Insulin Receptor Substrate-3 Functions as Transcriptional Activator in the Nucleus*

Received for publication, July 25, 2001, and in revised form, November 9, 2001
Published, JBC Papers in Press, November 27, 2001, DOI 10.1074/jbc.M107058200

Tomohiro Kabutaš, Fumihiko Hakunoš, Tomoichiro Asanôš, and Shin-Ichiro Takahashi¶¶

From the ¶Departments of Animal Sciences and Applied Biological Chemistry, Graduate School of Agriculture and Life Sciences and the §Department of Internal Medicine, School of Medicine, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

The family of insulin receptor substrates (IRSs) has been reported to play important roles for signal transduction of various hormones. Four members of the IRS family have been described. Each IRS is believed to have different functions; however, the distinct physiological roles of each IRS are unclear. This study was undertaken to determine the intracellular localization of IRS-3. IRS-3 was expressed in COS-7 cells as fusion with a green fluorescent protein (GFP), and subcellular localization of the chimera protein was analyzed by fluorescent microscopy. Surprisingly, GFP-IRS-3 was localized not only adjacent to the plasma membrane but also in the nucleus. We confirmed by immunostaining with anti-IRS-3 antibody that non-fused IRS-3 protein is also localized in the nucleus of COS-7 cells that were transfected with IRS-3 cDNA. In addition, we detected endogenous IRS-3 in the nucleus of isolated rat adipocytes. We then studied subcellular localization of deletion mutants and fragments of IRS-3 fused with GFP. We found that the region corresponding to amino acid residues 192–223 in the phosphotyrosine binding domain played an important role in nuclear localization. This region includes sequences that are unique to IRS-3. We then investigated intracellular localization of other IRSs fused with GFP. GFP-IRS-1, GFP-IRS-2, and GFP-IRS-4 were mainly localized in the cytosol or plasma membranes. Chimeric protein, Gal4 DNA binding domain fused with IRS-3 C-terminal region, increased transcription of the reporter gene containing Gal4 binding site in human embryonic kidney 293 cells. These results suggest that intracellular localization of IRS-3 is determined by a different mechanism from other IRS proteins, and that IRS-3 possesses a transcription-regulating activity.

Insulin receptor substrates (IRSs) have been shown to mediate many actions stimulated by insulin, insulin-like growth factor, growth hormone, or various cytokines through complex interactions following ligand receptor binding (1). The IRS family currently consists of four proteins from IRS-1 to IRS-4. Each IRS possesses high homology regions, a pleckstrin homology domain and a phosphotyrosine binding (PTB) domain (2). These regions are thought to be important for interaction with cell surface receptors (3, 4). The C-terminal region of IRS proteins is poorly conserved (2), suggesting that this region mediates different biological activities of each IRS. IRS proteins have multiple tyrosine phosphorylation sites that form the binding motifs, and they are recognized by Src homology 2 domain-containing signaling molecules such as Grb2, the p85 regulatory subunit of phosphatidylinositol 3-kinase, Nck, and SH2-containing protein tyrosine phosphatase. These molecules are reported to mediate metabolic, differentiating, and growth-promoting functions (3–5).

Subcellular localization of IRS proteins has been analyzed by biochemical methods. These reports indicated that IRS-1 and IRS-2 are localized in the low-density microsome fraction and that IRS-3 is located mainly to the plasma membranes (6). Recently, Fantin et al. reported that IRS-4 was concentrated at the cytoplasmic surface of the plasma membrane (7). There are few reports of IRS localization using imaging methods. In this study, we studied IRS-3 localization using green fluorescent protein (GFP)-IRS-3 chimera proteins. We found that IRS-3 was located to the nucleus, and we went on to define the regions of IRS-3 critical for nuclear localization. In addition, we investigated the function of IRS-3 in the nucleus and found that IRS-3 possesses transcription-regulating activity.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Nissui (Tokyo). Calf serum and fetal bovine serum were obtained from JRH Bioscience (Tokyo). Penicillin and streptomycin were obtained from Ban’yu Pharmaceutical Co. (Tokyo). Leupeptin and pepstatin were generous gifts from Dr. Takaaki Aoyagi (Institute of Microbial Chemistry, Tokyo). Anti-phosphotyrosine monoclonal antibody PY20 was from ICN (Irvine, CA), and anti-Gal4 DNA binding domain antibody was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Polyclonal anti-IRS-3 antibody was prepared by immunizing rabbits with synthetic peptides as reported previously (6). All dishes, plates, and flasks were obtained from IWAKI (Tokyo). Other chemicals were of the reagent grade available commercially.

Construction of Various Plasmids—Rat IRS-3 cDNA was prepared as described previously (6). To construct the GFP-IRS-3 vector, an EcoRI-EcoRI fragment encoding IRS-3 was inserted into an EcoRI-digested pEGFP-C1 vector (CLONTECH, Palo Alto, CA). The pIRS-3-GFP vector was prepared as follows. A BamHI-NotI fragment encoding IRS-3 was inserted into a BamHI-NotI-clut pBlueScriptKS+ (Stratagene, La Jolla, CA). An IRS-3 fragment was amplified by PCR from this pBlueScript/IRS-3 vector. The PCR product was digested with EcoRI and SalI, and then cloned into an EcoRI-SalI-cut pEGFP-N1 (CLONTECH) vector. The primers used for this experiment were 5’-TTTCACACAGGAAACGCTATGAC-3’ and 5’-GAAAGGTCGACAATTGATGCTGGCA-3’. The latter primer was used to remove the TAG (termination) site. The mammalian expression vector pCMV was constructed from pEGFP-N1 by deleting BamHI-NotI fragment encoding GFP. The pIRS-3 was constructed by inserting the aforementioned EcoRI-EcoRI fragment encoding IRS-3 at an EcoRI site of pCMV vector. The vectors of

* 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. Tel.: 81-3-5841-5184; Fax: 81-3-5841-8038; E-mail: aktskshin@mail.ecc.u-tokyo.ac.jp.

† The abbreviations used are: IRS, insulin receptor substrate; PTB, phosphotyrosine binding; GFP, green fluorescent protein; DMEM, Dulbecco’s modified Eagle’s medium; HEK, human embryonic kidney; PBS, phosphate-buffered saline; DF, detergent-free; DAPI, 4’,6-diamidine-2’-phenylindole dihydrochloride.
pGFP-IRS-3 deletion mutants, D1, D3, D4, D5, and D6 were constructed by ligation each fragment in pEGFP vector using appropriate restriction enzymes. Before D4 and D6 constructs were made, site-directed mutagenesis was performed using the Kunkel method (8) to yield PmnCl and ScaI sites in the sequenced amplifying primers (30). The primers 5'-CCGCTTCCCGACATGCTGTCAGGGCC-3' and 5'-GCAGAAGCCGACTGTTGCTGGCCG-3' were used for this experiment. The rat IRS-1 cDNA (9) was kindly donated by Dr. Takashi Kadowaki (The University of Tokyo School of Medicine, Tokyo). pGFP-IRS-1 vector was constructed by ligation of HindIII-BamHI and BamHI-HindIII fragments in a HindIII-BamHI-cut pEGFP vector. The human IRS-2 and IRS-4 cDNAs were prepared as described elsewhere (10). The pGFP-IRS-2 vector was constructed by ligation of an EcoRI fragment in an EcoRI-cut pGFP vector. Transformation of E. coli strain DH5 was performed using the CaCl2 method (13). The medium was replaced. Rat pIRS-3 vector or rat adipocytes were washed with phosphate buffered saline (PBS) and fixed with 3.7% formaldehyde for 10 min. Cells were washed with PBS again and permeabilized with methanol for 2 min. Cells were first washed with PBS and then with 3% bovine serum albumin in PBS before incubating for 1 h with anti-rat IRS-3 antibody or anti-rat IRS-3 antibody that had been neutralized with the synthetic peptides used for raising this antibody. Cells were washed with 1% Triton X-100 in PBS and then incubated with fluorescent isothiocyanate-labeled anti-rabbit IgG antibody (Biomedical Technologies Inc., Stoughton, MA) for 30 min. Finally, cells were washed with PBS and then incubated with 10% PBS and 0.1% saponin [Sigma] in glycerol. Immunofluorescence analysis was performed.

Cell Culture and Transient Transfection of COS-7 Cells—COS-7 cells were generously provided by Dr. Hiroshi Kataoka (Graduate School of Frontier Sciences, The University of Tokyo, Tokyo). Cells were maintained at 37 °C in a humidified CO2-controlled atmosphere in DMEM supplemented with 10% calf serum, 0.1% NaHCO3, 50 IU/ml penicillin, 50 μg/ml streptomycin, 50 μg/ml amphotericin B (Sankyo, Tokyo). The cells were seeded on 60-mm plates at approximately 1×105 cells per plate in 10 ml DMEM containing 5% fetal saline solution (NISSUI) and cultured in 4 ml of growing medium for an experiment. An IRS-3N or IRS-3C fragment was amplified by PCR from pBluescript/IRS-3 vector, digested with EcoRI and XhoI, and inserted into an EcoRI-XhoI digested pEGFP (Promega) vector. Fusion protein in the DNA fragment was confirmed by sequencing. The primers 5'-TGCGGATCCGACCAGGATTGCTGTC-3' and 5'-CGAGGTACTATTGACGTCTGTTGTTG3' were used for this experiment. An IRS-3N or IRS-3C fragment was amplified by PCR, digested with EcoRI and XhoI and inserted into a pbind vector to yield the pBIND-IRS-3N or pBIND-IRS-3C vector. We used the following primers in this procedure: 5'-TGCGGATCCGACCAGGATTGCTGTC-3' and 5'-ACTGATATCCAGTCTCTTCATGGCAG-3' for IRS-3N and 5'-AGATATCAGGGGAGCAGACGACGAGCC-3' and 5'-CGAGGTACTATTGACGTCTGTTGTTG3' for IRS-3C. The pBIND-IRS-3C, pBIND-IRS-3, and pBIND-IRS-4C vectors were constructed by ligation each fragment in pBIND vector frame using appropriate restriction enzymes.

Isolation of Adipocytes—Adipocytes from the epididymal fat pads of rats were not changed artificially. To generate the D2 vector, a D2 fragment in an IRS-3C fragment was amplified by PCR, digested with EcoRI, KpnI, and XhoI, and inserted into an EcoRI-KpnI-XhoI digested pEGFP-C1 vector. The pGFP-IRS-2 vector was constructed by ligation of an EcoRI-EcoR vector in an EcoRI-cut pGFP-C1 vector. To construct the pBIND-IRS-1C vector, an IRS-1C fragment was amplified by PCR, digested with EcoRV and KpnI, and inserted into an EcoRV-KpnI digested pBIND vector (Promega) vector. Fusion protein in the DNA fragment was confirmed by sequencing. The primers 5'-TGGATTACGATCCGACCAGGATTGCTGTC-3' and 5'-ACTGATATCCAGTCTCTTCATGGCAG-3' were used for this experiment. An IRS-3N or IRS-3C fragment was amplified by PCR from pBluescript/IRS-3 vector, digested with EcoRI and XhoI, and inserted into a pbind vector to yield the pBIND-IRS-3N or pBIND-IRS-3C vector. We used the following primers in this procedure: 5'-TGGATTACGATCCGACCAGGATTGCTGTC-3' and 5'-ACTGATATCCAGTCTCTTCATGGCAG-3' for IRS-3N and 5'-AGATATCAGGGGAGCAGACGACGAGCC-3' and 5'-CGAGGTACTATTGACGTCTGTTGTTG3' for IRS-3C. The pBIND-IRS-3C, pBIND-IRS-3, and pBIND-IRS-4C vectors were constructed by ligation each fragment in pBIND vector frame using appropriate restriction enzymes.

Cell Culture and Transient Transfection of COS-7 Cells—COS-7 cells were transfected with the pSV-luc vector (Promega), pG5-luc (Promega), and each pBIND vector, 5 μl of transfection buffer (50 mm Tris-HCl, pH 7.4, 10 mm KCl, 5 mm EDTA, 50 μM Na2VO4, 10 μg/ml leupeptin, 5 μg/ml pepstatin, 20 μg/ml PMSF, 100 kallikrein-inactivating unit/ml aprotinin, 10 mg/ml p-nitrophenyl phosphate). The lysates were homogenized by 20 strokes in a Dounce homogenizer with a loose pestle (Wheaton, NJ). The homogenates were centrifuged at 10,000 × g for 5 min, and the supernatant was obtained as a cytosolic fraction. After washing twice with DF harvesting buffer, the initial pellet was suspended in 200 μl of Triton lysis buffer (0.1% Triton X-100 in DF harvesting buffer), incubated for 15 min, and centrifuged at 10,000 × g for 5 min. The supernatant was obtained as a membrane fraction. Finally, after washing twice with Triton lysis buffer, the pellet was suspended in 200 μl of radiomimimic precipitation lysis buffer (1% deoxycholic acid sodium salt in water), incubated for 15 min, and centrifuged at 10,000 × g for 5 min. The supernatant was obtained as a nuclear fraction. In some experiments, the pellet was suspended in Triton lysis buffer with the exception that the concentration of Triton X-100 is 1% instead of 0.1% and homogenized vigorously to break up the nucleus. The mixture was centrifuged at 10,000 × g for 5 min, and the supernatant was recovered as a nuclear fraction. The activity of alkaline phosphatase, a plasma membrane enzyme marker, was measured in each fraction prepared without SDS and EDTA according to the method reported by Koyama et al. (15). The contents of DNA in each fraction were measured as a nuclear marker by the modified methods of Sambrook et al. (16). 100 μl of concentrated 3× Laemmli sample buffer solution (9% SDS, 15% glycerol, 30 mm Tris-HCl, pH 7.8, 0.65% bromophenol blue, 6% 2-mercaptoethanol) was then added to 200 μl of each fraction, and the mixture was incubated for 5 min at 100 °C. These samples were then stored at −80 °C until electrophoresis.

Isolation of Nuclei—Isolated nuclei were prepared from rat adipose tissues basically according to the methods of Lynch et al. (17). All steps were carried out at 0 °C. Rat adipose tissue (−4 g) were rinsed in 0.3 μm sucrose and minced with dissecting scissors in 15 volumes of 0.3 μm sucrose/0.01% CaCl2. The homogenization was then performed in a Dounce homogenizer with a loose pestle. The homogenate was passed through 250-μm piece of nylon mesh, and the filtrate was centrifuged at 750 × g for 4 min. The supernatant was discarded, and the pellet was resuspended in 7 ml of 2 μm sucrose, 1 μm CaCl2. The suspension was then layered over 6 ml of 2.2 μm sucrose, and the nuclei were collected by centrifugation for 30 min at 40,000 × g. Finally, the nuclei were suspended in 4 ml of growing medium for 40 min. The supernatant was removed, and the nuclei were suspended in 20 μl of concentrated 3× Laemmli sample buffer solution. The mixture was incubated at 5 min at 100 °C, and the immunoblotting analysis of these samples was performed as described above. In some experiments, the nuclei were suspended in 1% Triton lysis buffer, and the nuclear extracts were prepared as described above followed by measurement of the alkaline phosphatase activity.

Immunoblotting Analysis—The same volume of the samples was subjected to 9% SDS-PAGE and transferred to membranes based on standard electrophoretic procedures as described previously (18). The membrane was then incubated for at least 1.5 h at room temperature in the blocking buffer containing anti-IRS-3 antibody (1:3000) or a monoclonal anti-phosphotyrosine antibody PY20 (1:3000). Specific proteins were detected using an ECL kit according to the directions by the manufacturer (DuPont). The results were quantified using NIH image computer program (version 1.61).

Immunoprecipitation—After cell fractionation, 60 μl of each fraction was diluted to 1 ml with DF harvesting buffer. This solution was incubated with anti-IRS-3 antibody (10 μl) for 12 h at 4 °C, 40 μl of protein A-Sepharose (50% (w/v), Amersham Biosciences, Inc.) was then added, and incubation was continued for 2 h. Immunoprecipitation was then performed by immunoblotting as described previously (18).

Luciferase and β-Galactosidase Assay—HEK 293 cells were co-transfected with 2 μg of pG5-luc, pSV-β-galactosidase, and each pBIND fusion construct as described above. In the case of a positive control experiment, we transfected cells with 2 μg of pACT-MyoD, which con
A Novel Function of IRS-3 in the Nucleus

RESULTS AND DISCUSSION

To determine the subcellular localization of IRS-3, we expressed IRS-3 in a COS-7 cells as a fusion protein with a GFP and analyzed the subcellular location of the chimera by fluorescence microscopy. When we introduced the pEGFP-C1 vector encoding GFP into COS-7 cells, the fluorescence was dispersed in the cytosol (Fig. 1, top panels). As shown in Fig. 1, middle panels, IRS-3 labeled with GFP at its N-terminal end (GFP-IRS-3) was localized not only to the plasma membranes but also in the nucleus. We identified this compartment as the nuclei, because the compartment is exclusively stained by 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI). A chimera protein labeled with GFP at the IRS-3 C-terminal end (IRS-3-GFP), showed the same distribution pattern as that seen using IRS-3 labeled at its N-terminal end (Fig. 1, bottom panels).

We then sought to determine whether non-fused wild-type IRS-3 is localized in the nucleus of COS-7 cells that do not express IRS-3. We transiently transfected cells with pIRS-3 and immunostained with anti-IRS-3 antibody (Fig. 2A). The IRS-3 signal was detected in the nucleus. In the presence of the peptide used to raise the anti-IRS-3 antibody, these signals were completely obliterated. To further confirm the presence of IRS-3 in the nucleus, COS-7 cells expressing IRS-3 were homogenized and fractionated into the cytosol, membrane, and nuclear fractions. Contamination of the cytosol or nuclear fractions by membrane proteins was minimal, because we detected only 2.6 or 1.5% alkaline phosphatase activity in the cytosol and nuclear fractions, respectively, that was found in the membrane fraction. In addition, we measured the DNA contents of each fraction and lysates of the cytosol, membrane, and nuclear fractions containing 0.00, 0.00, and 0.41 μg of DNA, respectively. Each fraction was subjected to immunoblotting with anti-IRS-3 antibody. As shown in Fig. 2B, IRS-3 was clearly detected in all of three fractions. Based on the density of signals, the relative ratios of IRS-3 in the cytosolic, membrane, and nuclear fractions were calculated as 5:12:5. Because IRS-3 is known to be tyrosine-phosphorylated, aliquots of each fraction were also immunoprecipitated with anti-IRS-3 antibody.
followed by immunoblotting with anti-phosphotyrosine antibody (Fig. 2B). We found that IRS-3 in membrane and nuclear fractions was tyrosine-phosphorylated, although IRS-3 in cytosolic fraction was not phosphorylated. To analyze whether the factors in serum including insulin and insulin-like growth factors induce nuclear import and tyrosine phosphorylation of IRS-3, we transfected cells with pIRS-3 under serum-free conditions. We found that IRS-3 was localized into the nucleus and phosphorylated even under serum-free conditions (data not shown). Thus, the stimuli leading to nuclear import of IRS-3 and its tyrosine phosphorylation remain to be determined.

We then investigated the subcellular localization of endogenous IRS-3 by immunostaining of rat adipocytes, a tissue known to express IRS-3 in the rat (19). As shown in Fig. 3A, endogenous IRS-3 was detected in the nucleus of isolated rat adipocytes. Immunoneutralized serum (Fig. 3A) or non-immune serum (data not shown) failed to yield a signal in the nucleus, although modest signals on the plasma membranes were observed. In addition, we found IRS-3 in the nuclear fractions after cell fractionation of adipocytes (Fig. 3B). In this case, alkaline phosphatase activity was not detected in the nuclear extracts. These experiments indicate that endogenous IRS-3 is localized to the nucleus, and that our results are not an artifact of overexpression.

To identify the region of IRS-3 that directs nuclear localization, we prepared deletion mutants and fragments of IRS-3 fused with GFP and then performed subcellular localization of each protein. First of all, signals of GFP fused with N-terminal or C-terminal regions including PTB domain were observed in the nuclei (Fig. 4, D1 and D2). In addition, we found that a portion of IRS-3 that includes the PTB domain fused with GFP localized predominately to the nuclei (Fig. 4, D3). We then introduced a mutant of the PTB domain deleting amino acids 194–213. The localization of this mutant exclusively took place in the cytosol (Fig. 4, D4), whereas the 192–223 fragment fused with GFP accumulated in the nuclei (Fig. 4, D5). Lastly, the mutant with a deletion of amino acids 194–213 from intact IRS-3 was observed only in cytosol (Fig. 4, D6). Taken together, these data suggest that region 192–223 functions as a nuclear localization signal.

In Fig. 5A, we aligned the amino acid sequences of each IRS.
A Novel Function of IRS-3 in the Nucleus

The putative nuclear localization signals in PTB domain have a unique amino acid sequence from amino acids 192 to 223, suggesting that these sequences may be important for IRS-3 localization. This region contains scattered basic amino acids clusters, suggesting that these regions may provide nuclear localization signals (20). We then asked whether IRS-1, IRS-2, or IRS-4 could exhibit nuclear localization. The N-terminal GFP fusion protein of each IRS was expressed in COS-7 cells, and the subcellular localization of each chimeric protein was examined to nuclei, and unique amino acid sequences in IRS-3 appeared to be involved in the nuclear localization of IRS-3. The putative nuclear localization signals in PTB domain were recognized as amino acids 192 to 223, suggesting that these sequences may be important for IRS-3 localization.

Finally, to investigate the function of IRS-3 in the nucleus, we measured transcriptional activity of IRS-3 by Gal4 chimeric transcriptional regulator assay using HEK 293 cells (Fig. 6). To analyze the ability of IRS-3 as a potential transcriptional activator, we generated the construct Gal4-full-length IRS-3, N-terminal region of IRS-3 (amino acid residues 1–270), or C-terminal region of IRS-3 (amino acids 271–494) by fusing IRS-3 N-terminally to the Gal4-DNA binding domain. Each construct was transiently transfected into HEK 293 cells together with the reporter construct (Fig. 6A). The Gal4-C-terminal region of IRS-3 potently stimulated the transcription of the reporter gene, although Gal4-full-length IRS-3 or N-terminal region of IRS-3 did not affect transcriptional activity. We concluded that the C-terminal region of IRS-3 has transcription activity that can be unmasked by the removal of the N-terminal region. Similar results were reported in the case of Smad1 (21). We then compared the transcriptional activity of the C-terminal region of each IRS. As shown in Fig. 6B, only the C-terminal region of IRS-3 worked as a transcriptional activator. The expression of each construct was confirmed by immunoblotting with anti-Gal4 antibody as well as anti-IRS-antibodies (data not shown). As discussed above, the C-terminal domains of each IRS exhibit low homology, suggesting that among the proteins of the IRS family the C-terminal region of IRS-3 possessed transcription-regulating activity.

IRS-1 and IRS-2 have important roles in signal transduction for hormones or cytokines including insulin and insulin-like growth factor-I. The physiological relevance of IRS-3 is less clear. Zhou et al. (22) reported that IRS-3 is capable of mediating phosphatidylinositol 3-kinase-dependent metabolic actions of insulin in adipose tissues. In addition, Anai et al. (6) demonstrated that IRS-3 has a different role from IRS-1 and IRS-2 in insulin signaling in rat adipocytes. Razzini et al. (23) recently showed that the pleckstrin homology domains of IRS-1, IRS-2, and IRS-3 bound with different specificities to the 3-phosphorylated phosphoinositides, and the localization of pleckstrin homology domains of IRS-1 and IRS-2 was different from IRS-3. Taken together, these results imply that IRS-3 has a different physiological function than other IRS proteins. IRS-3 does not appear essential for normal growth, glucose homeostasis, and glucose transport, because irs-3 null mutant mice do not demonstrate a phenotype consistent with such abnormalities (24). Nevertheless, IRS-3 is highly expressed at 7 days of mouse embryonic life (25), suggesting that IRS-3 has a unique physiological role in the nuclei such as regulation of transcription.

Our studies demonstrate three novel findings. IRS-3 is localized to nuclei, and unique amino acid sequences in IRS-3 appear to function as nuclear localization signals. In addition, the C-terminal domain of IRS-3 has transcriptional activity as demonstrated when it was fused to the Gal4 DNA binding domain. These results suggest that intracellular localization of IRS-3 is different from that of other IRS proteins, and that IRS-3 possesses a novel nuclear function in addition to its physiological role as one of the insulin receptor kinase substrates.

Acknowledgments—We thank Dr. Takaaki Aoyagi (Institute of Microbial Chemistry, Tokyo) for leupeptin and pepstatin and Dr. Takashi Kodawaki (The University of Tokyo School of Medicine, Tokyo) for cDNA of rat IRS-1. We acknowledge Drs. Hiroshi Kataoka and Kunio Shiota (Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo) for donating COS-7 cells and HEK 293 cells and Dr. Masugi Nishihara for Wistar strain rats. We appreciate a helpful discussion while performing the experiments with Dr. Kazuhiro Chida (Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo), Dr. Marco Conti (Stanford University, Palo Alto, CA), Dr. Steven C. Boyages (Westmead Hospital, University of Sydney, New South Wales, Australia), and Dr. Akiko Takenaka (Section of Biosource Utilization, Yamagata University, Yamagata, Japan). Lastly, we deeply acknowledge Drs. A. Joseph D’Ercole and Judson J. Van Wyk (The University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC) for many suggestions during the preparation of this manuscript.

REFERENCES

1. Cheatham, B., and Kahn, C. R. (1995) Endoer. Rev. 16, 117–142
2. Lavan, B. E., Fantin, V. R., Chang, E. T., Lane, W. S., Keller, S. R., and Lienhard, G. E. (1997) J. Biol. Chem. 272, 21403–21407
3. Leboit, D., Werner, H., Beiser, J. D., and Roberts, C. J. (1995) Endoer. Rev. 16, 143–163
4. White, M. F. (1997) Diabetologia 40, S2–S17
5. Marte, B. M., and Downward, J. (1997) Trends Biochem. Sci. 22, 355–358
6. Anai, M., Oto, H., Funaki, M., Fukushima, Y., Inukai, K., Ogihara, T., Sakoda,
A Novel Function of IRS-3 in the Nucleus

H., Onishi, Y., Yazaki, Y., Kikuchi, M., Oka, Y., and Asano, T. (1998) J. Biol. Chem. 273, 29686–29692
7. Fantin, V. R., Sparling, J. D., Slot, J. W., Keller, S. R., Lienhard, G. E., and Lavan, B. E. (1998) J. Biol. Chem. 273, 10726–10732
8. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, pp. 15.74–15.79, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
9. Ueno, H., Kondo, E., Yamamoto-Honda, R., Tobe, K., Nakamoto, T., Sasaki, K., Mitani, K., Furusaka, A., Tanaka, T., Tsujimoto, Y., Kasowaki, T., and Hirai, H. (2000) Mol. Biol. Cell 11, 735–746
10. Anai, M., Funaki, M., Ogiwara, T., Terasaki, J., Inukai, K., Katagiri, H., Fukushima, Y., Yazaki, Y., Kikuchi, M., Oka, Y., and Asano, T. (1998) Diabetes 47, 13–23
11. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, pp. 16.41–16.45, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
12. Graham, F. L., and van der Eb, A. J. (1973) Virology 52, 456–467
13. Simpson, I. A., Yver, D. R., Hissin, P. J., Wardzala, L. J., Karmeli, E., Salans, L. B., and Cushman, S. W. (1983) Biochim. Biophys. Acta 763, 393–407
14. Jin, C. S.-L., Bushnik, T., Lan, L., and Conti, M. (1998) J. Biol. Chem. 273, 19672–19678
15. Koyama, H., and Ono, T. (1976) J. Cell. Physiol. 88, 49–56
16. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, pp. 9.16–9.17, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Lynch, W. E., Brown, R. F., Umeda, T., Langreth, S. G., and Lieberman, I. (1970) J. Biol. Chem. 245, 3911–3916
18. Ariga, M., Nedachi, T., Akahori, M., Sakamoto, H., Ito, Y., Hakune, F., and Takahashi, S.-I. (2000) Biochem. J. 348, 409–416
19. Lavan, B. E., Lane, W. S., and Lienhard, G. E. (1997) J. Biol. Chem. 272, 11439–11443
20. Boulikas, T. (1993) Crit. Rev. Eukaryotic Gene Expression 3, 193–227
21. Liu, F., Hata, A., Baker, J. C., Doody, J., Carcamo, J., Harland, R. M., and Massague, J. (1996) Nature 381, 620–623
22. Zhou, L., Chen, H., Xu, P., Cong, L.-N., Sciacchitano, S., Li, Y., Graham, D., Jacobs, A. R., Taylor, S. I., and Quon, M. J. (1999) Mol. Endocrinol. 13, 505–514
23. Razzini, G., Ingrasso, A., Brancaccio, A., Sciacchitano, S., Esposito, D. L., and Falasca, M. (2000) Mol. Endocrinol. 14, 823–836
24. Liu, S. C., Wang, Q., Lienhard, G. E., and Keller, S. R. (1999) J. Biol. Chem. 274, 18093–18099
25. Sciacchitano, S., and Taylor, S. I. (1997) Endocrinology 138, 4981–4940
Insulin Receptor Substrate-3 Functions as Transcriptional Activator in the Nucleus
Tomohiro Kabuta, Fumihiko Hakuno, Tomoichiro Asano and Shin-Ichiro Takahashi

J. Biol. Chem. 2002, 277:6846-6851.
doi: 10.1074/jbc.M107058200 originally published online November 27, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M107058200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 22 references, 10 of which can be accessed free at http://www.jbc.org/content/277/9/6846.full.html#ref-list-1