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κB enhancer-binding complexes that do not contain NF-κB are developmentally regulated in mammalian brain

(transcription/UV crosslinking/neural plasticity)

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ABSTRACT **DNA-binding complexes which are temporally regulated in developing rat brain have been identified by their ability to interact with the κB enhancer sequence in electrophoretic mobility-shift assays. These complexes, referred to as developing-brain factors (DBFs) 1 and 2, are abundant in nuclear extracts from developing rat brain through postnatal day 2 and decline to nearly undetectable levels by postnatal day 7. DBFs were not detected in extracts from cultured cell lines or in tissues other than the developing brain. The highest level of DBF DNA-binding activity was observed in developing cortex, and the lowest in cerebellum. In UV crosslinking experiments, a labeled κB oligonucleotide probe crosslinked 110- and 115-kDa proteins from DBF complexes. DBFs are likely to be involved in the regulation of transcriptional events which take place during brain development.**

Plasticity in the central nervous system, in response to environmental stimuli and during the course of development or regeneration, requires modulation of gene expression. Such changes in gene expression are likely to be governed by specific transcription factors. Thus, the factors which regulate gene expression in the central nervous system are of crucial interest in understanding the molecular basis of neural plasticity. The κB enhancer element is necessary for the inducible expression of a number of genes, many of which are involved in the immune response (1). In this study we were interested in identifying factors expressed in brain which bind to, and can potentially regulate transcription from, the κB enhancer. Here we report the identification of two κB-binding complexes, developing-brain factors (DBFs) 1 and 2, which are found exclusively in developing brain through postnatal day 7. Unlike other κB-binding proteins, DBFs are identified only in the nuclear fraction of cell extracts. By UV crosslinking, DBFs were shown to include 110- and 115-kDa proteins which are distinct from the previously identified p100 and p105 NF-κB precursor proteins (2, 3).

MATERIALS AND METHODS

Binding-Site Probes and Competitor DNAs. The double-stranded oligonucleotide containing the human immunodeficiency virus (HIV) κB, the κ immunoglobulin light-chain enhancer, and the major histocompatibility complex (MHC) κB have been described (4, 5). The palindromic κB-site oligonucleotide probe used for UV crosslinking was generated by a fill-in reaction with Klenow DNA polymerase (6).

Cell Extract Preparation and Electrophoretic Mobility-Shift Assays (EMSAs). Nuclear extracts from cultured cells were prepared as described (7). Briefly, whole brains (or other tissues) were removed and immediately homogenized with six strokes of the B pestle in a Dounce homogenizer in 4 volumes of 0.25 M sucrose/15 mM Tris·HCl, pH 7.9/60 mM KCl/15 mM NaCl/5 mM EDTA/1 mM EGTA/0.15 mM spermine/0.5 mM spermidine containing protease inhibitors: 1 mM dithiothreitol, 0.1 mM phenylmethanesulfonyl fluoride, leupeptin (2 μg/ml), and aprotinin (5 μg/ml). Cells were collected by centrifugation at 5000 rpm in a Sorvall SS34 rotor for 10 min, suspended in 4 volumes of 10 mM Hepes, pH 7.9/1.5 mM $MgCl_2$/10 mM KCl/protease inhibitors. Nuclei were pelleted, again in an SS34, for 10 min at 6000 rpm. The supernatant from this centrifugation was taken as the cytoplasmic fraction. The pellet was suspended in 0.5 M Hepes, pH 7.9/0.75 mM $MgCl_2$/0.5 mM EDTA/0.5 M KCl/12.5% (vol/vol) glycerol/protease inhibitors and incubated for 30 min. After 30 min of salt extraction, nuclei were collected by centrifugation at 14,000 rpm in an SS34 for 30 min, and the supernatant was dialyzed against 10 mM Tris·HCl, pH 7.9/1 mM EDTA/5 mM $MgCl_2$/10 mM KCl/10% glycerol/protease inhibitors. The entire procedure was carried out at 4°C. After 6–12 hr of dialysis, extract was again centrifuged at 14,000 rpm in an SS34, aliquoted, and frozen at −70°C. Protein concentrations were determined by Bradford assay (Bio-Rad).

EMSAs with or without deoxycholate (DOC) were performed as described (8). Methylation interference assays were performed as described (9).

UV Crosslinking. After the standard incubations with about 5×10^5 cpm of palindromic probe, the reaction mixture was transferred to a Nunc cryostat tube, and the tube was covered with SaranWrap, and placed under an inverted UV light box as described (9). After 1 hr of crosslinking, the binding reaction mixture was mixed with equal volume of 2× Laemmli sample buffer, boiled for 5 min, and electrophoresed in an SDS/10% polyacrylamide gel. For crosslinking in the gel, 1×10^6 cpm of palindromic probe was used and protocols were followed as described (10).

***In Vitro* Translation and Immunoprecipitation.** *In vitro* translation of p100 and p105 in pBluescript vectors (Stratagene) was carried out in TNT lysate (Amersham). ^{35}S-labeled protein was immunoprecipitated with antibodies to p100 or p105. Polyclonal p100 antiserum was raised against a synthetic peptide. p105 antiserum was raised against a peptide (antiserum 5177) or total protein expressed in bacteria (antiserum 5389). Protein A-Sepharose was used in all cases. Immunoprecipitation of UV-crosslinked proteins was carried out from the solution-phase crosslinking reaction mixture. Extract was crosslinked to ^{32}P-labeled HIV κB probe and immunoprecipitated with protein A-Sepharose.

RESULTS

NF-κB Is Present in an Inactive Form in Brain Extracts. The NF-κB complexes in nuclear and cytoplasmic extracts of

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Abbreviations: DBF, developing-brain factors; DOC, deoxycholate; EMSA, electrophoretic mobility-shift assay; HIV, human immunodeficiency virus; MHC, major histocompatibility complex.

adult rat brain tissue were identified by EMSA (10). Little constitutive specific κB enhancer-binding activity in adult rat brains was observed with the HIV κB enhancer sequence (12) (Fig. 1, lane 6). However, DNA-binding activity was detected with the addition of a detergent such as DOC, which presumably activates the κB complex (Fig. 1, compare lanes 2 and 3; ref. 8). An unlabeled κB oligonucleotide efficiently competed with the DOC-induced κB activity (lane 4), but a mutant oligonucleotide did not (lane 5). Nuclear extract from phorbol ester-stimulated 70Z/3 pre-B cells provided a positive control for NF-κB binding activity (Fig. 1, lane 1).

When cytoplasmic extracts from adult rat brains were incubated with the HIV κB probe in the presence of 1% DOC, a prominent band appeared (Fig. 1, compare lanes 7 and 8). Competition experiments with unlabeled wild-type and mutant HIV κB probes demonstrated that this induced activity was specific for the κB enhancer sequence (lanes 9 and 10). The upper DOC-induced complex in phorbol ester-stimulated mouse pre-B cell line 70Z/3 (lane 1) comigrated with the p50/p65 form of NF-κB (11). Thus, by EMSA, cytoplasmic and nuclear localization, and DOC inducibility, the NF-κB-like activity observed in extracts of adult rat brains was indistinguishable from the activity characterized in the lymphoid cell line 70Z/3.

κB Enhancer-Binding Activities Are Present in Developing Rodent Brains. We next asked whether κB enhancer-binding activities undergo changes paralleling development or differentiation in brain. EMSAs of nuclear extracts from developing rat brains (2 days postnatal) showed a prominent DNA-binding activity, appearing as a doublet (Fig. 2*A*, lane 3), which was virtually undetectable in adult rat brain nuclear extracts (lane 1). This binding activity migrated slower than the NF-κB activity observed by EMSA (Fig. 1). These slower moving complexes, termed DBFs, localized exclusively to the nuclear fraction (Fig. 2*B*, lane 3 versus lane 7) and were constitutively bound to the DNA probe. In fact, DBFs did not bind to DNA in the presence of 1% DOC, unlike the other NF-κB proteins. Comparison of DOC-activated extracts from neonatal and adult rat brain (Fig. 2*A*, even-numbered

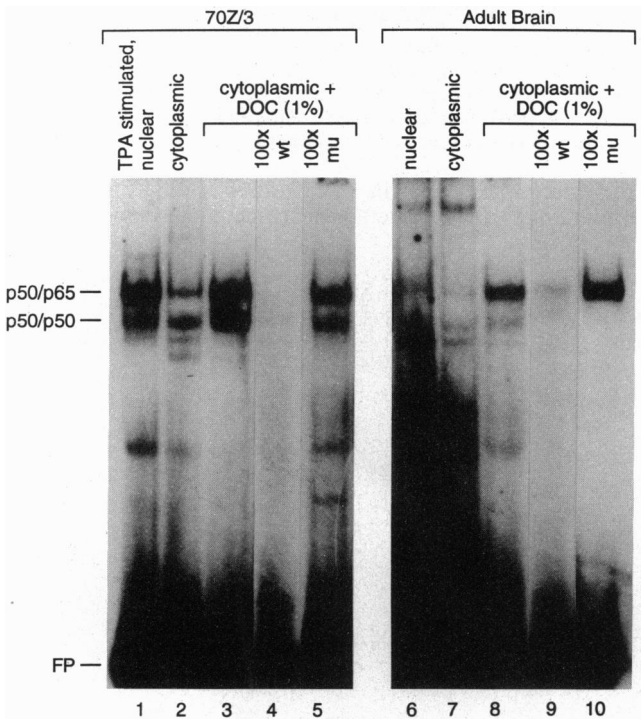

FIG. 1. The NF-κB-like activity found in brain extracts is indistinguishable from NF-κB as shown by EMSA with HIV κB probe. Lanes 1–5, 70Z/3 pre-B cell extracts. Lane 1, nuclear extract from cells stimulated with phorbol 12-myristate 13-acetate ("tetradecanoylphorbol acetate," TPA); lane 2, cytoplasmic extract; lane 3, cytoplasmic extract plus 1% DOC (wt/vol for sodium salt of DOC); lane 4, cytoplasmic extract plus 1% DOC, plus 100× unlabeled wild-type (wt) HIV κB probe; lane 5, cytoplasmic extract plus 1% DOC plus 100× unlabeled mutant (mu) HIV κB probe. Lanes 6–10, adult rat brain extracts. Lane 6, nuclear extract; lane 7, cytoplasmic extract; lane 8, cytoplasmic extract plus 1% DOC; lane 9, cytoplasmic extract plus 1% DOC plus 100× unlabeled wild-type HIV κB probe; lane 10, cytoplasmic extract plus 1% DOC plus 100× unlabeled mutant HIV κB probe. FP, free probe.

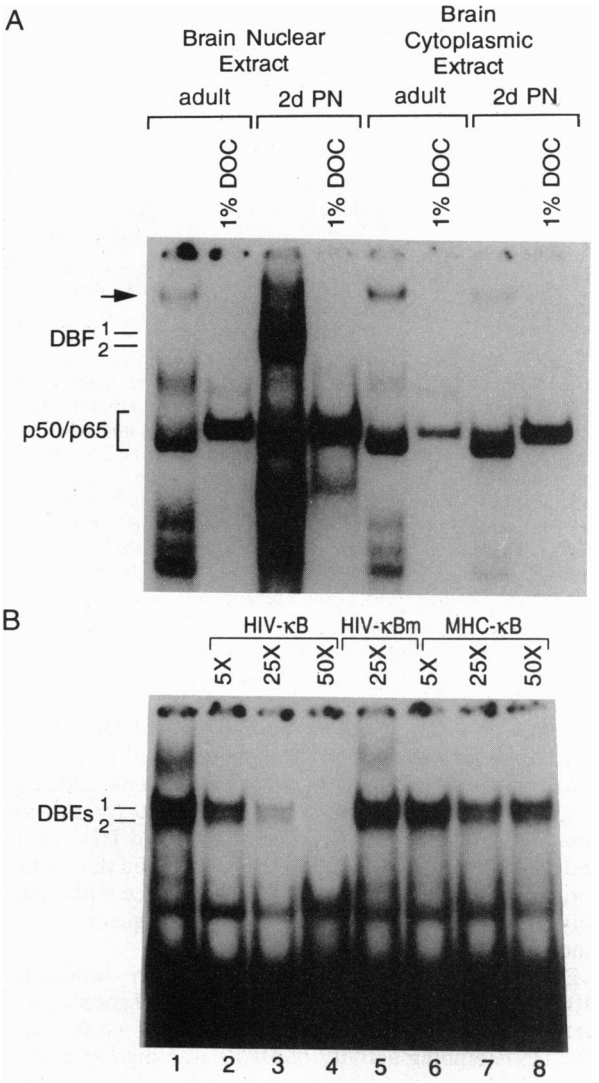

FIG. 2. (*A*) DBFs, κB enhancer-binding activities distinct from NF-κB, are present in developing brain as shown by EMSA of brain cellular extracts with HIV κB probe. Lane 1, nuclear extract from adult rat brain; lane 2, adult brain nuclear extract with 1% DOC in the binding reaction mixture; lane 3, nuclear extract from rat brain at postnatal day 2 (2d PN); postnatal day 2 brain nuclear extract with 1% DOC in the binding reaction mixture; lane 5, cytoplasmic extract from adult rat brain; lane 6, adult brain cytoplasmic extract with 1% DOC in the binding reaction mixture; lane 7, cytoplasmic extract from postnatal day 2 brain; lane 8, postnatal day 2 brain cytoplasmic extract with 1% DOC in the binding reaction mixture. (*B*) DBF activity is specific for HIV κB sites as shown by EMSA of neonatal brain (postnatal day 2) nuclear extracts with the HIV κB probe. Lane 1, no competitor; lanes 2–4, 5×, 25×, and 50× unlabeled competitor HIV κB oligonucleotide, respectively; lane 5, 25× mutant HIV κB oligonucleotide; lanes 6–8, 5×, 25×, and 50× MHC κB oligonucleotide (5′-GGGGATTCCCC-3′; ref. 5). Complex labeled with an arrow, upper left, is a candidate for the BETA factor (12).

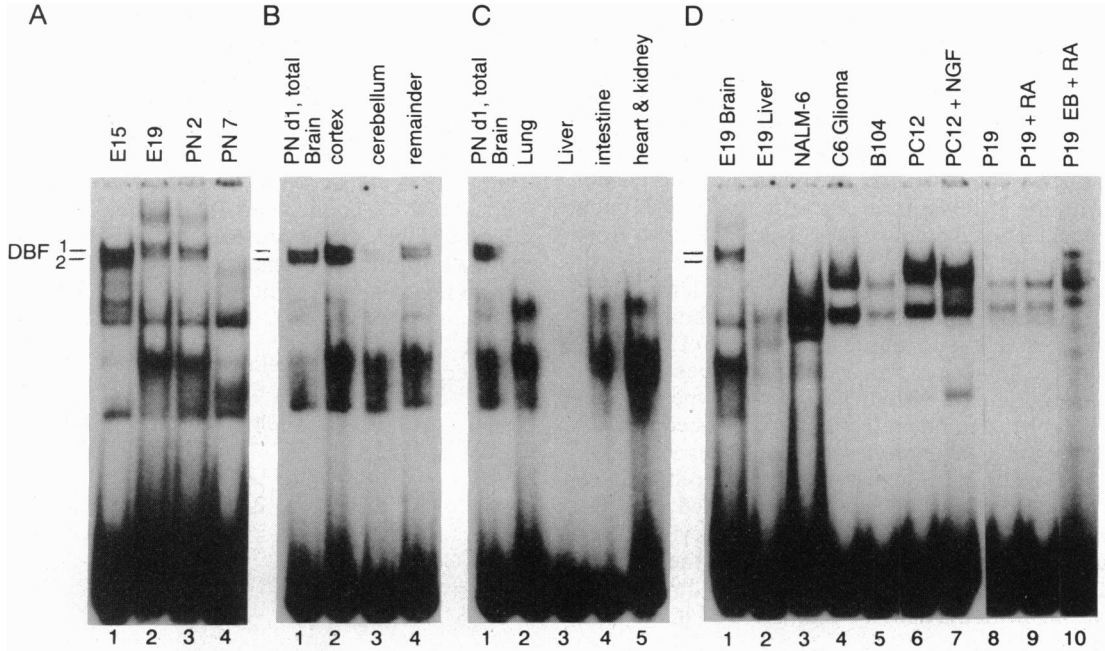

FIG. 3. (*A*) Time course of DBF expression in developing rat brain as shown by EMSA with nuclear extracts from rat brain at various times in development with the HIV κB element as probe. Lane 1, embryonic day 15, whole heads; lane 2, embryonic day 19, brains; lane 3, postnatal day 2 brains; lane 4, postnatal day 7 brains; lane 5, adult brain. The small difference in complex size between embryonic days 15, 19, and postnatal day 2 DBF complexes is not reproducible. Bands appearing in the lower half of the gel are nonspecific. (*B*) DBF expression in developing brain regions (postnatal day 1). Lane 1, whole brains; lane 2, cortex; lane 3, cerebellum; lane 4, remainder of the brain. (*C*) DBF expression in neonatal tissues (postnatal day 1). Lane 1, whole brain; lane 2, lung; lane 3, liver; lane 4, intestine; lane 5, heart and kidney. (*D*) DBF expression in cultured cell lines. DBF is not detectable in nuclear extracts from embryonic rat liver or in cell lines by EMSA with the HIV κB probe. Lane 1, embryonic day 19 brain; lane 2, embryonic day 19 liver; lane 3, Nalm-6 (a human B-cell line); lane 4, C6 glioma; lane 5, B104 (a rat neuroblastoma line); lane 6, PC12 (a rat pheochromocytoma line); lane 7, PC12 after 3 days with nerve growth factor (NGF) at 50 ng/ml; lane 8, P19 (a mouse embryonal carcinoma line); lane 9, monolayer P19 after 3 days in 1 μM retinoic acid (RA); lane 10, P19 cells cultured in suspension for 4 days as embryoid bodies (EB) in the presence of 1 μM retinoic acid, followed by growth as adherent cells for 4 days.

lanes) suggests that the net amount of DOC-releasable, NF-κB-like activity was not regulated over this developmental period.

Competition assays using an end-labeled HIV κB probe and excess unlabeled HIV κB oligonucleotide (Fig. 2*B*, lanes 2–4) or mutant oligonucleotide (lane 5) demonstrated that the DNA-binding activity of DBFs is specific for the κB sequence. Competition assays using a labeled HIV κB probe and excess unlabeled MHC κB probe showed that both DBF complexes recognized the HIV κB sequence with considerably higher affinity than the MHC κB sequence (Fig. 2*B*, lanes 6–8).

Expression of DBFs. Fig. 3 shows the expression pattern of DBFs during development and in a variety of somatic tissues. DBFs were expressed at the earliest time of assay (Fig. 3*A*). The DNA-binding activity of DBFs was high at embryonic day 15 and remained high through postnatal day 2, declining to nearly undetectable levels by postnatal day 7. Throughout the time course DBFs remained as two distinct bands of roughly equal intensity. EMSA of nuclear extracts from different regions of developing rat brains (postnatal day 1) showed that DBFs were present in developing cortex and cerebellum, as well as the remainder of the brain (Fig. 3*B*). The highest concentration of activity (per microgram of protein) was found in developing cortex, the lowest in cerebellum (Fig. 3*B*, lanes 2 and 3). DBFs could not be detected by EMSAs of postnatal day 1 lung, liver, intestine, or heart and kidney (Fig. 3*C*). DBFs could not be detected in a variety of tissues from adult rats (data not shown).

A survey of cultured cell lines (Fig. 3*D*) showed that DBFs could not be detected by EMSAs of nuclear extracts from Nalm-6 (a human B-cell line), C6 glioma, B104 (a rat neuroblastoma-derived cell line), PC12 (a rat pheochromocytoma cell line), and PC12 cells after 3 days of exposure to nerve growth factor. No detectable DBF could be detected in P19

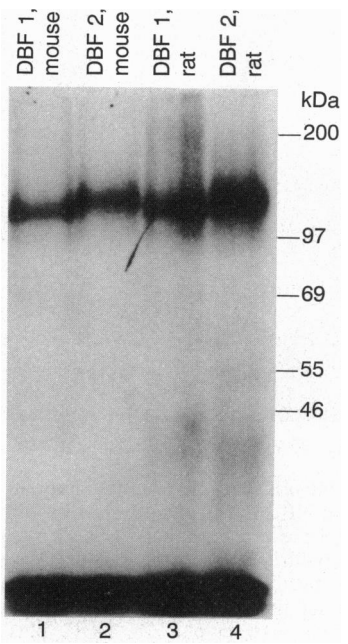

FIG. 4. The two DBF complexes include DNA-binding proteins of slightly different molecular masses, both about 110 kDa. Preparative EMSA was done with the bromodeoxyuridine-labeled palindromic κB-site probe. Crosslinking was performed in the EMSA gel, and the two DBF complexes were analyzed separately by SDS/7.5% PAGE. The results are the same for both rat and mouse.

cells (a mouse embryonal carcinoma line), which express endodermal markers in response to retinoic acid. However, when P19 cells were exposed to retinoic acid and cultured in suspension as embryoid bodies, an induced κB-binding activity appeared which comigrated with the upper DBF complex (Fig. 3*D*, lane 10).

Molecular Size of DBFs by UV Crosslinking Analysis. To determine the molecular size of the protein constituents of the DBFs, we carried out UV crosslinking studies, and each of the DBF complexes was excised from the gel and analyzed by SDS/PAGE. A single prominent band from each DBF mobility-shift complex was identified (Fig. 4). The crosslinked proteins migrated at 110–115 kDa. Interestingly, the more rapidly migrating DBF complex (DBF 2) crosslinked a slightly larger protein (≈115 kDa) than the more slowly migrating complex (DBF 1) (Fig. 4).

Characterization of DBF Binding Site. To determine the nucleotide contact points of the DBFs on the κB enhancer, methylation interference assays were carried out. The overall interference patterns determined for NF-κB and the DBFs were very similar (Fig. 5). The DNA-binding capability of both DBF complexes was reduced by the presence of methyl groups on any of the three designated guanosines previously shown to be critical for the binding of NF-κB (Fig. 5; ref. 10). The two DBF complexes gave virtually identical methylation interference patterns. Site-directed mutagenesis of the HIV κB oligonucleotide probe followed by EMSA revealed that overall binding-site specificity of DBF complexes is very similar to that of NF-κB (data not shown).

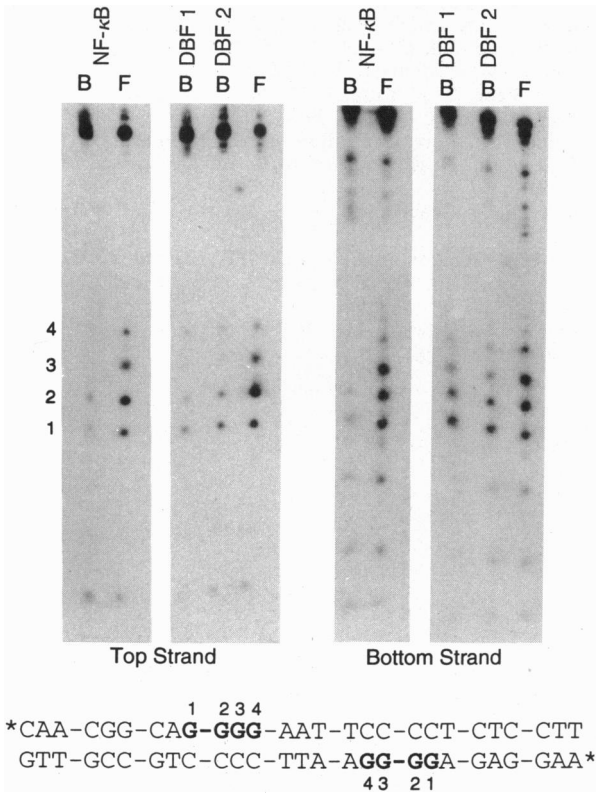

FIG. 5. Methylation interference assays carried out on DBF-bound and free palindromic κB oligonucleotide probes. Nuclear extract from phorbol ester-stimulated 70Z/3 cells was the source of p50/p65 NF-κB complex; DBF was from embryonic day 19 rat brain nuclear extracts. The two DBF complexes were mapped separately. Either the top (lanes 1–5) or the bottom (lanes 6–10) strand of the oligonucleotide was end-labeled prior to annealing, and interference patterns were generated for both DNA strands. NF-κB p50/p65, DBF 1, and DBF 2 were analyzed with bound (B) or free (F) probe.

DBFs Are Not Related to the p100 or p105 Precursors of NF-κB. The crosslinked proteins from DBFs 1 and 2 are very similar in size to the p100 and p105 proteins identified as precursors of the p52 and p50 DNA-binding components of NF-κB complexes (2, 3). We therefore undertook an immunoprecipitation analysis of the proteins or the DBF 1 and 2 complexes. p100 and p105 translated *in vitro* (Fig. 6, lanes 1 and 2) were immunoprecipitated with antibodies raised against p100 (lane 4) and p105 (lanes 5 and 6). However, none of these antibodies identified either DBF 1 or 2 (Fig. 6*B*). Additionally, p100- and p105-specific antibodies did not influence the DNA-binding complexes observed with DBFs 1 and 2 (data not shown). We therefore conclude that DBFs 1 and 2 are distinct κB-binding proteins.

DISCUSSION

We have identified DBFs 1 and 2, two κB enhancer-binding proteins which are developmentally regulated in rodent brain. These proteins are present in nuclear extracts of developing rat brains through several days following birth, declining to nearly undetectable levels by postnatal day 7. DBFs were not detected in EMSAs of nuclear extracts from other tissues or cell lines. DBFs 1 and 2 from neonatal brain extracts recognize the κB enhancer motif, with binding specificity very similar to that of NF-κB, but show a clear preference for the HIV κB over the MHC κB sequence (Fig. 2*B*). The two DBFs were indistinguishable with respect to DNA sequence recognition and gross temporal and spacial expression in the developing brain. In UV crosslinking analysis, each of the two gel shift bands crosslinked a single protein. The two identified proteins migrate slightly differently in SDS/polyacrylamide gels; the upper complex, DBF 1, crosslinks a protein of 110 kDa, while the lower complex (DBF 2) crosslinks a protein of 115 kDa. The different sizes of these two proteins argue that the two DBF complexes are not simple monomer and dimer forms of the same protein

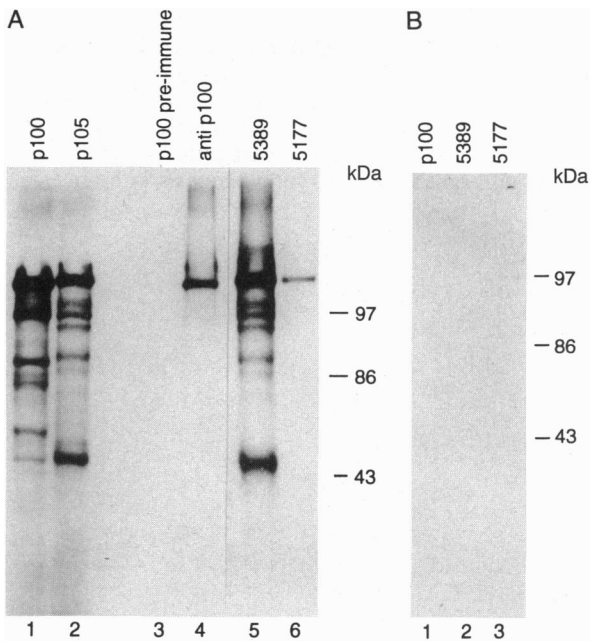

FIG. 6. Crosslinked DBF proteins are not recognized by antibodies to p100 or p105. (*A*) p100 and p105 proteins were translated *in vitro*, and ^{35}S-labeled proteins were visualized (lanes 1 and 2). Antiserum to p100 peptide specifically immunoprecipitated p100 (lanes 3 and 4). Antiserum 5389, to bacterial p105 (lane 5), or antiserum 5177, to p105 peptide (lane 6) specifically precipitated p105. (*B*) These antisera were not reactive in immunoprecipitation experiments with UV-crosslinked proteins from DBF complexes.

subunits. It is possible, however, that these two proteins represent the modified products of a single gene.

There are two major classes of κB enhancer-binding proteins: the Rel-related proteins, including c-Rel, p50, and p65, and the zinc finger proteins such as MBP-1 and MBP-2 (1, 13, 14). MBP-1 and MBP-2 are 298- and 275-kDa proteins, respectively (15, 16). The molecular mass of the two proteins crosslinked from DBF complexes (≈110 kDa) places them outside either of these two classes. The DBF proteins could represent precursor forms of a Rel family member, such as p105 (2) or Lyt-10 (3), but the following arguments make it unlikely. (*i*) Though purified p100 has recently been shown to bind MHC κB sites with high affinity (19), these precursors are thought to be cytoplasmic proteins; p105 is incapable of efficiently binding DNA (17, 18) until it is proteolytically processed. (*ii*) Antibodies capable of supershifting c-Rel or p50 in mobility-shift assays have not been successful in supershifting DBF complexes (data not shown). (*iii*) Amino- and carboxyl-terminal antibodies to p105, shown to immunoprecipitate p105 synthesized by *in vitro* translation, fail to immunoprecipitate proteins from UV-crosslinked embryonic brain nuclear extracts (Fig. 6). Similarly, antibodies to p100 (Lyt-10) also fail to immunoprecipitate crosslinked proteins from these extracts. Therefore, the unique patterns of the mobility-shift complexes and UV-crosslinked proteins argue that DBFs and their constituent DNA-binding protein(s) are distinct κB-binding activities.

A brain-specific κB enhancer-binding protein, termed BETA, from adult brains has been reported (12). The BETA complex can be supershifted in EMSA with antibodies to AGIE BP-1/MBP-2 (14, 20). In contrast to the DBFs described here, AGIE BP-1/MBP-2 is a zinc finger protein of 210 kDa which binds to MHC κB sites (14). The gel shift conditions used in our studies may not be optimal for detection of BETA. The slowest migrating complex from adult brain extracts may be the BETA complex (arrow, upper left in Fig. 2*A*). The role of DBFs in the regulation of transcription remains to be determined. The time course of DBF activities in brain is too protracted for a simple correlation with cell division. Cell density and division in brain around the time of birth are high among granule cells of the cerebellum, yet DBF DNA-binding activities are not enriched in this brain region (Fig. 3*B*). The activity of DBFs therefore may correlate with a particular phase of neural plasticity, differentiation, or synaptogenesis in brain.

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1. Baeuerle, P. A. (1991) *Biochim. Biophys. Acta* **1072**, 63–80.
2. Kieran, M., Blank, V., Logeat, F., Vandekerckhove, J., Lottspeich, F., LeBail, O., Urban, M. B., Kourilsky, P., Baeuerle, P. & Israel, A. (1990) *Cell* **62**, 1007–1018.
3. Neri, A., Chang, C.-C., Lombardi, L., Salina, M., Corradini, P., Maiolo, A. T., Chaganti, R. S. K. & Dalla-Favera, R. (1991) *Cell* **67**, 1075–1087.
4. Urban, M. B. & Baeuerle, P. A. (1990) *Genes Dev.* **4**, 1975–1984.
5. Baldwin, A. S. & Sharp, P. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 723–727.
6. Ballard, D. W., Walker, W. H., Doerre, S., Sista, P., Molitor, J. A., Dixon, E. P., Peffer, N. J., Hannink, M. & Greene, W. C. (1990) *Cell* **63**, 803–814.
7. Sonnenberg, J. L., Macgregor-Leon, P. F., Curran, T. & Morgan, J. (1989) *Neuron* **3**, 359–365.
8. Baeuerle, P. A. & Baltimore, D. (1988) *Cell* **53**, 211–217.
9. Ausubel, F. M., ed. (1991) *Current Protocols in Molecular Biology* (Wiley, New York).
10. Sen, R. & Baltimore, D. (1986) *Cell* **46**, 705–716.
11. Sen, R. & Baltimore, D. (1986) *Cell* **47**, 921–928.
12. Korner, M., Rattner, A., Mauxion, F., Sen, R. & Citri, Y. (1989) *Neuron* **3**, 563–572.
13. Schmid, R. M., Perkins, N. D., Duckett, C. S., Andrews, P. C. & Nabel, G. J. (1991) *Nature (London)* **352**, 733–736.
14. Rustgi, A. K., van't Veer, L. J. & Bernards, R. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8707–8710.
15. Fan, C.-M. & Maniatis, T. (1990) *Genes Dev.* **4**, 29–42.
16. van't Veer, L. J., Lutz, P. M. & Bernards, R. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 8971–8975.
17. Inoue, J.-I., Kerr, L. D., Ransone, L. J., Bengal, E., Hunter, T. & Verma, I. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3715–3719.
18. Rice, N. R., MacKichan, M. L. & Israel, A. (1992) *Cell* **71**, 243–253.
19. Potter, D. A., Larson, C. J., Eckes, P., Schmid, R. M., Nabel, G. J., Verdine, G. L. & Sharp, P. A. (1993) *J. Biol. Chem.* **268**, 18882–18891.
20. Rattner, A., Korner, M., Walker, M. D. & Citri, Y. (1993) *EMBO J.* **12**, 4261–4267.