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Integrator complex plays an essential role in adipose differentiation

Yuichiro Otani [a], Yusuke Nakatsu [a], Hideyuki Sakoda [b], Toshiaki Fukushima [a], Midori Fujishiro [b], Akifumi Kushiyama [c], Hirofumi Okubo [a], Yoshihiro Tsuchiya [a], Haruya Ohno [a], Shin-Ichiro Takahashi [d], Fusanori Nishimura [e], Hideaki Kamata [a], Hideki Katagiri [f], Tomoichiro Asano [a,]*

[a] *Department of Medical Chemistry, Division of Molecular Medical Science, Graduate School of Biomedical Sciences, Hiroshima University, Japan*
[b] *Department of Internal Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan*
[c] *Department of Internal Medicine, The Institute for Adult Diseases, Asahi Life Foundation, Tokyo, Japan*
[d] *Graduate School of Agriculture and Life Sciences, University of Tokyo, Tokyo, Japan*
[e] *Department of Dental Science for Health Promotion, Division of Cervico-Gnathostomatology, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan*
[f] *Division of Molecular Metabolism and Diabetes, Tohoku University Graduate School of Medicine, Sendai, Japan*

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ABSTRACT

The dynamic process of adipose differentiation involves stepwise expressions of transcription factors and proteins specific to the mature fat cell phenotype. In this study, it was revealed that expression levels of IntS6 and IntS11, subunits of the Integrator complex, were increased in 3T3-L1 cells in the period when the cells reached confluence and differentiated into adipocytes, while being reduced to basal levels after the completion of differentiation. Suppression of IntS6 or IntS11 expression using siRNAs in 3T3-L1 preadipocytes markedly inhibited differentiation into mature adipocytes, based on morphological findings as well as mRNA analysis of adipocyte-specific genes such as Glut4, perilipin and Fabp4. Although Pparγ2 protein expression was suppressed in IntS6 or IntS11-siRNA treated cells, adenoviral forced expression of Pparγ2 failed to restore the capacity for differentiation into mature adipocytes. Taken together, these findings demonstrate that increased expression of Integrator complex subunits is an indispensable event in adipose differentiation. Although further study is necessary to elucidate the underlying mechanism, the processing of U1, U2 small nuclear RNAs may be involved in cell differentiation steps.

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1. Introduction

White adipose tissue (WAT) is a major energy reserve in higher eukaryotes, and storing triacylglycerol in periods of energy excess and its mobilization during energy deprivation are its primary roles. Understanding the mechanisms of adipogenesis is of major relevance to human disease, as adipocyte dysfunction is a major contributor to metabolic disease in obesity [1,2]. For the past two decades, many intensive studies have revealed the process of stepwise expressions of transcription factors and proteins which take place during the transition from preadipocytes into mature adipocytes. Briefly, growth arrested 3T3-L1 preadipocytes treated with differentiation inducers immediately express C/ebpβ, and C/ebpβ triggers transcription of Pparγ and C/ebpα, which in turn induce adipocyte-specific genes such as Glut4, perilipin and Fabp4 in a coordinate fashion [3–5].

While the aforementioned transcriptional cascade itself has been largely clarified, much attention is now being given to newly identifying the proteins which regulate and/or initiate these processes. For example, upregulation of Pin1 is essential for the differentiation of preadipocytes into adipocytes, although it is still unclear how Pin1 affects the upregulation of adipocyte-specific gene expressions [6,7]. In this study, we attempted to screen the proteins associated with AS160 reportedly playing a role in the process from Akt activation to the translocation of GLUT4 to the cell surface [8] and IntS6 was identified as one of these proteins. IntS6 is one of the components constituting the Integrator complex, which plays critical roles in the 3′ end processing of U1 and U2 small nuclear (sn) RNAs. Subsequently, we planned to perform experiments examining whether or not this association is critical for GLUT4 translocation. However, unexpectedly, it was revealed that gene silencing of IntS6 using small interfering RNAs (siRNA) suppresses the adipose differentiation of 3T3-L1 preadipocytes. Therefore, we instead focused on this effect, and herein show the critical role of the Integrator complex in adipose differentiation.

2. Materials and methods

2.1. Antibodies and adenoviruses

Anti-IntS6 antibody was generated by immunization of rabbits with the glutathione S-transferase-fused COOH-terminal 98 amino acids of IntS6. Anti-actin antibody was purchased from Fabgennix

* Corresponding author.
E-mail address: tasano@hiroshima-u.ac.jp (T. Asano).

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(Mississauga, ON, Canada). anti-IntS11 antibodies were from Abcam. Anti-GLUT4, anti-C/EBPα, anti PPAR-γ, anti-Fabp4, anti C/EBPδ and anti-perilipin were from Cell Signaling Technology. Anti-C/EBPβ was from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-rabbit and anti-mouse horseradish peroxidase-conjugated antibodies were purchased from GE Healthcare (Buckinghamshire, UK). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA). Recombinant adenoviruses encoding mouse PPARγ2 and green fluorescent protein (GFP) were prepared according to the instruction manual of the Adenovirus Dual Expression Kit (TaKaRa Bio). Adenovirus encoding GFP alone served as a control.

2.2. Cell culture and induction of differentiation

3T3-L1 preadipocytes were propagated and maintained in DMEM containing 10% (vol/vol) calf serum. After the cells reached 100% confluence, the culture medium was changed to DMEM containing 10% FBS and 4500 mg/L of D-glucose. After two days (designated day 0), the cells were induced to differentiate with DMEM containing 10% FBS, 1 μM dexamethasone, and 0.5 mM 3-isobutyl-1-methyl-xanthine, and 1 μg/ml insulin. After two days, the cells were maintained in DMEM supplemented with 10% FBS and 1 μg/ml insulin until day 4, after which DMEM containing 10% FBS and 4500 mg/L of D-glucose was added daily.

2.3. Oil red O staining

3T3-L1 preadipocytes which had differentiated for 6 days were subjected to visualization of intracellular lipid droplets by oil red O staining. Cells were washed three times with phosphate-buffered saline (PBS) and then fixed for 2 min with 3.7% formaldehyde. Oil red O (0.5% in isopropanol) was diluted with water (3:2), filtered through a 0.45-μm filter, and incubated with the fixed cells for 1 h at room temperature. Cells were washed with water, and the stained fat droplets in the cells were then visualized by light microscopy.

2.4. Western blot analysis

The 3T3-L1 cells at different time-points after induction were rinsed twice with PBS, and then lysed in Laemmli buffer (containing 4% SDS 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue, 0.125 M Tris–HCl). Equal amounts of protein lysates were separated by SDS–PAGE and electrophoretically transferred to polyvinylidene difluoride membranes in a transfer buffer consisting of 20 mM Tris–HCl, 150 mM glycine and 20% methanol.

On the other hand, to detect the large Integrator complex consisting of many subunits, the blue native-polyacrylamide gel electrophoresis (BN-PAGE) method was adopted. In order to prepare samples for BN-PAGE analysis, cells were washed with ice-cold PBS and lysed on ice with 1% digitonin-buffer (50 mM Bis–Tris–HCl pH 7.2, 1% digitonin, 50 mM NaCl, 10% glycerol, 0.001% Ponceau S) or 1% Triton ×-100-buffer (50 mM Tris–HCl pH 7.5, 1% Triton ×-100, 150 mM NaCl, 50 mM NaF, 1 mM EDTA, 1 mM EGTA). Before use, each buffer was mixed with 500 μM Na_3VO_4, 100 kallikrein-inactivating units (KIU)/ml aprotinin, 20 μg/ml phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml leupeptin and 5 μg/ml pepstatin. The lysates were centrifuged at 15,000g for 10 min at 4 °C, and 10 μg of the protein of each supernatant were mixed with BN-PAGE sample buffer (Invitrogen) and Coomassie brilliant blue G-250, according to the manufacturer's recommended protocols.

The membranes were blocked with 3% nonfat dry milk or 5% BSA in Tris-buffered saline with 0.1% Tween 20 and incubated with specific antibodies, followed by incubation with horseradish peroxidase-conjugated secondary antibodies. The antigen–antibody interactions were visualized by incubation with ECL chemiluminescence reagent (GE Healthcare).

2.5. Small interfering RNA transfection

The siRNAs against IntS6 and IntS11 were purchased from Invitrogen (Stealth/siRNA duplex oligoribonucleotides), and the transfection of these siRNAs was performed using Lipofectamine RNAi Max (Invitrogen) according to the manufacturer's instructions. After 3T3-L1 preadipocytes had been plated on collagen-I coated dishes, cells were cultured to 50–70% confluence in DMEM containing 10% FBS. Then, the transfection reagents were added to the dishes to give a final concentration of 50 nM of siRNA. Transfection was consistently performed 4 days prior to the induction of adipocyte differentiation.

2.6. RT-PCR and real-time quantitative PCR

Total RNA was isolated using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Reverse transcription was performed using a verso cDNA synthesis kit (Thermo Scientific). Quantitative PCR was performed using the Opticon Monitor (version 3; Bio-Rad). Cycling conditions comprised a 3-min denaturation step at 95 °C, followed by 40 cycles of denaturation (95 °C for 15 s), annealing (60 °C for 30 s), and extension (72 °C for 30 s). After amplification, melting curve analysis was performed. Each sample was amplified in triplicate. The primer sets for mouse genes were as follows.IntS6

For 5′: GCTGGATGGAAAGAAAACCA3′
Rev 5′: TTCTTCCCTGCCCATAGTTG3′

C/ebpβ

For 5′: CAAGCTGAGCGACGAGTACA3′
Rev 5′: AGCTGCTCCACCTTCTTCTG3′

Pparγ2

For 5′: TGGGTGAAACTCTGGGAGATTC3′
Rev 5′: GAGAGGTCCACAGAGCTGATTCC3′

C/ebpα

For 5′: TGGACAAGAACAGCAACGAG3′
Rev 5′: CCTTGACCAAGGAGCTCTCA3′

Glut4

For 5′: CAGATCGGCTCTGACGATG3′
Rev 5′: GGCATTGATAACCCCAATGT3′

Fabp4

For 5′: CATCAGCGTAAATGGGGATT3′
Rev 5′: TCGACTTTCCATCCCACTTC3′

Perilipin

For5′: GATCGCCTCTGAACTGAAGG3′
Rev5′: CTTCTCGATGCTTCCCAGAG3′

3. Results

3.1. Elevated expressions of IntS6 and IntS11 during the early phase of adipose differentiation in 3T3-L1 cells

IntS6 was identified as one of the candidates for binding to AS160 [8], by yeast two hybrid screening. Unfortunately, no endogenous associations between IntS6 and AS160 were observed, though we examined the expression of IntS6 during adipose differentiation of 3T3-L1 cells. The time-dependent expression patterns

of C/EBPδ, C/ebpβ, PPARγ and GLUT4 after stimulation with differentiation-inducing medium containing 10% FBS, 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methyl-xanthine and 1 μg/ml insulin indicated successful induction of adipose differentiation of 3T3-L1 cells (Fig. 1). IntS6 expression was detected when the cells reached confluence (day 1), and high expression levels continued for several days after the induction of differentiation (day 0), while being reduced after the completion of differentiation (day 7,8). Similar high expression during the early step of adipose differentiation was also observed for IntS11, a catalytic subunit of the Integrator complex [9,10], although the induction of IntS11 took place significantly earlier than that of IntS6.

3.2. Expressions of adipose specific proteins were suppressed in 3T3-L1 cells treated with IntS6 or IntS11 siRNA

Since the expressions of IntS6 and IntS11 were transiently elevated during the process of adipocyte differentiation, the roles of these components of the Integrator complex were investigated by suppressing their expressions using specific siRNAs. Treatment with IntS6 siRNA markedly suppressed the amount of IntS6 (Fig. 2A) but did not affect that of IntS11 (Fig. 2B). Expression levels of the major transcriptional factors associated with adipose differentiation were investigated using western blotting and quantitative PCR, which revealed abundant expressions of Glut4, Fabp4 and perilipin in control cells on day 6 after induction while these expressions were markedly blunted in IntS6 siRNA-treated 3T3-L1 cells (Fig. 2A and C). Very similar results were obtained for IntS11 siRNA. Treatment with IntS11 siRNA almost completely abolished the expression of IntS11 protein (Fig. 3A) but not that of IntS6 (Fig. 3B), and suppressed the induction of adipocyte-specific proteins and mRNAs such as those of Glut4, Fabp4 and perilipin (Fig. 3A). In good agreement with these results, oil-red O staining revealed markedly suppressed lipid accumulation in 3T3-L1 cells treated with IntS6 or IntS11 siRNA (Fig. 2D). We also employed other siRNAs, one each for IntS6 and IntS11, and obtained essentially the same results (data not shown).

3.3. Treatment with IntS11 siRNA reduced the IntS6-containing complex and suppressed adipogenic differentiation in 3T3-L1 cells

To examine whether or not the amount of Integrator complex is truly reduced by treatment with IntS6 or IntS11 siRNA, the cell lysates were subjected to undenatured BN-PAGE analysis without using SDS, followed by immunoblotting with anti-IntS6 antibody. In this analysis, a high-molecular-mass band was detected by immunoblotting with anti-IntS6 but not with anti-IntS11 antibody. This band had a size of approximately 1000 kDa, which likely corresponds to the large Integrator complex (Fig. 3C). The Ints-6-containing large complex was markedly reduced by treatment with IntS11 siRNA (Fig. 3C), although IntS11-mediated gene knockdown did not affect the protein level of IntS6 (Fig. 2B). Unfortunately, the anti-IntS11 antibody did not recognize the IntS11-containing large complex electrophoresed by the undenatured BN-PAGE analysis, probably because the epitope region of IntS11 recognized by this antibody would be masked when present as a large complex. Nevertheless, it was demonstrated that knock-down of IntS11 reduces the amount of the large Integrator complex.

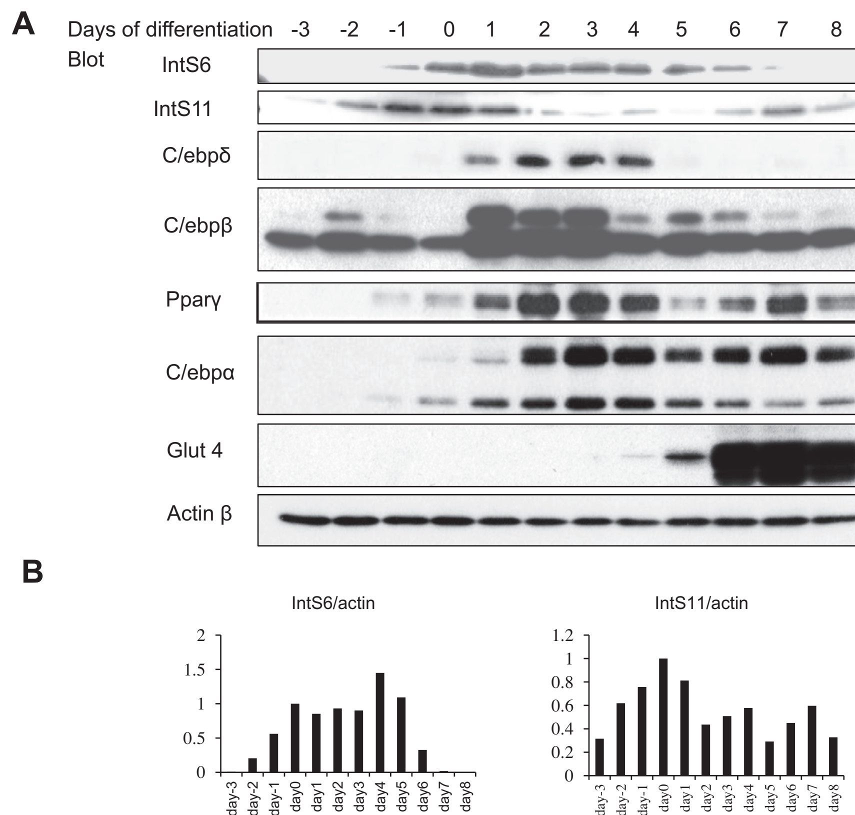

Fig. 1. High expression levels of IntS6 and IntS11 in the early phase of adipose differentiation in 3T3-L1 cells. 3T3-L1 preadipocytes were induced to differentiate into mature adipocytes with DMI and proteins were isolated at various time points (from day 3 to day 8). (A) The protein expressions of IntS6, IntS11, C/ebpδ, β, Pparγ, C/ebpα, Glut4 and β actin were examined by Western blot analysis as described in Section 2. (B) IntS6 and IntS11 expression levels at various time points were quantified. Results are the means of three separate experiments, and one representative blotting dataset is shown.

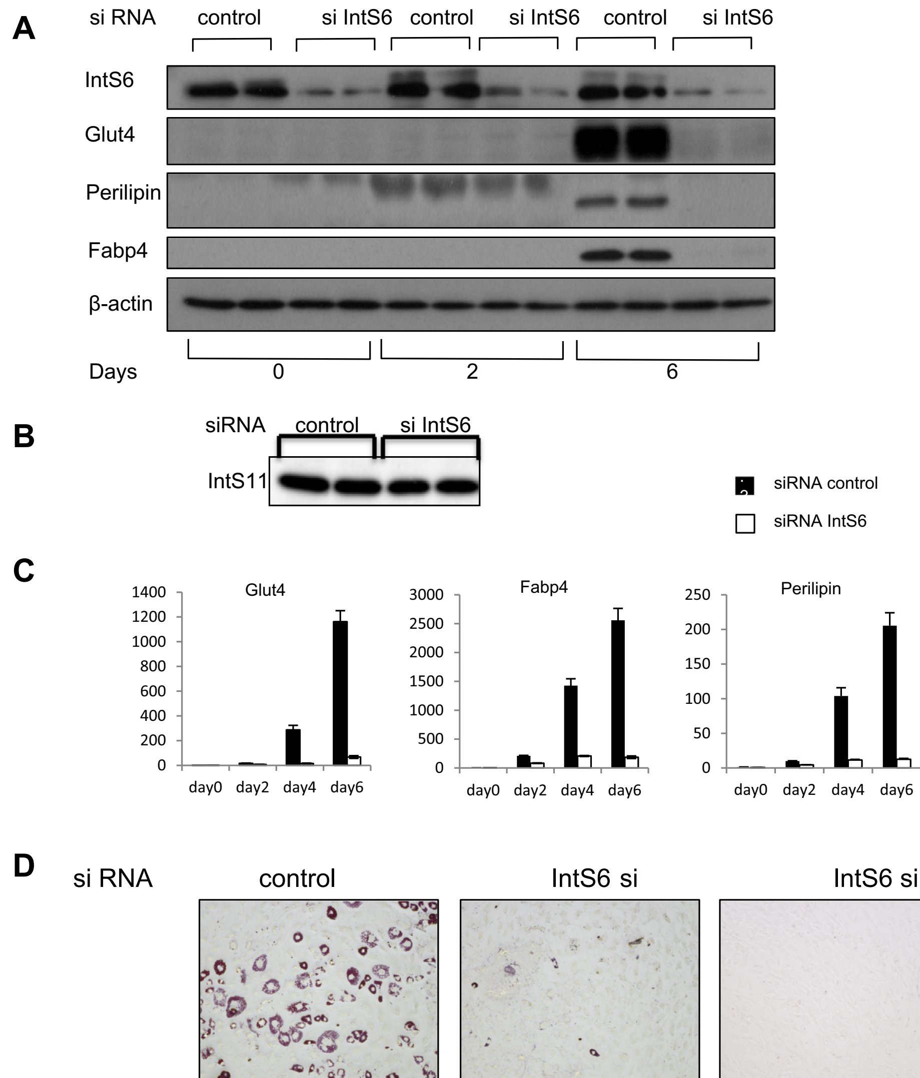

Fig. 2. Effects of IntS6 siRNA treatment on the induction of adipose specific proteins and mRNAs. The expression levels of Glut4, perilipin, and Fabp4 were compared between control siRNA and IntS6 siRNA-treated cells by western blotting (A) (on days 0, 2, 6) and quantitative PCR (qPCR) (C) (on days 0, 2, 4, 6) using their specific primers as described in Section 2. (B) The IntS11 expression level was not affected by treatment with IntS6 siRNA. Results are means of three separate experiments, and one representative blotting dataset is shown. (D) Control, IntS6 or IntS11 siRNA-treated 3T3-L1 cells were cultured in differentiation-inducing medium for 6 days and the accumulation of intracellular lipid was examined by oil red O staining.

3.4. Overexpression of Pparγ2 did not restore the adipose differentiation ability of IntS6 siRNA-treated 3T3-L1 cells

To investigate the mechanism underlying the suppression of adipose differentiation by IntS6 or IntS11 siRNA, the inductions of Pparγ2 and C/EBPβ expressions were investigated. Quantitative PCR showed marked suppression of Pparγ2 induction in 3T3-L1 cells treated with IntS6 siRNA (right panel of Fig. 4A) or IntS11 siRNA (data not shown), while the alteration in C/EBPβ expression was minimal (left panel of Fig. 4A). Thus, we speculated that suppressed induction of Pparγ2 may be the origin of IntS6 or IntS11 siRNA-induced inhibition of adipose differentiation. To assess this possibility, Pparγ2 or control GFP was overexpressed in IntS6 siRNA-treated 3T3-L1 cells, and these cells were incubated with differentiation-inducing medium. The amount of overexpressed Pparγ2 was far higher than that of endogenously expressed Pparγ2 during adipose differentiation (Fig. 4B). However, IntS6 siRNA-treated 3T3-L1 cells failed to differentiate into adipocytes even with high Pparγ2 overexpression (Fig. 4C). This result indicates that the inhibitory effect of IntS6 siRNA treatment on adipose differentiation cannot be attributed solely to the suppressed induction of Pparγ2.

4. Discussion

The splicing machinery is a cornerstone of the diversity of gene expressions. In addition, removal of introns is essential for producing the mature mRNA needed for protein production, and alternative splicing of genes contributes to the generation of protein isoforms with different functions [11,12]. The Integrator complex consisting of 12 subunits from IntS1 to IntS12 in descending order of molecular weight [9] directly associates with the C-terminal domain of RNA polymerase II and mediates processing of the 3′ ends of U1 and U2 sn RNAs, the leading actors in pre-mRNA splicing [13–15], thereby inducing many biological processes. However the presence of other function(s) of this complex cannot be ruled out.

In this study, it was demonstrated that IntS6 and IntS11, components of the Integrator complex, are upregulated after the cells

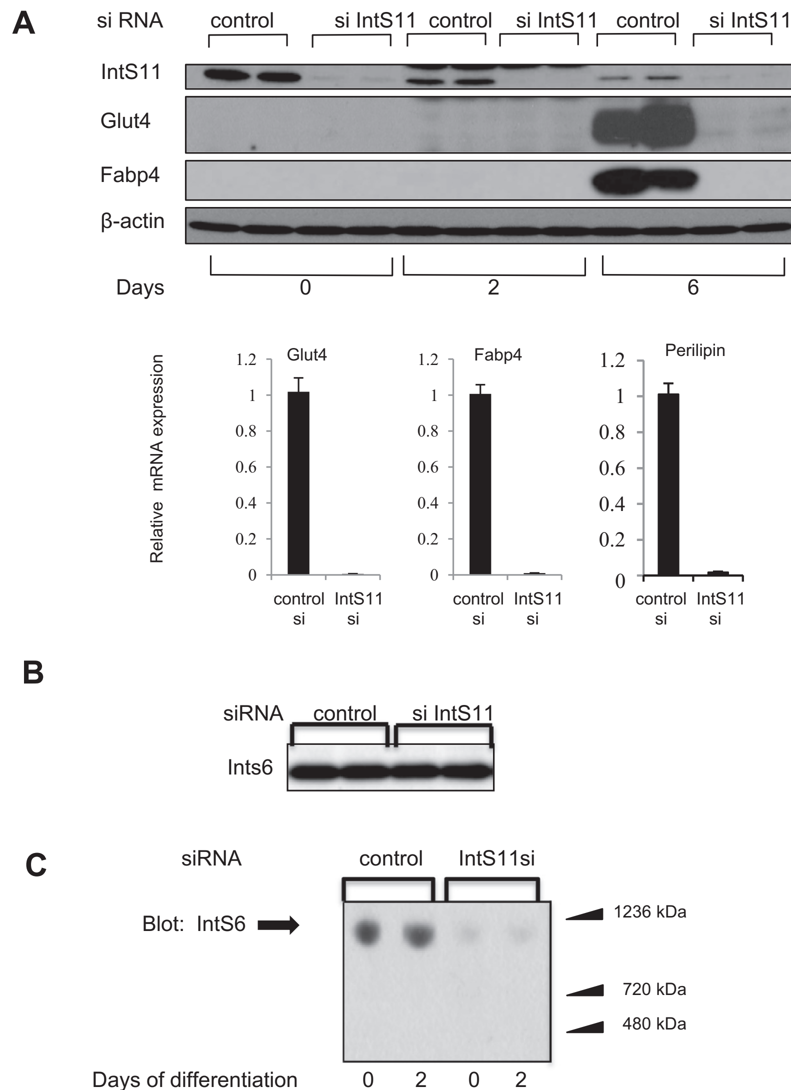

Fig. 3. Effects of IntS11 siRNA treatment on the induction of adipose specific proteins and mRNAs and Integrator complex formation. (A) The expression levels of Glut4, perilipin, and Fabp4 were compared between control siRNA and Ints11 siRNA-treated cells by Western blotting (on days 0, 2, 6) and quantitative PCR (qPCR) (on day 6) using their specific primers as described in Section 2. (B) The IntS6 expression level was not affected by treatment with IntS11 siRNA. (C) siRNA-mediated IntS11 gene knockdown disrupted formation of the Integrator complex. The cell lysates were prepared at the indicated time-points, and subjected to non-denatured BN-PAGE and subsequent immunoblotting with the anti-IntS6 antibody. Results are means of three separate experiments, and one representative blotting dataset is shown.

nearly reached confluence and its high expression levels continues during the early phase of adipose differentiation in the 3T3-L1 cells. In contrast, expression levels of IntS6 and IntS11 were very low, when 3T3-L1 cells are proliferating as fibroblasts (day 3, Fig. 1A). Treatment with IntS11 siRNA reduced not only the amount of IntS11 itself but also the amount of the IntS6-containing complex detected by native-PAGE electrophoresis and subsequent immunoblotting with anti-IntS6 antibody (Fig. 3C), indicating disrupted formation of the Integrator complex due to lack of IntS11. In addition, taking into consideration that gene silencing of either IntS6 or IntS11 markedly inhibited the adipose differentiation of 3T3-L1 cells, it is reasonable to speculate that the Integrator complex plays a critical role in adipose differentiation but not in proliferation.

Furthermore, interestingly, restoration of PPARγ expression cannot compensate for inhibition of adipogenesis by IntS6 or IntS11 knockdown. Because adipogenesis is fully induced by both PPARγ-dependent and -independent pathways, the Integrator complex apparently plays a vital role in the latter pathway. In contrast, gene silencing of IntS6 with siRNA treatment did not affect thymidine incorporation into 3T3-L1 preadipocytes (data not shown). Taken together, these observations indicate that IntS6, or more probably the Integrator complex, is likely to play an indispensable role in the process from C/EAPδ expression to the final downstream induction of adipose differentiation, but not in cell proliferation.

In terms of the relationship of the subunits of the Integrator complex with other forms of cell differentiation, a recent study showed that knockdown of IntS5 and IntS11 in zebrafish embryos led to aberrant splicing of smad1 and smad5 RNA and thereby impaired red blood cell differentiation [10]. Another study showed that targeted disruption of mouse IntS1, the largest subunit of the complex and presumed to act as a scaffold for the complex, causes impaired processing of U2 sn RNA and growth arrest in early blastocyst stage embryos [16]. Furthermore, IntS6 was identified as a tumor suppressor gene and termed "deleted in cancer 1" (Dice1) since it was lost or highly downregulated in non-small cell lung carcinomas, indicating that the presence of IntS6 is not essential for cell survival. These data suggest that the Integrator complex plays critical roles in the regulation of certain types of

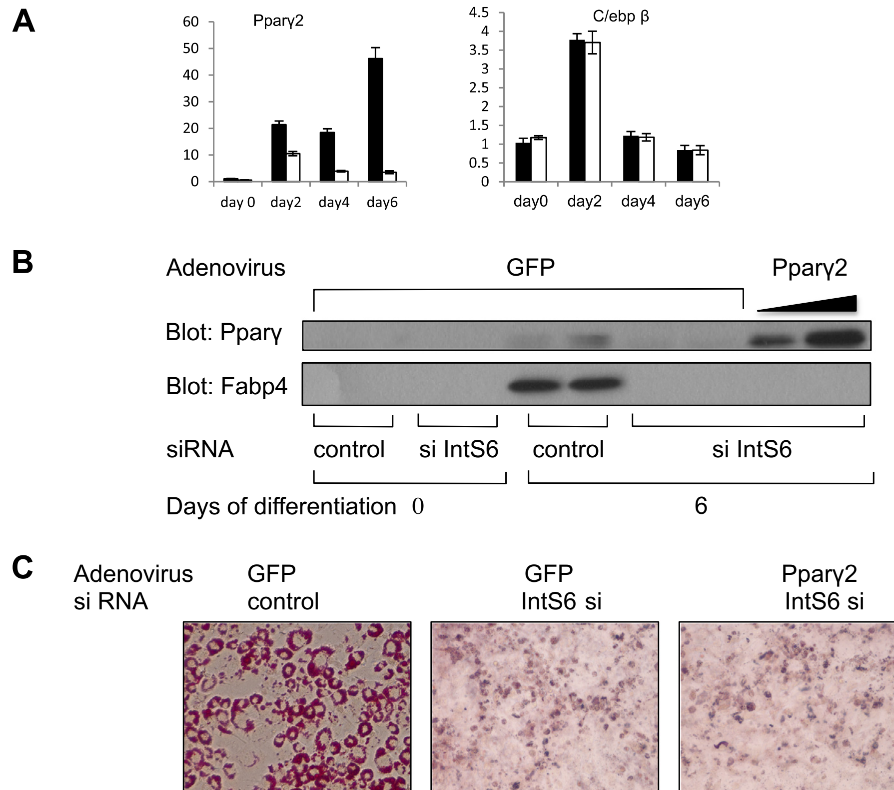

Fig. 4. Suppression of adipose differentiation with IntS6 or IntS11 siRNA was not restored by adenoviral overexpression of Pparγ2. (A) Pparγ2 and C/ebpβ mRNA levels were examined in 3T3-L1 cells treated with control or IntS6 siRNA (on days 0, 2, 6) by real time PCR analysis. (B, C) Adenovirus expressing Pparγ2 was transfected into IntS6 siRNA treated cells one day prior to adding differentiation medium (DMI) and incubated for six days. The expression levels of Pparγ2 and Fabp4 were examined by Western blotting, and lipid accumulation in the cells was analyzed by oil-red O staining. Results are means of three separate experiments, and one representative blotting and staining dataset is shown.

differentiation, although it is unclear whether or not these functions are mediated by U1 and U2 sn RNA processing and its resultant splicing machinery.

In conclusion, the expression levels of Integrator complex components, IntS6 and IntS11, increase during adipose differentiation. However, details of the functions of the Integrator complex in the regulation of cell differentiation remain to be elucidated. This is the first study strongly suggesting a critical role of the Integrator complex in adipose differentiation, and further studies are necessary to reveal the underlying molecular mechanisms.

References

[1] A.R. Saltiel, You are what you secrete, Nat. Med. 7 (2001) 887–888.
[2] B.M. Spiegelman, J.S. Flier, Adipogenesis and obesity: rounding out the big picture, Cell 87 (1996) 377–389.
[3] R.P. Brun, J.B. Kim, E. Hu, B.M. Spiegelman, Peroxisome proliferator-activated receptor gamma and the control of adipogenesis, Curr. Opin. Lipidol. 8 (1997) 212–218.
[4] E.D. Rosen, C.J. Walkey, P. Puigserver, B.M. Spiegelman, Transcriptional regulation of adipogenesis, Genes Dev. 14 (2000) 1293–1307.
[5] P. Cornelius, O.A. MacDougald, M.D. Lane, Regulation of adipocyte development, Annu. Rev. Nutr. 14 (1994) 99–129.
[6] Y. Nakatsu, H. Sakoda, A. Kushiyama, H. Ono, M. Fujishiro, N. Horike, M. Yoneda, H. Ohno, Y. Tsuchiya, H. Kamata, H. Tahara, T. Isobe, F. Nishimura, H. Katagiri, Y. Oka, T. Fukushima, S. Takahashi, H. Kurihara, T. Uchida, T. Asano, Pin1 associates with and induces translocation of CRTC2 to the cytosol, thereby suppressing cAMP-responsive element transcriptional activity, J. Biol. Chem. 285 (2010) 33018–33027.
[7] Y. Nakatsu, H. Sakoda, A. Kushiyama, J. Zhang, H. Ono, M. Fujishiro, T. Kikuchi, T. Fukushima, M. Yoneda, H. Ohno, N. Horike, M. Kanna, Y. Tsuchiya, H. Kamata, F. Nishimura, T. Isobe, T. Ogihara, H. Katagiri, Y. Oka, S. Takahashi, H. Kurihara, T. Uchida, T. Asano, Peptidyl-prolyl cis/trans isomerase NIMA-interacting 1 associates with insulin receptor substrate-1 and enhances insulin actions and adipogenesis, J. Biol. Chem. 286 (2011) 20812–20822.
[8] H. Sano, S. Kane, E. Sano, C.P. Mîinea, J.M. Asara, W.S. Lane, C.W. Garner, G.E. Lienhard, Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation, J. Biol. Chem. 278 (2003) 14599–14602.
[9] I. Wieland, K.C. Arden, D. Michels, L. Klein-Hitpass, M. Böhm, C.S. Viars, U.H. Weidle, Isolation of DICE1: a gene frequently affected by LOH and downregulated in lung carcinomas, Oncogene 18 (1999) 4530–4537.
[10] S. Tao, Y. Cai, K. Sampath, The Integrator subunits function in hematopoiesis by modulating Smad/BMP signaling, Development 136 (2009) 2757–2765.
[11] A. Kalsotra, T.A. Cooper, Cooper functional consequences of developmentally regulated alternative splicing, Nat. Rev. Genet. 12 (2011) 715–729.
[12] H. Keren, G. Lev-Maor, G. Ast, Alternative splicing and evolution: diversification, exon definition and function, Nat. Rev. Genet. 11 (2010) 345–355.
[13] S.B. Patel, M. Bellini, The assembly of a spliceosomal small nuclear ribonucleoprotein particle, Nucleic Acids Res. 36 (2008) 6482–6493.
[14] D. Baillat, M.A. Hakimi, A.M. Naar, A. Shilatifard, N. Cooch, R. Shiekhattar, Integrator a multiprotein mediator of small nuclear RNA processing, associates with the C-terminal repeat of RNA polymerase II, Cell 123 (2005) 265–276.
[15] M.B. Warf, J.A. Berglund, Role of RNA structure in regulating pre-mRNA splicing, Trends Biochem. Sci. 35 (2010) 169–178.
[16] T. Hata, M. Nakayamaa, Targeted disruption of the murine large nuclear KIAA1440/Ints1 protein causes growth arrest in early blastocyst stage embryos and eventual apoptotic cell death, Biochim. Biophys. Acta 1773 (2007) 1039–1051.