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Nuclear Factor 1 Is a Component of the Nuclear Matrix

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Abstract Chicken histone H5 is an H1-like linker histone that is expressed only in nucleated erythrocytes. The histone H5 promoter has binding sites for Sp1 (a high affinity site) and UPE-binding protein, while the 3′ erythroid-specific enhancer has binding sites for Sp1 (one moderate and three weak affinity), GATA-1, and NF1. In this study we investigated whether *trans*-acting factors that bind to the chicken histone H5 promoter or enhancer are associated with adult chicken immature and mature erythrocyte nuclear matrices. We show that NF1, but not Sp1, GATA-1, or UPE-binding protein, is associated with the internal nuclear matrices of these erythroid cells. Further, we found that a subset of the NF1 family of proteins is bound to the mature erythrocyte nuclear matrix. These results suggest that in chicken erythrocytes NF1 may mediate an interaction between the histone H5 enhancer and the erythroid internal nuclear matrix. NF1 was also present in the internal nuclear matrices of chicken liver and trout liver. The observations of this study provide evidence that NF1 may have a role in a variety of cell types in targeting specific DNA sequences to the nuclear matrix.

Key words: NF1, nuclear matrix, histone H5, transcription, transcription factors

The nuclear matrix is operationally defined as the nuclear structure that remains following the salt extraction of nuclease treated nuclei. This structure consists of residual nucleoli, nuclear pore-lamina complex, and internal nuclear matrix [Belgrader et al., 1991b; Berezney, 1991; He et al., 1990]. Besides its role in organizing nuclear DNA, the nuclear matrix is involved in several nuclear functions, including replication, transcription, and RNA splicing. It is becoming increasingly evident that nuclear processes are not occurring haphazardly in the nucleoplasm but are localized to distinct regions in the nucleus. Focal sites of DNA synthesis have been visualized [Hozák et al., 1993]. These replication factories are attached to the nuclear matrix [Pienta et al., 1991]. Transcriptionally active genes and RNA transcripts are found in discrete "transcript domains" [Carter et al., 1991, 1993; Carter and Lawrence, 1991; Jackson et al., 1993; Xing et al., 1993]. Xing and Lawrence [1991] showed that this specific RNA distribution is preserved within nuclear matrices. RNA is transcribed, processed, and spliced at distinct foci and along discrete tracks within the nucleus [Carter et al., 1993; Lawrence et al., 1989; Xing et al., 1993]. There is also evidence of polarity in the sequence of processing events along these tracks implying that newly synthesized RNA molecules are transported from their site of synthesis and processed in an "assembly line" fashion where specific enzymes are spatially ordered along this track [Xing et al., 1993]. Several reports have provided evidence that transcribed and nontranscribed sequences are precisely compartmentalized within the nucleus [Andreeva et al., 1992; Delcuve and Davie, 1989]. These observations are consistent with the idea that mechanisms exist that specifically target transcriptionally active gene chromatin to the nuclear matrix.

We have shown that the histone deacetylase is a nuclear matrin [Hendzel and Davie, 1992; Hendzel et al., 1991], a component of the internal nuclear matrix [Belgrader et al., 1991a,b; Nakayasu and Berezney, 1991]. Since transcriptionally active DNA is complexed with histones undergoing dynamic acetylation [Boffa et al., 1990; Hendzel et al., 1991], histone deacetylase may provide a mechanism by which transcribed genes are associated with the internal nuclear matrix [Hendzel and Davie, 1992; Hendzel et al., 1991]. Recent reports displaying that transcrip-

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tion factors are associated with the nuclear matrix suggest another way by which transcribed genes are selectively attracted to the nuclear matrix [Bortell et al., 1992; Dworetzky et al., 1992; Isomura et al., 1992; Waitz and Loidl, 1991]. Sequence-specific DNA-binding proteins that are associated with the nuclear matrix include the estrogen receptor, NMP-1 (possible member of ATF transcription factor family), RAP-1 (repressor-activator binding protein-1), factor F6, c-*Myc*, and RFP [Dworetzky et al., 1992; Gasser et al., 1989; Isomura et al., 1992; Vassetzky et al., 1993; Waitz and Loidl, 1991].

Chicken histone H5 is an H1-like linker histone that is expressed only in nucleated erythrocytes [Neelin et al., 1964]. Levels of histone H5 protein increase during the differentiation and maturation of the chicken erythroid cell, and this increase is correlated with chromatin condensation, the shutdown of replication, and the repression of gene expression [Sun et al., 1989]. However, this accumulation of linker histones does not prevent the transcription of the erythroid-specific histone H5 gene in adult chicken immature and perhaps mature erythrocytes [Affolter et al., 1987; Sun et al., 1993]. Further, the histone H5 gene chromatin of chicken immature and mature erythrocytes has an insoluble character suggesting an association with the nuclear matrix [Delcuve and Davie, 1989]. Using adult immature and mature erythrocyte nuclear extracts, we showed that the histone H5 gene promoter has binding sites for Sp1 (high affinity) and an upstream promoter element (UPE)-binding protein, while the 3′ enhancer contains binding sites for Sp1 (one medium and three weak affinity, one of which may also bind the CACCC factor), GATA-1, and NF1 [Sun et al., 1992, 1993]. Similar results were recently reported by Rousseau et al. [1993]. The relative levels of Sp1 and GATA-1, but not NF1, are lower in mature nuclear extracts than those in immature nuclear extracts [Sun et al., 1993].

In this study, we investigated whether *trans*-acting factors that bind to the *cis*-acting DNA elements of the histone H5 gene were associated with adult chicken immature and mature erythrocyte nuclear matrices. We show that NF1, but not Sp1, GATA-1, or UPE-binding protein, was associated with the internal nuclear matrices of adult chicken immature and mature erythrocytes. NF1 was also found in the internal nuclear matrices of chicken liver and trout liver. These observations provide evidence that NF1 has a role in a variety of cell types in targeting specific *cis*-acting DNA sequences to the nuclear matrix.

MATERIALS AND METHODS

Preparation of Nuclear and Nuclear Matrix Extracts

Adult White Leghorn chickens were made anaemic by injections of phenylhydrazine hydrochloride. Blood was collected and nuclei were isolated from immature and mature erythrocytes as described previously [Sun et al., 1992]. Trout and chicken livers were scissor-minced and then homogenized in 100 mM KCl, 10 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 10 mM sodium butyrate, 0.25% (v/v) Nonidet P-40 (buffer A) with 1 mM phenylmethanesulfonyl fluoride added fresh from a 100 mM stock. For some preparations the livers were homogenized in RSB (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$, 10 mM sodium butyrate, 1 mM phenylmethanesulphonyl fluoride). The nuclei were pelleted by centrifugation at 2,500 rpm for 10 min in an SS34 rotor. The nuclear pellet was resuspended in a small volume of 0.25 M sucrose, 50 mM Tris-HCl, pH 7.5, 150 mM KCl, 10 mM sodium butyrate, 5 mM $MgCl_2$, and 1 mM phenylmethanesulfonyl fluoride (buffer B) and homogenized by three passages through a 22 guage needle. The suspension was layered onto 30 ml of buffer B containing 0.7 M sucrose, and the nuclei were collected by centrifugation at 4,500 rpm for 10 min in an SS34 rotor. Nuclear matrices were prepared according to published methods [Belgrader et al., 1991b; He et al., 1990]. Briefly, nuclei were resuspended in digestion buffer (10 mM 1,4-piperazine-diethanesulfonic acid (PIPES, pH 6.8), 50 mM NaCl, 300 mM sucrose, 3 mM $MgCl_2$, 1 mM EGTA, and 0.5% v/v Triton X-100) at a DNA concentration of 1 mg/ml. DNase I was added to a final concentration of 100 μg/ml, and the nuclei were digested for 2 h at 23°C. Ammonium sulfate was added dropwise from a 4 M stock to a final concentration of 0.25 M, and the nuclei were pelleted by centrifugation at 2,500 rpm for 10 min in an SS34 rotor. The ammonium sulfate–extracted nuclear matrix (P1 in Fig. 1) was resuspended in digestion buffer and reextracted by slowly adding NaCl to a final concentration of 2.0 M from a 4.0 M stock solution while mixing. This was centrifuged at 3,500 rpm for 10 min in an SS34 rotor to produce a soluble (NME2 in Fig. 1) and an insoluble fraction (nuclear core filaments plus nuclear pore-lamina complex; P3

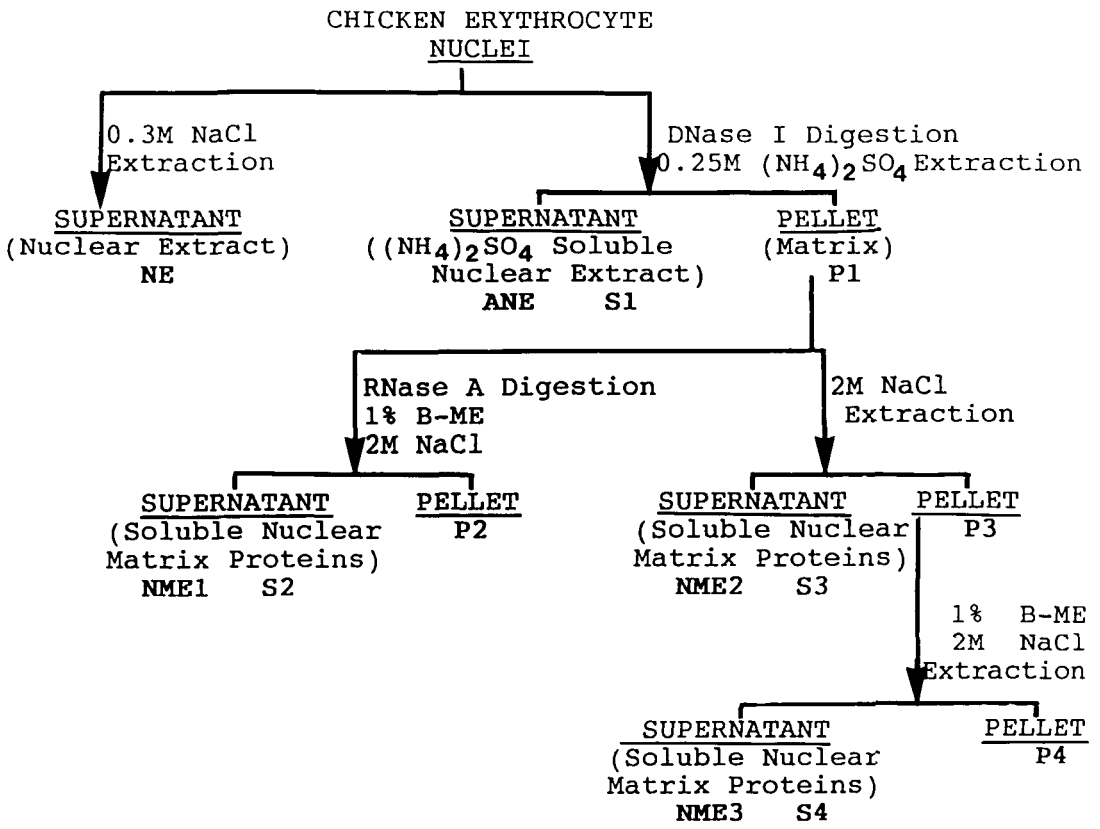

Fig. 1. Experimental procedures to isolate nuclear matrices. Nuclear proteins were extracted with 0.3 M NaCl (NE) or 0.25 M ammonium sulfate (ANE). Nuclear matrix proteins were extracted with 2 M NaCl ± β-mercaptoethanol (NME1, NME2, NME3). For details of these procedures, see Materials and Methods.

in Fig. 1). The ammonium sulfate–extracted nuclear matrices (P1) or 2 M NaCl-extracted nuclear matrices (P3) were treated with 10 μg/ml RNase A at room temperature for 10 minutes (P1 only) and then extracted with 1% v/v β-mercaptoethanol and 2 M NaCl for 30 min on ice. The soluble nuclear matrix extracts (NME1 or NME3) and insoluble (P2 or P4) fractions were separated by centrifugation at 10,000 rpm for 10 min in an SS34 rotor.

DNA Fragments and Oligonucleotides

DNA fragments spanning the histone H5 5′ promoter and 3′ enhancer of H5 gene (see Fig. 2) were isolated as described previously [Sun et al., 1992]. Oligonucleotides containing the histone H5 GC-box (Sp1-oligonucleotide), which has the sequence of a high affinity Sp1 binding site in the promoter region, upstream promoter element (UPE-oligonucleotide), and GATA site (GATA-oligonucleotide) were synthesized as described [Sun et al., 1992]. The following oligonucleotides were synthesized:

H5-NF1-for, 5′-TCGAGGGCTTGGCACAGCCCCAAGACCA-3′;
H5-NF1-rev, 5′-GTGGTCTTGGGGCTGTGCCAAGCCCTCG-3′;
M1-for, 5′-ATTTTCGCTTGAAGCCAATATGC-3′;
M1-rev, 5′-GGCATATTGGCTTCAAGCGAAA-3′;
M2-for, 5′-ATTTTGGCTTGAAGCCCATATGC-3′;
M2-rev, 5′-GGCATATGGGCTTCAAGCCAAA-3′;
c-*myc* C1-for, 5′-TGCTGCTTTGGCAGCAAATTGGGGGACT-3′;
c-*myc* C1-rev, 5′-CTGAGTCCCCCAATTTGCTGCCAAAGCA-3′;
c-*myc* C4-for, 5′-CAGTCTGGGTGGAAGGTATCCAATCCAGAT-3′;
c-*myc* C4-rev, 5′-GCTATCTGGATTGGATACCTTCCACCCAGA-3′.

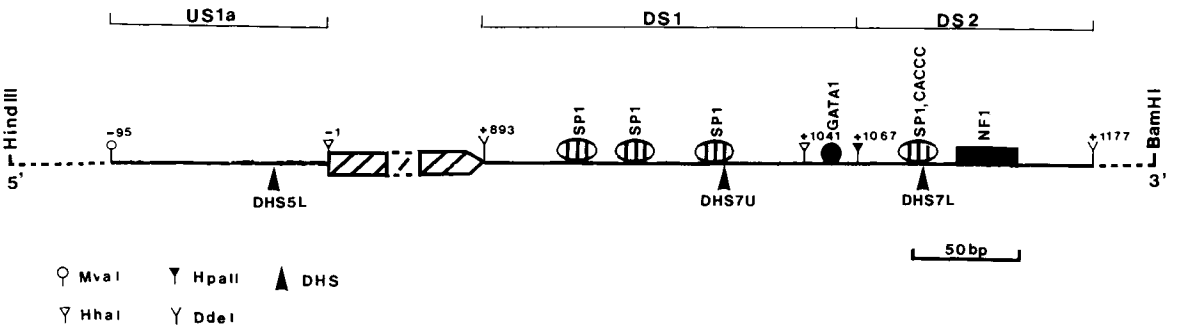

Fig. 2. Map of the chicken histone H5 gene and flanking regions. The sequence-specific DNA-binding proteins of adult chicken immature and mature erythrocyte nuclear extracts that bind to downstream enhancer region of the histone H5 gene are indicated. The DNA fragments used in this study (US1a, DS1, and DS2) are shown. The arrowheads show the location of the DNase I hypersensitive sites (DHS) in the erythroid histone H5 gene chromatin.

The NF1 oligonucleotide 5′-ATTTTGGCTT-GAAGCCAATATG-3′ (for) and 5′-CATATTG-GCTTCAAGCCAAAAT-3′ (rev) were obtained from the Hot Footprinting Kit (Stratagene, La Jolla CA).

DNA-Binding Assays

DNA end-labelling, gel mobility-shift (EMSA), competition experiments, and DNase I protection (footprinting) assays were done as described previously [Penner and Davie, 1992; Sun et al., 1992]. The molecular mass determinations were done as described by Bading [1988].

Purification of Nuclear Factor 1

NF1 was purified from chicken mature erythrocytes using a procedure similar to that described previously [Jones et al., 1987]. Nuclear extracts (NE) and nuclear matrix (NME1) were dialysed against buffer Z (20 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES pH 7.9), 12.5 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 20% glycerol, and 0.1% Nonidet P-40). The dialyzed extracts were passed through a Heparin-Agarose (Sigma, St. Louis, MO) column. The proteins were eluted with a gradient of 0.2–0.5 M KCl in buffer Z. EMSA with an end-labelled H5-NF1 oligonucleotide was used to monitor the NF1 activity of each fraction. The 0.4 M KCl fraction, which contained the highest NF1 activity, was passed through a Sephacryl S-300 (Sigma) (1.6 × 100 cm) column in buffer Z with 0.1 M KCl at a flow rate of 0.8 ml/min. The fractions containing NF1 activity were pooled and concentrated. A NF1 affinity column was prepared as described previously [Kadonaga, 1991]. H5-NF1 oligonucleotides 5′-GCCCTCGATGGTCTTGGGGCTGTGC-CAA-3′ (rev) and 5′-TCGAGGGCTTGGCA-CAGCCCCAAGACCA-3′ (for) were annealed, ligated, and combined with CNBr-activated sepharose 4B resin (Sigma). The pooled concentrated fractions from the Sephacryl S-300 column were made 8 μg/ml in poly dI.dC and 2 μg/ml calf thymus DNA and incubated on ice for 10 min before being passed through the NF1 affinity column. A gradient of 0.2–0.6 M KCl in buffer Z was used (flow rate 0.1 ml/min). Five μl of each fraction was used to check the activity; 100 μl of each fraction was precipitated with TCA. Samples were analyzed by 10% polyacrylamide SDS gel electrophoresis. The gels were stained with Commassie blue and then silver [Davie and Murphy, 1990].

RESULTS

Identification of Transcription Factors Associated With Adult Chicken Immature and Mature Nuclear Matrices

The internal nuclear matrix of adult chicken immature and mature erythrocyte nuclei is a thermal labile structure [Hendzel and Davie, 1992]. Nevertheless, when precautions are taken to avoid low temperature digestions with DNase I and using a relatively gentle method to isolate nuclear matrices [Belgrader et al., 1991b; He et al., 1990] (see Fig. 1), we have shown by electron microscopy that it is possible to isolate erythroid nuclear matrices with internal structures [Hendzel, Rattner, Sun, and Davie, manuscript in preparation]. The DNase I–digested, 0.25 M ammonium sulfate–extracted immature nuclei (P1) consisted of a nuclear pore-lamina structure and an internal fibrogranular network. Similar preparations of mature erythroid nuclei had an internal network of thin filaments. Further ex-

traction of the ammonium sulfate nuclear matrices with 2 M NaCl generated immature nuclear matrices (P3) with internal structures consisting of fibrogranular and thin filament components. The 2 M NaCl-extracted mature erythroid nuclear matrices (P3) had internal structures similar to those extracted with only 0.25 M ammonium sulfate. The 2 M NaCl-extracted nuclear matrices were depleted of histones and DNA [Belgrader et al., 1991b]. Extraction of these nuclear matrices with 2 M NaCl and 1% β-mercaptoethanol resulted in the solublization of the internal nuclear structure of both immature and mature erythrocytes, leaving empty shells of nuclear pore-lamina complexes. As has been shown by several groups, treatment of nuclear matrices with β-mercaptoethanol is an effective method to solubilize nuclear matrins [Hendzel and Davie, 1992; Kaufmann et al., 1983; Payrastre et al., 1992; von Kries et al., 1991].

The adult chicken immature and mature erythroid nuclear factors that bind to the histone H5 3′ enhancer region in vitro are shown in Figure 2. The downstream enhancer has binding sites for GATA-1, NF1, and Sp1. The Sp1 binding sites were of medium and weak affinity [Sun et al., 1992]. The promoter region has a high affinity Sp1 binding site and a site that binds to the UPE-binding protein [Rousseau et al., 1993; Sun et al., 1992, 1993].

To detect nuclear matrins of immature and mature erythrocytes that interacted with the promoter or enhancer regions of the histone H5 gene, electrophoretic gel mobility-shift assays (EMSA) with DNA fragments US1a, DS1, and DS2 (Fig. 2) and nuclear extracts or nuclear matrix extracts (NME1) (see Fig. 1) were performed. The only DNA fragment that generated a protein-DNA complex with mature and immature erythrocyte nuclear matrix proteins was DS2 (Fig. 3). Since US1a and DS1 did not form protein-DNA complexes with erythrocyte nuclear matrix proteins, this provided evidence that UPE-binding protein, GATA-1, and Sp1 were not associated with the erythrocyte nuclear matrix. The immature nuclear extracted proteins that interact with DS2 included Sp1, CACCC factor, which binds to the Sp1 binding site, and NF1 [Sun et al., 1992] (Fig. 2). However, in mature nuclear extracts the DNA binding activity of Sp1 was reduced, leaving NF1 as the major DS2 DNA-binding activity [Sun et al., 1993]. Accordingly, competition experiments with the H5-NF1 oligonucleotide demonstrated that the majority of the complexes formed with the DS2 DNA fragment and mature erythrocyte nuclear extracts was competed for with the H5-NF1

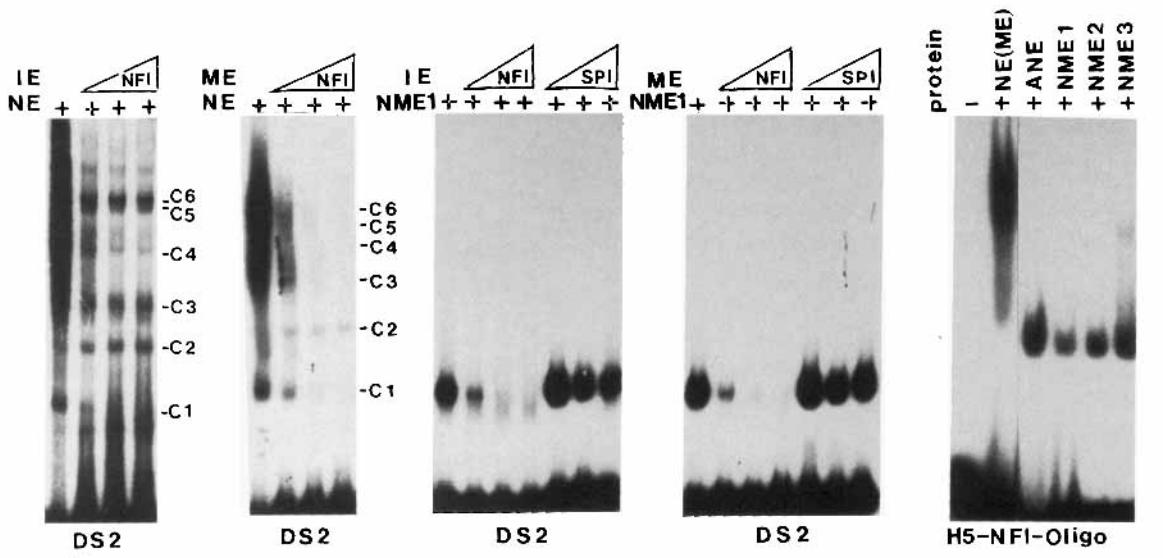

Fig. 3. A NF1 DNA-binding activity is a component of the adult erythrocyte mature and immature nuclear matrix. The DNA fragment (DS2) or oligonucleotide (H5-NF1) used in the electrophoretic gel mobility-shift assays are indicated at the bottom of each panel. End-labelled (1 ng) DNA fragment DS2 was incubated with 10 μg of nuclear extracted protein (NE or ANE; see Fig. 1) or 10 μg of nuclear matrix extracted protein (NME1, NME2, NME3; see Fig. 1) that had been isolated from immature (IE) or mature (ME) erythrocytes. The amount in molar excess of competitor oligonucleotide (H5-NF1 or Sp1), as indicated at the top of the panel, was 12.5, 50, and 100. The end-labelled H5-NF1 oligonucleotide (0.5 ng) was incubated with nuclear or nuclear matrix extracted protein (5 μg).

oligonucleotide. In contrast, not all of the complexes generated with DS2, and immature erythrocyte nuclear extracts were competed for with the H5-NF1 oligonucleotide.

Several NF1-related complexes were formed with the DS2 DNA fragment and mature erythrocyte nuclear extracts, including C1 and C3–C6. We have observed that the level of C1 formed was dependent upon the amount protein added to the assay. As the protein concentration was increased, the level of C1 decreased. There was also a corresponding increase in the amount of complexes C3–C6 formed.

For both mature and immature erythrocyte nuclear matrix extracts (NME1), one complex was typically generated with the DS2 fragment. The formation of this complex, which had the same electrophoretic mobility as complex C1, was prevented when H5-NF1 oligonucleotides, but not Sp1 oligonucleotides (or GATA-1, UPE), were added to the assay (Fig. 3).

To provide further evidence that the nuclear matrix protein was binding to the H5-NF1 site, DNase I footprinting studies with immature erythrocyte nuclear extracts and nuclear matrix extracts were performed. Figure 4 shows that nuclear-extracted proteins protected the GATA, Sp1, and NF1 binding sites, while the nuclear matrix–extracted proteins only bound to the NF1 site. EMSAs with the H5-NF1 oligonucleotide and mature or immature nuclear matrix extracts were also done (Fig. 3). As with the DS2 DNA fragment, several complexes were formed with the H5-NF1 oligonucleotide and mature (and immature) nuclear-extracted proteins. The amount of fastest migrating complex (C1 complex) generated with the mature erythrocyte nuclear extract was low. However, when lower amounts of protein were used in the assay, the abundance of the C1 complex increased (not shown). Similar to the results with DS2 and nuclear matrix extracts, one complex, which had a mobility similar to that of the fastest migrating C1 complex, was formed with the H5-NF1 oligonucleotide and mature (and immature) nuclear matrix extract (Fig. 3). Together, these observations suggested that NF1 was the nuclear matrix protein that was binding to the DS2 DNA fragment.

The preceding experiments provide evidence that NF1 is attached to the internal nuclear matrix of chicken mature and immature erythrocytes. However, analysis of the ammonium sulfate extract of DNase I–digested nuclei (ANE, Fig. 1), which contained the bulk of the chromatin fragments, showed that NF1 binding activity was also present in this fraction (Fig. 3, EMSA with H5-NF1 oligonucleotide). These observa-

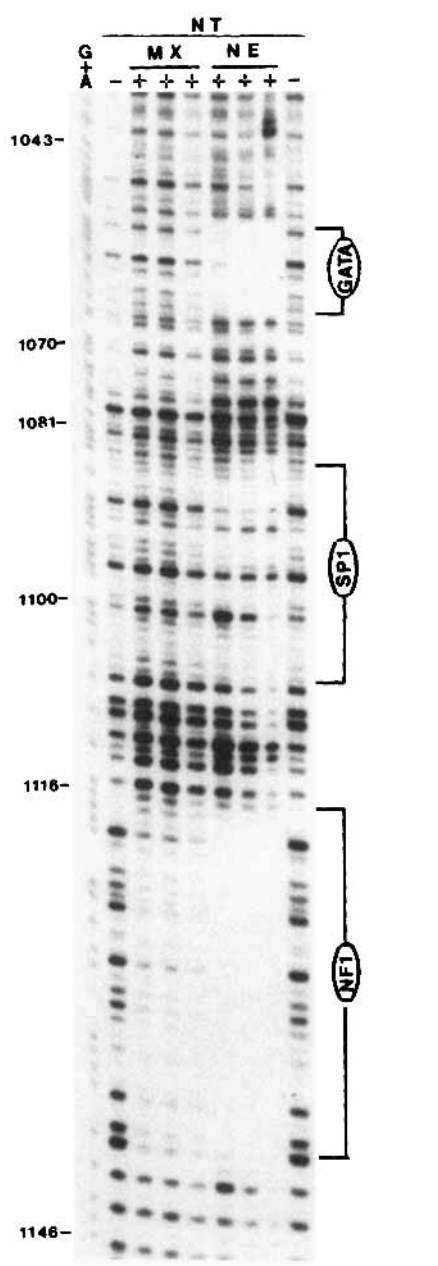

Fig. 4. DNase I footprint analysis of adult chicken immature nuclear extracted proteins interacting with the histone H5 enhancer region. DNA fragment DS1 + DS2 (1–2 ng) was end-labelled on the nontemplate (NT) strand and incubated with (+) or without (−) 10–30 μg nuclear (NE) or nuclear matrix fraction NME1 (MX) protein isolated from immature erythrocytes. The G + A chemical sequencing reaction is indicated. The amount of DNase I added to 50 μl was as follows (left to right): 0.2, 0.1, and 0.01 units. The factor binding sites are indicated.

tions suggested that NF1 was associated with chromatin and the internal nuclear matrix. Although the 0.25 M ammonium sulfate nuclear extract (fraction ANE) should contain a similar spectrum of proteins as those of the 0.3 M NaCl nuclear extracts, a single complex that comigrates with complex C1, but not the slower migrating complexes, was generated with fraction ANE proteins.

To find whether the NF1 activity attached to the nuclear matrix was resistant to 2 M NaCl extraction, the NF1 activities in a 2 M NaCl extract (fraction NME2; see Fig. 1) of the ammonium sulfate nuclear matrices and internal nuclear matrix extract (fraction NME 3; see Fig. 1) of 2 M NaCl nuclear matrices were analyzed by EMSA and H5-NF1 oligonucleotide. Figure 3 shows that some NF1 activity was extracted from the ammonium sulfate nuclear matrices with 2 M NaCl, while the remainder resisted 2 M NaCl extraction. These results demonstrate that NF1 DNA-binding activity remained bound to internal nuclear matrices that had been extracted sequentially with 0.25 M ammonium sulfate and 2 M NaCl.

The consensus binding sequence for NF1 is T/CGGA/CN_{5-6}GCCAA [Faisst and Meyer, 1992]. The H5-NF1 binding sequence (TGGCN_5CCCAA) deviates slightly from the consensus sequence. To provide further evidence that the nuclear matrin binding to the H5-NF1 oligonucleotide was NF1, oligonucleotides containing the consensus (NF1 oligonucleotide) or mutated NF1 (M1, TCGCN_5GCCAA; M2, TGGCN_5GCCCA) binding sequence were used in EMSA with nuclear matrix extracts (NME1) from chicken mature and immature erythrocytes. The point mutations in the NF1 consensus binding sequence (M1 and M2 oligonucleotides) have been shown to reduce the NF1 binding. For example, the point mutation in oligonucleotide M1 reduced the NF1 binding by 100-fold [Goyal et al., 1990; Jones et al., 1987]. Figure 5 shows that a protein-DNA complex formed with the H5-NF1 and NF1 oligonucleotides, but only very low amounts of this complex were generated with M1 and M2 oligonucleotides. Two oligonucleotides that corresponded to NF1 binding sites within the P0 promoter of the human c-*myc* gene were also tested in EMSA. Oligonucleotide C4 (TGGAN_5TCCAA) has a sequence that differs in one position from the consensus NF1 binding sequence, while oligonucleotide C1 (TGGCN_5ATTGG) has a weak

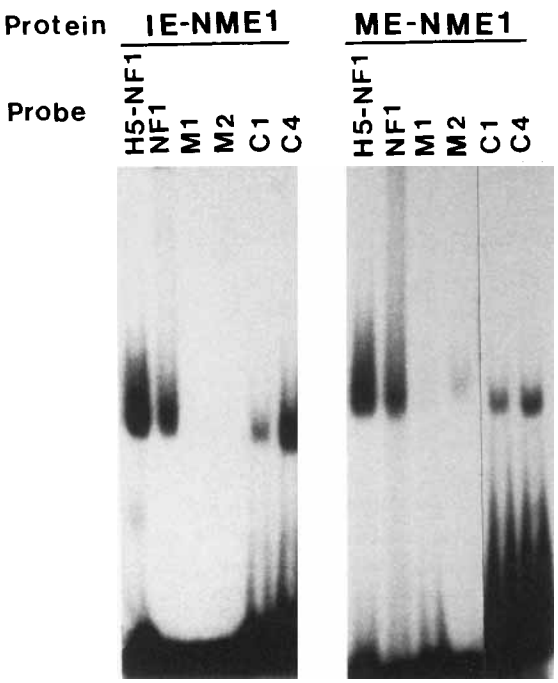

Fig. 5. Evidence that the nuclear matrix protein recognizes the NF1 binding site. Various oligonucleotides (0.5 ng, end-labelled) containing the consensus NF1 site or point mutations were used in EMSA with nuclear matrix extracts (NME1, 5 μg) that were isolated from chicken immature (IE) or mature (ME) erythrocytes. Oligonucleotide NF1 contains the consensus binding sequence for NF1 (TGGCN_5GCCAA). The H5-NF1, M1, and M2 oligonucleotides have the following sequences: H5-NF1, TGGCN_5CCCAA; M1, TCGCN_5GCCAA; M2, TGGCN_5GCCCA. The two oligonucleotides that corresponded to NF1 binding sites within the P0 promoter of the human c-*myc* gene were oligonucleotide C4 (TGGAN_5TCCAA) and oligonucleotide C1 (TGGCN_5ATTGG).

binding sequence for NF1 [Lang et al., 1991]. Both oligonucleotides formed a complex with the mature and immature erythrocyte nuclear matrix extracts (Fig. 5). Following their relative affinities for NF1, oligonucleotide C4 formed more complex than did oligonucleotide C1.

NF1 Is Associated With Nuclear Matrices of Chicken Liver and Trout Liver

To decide whether NF1 was attached to internal nuclear matrices of other cell types, nuclear matrix extracts (NME1) from chicken liver and trout liver were analyzed. Both nuclear matrix extracts formed protein-DNA complexes with oligonucleotides H5-NF1, NF1, and C4 (Fig. 6). Few complexes were generated with oligonucleotides C1, M2, and M1. The electrophoretic mobilities of the complex generated with chicken liver and erythrocyte nuclear matrix extracts were identical. In contrast to chicken liver and chicken

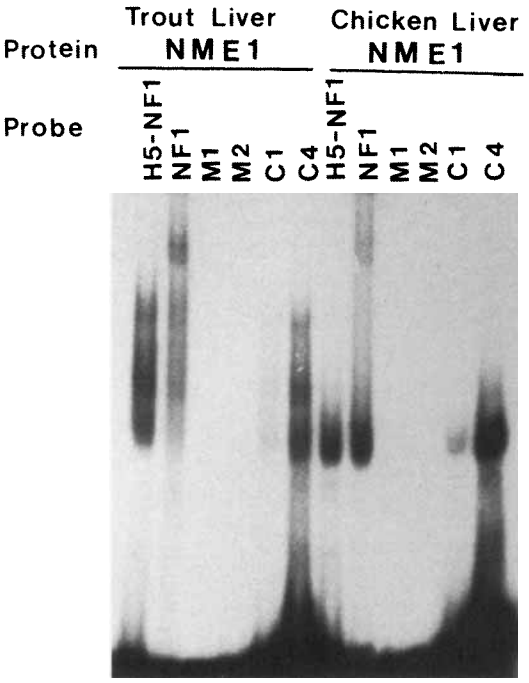

Fig. 6. NF1 is attached to the nuclear matrices of chicken and trout liver. Oligonucleotides (0.5 ng, end-labelled) containing the consensus or point mutations of the NF1 binding sequence (see legend to Fig. 5) were used in EMSA with nuclear matrix extracts (NME1) that were isolated from either trout liver or chicken liver.

erythrocyte nuclear matrix extracts, trout liver nuclear matrix extracts formed several protein-DNA complexes with the NF1 oligonucleotides. As with the chicken erythrocytes, NF1 activity was also detected in the ANE fraction from DNase 1–digested chicken liver and trout liver nuclei (not shown). These observations suggest that the association of NF1 with the internal nuclear matrix is neither tissue nor species specific.

Purification of NF1 Proteins From Mature Erythrocyte Nuclear and Nuclear Matrix Extracts

NF1 typically consists of a family of related proteins [Goyal et al., 1990; Jones et al., 1987; Rosenfeld and Kelley, 1986]. To find out whether the nuclear matrix NF1 activity contained the same spectrum of proteins that were present in the nuclear extracts, NF1 proteins were purified from nuclear matrix (NME1; see Fig. 1) and nuclear extracts of chicken mature erythrocytes. The purification of NF1 was monitored using EMSA with the H5-NF1 oligonucleotide, and the fractions with NF1 activity were analyzed by SDS gel electrophoresis and silver staining (Fig. 7). The affinity-purified NF1 fractions obtained from the nuclear extract contained a family of polypeptides with molecular masses ranging from 53–64 kDa, similar to NF1 proteins isolated from HeLa cells [Goyal et al., 1990; Jones et al., 1987; Rosenfeld and Kelley, 1986]. The nuclear matrix affinity-purified NF1 fraction contained a subset of the NF1 proteins (64, 58, and 59 kDa) that were present in the nuclear extract. Further, for nuclear matrix purified NF1, the amount of the 58/59 kDa was typically similar to that of the 64 kDa NF1 protein. This was different from the relative levels of the 58/59 kDa NF1 forms, which were always lower than that of the 64 kDa form, in the nuclear extract isolated NF1 species.

DISCUSSION

We show in this study that the transcription factor NF1 is attached to the internal nuclear matrices of adult chicken immature and mature erythrocyte. The observation that NF1 activity was extracted with β-mercaptoethanol from the 0.25 M ammonium sulfate/2 M NaCl nuclear matrices of mature and immature erythrocytes suggests that NF1 proteins are associated with the internal nuclear matrix. However, NF1 is also located in the nonmatrix, presumably chromatin-bound, fraction. The nuclear matrix protein bound specifically to the H5-NF1 oligonucleotide. Since the H5-NF1 site deviates slightly from the consensus NF1 site, we also did EMSA with oligonucleotides containing consensus and mutated NF1 sites. The results of this analysis prove that the chicken erythrocyte nuclear matrix protein recognized the NF1 site. NF1 binding activity was also present in nuclear matrices of chicken liver and trout liver. These observations suggest that the association of NF1 with the nuclear matrix is cell type and species independent. Thus, NF1 can be added to the growing list of transcription factors that are associated with the nuclear matrix.

Four chicken NF1 genes have been identified, NF1-A, -B, -C, and -X [Kruse et al., 1991; Rupp et al., 1990] which code for proteins with molecular masses ranging from 46–62 kDa. Differential splicing also contributes to the heterogeneity in the NF1 family [Faisst and Meyer, 1992; Rupp et al., 1990]. Purification of NF1 from chicken mature erythrocytes yielded several polypeptides, ranging in size from 53–64 kDa which is similar to the molecular masses of the mammalian family of NF1 proteins [Jones et al., 1987;

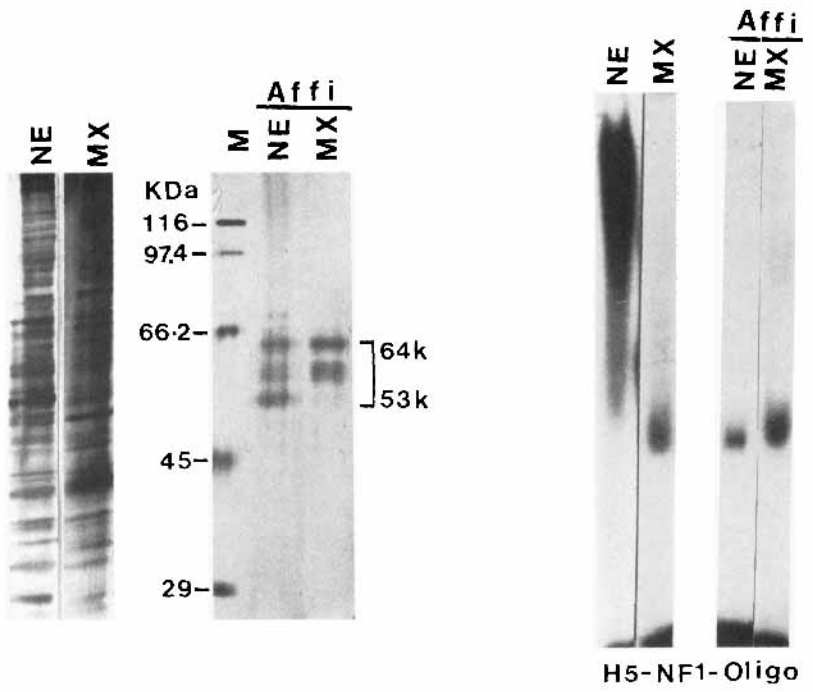

Fig. 7. Purification of NF1 proteins from chicken mature nuclear and nuclear matrix extracts. Silver-stained 10% polyacrylamide SDS gel patterns of proteins of nuclear extract (NE), nuclear matrix fraction NME1 (MX) protein, and affinity-purified (Affi) NF1 fractions are shown on the left of the figure. NE and MX lanes contained 10 μg protein, and the Affi NE and MX lanes contained 0.2 μg affinity-purified NF1 proteins. Lane M contains molecular weight markers (Sigma). For EMSA, end-labelled (0.5 ng) H5-NF1 oligonucleotide was incubated with nuclear extract protein (5 μg), nuclear matrix fraction NME1 protein (5 μg), or affinity-purified (Affi) column fractions (approximately 30 ng protein) (panels shown on the right).

Rosenfeld and Kelley, 1986]. We found that a subset of the chicken erythrocyte NF1 protein family was associated with adult erythrocyte mature nuclear matrices. The 58/59 kDa NF1 appeared to be enriched in nuclear matrix extracts which also contained the 64 kDa NF1 form. Our results show that the 53 kDa NF1 species was not associated with the nuclear matrix.

Recently, van Wijnen et al. [1993] reported that Sp1, ATF, CCAAT, C/EBP, Oct 1, and AP1 DNA-binding activities are associated with nuclear matrices. Some factors (Sp1, ATF) were associated with the nuclear matrices of several cell types (e.g., HeLa, a human cervical carcinoma, and ROS 17/2.8, a rat osteosarcoma), while others were found to be cell type specific (e.g., CCAAT, C/EBP). Our results suggest that the association of Sp1 with the nuclear matrix is also cell type specific since it is not found in the nuclear matrices of chicken erythrocytes. These observations provide evidence that the nuclear matrices of different cells selectively localize specific transcription factors (e.g., specific members of the NF1 family) which would contribute to the observed cell type specific nuclear matrix protein composition [Cupo, 1991]. Such observations further strengthen the hypothesis that the nuclear matrix is involved in transcriptional control [Stein et al., 1991; van Wijnen et al., 1993].

Several complexes were formed with the NF1-oligonucleotide and NF1 proteins of nuclear extracts (see Fig. 3) similar to the observations of others [Goyal et al., 1990]. However, with nuclear matrix extracts (NME1, NME2, and NME3) or the ammonium sulfate soluble nuclear extract (fraction ANE; see Fig. 3), one complex (C1) was generated with the H5-NF1 oligonucleotide (Figs. 3, 5). Also, the affinity purified NF1 proteins that were isolated from nuclear extracts or nuclear matrix extracts generated only this C1 complex. Using the Bading procedure to calculate the molecular weight of DNA-bound protein(s) [Bading, 1988; Sun et al., 1992], the approximate molecular mass of the proteins of the C1 complex was 112 kDa. The sizes of the chicken NF1 proteins ranged from 53–64 kDa, suggesting that a NF1 homo- or heterodimer is bound to the H5-NF1 oligonucleotide. The higher molecular weight complexes may be formed by

proteins associating with NF1 by protein-protein interactions dimers [Goyal et al., 1990; Martin, 1991]. Elevated ionic strength (e.g., 0.25 M ammonium sulfate) may disrupt the protein-protein interactions, dissociating the high molecular weight complexes.

Transcriptionally active histone H5, but not the inactive, DNA sequences were enriched in the chromatin fragments bound to the low salt-insoluble nuclear material of adult erythrocyte immature and mature erythrocytes [Delcuve and Davie, 1989]. The demonstration that NF1 is a component of the erythrocyte nuclear matrix suggests that NF1 may contribute to the association of the histone H5 gene 3′ enhancer with the nuclear matrix. However, it is doubtful that NF1 is solely responsible for an attachment of the histone H5 DNA sequences to the nuclear matrix. Other proteins that may reinforce the interaction of the transcriptionally active chromatin with the nuclear matrix are the histone acetyltransferase and deacetylase [Hendzel and Davie, 1992; Hendzel et al., 1991]. These nuclear matrins catalyze the dynamic acetylation of the histones bound to transcriptionally active DNA [Boffa et al., 1990; Hendzel et al., 1991]. Several indirect and direct interactions of transcriptionally active DNA sequences with nuclear matrins are probably involved in bringing the transcriptionally active histone H5 gene chromatin to the nuclear matrix.

The nuclear matrix is thought to be the site of transcription, RNA processing, and DNA replication [Berezney, 1991; Carter et al., 1993; Cook, 1989, 1990; Xing et al., 1993]. NF1 activates both transcription and DNA replication [Jones et al., 1987]. In this study, we show that some members of the NF1 family of proteins are nuclear matrins. The NF1 nuclear matrins may aid in the targeting of specific genes (e.g., histone H5 in erythrocytes, c-*myc* in dividing cells) to sites on the nuclear matrix that contain the transcription machinery and RNA processing machinery (e.g., transcription foci; see Jackson et al. [1993]). It should be noted that nucleosomes prevent NF1 from binding to its DNA binding site [Archer et al., 1992]. Thus only *cis*-acting DNA elements with NF1 binding sites that are not in nucleosomes will have the opportunity to associate with NF1 and the nuclear matrix. An interesting possibility is that NF1 may also play a role in directing the adenovirus origin of replication to nuclear matrix sites harboring the DNA replication machinery. In this regard the results of Bosher et al. [1992] are of particular interest. These investigators showed that upon infection of A549 (human lung carcinoma cell line) with adenovirus type 2, NF1 was recruited to replication foci. These replication factories are attached to the nuclear matrix [Hozák et al., 1993]. These results suggest that NF1 is actively recruited from one nuclear site to another (e.g., chromatin sites, transcription foci, replication factories).

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