Biochemical and Biophysical Research Communications 421 (2012) 591–598

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Feed-back regulation of disabled-2 (Dab2) p96 isoform for GATA-4 during differentiation of F9 cells

Jung Ah Kim [a], Seong Ho Bae [a], Young Joon Choi [a], Kyung Hyun Kim [b], Sung Soo Park [a,*]

[a] School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Republic of Korea
[b] Department of Biotechnology and Bioinformatics, College of Science and Technology, Korea University, Chungnam, South Korea

ARTICLE INFO

Article history:
Received 27 March 2012
Available online 14 April 2012

Keywords:
Disabled-2 (Dab2)
Extra-embryonic endoderm (ExE)
Feed-back loop
GATA-4

ABSTRACT

F9 embryonic carcinoma (EC) cells undergo extra-embryonic endodermal (ExE) differentiation in response to retinoic acid (RA) treatment, which induces the expression of two isoforms (p96 and p67) of the adaptor protein, Disabled-2 (Dab2). In the current study, constitutive and ectopic expression of the p96 isoform induced ExE differentiation in F9 EC cells in the absence of RA treatment via the activation of GATA-4 by p96. During the RA-induced differentiation process, Dab2 expression is induced by the GATA factors in a coherent feed-forward loop; on the other hand, we showed that p96 regulates GATA-4 in a positive feed-back manner in this study. Our results indicate that p96 Dab2 plays a key role in the ExE differentiation process.

1. Introduction

F9 embryonic carcinoma (EC) cells resemble the pluripotent stem cells of the inner cell mass (ICM) of blastocysts. Depending on the culture conditions, retinoic acid (RA) induces differentiation of F9 cells into three different cell types, primitive endoderm (PrE), parietal endoderm (PE) and visceral endoderm (VE) [1,2], mimicking the early events that occur in 3–5 days blastocysts.

The zinc finger-containing transcription factors, GATA-4, 5 and 6, are expressed in the extra-embryonic endoderm (ExE) lineages during early mouse development [3,4]. GATA-4 and GATA-6 are expressed in the PrE and the derived VE and PE of the ExE [3,5]. In cell culture studies, either GATA-4 or GATA-6 overexpression in ES cells induces differentiation toward ExE [6]. In addition, it was reported that GATA-4 is required for GATA-6-mediated Disabled-2 (Dab2) induction, since the transfection of GATA-6 into wild-type ES cells induced Dab2 expression, while transfection into GATA-4 (−/−) ES cells did not [7].

Dab2 is a multifunctional protein involved in several processes including cellular signaling, oncogenesis, endocytosis, cell positioning and embryonic development [8–14]. Furthermore, the *Dab2*$^{-/-}$ embryo is early embryonic lethal [11].

The two spliced isoforms (p96, p67) of Dab2 are both strongly up-regulated during F9 cell differentiation by RA [15] and are differentially expressed in a tissue-specific manner. We reported previously that both major isoforms (p96 and p67) are expressed during RA-induced differentiation of F9 cells [16,17]. However, the specific function of each Dab2 isoform in F9 cells is still unclear.

In the current study, small interfering RNA (siRNA) was used to knockdown each Dab2 isoform to determine its role in RA-induced F9 cell differentiation. The data demonstrated that each isoform plays a specific role in F9 differentiation and that the p96 isoform is a key molecular regulator of ExE differentiation and can induce differentiation in F9 cells in the absence of RA.

2. Materials and methods

2.1. Cell culture and transient transfection

F9 mouse EC cells were cultured as monolayers on gelatinized surfaces as described previously [18]. Wild-type F9 and 96+ cells were cultured and differentiated with all-*trans*-RA (1 μM, Sigma) alone [15,19]. Transfections were performed using Lipofectamine 2000 reagent according to the manufacturer's instructions (Invitrogen) [20].

2.2. DNA constructs and antibodies

Expression constructs, pEF-Myc/his-p96 and pEF-Myc/His-p67, were cloned into pEF-Myc/His vectors (Invitrogen). Anti-GATA-4 antibody was purchased from Santa-Cruz Biotechnology

Abbreviations: RA, all-*trans*-retinoic acid; PrE, primitive endoderm; ExE, extra-embryonic endoderm; VE, visceral endoderm; PE, parietal endoderm; dbcAMP, dibutyryl cyclic AMP.

* Corresponding author. Address: The Laboratory of Developmental Genetics, School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Republic of Korea. Fax: +82 2 929 1864.
E-mail address: sspark@korea.ac.kr (S.S. Park).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.04.051

(Delaware, CA, USA), the anti-Dab2 antibody from Transduction Laboratories (San Jose, CA, USA), and the anti-α-tubulin and β-actin antibodies from Sigma–Aldrich (St. Louis, MO, USA).

2.3. Semi-quantitative RT-PCR and quantitative real-time RT-PCR

Semi-quantitative RT-PCR analysis was described previously [6] and quantitative real-time RT-PCR detailed in supplemental Materials and Methods. All data were obtained from PCR reactions performed in triplicate for four different cultures.

2.4. siRNA experiments

RNA interference (RNAi) was used to knockdown each Dab2 isoform and detailed in supplemental Materials and Methods.

2.5. Establishment of stable cell lines expressing each isoform of Dab2

To establish stable cell lines, clones encoding *p96* and *p67* in pEF-Myc/his were transfected into cultured F9 EC cells without RA, and about 60 neomycin-resistant colonies were selected. After expansion of the transfected clones, cell lines, each expressing one of the two Dab2 isoforms, were characterized for Dab2 expression using Western Blot analysis.

3. Results

3.1. Inhibition of RA-induced differentiation in F9 EC cells by p96 knock-down

F9 EC cells are induced to differentiate into PrE or VE depending on the length of the RA treatment period, or into PE following the addition of dbcAMP [2,21,22]. The Dab2 protein was observed strongly in its two major isoforms (p96 and p67) after 3 days after treatment with RA [15] (Supplementary Fig. S1).

To determine the role of each Dab2 isoform in RA-induced F9 cell differentiation, RA-treated F9 cells were transiently transfected with an siRNA specific for the p96 isoform (si96), an siRNA specific for the p67 isoform (si67), or an siRNA directed against both isoforms of Dab2 (siDab2) to knockdown the p96, p67 or both isoforms, respectively. A scrambled siRNA was used as the negative

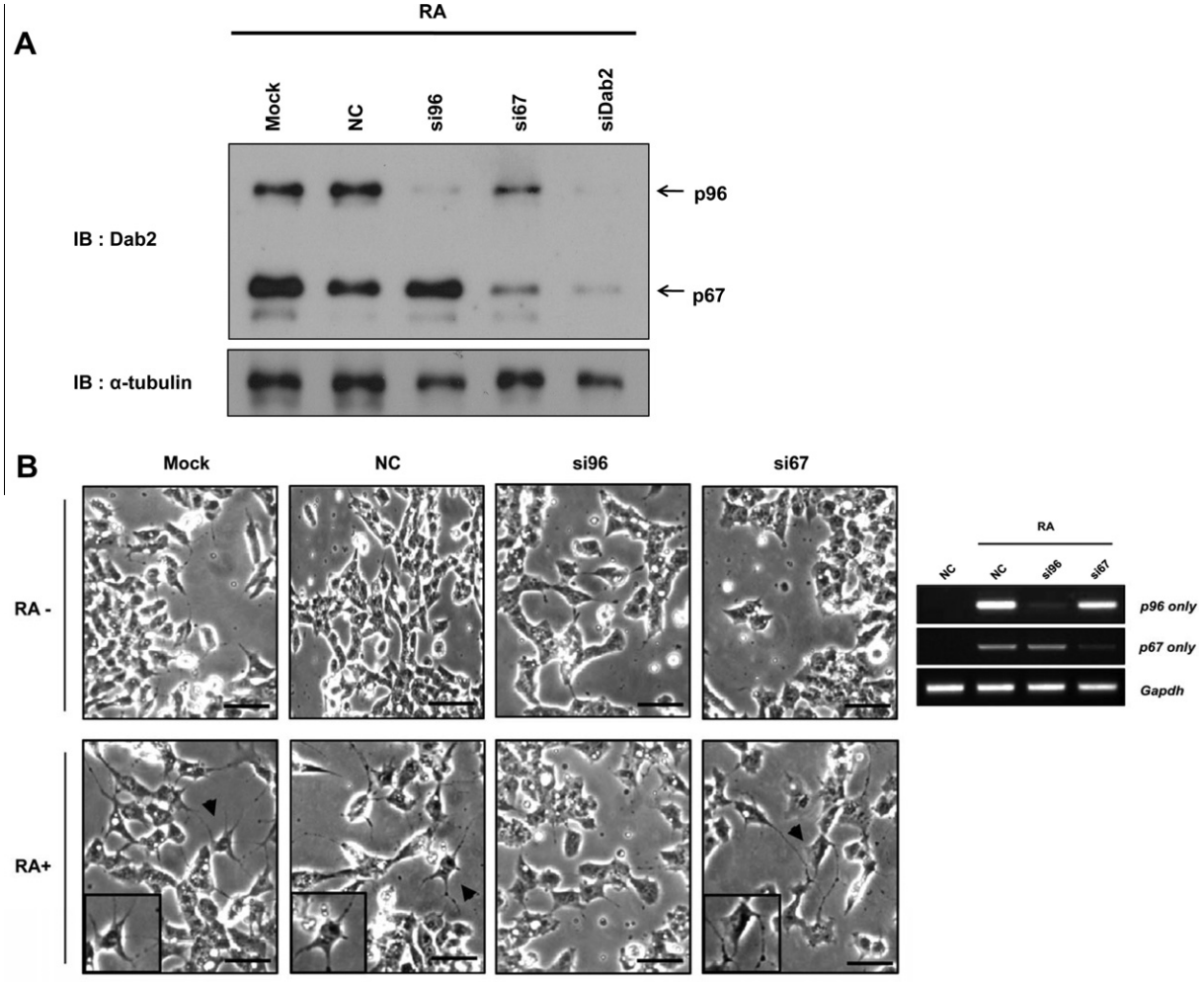

Fig. 1. The effect of p96 knockdown on RA-induced differentiation of F9 EC cells. (A) Knockdown of Dab2 isoform expression by siRNA in RA-treated F9 cells: F9 cells were treated with RA for 1 day. After transfection with each siRNA, the cells were cultured with RA for 2 days. Dab2 expression was monitored by Western blot analysis. (B) Morphological changes due to the silencing of each Dab2 isoform in differentiated F9 cells following RA treatment: The specific siRNAs were transfected separately into F9 cells and then, the cells were incubated for 2 days in the absence (upper panel) or presence (lower panel) of RA. *Bar*, 50 μm. Arrows indicated below boxed regions are shown at higher magnification. The silencing of Dab2 expression was analyzed by RT-PCR analysis. (C) Quantitative real-time PCR analysis of *GATA-4* and *GATA-6* gene expression in Dab2 knockdown F9 cells: Following transfection of specific siRNAs, the cells were incubated with RA for 2 days or without RA. The relative expression of all mRNA levels was normalized to the endogenous reference gene, *gapdh*. The mRNA expression of level of each gene in NC-transfected cells which were cultured in the absence of RA was arbitrarily set at 1. Data represent the mean ± s.d. of four independent experiments performed in triplicate. The silencing of Dab2 expression was analyzed by RT-PCR analysis.

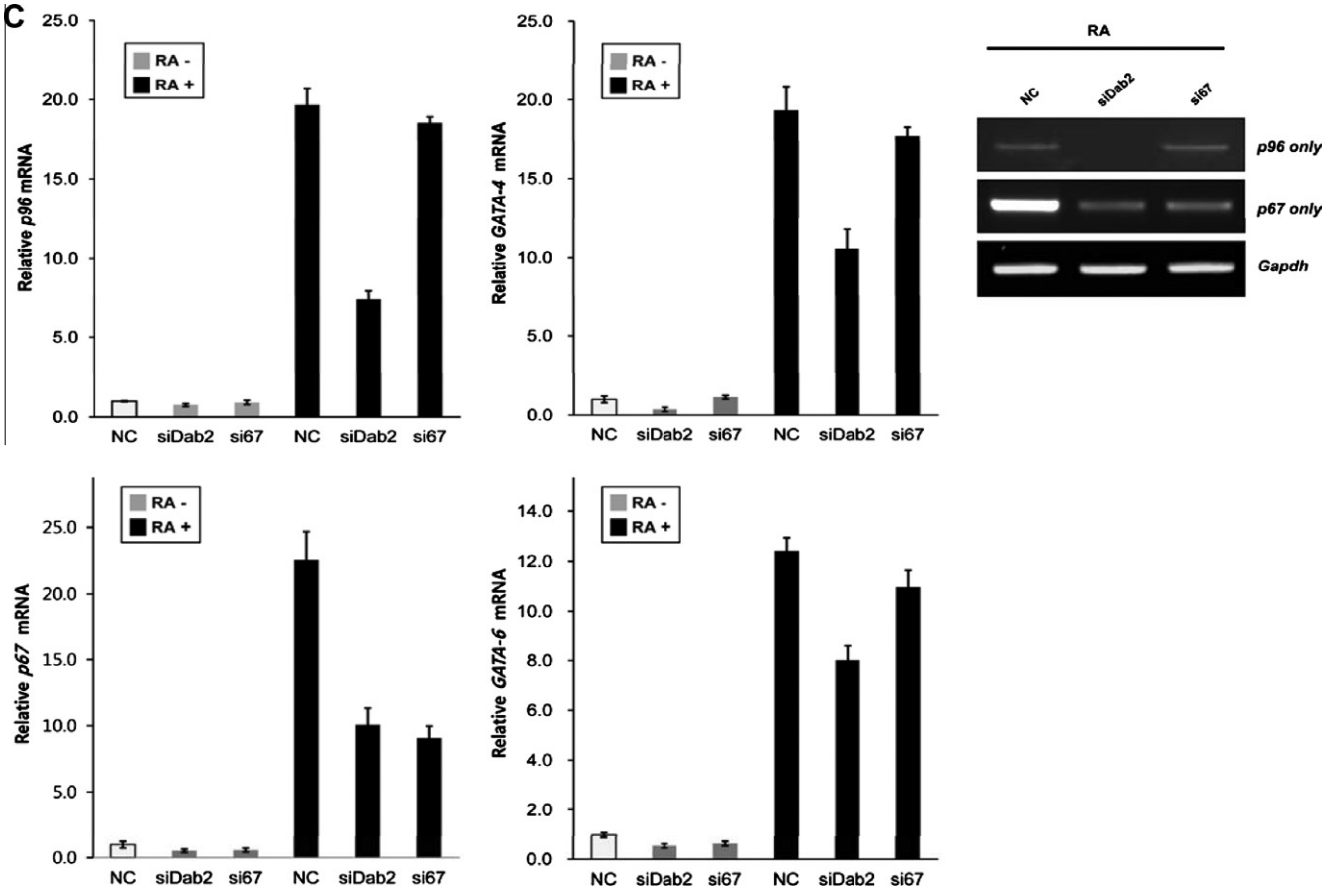

Fig. 1 (*continued*)

control (NC). Expression of both Dab2 isoforms decreased in si-Dab2-transfected F9 cells, whereas expression of the p67 isoform was reduced in si67-transfected F9 cells, respectively (Fig. 1A).

RA treatment of F9 cells induced morphological changes, which manifested as an elongated and stretched polygonal morphology. RA-untreated cells transfected with specific siRNA showed no morphological changes (Fig. 1B; upper panel). However, the morphological changes in si96-transfected cells were either significantly inhibited or absent, compared with those in the NC and si67-transfected cells (Fig. 1B; lower panel). The data suggested that, following knockdown of p96, F9 cells failed to differentiate in response to RA.

To investigate whether the knockdown of p96 Dab2 affected the RA-induced differentiation process, semi-quantitative RT-PCR was used to analyze the expression of the differentiation markers *GATA-4, 6*, and *Collagen IV* [3,4,23]. The endogenous expression levels of *GATA-4, 6*, and *Collagen IV* in si96-transfected cells were reduced compared with those in NC- and si67-transfected cells (Supplementary Fig. S2).

To investigate whether the simultaneous knockdown of two isoforms of Dab2 affected the RA-induced differentiation process, real-time RT-PCR was used to analyze the expression of the differentiation markers. Semi-quantitative RT-PCR using p96 and p67 isoform-specific primers revealed that both isoforms and the p67 isoform were down-regulated in siDab2- and si67-transfected cells, respectively (Fig. 1C). As shown in Fig. 1C, the endogenous expression levels of *GATA-4* and *GATA-6* in siDab2-transfected cells were reduced compared with those in si67-transfected cells (Fig. 1C). Similar results were observed by analyzing the expression of the endoderm differentiation marker in si96-transfected cells (Supplementary Fig. S2). These data indicated that p96, rather than p67, is involved in promoting RA-induced differentiation in F9 EC cells.

3.2. The induction of GATA-4 following transient expression of p96 in F9 EC cells

Master transcriptional regulators, such as the GATA factors, regulate the pluripotency and differentiation of stem cells [6]. To investigate whether p96 is involved in regulating the expression of the master regulator candidates, GATA-4, 6, Oct3/4, Nanog and Sox-2, during F9 cells differentiation [7,24–26], the endogenous mRNA levels of each of the potential master regulators were analyzed following the transient expression of each of the two Dab2 isoforms. RA-untreated F9 cells were transiently transfected with plasmids encoding either p96 or p67, and semi-quantitative RT-PCR was used to determine the gene expression using specific primers (Fig. 2A). The p96 and p67 isoform-specific primers confirmed that p96 and p67 were expressed in the respective transfected cells. It is worth noting that *GATA-4* mRNA was detected in p96-tranfected cells (Fig. 2A). These data indicated that expression of p96 can induce the expression of *GATA-4* in F9 cells without RA.

To investigate whether p96 is involved in regulating the expression of GATA-4 in RA-treated F9 cells, endogenous GATA-4 protein levels were determined by transient expression of the p96 isoform of Dab2 (Fig. 2B) and using siRNAs specific to Dab2 (si96 or si67) (Fig. 2C). Endogenous GATA-4 expression in p96-transfected cells showed greater upregulation than that in cells transfected with empty vectors, in comparison with α-tubulin expression levels. There were no obvious differences between GATA-4 protein

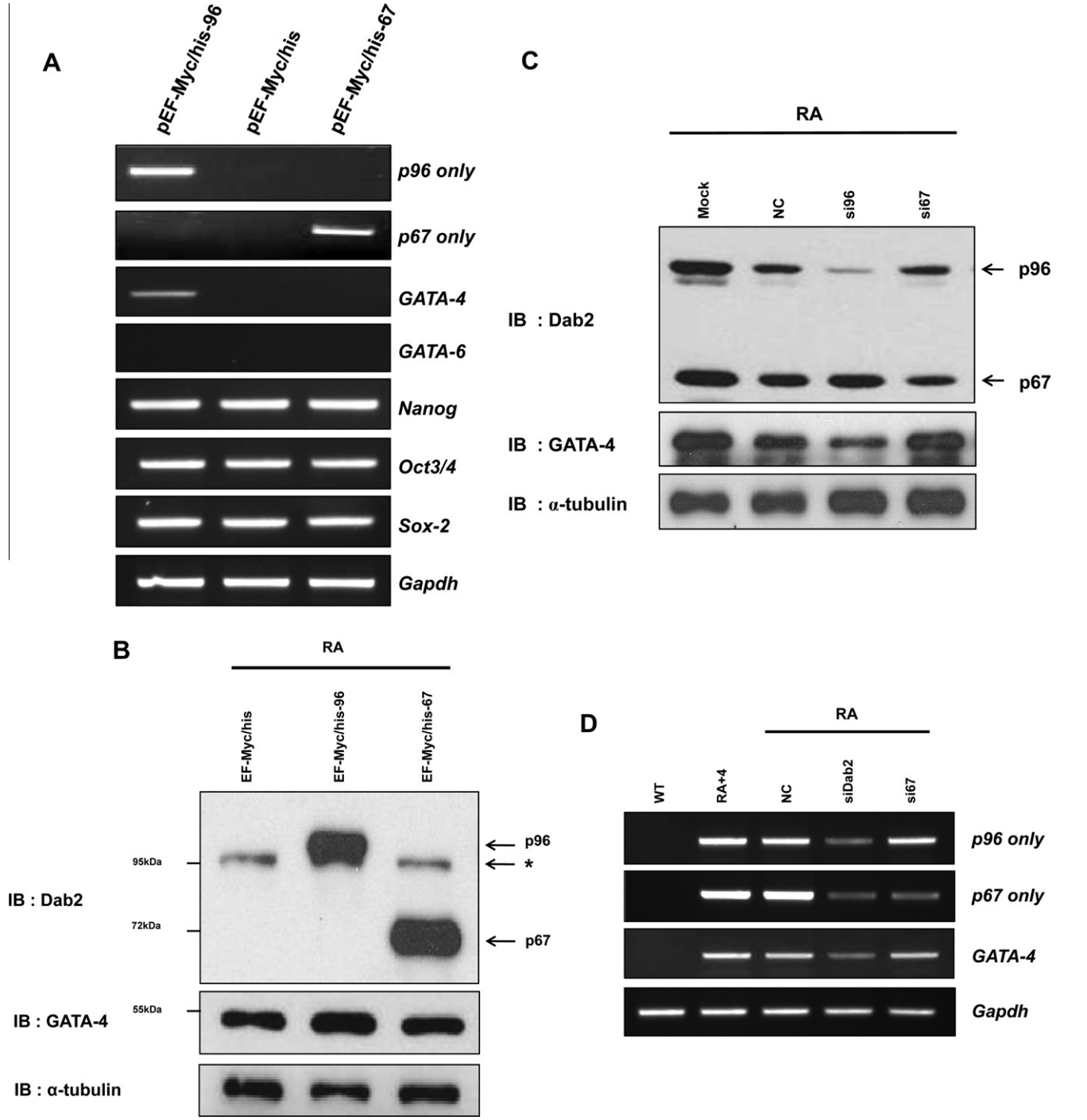

Fig. 2. Induction of GATA-4 by transient expression of p96 without RA. (A) Transient expression of p96 induces GATA-4 expression: RA-untreated F9 cells were transfected with pEF-Myc/his- *p96* or -*p67* cDNA for 4 days and analyzed using semi-quantitative RT-PCR. (B) Transient expression of p96 induces GATA-4 expression in RA-treated F9 cells: RA-treated F9 cells for 1 day were transfected with either pEF-Myc/his-p96 or -p67. Following transfection, the cells analyzed by Western Blot. (C) The effect of p96 knockdown on the expression of *GATA-4*: After transfection of each siRNA into RA-treated F9 cells for a day, cells were incubated with RA for an additional 1 day (RA). Total cell lysates were subjected to Western blot. The asterisk marks a nonspecific band. (D) The effect of Dab2 knockdown on the expression of *GATA-4*: As a positive control, wt F9 cells were cultured with 1 μM RA for 4 days (RA+4). RA-treated for F9 cells for 2 days were transfected with each siRNAs. After transfection, the cells were incubated with RA for an additional 2 days (RA) and analyzed by using semi-quantitative RT-PCR.

expression levels in p67 transiently-transfected cells and controls (Fig. 2B). In contrast, the endogenous expression level of the GATA-4 protein in si96-transfected F9 cells was lower than in cells transfected with si67 and NC (Fig. 2C). Similar results were observed by analyzing the expression of GATA-4 in siDab2-transfected cells, compared with NC and si67-transfected cells (Fig. 2D). These data implied that GATA-4 expression is affected by the p96 Dab2 in RA-induced differentiation of F9 cells.

3.3. Induction of F9 EC cell differentiation following stable p96 expression

To examine the effect of Dab2 expression on cell differentiation, stable cell lines, each expressing one of the Dab2 isoforms, were established without RA treatment (Fig. 3A). Interestingly, the stable p96-expressing cells (96+ cells) expressed endogenous p67 in addition to p96, although they were transfected only with the

Dab2 p96; in contrast, the stable p67-expressing cells (67+ cells) only expressed p67 compared with undifferentiated wild-type F9 cells (wt cells) and empty vector-transfected F9 cells (control cells) as verified by Western Blot (Fig. 3A) and semi-quantitative RT-PCR analysis (Supplementary Fig. S3). In addition, the 96+ cells had an enlarged morphology, while there were no morphological changes in control, wt, or 67+ cells (data not shown). These results suggested that stable expression of p96 in F9 cells, without RA treatment, leads to the expression of endogenous Dab2 and to changes in cell morphology.

To determine whether stable expression of each isoform of Dab2 in F9 cells was linked to cellular differentiation, real-time RT-PCR was used to analyze the expression of several differentiation markers [6], including the stem cell marker, *Oct3/4*, and the PrE differentiation marker, *GATA-4*, compared with wt, control cells and RA-treated F9 cells for 4 days (RA+4) (Fig. 3B). The 96+ cells showed significant expression of *GATA-4* and *GATA-6* (at similar levels to those seen in RA-treated F9 cells) compared with control and 67+ cells (Fig. 3B). Similar result was observed by analyzing the expression of VE endoderm differentiation marker, *alpha-fetoprotein* (*AFP*) (data not shown). The abrogated expression of Oct3/4 in 96+ cells implies that the cells had undergone differentiation (Fig. 3B). These data suggested that stable expression of p96 in F9 cells can induce differentiation even in the absence of RA.

To examine whether stable p96-expressing F9 cells differentiated to the ExE lineage without RA, semi-quantitative RT-PCR was used to analyze the expression of various differentiation markers [6] (Fig. 3C). The transcript expression levels of the stem cell markers *UTF-1* and *Rex1* decreased significantly in 96+ cells compared with RA-untreated wt and control cells (Fig. 3C). The expression of *transthyretin* (*Ttr*) and *AFP*, both VE markers in differentiated cells, was detected in RA-untreated 96+ cells and RA+4 cells (Fig. 3C). In the condition of treatment of RA, the expression levels of *TM* (PE differentiation markers) has no obvious differences, compared to *AFP* and

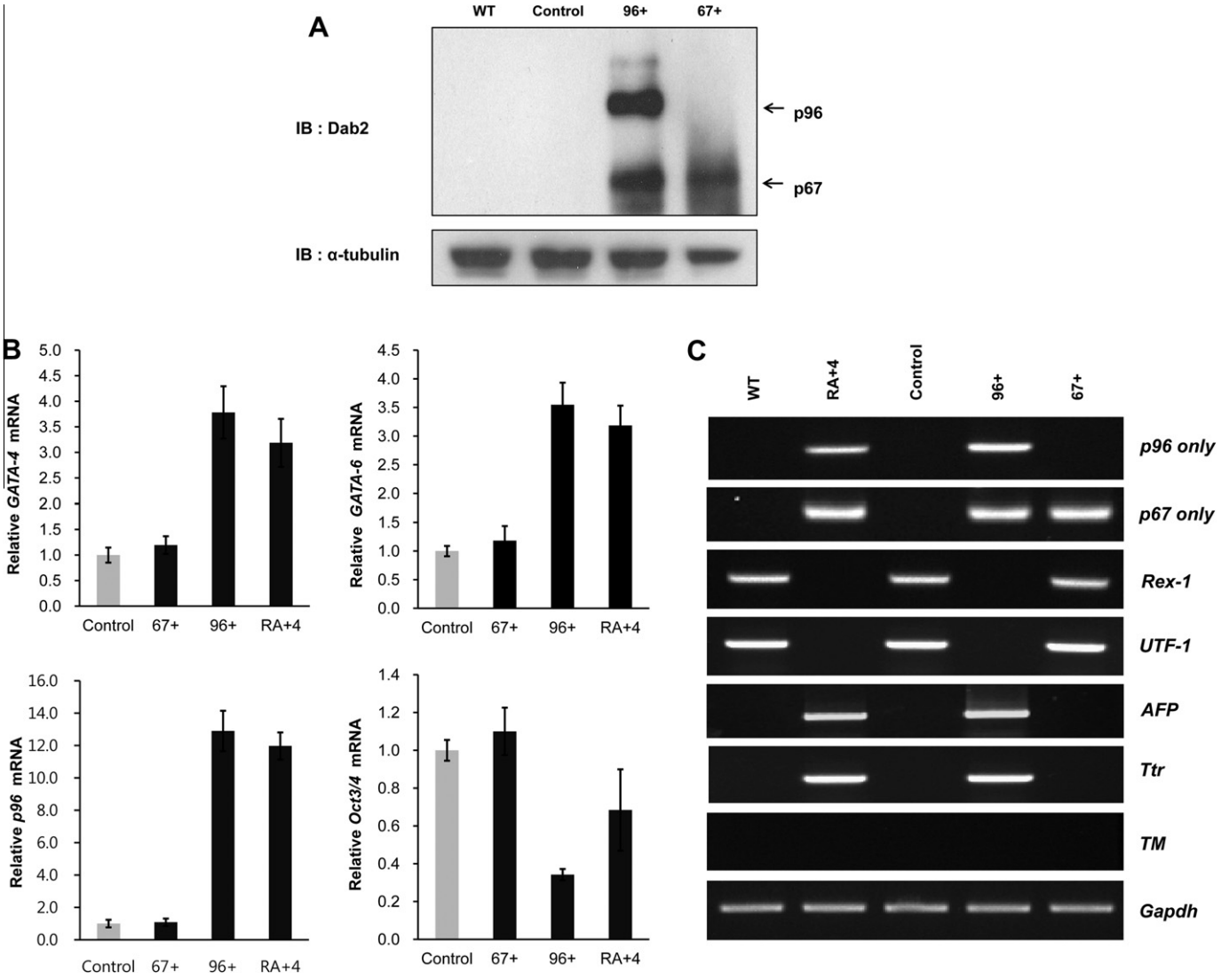

Fig. 3. Construction of stable Dab2-expressing cell lines in RA-untreated F9 cells. (A) Construction of stable p96- and p67-expressing cell lines in RA-untreated F9 cells: 96+, 67+, Control, and WT cells were cultured without RA and were subjected to Western blotting. (B) Quantitative real-time PCR analysis of *GATA-4* and *GATA-6* gene expression in stable cell lines: The 96+, 67+and Control cells were cultured without RA, while wt F9 was treated with RA for 4 days (RA+4) to analyze the expression level of the differentiation markers. The relative gene expression mRNA levels were normalized to *gapdh*. The mRNA expression of level of each gene in control cells was arbitrarily set at 1. Data represent the mean ± s.d. of four independent experiments performed in triplicate. (C) Stable-expression of the Dab2 p96 in F9 cells promotes differentiation of extra-embryonic endoderm (ExE) without RA: RA+4, WT, Control, 67+ and 96+ cells were cultured without RA and then analyzed by semi-quantitative RT-PCR for differentiation markers.

Ttr mRNA transcripts notably upregulated in 96+ cells (Fig. 3C). These data indicated that stable p96 Dab2-expressing F9 cells can enhance VE differentiation in the absence of RA and suggested that stable p96 expression in F9 cells could be involved in promoting the differentiation of ExE.

3.4. Role of p96 during the early stages of F9 cell differentiation

Our data suggest that the p96 is critical for the early process of ExE differentiation. It, however, was not available to investigate the triggering mechanism for the differentiation process in the stable cell line, since the expression of p96 was already in steady-state level and could not be controlled step by step at an early stage of differentiation.

To mimic the early stages of the differentiation process mediated by stable expression of p96, a plasmid encoding p96 was transiently transfected into RA-untreated F9 cells (Fig. 4A; left panel). The expression of *GATA-4* was detected in p96-transfected wild-type F9 cells (Fig. 4A; left panel). In addition, expression of the *Ttr* and *GATA-6* transcripts was not observed. These data suggested that GATA-4 expression is mediated by p96 expression and the RA-independent differentiation by stable p96 expression might be mediated through GATA-4 expression at the early stage.

We have observed that the withdrawal of RA after treatment for only 1 day did not reverse but did retard the ExE differentiation process (data not shown). To investigate the role of Dab2 expression during the early stages of RA-induced differentiation, F9 cells treated with RA for 1 day were transiently transfected with a plasmid encoding p96, after which RA treatment was stopped (Fig. 4A; right panel). The expression levels of *GATA-4, -6* and *Ttr* mRNAs in p96-transfected cells in spite of removal of RA treatment showed a marked difference from those in vector-transfected cells (Fig. 4A; right panel). Notably, both isoforms of Dab2 were expressed in p96 only-transfected cells compared to in empty vector-transfected cells (Fig. 4A; right panel) and p96-transfected in wild type F9 cells (Fig. 4A; left panel). These data indicated that the expression of p96 in RA-stimulated F9 cells can induce normally the differentiation process in spite of the withdrawal of RA.

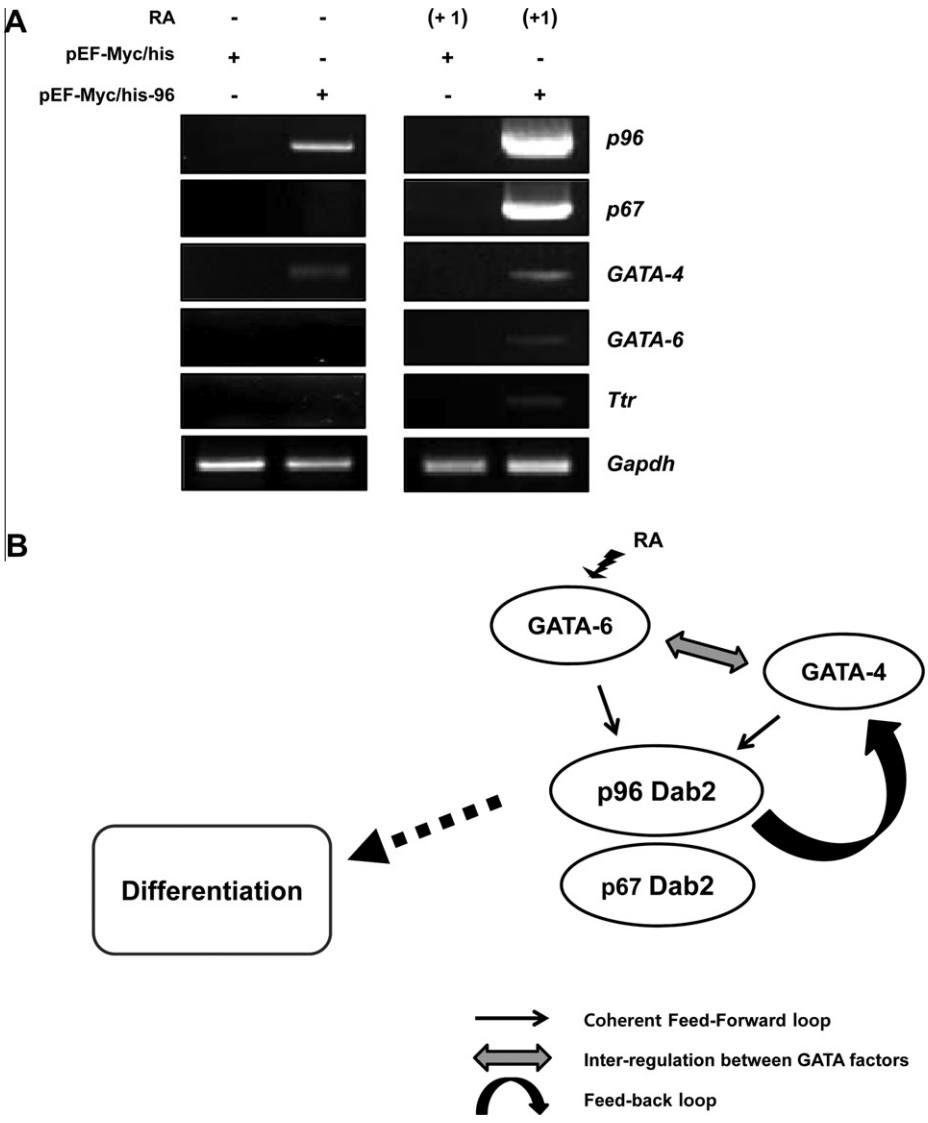

Fig. 4. Role for p96 in differentiation in F9 cells through the regulation of GATA-4 expression. (A) Promotion to differentiation process by transient expression of p96 in F9 cells: RA-untreated or RA-treated F9 cells for 1 day (+1) were transfected with pEF-Myc/his-96. After transfection of expression vector, the transfected F9 cells were cultured for an additional 2 days. RA was removed from the culture medium (+1) after transfection. Gene expression was analyzed by semi-quantitative RT-PCR. (B) Schematic diagram of the coherent feed-forward and feed-back loops in the RA-induced differentiation pathway: Each regulatory mode is denoted by different arrows.

4. Discussion

GATA factors (GATA-4 and -6), which contain zinc-finger motifs, are expressed first and followed by Dab2 in the RA-induced differentiation pathway of ES or EC cells [6,23] (Supplementary Fig. S1). In a previous report, GATA-6 was proposed as an upstream regulator of GATA-4 following the analysis of GATA-6 null mouse and GATA-6 (−/−) ES cells [4,5,7]. GATA-6 responds to RA and then activates GATA-4 and Dab2 in parallel [7] in a typical coherent feed-forward loop, widely found in various developmental pathways in nature [27]. Interestingly, in the present study, the silencing of p96 induced to reduce GATA-4 and GATA-6 in RA-treated F9 cells (Figs. 2C and S2). Moreover, the constitutive and ectopic expression of p96 induced EC cells to express GATA-4/6 and Ttr, as well as PrE and VE differentiation markers of the ExE differentiation lineage without RA (Fig. 3B, C). From these data, we proposed that two conclusions are inferable; (i) p96 regulates GATA-4 and GATA-6 in a feed-back loop and (ii) p96 plays key roles in the ExE differentiation process, rather than p67.

Our observation that the transient transfection of p96 in RA-treated EC cells resulted in the increase of GATA-4 supports the existence of a feed-back loop between p96 and the GATA-factors (Fig. 2B), which is thought to operate by p96 expression. Surprisingly, in 96+ cells, differentiation markers which lie downstream of the GATA factors were co-induced with GATA factors in spite of the fact that p96 was expressed alone (Fig. 3B, C). The result implied that the p96 isoform of Dab2 plays key roles in regulating the developmental stage.

In contrast to the results obtained for p96, we gained no insight into the function of p67 in the regulatory mechanism operating between the GATA factors and p96. In 96+ cells, endogenous p67 was the dominant Dab2 isoform as in the RA-treated F9 cells (data not shown). Thus, we presumed that p67 is somehow required for the ExE differentiation pathway. Further research is required to elucidate the role.

To determine how the feed-back loop is triggered at an early stage during construction of the stable cell line, p96 was expressed transiently in RA-untreated cells. Interestingly, only GATA-4 was expressed following the transient expression of p96 alone (Fig. 4A; left panel). It is probable that the feedback regulation loop is initiated following the activation of GATA-4 by p96. The expression of GATA-6 and Ttr was not detected in this experiment using a transient expression system (Fig. 4A, left panel), compared with 96+cells (Fig. 3B, C); however, it is assumed that GATA-6 would be induced by GATA-4 which was expressed through the feed-back loop if the expression of p96 was prolonged further, since GATA-4 and 6 activate each other [5,7]. Thus, GATA-4 expression by transient expression p96 in RA-untreated F9 cells might have been insufficient to accumulate enough GATA-4 to reach the threshold level required to stimulate GATA-6 and Dab2 and launch the differentiation pathway, compared with 96+ cells.

On the other hand, in experiments that mimicked the early stages of RA-induced endodermal differentiation, brief RA stimulation in EC cells was not sufficient to induce the differentiation pathway. The differentiation process was inhibited in empty vector-transfected cells (Fig. 4A; right panel); however, transient transfection of p96 alone recovered this inhibition, allowing the EC cells to differentiate into the ExE lineage (Fig. 4A, right panel). Transient transfection of p96 triggered the operation of the feed-back loop, which activated GATA-4. GATA-4 then accumulated and activated GATA-6 via inter-regulation and Dab2 via the feed-forward loop, finally leading to activate the endogenous Dab2 and launch the differentiation pathway (Fig. 4B).

Combining all the data, we propose that feed-forward and feed-back loops are the main regulatory circuits involved in RA-induced ExE differentiation and that the expression level of p96 through such a regulatory circuit is critical for the process (Fig. 4B).

Acknowledgments

This study was mostly aided by Grant No. (R01-2006-000-11256-0) from the Basic Research Program of the Korea Science & Engineering Foundation (KOSEF) and was supported in part by professor research fund (2011) provided from Korea University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.04.051.

References

[1] J.F. Boylan, L.J. Gudas, Overexpression of the cellular retinoic acid binding protein-I (CRABP-I) results in a reduction in differentiation-specific gene expression in F9 teratocarcinoma cells, The Journal of Cell Biology 112 (1991) 965–979.
[2] S. Strickland, V. Mahdavi, The induction of differentiation in teratocarcinoma stem cells by retinoic acid, Cell 15 (1978) 393–403.
[3] R.J. Arceci, A.A. King, M.C. Simon, S.H. Orkin, D.B. Wilson, Mouse GATA-4: a retinoic acid-inducible GATA-binding transcription factor expressed in endodermally derived tissues and heart, Molecular and Cellular Biology 13 (1993) 2235–2246.
[4] E.E. Morrisey, H.S. Ip, M.M. Lu, M.S. Parmacek, GATA-6: a zinc finger transcription factor that is expressed in multiple cell lineages derived from lateral mesoderm, Developmental Biology 177 (1996) 309–322.
[5] E.E. Morrisey, Z. Tang, K. Sigrist, M.M. Lu, F. Jiang, H.S. Ip, M.S. Parmacek, GATA6 regulates HNF4 and is required for differentiation of visceral endoderm in the mouse embryo, Genes & Development 12 (1998) 3579–3590.
[6] J. Fujikura, E. Yamato, S. Yonemura, K. Hosoda, S. Masui, K. Nakao, J.-i. Miyazaki, H. Niwa, Differentiation of embryonic stem cells is induced by GATA factors, Genes & Development 16 (2002) 784–789.
[7] C.D. Capo-chichi, M.E. Rula, J.L. Smedberg, L. Vanderveer, M.S. Parmacek, E.E. Morrisey, A.K. Godwin, X.-X. Xu, Perception of differentiation cues by GATA factors in primitive endoderm lineage determination of mouse embryonic stem cells, Developmental Biology 286 (2005) 574–586.
[8] X.-X.Y. Xu, B. Taolin, J.D. Tang, Lambeth, disabled-2 (Dab2) is an SH3 domain-binding partner of Grb2, Oncogene 16 (1998) 1561–1569.
[9] C.-P. Tseng, C.-L. Huang, C.-H. Huang, J.-C. Cheng, A. Stern, C.-H. Tseng, D.T.-Y. Chiu, Disabled-2 small interfering RNA modulates cellular adhesive function and MAPK activity during megakaryocytic differentiation of K562 cells, FEBS Letters 541 (2003) 21–27.
[10] J. Zhou, J.-T. Hsieh, The inhibitory role of DOC-2/DAB2 in growth factor receptor-mediated signal cascade, Journal of Biological Chemistry 276 (2001) 27793–27798.
[11] S.M. Morris, M.D. Tallquist, C.O. Rock, J.A. Cooper, Dual roles for the Dab2 adaptor protein in embryonic development and kidney transport, EMBO Journal 21 (2002) 1555–1564.
[12] D.-H. Yang, E.R. Smith, I.H. Roland, Z. Sheng, J. He, W.D. Martin, T.C. Hamilton, J.D. Lambeth, X.-X. Xu, Disabled-2 is essential for endodermal cell positioning and structure formation during mouse embryogenesis, Developmental Biology 251 (2002) 27–44.
[13] C.-L. Huang, J.-C. Cheng, K. Kitajima, T. Nakano, C.-F. Yeh, K.-Y. Chong, C.-P. Tseng, Disabled-2 is required for mesoderm differentiation of murine embryonic stem cells, Journal of Cellular Physiology 225 (2010) 92–105.
[14] M.E. Maurer, J.A. Cooper, Endocytosis of megalin by visceral endoderm cells requires the Dab2 adaptor protein, Journal of Cell Science 118 (2005) 5345–5355.
[15] S. Cho, S. Cho, S. Lee, S. Park, Differential expression of mouse disabled 2 gene in retinoic acid-treated F9 embryonal carcinoma cells and early mouse embryos, Molecules and Cells 9 (1999) 179–184.
[16] Y.-J. Choi, S.-Y. Cho, H.-W. Kim, J.-A. Kim, S.-H. Bae, S.-S. Park, Cloning and characterization of mouse disabled 2 interacting protein 2, a mouse orthologue of human NOSTRIN, Biochemical and Biophysical Research Communications 326 (2005) 594–599.
[17] S. Cho, J. Jeon, S. Lee, S. Park, P67 isoform of mouse disabled 2 protein acts as a transcriptional activator during the differentiation of F9 cells, Biochemical Journal 15 (2000) 645–650.
[18] J. Boylan, D. Lohnes, R. Taneja, P. Chambon, L. Gudas, Loss of retinoic acid receptor g function in F9 cells by gene disruption results in aberrant Hoxa-1 expression and differentiation upon retinoic acid treatment, Proceeding of the National Academy of Sciences of the United States of America 90 (1993) 9601–9605.
[19] M.B. Rogers, S.C. Watkins, L.J. Gudas, Gene expression in visceral endoderm: a comparison of mutant and wild-type F9 embryonal carcinoma cell differentiation, The Journal of Cell Biology 110 (1990) 1767–1777.

[20] B.A. Hocevar, F. Mou, J.L. Rennolds, S.M. Morris, J.A. Cooper, P.H. Howe, Regulation of the Wnt signaling pathway by disabled-2 (Dab2), EMBO Journal 22 (2003) 3084–3094.

[21] G. Bour, R. Taneja, C. Rochette-Egly, Mouse embryocarcinoma F9 cells and retinoic acid: a model to study the molecular mechanisms of endodermal differentiation, in: T. Reshma (Ed.), Advances in Developmental Biology, Elsevier, 2006, pp. 211–253.

[22] D.R. Soprano, B.W. Teets, K.J. Soprano, Role of retinoic acid in the differentiation of embryonal carcinoma and embryonic stem cells, Vitamins and Hormones 75 (2007) 69–95.

[23] E.E. Morrisey, S. Musco, M.Y.Z. Chen, M.M. Lu, J.M. Leiden, M.S. Parmacek, The gene encoding the mitogen-responsive phosphoprotein Dab2 is differentially regulated by GATA-6 and GATA-4 in the visceral endoderm, Journal of Biological Chemistry 275 (2000) 19949–19954.

[24] F. Cesari, Stem cells: a samurai without a master, Nature Reviews Molecular Cell Biology 9 (2008) 587.

[25] Q. Zhou, H. Chipperfield, D.A. Melton, W.H. Wong, A gene regulatory network in mouse embryonic stem cells, Proceedings of the National Academy of Sciences 104 (2007) 16438–16443.

[26] F.-K.C. De-Liang Hu, Yao-Qiu Liu, Yan-Hui Sheng, Rong Yang, Xiang-Qing Kong, Ke-Jiang Cao, Hai-Tao Gu, Ling-Mei Qian, GATA-4 promotes the differentiation of P19 cells into cardiac myocytes International, Journal of Molecular Medicine 26 (2010) 365–372.

[27] U. Alon, Network motifs: theory and experimental approaches, Nature Reviews Genetics 8 (2007) 450–461.