FEBS Letters 576 (2004) 320–324 FEBS 28857

Identification of genes regulated by oleic acid in Jurkat cells by suppressive subtractive hybridization analysis

Thais Martins de Lima[a], Leonardo de Oliveira Rodrigues[b], Mario Henrique Bengtson[b], Mari Cleide Sogayar[b], Camila Nogueira Alves Bezerra[a], Nancy Amaral Rebouças[a], Rui Curi[a,*]

[a]*Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, Av. Professor Lineu Prestes, 1524, Butantã, São Paulo, SP 05508-900, Brazil*

[b]*Department of Biochemistry, Chemistry Institute, University of São Paulo, Av. Professor Lineu Prestes, 748, Butantã, São Paulo, SP 05508-900, Brazil*

Received 15 June 2004; revised 20 August 2004; accepted 6 September 2004

Available online 25 September 2004

Edited by Lukas Huber

Abstract In this study, the effect of oleic acid (50 μM) on gene expression of Jurkat cells (human T lymphocytes cell line) was examined using the suppressive subtractive hybridization approach. This technique allowed us to identify genes with higher or lower expression after cell treatment with oleic acid as compared to untreated cells. Oleic acid upregulated the expression of the translation elongation factor alpha 1 and ATP synthase 8 and downregulated gp96 (human tumor rejection antigen gp96), heat-shock protein 60 and subtilisin-like protein 4. These results suggest that oleic acid, at plasma physiological concentration, can regulate the expression of important genes to maintain the machinery that ensures cell functioning.

Keywords: Gene expression; Jurkat cell; Lymphocyte; Oleic acid; Subtractive hybridization

1. Introduction

Both the amount and the type of dietary fat play a role in the health of individuals. Consumption of lipids with a high proportion of saturated fatty acids (SFA) has been associated with increased risk of developing coronary heart disease, as shown by the Seven Country Study [1]. Subsequent studies showed that replacement of SFA with either mono- or polyunsaturated fatty acids (PUFA or MUFA) might be beneficial [2,3]. The Mediterranean diet has been widely recognized as a model for a healthy diet, since these countries display the lowest rate of chronic heart diseases [4]. This diet is characterized by a high consumption of olive oil, which is rich in oleic acid, a MUFA. There has been great interest in the effects of oleic acid on lipoprotein metabolism and atherosclerosis [3]. Although cells of the immune system are an inherent part of the inflammatory events involved in the development and progression of atherosclerosis, less attention has been paid to the effects of these fatty acids (FA) on leukocytes.

Olive oil has classically been used as placebo in studies investigating the effects of fish oils on immune function, since MUFA has typically been regarded as being neutral [5,6]. However, some clinical trials have reported the effects of olive oil treatment that are equal or similar to the effects of fish oil treatment [7–9]. Rats fed with a diet rich in oleic acid present low rate of proliferation of mesenteric lymph node lymphocytes [10,11], suppressed NK cell activity [12] and low expression of the adhesion molecules CD2, ICAM-1 and LFA-1 [13]. Similar results were obtained in human studies [14,15].

Several mechanisms have been proposed to explain FA modulation of the immune response. Although some of the effects may be brought about by modulation of the amount and type of eicosanoids produced, FA also elicit some of their effects by arachidonic acid-independent mechanisms, including actions upon intracellular signaling pathways and transcription factor activity [16,17]. In addition, FA are also known to regulate gene expression [18–20]. Therefore, the beneficial effects of the Mediterranean diet may be related to changes in gene expression induced by oleic acid.

In order to investigate oleic acid-regulated genes, Jurkat cells (a human T-lymphocyte cell line) were treated with this FA in a concentration within the plasma physiological range (50 μM), for a sufficient exposure time (24 h) and subjected to the suppressive subtractive hybridization (SSH) technique to isolate genes whose expression was regulated by oleic acid treatment. In SSH, differentially expressed sequences are enriched and the concentration of high- and low-abundance sequences is equalized. SSH is a highly efficient and widely used polymerase chain reaction (PCR)-based method for identifying differentially expressed genes [21–24].

2. Material and methods

2.1. Chemicals

Cell culture medium, antibiotics, fetal calf serum and TRIzol reagent were obtained from Invitrogen (Grand Island, NY, USA). All other reagents were obtained from Sigma (St. Louis, MO).

2.2. Cell culture conditions

Jurkat (human T-lymphocytes) cells were obtained from the Dunn School of Pathology (Oxford University, UK). Cells maintained in log-phase growth at 37 °C and 5% CO_2 humidified atmosphere were grown

* Corresponding author. Fax: +55-11-3091-7245.
E-mail address: ruicuri@fisio.icb.usp.br (R. Curi).

Abbreviations: EF-1α, translation elongation factor 1 alpha; EFA, essential fatty acids; FA, fatty acid; MUFA, monounsaturated fatty acid; NK, natural killer; PCR, polymerase chain reaction; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acids; SPC4, subtilisin-like protein 4

0014-5793/$22.00
doi:10.1016/j.febslet.2004.09.025

in RPMI-1640 medium supplemented with 10% fetal calf serum, 20 mM HEPES, 10 U/ml ampicillin and 10 μg/ml streptomycin. The cell population was maintained at densities between 1×10^5 and 1×10^6 cells/ml.

2.3. Cell treatment

Cells were diluted to 2×10^5 cells/ml in 75 cm^3 bottles. On the following day, when the number of cells reached 4×10^5 cells/ml, oleic acid, diluted in ethanol, was added to the final concentration of 50 μM. Previous study has shown that higher concentrations of oleic acid are toxic to Jurkat cells [25]. Control cells were treated with the same amount of ethanol, which was always lower than 0.05%. This concentration of ethanol has shown to be non-toxic for the cells. The cells were cultured with the FA at 37 °C and 5% CO_2 humidified atmosphere for 24 h.

2.4. RNA extraction

Total RNA from control (untreated) and oleic acid-treated cells was extracted using TRIzol reagent. Poly(A)$^+$ RNA was isolated using the QuickPrep™ mRNA Purification Kit* (Amersham Biociences, Freiburg, Germany).

2.5. Suppression subtractive hybridization (SSH) and cDNA library construction

cDNA synthesis and SSH were carried out using the PCR-Select™ cDNA Subtraction Kit (CLONTECH Laboratories, California, USA) with the modified protocol of Rebrikov et al. [26]. Two micrograms of poly(A)$^+$ RNA from control and oleic acid-treated cells was used for cDNA synthesis. For the forward subtraction, cDNA from control cells was used as the driver and cDNA from treated cells was used as the tester. The opposite was used for the reverse subtraction. The forward subtraction was performed to isolate genes whose expression increased after the treatment. The reverse library contains genes whose expression is decreased upon treatment. After digestion with *Rsa*I, tester cDNA preparations were divided into two subpopulations, which were annealed to different adaptors. The two sub-populations were then hybridized with an excess amount of driver cDNA, after which they were combined and hybridized again in the presence of driver cDNA, without denaturing the DNA before the second hybridization. Following the second hybridization, two PCR rounds were performed to enrich and amplify the differentially expressed sequences.

The PCR products obtained from the forward and the reverse subtractions were cloned into the pUC18 plasmid (Amersham Biociences, Freiburg, Germany). Randomly selected clones were arrayed in 96 well microtiter dishes with 200 μl Luria–Bertrani broth containing ampicillin (100 μM/ml) and grown overnight on a shaker.

2.6. Reverse Northern blotting

One microliter of the bacterial cultures was used as the template in PCRs and the products were spotted onto nylon membranes using the Exprecision™ DNA Arraying System (Bioinformática, Campinas, Brazil). Reverse Northern blotting was performed with [^{32}P]dATP-labeled subtracted cDNA from both forward and reverse subtractions.

The arrayed membranes were pre-hybridized at 42 °C for 3 h in hybridization solution (50% formamide, 6× SSC, 5× Denhardt's solution, 1% SDS, 0.5 μg/ml poly-dA and 0.5μg/ml Cot1-DNA), after which the denatured probe was added to the solution and hybridized to the membranes at 42 °C overnight. Following hybridization, the membranes were washed twice in 2× SSC/1% SDS at room temperature for 15 min, twice in 0.1× SSC/0.5% SDS at 65 °C for 20 min, and once in 0.1× SSC/0.5% SDS at 68 °C for 15 min. After the final wash, the membranes were exposed to Phosphoimager screens (Molecular Dynamics Inc., Sunnyvale, CA) for 5–7 days.

2.7. Data analysis

Hybridization signals were detected and analyzed using a Phosphoimager scanner and the ImageQuanNT software program (Molecular Dynamics).

2.8. Northern blot hybridization

cDNAs displaying differential expression between control and oleic acid-treated cells were identified and the results were confirmed by Northern blotting. Twenty micrograms of total RNA from control and oleic acid-treated cells were fractionated by electrophoresis through agarose-formaldehyde gels and blotted onto nylon membranes as previously described [27]. The PCR products derived from the selected clones and from the β-actin gene were labeled with [α-^{32}P]dATP and used as probes to hybridize with the nylon membranes. Hybridization and data analysis were carried out as described before.

2.9. DNA sequencing

Sequencing of the cDNA clones was performed with the M13 primer, using an ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Perkin–Elmer Corp., Foster City, CA) and an ABI 310 sequencer (Perkin–Elmer).

3. Results

3.1. Screening of the recombinant plasmids obtained after forward and reverse cDNA subtractions

The PCR products obtained from the forward and the reverse subtractions were cloned into pUC18 plasmids. We obtained 14 microtiter (96 well) dishes of clones from the forward subtraction and 16 microtiter dishes of clones from the reverse subtraction, corresponding to 1344 genes whose expression increased and 1536 genes whose expression decreased after oleic acid treatment. Due to the possible presence of the so-called "false positive" clones, recombinant plasmids were first submitted to a primary screening by reverse Northern blotting.

All PCR products were immobilized in nylon membranes using the Exprecision™ DNA Arraying System (Bioinformática, Campinas, Brazil). We constructed two distinct membranes with the forward subtraction clones and two other membranes with the reverse subtraction clones. All clones were spotted in duplicate. An exact copy of each membrane was generated to allow simultaneous hybridization with [^{32}P]dATP-labeled subtracted cDNA probes from both the forward and the reverse subtractions (Fig. 1).

The signal intensities of all PCR products were analyzed and only those with a 5-fold difference between forward and reverse probe hybridization were considered as candidates to be regulated by oleic acid treatment. Similar protocols were used by others [27,28]. Only 36 clones (2.7%) from the forward subtraction and 25 (1.6%) from the reverse subtraction presented the 5-fold signal difference. Most clones obtained were "false positives".

3.2. Northern blot analysis

All clones selected by the reverse Northern blotting methodology were PCR-amplified and the products were labeled with [^{32}P]dATP. These probes were used to hybridize to nylon membranes that contained 20 μg of total RNA from Jurkat cells treated with ethanol (control) or with oleic acid. The β-actin gene was also amplified and used as a control housekeeping gene.

The signal intensities were normalized in comparison with the β-actin gene signal and only clones B4/R15, E10/F9, F5/R10, G9/F14 and H11/R13 had their expression significantly altered (over 50%) after the treatment with oleic acid. These results are presented in Fig. 2.

The sequences of these clones were determined using automated cycle sequencing and compared with previously identified genes deposited in the GenBank. Table 1 shows the results obtained.

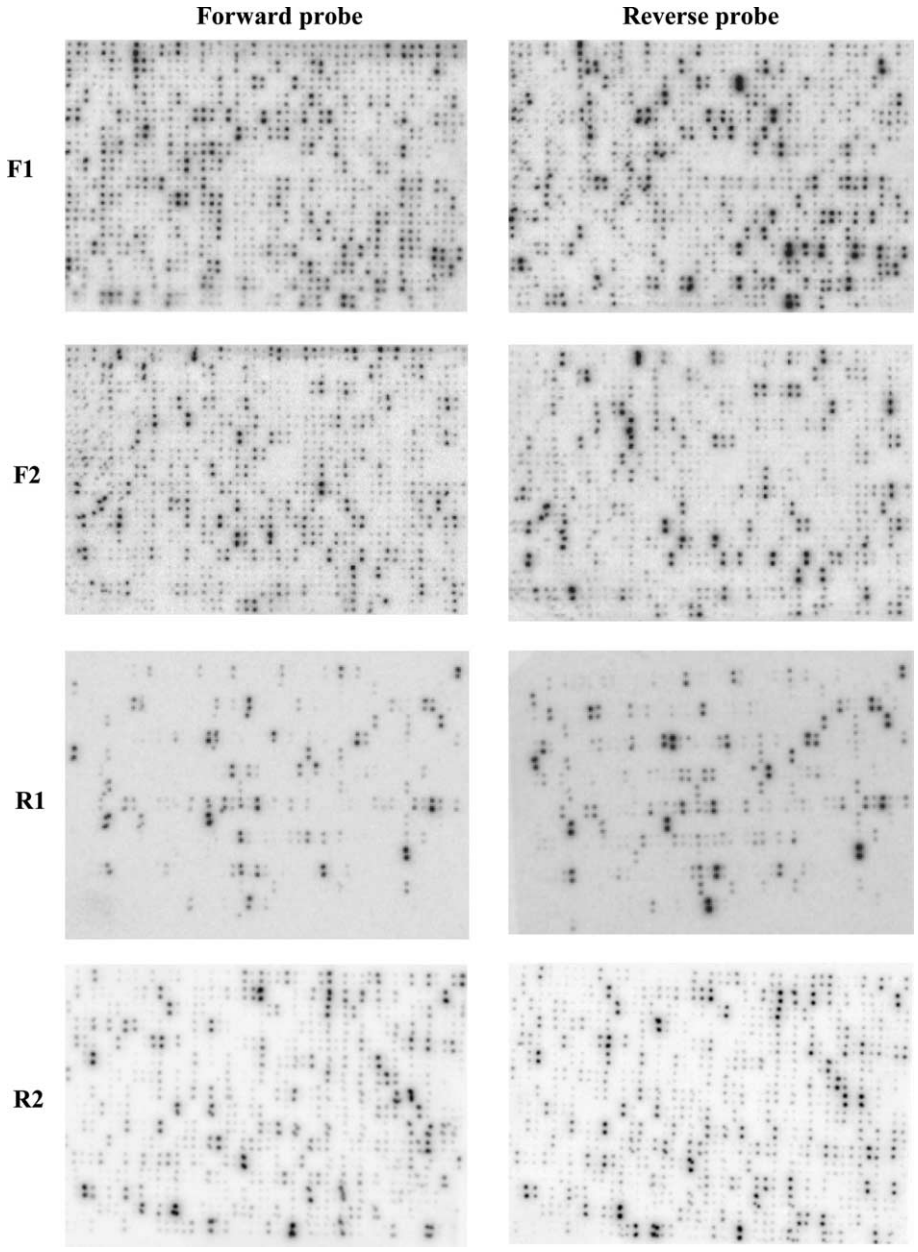

Fig. 1. Reverse Northern blotting analysis of clones obtained after forward and reverse cDNA subtractions from Jurkat cells treated with ethanol (vehicle) or oleic acid. Each membrane contained clones plotted in duplicate. They were hybridized with [^{32}P]dATP-labeled cDNA from both forward and reverse subtractions (forward and reverse probes).

4. Discussion

FA are very important for normal physiological functioning. Saturated FA are involved in energy production, energy storage, synthesis of phospholipids and sphingolipids required for membrane synthesis, and covalent modification of several regulatory proteins. MUFA are also involved in many of these processes and play a key role in maintaining optimal fluidity of the membrane lipid bilayer [29]. Although these are vital physiological processes, the term ''essential'' is not applied to saturated or MUFA. The designation is reserved for those PUFA that are required for good health but cannot be completely synthesized in the body.

Recent reviews have proposed a new terminology for the so-called ''essential'' FA (EFA), based on their conditional requirement during specific situations throughout individuals' life time (such as childhood, pregnancy, lactation and cancer) [29–31]. Essentiality connotes a more important role for these FA than for those that can be endogenously produced. For example, FA known as essential, such as linoleate, α-linolenate and docosahexaenoate contribute significant amounts of carbon to the synthesis of non-essential fatty acids [32,33]. Moreover, under certain circumstances, EFA can be preferentially oxidized relative to non-EFA [34,35]. Thus, there are circumstances in which EFA seem less important (less conserved) than non-EFA. There is also evidence that oleate cannot be synthesized in sufficient amounts to maintain tissue levels that are routinely observed when it is present in the diet [36]. Reduced tissue levels of a FA, by itself, constitute an insufficient justification for a dietary requirement but this is a

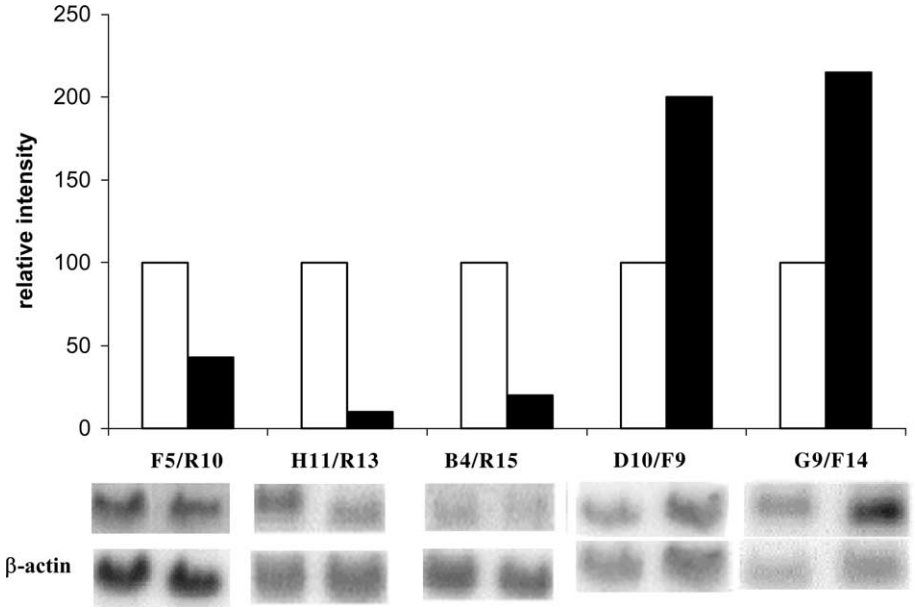

Fig. 2. Northern blotting analysis of clones identified by reverse northern blotting. Total RNA from Jurkat cells treated with ethanol (open bars) or oleic acid (hatched bars) was immobilized in nylon membranes and hybridized with [^{32}P]dATP-labeled PCR products of the chosen clones. Equal loading of samples was confirmed by hybridization with a [^{32}P]dATP-labeled β-actin probe. Hybridization signals were detected and analyzed using a Phosphoimager scanner and ImageQuanNT software program (Molecular Dynamics). The results of gene expression were calculated in relative values (relative expression) between OD of interest gene and that of β-actin.

Table 1
Genes with altered expression upon treatment of Jurkat cells with oleic acid (50 μM)

Clone	Identity	Expression variation (%)	GenBank Accession No.
B4/R15	Human tumor rejection antigen gp96	−80	CO960795
F5/R10	Heat shock protein 60 kDa	−57	CO960796
H11/R13	Subtilisin-like protein 4 (SPC 4)	−90	CO960797
D10/F9	Translation elongation factor 1 alpha 1	+100	CO960798
G9/F14	ATP synthase subunit 8	+115	CO960799

potential example of conditional essentiality. Essentiality should then be replaced by conditional requirement, a concept that comprises the balance between production and consumption of a specific FA in a particular situation.

Our study presents evidence that oleic acid, a so-called non-EFA, has an important role in maintenance of T-lymphocyte homeostasis. We show that the treatment of Jurkat cells with 50 μM of oleic acid increases the expression of translation elongation factor 1 alpha 1 (EF-1α, 100%) and of ATP synthase subunit 8 (115%). The expression of human tumor rejection antigen gp96, heat-shock protein 60 kDa and subtilisin-like protein (SPC4) was suppressed by oleic acid treatment, by 80%, 57% and 90%, respectively.

The ATP synthases comprise a large family of enzymes, which members are found in the bacterial cytoplasmic membrane, the inner membrane of mitochondria and the thylakoid membrane of chloroplasts. This enzyme is responsible for the production of ATP, the universal carrier of cell energy, from ADP and phosphate, using free energy from an electrochemical gradient of protons. The energy provided by ATP hydrolysis is necessary for active transport of ions and molecules and macromolecules synthesis [37]. Increased expression of ATP synthase, as induced by oleic acid treatment, ensures adequate energy supply for the cells' regular functions.

The EF-1α is a nucleotide exchange protein that binds GTP and aminoacyl-tRNAs. It is responsible for their codon-dependent placement at the A-site of the ribosome [38,39] and guarantees translation fidelity. EF-1α is physically associated and may be an important element in mitotic spindle formation [40], possibly playing a role in growth control, cell division and survival. Overexpression of EF-1α has been associated with extended lifespan in flies [41] and suppression of non-sense mutations in yeast [42]. Thus, its increased expression upon oleic acid treatment may have a protective effect on T-lymphocytes.

Proteolytic processing is a post-translational modification by which the cell can diversify and regulate the levels of gene products. In fact, processing is important in zymogen activation, mature peptide hormone generation, complement activation, clot formation and lysis, angiogenesis and tissue remodeling. In mammalian species, this catalytic function is carried out by a family of subtilisin-related pro-protein convertases, among which is SPC4 [43]. They appear to be highly specific enzymes, cleaving pro-protein precursors at specific positively charged amino acids, usually to produce biologically active products. Among the reported SPC4 substrates are pro-neurotrophin 3 [44], the insulin receptor [45], pro-nerve growth factor [46], pro-somatostatin [47] and human serum albumin

[48]. But the role of SPC4 in vivo remains unclear, as none of the precursors cited above demonstrates a clear preferential susceptibility to SPC4 activity.

Human tumor rejection antigen gp96 and heat-shock protein 60 kDa are members of the protein family known as chaperones. These proteins play a crucial role in cell recovery from stress and in cytoprotection, guarding cells from subsequent insults. An increase in the expression of these proteins is observed upon stress conditions [49,50]. They protect stressed cells by recognizing nascent polypeptides, unstructured regions of proteins and exposed hydrophobic stretches of amino acids. In doing so, chaperones hold, translocate or refold stress-denaturated proteins and prevent their irreversible aggregation with other proteins in the cell [50,51]. The reduced expression of these proteins upon oleic acid treatment may indicate that the cells do not consider oleic acid supplementation as a stress condition. This issue deserves to be further investigated.

The results presented herein led us to postulate that oleic acid, at plasma physiological concentration, may regulate the expression of important genes to maintain the machinery that ensures cell functioning. In addition, our results corroborate with the discussion on the importance of the so-called non-essential FA to ensure the normal cell functioning.

Acknowledgements: The authors are grateful to the technical assistance of G. de Souza, J.R. Mendonça, E. Portioli, Z. Mendonça, S.R. Souza and D.C. Costa. This research was supported by FAPESP, CNPq, Pronex, Capes and The British Council. The authors thank Drs. Rozangela Verlengia, Célio K. Miyasaka and Celine Pompéia for helpful discussions.

References

[1] Keys, A. (1970) Circulation 41, 1–211.
[2] Mensink, R.P. and Kata, M.B. (1987) Lancet 1, 122–125.
[3] Mata, P., Alvarez-Silva, L.A., Rubio, M.J., Nuno, J. and De Oya, M. (1992) Am. J. Clin. Nutr. 55, 846–850.
[4] Ferro-Luzzi, A. and Branca, F. (1995) Am. J. Clin. Nutr. 61S, 1338–1345.
[5] Cleland, L.G., French, J.K., Betts, W.H., Murphy, G.A. and Elliot, M.J. (1988) J. Rheum. 15, 1471–1475.
[6] Virella, G., Fourspring, K., Hyman, B., Haskill-Stround, R., Long, L., Virella, I., La Via, M., Gross, A.J. and Lopes-Virella, M. (1991) Clin. Immunol. Immunopathol. 61, 161–176.
[7] Dehmer, G.J., Popma, J.J., van den Berg, E.K., Eichhorn, E.J., Prewitt, J.B., Campbell, W.B., Jennings, L., Willerson, J.T. and Schmitz, J.M. (1988) New Engl. J. Med. 319, 733–740.
[8] Kremer, J.M., Lawrence, D.A., Jubiz, W., DiGiacomo, R., Rynes, R., Bartholomew, L.E. and Sherman, M. (1990) Arthr. Rheum. 33, 810–820.
[9] Linos, A., Kaklamanis, E., Kontomerkos, Y., Gazi, S., Vaiopoulos, G., Taokos, G.C. and Kaklamanis, P.H. (1991) Scand. J. Rheumat. 20, 419–426.
[10] Yaqoob, P., Newsholme, E.A. and Calder, P.C. (1995) Nutr. Res. 15, 279–287.
[11] Jeffery, N.M., Cortina, M., Newsholme, E.A. and Calder, P.C. (1997) Br. J. Nutr. 77, 805–823.
[12] Yaqoob, P., Newsholme, E.A. and Calder, P.C. (1994) Immunol. Lett. 41, 241–247.
[13] Sanderson, P., Yaqoob, P. and Calder, P.C. (1995) J. Nutr. Envir. Med. 5, 119–132.
[14] De Caterina, R., Cybulsky, M.I., Clinton, S.K., Gimbrone Jr., M.A. and Libby, P. (1994) Arther. Tromb. 14, 1829–1836.
[15] Yaqoob, P., Knapper, J.A., Webb, D.H., Williams, C.M., Newsholme, E.A. and Calder, P.C. (1998) Am. J. Clin. Nutr. 67, 129–135.
[16] Thewke, D., Kramer, M. and Sinensky, M.S. (2000) Biochem. Biophys Res. Commun. 273, 1–4.
[17] Haber, E.P., Hirabara, S.M., Gomes, A.D., Curi, R., Carpinelli, A.R. and Carvalho, C.R. (2003) FEBS Lett. 544, 185–188.
[18] Berard, A.M., Dumon, M.F. and Darmon, M. (2004) FEBS Lett. 559, 125–128.
[19] Verlengia, R., Gorjao, R., Kanunfre, C.C., Bordin, S., de Lima, T.M., Newsholme, P. and Curi, R. (2003) Lipids 38, 1157–1165.
[20] Verlengia, R., Gorjao, R., Kanunfre, C.C., Bordin, S., de Lima, T.M. and Curi, R. (2003) Life Sci. 73, 2939–2951.
[21] Diatchenko, L., Lau, Y.F., Campbell, A.P., Chenchik, A., Moqadam, F., Huang, B., Lukyanov, S., Lukyanov, K., Gurskaya, N., Sverdlov, E.D. and Siebert, P.D. (1996) Proc. Natl. Acad. Sci. USA 93, 6025–6030.
[22] Porkka, K.P. and Visakorpi, T. (2001) J. Pathol. 193, 73–79.
[23] Zendman, A.J., Cornelissen, I.M., Weidle, U.H., Ruiter, D.J. and van Muijen, G.N. (1999) FEBS Lett. 446, 292–298.
[24] Okuno, M., Arimoto, E., Nishizuka, M., Nishihara, T. and Imagawa, M. (2002) FEBS Lett. 519, 108–112.
[25] Lima, T.M., Kanunfre, C.C., Pompeia, C., Verlengia, R. and Curi, R. (2002) Toxicol in vitro 16, 741–747.
[26] Rebrikov, D.V., Britanova, O.V., Gurskaya, N.G., Lukyanov, K.A., Tarabykin, V.S. and Lukyanov, S.A. (2000) Nucleic Acids Res. 28, 90–96.
[27] Sambrook, J., Fritsch, E.F. and Maniats, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Edn. Cold Spring Harbor, New York.
[28] Kang, D., LaFrance, R., Su, Z. and Fisher, P.B. (1998) Proc. Natl. Acad. Sci. USA 95, 13788–13793.
[29] Spector, A.A. (1999) Lipids 34, S1-3.
[30] Cunnane, S.C. (2000) Br. J. Nutr. 84, 803–812.
[31] Cunnane, S.C. (2003) Prog. Lipid Res. 42, 544–568.
[32] Cunnane, S.C., Belza, K., Anderson, M.J. and Ryan, M.A. (1998) J. Lipid Res. 39, 2271–2276.
[33] Cunnane, S.C., Trotti, D. and Ryan, M.A. (2000) J. Lipid Res. 41, 1808–1811.
[34] Chen, Z.Y. and Cunnane, S.C. (1993) Br. J. Nutr. 42, 1206–1211.
[35] Chen, Z.Y., Menard, C.R. and Cunnane, S.C. (1996) Br. J. Nutr. 75, 583–591.
[36] Bourre, J.M., Dumont, O., Clement, M.E. and Durand, G.A. (1997) J. Nutr. 127, 488–493.
[37] Dimroth, P., Kaim, G. and Matthey, U. (2000) J. Exp. Biol. 203, 51–59.
[38] Riis, B., Rattan, S.I., Clark, B.F. and Merrick, W.C. (1990) Trends Biochem. Sci. 15, 420–424.
[39] Sonenberg, N. (1993) Opin. Cell Biol. 5, 955–960.
[40] Marchesi, V.T. and Ngo, N. (1993) Proc. Natl. Acad. Sci. USA 90, 3028–3032.
[41] Shepherd, J.C., Walldorf, U., Hug, P. and Gehring, W.J. (1989) Proc. Natl. Acad. Sci. USA 86, 7520–7521.
[42] Song, J.M., Picologlou, S., Grant, C.M., Firoozan, M., Tuite, M.F. and Liebman, S. (1989) Mol. Cell Biol. 9, 4571–4575.
[43] Bergeron, F., Leduc, R. and Day, R. (2000) J. Mol. Endocrinol. 24, 1–22.
[44] Seidah, N.G., Benjannet, S., Pareek, S., Chretien, M. and Murphy, R.A. (1996) FEBS Lett. 379, 247–250.
[45] Alarcon, C., Cheatham, B., Lincoln, B., Kahn, C.R., Siddle, K. and Rhodes, C.J. (1994) Biochem. J. 301, 257–265.
[46] Seidah, N.G., Benjannet, S., Pareek, S., Savaria, D., Hamelin, J., Goulet, B., Laliberte, J., Lazure, C., Chretien, M. and Murphy, R.A. (1996) Biochem. J. 314, 951–960.
[47] Brakch, N., Galanopoulou, A.S., Patel, Y.C., Boileau, G. and Seidah, N.G. (1995) FEBS Lett. 362, 143–146.
[48] Mori, K., Imamaki, A., Nagata, K., Yonetomi, Y., Kiyokage-Yoshimoto, R., Martin, T.J., Gillespie, M.T., Nagahama, M., Tsuji, A. and Matsuda, Y. (1999) J. Biochem. (Tokyo) 125, 627–633.
[49] Parsell, D.A. and Lindquist, S. (1993) Annu. Rev. Genet. 27, 437–496.
[50] Morimoto, R.I. (1998) Genes Dev. 12, 3788–3796.
[51] Bukau, B. and Horwich, A.L. (1998) Cell 92, 351–366.