The promoter of the amyloid β-protein precursor (APP) gene directs high levels of cell type-specific transcription with 94 base pairs’ 5′ to the main transcriptional start site. An essential activator domain in this proximal APP promoter is a nuclear factor binding site designated as APBβ. The recognition domain for the APBβ binding factor is located between position −93 and −82 relative to the main transcriptional start site.

The nuclear factor that binds to the APBβ site was partially purified by multiple steps of ion exchange and hydroxyapatite chromatography. Based on UV cross-linking results, a protein with an apparent molecular mass of 140 kDa was selected as the putative APBβ binding protein. After the final purification step consisting of preparative SDS-polyacrylamide gel electrophoresis, partial peptide sequences were obtained that completely matched the transcriptional factor CTCF. This protein is a known regulator of c-myc and lysozyme gene expression, and it binds to a variety of diverse DNA sequences.

The binding of CTCF to the APBβ domain was further established by competition with CTCF binding oligonucleotides in mobility shift electrophoresis. The identity was also confirmed by the observation that the APBβ binding factor is recognized by antibodies against C- and N-terminal sequences of CTCF. In addition, oligonucleotide competition during in vitro transcription affirmed that CTCF acts as a transcriptional activator in the APP gene promoter.

A characteristic pathological feature of Alzheimer’s disease and Down’s syndrome is the extracellular deposition of aggregated amyloid β-protein in the brain and cerebrovasculature (1–3). The amyloid β-protein is derived from a group of larger transmembrane glycoproteins, the amyloid β-protein precursors (APP)1 (4). The APP gene is expressed in all major tissues including brain, and the level of APP gene transcript is increased in Down’s syndrome and in certain areas of the brain in Alzheimer’s disease (5–8). This suggests that in some cases overexpression of the APP gene could play a contributing role in the pathological processes leading to amyloid depositions.

The mechanism of APP gene expression has been the subject of extensive studies (for review, see Ref. 9). The APP promoter extending to 3300 base pairs upstream from the transcriptional start site was found to confer cell type-specific expression of a reporter gene in transgenic mice (10). The distal section of the APP promoter contains numerous putative binding sites for regulatory transcription factors (11–15). Among these the hox1.3 (16), the heat shock (17), and the AP-1 (18–20) domains have been associated with functional relevance.

However, analyses of 5′ deletions of the human, rat, and mouse APP promoters demonstrated that 94–100 base pairs upstream from the transcriptional start site are sufficient for high levels of expression in numerous cell lines (11, 12, 21–23). The APP promoter is devoid of a recognizable TATA element but contains a prominent initiator element associated with the primary transcriptional start site at position +1 (Fig. 1). The integrity of the initiator element is crucial for promoter activity since transcription is disrupted and the start sites altered if the sequence of the initiator element is mutated (24). In addition, sequence elements immediately upstream of the initiator element to some degree affect both the transcriptional start site and transcriptional activity. Both the initiator element and the upstream element are associated with DNase I protected domains suggesting that nuclear factors occupy these sites (24) (Fig. 1).

The proximal APP promoter region also contains an sp1 site (25, 26) and two nuclear factor binding domains designated as APBo and APBβ (27). The factors that bind to these domains display sequence specificity, and it was demonstrated that the primary cellular factor that binds to APBβ is a heterodimer of USF43 and USF44 (25, 28–30). However, the primary activator domain in the proximal APP promoter is APBβ, which binds a nuclear factor to the recognition sequence GCGCTAGGGGT (position −93 to −82), and elimination of this site reduces total transcriptional activity from the proximal APP promoter by 70–90% (27). We report here on the purification of the APBβ binding factor and its identification as CTCF, an 11 zinc finger protein that is also a regulator of chicken c-myc and lysozyme gene expression (31–34). CTCF has a molecular mass of 82 kDa, and it binds divergent DNA sequences by utilizing different combinations of zinc fingers (32, 35).

MATERIALS AND METHODS

Plasmids and Oligonucleotides—Plasmid pCAT2bGAL as well as primers used for in vitro transcription have been described elsewhere (23, 24). Oligonucleotides synthesized by Genosys were deblocked and gel-purified prior to labeling and hybridization. Sequences of double-stranded oligonucleotides contained an additional 5′ single-stranded overhang of 1–2 bases on each end to maximize phosphorylation efficiency.

Nuclear Extracts, Binding Reactions, and Mobility Shift Electrophoresis—Nuclear extracts were prepared from HeLa cells grown in...
Purification of APBβ-binding Nuclear Factor—Partially purified nuclear factor for the optimization of APBβ binding conditions and UV cross-linking was obtained by a single step cation exchange chromatography procedure. Two milliliters of crude nuclear extract was loaded on a 1-ml Econo-Pac CM cation exchange cartridge (Bio-Rad) that had been preequilibrated with buffer D. The cartridge was washed with 5 ml of buffer D. Proteins were eluted with a 20-ml linear KCl gradient (100–500 mM) in buffer D, and 1-ml fractions were collected. The APBβ binding factor eluted around 450 mM KCl concentration.

The APBβ binding factor was purified for microsequencing in a multi-step procedure (Fig. 4A). Eight milliliters of crude nuclear extract were obtained from 16 liters of HeLa cell suspension culture (5 × 10⁶ cells/ml). This amount was loaded on a 1.5-ml Pharmacia HiTrap SP anion exchange column (Pharmacia Biotech Inc.) preequilibrated with buffer D containing 220 mM KCl. The column was washed with 5 ml of the same buffer and then with 2 ml of buffer D containing 400 mM KCl and 2.5% CHAPS. The APBβ binding factor eluted from the SP-Sepharose column. Therefore, the same final concentration and purification step with a hydroxypatite microcolumn was adopted without modifications.

UV Cross-linking—UV cross-linking efficiency is significantly improved when the thymidine analog 5-bromodeoxyuridine is introduced into the binding site (40). To increase the number of available thymidine residues, the transverse block mutation Mβ1 (Table I) was used for cross-linking studies. This mutation introduces four more thymidine residues in the vicinity of the binding domain and has no negative effect on binding activity (27). α-[32P]Deoxyxytidine and 5-bromo-2'-deoxyuridine were introduced in the coding strand of oligonucleotide Mβ1 (Table I) by primer extension with modified T7 DNA polymerase (Sequence34). The binding reaction was performed in 96-well plates either with crude nuclear extract from HeLa cells or with CM-purified binding factor as described above. Samples were covered with plastic wrap and UV-irradiated in a Stratalinker 2400 apparatus (Stratagene) for 10 min, supplemented with 5 mM MgCl₂ and 2 mM CaCl₂, and treated with 5–20 units of DNase I for 20 min at 37 °C. The reaction was stopped with 20 mM EDTA and 1% SDS, and the products were precipitated with 5 volumes of acetone at −20 °C for at least 6 h. Alternatively, samples were subjected to mobility shift agarose gel electrophoresis without prior 5-bromo-2'-deoxyuridine in the DNA probe, although with lower efficiency.

Antibodies and Western Blotting—Peptides denoting N-terminal amino acids 2–15 (EGDAVEAIVEEE) and C-terminal amino acids 708–721 (CATDAONGDLPFEMI) of human CTCF (35) were synthesized and purified to 95% homogeneity. These peptides were conjugated through their terminal cysteines to keyhole limpet hemocyanin and used for antibody production in rabbits. Peptide antibody production was performed by Coast Scientific (San Diego, CA). Western transfer to PVDF (Bio-Rad) membrane and antigen-specific protein detection was performed with the Amersham ECL system according to the manufacturer's instructions. The primary antiserum was used at a dilution of 1:400.

Mobility Shift Electrophoresis Followed by Western Transfer—APBβ binding factor that had been partially purified by CM cation exchange chromatography was concentrated 10-fold using a Microcon-30 device (Amicon). Four microliters of concentrated factor were incubated with 500,000 cpm (10 ng) of [32P]-Deoxycytidine and 5-bromo-2'-deoxyuridine without prior 5-bromo-2'-deoxyuridine substitution. The complex was UV cross-linked as described above and analyzed by mobility shift electrophoresis. Alternatively, 100 ng of unlabeled oligonucleotide was used in the binding reaction without subsequent UV cross-linking. Mobility shift electrophoresis was performed in a 5% polyacrylamide gel containing 0.5 × Tris borate/EDTA. The gel content was transferred to PVDF membrane (39) and probed with antiserum as described above.

In Vitro Transcription—The transcription reaction was carried out with HeLa cell nuclear extract and followed by primer extension as described elsewhere (24, 41) except in reactions where binding competition was performed. In such experiments 2 μg of plasmid was pre-

**TABLE I**

| Sequences of oligonucleotides used as double-stranded competitors or probes |
|---|
| MYC-80 | ggcctcaagctctgaagCCCCTTGGGCCCCCTTGCGCGGGGGCTCTTGTCGGCCTTctgctagggatccggg |
| APBβ-80WT | tgggctcaagctctgaagSCAGTTTCCCCGGGCGGGGCGGGGGGTTCCTGGGGTCCGAGCtagggatccggg |
| APBβ-80Mβ2 | tgggctcaagctctgaagSCAGTTTCCCCGGGCGGGGCGGGGGGTTCCTGGGGTCCGAGCtagggatccggg |
| APBβ-80WT | CAGTTTCCCCGGGCCCCCTGGGATTTCTCGGTCGGAGC |
| APBβ-80Mβ2 | CAGTTTCCCCGGGCCCCCTGGGATTTCTCGGTCGGAGC |
| APBβ-80WT | CAGTTTCCCCGGGCCCCCTGGGATTTCTCGGTCGGAGC |
| APBβ-80Mβ2 | CAGTTTCCCCGGGCCCCCTGGGATTTCTCGGTCGGAGC |

**Fig. 1. Sequence of the proximal AP promoter.** The previously characterized nuclear factor binding sites s1 (26), APBβ (USF) (30), and APBβ (27) are boxed, along with the DNP-I-protected domains USF and INr (24). Selected restriction sites and the position of relevant nucleotides are indicated relative to the position of the main transcription start site +1.
mixed with 2 μg of 80-mer oligonucleotides (APBβ-80WT, APBβ-M62, or MYC-80) prior to incubation with nuclear extract. This amount represents an approximately 100-fold molar excess of binding sequence.

RESULTS

Binding of Partially Purified APBβ Binding Factor Requires CHAPS and Bovine Serum Albumin—Preliminary efforts to purify the APBβ binding protein revealed that it bound to a variety of chromatographic supports such as DEAE-Sepharose, CM-agarose, and hydroxyapatite. However, when crude nuclear extract was loaded on a Bio-Rad Econo-Pac CM cation exchange cartridge and eluted with a 100–500 mM linear concentration gradient of KCl, for example, no mobility shift activity was observed in either the flow-through or in any of the elution fractions under standard assay conditions. Conversely, if the elution fractions were combined with flow-through material, a peak of binding activity was detected that eluted around 450 mM KCl concentration (not shown). We found that the flow-through fraction could be omitted if the binding reaction was supplemented with 2.5% CHAPS and 4 mg/ml bovine serum albumin. A mobility shift with whole nuclear extract (w.c.e.) is shown for reference. The positions of the bound (b) and free (f) oligonucleotides are indicated by brackets. No mobility shift activity could be detected in the flow-through (f.t.) fraction. A peak of nuclear factor binding activity was observed that eluted around 450 mM KCl concentration, which is represented by fractions 14–16.

UV cross-linked protein is indeed the specific APBβ binding protein, we first performed mobility shift agarose gel electrophoresis with the cross-linked products of the binding reaction. Thereafter, the binding complex was excised from the gel, electroeluted, treated with DNase I, and analyzed by SDS-polyacrylamide gel electrophoresis. In this instance the same major 140-kDa band was observed on the autoradiograph (Fig. 3, lane 3).

Partially Purified Nuclear Extract Fractions That Display APBβ Binding Activity Contain a 140-kDa Protein Identified as CTCF—The nuclear factor that binds to the APBβ element was partially purified by several steps of chromatography (Fig. 4A). APBβ binding activity in the fractions was monitored by mobility shift electrophoresis. In the initial purification step, crude nuclear extract was loaded on a DEAE-Sepharose column. The APBβ binding activity eluted at an approximate concentration of 220 mM KCl. The electrophoretic protein distribution pattern after this initial column shows the presence of numerous bands with little evidence for specific enrichment (Fig. 4B, lane 1).

After this initial step, two alternative schemes of purification were adopted. The first scheme takes advantage of the ability of the APBβ binding factor to bind with relatively high affinity to both anion and cation exchange resins. Fractions from the DEAE-Sepharose chromatography that contained APBβ binding activity were pooled and loaded on an SP anion exchange column. The APBβ binding activity eluted at a 500 mM KCl concentration. After SP-Sepharose chromatography only a few isolated protein bands were detected in the purified material (Fig. 4B, lane 2). Specifically, only one protein band with the apparent molecular mass of 140 kDa was detected in the molecular mass range defined by UV cross-linking.

To concentrate and to enrich further the APBβ binding factor, we loaded the SP-Sepharose purified material on a column packed with ceramic hydroxyapatite. The peak APBβ binding activity eluted at about 60 mM potassium phosphate concentration. The same protein band with the molecular mass of 140 kDa was observed by SDS-polyacrylamide gel electrophoresis.
...The bound (140 kDa protein.

D) indicates position of 140-kDa protein. D, same as in C except that the elution fractions were analyzed by mobility shift electrophoresis. The bound (b) and the free (f) fragments are indicated.

(Fig. 4B, lane 3). The concentration of binding protein that was achieved by the hydroxyapatite chromatography step provided an amount of protein in the band that was sufficient for microsequencing.

In an alternative scheme, the fractions with APβ binding activity from DEAE-Sepharose chromatography were pooled and first loaded on a heparin column. Also in this instance the APβ binding factor eluted approximately at a 500 mM KCl concentration. After final purification and concentration by hydroxyapatite chromatography, eluted material from the heparin column contained more protein bands than material eluted from the SP anion exchange column (Fig. 4B, lanes 3 and 4). However, in both cases a major protein band with the same molecular mass of 140 kDa was observed by SDS-polyacrylamide gel electrophoresis. Additional column combinations were also used in the attempt to purify the binding factor. For example, nuclear extract was subjected to SP anion exchange followed by hydroxyapatite chromatography. Also in this instance multiple protein bands are observed, but a comparison of mobility shift activity and protein composition of each elution fraction shows that the binding activity largely coincides with the presence of the 140-kDa protein (Fig. 4, C and D). Although the protein composition varied between different purification schemes, all elution fractions with APβ binding activity contained the 140-kDa protein. We therefore designated this protein as the putative APβ binding factor.

Preparative SDS-polyacrylamide gel electrophoresis was used as the final step to purify this protein for sequence analysis. After hydrolysis with trypsin, two of the resulting peptides were microsequenced. A search in the GenBank sequence data base revealed that both peptide sequences, YCDAVFHER and IQHQK, corresponded completely to the sequence of transcriptions factor CTCF, which was originally described as a regulator of the chicken c-myc gene (33).

The APβ Domain of the APP Promoter and the CTCF Binding Site of the Chicken c-myc Promoter Compete for the Same Nuclear Factor—The binding site of CTCF in the chicken c-myc promoter has been extensively characterized (33, 35). To demonstrate that CTCF is the protein that binds to the APβ element, we performed a mobility shift competition assay (Fig. 5). As a DNA fragment containing a well characterized CTCF-binding site we used the 80-bp double-stranded oligonucleotide here referred to as MYC-80, which reproduces the CTCF-binding DNA fragment designated as FPV by Lobanenkov et al. (34).

For a direct comparison between the APβ binding activity and CTCF binding to MYC-80, we increased the length of the APβ element to 80 bp by flanking the previously used 45-bp oligonucleotide with the same polylinker sequence that flanked the recognition sequence in oligonucleotide MYC-80 (Table I), and it will here be referred to as APβ-80WT. A transverse mutation designated Mβ2 was previously identified that abolished nuclear factor binding to the APβ domain (27). This mutation was introduced into APβ-80WT thereby generating oligonucleotide APβ-80Mβ2. Despite its longer flanking sequence, oligonucleotide APβ-80Mβ2 showed no evidence of nuclear factor binding (not shown).

Mobility shift electrophoresis (Fig. 5A, lanes 1–4) revealed that oligonucleotide APβ-80WT competed for nuclear factor binding to the APβ sequence with similar efficiency as oligonucleotide MYC-80 (Fig. 5A, lanes 2 and 4). In contrast, no competition was observed with mutation APβ-80Mβ2 (Fig. 5A, lane 3). This was further confirmed by reciprocal competition, which was carried out with labeled oligonucleotide...
Antibodies Against CTCF Peptides Recognize the Protein That Binds to the APBβ Element—Additional evidence that CTCF binds to the APBβ element was provided by antibodies against two peptides from the N- and C-terminal portions of the CTCF sequence.

Partially purified APBβ binding protein was separated by SDS-PAGE and transferred to PVDF membrane. The antisera against both N- and C-terminal peptides recognized the same 140-kDa protein band originally isolated for sequencing (Fig. 5, lanes 1 and 2). Mobility shift electrophoresis in a 6% acrylamide gel of 32P-end-labeled oligonucleotide APBβ-80WT competed for factor binding to the c-myc sequence, whereas oligonucleotide APBβ-Mβ2 showed no competition. Similar results were obtained when partially purified APBβ binding protein was used instead of whole nuclear extract (not shown). This illustrates that despite widely divergent nucleotide sequences, the CTCF binding site element for the binding of the same nuclear factor.

Antibodies Against CTCF Peptides Recognize the Protein That Binds to the APBβ Element—Additional evidence that CTCF binds to the APBβ element was provided by antibodies against two peptides from the N- and C-terminal portions of the CTCF sequence.

Partially purified APBβ binding protein was separated by SDS-PAGE and transferred to PVDF membrane. The antisera against both N- and C-terminal peptides recognized the same 140-kDa protein band originally isolated for sequencing (Fig. 4), whereas preimmune serum showed no reactivity (Fig. 6).

To examine whether these antibodies recognized the native APBβ binding protein, they were initially analyzed by mobility supershift electrophoresis. However, no supershift activity was observed (not shown), which suggested that either CTCF did not bind to the APBβ site or the antigenic determinants were not accessible in the native, nondenatured protein. To distinguish between these possibilities, the binding protein was analyzed by a combination of mobility shift electrophoresis and Western blotting. Specifically, mobility shift electrophoresis was performed in polyacrylamide gels (Fig. 7, lanes 1 and 2), and the content of the gel was transferred to PVDF membrane. However, as might be expected, the membrane did not retain the oligonucleotide, and it was therefore not possible to unequivocally identify the position of the shifted binding complex. To circumvent this problem, the APBβ binding protein was UV cross-linked to the DNA prior to mobility shift electrophoresis. As a result, the cross-linked complex was retained on the PVDF membrane, and a radiolabeled band could be observed on the blot, marking the position of the APBβ DNA-protein complex (Fig. 7, lane 3). Probing of this radiolabeled, UV cross-linked complex with anti-C-terminal CTCF antiserum displayed an immunoreactive band that precisely overlapped with the radio-

By having ascertained the appropriate position of the binding complex, mobility shift electrophoresis was also carried out with protein that was not UV cross-linked to the DNA. By this method an immunoreactive band was detected by both N- and C-terminal antibodies that migrated to the same position as the UV cross-linked complex (Fig. 7, lanes 5 and 7). If no oligonucleotide was added to the protein prior to the electrophoresis, no such band was observed (Fig. 7, lane 6 and 8). As an additional negative control preimmune serum was used to probe the blot (Fig. 7, lane 9). No immunoreactive band is apparent at the position of the complex in this lane.

Thus the APBβ binding factor that migrated on the mobility shift gel as part of the DNA-protein binding complex was detected by CTCF-specific antibodies after it was denatured by Western transfer.

CTCF Binding Activates in Vitro Transcription from the APP Gene Promoter—To correlate CTCF with functional activity, competition for CTCF binding to APBβ was performed during in vitro transcription with nuclear extract from HeLa cells. The template DNA used for the transcription reaction was the plasmid designated pCAT2bGAL (23). This plasmid contains two reporter genes chloramphenicol acetyltransferase and β-galactosidase. The β-galactosidase gene serves as an internal control for experimental variations, and it is transcribed from the largely constitutive β-actin promoter (36, 37). The chloramphenicol acetyltransferase gene is transcribed from the APP promoter extending from position −458 to position +100 (Fig. 1).

In vitro transcription from plasmid pCAT2bGAL generated two sets of transcripts originating from the β-actin and APP promoters. The β-actin promoter produced one major transcriptional start site, whereas transcription from the APP promoter initiated at three start sites at positions +1, −1, and −4 (Fig. 8, lane 1). A more detailed rendition on in vitro transcription from the APP promoter has been described elsewhere (24).
transcription reaction was performed either in the absence or in the presence of a 100-fold molar excess of oligonucleotides APB\(^\beta\)-80WT (lane 2), APB\(^\beta\)-80M\(\beta\)2 (lane 3), and MYC-80 (lane 4). The transcriptional start sites initiated from the APP (-4 and +1) and \(\beta\)-actin promoters are indicated by arrowheads.

During the initial attempts to purify the APB\(^\beta\) binding factor by a range of chromatographic methods, removal of the binding activity from crude nuclear extracts could be easily achieved. However, recovery of the activity after elution from the column was unsatisfactory unless individual fractions were supplemented with CHAPS and BSA. Such loss of activity following protein purification could be linked to destabilization of the binding protein or to nonspecific adsorption as a consequence of dilution. For example, non-ionic detergents are frequently included in mobility shift electrophoresis and the zwitterionic detergent CHAPS has been shown to significantly improve nuclear factor binding to DNA in mobility shift assays (44). In addition, CHAPS has been shown to inhibit nonspecific adsorption and to stabilize protein complexes (45).

The protein with direct contact points to the APB\(^\beta\) site was identified by UV cross-linking, and it was purified by multiple steps of chromatography. A band common to all fractions containing binding activity was found to have the approximate molecular mass of 140 kDa. This was the only protein in the same molecular weight range as observed by UV cross-linking, and it was collected for microsequencing. Two peptides were independently sequenced, and the obtained sequence showed a 100% match with the previously identified protein CTCF, which is a transcriptional regulator containing 11 zinc fingers (35). Specifically, one peptide with the sequence YCDAVFHER is located within zinc finger 7 and the other peptide with the sequence IQHQK is located between zinc fingers 7 and 8.

The direct binding of CTCF to the APB\(^\beta\) site was verified by generating antibodies against peptides representing the N- and C-terminal parts of the CTCF sequence. These antibodies specifically recognized the protein bound to the APB\(^\beta\) site in mobility shift electrophoresis. The conclusion that CTCF binds to the APB\(^\beta\) sequence was further supported by mobility shift competition assays in which a well characterized CTCF binding sequence from the chicken c-\(\text{myc}\) gene was shown to act as an efficient competitor for binding to the APB\(^\beta\) site and vice versa. The functional role of CTCF binding to the APP promoter was confirmed by \textit{in vitro} transcription assays. The APB\(^\beta\) nuclear factor binding domain had been previously demonstrated as essential for high levels of transcriptional activity from the APP promoter. This was determined \textit{in vitro} by transient transfection in HeLa and PC-12 cells and \textit{in vitro} by cell-free transcription with HeLa cell nuclear extract (24, 27). Those results were also confirmed here by competition for factor binding to the APB\(^\beta\) binding site during \textit{in vitro} transcription (Fig. 8). Both the oligonucleotides containing the CTCF recognition sequence from the chicken c-\(\text{myc}\) promoter and the APB\(^\beta\) sequence compete for binding of CTCF to the APP promoter in the plasmid CAT2bGAL, thereby inhibiting transcription, indicating that CTCF acts as a transcriptional activator in the APP promoter. In contrast, transcription from the \(\beta\)-actin promoter, which depends on a CCAAT, a serum response, and a TATA element (36, 46), was unaffected by the competition with CTCF binding oligonucleotides.

CTCF is a highly conserved DNA binding protein, and it binds to the promoter of the chicken c-\(\text{myc}\) gene between position –180 and –230 upstream from the transcriptional start site. It may act either as a transcriptional repressor or activator depending on what cell background the promoter is analyzed (33, 34, 42). The recognition sequence for CTCF in the chicken c-\(\text{myc}\) promoter is composed of several CCCTC repeats. In the human and mouse c-\(\text{myc}\) genes CTCF binds to divergent recognition sequences coinciding with RNA polymerase pausing sites in the transcribed region of the genes (35). In addition, a protein designated NeP1 was identified that binds to the chicken lysozyme silencer 2.4 kilobase pairs upstream from the transcriptional start site and synergistically represses transcription in conjunction with \(\nu\)-ERBA, the thyroid hormone receptor, or the retinoic acid receptor (31, 43). NeP1 has since been found to be identical to CTCF (32). However, its recognition sequence in the lysozyme silencer domain bears no apparent resemblance to any of the c-\(\text{myc}\) recognition sequences. Indeed, it has been demonstrated that CTCF binds variable sequences by employing different combinations of zinc fingers (32, 35). Therefore, it is not surprising that the APB\(^\beta\) site in the APP gene promoter is dissimilar from other known CTCF binding sites.

CTCF displays some other unusual properties, all of which are also evident within the context of its binding to the APB\(^\beta\) element. For example, the CTCF binding domain in the chicken c-\(\text{myc}\) promoter is unusually large in the sense that its DNase I footprint extends over approximately 50 bp (33). A similar footprint was observed in the APP promoter binding domain (not shown). Moreover, for successful CTCF binding in mobility shift assays, a DNA fragment exceeding 44 bp was required (33). Similarly, in the APP promoter the sequence surrounding the 12-base pair core APB\(^\beta\) recognition domain could be widely varied with little or no effect on factor binding in mobility shift assays (27). However, reducing the length of the 45-bp fragment (APB\(^\beta\)-WT, Table I) on either side of the core domain eliminated factor binding (not shown). CTCF also exhibits

![Fig. 8. Competition for APB\(^\beta\) binding during \textit{in vitro} transcription. The plasmid CAT2bGAL containing the APP promoter from position +100 to –488 was transcribed in HeLa cell nuclear extract either without competitor (lane 1) or with a 100-fold molar excess of oligonucleotides APB\(^\beta\)-80WT (lane 2), APB\(^\beta\)-80M\(\beta\)2 (lane 3), and MYC-80 (lane 4). The transcriptional start sites initiated from the APP (-4 and +1) and \(\beta\)-actin promoters are indicated by arrowheads.](image-url)
anomalous behavior in SDS-PAGE. As deduced from the sequence of its cDNA, it has a molecular mass of 82 kDa (34). However, by different accounts it migrates in SDS-polyacrylamide gel electrophoresis like a protein with the much larger molecular mass of 130–160 kDa (33, 35, 44), and our determination of the apparent molecular mass of the APBβ binding protein (140 kDa) is also within that range. The aberrant electrophoretic migration was traced to the N- and C-terminal portions of the CTCF molecule (42).

A defining pathological characteristic of Alzheimer’s disease is the extracellular deposition of aggregated amyloid β-protein in the brain and cerebrovasculature (1, 2). Another prominent feature of the disease is a substantial loss of neurons in the hippocampus and cerebral cortex, and several lines of evidence support the hypothesis that this loss is attributed to apoptotic neuronal cell death promoted by amyloid β-protein (47, 48). Incidentally, the c-myc gene has been implicated in playing a key role in the promotion of apoptosis (49). Furthermore, overexpression of the APP gene might be considered as one of the factors leading to the accumulation of amyloid β-protein in the affected areas of the brain. This study identifies the transcription factor CTCF as a major contributor to the activation of the APP promoter, and it is also a regulator of c-myc gene expression. Therefore, the transcriptional regulation of both the APP and c-myc genes by CTCF provides a new and intriguing, albeit as yet speculative, link between apoptosis and Alzheimer's disease.

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