Statin Induction of Liver Fatty Acid-Binding Protein (L-FABP) Gene Expression Is Peroxisome Proliferator-activated Receptor-α-dependent

Jean-François Landrier‡, Charles Thomas‡, Jacques Grober‡, Hélène Duez‡, Frédéric Percevault†, Maâmâ Souidi§, Christine Linard¶, Bart Staels§, and Philippe Besnard‡

From the ‡Physiologie de la Nutrition, Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à l’Alimentation (ENSBANA), UMR 5170 Centre Européen des Sciences du Goût CNRS/Institut National de la Recherche Agronomique, Université de Bourgogne, 1 Esplanade Erasme, F-21000, Dijon, France, §UR545 INSERM, Institut Pasteur de Lille, 1 Rue Calmette, F-59019, and Faculté de Pharmacie, Université de Lille 2, Lille, France, and ¶Institut de Radio-protection, Département de Radio-protection de l’Homme, Institut de Radioprotection et de Sureté Nucléaire B.P. N° 17, F-92262, Fontenay-aux-Roses Cedex, France

Statin Induction of Liver Fatty Acid-Binding Protein (L-FABP) Gene Expression Is Peroxisome Proliferator-activated Receptor-α-dependent*

Received for publication, July 6, 2004, and in revised form, August 25, 2004 Published, JBC Papers in Press, August 27, 2004, DOI 10.1074/jbc.M407461200

Statin Induction of Liver Fatty Acid-Binding Protein (L-FABP)

Gene Expression Is Peroxisome Proliferator-activated Receptor-α-dependent

The abbreviations used are: SREBP, sterol responsive element-binding protein-2; SREBP-2, which up-regulates several genes controlling cholesterol homeostasis, including the LDL receptor (LDLR) (1). Induction of LDLR in the liver enhances clearance of circulating LDL resulting in decreased plasma LDL-cholesterol levels. Statins also increase, at least in part via a PPARα-dependent mechanism (2), the level of high density lipoproteins (3, 4). As a consequence, statins improve the blood cholesterol profile and markedly reduce cardiovascular mortality and morbidity in dyslipidemic patients (5–7). Statins also influence plasma TG and NEFA levels in rats and humans (4, 8–11) through mechanisms not yet fully elucidated. Sustained hepatic clearance of TG-rich very low density lipoproteins by the LDLr (12), statin-dependent up-regulation of the lipoprotein lipase (LPL) gene, and down-regulation of the LPL inhibitor apolipoprotein C-III (13) may all contribute to the TG-lowering effect of statins. By contrast, it is not known if statins control the cellular uptake and the metabolic fate of lipids. Moreover, the NEFA lowering effect of statins is not yet explained.

Liver fatty acid-binding protein (L-FABP) is a cytoplasmic protein exhibiting a strong affinity for long-chain fatty acids (LCFA). It is highly expressed in liver, where it represents more than 5% of cytosolic proteins (14). The transcription rate of the L-FABP gene is tightly regulated and induced by both fibrate hypolipidemic drugs and LCFA through a peroxisome proliferator-activated receptor (PPAR)-responsive element located in the proximal part of the promoter. Several lines of evidence show that L-FABP participates in the cellular uptake and trafficking of LCFA. Using stably transfected L-cell fibroblasts it was shown that L-FABP overexpression leads to an increased uptake of LCFA (15). Conversely, antisense L-FABP knock-down reduced cellular LCFA influx in a dose-dependent manner in HepG2 cells (16). L-FABP expression can also affect the metabolic fate of LCFA. Indeed, overexpression of L-FABP was reported to be associated with an induced β-oxidation activity in Mca-RH7777 hepatoma cells (17). In the mouse, targeted deletion of the L-FABP gene resulted in a reduced rate of LCFA uptake by the liver under conditions of high lipid uptake.

* This work was supported by funds from the Conseil Régional de Bourgogne (to P. B.) and the Leducq Foundation (to B. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Physiologie de la Nutrition, ENSBANA, 1 Esplanade Erasme, F-21000 Dijon, France. Tel./Fax: 33-03-80-39-66-91; E-mail: pbsnard@u-bourgogne.fr

‡ The abbreviations used are: SREBP, sterol responsive element-binding protein; LDL, low density lipoproteins; LDLr, LDL receptor; TG, triglycerides; NEFA, non-esterified fatty acids; LPL, lipoprotein lipase; L-FABP, liver fatty acid-binding protein; LCFA, long-chain fatty acid; PPAR, peroxisome proliferator-activated receptor; WT, wild type; FCS, fetal calf serum; ACO, acyl-CoA oxidase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PPRE, PPAR-responsive element; CAT, chloramphenicol acetyltransferase; SRE, sterol regulatory element.
supply (e.g. intravenous LCFA bolus or prolonged fasting) (18, 19). Fasted L-FABP-null mice were characterized by lower hepatic TG synthesis and fatty acid oxidation than wild-type controls (19, 20). This phenotype is likely caused by the limitation of cellular LCFA availability for further metabolic use (i.e. esterification and oxidation) under high fatty acid load.

Altogether, these findings highlight the pivotal role exerted by L-FABP in hepatic lipid disposal and metabolic utilization. In the present study, we evaluated whether the hypolipidemic effect of statins is associated with an up-regulation of L-FABP expression in liver.

MATERIALS AND METHODS

Materials—Simvastatin was kindly provided by Merck Research Laboratories ( Rahway, NJ). Fenofibrate and Wy14,643 were purchased from Sigma. All products for cell culture were purchased from Invitrogen.

Animals and Experimental Treatments—French guidelines for the use and care of laboratory animals were followed. Wild-type or PPARα-null mice on the 129/SvJae background (a kind gift of F. Gonzalez, Laboratory of Metabolism, National Institutes of Health, Bethesda, MD) were used. Animals were housed individually in a controlled environment (constant temperature and humidity, darkness from 8 p.m. to 8 a.m.) and fed ad libitum a standard chow (UAR A04, Usine d’Alimentation Rationnelle). PPARα+/+ and PPARα−/− mice were force-fed for 5 days with simvastatin (100 mg/kg) and/or fenofibrate (40 mg/kg). Controls received the vehicle alone (1% carboxymethyl cellulose) by the same route. After sacrifice, livers were removed, snap frozen in liquid nitrogen, and stored at −80 °C until RNA extraction.

Cell Culture and Treatments—Rat hepatocytes were isolated by collagenase perfusion according to the modified procedure of Seglen (21). Hepatocytes were plated in 60-mm dishes in Williams E medium (Invitrogen) containing 100 units/ml penicillin, 100 μg/ml streptomycin, and 1% fetal calf serum (FCS). After an attachment period of 6 h, the culture medium was replaced by serum-free medium, and cells were incubated for an additional 24 h with 2.4–24 μM simvastatin. Control cultures received the vehicle alone (2 μM ethanol).

Rat Fao hepatoma cells (passages 20–35), used for transfection experiments, were cultured in a controlled environment (37 °C, 5% CO2) in Ham’s F-12 medium, 100 units/ml penicillin, 100 μg/ml streptomycin supplemented with 10% FCS. On the first day of confluence, cells were incubated for 24 h in the culture medium in the presence of Wy14,643 (10 μM) or simvastatin (12 μM) alone or in combination. Control cultures received the vehicle alone (2 μM ethanol).

Caco-2 cells (passages 35–45), used for transfection experiments, were cultured in a controlled environment (37 °C, 5% CO2) in Dulbecco’s modified Eagle’s medium, 4 mM glutamine, 1% non-essential amino acids, 100 units/ml penicillin, 100 μg/ml streptomycin supplemented with 20% FCS. Medium was changed every 2 days.

Northern Blotting—Total RNAs were extracted with TRIzol reagent (Invitrogen) and electrophoresed on a 1% agarose gel. After blotting, RNAs were hybridized with the PPAR and γ-glycerophosphate-δ-phosphate dehydrogenase (GAPDH) mRNAs or 28 S rRNA levels were determined by real-time reverse transcriptase-PCR.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA or 28 S rRNA levels were determined by real-time reverse transcriptase-PCR. Controls received the vehicle alone (2 μM ethanol).

Rat Fao hepatoma cells (passages 20–35), used for transfection experiments, were cultured in a controlled environment (37 °C, 5% CO2) in Ham’s F-12 medium, 100 units/ml penicillin, 100 μg/ml streptomycin supplemented with 10% FCS. On the first day of confluence, cells were incubated for 24 h in the culture medium in the presence of Wy14,643 (10 μM) or simvastatin (12 μM) alone or in combination. Control cultures received the vehicle alone (2 μM ethanol).

Caco-2 cells (passages 35–45), used for transfection experiments, were cultured in a controlled environment (37 °C, 5% CO2) in Dulbecco’s modified Eagle’s medium, 4 mM glutamine, 1% non-essential amino acids, 100 units/ml penicillin, 100 μg/ml streptomycin supplemented with 20% FCS. Medium was changed every 2 days.

Plasmid Construction—The −4000/+22 rat L-FABP promoter (kindly provided by Dr. J. I. Gordon, St. Louis, MO) was cloned into the pCAGGS basic vector (Promega). L-FABP 4000 mut was used to generate the following promoter sequences by PCR: L-FABP 275 mut, L-FABP 225 mut, L-FABP 175 mut, L-FABP 125 mut, and L-FABP 75 mut. Constructs harboring mutations of the PPAR-responsive element (PRE) were generated by site-directed mutagenesis (QuickChange®; Stratagene) using the following oligonucleotides: 5’-GCGACACTCTAGGCCCCGCGCTATTGGAAAGGAAAGCCG-3’ for L-FABP 275 mut (PRE in bold, point mutations are underlined) and 5’-GGCCCTAAGAGAAATCTTATTTGAGGGCGGAAAGCC-3’ for L-FABP 225 mut. All constructs were checked by restriction digestion analysis. The −1000/+60 (mPPARα) bp fragment of the mouse PPARα promoter (24) was subcloned into pCAGGS basic vector (upper panel). The bar graph represents L-FABP data normalized to 18 S RNA. Means ± S.E., n = 3; **, p < 0.01.
was supplemented with 1% of lipoprotein-depleted serum alone or with various simvastatin concentrations (from 2.4–24 μM) was added to the cells for an additional 24-h period. Cell extracts were prepared and assayed for CAT and β-galactosidase activities.

**Statistical Analysis**—The results are expressed as means ± S.E. Statistically, significant differences between groups were determined by Student’s t test.

**RESULTS**

**Simvastatin Induces L-FABP mRNA Levels in Primary Rat Hepatocytes**—To determine whether the expression of L-FABP gene is regulated by statins, rat hepatocytes were cultured in the presence of various concentrations of simvastatin. As shown in Fig. 1, L-FABP mRNA levels were increased in a dose-dependent manner by simvastatin. Indeed, a 1.9- and 2.9-fold rise of L-FABP mRNA was found after incubation with 12 and 24 μM simvastatin, respectively.

**Simvastatin-responsiveness of the L-FABP Promoter Is Mediated by Its PPRE**—Statins act on gene transcription by activating SREBP-2, which specifically up-regulates genes containing sterol regulatory elements (SRE) and/or E-boxes (1, 25). Three putative SREBP-binding sites were localized in the rat L-FABP promoter by *in silico* analysis: two SRE-like sequences located at positions −801/−790 and −245/−233 and one E-box at −306/−301. The previously identified PPRE is located at position −67/−55. B, Caco-2 cells were transiently transfected with 5′-progressive deleted constructs of the L-FABP promoter (L-FABP 4000wt, L-FABP 275wt, L-FABP 225wt, L-FABP 175wt, and L-FABP 75wt). C, Caco-2 cells were transiently transfected with the various constructs harboring punctual or total mutations of the PPRE (L-FABP 275mut, L-FABP 275del). Cells were transfected in 10% FCS medium for 12 h. Cells were cultured for an additional 24 h in medium containing 1% lipoprotein-deprived serum in presence or absence of 12 μM simvastatin. CAT activity is normalized to β-galactosidase activity. Results are represented as arbitrary units of normalized CAT activity. Means ± S.E., n = 3.

**Fig. 2.** The PPAR-responsive element mediates statin induction of rat L-FABP promoter activity. A, the rat L-FABP promoter sequence was analyzed *in silico* using MatInspector. The proximal part of promoter (−821/+19) revealed the existence of two putative sterol responsive elements (SRE 1 and 2) located at position −801/−790 and −245/−233 and one E-box at −306/−301. The previously identified PPRE is located at position −67/−55. B, Caco-2 cells were transiently transfected with 5′-progressive deleted constructs of the L-FABP promoter (L-FABP 4000wt, L-FABP 275wt, L-FABP 225wt, L-FABP 175wt, and L-FABP 75wt). C, Caco-2 cells were transiently transfected with the various constructs harboring punctual or total mutations of the PPRE (L-FABP 275mut, L-FABP 275del). Cells were transfected in 10% FCS medium for 2 h. Cells were cultured for an additional 24 h in medium containing 1% lipoprotein-deprived serum in presence or absence of 12 μM simvastatin. CAT activity is normalized to β-galactosidase activity. Results are represented as arbitrary units of normalized CAT activity. Means ± S.E., n = 3.

Analysis. Surprisingly, activation by simvastatin was observed even when constructs that did not contain any SRE or E-box (Fig. 2B). This unexpected result demonstrates that these putative sequences are not SREBP-binding sites. The fact that the L-FABP 75wt sequence contains a PPRE known to be crucial for the regulation of the L-FABP gene by fibrates led us to evaluate the possible involvement of this PPAR-binding site in this regulation. Interestingly, mutations in the PPRE (L-FABP 275mut and L-FABP 275del) fully abolished the activation of the L-FABP promoter by simvastatin (Fig. 2C). This unresponsive-ness was not caused by functional alterations of the promoter constructs used because the L-FABP gene regulator hepatocyte nuclear factor 1 (26) was still able to transactivate a reporter gene driven by the mutated or deleted promoters (data not shown). Taken together these data suggest that the PPRE is implicated in the up-regulation of L-FABP by simvastatin. L-FABP Gene Expression Is Synergistically Induced by Simvastatin and the PPARα Agonist Wy14,643—To assess the involvement of PPARα in the statin-mediated induction of L-FABP gene expression, FAO cells were cultured with simvastatin and/or the specific PPARα agonist Wy14,643. This cell line was chosen because it was previously used to study PPAR-mediated regulation of the L-FABP gene by LCFA (27). As observed in primary rat hepatocyte cultures, simvastatin robustly induced L-FABP mRNA levels to an extent similar as Wy14,643. Co-treatment with simvastatin and the PPARα ag-
onist triggered a synergistic rise of L-FABP mRNA levels, which were induced by more than 60-fold compared with controls (Fig. 3). Interestingly, similar effects were also found for ACO, the rate-limiting enzyme of peroxisomal \( \beta \)-oxidation, a prototypical PPAR \( \alpha \)-oxidative pathways through mechanisms that reiterate the physiological relevance of these in vitro data, wild-type and PPAR \( \alpha \)-null mice were force-fed for 5 days with simvastatin and/or fenofibrate. In wild-type mice, in line with the results obtained in Fao cells, simvastatin and fenofibrate alone up-regulated L-FABP mRNA levels, and co-treatment exerted a drastic synergistic effect (Fig. 4A). These changes were associated with a rise in L-FABP protein amounts, although combined treatment did not produce an additive induction (Fig. 4B). These treatments also induced ACO mRNA levels (Fig. 4C). Interestingly, whereas plasma triglycerides did not change when simvastatin or fenofibrate were given alone, combined treatment resulted in a significant drop in plasma TG levels (81 ± 5 in simvastatin/fenofibrate treated animals versus 117 ± 12 mg/dl in controls, Fig. 5). By contrast, deletion of the PPAR \( \alpha \) gene fully abolished the statin-mediated up-regulation of L-FABP expression (mRNA and protein levels, Fig. 4, A and B), ACO mRNA levels (Fig. 4C) and the decrease of plasma TG (Fig. 5), demonstrating the crucial role of PPAR \( \alpha \) in the induction of L-FABP gene expression by statins.

Simvastatin Induces PPAR \( \alpha \) mRNA Levels and PPAR \( \alpha \) Promoter Activity—The effect of simvastatin on PPAR \( \alpha \) mRNA levels was evaluated both in vitro and in vivo. As shown in Fig. 6A, expression of this nuclear receptor was significantly induced by simvastatin in Fao cells as well as in wild-type mice. To further investigate the mechanism by which this regulation takes place, transfection studies were performed using a construct containing the mouse PPAR \( \alpha \) promoter cloned upstream of the CAT reporter gene. Increasing concentrations of simvastatin produced a dose-dependent transactivation of the reporter gene (Fig. 6B), which strongly supports the hypothesis of a transcriptional control of PPAR \( \alpha \) gene expression by statins.

**DISCUSSION**

3-Hydroxy-3-methylglutaryl CoA reductase inhibitors (statins) are widely used to correct hypercholesterolemia in humans. Statins act through the activation of SREBP-2 that controls genes involved in cholesterol homeostasis (1). Statin treatment also normalizes blood TG and NEFA levels, but the mechanism is not yet fully understood. Although the expression of the LDLr, known to be involved in the LDL clearance, is induced by statins (29), this change cannot fully explain the hypotriglyceridemic action of these drugs. Indeed, TG levels are still decreased by statins in patients suffering from familial hypercholesterolemia in which the LDLr is not functional (30, 31). This observation suggests the existence of additional statin target genes that specifically control TG metabolism. In line with this hypothesis, statins are reported to induce the expression of LPL, an endothelial enzyme responsible for the hydrolysis of TG-rich lipoproteins, chylomicrons, and very low density lipoproteins (13, 32, 33). This effect is further enhanced by a statin-mediated decreased in expression of apolipoprotein CIII (apo CIII), a potent inhibitor of LPL activity (13). Interestingly, both LPL (positive) and apoC-III (negative) are PPAR \( \alpha \) target genes (34–37). In hepatocytes, statins also inhibit the microsomal triglyceride transfer protein (MTP) gene that encodes a lipid-binding protein implicated in very low density lipoproteins assembly (38, 39). Moreover, statins stimulate the \( \beta \)-oxidative pathways through mechanisms that remain to be determined (40, 41). In the present study, we demonstrate for the first time that L-FABP gene expression is also up-regulated by statins, an effect that might contribute to the hypolipidemic action of these drugs by facilitating LCFA influx and trafficking into hepatocytes. Collectively, these data strongly suggest that the hypotriglyceridemic effect of statins results from of an integrative regulatory pathway affecting a set of genes encoding for enzymes and lipid-binding proteins responsible for the hydrolysis of TG-rich lipoproteins and the cellular uptake and metabolic fate of LCFA.

In hepatocytes, the cholesterol depletion induced by statins triggers the proteolytic activation of the SREBP transcription factors, which after translocation into the nucleus modulate the transcription rate of sterol target genes (1). Surprisingly, although three putative SREBP-binding sites were identified by in silico analysis of the L-FABP gene, they were apparently non-functional in the context of the natural rat promoter. By contrast, statin regulation required the DR1–67/-55 sequence, which confers PPAR-responsiveness to the L-FABP gene (42). The physiological relevance of this finding was confirmed using PPAR \( \alpha \)-null mice. Indeed, deletion of the PPAR \( \alpha \) gene fully abolished the statin-mediated up-regulation of L-FABP expression (mRNA and protein levels) demonstrating a crucial role for PPAR \( \alpha \).

PPAR \( \alpha \) activity is regulated at various levels including at the...
transcriptional level by ligand activation, cofactor recruitment, and by post-transcriptional modification, such as ubiquitination and phosphorylation (43). The inhibition of PPARα/phosphorylation by cerivastatin was recently reported to be responsible for the induction of apolipoprotein A-I gene expression (2). This regulation was accounted for by a statin-dependent inhibition of geranylgeranylation of Rho-A protein (2), known to play a role in the c-jun NH₂-terminal kinase and p38 mitogen-

FIG. 4. PPARα mediates the synergistic up-regulation of L-FABP gene expression by simvastatin/fenofibrate. SV129 PPARα (+/+) or PPARα (−/−) mice (n = 6/group) were force fed daily with 200 μl of simvastatin (100 mg/kg, Sim) and/or fenofibrate (40 mg/kg, Feno) for 5 days. Controls received the vehicle alone (1% carboxymethyl cellulose). A, L-FABP mRNA levels were evaluated by Northern blotting using 20 μg of total RNAs from liver. The bar graph represents L-FABP data normalized to 18 S rRNA. B, L-FABP protein expression was determined by enzyme-linked immunosorbent assay. C, ACO mRNA levels were evaluated by Northern blot analysis using 20 μg of total RNAs from liver. Data are normalized to 18 S rRNA. Means ± S.E.; *, p < 0.05; ***, p < 0.001.
activated protein kinase cascades (44, 45). It is likely that such a regulation might also occur for other genes including L-FABP. However, the induction of PPARα gene expression by statins might constitute an alternative complementary mechanism. Indeed, simvastatin induced a significant rise in PPARα mRNA levels both in vitro and in vivo (Fig. 6). These data are in line with previously published results obtained in endothelial cells (46) and in hypertriglyceridemic rats treated with statins (9). A transcriptional mechanism for this regulation is suggested by the fact that simvastatin is able to transactivate the PPARα promoter (Fig. 6). Further experiments are required to determine whether PPARα is regulated by a direct SREBP-dependent mechanism, as shown for PPARγ (47), or whether an indirect regulation occurs.

A synergistic action on L-FABP mRNA levels was observed upon combination treatment (statins plus PPARα agonists) in both hepatoma Fao cells and in livers from wild-type mice (Fig. 4). Such an effect, which has already been pointed out by others (2, 48), is consistent with the existence of a cross-talk between the PPARα and statin signaling pathways. This observation
could provide a molecular basis and a scientific rationale for the therapeutic association of these drugs. Because PPARα controls fatty acid β-oxidation, we speculate that the unexplained rise in LCFAs oxidation activity by statins (40, 49, 50) is at least in part mediated via PPARα activation. This hypothesis is strongly supported by the fact that the induction of ACO mRNA levels by simvastatin is fully suppressed in PPARα-null mice (Fig. 4C). Statin-mediated induction of L-FABP might contribute to the metabolic utilization of NEFAs because L-FABP is required for NEFA uptake and its subsequent β-oxidation (20). Moreover, hepatic TG synthesis is positively correlated with hepatic LCFAs concentration (51). L-FABP up-regulation by statins might also contribute to a decrease of TG synthesis and thereby to the reduction of plasma very low density lipoproteins concentrations. Therefore, the regulation of L-FABP by statins could also contribute to the effects of statins on blood NEFA concentrations, which could be beneficial in patients suffering from diabetes or the metabolic syndrome, pathologies that are characterized by dyslipidemia and high NEFA levels (52, 53).

In conclusion, L-FABP induction by statin treatment might provide a mechanism contributing to the hypolipidemic effect of these drugs. Our data strongly suggest that PPARα is involved in this phenomenon, which could be caused at least in part by a transcriptional induction of the PPARα gene by statins.

Acknowledgment—We thank Dr Jérôme Bellenger (Université de Bourgogne, Dijon, France) for the generous gift of rat hepatocytes.

REFERENCES

1. Brown, M. S., and Goldstein, J. L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 11041–11048
2. Martin, G. S., Duez, H., Blanquart, C., Berezowski, V., Pouliain, P., Fruchart, J. C., Najib-Fruchart, J., Glineur, C., and Staels, B. (2001) J. Clin. Invest. 107, 1423–1432
3. Schaefer, J. R., Schweer, H., Ikewaki, K., Stracke, H., Seyberth, H. J., Kaf- farnik, H., Maisch, B., and Steinmetz, A. (1999) Arterioscler. Thromb. Vase. Biol. 21, 1712–1719
4. Saposnik, A., Santos, R. D., Amancio, R. F., Ramires, J. A., John Chapman, M., and Maranhao, R. C. (2003) Arterioscler. Thromb. Vase. Biol. 23, 301–306
5. Boux, M. N., and Parent, P. (2003) Atherosclerosis 166, 311–312
6. Lehnung, D., Lindberg, K., Oscarsson, J., Claesson, C., Asp, L., Li, L., Gustafsson, M., Boren, J., and Olsson, S. O. (2002) J. Biol. Chem. 277, 23044–23053
7. Martin, G. S., Danneberg, H., Kumar, L. S., Atshaves, B. P., Erol, E., Bader, M., Schroeder, F., and Binaz, B. (2003) J. Biol. Chem. 278, 21429–21434
