Identification of a Zinc Finger Protein That Binds to the Sterol Regulatory Element

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Cholesterol balance in mammalian cells is maintained in part by sterol-mediated repression of gene transcription for the low density lipoprotein receptor and enzymes in the cholesterol biosynthetic pathway. A promoter sequence termed the sterol regulatory element (SRE) is essential for this repression. With the use of an oligonucleotide containing the SRE to screen a human hepatoma complementary DNA expression library, a clone for a DNA binding protein was isolated that binds to the conserved SRE octanucleotide in both a sequence-specific and a single-strand–specific manner. This protein contains seven highly conserved zinc finger repeats that exhibit striking sequence similarity to retroviral nucleic acid binding proteins (NBPs). We have designated the protein "cellular NBP" (CNBP). CNBP is expressed in a wide variety of tissues, is up regulated by sterols, and exhibits binding specificity that correlates with in vivo function. These properties are consistent with a role in sterol-mediated control of transcription.

THE EXPRESSION OF THE LOW DENSITY lipoprotein (LDL) receptor and enzymes involved in cholesterol biosynthesis is coordinately regulated. Negative feedback inhibition by sterols derived both from exogenous LDL and endogenous synthesis is the chief means by which cholesterol homeostasis is maintained in mammalian cells (*1*). A sequence essential for sterol-mediated repression of transcription has been defined through studies of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (*2, 3*) and LDL receptor gene promoters (*4, 5*). Referred to as the sterol regulatory element (SRE), this sequence contains an octanucleotide motif, GTG$^{C}_{G}$GGTG, hypothesized to be the core binding site for a sterol-dependent transacting factor (*3*). Gil *et al.* (*6*) have isolated two proteins in the nuclear factor 1 (NF1) family that bind specifically to six deoxyribonuclease I footprint positions along the HMG-CoA reductase promoter, including a sequence overlapping the SRE octanucleotide. Neither of these proteins is responsible for sterol-mediated repression, but the location of the binding sites suggests the possibility of interaction or competition with the repressor.

In an attempt to identify the repressor, we used an oligonucleotide probe [56 nucleotides (nt)] containing the HMG-CoA reductase SRE to screen a λgt11 cDNA library from human hepatoma (HepG2) cells. The method of Singh *et al.* (*7*) was followed with modifications (*8*). A positive clone, cellular nucleic acid binding protein (CNBP), was isolated and confirmed by secondary and tertiary screenings. The complete nucleotide and derived amino acid sequence of the 1500-nt CNBP cDNA insert is shown in Fig 1A. Observation of a 1.5-kb mRNA species in Northern blot analysis suggests that this CNBP cDNA clone is essentially full length. The 5′ end represents an open reading frame continuous with *lac Z*. However, the presence of the most optimal translation initiation consensus sequence (*9*) around the first methionine codon at nucleotides 103 to 105 suggests that this ATG is the initiation codon of the native CNBP gene. Prediction of termination at the TAA codon at position 634 to 636 was confirmed by expression of the expected 5-kD CNBP-encoded peptide from a TrpE expression vector (*10*) containing the CNBP Eco RI fragment extending from nucleotides 472 to 1500. Hence, the sequence suggests that CNBP is a 177–amino acid polypeptide. In keeping with this, we found by immunoblot analysis with an antibody against *Escherichia coli* β-galactosidase that CNBP contributes about 20 kD to the mass of the β-galactosidase fusion protein. Moreover, in vitro translation of RNA transcribed from the complementary DNA yielded the expected 19-kD protein product (Fig. 2B). The CNBP mRNA has a long 3′ untranslated region of at least 870 nt (Fig. 1A).

The predicted amino acid sequence of CNBP reveals the presence of seven tandem repeats of 14 amino acids, indicated by the underlined regions in Fig. 1A. The first repeat is separated from the other six by a stretch of 32 amino acids rich in glycine and arginine, and punctuated by prolines. The highly repetitive nature of the CNBP protein sequence is shown in Fig. 1B. The individual repeat sequences can be aligned into zinc finger motifs as reported for numerous DNA binding proteins (*11*). The importance of zinc, coordinated tetrahedrally by four cysteines or a combination of cysteines and histidines, has been directly demonstrated in the zinc fingers of several proteins (*11*). A search of the GenBank and National Biomedical Research Foundation (NBRF) libraries indicated that CNBP shares both nucleotide and amino acid sequence homologies with a number of nucleic acid binding proteins, including the *Drosophila* retrotransposon copia, a Syrian hamster intracisternal-A particle, and the finger portion of the coat protein of the cauliflower mosaic virus. The most striking similarity is seen with the nucleic acid binding proteins (NBPs) encoded by the *gag* gene of retroviruses. The zinc finger consensus sequence of CNBP, $CYXCGX_2GHXAX_2C$, is almost identical to that of the NBPs (*12*) (Fig. 1B). However, whereas the NBPs of retroviruses contain either one or two copies of the finger repeat separated by 5 to 11 residues, CNBP contains seven copies of the finger sequence separated by 4 to 32 residues. Therefore, CNBP apparently belongs to a subfamily of the Cys/Cys-His/Cys class of zinc finger proteins (*12*). Since retroviral NBPs in this class bind to single-stranded DNA and to RNA, we speculated that CNBP might be a single-stranded DNA binding protein.

To examine this possibility and to test the DNA sequence binding specificity of the CNBP fusion protein, we carried out blotting analysis. A single-stranded DNA probe (25 nt) containing the GTGCGGTG sequence bound specifically to the 135-kD CNBP fusion protein (Fig. 2A, lane 2). Competition for binding of this end-labeled probe was demonstrated with an excess of unlabeled probe. However, both a single-stranded oligonucleotide containing the oc-

Table 1. Correlation of DNA sequences for ability to confer sterol responsiveness and to bind CNBP.

Oligonucleotide sequence	Sterol responsiveness	CNBP binding
GTGCGGTG*	+	+
GTGGGGTG†	+	+
CTGGGGTG‡	+	+
GTGGCGCG§	+	+
GTGGGTAT‖	–	–
CATAGGTG‖	–	–
CATATCAT‖	–	–

*The human and hamster HMG-CoA reductase SRE sequences (*3*). †The human LDL receptor (repeat 2) SRE sequence (*5*). ‡An SRE-like sequence present in the promoter of mouse apolipoprotein AIV (*16*). §An SRE-like sequence present in the promoter of rat farnesyl pyrophosphate synthetase (*17*). ‖Octamer sequences that fail to confer sterol responsiveness when substituted for the LDL receptor SRE core sequence (*5*).

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tamer complement sequence CACGCCAC (lane 3), and the corresponding double-stranded oligonucleotide (lane 4) failed to bind to the fusion protein. Several additional single- and double-stranded oligonucleotides of related or unrelated sequence also failed to bind CNBP (for example, lane 6). Lysate from the wild-type λgt11 lysogen served as a negative control for the CNBP fusion protein and contained no proteins of 135 kD capable of binding the probe (lane 1). Thus, CNBP is indeed a single-stranded DNA binding protein with preferential specificity for the GTGCGGTG sequence. The original isolation of the cDNA for this protein was possible because of the partially single-stranded nature of the probe used for screening.

The binding specificity of the CNBP protein was further examined by testing a series of single-stranded 25-nt DNA probes containing various modifications of the octamer sequence. The related octanucleotide sequence, GTGGGGTG [present in the LDL receptor and HMG-CoA synthase promoters (*4*)], exhibited strong binding to CNBP (Fig. 2A, lane 5). Also, CNBP bound strongly to related sequences in the promoters for two additional sterol-responsive genes, rat farnesyl pyrophosphate synthetase and mouse apolipoprotein AIV (Table 1), but not their complementary sequences. In contrast, no CNBP binding was observed with several sequences that are incapable of conferring sterol-mediated repressibility on a reporter gene (Table 1). These results, indicating a correlation between the effect of the related octamer sequences on transcription and their ability to bind the CNBP protein, are consistent with the concept that CNBP is involved in sterol-mediated repression.

Blotting studies revealed a protein in HepG2 nuclear extracts that resembles CNBP in DNA binding specificity and size (Fig. 2B). The protein comigrated on denaturing gels with in vitro translated CNBP (apparent molecular mass, 19 kD) and was specific for the single-stranded SRE core sequence. These results also indicate that CNBP is itself regulated by sterols. When cholesterol synthesis of HepG2 cells was down regulated by treatment for 14 hours with 25-hydroxycholesterol (1 μg/ml) and mevalonic acid (10 m*M*), there was a four-fold increase in CNBP binding activity (Fig. 2B). In contrast, a 34-kD nuclear protein that also bound the labeled oligonucleotide probe was unaffected by the sterol treatment (Fig. 2B). A protein resembling CNBP in DNA binding specificity was also detected in nuclear extracts by gel retardation analysis, although these results were complicated by the presence of multiple bands.

The entire CNBP cDNA insert was used as a probe in Southern blot analysis of human and mouse genomic DNA. Under stringent hybridization conditions, each of a number of restriction digests gave a simple hybridization pattern consistent with CNBP being a single copy gene highly conserved in

A

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                                                    5'- GAATTCCAAACAGCCTCTACCTT   23
GCGAGCCGTCTTCCCCAGGCCTGCGTCCGAGTCTCCGCCGCTGCGGGCCCGCTCCGACGCGGAAGATCTGACTGCAGCC   102
ATG AGC AGC AAT GAG TGC TTC AAG TGT GGA CGA TCT GGC CAC TGG GCC CGG GAA TGT CCT   162
Met Ser Ser Asn Glu Cys Phe Lys Cys Gly Arg Ser Gly His Trp Ala Arg Glu Cys Pro
                                    10
ACT GGT GGA GGC CGT GGT CGT GGA ATG AGA AGC CGT GGC AGA GGT GGT TTT ACC TCG GAT   222
Thr Gly Gly Gly Arg Gly Arg Gly Met Arg Ser Arg Gly Arg Gly Gly Phe Thr Ser Asp
                                    30
AGA GGT TTC CAG TTT GTT TCC TCG TCT CTT CCA GAT ATT TGT TAT CGC TGT GGT GAG TCT   282
Arg Gly Phe Gln Phe Val Ser Ser Ser Leu Pro Asp Ile Cys Tyr Arg Cys Gly Glu Ser
                                    50
GGT CAT CTT GCC AAG GAT TGT GAT CTT CAG GAG GAT GCC TGC TAT AAC TGC GGT AGA GGT   342
Gly His Leu Ala Lys Asp Cys Asp Leu Gln Glu Asp Ala Cys Tyr Asn Cys Gly Arg Gly
                                    70
GGC CAC ATT GCC AAG GAC TGC AAG GAG CCC AAG AGA GAG CGA GAG CAA TGC TGC TAC AAC   402
Gly His Ile Ala Lys Asp Cys Lys Glu Pro Lys Arg Glu Arg Glu Gln Cys Cys Tyr Asn
                                    90
TGT GGC AAA CCA GGC CAT CTG GCT CGT GAC TGC GAC CAT GCA GAT GAG CAG AAA TGC TAT   462
Cys Gly Lys Pro Gly His Leu Ala Arg Asp Cys Asp His Ala Asp Glu Gln Lys Cys Tyr
                                   110
TCT TGT GGA GAA TTC GGA CAC ATT CAA AAA GAC TGC ACC AAA GTG AAG TGC TAT AGG TGT   522
Ser Cys Gly Glu Phe Gly His Ile Gln Lys Asp Cys Thr Lys Val Lys Cys Tyr Arg Cys
                                   130
GGT GAA ACT GGT CAT GTA GCC ATC AAC TGC AGC AAG ACA AGT GAA GTC AAC TGT TAC CGC   582
Gly Glu Thr Gly His Val Ala Ile Asn Cys Ser Lys Thr Ser Glu Val Asn Cys Tyr Arg
                                   150
TGT GGC GAG TCA GGG CAC CTT GCA CGG GAA TGC ACA ATT GAG GCT ACA GCC TAA TTATTTT   643
Cys Gly Glu Ser Gly His Leu Ala Arg Glu Cys Thr Ile Glu Ala Thr Ala ***
                                   170
CCTTTGTCGCCCCTCCTTTTTCTGATTGATGGTTGTATTATTTTCTCTGAATCCTCTTCACTGGCCAAAGGTTGGCAGA   722
TAGAGGCAACTCCCAGGCCAGTGAGCTTTACTTGCCGTGTAAAAGGAGGAAAGGGGTGGAAAAAAACCGACTTTCTGCA   801
TTTAACTACAAAAAAAGTTTATGTTTAGTTTGGTAGAGGTGTTATGTATAATGCTTTGTTAAAGAACCCCCTTTCCGTG   880
CCACTGGTGAATAGGGATTGATGAATGGGAAGAGTTGAGTCAGACCAGTAAGCCCGTCCTGGGTTCCTTGAACATGTTC   959
CCATGTAGGAGGTAAAACCAATTCTGGAAGTGTCTATGAACTTCCATAAATAACTTTAATTTTAGTATAATGATGGTCT  1038
TGGATTGTCTGACCTCAGTAGCTATTAAATAACATCAAGTAACATCTGTATCAGGCCCTACATAGAACATACAGTTGAG  1117
TGGGAGTAAACAAAAAGATAAACATGCGTGTTAATGGCTGTTCGAGAGAAATCGGAATAAAAGCCTAAACAGGAACAAC  1196
TTCATCACAGTGTTGATGTTGGACACATAGATGGTGATGGCAAAGGTTTAGAACACATTATTTTCAAAGACTAAATCTA  1275
AAACCCAGAGTAAACATCAATGCTCAGAGTTAGCATAATTTGGAGCTATTCAGGAATTGCAGAGAAATGCATTTTCACA  1354
GAAATCAAGATGTTATTTTTGTATACTATATCACTTAGACAACTGTGTTTCATTTGCTGTAATCAGTTTTTAAAAGTCA  1333
GATGGAAAGAGCAACTGAAGTCCTAGAAAATAGAAATGTAATTTTAAACTATTCCAATAAAGCTGGA- 3'          1500
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B

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1  M S S N E C F K C G R S G H W A R E C P  TGGGRGR
52   D I C Y R C G E S G H L A K D C D  LQE
72   D A C Y N C G R G G H I A K D C K  EPKRERE
96   Q C C Y N C G K P G H L A R D C D  HADE
117  Q K C Y S C G E F G H I Q K D C T  K
135  V K C Y R C G E T G H V A I N C S  KTSE
156  V N C Y R C G E S G H L A R E C T  IEATA *

Consensus
CNBP  X X C Y X C  G X X G H X A X X C X  X
NBPs  X X C X X C  G X X G H X X X X C X  X
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Fig. 1. (**A**) Nucleotide sequence and derived amino acid sequence of human CNBP cDNA. Nucleotides are numbered with position 1 corresponding to the first nucleotide of the 5′ Eco RI site of the cDNA insert. Amino acids are numbered beginning at the initiator methionine. Underlining highlights the zinc finger repeats. (**B**) Alignment of the zinc finger repeats of the human CNBP protein sequence. The amino acid positions of the repeats are on the left. The asterisk represents the predicted stop codon. The consensus sequence (bottom) shows the characteristic features of a typical repeat unit and is matched with the consensus sequence for zinc finger repeats of retroviral NBPs (*12*). Abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. The CNBP cDNA clone was isolated as follows: a 56-nt oligonucleo tide, 5′-GAAGC-TTCCGTGGTGAGAGAT-GGTGCGGTGCCTGTT-CTTGGCCCGGGAATTCTGCA-3′ and its complement were synthesized on the basis of HMG-CoA reductase promoter sequence (*18*) (underlined). The SRE octanucleotide is marked in bold lettering. The labeled double-stranded probe was prepared by annealing the complementary ^{32}P-end-labeled single-stranded 56-nt oligonucleotides. After ethanol precipitation in the presence of 2.5*M* ammonium acetate, the double-stranded oligonucleotides were treated with T4 DNA ligase for 12 hours at 15°C, and the resulting concatenated probe was purified over a Sephadex G-50 column. This probe, containing approximately 90% double-stranded and 10% single-stranded molecules, was used to screen a λgt11 HepG2 cDNA library (*19*). The phage library was plated on *E. coli* Y1090 at a density of 20,000 phage per 150-mm plate, and isopropylthiogalactoside-induced plaques were transferred to nitrocellulose filters. Filters were denatured in 6*M* guanidine hydrochloride in binding buffer (25 m*M* Hepes, *p*H 7.9, 40 m*M* KCl, 2m*M* $MgCl_2$, and 1 m*M* dithiothreitol) at 4°C, followed by renaturation in a series of 5-min rinses in four successive 1:1 dilutions of the guanidine solution in binding buffer. After two final 5-min washes in binding buffer alone, filters were blocked for 30 min in binding buffer containing 5% nonfat milk. After incubation with 2×10^6 cpm per milliliter of labeled probe in the presence of sonicated salmon sperm DNA (100 μg/ml) in binding buffer containing 0.25% nonfat milk at 4°C for 12 hours, filters were washed in binding buffer with 0.25% milk and autoradiographed overnight. A 25-nt probe containing only the octanucleotide GTGCGGTG from the HMG-CoA reductase promoter sequence also bound to the CNBP clone. For sequencing, the entire cDNA insert and both the large and small Eco RI fragments of CNBP were separately subcloned into the Eco RI site of phage M13mp19. The internal Eco RI site is at nucleotide 472. Sequencing of single-stranded templates was carried out by the dideoxy chain-termination method (*20*) with either the M13 universal primer or synthetic oligonucleotide primers corresponding to sequenced regions of the CNBP cDNA.

mouse and man. The expression of the CNBP gene was analyzed in Northern blot assays of total RNA from a variety of rat tissues (Fig. 3A). The 1.5-kb mRNA species recognized by the CNBP cDNA probe was present in all the tissues examined, as would be expected if CNBP were involved in regulation of cellular cholesterol levels. The highest level of CNBP mRNA was seen in the testes and adrenal glands. Northern blot analysis also revealed that repression of cholesterol synthesis by treatment of HepG2 cells for 9 or 14 hours with 25-hydroxycholesterol and mevalonic acid was accompanied by a three- to fivefold increase in CNBP mRNA (Fig. 3B). In contrast, the levels of a control mRNA (apolipoprotein E) were unaffected by sterol treatment. Thus, the increase in CNBP binding activity in response to sterol treatment (Fig. 2B) can be explained by an increase in CNBP mRNA levels.

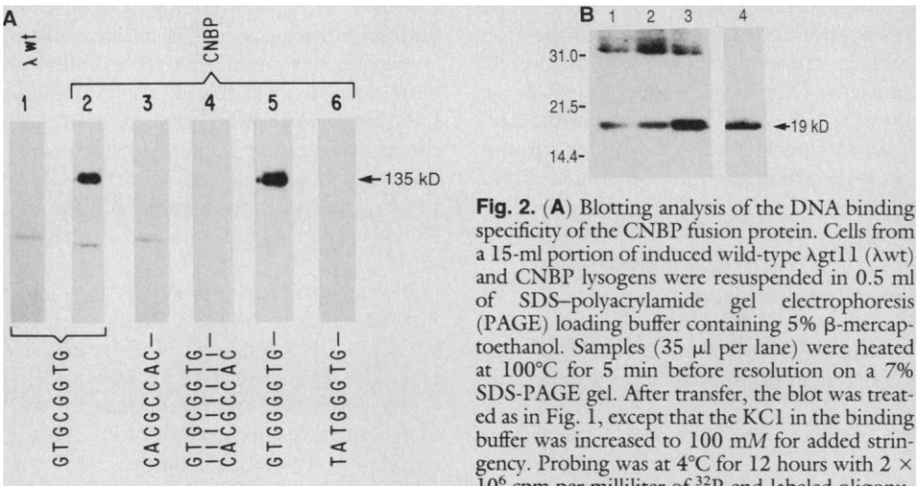

Fig. 2. (**A**) Blotting analysis of the DNA binding specificity of the CNBP fusion protein. Cells from a 15-ml portion of induced wild-type λgt11 (λwt) and CNBP lysogens were resuspended in 0.5 ml of SDS–polyacrylamide gel electrophoresis (PAGE) loading buffer containing 5% β-mercaptoethanol. Samples (35 μl per lane) were heated at 100°C for 5 min before resolution on a 7% SDS-PAGE gel. After transfer, the blot was treated as in Fig. 1, except that the KCl in the binding buffer was increased to 100 m*M* for added stringency. Probing was at 4°C for 12 hours with 2 × 10^6 cpm per milliliter of ^{32}P-end-labeled oligonucleotides, and salmon sperm DNA was omitted. The blot was washed at 4°C two times for 10 min. For lane 2, the 25-nt probe used was 5′-GAAGCTTGTGCGGTGGAATTCTGCA-3′ with the underlined octamer being the only sequence derived from HMG-CoA reductase promoter. The sequence of the 25-nt fragment used in the remaining lanes differed only within the octamer. Four probes were single-stranded (lanes 1 to 3, 5, and 6) and one double-stranded (lane 4). The position of the 135-kD CNBP fusion protein is marked. (**B**) Identification of a nuclear protein resembling CNBP. Nuclear extracts were prepared (*21*) from HepG2 cells grown in Dulbecco's modified Eagles medium (DMEM) with 5% fetal calf serum (lane 1) or treated in the following ways: intracellular cholesterol was depleted by incubation for 24 hours in DMEM with lipoprotein-deficient serum (LPDS) and mevinolin (2 μg/ml) (lane 2). Some of these cells were then treated with DMEM containing LPDS, 25-hydroxycholesterol (1 μg/ml), and mevalonic acid (10 m*M*) for 14 hours to repress cholesterol synthesis (lane 3). Samples of the extracts (40 μg of protein) were then resolved on 12% SDS-PAGE gels, transferred to nitrocellulose, and probed as described in (A) with an oligonucleotide containing the SRE core sequence GTGCGGTG. On the same gel we ran a sample of in vitro translated, [^{35}S]methionine-labeled CNBP. For these experiments, CNBP RNA was obtained by in vitro transcription of the full-length CNBP cDNA subcloned into the vector Bluescript (Stratagene). The RNA was capped during the transcription reaction and 1 μg of the product was translated in a rabbit reticulocyte lysate in the presence of [^{35}S]methionine. Before autoradiography, this lane was removed from the others, treated with sodium salicylate, and dried.

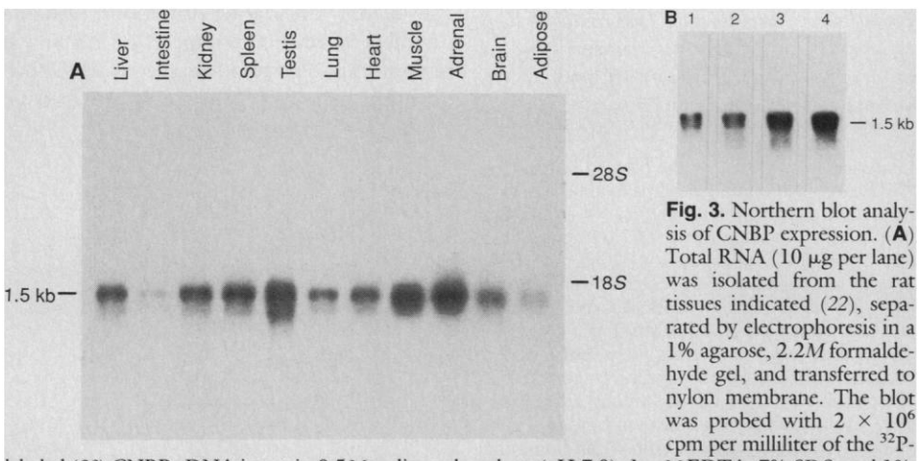

Fig. 3. Northern blot analysis of CNBP expression. (**A**) Total RNA (10 μg per lane) was isolated from the rat tissues indicated (*22*), separated by electrophoresis in a 1% agarose, 2.2*M* formaldehyde gel, and transferred to nylon membrane. The blot was probed with 2 × 10^6 cpm per milliliter of the ^{32}P-labeled (*23*) CNBP cDNA insert in 0.5*M* sodium phosphate (*p*H 7.0), 1 m*M* EDTA, 7% SDS, and 1% bovine serum albumin at 60°C. Washes were in 0.5× saline sodium citrate (SSC) and 0.1% SDS at 55°C. The 1.5-kb CNBP mRNA species is indicated, as is the migration of 28*S* and 18*S* ribosomal RNAs. (**B**) Northern blot analysis of total RNA from HepG2 cells was carried out as in (A). Cells were either maintained in DMEM with 5% fetal calf serum (lane 1), treated for 24 hours in DMEM with LPDS and mevinolin (2 μg/ml) (lane 2), or treated with DMEM containing LPDS, 25-hydroxycholesterol (1 μg/ml), and mevalonic acid (10 m*M*) for 9 hours (lane 3) or 14 hours (lane 4) to repress cholesterol synthesis.

Single-stranded DNA binding proteins are important in DNA replication, recombination, and repair in prokaryotes (*13*). Although several eukaryotic single-stranded DNA binding proteins have been identified (*14*), their biological functions are unclear. The apparent specificity of CNBP binding to the sterol regulatory octanucleotide sequence is suggestive of a role for this single-stranded DNA binding protein in sterol-mediated repression. If the NF1-like proteins that bind near the HMG-CoA reductase SRE function as positive transcription factors, CNBP may disrupt their binding, thereby repressing transcription of the gene. Supporting this speculation is a recent report showing that the estrogen receptor binds the "coding strand" of an estrogen-responsive element with about 60-fold higher affinity than the double-stranded element (*15*). This suggests strongly that single-stranded DNA binding proteins can function in transcriptional regulation. The estrogen receptor is also a zinc finger protein, and, like CNBP, it fails to bind to the complementary strand of target DNA (*15*). On the other hand, our studies provide only circumstantial evidence for the involvement of CNBP in sterol metabolism. Preliminary transfection studies failed to reveal any significant effects of the overexpression of CNBP on the expression of a reductase promoter-reporter gene construct. Given the complexity of sterol-mediated repression, definitive evidence may require the analysis of CNBP mutations.

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The Product of the *mos* Proto-Oncogene as a Candidate "Initiator" for Oocyte Maturation

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The endogenous c-*mos* product, pp39^{mos}, is required for progesterone-induced meiotic maturation in *Xenopus* oocytes. Treatment of oocytes with progesterone induced a rapid increase in pp39^{mos} that preceded both the activation of maturation promoting factor (MPF) and germinal vesicle breakdown (GVBD). Microinjection of synthetic *mos* RNA into oocytes activated MPF and induced GVBD in the absence of progesterone. Thus, the *mos* proto-oncogene product may qualify as a candidate "initiator" protein of MPF and is at least one of the "triggers" for G_2 to M transition.

IN MOST SPECIES, FULLY GROWN OOcytes are naturally arrested at the first meiotic prophase (or late G_2 phase) (*1, 2*). In *Xenopus*, this arrest is released by progesterone and is followed by the appearance of a cytoplasmic activity, MPF, that causes nuclear breakdown or GVBD and chromosome condensation (*3*). MPF was originally described as a cytosolic factor that, when microinjected, could induce G_2-arrested oocytes to enter meiosis in the absence of protein synthesis (*4, 5*). An identical activity was subsequently shown to exist in a variety of maturing oocytes and also in mitotic cells of many species (*6–9*). Recently, *Xenopus* MPF has been purified to near homogeneity and shown to contain two components (*10*). Importantly, one of the components, a 32-kD polypeptide, has been shown to be a *Xenopus* homolog of the fission yeast *cdc2* gene product, p34^{cdc2}, which is a key element controlling entry into mitosis (*11, 12*). MPF is therefore almost certainly a fundamental and universal regulator of M phase or G_2 to M transition in both meiosis and mitosis in eukaryotes (*2, 3*).

Xenopus MPF exists in immature oocytes in an inactive form (pre-MPF) (*2, 13, 14*), and during the first few hours after progesterone treatment, de novo protein synthesis is required for MPF activation (*2, 3*). An "initiator" for MPF activation (*15*) must be synthesized early and in low abundance because a detectable increase in protein synthesis occurs only after MPF is activated, at a time when cycloheximide no longer inhibits GVBD (*2, 3, 13*). Identification of the initiator is essential to understand the molecular mechanisms leading to activation of pre-MPF.

We have shown that *mos* proto-oncogene transcripts are expressed at high levels in adult gonadal tissue (*16–19*) and are specifically associated with germ cells (*20–23*). Synthesis of the endogenous *Xenopus mos* product, pp39^{mos}, occurs during progesterone-induced oocyte maturation and is required for GVBD (*18*). The *mos* product has

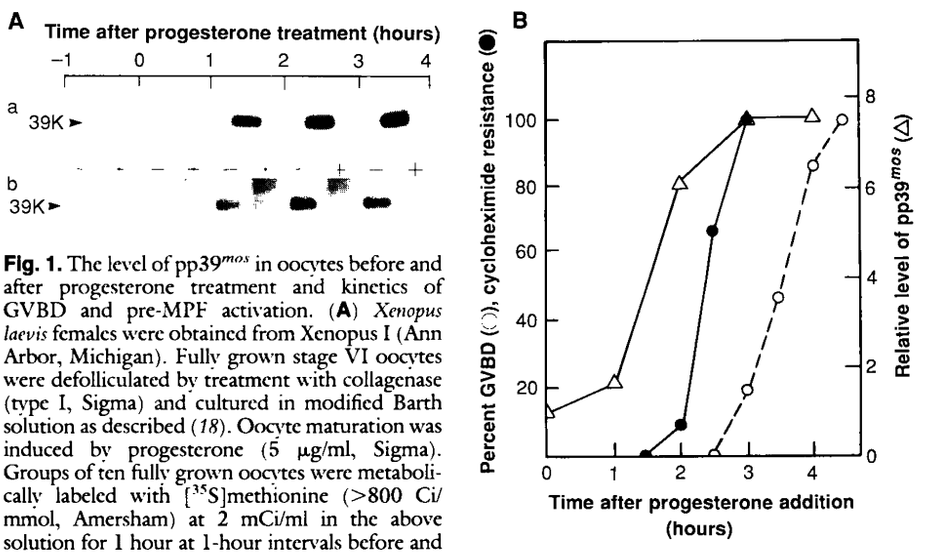

Fig. 1. The level of pp39^{mos} in oocytes before and after progesterone treatment and kinetics of GVBD and pre-MPF activation. (**A**) *Xenopus laevis* females were obtained from Xenopus I (Ann Arbor, Michigan). Fully grown stage VI oocytes were defolliculated by treatment with collagenase (type I, Sigma) and cultured in modified Barth solution as described (*18*). Oocyte maturation was induced by progesterone (5 μg/ml, Sigma). Groups of ten fully grown oocytes were metabolically labeled with [^{35}S]methionine (>800 Ci/mmol, Amersham) at 2 mCi/ml in the above solution for 1 hour at 1-hour intervals before and after progesterone treatment. Procedures for immunoprecipitation and SDS–polyacrylamide gel electrophoresis (PAGE) analyses have been described (*18*). Intracellular pools of free [^{35}S]methionine were estimated from aliquots before immunoprecipitations. Uptake of [^{35}S]methionine was similar up to 3 hours after progesterone treatment, but was 40% lower during the fourth hour, and this sample was normalized accordingly. Oocyte extracts from the equivalent of four oocytes were subjected to immunoprecipitation analysis with either (a) a monoclonal antibody (termed 5*S*) raised against the *Xenopus* c-*mos* product expressed in *Escherichia coli* (*18*), or (b) a peptide antiserum C232 (*18*) in the presence (+) or absence (−) of the peptide antigen. (**B**) Thirty oocytes of the same batch described above were scored for GVBD every 30 min after progesterone addition (○). Groups of 20 oocytes were also treated with cycloheximide (100 μg/ml) at 30-min intervals after progesterone addition. After 12 hours, oocytes were examined for percent of GVBD, which is expressed as cycloheximide resistance of GVBD (●). Densitometric measurements of autoradiographs as shown in (A) and performed in parallel is shown (△). These data are derived from three independent experiments.

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