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L. M. Heltemes
Iowa State University

C. K. Tuggle
Iowa State University

Susan J. Lamont
Iowa State University, sjlamont@iastate.edu

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Abstract

The octamer motif is important in transcriptional regulation of genes of the immune system in many species, including the chicken. Little is known, however, regarding octamer-binding protein expression in chicken tissues. We examined octamer-binding protein expression patterns in multiple chicken tissues (ovary, cerebrum, liver, lung, kidney, spleen, thymus, and bursa of Fabricius) plus two lymphocyte cell lines. Every tissue and cell line had multiple octamer-binding proteins. Seven distinct protein-DNA complexes were identified. Our results demonstrate that multiple octamer-binding proteins, exhibiting differential tissue expression, exist in a wide variety of tissues of the chicken.

Keywords

chicken, Oct-1, octamer motif, transcriptional regulation, differential tissue expression

Disciplines

Agriculture | Animal Sciences | Genetics | Poultry or Avian Science

Comments

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MOLECULAR BIOLOGY

Research Notes

Tissue Expression Patterns of Chicken Octamer-Binding Proteins[1]

L. M. HELTEMES,*,[2] C. K. TUGGLE,† and S. J. LAMONT*,†,[3]

Program of Immunobiology and †Department of Animal Science, Iowa State University, Ames, Iowa 50011

ABSTRACT The octamer motif is important in transcriptional regulation of genes of the immune system in many species, including the chicken. Little is known, however, regarding octamer-binding protein expression in chicken tissues. We examined octamer-binding protein expression patterns in multiple chicken tissues (ovary, cerebrum, liver, lung, kidney, spleen, thymus, and bursa of Fabricius) plus two lymphocyte cell lines. Every tissue and cell line had multiple octamer-binding proteins. Seven distinct protein-DNA complexes were identified. Our results demonstrate that multiple octamer-binding proteins, exhibiting differential tissue expression, exist in a wide variety of tissues of the chicken.

(*Key words*: chicken, Oct-1, octamer motif, transcriptional regulation, differential tissue expression)

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INTRODUCTION

The sequence-specific interaction between *trans*-acting proteins and *cis*-acting DNA elements is the foundation of transcriptional regulation and tissue-specific gene expression (Hatzopoulos *et al*., 1990). One well-recognized *cis*-acting DNA element involved in both tissue-specific and ubiquitous gene expression is the octamer motif (ATTTGCAT; Kemler and Schaffner, 1990). The importance of the octamer motif in the chicken has been demonstrated in the enhancer of both the U1 and U4B snRNA genes (Zamrod and Stumph, 1990; Cheung *et al*., 1993) and the immunoglobulin lambda light chain promoter (Bulfone-Paus *et al*., 1995; Heltemes *et al*., 1997b).

Several mammalian octamer-binding factors have been identified (Wegner *et al*., 1993), including a tissue-specific factor, Oct-2 (Muller *et al*., 1988) and a ubiquitously expressed factor, Oct-1 (Sturm *et al*., 1988). In the mouse, approximately 10 octamer-specific proteins, identified in a variety of tissues, are not only expressed in the mouse during postnatal development, but also differentially expressed during embryogenesis (Scholer *et al*., 1989; Scholer, 1991). To date, two octamer-specific genes (Oct-1 and Oct-11/Skn-1) have been described in the chicken (Petryniak *et al*., 1990; Heltemes *et al*., 1997a).

The electrophoretic mobility shift assay (EMSA) can be used to examine interactions between DNA elements and transcription factors, thereby revealing tissue expression patterns of DNA-binding proteins. It is based on the principle of DNA-protein interactions altering the mobility of DNA fragments in electrophoresis. The objective of this study was to define the expression pattern of octamer-binding proteins in multiple tissues and cell lines of the chicken.

MATERIALS AND METHODS

Cell Lines, Tissue Samples, and Cell Extracts

Two chicken lymphocyte lines, B cell line (DT40; Baba *et al*., 1985) and T cell line (MSB1; Akiyama and Kato, 1974) were made available by Craig Thompson, University of Chicago (5841 S. Maryland Ave., Chicago, IL 60637). They were cultured in Dulbecco's Modified Eagle's Medium with 10% newborn calf serum and 5% chicken serum at 42 C with 5% CO_2.

White Leghorn chicken tissues (juvenile; whole blood, cerebrum, ovary, thymus, liver, spleen, kidney, bursa, and lung) and mouse tissues (Day 15 embryo) were removed

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[2]Current address: L. M. Heltemes, Human Gene Therapy Research Institute, Department of Molecular Oncology, 1415 Woodland Avenue, Des Moines, IA 50309.

[3]To whom correspondence should be addressed: sjlamontaiastate.edu

Abbreviation Key: EMSA = electrophoretic mobility shift assay.

A. Octamer Oligonucleotides

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1)  5′  G CGC AGG GAG TTA TTT GCA TAG GGG GGC GTC  3′
2)  5′        TTC TAG TGA TTT GCA TTC GCC A        3′
3)  5′            TGT TAA TTT GCA TTT CTA A        3′
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B. Mutant Octamer Oligonucleotides

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1)  5′  G CGC AGG GAG TTA TTT GCA TAG GGG GGC GTC  3′
2)  5′  G CGC AGG GAG TTA ... ... TAG GGG GGC GTC  3′

3)  5′        TTC TAG TGA TTT GCA TTC GCC A        3′
4)  5′        TTC TAG TGg aTT GCA TTC GCC A        3′
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FIGURE 1. Sequence of normal and mutated octamer oligonucleotides. Only the sense strand of each oligonucleotide is shown. A) Oligonucleotide sequences aligned by the octamer sequence (ATTTGCAT; underlined sequence). 1) Sequence from chicken immunoglobulin lambda light chain promoter (Oligo1; McCormack *et al.*, 1989). 2) Oct-1 oligonucleotide from Santa Cruz Biotechnology (Oligo2). 3) Mouse octamer oligonucleotide (Oligo3; Tammy Nowling, Iowa State University). B) Comparison of regular oligonucleotide with mutated oligonucleotide. 1) Oligo3. 2) Mutated Chicken immunoglobulin lambda light chain oligonucleotide (MOligo1). 3) Oligo2 4) Santa Cruz Biotechnology mutant Oct-1 oligonucleotide (MOligo2).

using aseptic techniques, immediately frozen in liquid nitrogen, and stored at –70 C. Whole cell extracts from the tissues were isolated by the technique of Deryckere and Gannon (1994). From the cell lines, whole cell extracts were isolated as described by Dent and Latchman (1993). HeLa nuclear extracts were purchased.[4] Mouse embryo Day 12.5 nuclear extracts (Tammy Nowling, Iowa State University) were also used. Protein concentrations were determined by using the Bio-Rad protein assay.[5] Extracts were aliquoted and stored at –70 C.

Probes and Competitor DNA

Multiple octamer oligonucleotides were used (Figure 1). Two single-stranded oligonucleotides (sense and anti-sense) were designed from the published chicken immunoglobulin promoter sequence (McCormack *et al.*, 1989; Petryniak *et al.*, 1990) and synthesized (Nucleic Acid Facility, Iowa State University). A double-stranded octamer oligonucleotide (Oligo1) was created by annealing equal molar amounts of each single-stranded oligonucleotide (sense and anti-sense), heating to 70 C for 5 min, and cooling to room temperature. The overhang was then filled in by adding dNTPs and Klenow fragment[4] and incubating at 37 C for 15 min. The annealed oligonucleotides were then dephosphorylated by using shrimp alkaline phosphatase[6] following the manufacturer's recommendations. The oligonucleotide was phenol-chloroform extracted, ethanol precipitated, and resuspended in water. A mutant form of the same oligonucleotide (Moligo1) was also created in which the six internal base pairs of the octamer motif were deleted. Double-stranded Oct-1 oligonucleotide (Oligo2) and a double-stranded mutated Oct-1 oligonucleotide (MOligo2) were purchased.[7] The double-stranded mouse octamer oligonucleotide (Oligo3) was annealed as described above.

EMSA

DNA fragments were end-labeled by using T4 polynucleotide kinase and then passed through a Bio-Spin column[5] to remove unincorporated nucleotides. The sample (0.5 to 2.0 ng of labeled oligonucleotide, 1 μg poly [dI-dC·dI-dC], 1 to 5 μg of protein extract [25% glycerol, 29 m*M* HEPES pH 7.9, 420 m*M* NaCl, 1.2 m*M* $MgCl_2$, 0.2 m*M* EDTA, 0.5 m*M* DTT, 0.5 m*M* PMSF, 2 m*M* Benzamidine, and 5 μg/μL pepstatin, leupeptin, and aprotinin]) was brought to a final volume of 20 μL with sample buffer (10 m*M* Tris, 50 m*M* NaCl, 1 m*M* DTT, 1 μ*M* EDTA, and 5% glycerol) and incubated for 20 min at room temperature. The sample was loaded on a 5% polyacrylamide gel (29 acrylamide:1 bis-acrylamide). The gel was run at 150 V for 2 to 2.5 h, then vacuum dried on Whatman paper. The x-ray film then exposed to the gel.

Competition assays were done in the same way, except that 100 ng of the appropriate unlabeled, annealed oligonucleotide was added to the reaction mixture. For nonspecific competitor, mutated octamer oligonucleotides were used.

Supershift assays were done by preincubating 1 μg of anti-Oct-1 antibody[6] with cell extract and incubating for 30 min at 4 C, before performing the rest of the assay.

RESULTS AND DISCUSSION

Three different octamer oligonucleotides were used, in which the octamer sequence was identical but the flanking sequences were different (Figure 1). We were unable to detect any differences in banding pattern among any of the three oligonucleotides (data not shown). This result suggests that binding occurred only

[4]Promega, Madison, WI 53711.
[5]BioRad Laboratories, Richmond, CA 94804.
[6]United States Biochemical Corp., Cleveland, OH 44122.
[7]Santa Cruz Biotechnology, Santa Cruz, CA 95060.

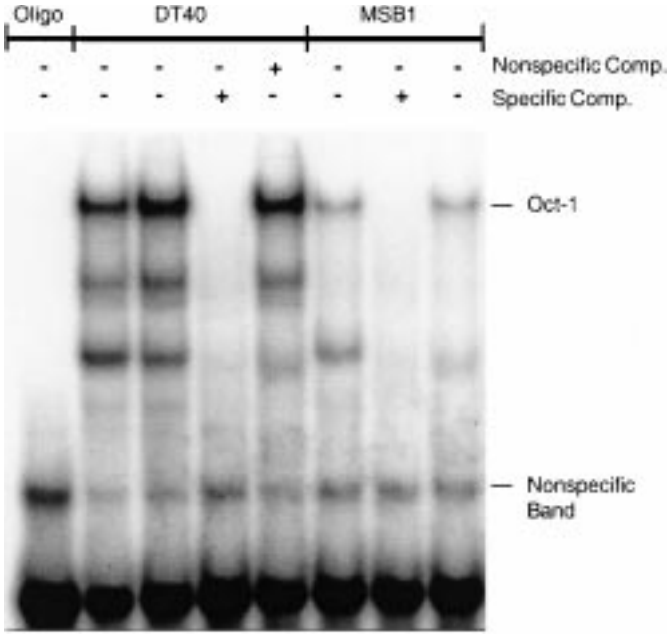

FIGURE 2. Competitor assay for MSB1 and DT40. The lane labeled oligo demonstrates the results of running the labeled oligo without any extract (negative control). Oct-1 is the top band and the other bands are unknown octamer binding proteins. The oligonucleotides used were Oligo2 (labeled oligo and specific oligo) and Moligo2 (nonspecific competitor).

at the octamer site and not in the flanking sequences. The results presented are those generated using Oligo2.

A chicken B cell line (DT40) and T cell line (MSB1) were used as references for chicken Oct-1 and Oct-2 proteins. These cell lines were previously used by Petryniak *et al.* (1990), who cloned chicken Oct-1, to show that DT40 B cells contained an Oct-2-like octamer-binding protein that was not present in MSB1 T cells. In their EMSA, they found two protein-DNA complexes using DT40 nuclear extract and only one protein-DNA complex using MSB1 nuclear extract present in their gels. In contrast, we demonstrated one additional band in each cell line that displays the same mobility. The difference between our results and those of the previous researchers may be due to the isolation of whole cell, instead of nuclear, extract. Petryniak *et al.* (1990) defined the uppermost complex seen in both cell line extracts to be Oct-1 (Figure 2) by the convention of numbering, starting with the slowest migrating complex (uppermost complex) being labeled as Oct-1.

To confirm the identity of the Oct-1-DNA interaction, a supershift assay (a variation of the EMSA) was used. By adding specific antibody to the reaction mix with the protein and DNA, the supershift assay utilizes specific protein-antibody binding either to decrease the normal migration of the DNA-protein interaction (supershift) or to interfere with the interaction between protein and DNA. The addition of mouse anti-human Oct-1 antibody resulted in a shift of the upper complex in the HeLa cell extract and in chicken B and T cells (Figure 3), confirming its identity as Oct-1. There is an apparent difference in the supershift band intensity from between the HeLa cell extract and the two chicken cell extracts, which could be a result of weaker interaction between the protein and the antibody. The anti-Oct-1 antibody is mouse, rat, and human Oct-1 reactive according to the manufacturer. Therefore, there may be a weaker interaction between this antibody and chicken Oct-1.

The tissues and cell types used in the EMSA examined a wider variety of cell types than have been previously reported. The EMSA showed differences in DNA binding to octamer-binding proteins in chicken tissues (blood, cerebrum, ovary, thymus, liver, spleen, kidney, bursa, and lung) and lymphocyte cell lines (B cells, T cells), which demonstrates tissue-specificity in the expression of chicken octamer-binding proteins. Figure 4 is a schematic presentation of the combined EMSA results and Figure 5 is an example of a typical, single assay result.

Competition assays were performed to determine whether the bands were a result of specific or nonspecific DNA binding. Competitor oligonucleotides (regular and mutant) were added to the binding reaction to compete away protein interactions with labeled DNA (Figure 2). The specific competitor resulted in the removal of virtually all the bands, but the nonspecific competitor (mutated octamer oligonucleotide) had little or no effect on the banding patterns. Therefore,

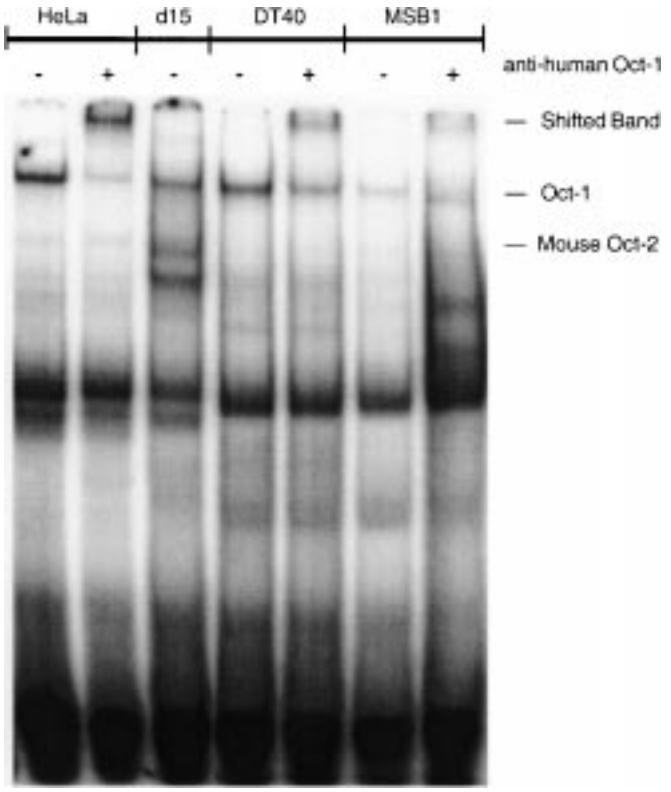

FIGURE 3. Antibodies to Oct-1 change the mobility of the DNA-protein complex demonstrating the presence of Oct-1 in chicken B (DT40) and T (MSB1) cells. HeLa cell extract was used as a positive control, and the mouse Day 15 embryo was included to show relative positions of additional bands. The oligonucleotide used was Oligo2.

competitor assays demonstrated that most of the bands seen in the EMSA are specific binding interactions with the octamer oligonucleotide. The EMSA results identified differences in tissue-expression patterns for chicken octamer-binding proteins. Expression differences in octamer-binding patterns have also been demonstrated in other species (Scholer *et al*., 1989; Scholer, 1991). There were a total of nine octamer-binding proteins identified in the chicken tissues examined. Many of these were expressed in multiple tissues. Only one band, identified as Oct-1, showed widespread expression; however, Oct-1 was not expressed in chicken liver. It cannot be determined from this assay whether these many binding reactions (bands) represent separate genes or distinct proteins created from the same gene (alternative splicing).

Comparison of octamer-binding patterns in the chicken with those of mammals shows several distinct differences. There is no DNA-protein complex in chicken with a migration pattern identical to that of mouse Oct-2. This finding suggests that if Oct-2 exists in chickens, it may be a different size than in mice. Interestingly, the octamer family genes identified thus far in the chicken share striking homology to their mammalian counterparts (Petryniak *et al*., 1990; Heltemes *et al*., 1997a). There is, however, a faster-migrating protein-DNA complex (Chick Band; Figure 4) that is present only in the B cell line, bursa, and brain. This expression pattern is typical of Oct-2 expression in mammals. Perhaps this represents Oct-2 in chickens. Additional differences exist in the octamer-binding patterns between chicken and mouse. The chicken liver has a protein-octamer complex larger in size than the mammalian Oct-1 complex and also one smaller in size. The larger complex is found only in liver, but the smaller complex is found in all tissues except the lymphoid cell lines. This result is in contrast to the mouse, in which only Oct-1 protein was detected in the liver (Scholer *et al*., 1989).

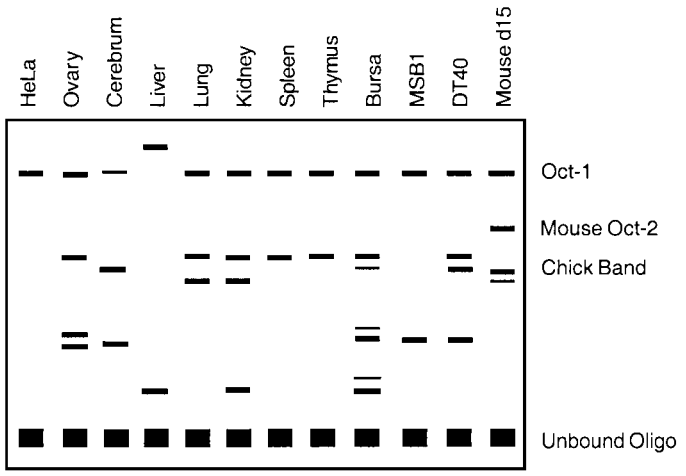

FIGURE 4. Schematic of tissue expression of chicken octamer-binding patterns, human HeLa cell extract and mouse Day 12.5 and Day 15 embryo extract. The DNA-protein complexes are represented by bars. All complexes represented were reproduced at least three times. Only octamer-specific complexes are shown, except for ovary and cerebrum, where competitor assays were not done.

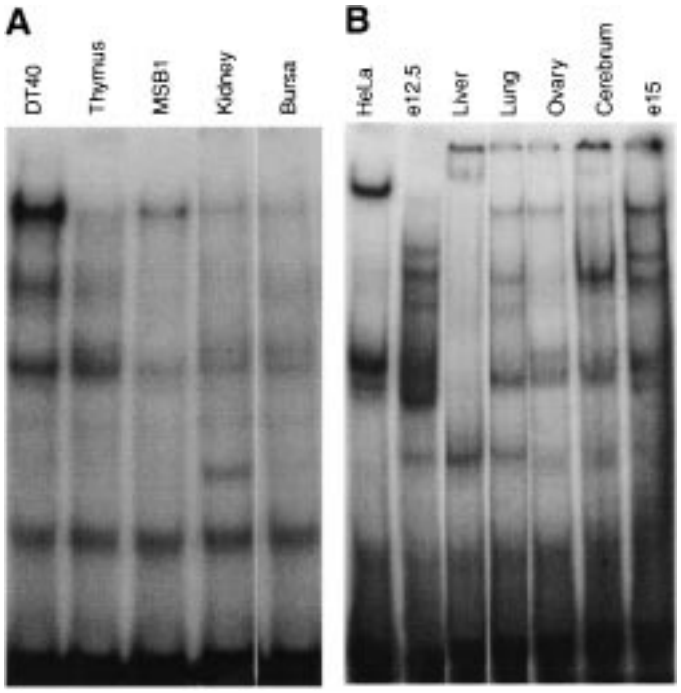

FIGURE 5. An assay demonstrating the octamer-binding protein patterns for multiple tissues. The oligonucleotide used was Oligo2. A) Assay demonstrating DNA-protein complexes for DT40, thymus, MSB1, kidney, and bursa of Fabricius. B) Assay demonstrating DNA-protein complexes for HeLA, mouse embryo (Day 12.5), liver, lung, ovary, cerebrum, and mouse embryo (Day 15).

We demonstrated the expression of Oct-1 in all the examined chicken tissues except liver, as well as the existence of multiple tissue-restricted octamer-binding proteins. Not only is the chicken Oct-1 protein sequence highly homologous to that of mammals (Petryniak *et al*., 1990), but the expression pattern, as determined in this study, is also very similar. We identified, however, several differences between chicken and mammals, including the existence of an octamer-binding protein in chicken with a migration pattern different than any seen in the mouse. In summary, this research has demonstrated the presence of multiple octamer-binding proteins with differential tissue expression in the chicken.

ACKNOWLEDGMENTS

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