# Age-dependent changes in the level of a 34 kDa DNA-binding protein in developing chick embryo liver

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The relative amounts of a DNA-binding protein of 34 kDa increased during the early stages of development of chick embryo liver. The content of this protein reached a maximum in 18–19-day-old embryonic livers and decreased afterwards in older embryonic and post-natal chick livers. The 34 kDa polypeptide is the major DNA-binding protein (DBP) of embryonic liver and it preferentially binds to single-stranded DNA. The quantity of the 34 kDa DBP was relatively very low in embryonic muscle, heart and brain.

*DNA-binding protein* *Growth-dependent change* *Chick embryo liver*

## 1. INTRODUCTION

Embryonic development is a highly organized regulatory process that results in the production of specialized organs and tissues. Several classes of proteins and enzymes controlling the process of cell differentiation and DNA-binding proteins (DBP) could be considered as an important class of regulatory proteins. In prokaryotes, several DBPs have been isolated and their biological functions established. Recent studies indicate the presence of distinct DBPs in eukaryotic cells [1–10]. However, the role of these proteins in gene expression has not been established although repressor-like proteins and non-histone chromosomal proteins are thought to be involved in the regulation of gene expression in eukaryotes.

Here, we have isolated and characterized a 34 kDa DBP from embryonic liver. This protein is the major DBP of embryonic liver and displays age-dependent variations in developing liver.

## 2. MATERIALS AND METHODS

Chick embryos and one-day-old chicken of the Samrat strain were used. The fertile eggs were incubated in a humidified incubator at 37°C. The embryos were dissected at 4°C and rinsed in cold phosphate-buffered saline (PBS).

### 2.1. *Preparation of cytoplasmic and nuclear fractions*

Following washing with PBS, the individual tissues (0.4–0.8 g) were resuspended in 10 ml sterile water. After 15 min, all tissues other than skeletal muscle were homogenized with 10 strokes in a tight-fitting Dounce homogenizer; muscle was homogenized in a Sorvall Omnimix using three 15-s bursts of the blender at low setting. In all cases the homogenate was then centrifuged at 7000 × *g* to pellet the nuclei. The supernatant was adjusted to 20 mM Tris (pH 8.1), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM $\beta$-mercaptoethanol ($\beta$ME) and centrifuged at 30000 × *g* for 25 min. The 30000 × *g* supernatant (S-30) fraction was frozen. The 34 kDa protein was isolated from the S-30 of individual tissues and fractionated on single-stranded DNA-cellulose columns as described below.

Purified nuclei [11] were washed 4 times with column buffer (20 mM Tris, pH 8.1, 1 mM EDTA, 1 mM PMSF, 1 mM $\beta$ME, 50 mM NaCl) and the final nuclear pellet was suspended in 5 vols extraction buffer (2 M NaCl, 10 mM Tris, pH 7.6,

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1 mM $MgCl_2$, 0.5 mM PMSF). The nuclear suspension was stirred for 4 h at 4°C to extract the nuclear proteins. DNA was precipitated from the nuclear extract by the addition of 10% polyethylene glycol 6000. After stirring for 15 min, the solubilized proteins were separated by centrifugation at 30000 × *g* for 30 min and the supernatant was extensively dialysed against column buffer.

2.2. *DNA-cellulose chromatography*

Native and denatured DNA-celluloses were prepared using either calf thymus or embryonic liver DNAs and DNA-cellulose chromatography was performed as described [8–10].

2.3. *Protein estimation*

Protein estimation was done according to Lowry et al. [12].

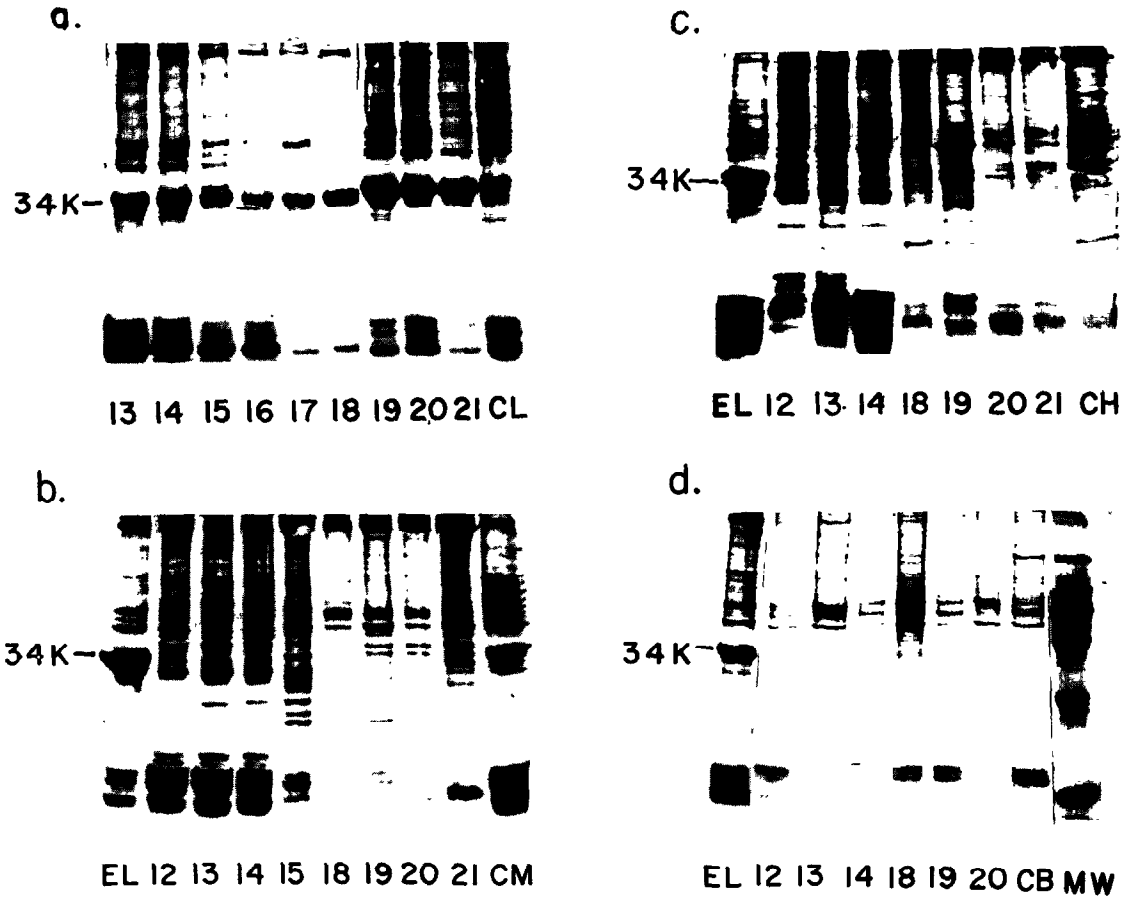


Fig.1. Tissue-specific distribution of the 34 kDa DBP. The DBPs of various tissue extracts were isolated by chromatography on denatured calf-thymus DNA-cellulose columns. The bound proteins were eluted with 2 M NaCl and electrophoresed in 12% SDS-polyacrylamide gels. The numbers below each lane indicate the age of the developing embryos in days. 21 refers to the embryos at the 21st day of development. CL, CM, CH and CB denote the eluates derived from 1-day-old chick liver, muscle, heart and brain, respectively; EL, 19-day embryonic liver. a–d indicate DBP patterns at different stages of development of embryonic liver, embryonic muscle, embryonic heart, and embryonic brain, respectively. Each lane of a–d contained samples equivalent to 170, 200, 150 and 40 $\mu$g proteins, respectively. Lane MW of d contains molecular mass markers: phosphorylase *a* (92 kDa), bovine serum albumin (68 kDa), $\gamma$-globulin heavy chain (50 kDa), ovalbumin (43 kDa), $\gamma$-globulin light chain (24 kDa) and cytochrome *c* (12 kDa). 34 K corresponds to the position of the 34 kDa DBP.

### 2.4. *Electrophoresis on SDS-polyacrylamide gels (SDS-PAGE)*

The proteins present in the eluates were processed and electrophoresed in 12% polyacrylamide gels as described [8–10].

## 3. RESULTS

### 3.1. *Variations in the level of 34 kDa DBP during development*

Age-dependent variations in the content of the 34 kDa DBP in developing embryonic liver are shown in fig.1. Notable increase in the level of the 34 kDa protein was observed in embryonic livers from the 13th day of development. The amount of the 34 kDa DBP reached a maximum in 19-day-old embryonic livers; the level of this protein declined thereafter and reached the lowest level in 1-day-old chick liver. Densitometric quantitations of fig.1a indicate that this protein consisted of 20, 52 and 5.6% of the total DBPs, respectively, of 13-day embryonic liver, 19-day embryonic liver, and 1-day chick liver. It is difficult to ascertain whether the 34 kDa DBP shows any age-dependent variations in embryonic muscle, heart and brain since the quantity of this protein was too low to quantitate in these tissues (fig.1b–d).

### 3.2. *Specificity of binding of the 34 kDa protein to double-stranded (ds) and single-stranded (ss) DNAs*

To determine the specificity of binding of the 34 kDa DBP to ds and ss DNA, aliquots containing 3 mg cytosolic proteins from 19-day embryonic livers were chromatographed separately on native and denatured chick embryo DNA-cellulose columns and the bound proteins eluted step-wise with different concentrations of NaCl. The DBPs present in the eluates were resolved by electrophoresis in SDS-PAGE. The results presented in fig.2 (lanes H and I) show the presence of large quantities of the 34 kDa DBP in the 0.6 and 2 M NaCl eluates obtained from the ss DNA-cellulose column indicating preferential affinity of this protein to ss DNA. Native chick embryo liver DNA-cellulose did not show appreciable binding of the 34 kDa protein (fig.2, lanes B–E).

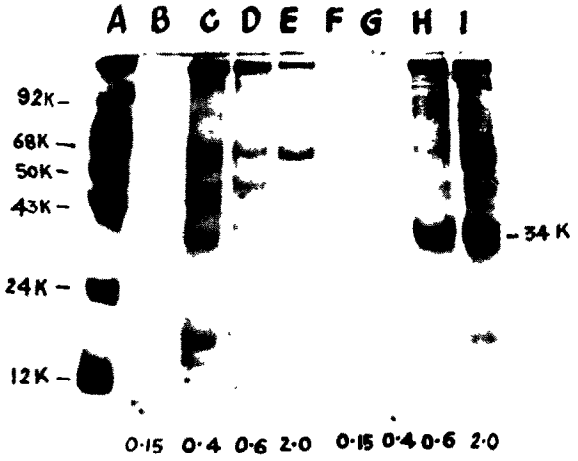


Fig.2. Comparison of binding of the 34 kDa embryonic liver protein to native (ds) and denatured (ss) DNA-cellulose columns. Cytoplasmic extracts corresponding to 3 mg protein were chromatographed on chick-embryo DNA-cellulose columns and the DBPs were eluted with different concentrations of NaCl. The numbers below the figure indicate the molar concentrations of NaCl. The proteins in the eluates were precipitated and aliquots corresponding to 50 $\mu$g protein were electrophoresed as described in section 2. (A) Molecular mass markers; (B–E) DBPs eluted from native DNA-cellulose; (F–I) DBPs eluted from denatured DNA-cellulose.

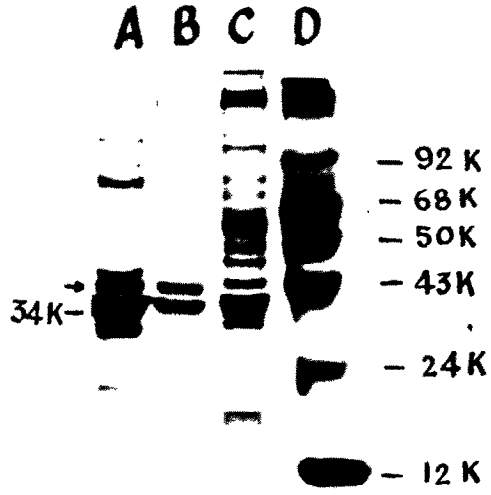


Fig.3. Nuclear DBPs of chick liver. Nuclear proteins were isolated, chromatographed on denatured calf-thymus DNA-cellulose columns and electrophoresed in 12% gels as described in section 2. Aliquots containing 50 $\mu$g proteins were used for lanes A and B while lane C contained 100 $\mu$g protein. (A) Nuclear DBPs from 19-day embryonic liver; (B) nuclear DBPs from 1-day-old chick liver; (C) DBPs from S-30 of 19-day embryonic liver; (D) molecular mass marker proteins.

### 3.3. *Comparison of nuclear and cytoplasmic DBPs*

Fig.3 shows the pattern of DBPs in embryonic liver. As in the cytosol, the 34 kDa DBP is the major ss DNA-binding protein of the nuclei. The relative amount of the 34 kDa DBP was lower in 1-day-old chick liver nuclei than in the 19-day embryonic liver nuclei while the level of the protein indicated by the arrow in fig.3 is constant in both.

## 4. DISCUSSION

Cell differentiation and embryonic development are known to be accompanied by changes in the level of histone and non-histone proteins [13–17]. To gain further insight into the role of non-histone proteins in the developmental process, we have attempted to identify non-histone proteins that may be involved in gene expression during development. We have used the technique of affinity chromatography to select proteins that bind specifically to ss DNA. The present results indicate that a 34 kDa protein of embryonic liver exhibits several properties that are consistent with regulatory roles. These are (i) preferential binding to ss DNA, (ii) tight binding to ss DNA as evidenced by the elution from the affinity column by 0.6 M and 2 M NaCl, (iii) abundance in embryonic liver, (iv) drastic reduction in the level during the development of embryonic liver to the newborn stage and (v) presence in nuclei. Two non-histone proteins of chick embryo of 35.5 and 125 kDa were shown to be lost during differentiation of precartilage mesenchyme to cartilage form [16]. A regulatory role was proposed for the 35.5 kDa cartilage protein since this protein was found to be associated near the DNase I-sensitive region of cartilage chromatin. The presence of large quantities of 34 kDa protein in liver or the 35.5 kDa protein in cartilage at early stages of development may activate transcription by unwinding or destabilizing the DNA duplex. Such DNA unwinding and helix destabilizing proteins are known to be present in eukaryotic cells [18,19]. Alternatively, these non-histone proteins may repress certain genes at early stages of development and the loss of the presumptive repressors at later stages may activate the genes. However, the roles of DBPs in gene expression during development are not known. Ongoing studies on the purification of 34 kDa DBP and determination of its influence on transcription would tell us whether the 34 kDa protein is an activator or repressor of gene expression.

## ACKNOWLEDGEMENTS

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