ELSEVIER

FEBS Letters 342 (1994) 115–118

FEBS 13838

FEBS LETTERS

Differential response of NF-κB1 p105 and NF-κB2 p100 to HTLV-I encoded Tax

Mitsuo Watanabe*,**, Masa-aki Muramatsu, Akio Tsuboi, Ken-ichi Arai*

Department of Molecular and Developmental Biology, Institute of Medical Science, The University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108, Japan

Received 16 February 1994

Abstract

In a previous study we found that HTLV-I encoded Tax *trans*activator, which binds to NF-κB1 p105, suppresses p105-mediated IκB activity, thereby allowing entry of NF-κB p65 (RelA) and p50 into the nucleus. In the present report, we compared the effect of Tax on NF-κB2 p100, which also binds to Tax, with that of p105 in transfected COS7 cells. While p105 is processed to the DNA binding form, p50, processing of p100 was much less efficient both in the presence and absence of Tax. Both p105 and p100 showed IκB activity in sequestering NF-κB p65 into the cytoplasm. However, only p105-mediated IκB activity, and not that of p100, was inhibited by Tax. Chimeric molecules between p100 and p105 suggested that inefficient processing of p100 can be attributed to the Rel homologous domain, rather than to the ankyrin repeat domain. p100, but not p105, potently suppressed Tax- and p65-induced *trans*activation of a GM-CSF promoter in Jurkat cells. Taken together, these results suggest that p105 and p100 may have distinct effects on Tax-induced *trans*activation events.

Key words: NF-κB; IκB; Rel; HTLV-I; Tax; *trans*Activation

1. Introduction

NF-κB transcription factor which consists of a heterodimer of two subunits, NF-κB p65 and p50 [1,2] is sequestered into the cytoplasm through an interaction with the inhibitory factor, IκB. Various stimuli, including phorbol ester of TNF-α treatment, lead to the dissociation of p65–p50 from IκB, then translocation into the nucleus and activation of NF-κB-responsive genes. Human T-cell leukemia virus type-I (HTLV-I)-encoded Tax protein also induces NF-κB in the nucleus [3–5]. Molecular cloning of cDNA encoding NF-κB p50 indicated that p50 is derived from a 105 kDa precursor, NF-κB1 p105, which has a Rel homology domain (RHD) and an ankyrin repeat domain (ARD) in the amino- and carboxy-terminal portions, respectively [6]. The precursor p105 itself possesses an IκB function retaining p50, p65 and c-Rel in the cytoplasm [7–9]. We recently reported that Tax relieves p105-mediated IκB activity and induces p50 and p65 in the nucleus [7]. This mechanism may, to some extent, account for the induction of NF-κB by Tax.

NF-κB2 p100, previously referred to as p100/p49, p50B or Lyt10, which is homologous to NF-κB1 p105, has been described [10–12]. p100 also contains a RHD and an ARD, retains IκB-function [13] and binds to Tax [14]. These lines of evidence prompted us to compare the properties of p100 and p105 with regard to the Tax response.

2. Materials and methods

2.1. Cells and transfection

COS7 cells and Jurkat cells were maintained in DMEM and RPMI1640 medium, respectively, supplemented with 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 mg/ml). Transfection was done using a DEAE-dextran method with chloroquine treatment [15]. The total amounts of the DNA transfected were adjusted by adding pME18S vector.

2.2. Plasmid construction

Construction of pME18S-p65, pcDSRα-p105, pcDSRα-p105ΔX and pcDSRα-Tax has been described elsewhere [7]. The cDNA of NF-κB2 p100 [11] was subcloned into the pME18S vector to generate pME18S-p100. pME18S-p100ΔXS has a truncation at the 3′ side of the *Xho*I site in the cDNA, which generates additional Leu–Val and a stop codon following Arg^{447} of p100. cDNAs for chimera-A -B proteins were constructed as follows. To generate chimera-A, the 1.37 kb *Eco*RI–*Rsa*I fragment from pcDSRα-p105, which corresponds to amino acids 1–400 of p105, was inserted into the 4.45 kb *Xma*I–*Eco*RI fragment of pME18S-p100, which includes the cDNA corresponding to amino acids 402–936 of p100. For chimera-B, the 1.04 kb *Eco*RI–*Afl*III fragment from pME18S-p100, which corresponds to amino acids 1–320 of p100, was first inserted into the *Eco*RI–*Sma*I site of pBluescript II SK$^+$ vector (Stratagene) to generate pBS-p100 (1–320). Then, the *Eco*RI–*Spe*I fragment from pBS-p100 (1–320) was inserted into the *Spe*I–*Eco*RI site of pcDSRα-p105, which corresponds to amino acids 342–969 of p105, to generate pSRα-chimera-A. Therefore, the resulting chimeric-B cDNA encodes polypeptides with amino acid 1–320 of p100, including the RHD, amino acid sequence -Gly-Gly-Ser- in the junction, and amino acids 342–969 of p105 including the ARD. The DNA sequence of the junctional portion was confirmed by sequencing.

*Corresponding author. Fax: (81) (3) 3443-5320.

***Present address*: Unitika Research and Development Center, Uji-kozakura, Uji-shi, Kyoto 611, Japan.

SSDI 0014-5793(94)00223-1

2.3. Immunoblotting analysis, electrophoretic mobility shift assay (EMSA) and luciferase assay

COS7 cells were harvested 42–48 h after transfection and nuclear and cytoplasmic fractions were prepared for immunoblotting or EMSA, as described elsewhere [7]. Luciferase assay was performed using Jurkat cells as hosts and a reporter gene, gm-95Luc with the 5′ promoter region of the mouse GM-CSF gene and the luciferase structure gene [16].

3. Results and discussion

3.1. Expression of NF-κB2 p100 cDNA in COS7 cells

NF-κB2 p100 cDNA was transfected into COS7 cells, with or without Tax cDNA and the nuclear extract was subjected to EMSA. No significant NF-κB binding activity was observed, either in the presence or absence of Tax cDNA co-expression (Fig. 1A). Therefore, the response of p100 and p105 clearly differs, since co-expression of p105 and Tax markedly enhanced the binding activity in the COS7 cell nuclear extracts [7]. Transfection of

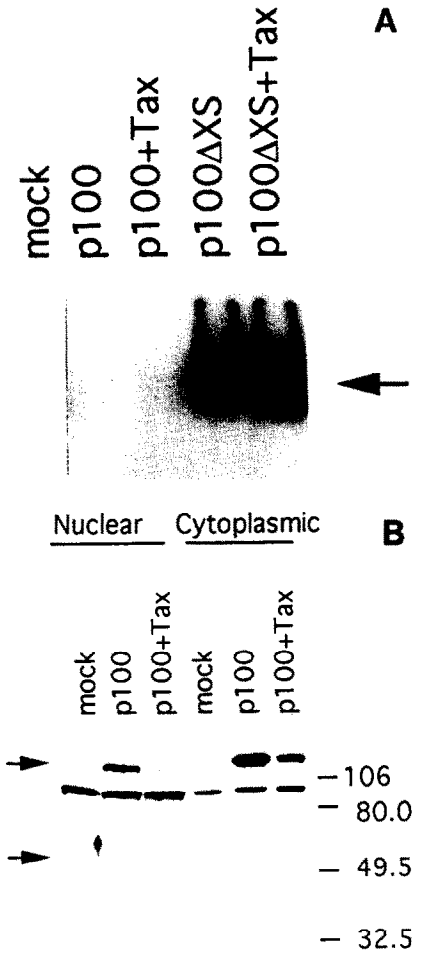


Fig. 1. (A) NF-κB binding activity of the nuclear extracts from COS7 cells transfected with cDNAs of p100 or p100ΔXS. COS7 cells were transfected with 5 μg of pME18S-p100 or pME18S-p100ΔXS, alone or in combination with 5 μg of pcDSRα-Tax, and EMSA was done as described in section 2. (B) Immunoblotting analysis of the nuclear and cytoplasmic fractions of COS7 cells transfected with pME18S-p100 (5 μg) with or without pcDSRα-Tax (5 μg).

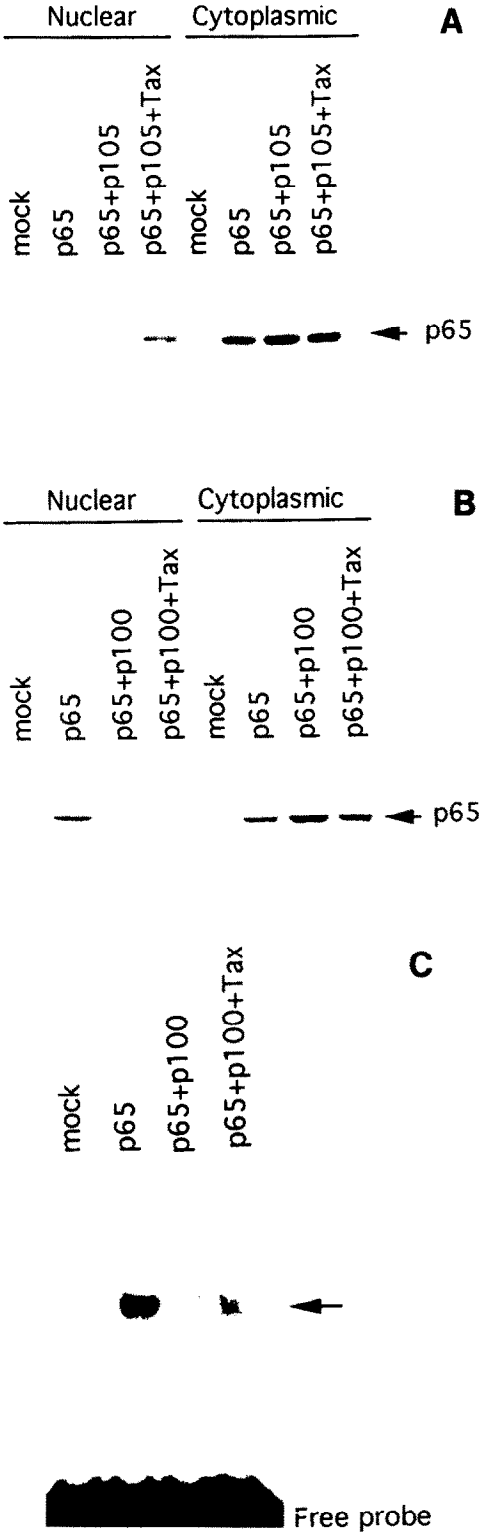


Fig. 2. Shift of subcellular localization of NF-κB p65. (A) COS7 cells were transfected with pME18S-p65 (2 μg), pSRα-p105 (8 μg), or pcDSRα-Tax (2 μg) in combination, as described in the figure, and the nuclear and cytoplasmic fractions were subjected to immunoblotting analysis using an anti-p65 antibody. (B) COS7 cells were transfected with pME18S-p65 (2 μg), pME18S-p100 (8 μg), or pcDSRα-Tax (2 μg) in combination and the nuclear and cytoplasmic fractions were subjected to immunoblotting analysis using an anti-p65 antibody, as described. (C) EMSA was performed with the nuclear extract used in (B) with an NF-κB probe.

p100ΔXS cDNA generated a dense shift band, indicating that the RHD of p100 retains binding activity. The level of NF-κB activity generated by p100ΔXS was not affected by Tax co-expression (Fig. 1A). Immunoblotting analysis of nuclear and cytoplasmic extracts of COS7 cells expressing p100 cDNA showed that p100 was present in both nuclear and cytoplasmic fractions (Fig. 1B, upper arrow), but the processed form, which should appear as a 52 kDa band, was not evident with immunoblotting (Fig. 1B, lower arrow). Co-expression of Tax cDNA slightly decreased the expression level of p100 but there was no evidence of a processed form (Fig. 1B). Inefficient processing of p100 is consistent with the observation in HeLa and Jurkat cells [17].

3.2. IκB function of NF-κB1 p105 and NF-κB2 p100 expressed in COS7 cells

Since IκB activity of p100 towards its own processed form could not be tested due to inefficient processing, we examined the IκB function towards NF-κB p65. Expression of p65 cDNA in COS7 cells generates p65, which is distributed in both nuclear and cytoplasmic fractions (Fig. 2A and B). Co-expression of either p105 or p100 cDNA reduced the amount of nuclear p65 and increased the amount of cytoplasmic p65 (Fig. 2A and B). This observation confirms that both p105 and p100 possess IκB activity [7,13]. When Tax was further co-expressed, the level of nuclear p65 was regained in cells expressing p65 and p105 (Fig. 2A). In contrast, the level of nuclear p65 was not regained in cells expressing p65 and p100 (Fig. 2B). EMSA of the nuclear extracts used in Fig. 2B also shows that NF-κB binding activity is inhibited by p100 but is not enhanced by further Tax expression (Fig. 2C). These results indicate that Tax inhibits IκB activity mediated by p105 but not by p100 in COS7 cells.

3.3. Chimera between NF-κB1 p105 and NF-κB2 p100 cDNAs

In an attempt to account for the different properties of p105 and p100, we constructed chimeric cDNAs by exchanging the amino-terminal region, including the RHD, and carboxy-terminal region, including the ARD (Fig. 3A). The chimera-A cDNA encodes the RHD of p105 and the ARD of p100, while chimera-B cDNA encodes the RHD of p100 and the ARD of p105. Chimera-A and -B possess the nuclear localization signal (NLS) of NF-κB1 and NF-κB2, respectively.

When chimera-A cDNA was expressed in COS7 cells, a high level of 55 kDa protein was generated in the nuclear and cytoplasmic fractions (Fig. 3B, lower arrow), but a product corresponding to the size of the unprocessed protein was barely detectable (Fig. 3B, upper arrow). This suggests that chimera-A protein is so efficiently processed that the precursor is not retained in a detectable amount. In contrast, expression of chimera-B cDNA generated a 105 kDa product corresponding to

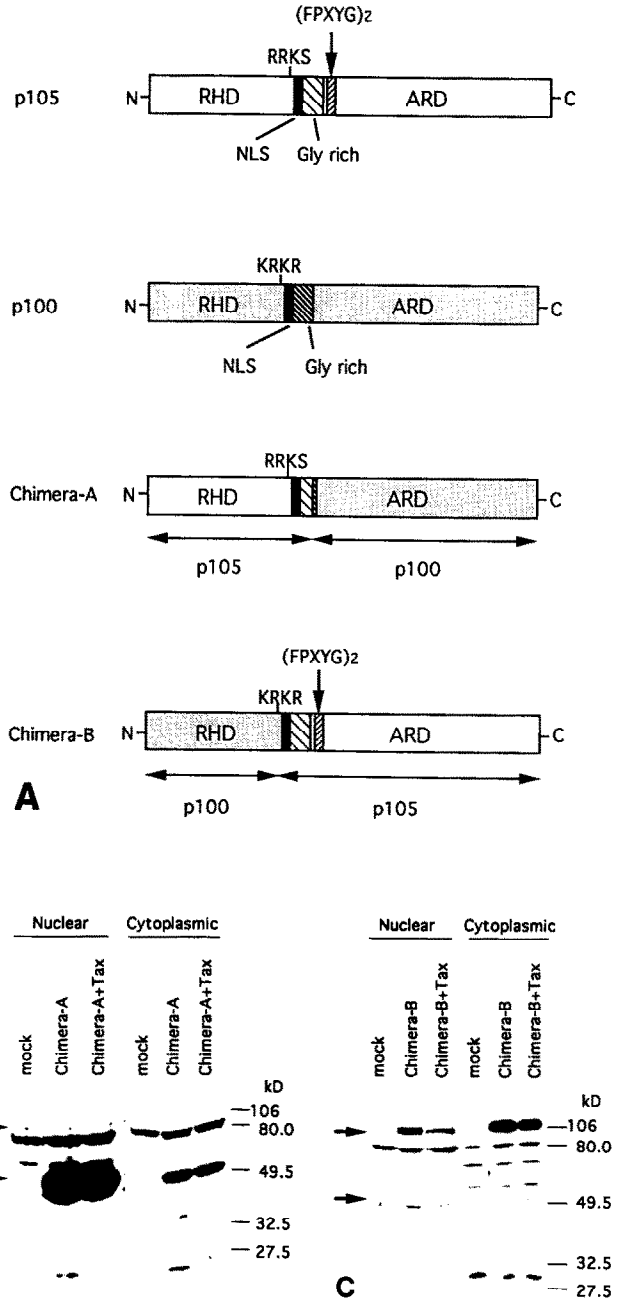
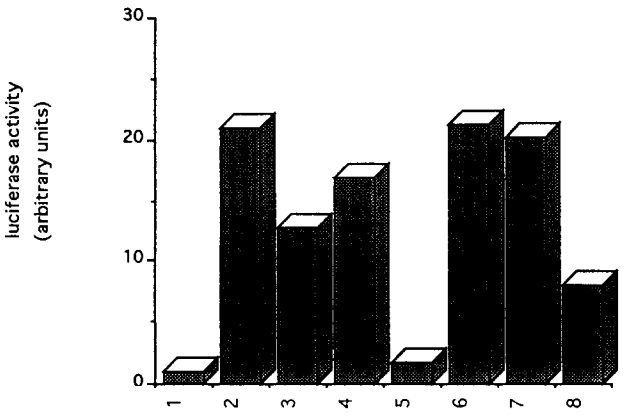


Fig. 3. (A) Structures of the chimeric proteins between NF-κB1 p105 and NF-κB2 p100. NLS, a putative nuclear localization signal; Gly rich, a polyglycine putative hinge domain; $(FPXYG)_2$, two copies of a sequence, FPXYG, which could serve as proteolytic cleavage signal; RRKS, a putative protein kinase A phosphorylation site in p105; KRKR, a sequence in p100 which resides in the analogous position of RRKS in p105. (B) Immunoblotting analysis of COS7 cells expressing chimera-A cDNA with or without Tax cDNA. (C) Immunoblotting analysis of COS7 cells expressing chimera-B cDNA with or without Tax cDNA.

the size of the intact chimera-B protein (Fig. 3C, upper arrow), but the processed form, which should appear as a 50–55 kDa band, was not detected (Fig. 3C, lower arrow). This indicates that chimera-B does not process efficiently. Co-expression of Tax cDNA with either chi-



gm-95luc	+	+	+	+	+	+	+	+
Tax + p65	−	+	+	+	+	+	+	+
others	pME 18S	pME 18S	p105	p105 ΔX	p100	p100 ΔXS	chimera A	chimera B

Fig. 4. Effect of p105, p100 and other constructs on Tax- and p65-induced *trans*activation of the GM-CSF promoter. Jurkat cells were transfected with gm-95Luc, pME18S-p65 and pcDSRα-Tax in combination with other constructs as shown in the figure. The luciferase activity of the extract from cells transfected with gm-95Luc and pME18S was arbitrarily assigned a value of 1 and the relative activities were calculated.

mera-A or -B cDNA did not significantly affect the processing or the subcellular localization of the chimera products (Fig. 3B and C). Since chimera-A and -B possess the glycine-rich region and the FPXYG motifs of p105 which contain the putative processing site [18], these regions cannot be attributed to processing efficiency. Combining the results of chimera-A and -B, it is likely that the RHD but not ARD of p100 has the inhibitory effects on the processing in COS7 cells.

3.4. *NF-κB2 p100 inhibits Tax- and NF-κB p65-induced* trans*activation of a GM-CSF promoter in Jurkat cells*

Next we examined the effect of p100, p105 and other constructs on Tax- and p65-induced *trans*activation of a GM-CSF promoter in Jurkat cells. The activity of the reporter gene, gm95-Luc, is at the basal level, and coexpression of Tax and p65 cDNAs enhanced the promoter activity (Fig. 4, columns 1 and 2). Further coexpression of p100 cDNA but not of p105 cDNA inhibited promoter activity (Fig. 4, columns 5 and 3). Coexpression with either p105ΔX, p100ΔXS, chimera-A or -B cDNAs (Fig. 4, columns 4, 6, 7, 8, respectively) did not significantly suppress Tax- and p65-induced *trans*activation. It thus appears that p100 inhibits Tax *trans*activation and that a proper structure of p100 is required for efficient inhibition.

In conclusion we obtained evidence that NF-κB1 p105 and NF-κB2 p100 show different responses to Tax. The interaction of Tax with p105 may be one mechanism by which Tax induces NF-κB activity in the nucleus. Recent data shows that Tax binds to ankyrin motifs of IκB and induces NF-κB proteins for transcriptional activation [19]. In contrast, the interaction of Tax with p100 cannot be interpreted in such a straightforward manner. Although over-expression of p100 has been shown to sequester Tax in the cytoplasm [14], the physiological significance remains to be determined. It is conceivable that these different effects may affect Tax-induced *trans*activation events, and might render complexities in the pathophysiological role of Tax in HTLV-I infected cells.

Acknowledgements: We are grateful to Drs. A. Israel, G. Nabel, and C.A. Rosen, for cDNAs of p105, p100, and p65, respectively. This work was supported in part by Unitika Ltd., Japan.

References

[1] Nolan, G.P. and Baltimore, D. (1992) Curr. Opin. Genet. Dev. 2211–220.
[2] Blank, V., Kourilsky, P. and Israël, A. (1992) Trends. Biol. Sci. 17, 135–140.
[3] Leung, K. and Nabel, G.J. (1988) Nature 333, 776–778.
[4] Lindholm, P.F., Marriott, S.J., Gitlin, S.D., Bohan, C.A. and Brady, J.N. (1990) New Biol. 2, 1034–1043.
[5] Lindholm, P.F., Reid, R.L. and Brady, J.N. (1992) J. Virol. 66, 1294–1302.
[6] Kieran, M., Blank, V., Logeat, F., Vanderkerckhove, J., Lottspeich, F., Le Bail, O., Urban, M.B., Kourilsky, P., Baeuerle, P. and Israël, A. (1990) Cell 62, 1007–1018.
[7] Watanabe, M., Muramatsu, M., Hirai, H., Suzuki, T., Fujisawa, J., Yoshida, M., Arai, K. and Arai, N. (1993) Oncogene 8, 2949–2958.
[8] Rice, N.R., MacKichan, M.L. and Israel, A. (1992) Cell 71, 243–253.
[9] Naumann, M., Wulczyn, F.G. and Scheidereit, C. (1993) EMBO J. 12, 213–222.
[10] Neri, A., Chang, C.-C., Lombardi, L., Salina, M., Corradini, P., Mariolo, A.T., Chaganti, R.S.K. and Dalla-Favera, R. (1991) Cell 67, 1075–1087.
[11] Schmid, R.M., Perkins, N.D., Duckett, C.S., Andrews, P.C. and Nabel, G.J. (1991) Nature 352, 733–736.
[12] Bours, V., Burd, P.R., Brown, K., Villalobos, J., Park, S., Ryseck, R.-P., Bravo, R., Kelly, K. and Siebenlist, U. (1992) Mol. Cell. Biol. 12, 685–695.
[13] Scheinman, R.I., Beg, A.A. and Baldwin Jr., A.S. (1993) Mol. Cell. Biol. 13, 6089–6101.
[14] Beraud, C., Sun, S.C., Ganchi, P., Ballard, D.W. and Greene, W.C. (1994) Mol. Cell. Biol. 14, 1374–1382.
[15] Takebe, Y., Seiki, M., Fujisawa, J., Foy, P., Yokota, T., Arai, K., Yoshida, M. and Arai, N. (1988) Mol. Cell. Biol. 8, 466–472.
[16] Tsuboi, A., Sugimoto, K., Yodoi, J., Miyatake, S., Arai, K. and Arai, N. (1991) Int. Immunol. 3, 807–817.18.17.
[17] Mercurio, F., DiDonato, J., Rosette, C. and Karin, M. (1993) Genes Dev. 7, 705–718.
[18] Baeuerle, P.A. (1991) Biochem. Biophys. Acta. 1072, 63–80.
[19] Hirai, H., Suzuki, T., Fujisawa, J., Inoue, J. and Yoshida, M. (1994) Proc. Natl. Acad. Sci. USA. (in press).