Singhofer-Wowra, M., Clayton, L., Dawson, P., Gull, K., & Little, M. (1986) *Eur. J. Biochem. 161*, 669–679.
Studier, F. W., Rosenberg, A. H., Dunn, J. J., & Dubendorff, J. W. (1990) *Methods Enzymol.* (in press).
Takagi, T., Konishi, K., & Mabuchi, I. (1988) *J. Biol. Chem. 263*, 3097–3102.
Towbin, H., Staehelin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.
Yonezawa, N., Nishida, E., & Sakai, H. (1985) *J. Biol. Chem. 260*, 14410–14412.
Yonezawa, N., Nishida, E., Ohba, M., Seki, M., Kumagai, H., & Sakai, H. (1989) *Eur. J. Biochem. 183*, 235–238.
Young, R. A., & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1194–1198.

Sequence of cDNAs Encoding Actin Depolymerizing Factor and Cofilin of Embryonic Chicken Skeletal Muscle: Two Functionally Distinct Actin-Regulatory Proteins Exhibit High Structural Homology[†,‡]

Hiroshi Abe,[§] Takeshi Endo,[§] Keiichi Yamamoto,[∥] and Takashi Obinata*[,§]

Department of Biology, Chiba University, Yayoi-cho, Chiba 260, Japan, and Faculty of Liberal Arts, The University of the Air, 2-11 Wakaba, Chiba 260, Japan

Received February 22, 1990; Revised Manuscript Received April 26, 1990

ABSTRACT: Two actin-regulatory proteins of 19 and 20 kDa are involved in the regulation of actin assembly in developing chicken skeletal muscle. They are homologous with actin depolymerizing factor (ADF) and cofilin, a pH-dependent actin-modulating protein, which were originally discovered in chicken and mammalian brain, respectively. In this study, full-length cDNA clones were isolated by screening a λgt11 cDNA library constructed from poly(A+) RNA of embryonic chicken skeletal muscle with the antibodies specific for each protein, and their complete sequences were determined. The chicken cofilin cDNA encoded a protein of 166 amino acids, the sequence of which had over 80% identity with that of porcine brain cofilin. The amino acid sequence of the ADF was 165 amino acids and showed about 70% identity with either chicken or mammalian cofilin, in spite of the fact that ADF and cofilin are functionally distinct. Like chicken and mammalian cofilin, ADF contained a sequence similar to the nuclear transport signal sequence of SV40 large T antigen. ADF and cofilin shared a hexapeptide identical with the amino-terminal sequence of tropomyosin as well as the regions homologous to other actin-regulatory proteins, including depactin, gelsolin, and profilin. The overall nucleotide sequences and Southern blot analysis of genomic DNA, however, indicated that the two proteins were derived from different genes.

Actin is a major constituent of thin filaments of cross-striated myofibrils in skeletal muscle. In the developing skeletal muscle, it is synthesized, polymerized, and organized into sarcomeric structures. Little is known as to how the polymerization of actin and the organization of actin filaments into the sarcomeric structures are regulated in developing muscle cells. Purified actin is known to be polymerized spontaneously and form actin filaments in vitro under physiological salt and pH conditions. Most actin in adult muscle is filamentous, and monomeric actin is present only at the level of critical concentration. However, in young embryonic muscle, actin is largely present in unpolymerized forms in the cytoplasm (Shimizu & Obinata, 1986). Since purified embryonic muscle actin is polymerizable to the same degree as adult muscle actin, regardless of the difference in major actin isoforms between embryonic and adult muscles (Hayward & Schwartz, 1986; Shimizu & Obinata, 1980), it was postulated that actin polymerization in developing muscle is controlled or suppressed by some regulatory proteins (Shimizu & Obinata, 1986). Recently, three actin regulatory proteins of 16, 19, and 20 kDa, respectively, have been isolated from chicken embryonic skeletal muscle. The first one, the 16K protein, was identified with profilin (Carlsson et al., 1977) from its functional characteristics and size (Ohshima et al., 1989). The second one, the 19K protein, was regarded as being the same as actin depolymerizing factor (ADF) of chicken brain (Bamburg et al., 1980) or its variant, because, like ADF, the 19K protein forms a 1:1 complex with G-actin to inhibit its polymerization, and quickly depolymerizes F-actin, and the antigenicity and peptide maps of this protein were similar to those of ADF (Abe & Obinata, 1989). The third one, the 20K protein, binds to both G- and F-actin, inhibits actin polymerization in a pH-dependent manner, and inhibits binding of tropomyosin to F-actin (Abe et al., 1989a,b). Since such characteristics have been detected in porcine brain cofilin (Nishida et al., 1984; Yonezawa et al., 1985), the 20K protein appears to be homologous to porcine cofilin. However, it has been reported that cofilin is absent from muscle cells (Yonezawa et al., 1987). To better understand the structures and functions of these actin binding proteins in embryonic muscle, we have isolated cDNA clones for their respective proteins from a cDNA library constructed from poly(A+) RNA of

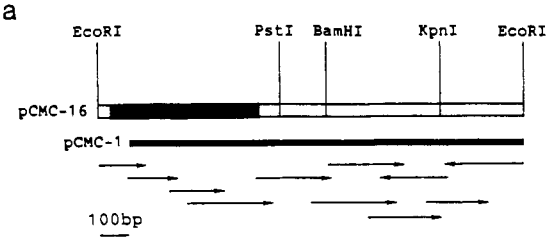
[†] This work was supported by research grants from the Ministry of Education, Science and Culture, the National Center of Neurology and Psychiatry (NCPN) of the Ministry of Health and Welfare of Japan, the Muscular Dystrophy Association of America (MDA), and the Naito Foundation.

[‡] The nucleic acid sequence in this paper has been submitted to GenBank under Accession Number J02915.

* To whom correspondence should be addressed.
[§] Chiba University.
[∥] The University of the Air.

0006-2960/90/0429-7420$02.50/0

a



b

```
CCCGCACCGCAGGCAGCCGCCGCCGCCCCGGACCCGGCGCCATGGCTTCTGGAGTAACAGTGAATGATGAAGTCATAAAGGTTTTTAATG   90
                                              M  A  S  G  V  T  V  N  D  E  V  I  K  V  F  N  D

ACATGAAAGTAAGAAAATCTTCAACCCCAGAAGAGATTAAAAAAAAGAAAGAAAGCCGTTCTCTTCTGCTTAAGCGATGACAAAAAGCAAA  180
  M  K  V  R  K  S  S  T  P  E  E  I  K  K  R  K  K  A  V  L  F  C  L  S  D  D  K  K  Q  I

TAATCGTAGAGGAGGCGACAAGGATACTGGTCGGTGACATCGGAGATACCGTGGAGGACCCCTATACGGCCTTTGTGAAGTTGCTACCGT  270
  I  V  E  E  A  T  R  I  L  V  G  D  I  G  D  T  V  E  D  P  Y  T  A  F  V  K  L  L  P  L

TGAACGATTGCCGATACGCTTTGTACGATGCCACGTATGAGACAAAGGAATCTAAGAAGGAAGACCTGGTATTTATATTCTGGGCTCCTG  360
  N  D  C  R  Y  A  L  Y  D  A  T  Y  E  T  K  E  S  K  K  E  D  L  V  F  I  F  W  A  P  E

AAAGCGCACCTTTAAAAAGCAAGATGATCTACGCAAGCTCTAAAGATGCCATTAAAAAGAAATTTACAGGTATTAAACATGAGTGGCAAG  450
  S  A  P  L  K  S  K  M  I  Y  A  S  S  K  D  A  I  K  K  K  F  T  G  I  K  H  E  W  Q  V

TAAATGGTTTGGATGATATTAAGGACCGTTCAACACTCGGAGAGAAACTGGGAGGCAACGTGGTAGTTTCACTTGAAGGAAAACCCTTAT  540
  N  G  L  D  D  I  K  D  R  S  T  L  G  E  K  L  G  G  N  V  V  V  S  L  E  G  K  P  L  *

AAAAAGACAGACAAGTGCCATCTGGATCTAAGGAGCTTCCATTTCTGCAGCTCGTTCAATTGGAATAGTATTAGTCTCCCTTTCTCCTTC  630

CCTCCTCAAAAATAAGCCCCTTCCCTTCTGCCCCTGAAGGAGATGTCATTGTTAAGCAGTCTACCAGTGATTGCCATTAGACTGTTTAAT  720

CCTGGTAGTTTTATGTAGGATCCAAGGAATGCTTTCACGTCATACTCTTAGCCAAAACTGACGCTGCATGCATTCCTTGCCACACGTACA  810

ATGAATGTGATAGTTAATGTGAATAGTCTAGCAGACAGCAAAGGGTAAGCTAATTGAATGCCTTGAAAGTATTGTCACTGGTGGGATGGT  900

AGACTCTATACAGTATTACTTACAGTTGCACTTGATTGCAGTTCCATGAGGCTCTTGTGCATTCATACACCTCACCTGCCTTGACAAGCC  990

TATTTTTGTGACATGGCAGCACACAACACTATGCATTTAAAGCACTTTTTTGTAATATGTTTGACCTGCCCCTCCCCCCCAAAGGAATGC 1080

CAATTAAGTGTGTAACTGTGTCACAATTATTGTGGTACCTCAGTTCATTCCTGTTACATGCATATCTTATAAATGAAGTAGCTGTTACGA 1170

TGCCTTTTGTTTTCCATTGAATGTACACTACTGAACAGGAGTAGAAGTTATCTGTTTACCATGTGAGTCTTGAAACACTAAAGTTTTGTA 1260

AAACATCGGTCATGATGGCAATTTCTGTATTAAAAAGAGCCTTAAATGGAACATTGTTTTTGAGATCAAAAACTGGCCACGTTGCAATAA 1350

AACTTGTGGCTTATTACAGAAAAAAAAAAAA 1381
```

FIGURE 1: Sequence of a chick cofilin cDNA and the deduced amino acid sequence. (a) The diagram at the top is a restriction map for the entire cDNA sequence. The closed and open boxes indicate the coding and noncoding regions, respectively. DNA sequencings proceeded in the direction of the horizontal arrows, and the distance determined by each sequencing procedure is indicated by the length of the arrows. (b) Nucleotide and amino acid sequence of the cDNA clone pCMC-16. The initiation codon ATG is boxed. The putative poly(A) additional signal AATAAA is underlined.

embryonic chicken skeletal muscle, and the whole sequences were determined.

Materials and Methods

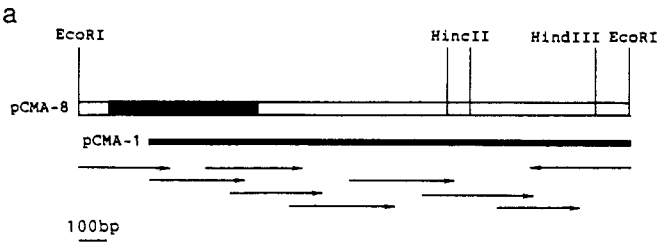
Construction and Screening of the cDNA Library. Cytoplasmic RNA was prepared from the skeletal muscle of 10-day-old chick embryos by the vanadyl ribonucleoside complex method (Favaloro et al., 1980), and poly(A+) RNA was isolated by oligo(dT)–cellulose column chromatography. A λgt11 expression library of cDNA was constructed using an oligo(dT) primer. Clone pCMC-1 was obtained with a monoclonal antibody for the 20K protein, MAB22 (Abe et al., 1989a,b), and clone pCMA-1 was isolated with the IgG fraction of rabbit polyclonal antibody for the 19K protein (ADF) (Abe & Obinata, 1989). The full-length cDNAs for the 20K protein (pCMC-16) and for ADF (pCMA-8) were cloned by hybridization with the pCMC-1 or pCMA-1 probe.

RNA and DNA Blot Analysis. RNA gel electrophoresis and hybridization were performed as described (Thomas, 1980). Chromosomal DNA from 10-day-old chicken liver was prepared as described by Blin and Stafford (1976) and 10 μg of DNA was digested by restriction endonucleases *Eco*RI, *Bam*HI, *Pst*I, and *Hin*dIII, electrophoresed on a 0.7% agarose gel, and transferred to a nitrocellulose filter. The filter was hybridized to 10^7 cpm of ^{32}P-labeled pCMC-16 or pCMA-8 insert and washed in several changes of 0.1 × SSC/0.2% SDS at 60 °C.

Nucleic Acid Sequence Analysis. *Eco*RI inserts of positive phage clones were subcloned into pBluescript II-KS$^+$, and the unidirectional deletions of the inserts were made by using exonuclease III and mung bean nuclease. The nucleotide sequence was determined by the dideoxy method (Sanger et al., 1977). Analysis of DNA sequence data was carried out with the Genetyx program (SDC Software).

Results

Cloning and Sequencing of Chicken Cofilin and ADF cDNAs. By screening the λgt11 cDNA library of embryonic muscle with a monoclonal antibody for the 20K protein (MAB-22) and an affinity-purified polyclonal antibody against ADF, partial-length cDNA clones, pCMC-1 for the 20K protein and pCMA-1 for ADF, were obtained. The full-length cDNA clone (pCMC-16) for the 20K protein and an ADF cDNA (pCMA-8) clone were then isolated by rescreening the library with these cDNA clones as probes. The complete nucleotide sequences of the full-length cDNAs were determined, and the amino acid sequences were deduced (Figures 1 and 2). The first ATG codon of the cDNA clone of each protein (boxed in Figures 1 and 2) lies within a sequence similar to the consensus sequence for translational initiation



b

```
GCGGCGCTCGGCTCGCGCCCTCGCCGCTTCCCTTCCTGCTGCCGGAGCCCGCGCCGGCCCAGCACCCGAGGGAAGCAGCAGCCGCCGCTC   90

CGCCGACACCATGGCATCTGGAGTACAAGTTGCCGATGAGGTATGCCGTATCTTCTACGACATGAAAGTGCGGAAATGCTCTACGCCTGA  180
          M  A  S  G  V  Q  V  A  D  E  V  C  R  I  F  Y  D  M  K  V  R  K  C  S  T  P  E

GGAAGTGAAGAAGAGGAAGAAGGCTGTCATCTTCTGCCTCAGTCCAGACAAAAAGTGCATTATCGTGGAAGAAGGCAAAGAGATTCTAGT  270
 E  V  K  K  R  K  K  A  V  I  F  C  L  S  P  D  K  K  C  I  I  V  E  E  G  K  E  I  L  V

GGGAGATGTTGGTGTGACGGTTACTGACCCTTTCAAGCACTTTGTCGAGATGCTTCCCGAGAAGGATTGCCGTTATGCCTTGTATGATGC  360
 G  D  V  G  V  T  V  T  D  P  F  K  H  F  V  E  M  L  P  E  K  D  C  R  Y  A  L  Y  D  A

AAGTTTTGAGACCAAGGAATCCAAAAAAGAAGAGCTGATGTTCTTCTTGTGGGCACCAGAACAAGCACCTCTCAAAAGTAAGATGATCTA  450
 S  F  E  T  K  E  S  K  K  E  E  L  M  F  F  L  W  A  P  E  Q  A  P  L  K  S  K  M  I  Y

TGCAAGCTCCAAGGATGCAATCAAAAAGAAGTTCCAAGGCATAAAGCATGAATGCCAAGCAAATGGGCCAGAGGACCTCAATCGAGCTTG  540
 A  S  S  K  D  A  I  K  K  K  F  Q  G  I  K  H  E  C  Q  A  N  G  P  E  D  L  N  R  A  C

TATTGCTGAGAAGCTAGGAGGCTCCCTAGTCGTAGCTTTTGAAGGAAGTCCTGTGTAGATGTCATACAGTGCCACAACTCTACAAGCATT  630
 I  A  E  K  L  G  G  S  L  V  V  A  F  E  G  S  P  V  *

CCATGTTTTAATATATCAGTTATGTAAAGCAACCATCCTAGGCAATGGGTTCACTAACTGTAGGGAAGTTGTCTTATAGAATAAAGTAGA  720

TTTTGGAGATGTAGGTCCTATGTATAAATGACCTTAAATAAATCAAATTAAGGATTTATCTTGGTGTATTAATATTTGCATTGGTTGTCT  810

ATCCCAGTACTATCCCCGATGAATTAACACTAAAGGTCTCCAAAAATTCTGATTCAGCAGTGTATCGTGGGATGAAGGGGGGGGTTGATG  900

AGAACAATGTGGTTCCTGTTCTCTTCTAAATTAAAATGAGCAAAATCATCAGTCTTTCTGAGGCTACTGGCCAAATTGCCAAACTCAAAA  990

CTTTCCCCTCGAACCGTTGAATGCAAGACTTAAAACCTGTAGTTCAAGTTAGGCACCTAAACAGAGCTAAATTCTTAGTGCTTGCAGTTG 1080

CCCGGCGTTACTAATAAGGGTTAATCAGGAGGTTAAAATAGAGATTAAAGTAGGTACGCTTTCCTGGAGAAAGTCCTAATCATCCTTGTA 1170

AACTCAGCGTGCAGTAATCTTTAAAGGTTTGCTTGTTCCTACAGGTTAACATTGGTTATGAAGTGTCTCGAGGTCTCCTCCAAGGCTGAA 1260

CGATTCTATTAAAATTGCTTTTCTTTTGGCATAGGTTAACTTAGGACCACCTTATTTCCACTAACACACTTAGTAGCGGATCAGACGCTT 1350

CTGAGGCTTTGACAAGTGTAGATCCTTCCGTTGGAGCTCTCCAGTGCTGGGCAGTACCATGAGAAAGAACCACTTGGAAAAACAGCAAAA 1440

GATCACAAGTGGTGTTCTCAGCCCCACTCTTTGGTCACTTGGTCCATTTGTCCGTAATTATTATTGATTTAACTTAGTAGAACCAATCAG 1530

CCTGACTAATATGGCTTGATCTGGGCTTTTTGTCTGTTAGCTTCTTTAATTCTGTAGAAATGCATGGAAATATCACCTACTAGAAAGTTT 1620

GACCTGAAATGCCTTTTGTATGGGGGGGGGGGGGAGGGGGGTAGAGAACATTCCTGTGCCAGCTACAGAGATCTTAAAAGCTTCCATGTT 1710

TACCAGTGGTTGATTAAAGCTGTTTCCTTTGTGTAATGTGTACATGGAAGTTGCATAGAGTTTTCCTGTATGTCTGTAGAGAAAAAAAAA 1800

AAAAGAAAAAAAACAAAAAAAAAAAAAAAAA 1831
```

FIGURE 2: Sequence of a chicken muscle ADF cDNA and the deduced amino acid sequence. (a) Restriction cleavage maps and sequencing strategy of pCMA-1 and pCMA-8. (b) Nucleotide and deduced amino acid sequence of the cDNA clone pCMA-8. The underlined amino acid sequence corresponds to that determined by amino acid sequencing of the proteolytic fragment of ADF. (For details, see Figure 1.)

(5′-CCA/GCCATGG-3′) (Kozak, 1984).

The open reading frame of pCMC-16 begining with ATG at position 42 and terminating at position 539 specified a protein of 166 amino acid residues. The 5′- and 3′-untranslated sequences excluding the poly(A) stretch consist of 41 and 846 nucleotides, respectively. A putative polyadenylation signal, AATAAA, is seen 19 nucleotides upstream from the poly(A) tail. The number of amino acid residues of this protein was exactly the same as that of porcine brain cofilin, and close (over 80%) homology was seen between this sequence and that of the porcine cofilin (Matsuzaki et al., 1988) (Figure 5). Several reported characteristics of the porcine cofilin sequence (Matsuzaki et al., 1986) were also detected in this sequence, as discussed later. Therefore, we designate the 20K protein encoded by pCMC-16 as chicken cofilin. The calculated molecular weight of the chicken cofilin is 18 662.

The translation initiation site for ADF was assigned to the first ATG codon at position 101. The open reading frame ends at position 595 and is followed by 1236 nucleotides of the 3′-untranslated region which is characterized by a putative signal for adenylation ATTAAA, located at 86 nucleotides upstream from the poly(A) tail. The sequence determined by amino acid sequencing of a peptide fragment of ADF was included in the deduced sequence (underlined in Figure 2). The deduced amino acid sequence of ADF is 165 residues long, and the calculated molecular weight is 18 532, which is close to the apparent molecular weight (19K) estimated by SDS–PAGE (Abe & Obinata, 1989).

Northern Blot Analysis of Cofilin and ADF mRNAs. RNA blot analysis of cytoplasmic RNA isolated from embryonic chicken skeletal muscle with ^{32}P-labeled cofilin cDNA and ADF cDNA is shown in Figure 3. Under conditions of high stringency, the cofilin cDNA probe hybridized strongly to a single discrete mRNA species of about 1.5 kb, while three rather faint additional bands were detected, the sizes of which were around 4–5 kb as judged by the position of ribosomal RNAs. On the other hand, the cDNA probe of ADF hybridized clearly to two mRNA species of about 2.1 and 0.9

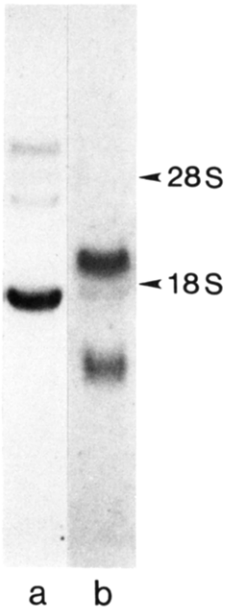


FIGURE 3: Northern blot hybridization analysis of mRNA from embryonic chicken skeletal muscle. Fifteen micrograms of total RNA from skeletal muscle of 12-day-old chick embryo was separated by electrophoresis in a denaturing formaldehyde/agarose gel, transferred to a nitrocellulose filter, and hybridized to ^{32}P-labeled pCMC-16 cDNA (lane a) or pCMA-8 cDNA (lane b) by nick translation. The blot was then washed in 0.1 × SSC/0.2% SDS at 60 °C. The positions of 28S and 18S ribosomal RNAs are indicated.

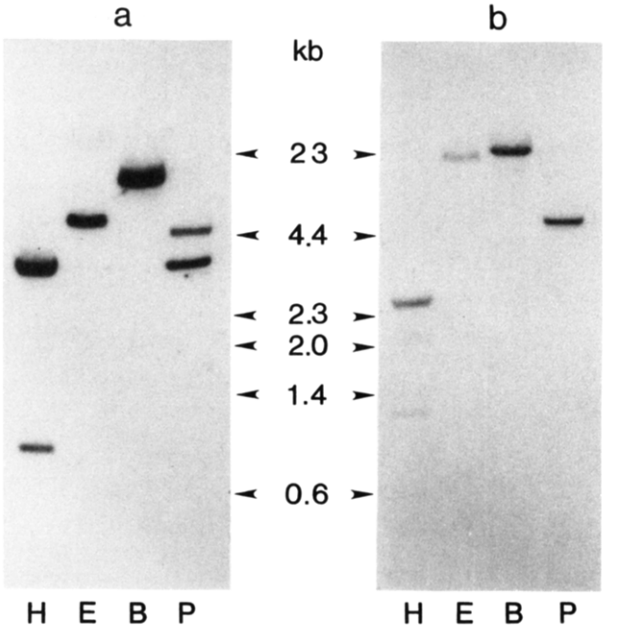


FIGURE 4: Southern blot analysis of ADF and cofilin in chicken genomic DNA. Embryonic chicken liver DNA was cleaved with *Hin*dIII (lane H), *Eco*RI (lane E), *Bam*HI (lane B), or *Pst*I (lane P). Each reaction product was size-fractionated on a 0.7% agarose gel. After transfer to a nitrocellulose filter by the procedure of Southern (1975), the filter was hybridized to ^{32}P-labeled pCMC-16 (a) or pCMA-8 cDNA (b) by nick translation. Hybridization was carried out in 6 × SSC, 1 × Denhardt's solution, 25 mM sodium phosphate (pH 7.2), 0.1% SDS, and 50% formamide at 50 °C. Washing was performed in 0.1 × SSC and 0.2% SDS at 65 °C. Mobilities of size markers are shown in kilobase pairs (kb).

kb, respectively. Therefore, it is likely that two different ADF transcripts are generated, and the former may correspond to pCMA-8.

Southern Blot Analysis of Chicken Genomic DNA. To determine the number of gene(s) corresponding to chicken cofilin and ADF in the chicken genomes, Southern blot analysis of chicken liver genomic DNA after digestion with several endonucleases was carried out. The probes used for hybridization were the full-length cDNA of each protein. As shown in Figure 4, the probe for ADF hybridized mainly to a single fragment digested by different restriction enzymes, although the probe hybridized weakly to an additional fragment only in the *Pst*I digest. The probe for chicken cofilin also exhibited strong hybridization to a single DNA fragment in each genomic DNA digest and weak hybridization to an additional fragment in the *Hin*dIII and *Pst*I digests. It therefore appears that there exists a single copy of the gene for each protein in the haploid chicken genome but there is also the possibility that genes related to the cDNAs of the two proteins exist. Further analysis will be needed to clarify whether such genes are also functional and encode the proteins related to cofilin and ADF. Similar results have been reported in mammals with cofilin cDNA probes (Matsuzaki et al., 1988).

Sequence Homology between Cofilin and ADF. When the ADF sequence was aligned along the sequences of chicken and mammalian cofilin sequences to determine the maximum homology, extraordinarily high sequence homology was observed between cofilin and ADF, although the two proteins are distinct in their effects on actin assembly (Nishida et al., 1984; Abe & Obinata, 1989; Abe et al., 1989a,b) (Figure 5). The homology between chicken cofilin and porcine cofilin was over 80%, while the ADF amino acid sequence showed about 70% identity with that of chicken cofilin. With the inclusion of the conservative exchange of amino acid residues, the homology between the two proteins reached 90%. A single residue at position 143 was skipped in the case of ADF (Figure 5). The two proteins differed in the number of cysteine residues; there were two cysteine residues in cofilin and seven in ADF. The sequence difference was distributed from the N-terminus to the C-terminus, and the deletion or insertion of specific sequences was not seen. Therefore, it is concluded that the two proteins are generated via transcription from different genes rather than from the alternative splicing of a transcript derived from a single gene.

It has been reported that porcine cofilin has a sequence of Pro-X-X-Lys-Lys-Arg-Lys-Ala-Val, which is homologous to the nuclear transport signal of SV40 large T antigen (Kalderon et al., 1984; Matsuzaki et al., 1988). We found the same sequence not only in chicken cofilin (residues 26–36) but also in ADF (residues 26–36) (Figure 5). It was observed in addition that chicken cofilin and ADF, like porcine cofilin (Matsuzaki et al., 1988), share a hexapeptide of Asp-Ala-Ile-Lys-Lys-Lys (residues 122–127 in both cofilin and ADF), which is completely identical with the amino-terminal sequence of tropomyosin (Stone & Smillie, 1985). Depactin in sea urchin is known to be functionally very similar to ADF (Mabuchi, 1983). Homology between depactin (Takagi et al., 1988), ADF, and cofilin was localized in residues 98–115 (depactin) and residues 103–120 (ADF and cofilin) (Figure 6), although the overall amino acid sequence of depactin shows only 30% homology with the other proteins. The C-terminal sequences, residues 148–162 of cofilin, and residues 147–161 of ADF were also homologous with other actin binding proteins, including gelsolin (Kwiatkowski et al., 1986) and profilin (Ampe et al., 1985), as pointed out by Matsuzaki et al. (1988).

Discussion

Previous investigations demonstrated that ADF (Bamburg & Bray, 1987; Abe & Obinata, 1989) and the 20K protein (cofilin-like protein) (Abe et al., 1989a,b) are involved in the

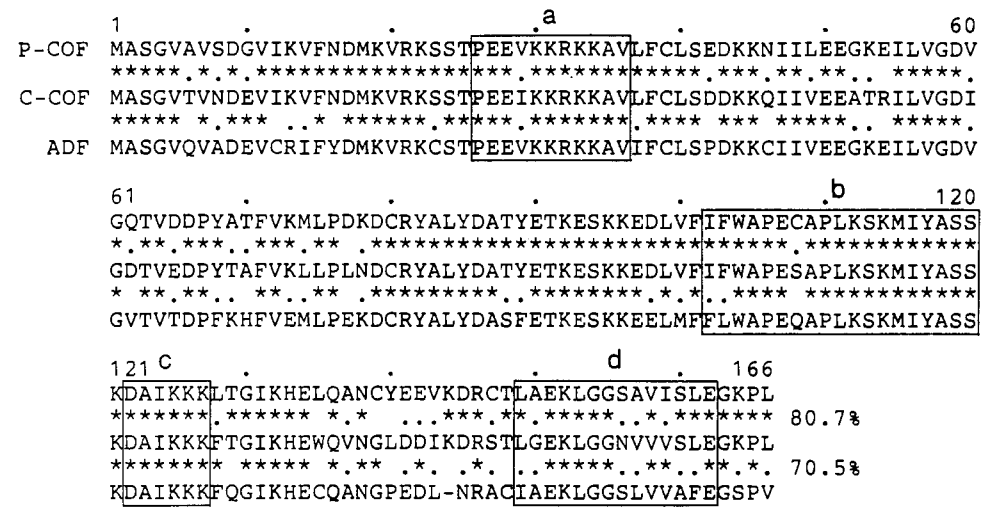


FIGURE 5: Comparison of the amino acid sequences of chicken ADF, chicken cofilin (C-COF), and porcine cofilin (P-COF). Sequence alignment was formulated in such a way as to maximize homology. 70.5% identity in amino acid residues was observed between ADF and chicken cofilin sequences, and 80.7% identity between chicken and porcine cofilin. Asterisks indicate identical amino acid residues, and dots represent conservative substitutions. A skip residue at position 144 is marked by a dash. The location of the putative nuclear localization signal transport is marked by box a. The sequences homologous to depactin and tropomyosin are indicated by boxes b and c, respectively. The sequences in box d exhibit some homology with other actin binding proteins, including gelsolin, profilin, and fragmin.

```
    C-COF FWAPESAPLKSKMIYASS  Residues 103-120
          **. *.* .* ** *.*.
depactin FWSMETANIKLKMKYSST  Residues  98-115
          .*. * * .* ** *.*.
      ADF LWAPEQAPLKSKMIYASS  Residues 103-120
```

FIGURE 6: Sequence similarity between chicken ADF, chicken cofilin, and sea urchin depactin. Overall sequence homology is low between depactin and ADF/cofilin, but a partial sequence with high homology was also detected, as shown. Asterisks, identical residues; dots, conservative substitutions.

regulation of actin assembly during myofibrillogenesis. There was ambiguity as to whether the latter was identical with cofilin, because, according to previous reports, cofilin was not detected in skeletal muscle of mammals (Yonezawa et al., 1987). As judged by the sequence determined in this study and the functional characteristics, we could identify the 20K protein from embryonic chicken skeletal muscle as chicken cofilin. ADF and cofilin are functionally distinct, and their expression is differently regulated during muscle development: the former appears transiently in embryonic stages in muscle, while the latter is expressed through developmental stages. Nevertheless, the two proteins exhibited high sequence homology. It is surprising that such high homology exists between functionally different proteins.

It is a matter of interest as to which regions of the sequence are involved in their functional difference. We can point out two regions where the homology is relatively low; they are residues 52–78 and residues 143–150. Computer analysis suggests that the secondary structure in residues 52–78 would be β-structure–random–α-helix in the case of ADF, while random–β-structure–random–β-structure in cofilin. The difference in the number of Cys residues may also be involved in the conformational difference between the two proteins. Yet another difference is seen in residues 143–150 of cofilin and residues 143–149 of ADF. In this region, one residue of cofilin which corresponds to residue 144, Lys, is missing in ADF. Serial arrangement of three charged amino acids exists in cofilin (Lys-Asp-Arg, residues 144–146), while ADF possesses only one charged residue in this region (Arg, residue 145). This region would be responsible for the difference in the pH responsiveness between cofilin and ADF. Analysis using site-directed mutant proteins would clarify the exact regions responsible for the different functions.

A putative nuclear transport signal was detected not only in chicken cofilin but also in ADF. Nishida et al. (1987) reported that porcine cofilin was located in nuclei to form actin–cofilin rods in dimethyl sulfoxide (DMSO-) or heat-treated mammalian nonmuscle cells. Chicken cofilin was also transported in nuclei to form rod structures together with actin, when chicken muscle cultures were incubated in the medium containing DMSO (Abe et al., 1990). However, ADF was not translocated at all under the same conditions, the presence of the putative nuclear transport sequence notwithstanding, suggesting that some additional signal(s) would be necessary for nuclear transportation of these proteins.

ACKNOWLEDGMENTS

When these investigations were completed, we became aware of complementary studies (Adams et al., 1990) on sequencing of ADF cDNA. Both laboratories have exchanged unpublished sequence information and agreed upon coordinate publication of the two manuscripts. We express our sincere appreciation to Drs. M. E. Adams and J. R. Bamburg, who shared with us their unpublished cDNA sequence data on chicken ADF.

Registry No. DNA (chicken muscle cofilin), 127997-37-9; cofilin (chicken muscle reduced), 127997-35-7; DNA (chicken muscle actin-depolymerizing factor), 127997-36-8; ADF (chicken muscle reduced), 127997-38-0; RNA (chicken clone pCMC-16 cofilin-specifying messenger), 127997-39-1.

REFERENCES

Abe, H., & Obinata, T. (1989) *J. Biochem. (Tokyo) 106*, 172–180.

Abe, H., Ohshima, S., & Obinata, T. (1989a) *J. Biochem. (Tokyo) 106*, 696–702.

Abe, H., Endo, T., & Obinata, T. (1989b) *J. Cell. Biochem. Suppl. 14A*, 11.

Adams, M. E., Minamide, L. S., Duester, G., & Bamburg, J. R. (1990) *Biochemistry* (preceding paper in this issue).

Ampe, C., Vandekerckhove, J., Brenner, S. L., Tobacman, L., & Korn, E. D. (1985) *J. Biol. Chem. 260*, 834–840.

Bamburg, J. R., & Bray, D. (1987) *J. Cell Biol. 105*, 2817–2825.

Bamburg, J. R., Harris, H. E., & Weeds, A. G. (1980) *FEBS Lett. 121*, 178–181.

Blin, N., & Stafford, D. W. (1976) *Nucleic Acids Res. 3*, 2303–2308.
Carlsson, L., Nystrom, L. E., Sundkvist, I., Markey, F., & Lindberg, U. (1977) *J. Mol. Biol. 115*, 465–483.
Favaloro, J., Freisman, R., & Kamen, R. (1980) *Methods Enzymol. 65*, 718–749.
Hayward, L. J., & Schwartz, R. J. (1986) *J. Cell Biol. 102*, 1485–1493.
Kalderon, D., Roberts, B. L., Richardson, W. D., & Smith, A. E. (1984) *Cell 39*, 499–509.
Kozak, M. (1984) *Nucleic Acids Res. 12*, 857–872.
Kwiatkowski, D. J., Stossel, T. P., Orkin, S. H., Mole, J. E., Colton, H. R., & Yin, H. L. (1986) *Nature (London) 323*, 455–458.
Matsuzaki, F., Matsumoto, S., Yahara, I., Yonezawa, N., Nishida, E., & Sakai, H. (1988) *J. Biol. Chem. 263*, 11564–11568.
Nishida, E., Maekawa, S., & Sakai, H. (1984) *Biochemistry 23*, 5307–5313.
Nishida, E., Muneyuki, E., Maekawa, S., Ohta, Y., & Sakai, H. (1985) *Biochemistry 24*, 6624–6630.
Nishida, E., Iida, K., Yonezawa, N., Koyasu, S., Yahara, I., & Sakai, H. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5262–5266.
Ohshima, S., Abe, H., & Obinata, T. (1989) *J. Biochem. (Tokyo) 105*, 855–857.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.
Shimizu, N., & Obinata, T. (1980) *Dev., Growth Differ. 22*, 789–796.
Shimizu, N., & Obinata, T. (1986) *J. Biochem. (Tokyo) 99*, 751–759.
Southern, E. M. (1975) *J. Mol. Biol. 98*, 507–517.
Stone, D., & Smillie, L. B. (1985) *J. Biol. Chem. 253*, 1137–1148.
Takagi, T., Konishi, K., & Mabuchi, I. (1988) *J. Biol. Chem. 263*, 3097–3102.
Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5201–5205.
Yonezawa, N., Nishida, E., & Sakai, H. (1985) *J. Biol. Chem. 260*, 14410–14412.
Yonezawa, N., Nishida, E., Koyasu, S., Maekawa, S., Ohta, Y., Yahara, I., & Sakai, H. (1987) *Cell Struct. Funct. 2*, 443–452.

Sequential Assignment of the ^{1}H Nuclear Magnetic Resonance Spectrum of Barnase†

Mark Bycroft,*,‡ Richard N. Sheppard,§ Frankie Tat-Kwong Lau,‡,∥ and Alan R. Fersht‡

MRC Unit for Protein Function and Design, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K., and Department of Chemistry, Imperial College of Science, Technology and Medicine, London SW7 2AY, U.K.

Received November 28, 1989; Revised Manuscript Received March 15, 1990

ABSTRACT: Two-dimensional nuclear magnetic resonance spectroscopy has been used to study the bacterial ribonuclease barnase (MW 12382). Resonance assignments have been made for protons in all of the 110 residues. Analysis of medium- and long-range contacts in NOESY spectra has demonstrated that the major elements of secondary structure in barnase in solution are essentially identical with those found in the crystal structure.

Barnase is a guanine-specific bacterial ribonuclease (MW 12382) containing 110 amino acids (Hartley, 1989). This protein is currently being studied in detail in this laboratory by using site-directed mutagenesis. A number of areas are being investigated including the factors affecting protein stability, the pathway of protein folding, and the mechanism of catalysis (Kellis et al., 1988, 1989; Sali et al., 1988; Matouschek et al., 1989; Mossakowska et al., 1989). NMR spectroscopy has the potential to provide useful information on all of the above areas (Jardetzky & Roberts, 1981). The availability of extensive assignments for barnase is, however, an essential prerequisite for any detailed study using NMR spectroscopy. In recent years, the development of two-dimensional NMR methods (Ernst et al., 1987) has enabled sequence-specific resonance assignments to be obtained for small proteins (Wüthrich, 1986). We report here the assignment of proton resonances in all of the 110 residues of barnase.

Experimental Procedures

Barnase was purified from cultures of *Escherichia coli* containing the plasmid pMT410 (Paddon & Hartley, 1987) as described previously (Mossakowska et al., 1989). Purified barnase was dialyzed exhaustively against distilled water and then lyophilized. NMR samples were prepared by dissolving lyophilized protein in 0.5 mL of 90% H_2O/10% D_2O or 100% D_2O and adjusting the pH or pD with DCl. Protein concentrations were between 4 and 6 mM. NMR spectra were

† This work was supported by the EEC (BAP) and the Medical Research Council of the U.K.
* Author to whom correspondence should be addressed.
‡ MRC Unit for Protein Function and Design, University Chemical Laboratory.
§ Department of Chemistry, Imperial College of Science, Technology and Medicine.
∥ Present address: Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge, MA.

0006-2960/90/0429-7425$02.50/0