n*-6 and 24:5*n*-3 as suggested elsewhere [15–17]. In spite of the similar retention times of 22:5*n*-6 and 14:0 under these HPLC conditions, the presence of 22:5*n*-6 in Chang cells was ruled out since the intermediate metabolite, 24:5*n*-6, was not detected as it was in ZR-75-1 cells.

When Chang cells were incubated with [1-^{14}C]20:3*n*-6, a higher percentage of radioactivity was detected in the direct elongation metabolite, 22:3*n*-6, than was in the ZR-75-1 cells (Table 5). Both cell lines were able to convert 20:3*n*-6 to 20:4*n*-6 by Δ-5 desaturase, followed by two successive rounds of elongation.

The Northern blots shown in Fig. 3 clearly indicate the significant presence of both fatty acid Δ-5 and Δ-6 desaturase transcripts in the ZR-75-1 cell line. Chang liver cells, on the other hand, expressed only the Δ-5 desaturase. The integrity and amount of RNA loaded on the gel was confirmed using the human GAPDH control probe.

4. Discussion

The uptake of PUFAs by these two cell lines was consistent with that observed in other mammalian cells in vitro [9,18]. The possibility of a different metabolic fate for each PUFA in Chang or ZR-75-1 cells due to a differential intracellular fatty acid concentration is unlikely since all the fatty acids tested showed similar uptake in both cell lines. As well, the fatty acid supplementation in the medium did not affect cell number (approximately 95% of cells were alive) nor the protein content at any time point (data not shown).

Our findings in Chang cells showing a lack of Δ-6 desaturase activity for 18:2*n*-6, 18:3*n*-3, 24:4*n*-6, and 24:5*n*-3 and a significant capability to produce 20:4*n*-6 by a Δ-5 desaturase system support and expand, although not completely, earlier reports. Indeed, the lack of Δ-6 desaturation on 18:2*n*-6 has been described in several malignant cells in culture and in microsomes from human tumors [19,20]. However, the direct or indirect test of Δ-6 desaturase activity on substrates such as 24:4*n*-6 and 24:5*n*-3 has been confined to rat liver hepatocytes and microsomes [1,16], rat seminiferous tubules [21], or rat testis cells [17]. It has also been reported that Chang cells as well as other cell lines (LM and CHO) were not able to produce higher homologue PUFAs from 18:2*n*-6 in spite of the presence of Δ-5 desaturation [22]. Nevertheless, our data point to an alternative explanation of PUFA metabolism in Chang

Table 3
Percent of radioactivity recovered in *n*-6 and other fatty acids of Chang and ZR-75-1 cells after a 12 h incubation with [1-^{14}C]24:4*n*-6

Cells	Distribution of radioactivity (%)						
	24:4	24:5	22:5	14:0	16:0	18:1	unknown
Chang	40.1 ± 2.8	ND	ND	17.6 ± 0.3	15.6 ± 2.5	26.7 ± 0.2	ND
ZR-75-1	20.8 ± 1.4	18.1 ± 1.6	10.6 ± 0.8*	–	16.7 ± 0.8	17.3 ± 1.7	16.5 ± 3.1

Values are the mean ± S.D. of at least three determinations. ND: not detected. * may represent the sum of 22:5*n*-6 and 14:0, since both FAME have similar retention times under the present HPLC conditions.

Table 4
Percent of radioactivity recovered in *n*-3 and other fatty acids of Chang and ZR-75-1 cells after a 12 h incubation with [1-^{14}C]24:5*n*-3

Cells	Distribution of radioactivity (%)					
	24:5	24:6	14:0	16:0	18:1	unknown
Chang	33.8 ± 2.3	ND	19.6 ± 1.6	16.4 ± 0.9	30.2 ± 1.5	ND
ZR-75-1	18.9 ± 3.1	15.8 ± 1.3	12.5 ± 0.8	19.3 ± 1.8	18.7 ± 1.3	7.8 ± 1.4

Values are the mean ± S.D. of at least three determinations. ND: not detected.

Table 5
Percent of radioactivity recovered in *n*-6 desaturation and elongation metabolites of [1-^{14}C]20:3*n*-6 incubated with Chang and ZR-75-1 cells for 12 h

Cells	Distribution of radioactivity (%)				
	20:3	20:4	22:3	22:4	24:4
Chang	71.1 ± 0.7	17.9 ± 0.5	4.0 ± 0.5	5.9 ± 0.3	Tr
ZR-75-1	70.8 ± 0.7	15.2 ± 0.3	2.03 ± 0.3	4.5 ± 0.4	5.7 ± 0.6

Values are the mean ± S.D. of at least three determinations. Tr: traces.

cells to that previously reported by Maeda et al. regarding the activities of both Δ-6 and Δ-5 desaturases on 18:3*n*-3 and 20:4*n*-3 (i.e. $\Delta^{8,11,14,17}$20:4), respectively [22]. These authors, using a combination of glass column gas chromatography and $AgNO_3$–silica gel thin layer plates found that the radioactivity from 18:3*n*-3 was distributed mainly in 20:3*n*-3 and 20:4*n*-3 (i.e. $\Delta^{8,11,14,17}$20:4) and slightly in 18:4*n*-3 indicating the presence of Δ-6 desaturase and the absence of Δ-5 desaturase activity for the *n*-3 PUFA series. Conversely, our data suggest that Chang cells cannot desaturate 18:3*n*-3 directly, but are able to elongate and further desaturate it to 20:3*n*-3 and then 20:4*n*-3 (i.e. $\Delta^{5,11,14,17}$20:4), indicating the absence of Δ-6 desaturase and the presence of Δ-5 desaturase activity for the *n*-3 PUFA series.

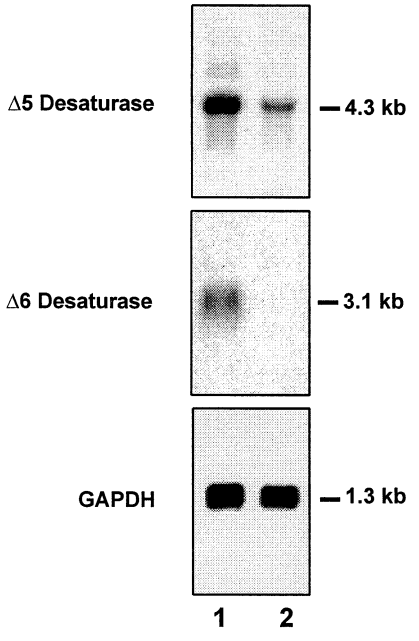

Fig. 3. mRNA expression of human Δ-5 and Δ-6 desaturase genes in (1) ZR-75-1 and (2) Chang cells by Northern blots. Each lane contains 5 μg of total RNA. Δ-5 and Δ-6 desaturase mRNA transcripts are shown at 4.3 and 3.1 kb, respectively. GAPDH was used as an internal RNA loading control.

We base the interpretation of our results on the fact that we were not able to detect 18:4*n*-3, even though under the same experimental conditions the chromatograms for ZR-75-1 cells showed a well defined peak identified as this fatty acid. Therefore, without 18:4*n*-3, no 20:4*n*-3 (i.e. $\Delta^{8,11,14,17}$20:4) could be produced, which is the substrate for Δ-5 desaturase in the standard pathway. As a result of these two observations, our data suggest that the isomer of 20:4*n*-3 in the chromatograms of Chang cells, produced by the Δ-5 desaturation of 20:3*n*-3, was $\Delta^{5,11,14,17}$20:4. In addition, the inability of Chang cells incubated with 24:5*n*-3 to synthesize the final *n*-3 metabolic product, 22:6*n*-3 (Table 4), is in good agreement with previous experiments which did not detect this fatty acid even when the cells were supplemented with 50 μM radiolabelled 20:5*n*-3 [22]. In fact, only a small number of cell lines, such as hepatocyte, astrocyte, retinoblastoma, and T-cell leukemia, show the capability to synthesize 22:6*n*-3 [23].

Overall, our studies suggest that alterations in fatty acid desaturase activities are not specific to any fatty acid series. Indeed, in Chang cells, the lack of activity on either *n*-6 substrate (i.e. 18:2*n*-6 or 24:4*n*-6) correlates with a corresponding lack of activity on its equivalent fatty acid substrates in the *n*-3 series (18:3*n*-3 or 24:5*n*-3). Data suggest that Δ-6 desaturase impairment in this cell line is not specific to any particular step of the PUFA metabolic pathways, which may diminish the rationale for the existence of at least two distinct enzymes [23]. To date, there is no definite proof that the Δ-6 desaturase which recognizes 24-carbon substrates is different from the enzyme that desaturates 18:2*n*-6 or 18:3*n*-3 [24]. In fact, only three human fatty acid desaturase genes have been cloned and according to Marquardt et al. the gene that has not yet been ascribed to a function is unlikely to represent a second human Δ-5 or Δ-6 desaturase [6]. Our data, however, do not rule out the possibility of the existence of a second Δ-6 desaturase which is regulated in the same fashion as the one described herein.

The use of two cell models with distinct PUFA metabolism allowed us to find a direct and positive correlation between mRNA presence and enzyme activity for Δ-5 and Δ-6 desaturases. The sizes of the transcripts at 4.3 kb and 3.1 kb, respectively, agree well with those reported earlier in human tissues [4–6]. Our findings in Chang cells showing the lack of Δ-6

desaturase activity for any substrate and a corresponding absence of mRNA, but, with significant Δ-5 desaturase activity and its mRNA, are the first to suggest that Δ-5 and Δ-6 desaturase genes have, at least in part, independent control mechanisms. However, our data are not sufficient to distinguish between direct transcriptional control or RNA transcript stability.

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