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Identification and Localization of a Novel Zinc Finger Gene in Developing Chick Skin and Feather Buds

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We have cloned and sequenced a cDNA encoding a novel zinc finger protein (Fzf-1) containing two tandem repeats of zinc finger motifs of the C2H2 type. The cDNA is 3.0 Kb long and has an open reading frame which codes for a protein of 789 amino acids. The expression pattern of the zinc finger gene was studied in chick embryonic skin and feathers by in situ hybridization. The expression of the gene is found to be temporally and spatially regulated. In stage 38 chick embryos, the transcripts are localized to the epidermis but in 10-day-old embryos, the signal is localized to the forming dermis. In 12-day-old chick, the transcripts are localized to the mesenchymal region of the elongated feather buds. Reverse transcription followed by Polymerase Chain Reaction (RT-PCR) did not detect the transcripts in any other tissues.

During development of multicellular organisms, cells carrying identical genetic information are differentiated into various types of cells like neuronal, cardiac and skin cells. This is the result of the expression of various combinations of genes in the cells as development progresses, leading to the synthesis of specialized proteins that give differentiated cells their distinctive properties. This combinatorial expression of various genes is achieved by the interaction of various DNA binding proteins to specific sites on the DNA through small, discrete domains such as helix-turn-helix, leucine zippers, β-ribbon recognition elements, helix-loop-helix and zinc finger motifs.

Numerous proteins having zinc finger motifs containing 2 to 37 tandem fingers have been identified in various species and they include transcription factors, hormone receptors, myc and erbA-onc proteins and several proteins encoded by the kinase family of oncogenes (1,2). In this study, we have identified a protein with two tandem repeats of zinc finger motifs (C_2H_2 type) and is found to be expressed in the skin and feather of chick.

Feather development has been used as a model to study the mechanisms of morphogenesis for several years (3,4). Recent studies shows that several molecules like Cell Adhesion Molecules, homeobox genes and matrix molecules are playing crucial roles in feather development. Most of these proteins show antero-posterior polarity in their expression pattern. (5) The expression pattern of the newly identified zinc finger gene (Fzf-1) presented here shows that it may play a role in skin and feather development.

MATERIALS AND METHODS

cDNA isolation. A cDNA library constructed from stage 23 whole chick embryonic poly (A) RNA was searched for transcription factor-like genes. (6). Initial sequencing of one of the clones showed that it has zinc finger like domains on one of the terminal sequences. This clone was used in this study for complete sequencing using the dideoxy sequencing procedure. (7)

Northern blot analysis. Total RNA was isolated from various embryonic tissues as described (8). Poly (A) RNA was selected by double passage over oligo dT columns as described. (9). Four μg of poly (A) RNA was run on a 0.8% Agarose gel, transferred on to zeta bind membrane (Cuno, Inc.,) and processed according to the manufacturer. A 1.2 Kb DNA fragment containing the 5′ region of the zinc finger cDNA was labeled with ^{32}-P dCTP using the random prime labeling kit (Boehringer Mannenheim Biochemicals) according to the manufacturer and was used as the probe.

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RT-PCR analysis. Total RNA from day 12 chick embryonic skin, heart, skinless cranial region and limb were reverse transcribed and PCR amplified using the RT-PCR kit (Perkin-Elmer Cetus) with two zinc finger gene specific oligonucleotides 5′-GAAGTTGGTAACGCTGCACA-3′ and 5′-CGCAGGTCCTTCTTGTTCTT-3′ according to the manufacturer.

In situ localization. In situ hybridization was done as previously described. (6). Briefly, Bouins fixed day 12 chick embryonic tissues were paraffin embedded and sectioned (7um). The sections were then dried onto Superfrost microscope slides (Fisher Scientific), dewaxed, treated with proteinase K and acetylated. The tissues were then hybridized at 50° C overnight in a humid chamber followed by stringent washings with 50% Formamide at 55° C, RNase treatments and 0.1 × SSC wash at 50° C. The probe used was a Pst I 480 bp fragment from the zinc finger cDNA cloned into the plasmid pTα19 (BRL) (See Fig 1). The sense or antisense strands were in vitro transcribed using t3 or t7 RNA polymerase (Stratagene). Exposure to photographic emulsion was for 8 days at 4°C followed by developing and staining the tissues with hematoxylin.

RESULTS

Homology searches against the GenBank data base with end sequences of a cDNA clone obtained from a stage 23 chick embryonic library showed that it has sequences similar to zinc

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   1 TCACTCAGCACATGAAGACACACAGCACGGAGAAGCCGCACATGTGTGACAAGTGTGGGAAGTCCTTTAAGAAGCGTTACACCTTCAAGATGCACCTGCTGACACATATCCAGGCCATTG
       T  Q  H  M  K  T  H  S  T  E  K  P  H  M  C  D  K  C  G  K  S  F  K  K  R  Y  T  F  K  M  H  L  L  T  H  I  Q  A  I  A

 121 CTAACCGCAGGTTCAAATGTGAGTTCTGTGACTACGTCTGCGAGGACAAAAAGGTGCTGCTGAACCACCAGCTGTCACATATGCTGCAAGCCTACAAGTGCAGCTTCTGCAAGTATTCCA
       N  R  R  F  K  C  E  F  C  D  Y  V  C  E  D  K  K  V  L  L  N  H  Q  L  S  H  M  L  Q  A  Y  K  C  S  F  C  K  Y  S  T

 241 CCTTCCGGGAGGACTTCCTGGCCTCACACATGGCCGTCAAGCACACGGGAGGGAAGCCCTTTGCCTGTGAGTTCTGTCACTTCACCACCAAGCACAAGAAGAACCTGCGCCTCCACGTGC
       F  R  E  D  F  L  A  S  H  M  A  V  K  H  T  G  G  K  P  F  A  C  E  F  C  H  F  T  T  K  H  K  K  N  L  R  L  H  V  H

 361 ACTGCCGCCACGCCGACTCCTTTGAGGAGTGGGCACAGAGGCACCCCGAGGAGCCGCCCTGCCGCCGCCGCCCCTTCTTCACCCTGCAGCAGATCGAGGAGCTGAAGCAGCAGCACCGCC
       C  R  H  A  D  S  F  E  E  W  A  Q  R  H  P  E  E  P  P  C  R  R  R  P  F  F  T  L  Q  Q  I  E  E  L  K  Q  Q  H  R  Q

 481 AAGTGCAGACCCCGGCTGAGCCAGAGCGCAGCCCAGCAGCCCCTCTTGGCCCTGTCACCTACCAGGCGGTGCAGGCGGTGCCTGGGTCGGAGCCTCCTATCCTtTCTCAGGATTCTCTGG
       V  Q  T  P  A  E  P  E  R  S  P  A  A  P  L  G  P  V  T  Y  Q  A  V  Q  A  V  P  G  S  E  P  P  I  L  S  Q  D  S  L  E

 601 AGGGGGCCACCATTATCTATGAACAAGGTGTGGATGGGTCAgcAGAACTGGCCACGCAGACCGCCCTGGATCTtTTGCTGAACATGAGTACTCAGCGAGAGCTTGCTGCGGGCTCCCTGC
       G  A  T  I  I  Y  E  Q  G  V  D  G  S  A  E  L  A  T  Q  T  A  L  D  L  L  L  N  M  S  T  Q  R  E  L  A  A  G  S  L  Q

 721 AGGTGGCGGTAGTGAAACCGGATGATTCAGGGGAGGTGCAGGCCTCTTGTGAAGCACAGGCACAGGAGGAAGGGACGGAGCTGGATTCCTCCGAGCAGCAGCAGCAGAAGTTGGTAACGC
       V  A  V  V  K  P  D  D  S  G  E  V  Q  A  S  C  E  A  Q  A  Q  E  E  G  T  E  L  D  S  S  E  Q  Q  Q  Q  K  L  V  T  L

 841 TGCACATGGCAGACCGCGGGGAGACGTTGGTGCAGGAGGCTACGAGGAGGCGGCCCTGGGTGGCTCAGAGCTGCAGCAGATCACTATTCCCTTTGGGGGGACAACGGAGTACAGCATCA
       H  M  A  D  R  G  E  T  L  V  Q  E  A  Y  E  E  A  A  L  G  G  S  E  L  Q  Q  I  T  I  P  F  G  G  T  T  E  Y  S  I  I
                                                                                  |⇒
 961 TTACACCCATCAGTGAGGAGATTCCGGCTCCAGGCACGCTGTACAGTGAGGAGGAGGGCTCTGCAGAGACTTCGCATGCAGTCGTGGTGAGCGAGGCTGTGATGACAGAGGTTCTGAAGG
       T  P  I  S  E  E  I  P  A  P  G  T  L  Y  S  E  E  E  G  S  A  E  T  S  H  A  V  V  V  S  E  A  V  M  T  E  V  L  K  D

1081 ACCATAACAATCACTACATCATGTCATCCAGCGTCCCAGGGAACCAGTTCCAACACATGGAGCCCCTCCAATGGAGATGCTGCCTTCCCTTTGCCGGCAGAGGGTCAGGAGGCACAGCCA
       H  N  N  H  Y  I  M  S  S  S  V  P  G  N  Q  F  Q  H  M  E  P  L  Q  W  R  C  C  L  P  F  A  G  R  G  S  G  G  T  A  N

1201 ACTGGTGTCAAGTGGCCCCTGGTGCAGTGTGTCACTCGGCAGCTCCAGAAGGACTCCAATTTGTCCCCCACCTCCGAAGGGCAAGAAGTCACATCCCCCAAGGTCAAATGGCCTGCAATC
       W  C  Q  V  A  P  G  A  V  C  H  S  A  A  P  E  G  L  Q  F  V  P  H  L  R  R  A  R  S  H  I  P  Q  G  Q  M  A  C  N  P

1321 CAAGGCATGGCCAAAAAGCTCTCGTGCAAGGTTTCCACAGCCAAGAAGCTCTCATGCAAGATTTCCACAGCCAAAAAGTTTTCCTGCAAGATTTGCACAGCCATGTTTACAGGGAGAGCA
       R  H  G  Q  K  A  L  V  Q  G  F  H  S  Q  E  A  L  M  Q  D  F  H  S  Q  K  V  F  L  Q  D  L  H  S  H  V  Y  R  E  S  R
                                                                                  ⇐|
1441 GAAATGGAGAGTCACAAGAGAGCCACATTGGGCCCAGCACCTTCAAGTGTCCTGACTGTCCGTTCACTGCAGCCCTCTGGCCGGAGGTTCGGAGCCACATGGTGCAGCATGCCAGCCTTC
       N  G  E  S  Q  E  S  H  I  G  P  S  T  F  K  C  P  D  C  P  F  T  A  A  L  W  P  E  V  R  S  H  M  V  Q  H  A  S  L  R

1561 GGCCACACAAGTGCACCCACTGCAGCTTTGCCTCCAAGAACAAGAAGGACCTGCGCAGACACATGTTAACACACACCAATGAGAAGCCCTTTGCCTGCCAGATTTGTGGGCAGAGGTTCA
       P  H  K  C  T  H  C  S  F  A  S  K  N  K  K  D  L  R  R  H  M  L  T  H  T  N  E  K  P  F  A  C  Q  I  C  G  Q  R  F  N

1681 ACCGTAATGGACACCTCAAGTTCCACATGCAGCGTTTGCACAGCTCAAAGGGGAAGAAGCCAGAGCGGCCTGCAGCTCCTGCCCAGCAGACCATCATACTGAACAGTGATGAGGACACGC
       R  N  G  H  L  K  F  H  M  Q  R  L  H  S  S  K  G  K  K  P  E  R  P  A  A  P  A  Q  Q  T  I  I  L  N  S  D  E  D  T  L

1801 TGGCCACCCTGCAGACGGCTCTGCAGTCCGGGCAGGCAGTGCTGGCCCCCGAAGCGGCTGCAGCAGGCACTGGGACAGGAGCACATCATCGTTGCTCAGGAGCAGAGCATTGCGAGCCAG
       A  T  L  Q  T  A  L  Q  S  G  Q  A  V  L  A  P  E  A  A  A  A  G  T  G  T  G  A  H  H  R  C  S  G  A  E  H  C  E  P  A

1921 CACCTGGCTCTCACTGGTTACAGGAGGAGGCGACGTACATTCAGGAGATCACTACTGCCGACGGACAGACAGTACAGCACTTAGTGACATCTGACAACCAGGTTCAGTACATCATTGCCC
       P  G  S  H  W  L  Q  E  E  A  T  Y  I  Q  E  I  T  T  A  D  G  Q  T  V  Q  H  L  V  T  S  D  N  Q  V  Q  Y  I  I  A  Q

2041 AGGATGGCGTACAGCACTTGCTTCCCCACGAGTACGTTGTTGTCCCAGAAGGACATCACATCCAGGTTCAGGATGGTCAGATCACTCACATTCAGTATGAACAAGGTAGCCAATTTCTCC
       D  G  V  Q  H  L  L  P  H  E  Y  V  V  V  P  E  G  H  H  I  Q  V  Q  D  G  Q  I  T  H  I  Q  Y  E  Q  G  S  Q  F  L  Q

2161 AGGAGCCACAGATCCAGTACATGCCTGTCTCACCTGAGCAGCAGCTTGTCACCCAGGCTCAGCTGGAAGCGGCTGCGCACTCAGCAGTCTCAGCAGTGGCTGATGCTGCCATGGCTCAGG
       E  P  Q  I  Q  Y  M  P  V  S  P  E  Q  Q  L  V  T  Q  A  Q  L  E  A  A  A  H  S  A  V  S  A  V  A  D  A  A  M  A  Q  A

2281 CACAGAGCGTGTTCGCCACGGAGGCAACGGCTGAGCAGATCCAGCAGTTGCAGCAGGGAATCCACTACGATGTCATCACGCTGGCAGATTAGCACTGGTGACAGGCCTGGCTGATGCTCA
       Q  S  V  F  A  T  E  A  T  A  E  Q  I  Q  Q  L  Q  Q  G  I  H  Y  D  V  I  T  L  A  D  *

2401 GGGACATCCGTGCTGAGCAGGACAGCGGCAACCAgCGGGAGCCCTCTCCAGCCAGCAGTTGCCTTATTGCTTTGGGGCTACGAGGGACGTGCCTGAGTGCGCGCCAGCAGGGGCGTGCTG

2521 CCACTCTGCTGTGCACGGCGAGGGTCCTGCGAGTCCTGCCCGGCACAGGAGGCTCTCCTGAGCAGAGACTGCGCCACAGCCGAAGCGCCGGGACGGTCTGAGCAGCGGCAGCACTCACGG

2641 GGCCCATCTCTGCCTGTTGCACTCGATGATGGTTTGAAATCCCTTTTCCTGAATCACGAGGAACTCGGCGCGGGGTGCGGGGATCCCGGGGGAGCTGTGGGGCCGAGCGCTGCCACGGGG

2561 TGCAGTGTGAGATGCTGAGGTGTGTTCAGCCTCAGCCCCCCACTCCTCGCTCCCGTGGGGCACACGGGCCGGGCTGAGGGCAGCCTCCCTCCCTTGGCTCCTCCGGCCTGCGGGCGGGCG

2681 GCGATAGGGCTTTGGACTGGAGGAACCTTCCGGAGTCAGAGGCCGTGTAGGTGGTGGAAGATGTTCTATTTTTTCTTTGGAAGAGGGATGGATGCTGTGGCAAAGTTGGTTTGGAAGTCT

3001 TACGGGAGGGGTTCGATTCTGACGTTCTCAAGCTTT
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FIG. 1. Nucleotide and predicted amino acid sequence of the Fzf-1 cDNA. The two zinc finger-like domains observed in two different regions in the cDNA are underlined. The region shown between the open-ended arrows was used in situ hybridization and RT-PCR experiments. (GenBank Accession No. U27196)

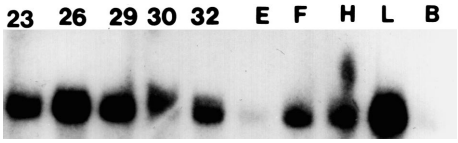

FIG. 2. Fzf-1 mRNA distribution during chick embryonic development. Northern blot analysis of total RNA (20 μg) extracted from chick embryos from stage 23 through stage 32 were hybridized to a Fzf-1-specific probe. Total RNA from Day 12 embryonic tissues were also hybridized to the same probe. E, eye; F, facial; H, whole head; L, limb; B, brain.

finger like domains. The cDNA sequence of the zinc finger cDNA is shown in Fig 1 and shows that it is 3.0 kb long. Translation of the cDNA reveals an open reading frame encoding a 789 amino acid protein.

The first 450 bp of the cDNA is found to encode four zinc finger domains of the class I type. It is highly likely that there are more zinc finger domains on the N-terminus as the amino acid sequence in the region of the first zinc finger motifs shows potential sequences. A second tandem of three Class I zinc finger domains is observed between amino acids 496 and 528. The amino acid sequences of the zinc finger motifs showed only less than 40% homology to previously reported zinc binding domains.

To gain further insight into the zinc finger gene functions, the expression of the gene was examined by northern blot analysis. Fig 2 illustrates that a 4.2 Kb mRNA is detected in stage 23

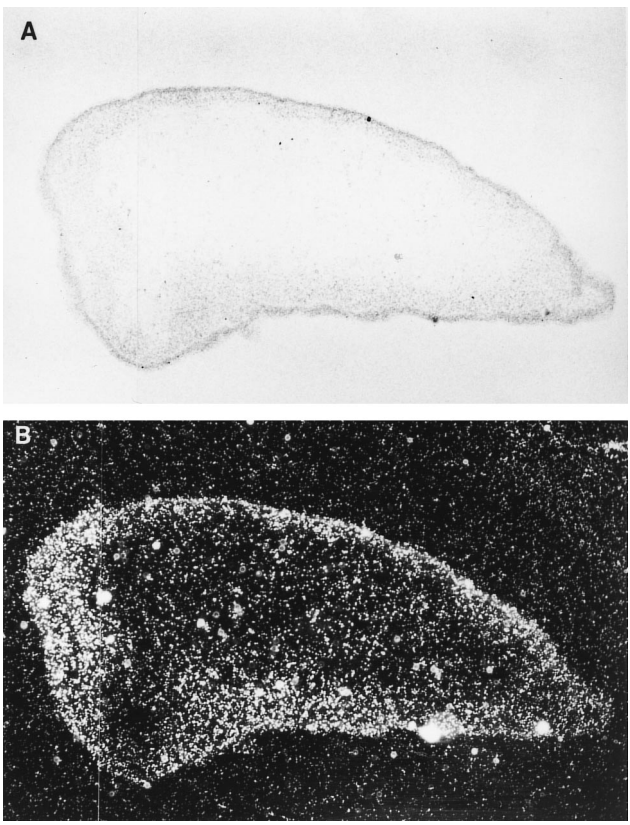

FIG. 3. Expression of Fzf-1 in skin dissected from the dorsal region of stage 38 chick embryo. (A) Bright field photograph of a histologic section of skin derived from the dorsal region. (B) The section shown in A is hybridized to an ^{35}S-labeled antisense probe to Fzf-1. (40×)

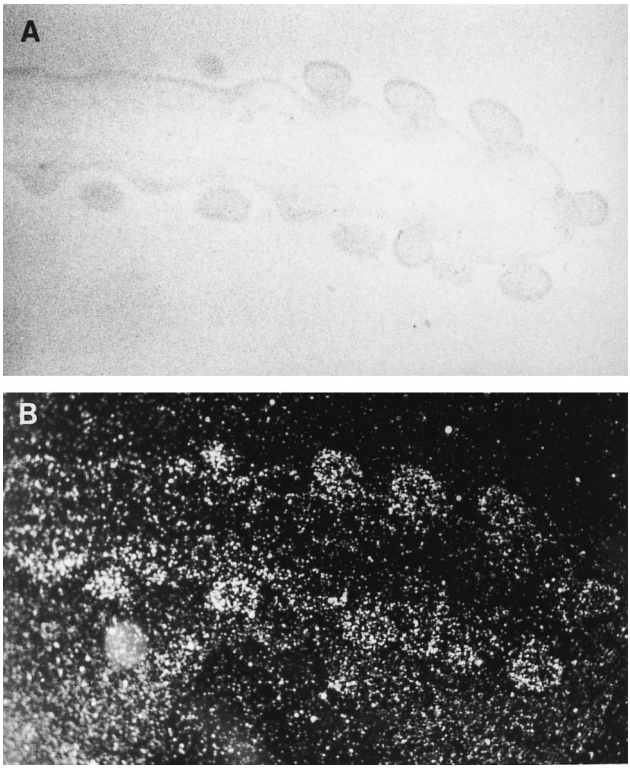

FIG. 4. Expression of Fzf-1 mRNA in 10-day-old chick embryonic skin derived from the dorsal region. (A) Bright field photograph of the Day 10 skin showing the forming dermis. (B) Localization of the Fzf-1 mRNA in the forming dermis shown by in situ hybridization of Fzf-1 specific probe. (40×)

through 32. A panel of 12 day old chick tissue RNA was examined. The transcript seems to be expressed primarily in the limb and head tissues. Upon further investigation using RT-PCR analysis and in situ hybridization, it was found that the message expression is limited to the skin and feathers and the expression found in head and limb tissues by Northern hybridization were due to the presence of skin tissues in these preparations. Reverse transcription of mRNA originating from day 12 chick skin, heart, and skinless cranial tissues and limb followed by PCR using zinc finger specific oligos showed that the expression of Fzf-1 is specific to skin tissues. (Data not shown).

An anti sense probe containing a non conserved region of the zinc finger cDNA (See Materials and Methods and Fig 1.) was hybridized to histologic sections of skin dissected from the dorsal region of stage 38 chick embryos. Expression of Fzf-1 is localized to the epidermis and the signal is found to be homogeneous and uniform (Fig 3).

In order to examine the expression of Fzf-1 gene in developing feather buds, hybridization was performed on serial sections of skin derived from the dorsal posterior region of 10 day old embryos. (Fig. 4). At this stage, the transcripts are localized to the forming dermis. It is interesting to note that no graded pattern of expression of the gene in the dorso-ventral or anterior-posterior axis is observed.

The expression pattern of the zinc finger gene in elongated feather buds was studied using 12 day old skin dissected from the wings. Upon hybridization of the probe, the transcripts are found in the mesenchymal region of the elongated feather bud. (Fig 5). No graded pattern of expression is observed at this stage.

DISCUSSION

We have isolated a cDNA for a novel zinc finger gene (Fzf-1) containing two tandem repeats of

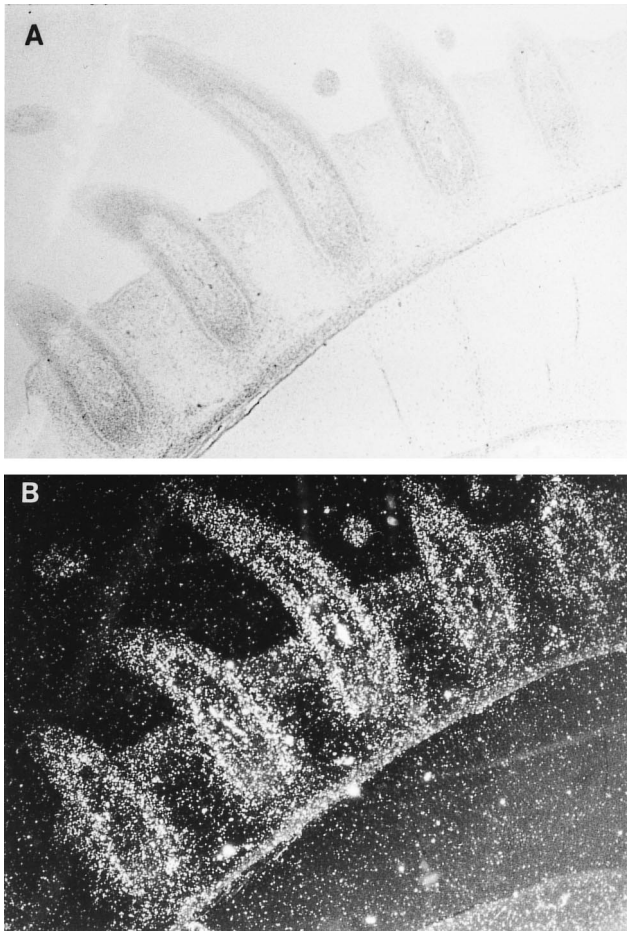

FIG. 5. In situ hybridization of the Fzf-1 probe to a section of skin derived from 12-day-old embryonic chick wings is shown in section B. Bright field photograph of the same section is shown in section A. (40×)

Class I (C2H2 type) zinc finger domains. The temporal and spatial expression pattern of the gene was studied. The Fzf-1 gene is found to be expressed in stage 38 chick skin and as development progresses and feather buds are formed, the transcripts are localized to the developing buds. The expression of the Fzf-1 gene is found to be in the mesenchymal cells of the elongated feather bud and no anterior-posterior or dorsal-ventral gradient is observed.

ACKNOWLEDGMENTS

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