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Regulation of NF-κB through the Nuclear Processing of p105 (NF-κB1) in Epstein-Barr Virus-immortalized B Cell Lines*

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Transcription factors of the NF-κB/Rel family are retained in the cytoplasm as inactive complexes through association with IκB inhibitory proteins. Several NF-κB activators induce the proteolysis of IκB proteins, which results in the nuclear translocation and DNA binding of NF-κB complexes. Here, we report a novel mechanism of NF-κB regulation mediated by p105 (NF-κB1) precursor of p50 directly at the nuclear level. In Epstein-Barr virus-immortalized B cells, p105 was found in the nucleus, where it was complexed with p65. In concomitance with NF-κB activation, mitomycin C induced the processing of p105 to p50 in the nucleus, while it did not affect the steady-state protein levels of IκBα and p105 in the cytoplasm. Differently, phorbol 12-myristate 13-acetate induced a significant proteolysis of both IκBα and p105 in the cytoplasm, while it did not affect the protein level of p105 in the nucleus. These results suggest that in Epstein-Barr virus-positive B cell lines the nuclear processing of p105 can contribute to NF-κB activation in response to specific signaling molecules, such as DNA-damaging agents.

Transcription factors of the NF-κB/Rel family regulate the expression of genes involved in the immune and acute-phase responses and cell growth and differentiation. This family includes different proteins which share a sequence homology over a 300-amino acid "Rel homology region," homo- or heterodimerize, and bind with different affinities to the κB sites in the enhancer of NF-κB-dependent genes (reviewed in Refs. 1–3). IκB proteins, such as IκBα (4), IκBβ (5), and the p105 (NF-κB1) (6–8) and p100 (NF-κB2) (9, 10) precursors of p50 and p52, respectively, play a crucial role in the regulation of NF-κB activity. These proteins carry ankyrin repeats, associate with Rel proteins in the cytoplasm, and inhibit the nuclear translocation and DNA binding of NF-κB complexes (1–3). NF-κB activation by different stimuli is mainly regulated through the proteolytic degradation of IκBα (11–19) and IκBβ (5), and the processing of p105 and p100 to p50 and p52, respectively (16, 17, 19, 20). These events result in the unmasking of the nuclear localization signal of Rel proteins and the nuclear translocation and DNA binding of NF-κB complexes.

In the present study, we have examined the mechanisms of NF-κB activation by two different NF-κB inducers. For this purpose, MC3 cells, an Epstein-Barr virus (EBV)[1]-immortalized B cell line, were exposed to treatment with mitomycin C, a DNA alkylating agents (21), and PMA, a protein kinase C-activator (22). Then, the fate of Rel proteins and IκBα inhibitor was analyzed at different times of treatment by immunoblotting analysis of cytosolic and nuclear extracts. Our data indicate that in MC3 cells p105 can be found in the cytoplasm as well as in the nucleus. In concomitance with NF-κB activation, mitomycin C induced the processing of p105 to p50 in the nucleus, while it did not significantly affect the steady-state protein level of IκBα and p105 in the cytoplasm. Concomitantly with NF-κB activation, the nuclear content of p65 and c-Rel was unmodified by the mitomycin C treatment. Differently, PMA caused a significant degradation of IκBα and the processing of p105 in the cytoplasm, while it did not affect the p105 protein level in the nucleus. Concomitantly with the PMA-mediated activation of NF-κB, both p65 and c-Rel proteins increased in the nucleus. Nuclear p105 was confirmed to occur exclusively in EBV-positive cell lines either by immunoblotting analysis of cell fractions or by cell immunostaining of different cell lines. Moreover, by immunoprecipitation, p105 was shown to be associated with p65, but not c-Rel, in the nucleus.

These findings indicate that in EBV-positive B cell lines p105 can down-regulate the NF-κB activity directly in the nucleus by associating with p65. Nuclear processing of p105 may be an additional mechanism of NF-κB activation in response to specific signals, such as DNA damage.

MATERIALS AND METHODS

Cells and Chemical Treatments—MC3 cells, an EBV-positive B lymphocytes cell line (21), were cultured in Dulbecco's modified Eagle's medium supplemented with 10% v/v heat-inactivated fetal calf serum (Flow Laboratories, Italy), 3 mM glutamine and 10 mM Hepes buffer, pH 7.2 (Life Technologies, Inc., Italy). Exponential phase cultures were treated with mitomycin C (10 μM) or PMA (100 ng/ml) for the indicated time or left untreated. Cell viability was determined by trypan blue exclusion.

Cell Extracts—Nuclear and cytosolic extracts were prepared as described previously (21). Briefly, cells (2×10^7) were harvested, washed twice in cold PBS, and resuspended in 200 μl of lysing buffer (10 mM Hepes buffer, pH 7.9, 1 mM EDTA, 60 mM KCl, 1 mM dithiothreitol, 1 mM PMSF, 50 μg/ml antipain, 40 μg/ml APMSF, 10 μg/ml aprotinin, 40 μg/ml bestatin, 20 μg/ml chymostatin, 0.2% v/v Nonidet P-40) for 5 min. Samples were centrifuged at 400 × *g* for 5 min. The supernatant (cytosolic fraction) was stored at −80 °C until use. The pelletted nuclei were gently rinsed twice with 1 ml of Nonidet P-40-free lysing buffer,

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[1] The abbreviations used are: EBV, Epstein-Barr virus; PMA, phorbol 12-myristate 13-acetate; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; APMSF, 4-amidinophenyl-methanesulfonylfluoride; HIV-1, human immunodeficiency virus, type 1.

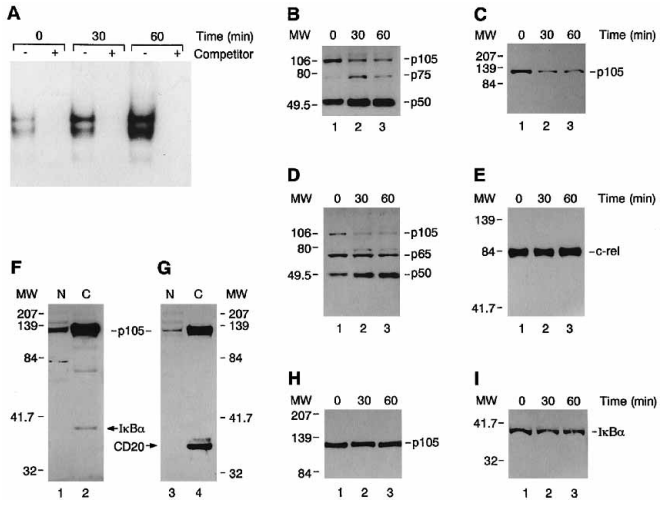


FIG. 1. **NF-κB activation by mitomycin C.** MC3 cells were treated with mitomycin C (10 μM) for the indicated time and nuclear and cytosolic extracts were performed and used in electrophoretic mobility shift and immunoblotting analysis as detailed under "Materials and Methods." *A*, kinetic of NF-κB activation by mitomycin C. Nuclear extracts (5 μg) were incubated with an NF-κB oligonucleotide in presence or absence of the unlabeled NF-κB oligonucleotide (competitor) used at 100-fold molar excess. *B–E*, fate of nuclear p105, p50, p65, and c-Rel. Nuclear extracts (20 μg) from MC3 cells treated with mitomycin C for the indicated time or left untreated were analyzed by immunoblotting using the antiserum 1141 raised against the N terminus of p105 (*B*), the antiserum 1140 raised against the C terminus of p105 (*C*), the antiserum 1226 raised against the C terminus of p65 (*D*), and the antiserum 265 raised against the C terminus of c-Rel (*E*). In *D*, the membrane filter shown in *B* was reprobed with anti-p65 antiserum 1226. *F* and *G*, cellular distribution of IκBα and CD20 proteins. Nuclear (*N*) and cytosolic (*C*) extracts from unstimulated MC3 cells (5×10^5 cells) were analyzed by immunoblotting using the anti-p105 antiserum 1140 and either the antiserum 1309 raised against the N terminus of IκBα (*F*) or an antiserum raised against an intracellular epitope of CD20 B cell antigen (*G*). *H*, fate of cytoplasmic p105. Cytosolic extracts (10 μg) were analyzed by immunoblotting using the anti-p105 antiserum 1140. *I*, fate of cytoplasmic IκBα. Cytosolic extracts (40 μg) were analyzed by immunoblotting using the anti-IκBα antiserum 1309. The values of densitometric analysis relative to each protein through *lanes 1* to *3* are: *B*, p105, 1.0, 0.5, 0.3; p75, 1.0, 12.0, 3.2; p50, 1.0, 2.1, 2.7; *C*, p105, 1.0, 0.2, 0.2; *D*, p65, 1.0, 1.1, 0.7; *E*, c-Rel, 1.0, 0.9, 1.1; *H*, p105, 1.0, 0.8, 1.1; and *I*, IκBα, 1.0, 0.8, 0.8. The values are expressed as the ratio between the treated samples (time 30 and 60 min) and the relative untreated control (time 0).

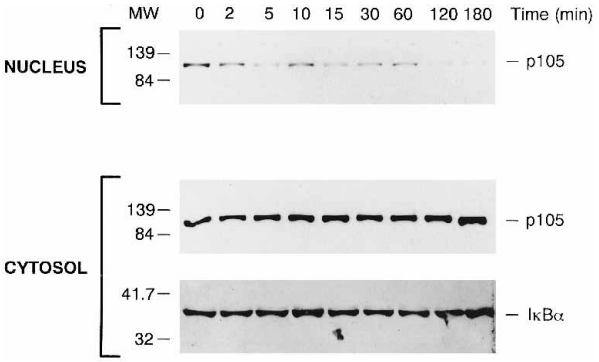


FIG. 2. **Fate of p105 and IκBα following the treatment with mitomycin C.** MC3 cells were treated with mitomycin C (10 μM) for the indicated time or left untreated. Nuclear and cytosolic extracts were performed and analyzed by immunoblotting analysis as detailed under "Materials and Methods." p105 was detected in the nuclear (10 μg) and cytosolic extracts (10 μg) using the anti-p105 antiserum 1140. IκBα was detected in the cytosolic extract (30 μg) using the anti-IκBα antiserum 1309. The values of densitometric analysis relative to each protein through *lanes 1–9* are: nuclear p105, 1.0, 0.6, 0.1, 0.6, 0.2, 0.3, 0.3, 0.1, 0.1; cytosolic p105, 1.0, 0.8, 1.3, 1.6, 1.6, 1.5, 1.5, 1.8, 2.5; and IκBα, 1.0, 0.8, 0.9, 1.3, 1.1, 1.0, 0.7, 1.9, 2.7. The values are expressed as the ratio between the treated samples (times 2 up to 180 min) and the relative untreated control (time 0).

resuspended in 300 μl of Nonidet P-40-free lysing buffer, and layered on top of 300 μl of same buffer containing 30% saccharose. After centrifugation at 2900 × *g* for 10 min, the supernatant was gently removed and the pelletted nuclei were resuspended in 200 μl of nuclear suspension buffer (250 mM Tris-HCl, pH 7.8, 60 mM KCl, 1 mM dithiothreitol, 1 mM PMSF, 50 μg/ml antipain, 40 μg/ml APMSF, 10 μg/ml aprotinin, 40 μg/ml bestatin, 20 μg/ml chymostatin). A small aliquot of nuclei preparation was stained with trypan blue and observed at the microscope to verify the integrity of nuclei. Then, the nuclei suspension was subjected to three cycles of freezing and thawing. The suspension was cleared by ultracentrifugation at 135,000 × *g* for 15 min, and the supernatant was stored at −80 °C until use.

Electrophoretic Mobility Shift Assays—The DNA band-shift analysis was performed as described previously (21). The HIV-1 NF-κB double-stranded oligonucleotide (5′-CAAGGGACTTTCCGCTGGGGACTTTC-CAG-3′) was end-labeled with [γ-^{32}P]ATP (Amersham International, Amersham, UK) using polynucleotide kinase (New England Biolabs, Beverly, MA). Nuclear extracts (5 μg of protein) were incubated in a 20 μl of reaction mixture containing 10% glycerol, 60 mM potassium chloride, 1 mM EDTA, 1 mM dithiothreitol, and 2 μg of poly(dI-dC) (Boehringer Mannheim, Germany) for 5 min on ice. One μl of γ-^{32}P-labeled double-stranded probe (0.2 ng, $4–6 \times 10^4$ cpm) was then added with or without a 100-fold molar excess of unlabeled oligonucleotide. The reactions were incubated at room temperature for 30 min and run on a 6% acrylamide/bis-acrylamide (30:1) gel in 22.5 mM Tris borate, 0.5 mM EDTA. Gels were dried and autoradiographed.

Immunoblotting Analysis—Immunoblotting analysis was performed as described previously (23). Briefly, nuclear or cytosolic proteins (5–40 μg) were separated by SDS-10% polyacrylamide gel electrophoresis, transferred onto a membrane filter (Cellulosenitrate, Schleider & Schuell, Germany), and incubated with the primary antiserum in PBS plus 5% dry milk for 2 h at room temperature. The blots were washed with PBS, incubated with peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (Boehringer Mannheim) at 1:2000 for 1 h and visualized by using the enhanced chemiluminescence system (Amersham). The specificity of detection was verified by the absence of immunostaining in presence of competitor peptide (10 μg/ml) or in presence of the second antibody alone. The polyclonal rabbit antisera anti-p105 1140 (amino acids 955–969 of human p105) and 1141 (amino acids 2–15 of human p105), anti-p65 1226 (amino acids 537–550 of human p65) and 1207 (amino acids 2–17 of human p65), anti-c-Rel 265 (amino acids 573–587 of human c-Rel), and anti-IκBα 1309 (amino acids 51–64 of human IκBα) were kindly provided by Dr. N. Rice (24). The monoclonal mouse antibody anti-human B cell CD20 was from Dako Corp., Carpinteria, CA. Densitometric evaluation of the protein content was performed by using an LKB 2202 ultrascan laser densitometer (Pharmacia Biotech Inc.) assisted by a Hewlett-Packard 3390A Integrator.

Immunoprecipitation—Nuclear extracts from unstimulated MC3 (1×10^8 cells) were incubated overnight in 500 μl of radioimmune precipitation buffer (50 mM Tris-HCl, pH 8.0, 150 mM sodium chloride, 1% Nonidet P-40, 1% sodium deoxycholate, 0.2% SDS, and 4 μg/μl bovine serum albumin) with the anti-p65 antiserum 1207 (5 μl), the anti-c-Rel antiserum 265 (5 μl), or a preimmune antiserum (5 μl). Immunocomplexes were precipitated with protein A-Sepharose (Sigma) by centrifugation at 720 × *g* for 10 min. The precipitate was rinsed twice by resuspension in radioimmune precipitation buffer followed by centrifugation. Immunoprecipitates were analyzed by immunoblotting with anti-p105 antiserum 1140, anti-p65 antiserum 1207, and anti-c-Rel antiserum 265.

Cell Immunostaining—Cytospins were air-dried, fixed in acetone for 1 min, rinsed in PBS, and incubated in PBS containing 5% dry milk for 30 min. Incubation with the anti-p105 antiserum 1140 was performed in PBS containing 5% dry milk for 30 min at room temperature. Immunocomplexes were revealed by peroxidase-labeled secondary antibody (Dako LSAB+ kit, peroxidase) according to the manufacturer's instructions. The competitor peptide was added to the incubation mixture at the final concentration of 10 μg/ml. Before analysis on the microscope, nuclei were counterstained blue with hematoxylin (Mayer). Immunostaining was evaluated by computer-assisted analysis using a Leica Quantimet 500C plus image analysis and processing system.

RESULTS

NF-κB Activation by Mitomycin C and PMA: Fate of Rel Proteins and IκBα Inhibitor—In MC3 cells, the NF-κB activity was constitutively expressed, and it was increased by treat-

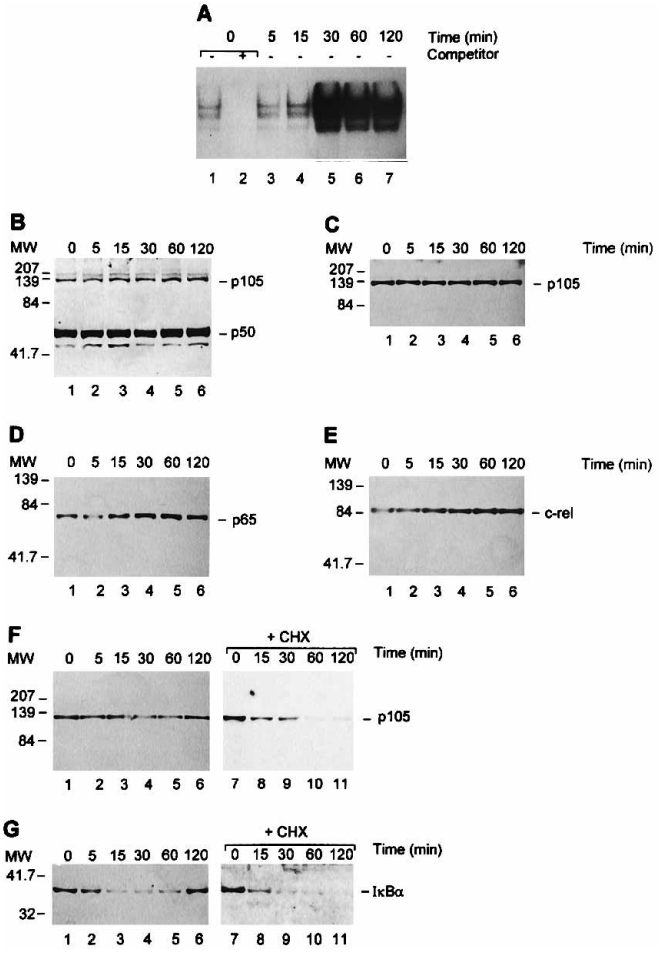


FIG. 3. **NF-κB activation by PMA.** MC3 cells were treated with PMA (100 ng/ml) for the indicated time or left untreated. Nuclear and cytosolic extracts were performed and analyzed in electrophoretic mobility shift and immunoblotting analysis as detailed under "Materials and Methods." *A*, kinetics of NF-κB activation by PMA. Nuclear extracts (5 μg) were incubated with a NF-κB oligonucleotide in the presence or absence of the unlabeled NF-κB oligonucleotide (competitor) used at 100-fold molar excess. *B–E*, fate of nuclear p105, p50, p65, and c-Rel. Nuclear extracts (20 μg) were analyzed with the anti-p105 antiserum 1141 (*B*), anti-p105 antiserum 1140 (*C*), the anti-p65 antiserum 1226 (*D*), or the anti-c-Rel antiserum 265 (*E*). *F*, fate of cytoplasmic p105. Cytosolic extracts (5 μg) from MC3 cells treated with PMA in the absence or presence of cycloheximide (50 μg/ml) were analyzed with the anti-p105 antiserum 1140. *G*, fate of cytoplasmic IκBα. Cytosolic extracts (40 μg) from MC3 cells treated with PMA in absence or presence of cycloheximide (50 μg/ml) were analyzed with the anti-IκBα antiserum 1309. The values of densitometric analysis relative to each protein through *lanes 1–6* are indicated as follows: *B*, p105, 1.0, 1.0, 1.4, 1.2, 1.4, 1.4 and p50, 1.0, 1.0, 1.2, 0.9, 1.4, 1.3; *C*, p105, 1.0, 1.3, 0.9, 0.8, 1.0, 0.9; *D*, p65, 1.0, 0.5, 1.9, 3.4, 3.7, 2.1; *E*, c-Rel, 1.0, 1.7, 2.7, 3.6, 3.9, 4.3; *F*, p105, 1.0, 0.8, 1.0, 0.3, 0.4, 1.0; and *G*, IκBα, 1.0, 0.8, 0.3, 0.1, 0.1, 1.3. The values are expressed as the ratio between the treated samples (times 5 up to 120 min) and the relative untreated control (time 0).

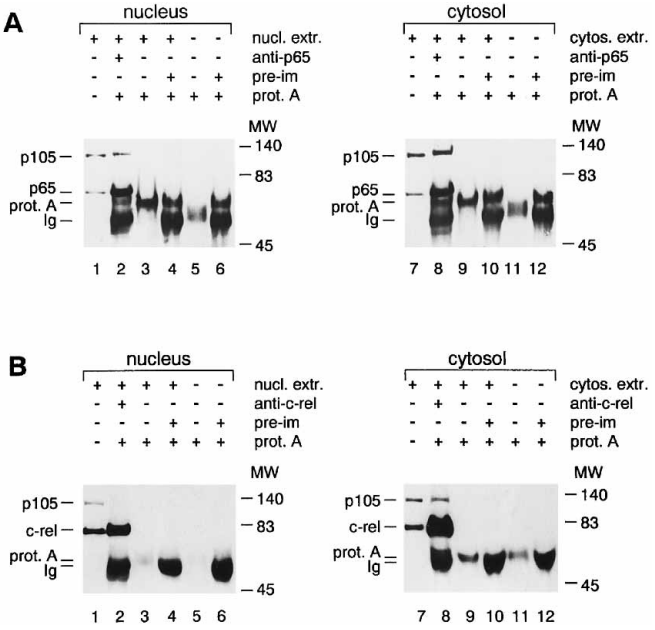


FIG. 4. **p105 is complexed with p65 in the nucleus.** *A*, nuclear or cytosolic extracts from MC3 cells (1×10^8) were precipitated with the anti-p65 antiserum 1207 (*lanes 2* and *8*), protein A-Sepharose (*lanes 3* and *9*), or a preimmune antiserum (*lanes 4* and *10*). The immunocomplexes were analyzed by immunoblotting using the anti-p105 antiserum 1140 and the anti-p65 antiserum 1207 (*lanes 1–12*). Nuclear and cytosolic extracts (1×10^6 cells) were also analyzed in the absence of incubation with antisera and protein A-Sepharose (*lanes 1* and *7*). *B*, nuclear and cytosolic extracts from MC3 cells (1×10^8) were precipitated with the anti-c-Rel antiserum 265 (*lanes 2* and *8*), protein A-Sepharose (*lanes 3* and *9*), or a preimmune antiserum (*lanes 4* and *10*). The immunocomplexes were analyzed by immunoblotting using the anti-p105 antiserum 1140 and the anti-c-Rel antiserum 265 (*lanes 1–12*). Nuclear and cytosolic extracts (1×10^6 cells) were also analyzed in the absence of incubation with antisera and protein A-Sepharose (*lanes 1* and *7*).

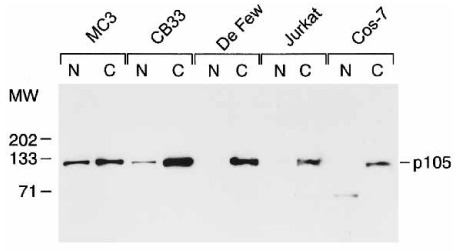
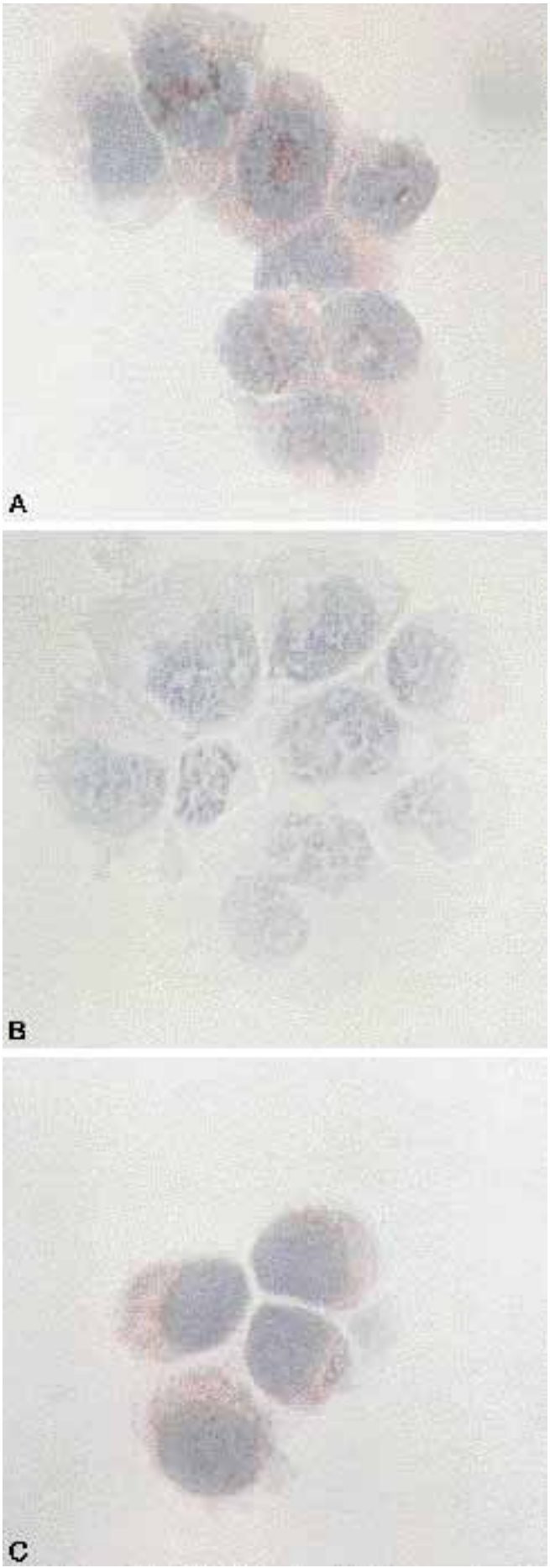


FIG. 5. **Subcellular distribution of p105 in different cell lines.** Nuclear (*N*) and cytosolic (*C*) extracts obtained from MC3 and CB33 (2×10^5 cells), De Few and Jurkat (2×10^6 cells), and Cos7 (4×10^5 cells) were analyzed by immunoblotting analysis using the anti-p105 antiserum 1140 as detailed under "Materials and Methods."

ment with mitomycin C at 30 and 60 min (Fig. 1*A*). p105 and p50 were both detected in the nuclear extracts of unstimulated cells by immunoblotting analysis using an antiserum raised against the N terminus of p105 (Fig. 1*B*, *lane 1*). At 30 and 60 min of mitomycin C treatment, p105 significantly decreased, while p50 increased (Fig. 1*B*, *lanes 2* and *3*). By the same antiserum, a 75-kDa protein (referred to as p75) was sometimes detected (Fig. 1*B*, *lanes 2* and *3*), being a possible intermediate product of p105 proteolysis. The nuclear location and the time-dependent proteolysis of p105 was confirmed by an antiserum raised against the C terminus of p105 (Fig. 1*C*). p65 and c-Rel were abundantly detected by specific antisera in the nucleus of unstimulated cells, and their content did not significantly change after chemical treatment (Fig. 1, *D* and *E*). IκBα was undetected in the nuclear extracts, while it was clearly revealed in the cytosolic extracts of unstimulated cells (Fig. 1*F*). An intracellular epitope of B cell-associated CD20 antigen (25) was also exclusively detected in the cytosolic extracts, providing a further control of the absence of cytosolic contamination of nuclear preparations (Fig. 1*G*). In concomitance with the NF-κB activation by mitomycin C, the steady-state protein levels of p105 and IκBα in the cytoplasm were not significantly affected (Fig. 1, *H* and *I*). The proteolysis of p105 in the nucleus was observed at times of treatment with mitomycin C as soon as 2 min and up to 3 h (Fig. 2). Concomitantly, in the cytoplasm the p105 and IκBα content did not significantly change up to 60 min of mitomycin C treatment, showing a slight increase at 2



and 3 h (Fig. 2).

In the case of PMA, NF-κB activity was induced at 15 and up to 120 min of treatment with a maximum at 30 min (Fig. 3*A*). In concomitance with NF-κB activation by PMA, the processing of p105 did not occur in the nucleus (Fig. 3, *B* and *C*), and the nuclear content of p50 was unchanged (Fig. 3*B*). In the nucleus, p65 increased at 15 and up to 60 min of PMA treatment followed by a slight decrease at 120 min (Fig. 3*D*). Nuclear c-Rel also progressively increased at 15 and up to 120 min of PMA treatment (Fig. 3*E*). In the cytoplasm, a significant proteolysis was observed for p105 at 30 and up to 60 min of PMA treatment (Fig. 3*F*) and for IκBα at 15 and up to 60 min of PMA treatment (Fig. 3*G*). Following degradation, both p105 and IκBα increased in the cytosol at 120 min (Fig. 3, *F* and *G*, *lane 6*). This increase was not observed in the presence of cycloheximide (Fig. 3, *F* and *G*, *lane 11*), suggesting that both NF-κB inhibitors were resynthesized at 120 min of PMA treatment.

p105 Associates with p65, but Not c-Rel, in the Nucleus—To test whether p105 could be associated with Rel proteins in the nucleus, nuclear extracts from MC3 cells were immunoprecipitated with anti-p65 or anti-c-Rel antiserum, and the immunocomplexes were analyzed for the presence of p105. As shown in Fig. 4, p105 was co-precipitated with p65 (*A*, *lane 2*), but not with c-Rel (*B*, *lane 2*). However, in accordance with previous reports (20, 24), p105 was co-precipitated with p65 and c-Rel from cytosolic extracts of MC3 cells (Fig. 4, *A* and *B*, *lane 8*). These results indicate that in MC3 cells nuclear p105 was preferentially associated with p65.

Nuclear Distribution of p105 Occurs in EBV-positive B Cell Lines—p105 was described as retained in the cytoplasm due to the C-terminal ankyrin repeats masking the nuclear localization signal of the protein (26, 27). However, in MC3 cells p105 was detected at significant levels both in the nucleus and the cytoplasm. To investigate the nuclear distribution of p105 in different cell lines, nuclear and cytosolic extracts from EBV-positive B cells (MC3 and CB33), EBV-negative Burkitt lymphoma (De Few), T cells (Jurkat), and epithelial cells (Cos7) were analyzed by immunoblotting for the presence of p105. As shown in Fig. 5, p105 was present in the cytosolic extracts of all examined cell lines, but it was found in the nucleus only in EBV-positive B cell lines. These findings were confirmed by immunostaining of MC3 and Cos7 cells with an antiserum raised against the C terminus of p105. p105 was stained red, and it was clearly detected both in the cytoplasm and the nucleus of MC3 cells, being homogeneously diffused in the cytoplasm and patchy in the nucleus (Fig. 6*A*). Specificity of immunostaining was verified by the lack of p105 staining in the presence of the antagonist peptide (Fig. 6*B*). In Cos7, p105 was localized exclusively in the cytoplasm (Fig. 6*C*).

DISCUSSION

Processing of p105 to p50 was described previously as a regulatory mechanism of NF-κB activity at the cytoplasmic level (16, 17, 19, 20). We now report that p105 is present at significant levels in the nucleus of EBV-positive B cell lines, where it can contribute to the regulation of NF-κB. This is the first report describing the nuclear distribution of p105 in human B cell lines. Alternative spliced forms of p105 were recovered in the nucleus of murine B cells (28). Moreover, in Cos7, C terminus-deleted p105 efficiently translocated into the nucleus (26, 27). In our case, we rule out that the nuclear p105 detected

Fig. 6. **Cell immunostaining.** MC3 (*A*) and Cos7 (*C*) cells were immunostained with the anti-p105 antiserum 1140 as detailed under "Materials and Methods." p105 is stained *red*. As a control, MC3 cells were immunostained with the anti-p105 antiserum 1140 in presence of the antagonist peptide (*B*). Magnification, × 40.

in EBV-positive B cells was an alternative spliced form or a C terminus-deleted form of p105. In fact, the nuclear p105 was detected as a unique protein of 105 kDa using an antiserum directed against the C-terminal amino acids 955–969 of p105, which are usually lost in the alternative spliced forms (28) or the C terminus-deleted forms of p105 (26, 27). Moreover, the nuclear p105 had the same size as the cytoplasmic p105 revealed in different cell lines (Fig. 5). In Cos7, p105 was retained in the cytoplasm due to the presence of the C-terminal ankyrin repeats, which were assumed to mask the nuclear localization signal of p105 (26, 27). However, ankyrin repeats did not interfere with the nuclear translocation of bcl-3 (29, 30) and IκBα (31). In the examined EBV-positive B cells (MC3 and CB33), cell type-specific factors as well as EBV proteins may induce post-translational modifications of p105 resulting in the unmasking of the nuclear localization signal of the protein. Alternatively, p105 may be translocated into the nucleus by direct interaction with EBV proteins or EBV-induced cellular factors. These possibilities are under investigation.

In addition, we show that p105 was associated with p65, but not c-Rel, in the nucleus. This association could potentially serve to down-regulate the p65-mediated gene expression, as p105 inhibits the DNA binding of NF-κB subunits (20, 24). This suggests that B cell lines, such as EBV-positive B cells, which have a constitutive NF-κB activity and a high content of nuclear NF-κB/Rel proteins, may have developed an additional control of NF-κB activity mediated by p105 directly at the nuclear level.

Interestingly, concomitantly with NF-κB activation by mitomycin C, p105 was processed to p50 in the nucleus but not in the cytoplasm. Moreover, the IκBα protein level was not significantly modified in the cytoplasm by the DNA alkylating agent. These events were strictly associated with the nuclear increase in p50, but not in p65 and c-Rel. Altogether these results indicate that NF-κB activation by mitomycin C was mainly due to the generation of active NF-κB complexes directly in the nucleus through the processing of nuclear p105 rather than to the nuclear translocation of NF-κB complexes released by the proteolysis of IκB inhibitors in the cytoplasm. Differently, PMA preferentially acted at the cytoplasmic level by inducing the degradation of IκBα and, to a less extent, the proteolysis of p105, which resulted in the nuclear translocation of p65 and c-Rel. Nuclear p105 content was unaffected by the PMA treatment. These findings clearly indicate the existence of different signal transduction pathways in MC3 cells leading to the degradation of specific NF-κB inhibitors either at the nuclear or at the cytoplasmic level, depending on the nature of the signaling molecules. Accordingly, a signal-specific degradation of IκBβ was reported (5). Thus, by targeting specific IκB proteins, NF-κB activators could differently modulate the nuclear content of Rel/NF-κB factors and determine the composition of nuclear NF-κB complexes.

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