Volume 326, number 1,2,3, 25–28 FEBS 12650 July 1993


# Molecular cloning of PARP (proline/arginine-rich protein) from human cartilage and subsequent demonstration that PARP is a fragment of the $NH_2$-terminal domain of the collagen α2(XI) chain

Natalia I. Zhidkova, Randolph G. Brewton and Richard Mayne

*Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294, USA*

Received 6 May 1993

We report the molecular cloning of a proline/arginine-rich protein (called PARP) from human cartilage using the polymerase chain reaction (PCR) and degenerate oligonucleotides based on the previously published amino acid sequence of bovine PARP [1]. Subsequently, a reverse transcription-polymerase chain reaction (RT-PCR) was performed with poly(A)-rich RNA from human cartilage using a sense oligonucleotide derived from PARP and an anti-sense oligonucleotide derived from the known sequence of the human collagen α2(XI) chain [2]. Nucleotide sequencing of the PCR product demonstrated that PARP is a fragment of the $NH_2$-terminal non-collagenous (NC3) domain of the collagen α2(XI) chain.

Cartilage; Human collagen type XI; Polymerase chain reaction; Nucleotide sequence

## 1. INTRODUCTION

In 1990, a proline/arginine-rich protein (called PARP) was isolated from bovine cartilage and the complete amino acid sequence determined [1]. PARP was found to be related to the $NH_2$-terminal domain of several collagen types, being most closely related (49% identity) to the $NH_2$-terminal domain of the collagen α1(XI) chain [1,3,4]. We now report the molecular cloning of human PARP using RT-PCR performed with degenerate oligonucleotide primers designed from the published amino acid sequence of bovine PARP. A subsequent RT-PCR reaction was performed with a sense primer derived from human PARP and an anti-sense primer derived from the published nucleotide sequence of the human α2(XII) collagen chain [2]. Cloning and nucleotide sequencing of the 1.86 kbp PCR product demonstrated that PARP is a fragment of the amino-terminal non-collagenous (NC3) domain of the collagen α2(XI) chain.

*Correspondence address:* R. Mayne, Department of Cell Biology, University of Alabama at Birmingham, Box 302-VH, Birmingham, AL 35294, USA. Fax: (1) (205) 934-7029.

*Abbreviations:* PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; PARP, proline/arginine-rich protein.

The sequence described in this paper has been submitted to the GenBank/EMBL Data Library under the accession number L 18987.

## 2. MATERIALS AND METHODS

### 2.1. *Isolation of RNA and first strand cDNA synthesis*

Chondrocytes were isolated from sternal cartilage obtained from an eight-year-old white female following corrective surgery for pectus excavatum. Minced cartilage was incubated in digestion medium (MEM, 5% L-glutamine, 2% antibiotic/antimycotic containing 127 U/ml collagenase (Worthington Biochemical Corp., CLS1) overnight at 37°C with shaking. Chondrocytes were freed from the matrix by repeated pipetting, filtered through lens paper, and centrifuged to a pellet. The cells were resuspended in MEM supplemental with 10% fetal bovine serum, washed three times with MEM and the pellet subsequently solubilized in 4.0 M guanidine isothiocyanate, 0.1 M Tris-HCl, pH 7.4, 0.1 M 2-mercaptoethanol and 0.5% *N*-lauryl-sarcosine. Total RNA was obtained by the single step acid-guanidine method [5] and poly(A)-rich RNA selected by oligo(dT)-cellulose chromatography [6]. First strand cDNA for PCR analysis was prepared as described previously [7].

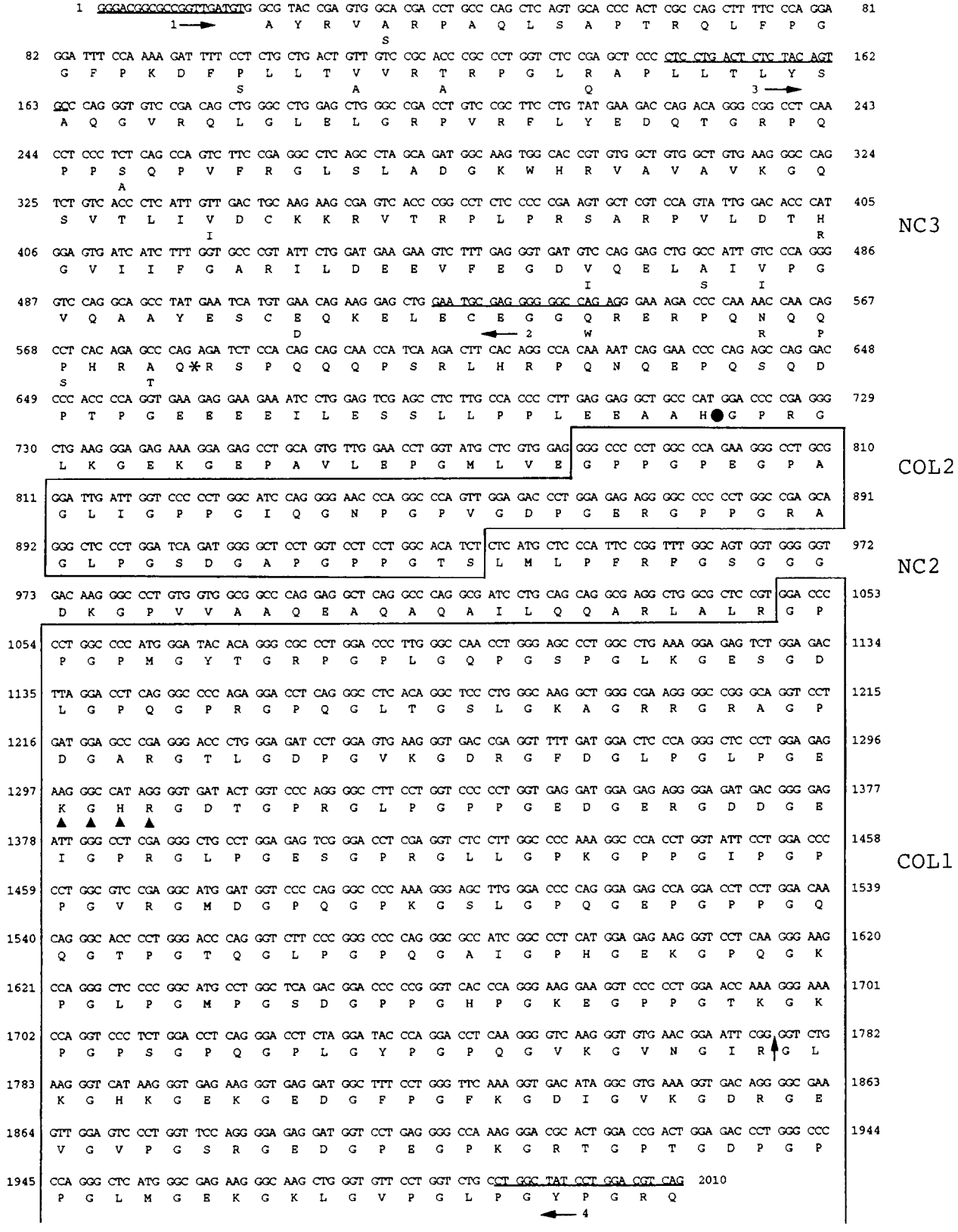
### 2.2. *PCR conditions and cloning of PCR products*

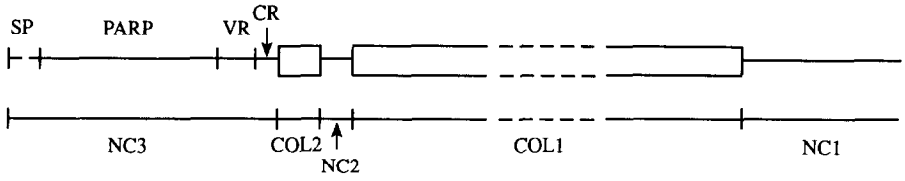
Conditions for all PCR reactions were: denaturation, 92°C, 1 min; annealing, 58°C, 1 min; polymerization, 72°C, 3 min; for 30 cycles as described [7]. After agarose gel electrophoresis, individual bands were recovered using Geneclean (BIO 101, LaJolla, CA) as described [7].

To clone PARP, RT-PCR was performed using degenerate 20-mer oligonucleotides designed from the bovine amino acid sequence. Sense and antisense primers were: 5′-GGIACIGCICC(ACGT)GT(ACGT)-GA(CT)GT-3′ and 5′-C(GT)CCAICCICC(CT)TC(AG)CA(CT)TC-3′ (Primers 1 and 2; for the location of these primers see Fig. 1) in which redundancy was reduced by incorporation of inosine at several sites [8].

To obtain a PCR product bridging PARP and the α2(XI) chain the sense primer from PARP was 5′-CTCCTGACTCTCTACAGTGC-3′ (Primer 3; Fig. 1) and the antisense primer was 5′-CTGACGTCCAG-GATAGCCAG-3′ (Primer 4; positions 953–972 of the published nucleotide sequence [2]).

Products of PCR were cloned into the pCRII vector using the TA Cloning kit as described by the manufacturer (Invitrogen, San Diego, CA).


NC3
COL2
NC2
COL1




Total amino acids/domain (from references 2, 9-12).

| | NC3 | | | | COL2 | NC2 | COL1 | NC1 |
|---|---|---|---|---|---|---|---|---|
| | SP | PARP | VR | CR | | | | |
| α2(XI) | - | 218 | 45 | 22 | 51 | 37 | 1014 | 190 |
| α1(XI) | 36 | 225 | 159 | 22 | 51 | 36 | 1014 | 264 |
| α1(V) | 36 | 225 | 185 | 22 | 51 | 39 | 1014 | 266 |

SP = signal peptide; PARP = proline/arginine rich peptide; VR = variable region; CR = constant region

Fig. 2. A model for the organization of the domains and sub-domains of the α2(XI), α1(XI) and α1(V) chains.

2.3. *DNA sequencing*

Dideoxynucleotide sequencing was performed on either single or double stranded DNA obtained after cloning PCR products in the pCRII vector. For PARP clones, Sequenase 2.0 (US Biochemicals) was used and for PARP-α2(XI) bridging clones Taq polymerase was used (TAQuence 2.0, US Biochemicals). Nucleotide sequences of PARP were obtained from a single clone (NZ1), and for PARP-α2(XI) bridging clones two independent clones (called NZ2 and NZ3) were sequenced.

## 3. RESULTS

In order to clone PARP, RT-PCR was initially performed with degenerate primers designed from the protein sequence of bovine PARP. The location of these primers (called Primer 1 and 2) and the nucleotide sequence for a clone of the PCR product called NZ1 is shown in Fig. 1. Primer 1 was a degenerate primer designed from the amino terminal sequence of bovine PARP (GTAPVDV [1]). However, subsequent nucleotide sequencing of NZ1 demonstrated that priming had occurred 24 amino acids further downstream at the sequence GICPADV.

To obtain a clone bridging between PARP and the α2(XI) chain, RT-PCR was performed using sense and anti-sense primers located at sites 3 and 4 in Fig. 1. The nucleotide sequence of this clone (called NZ2) is also shown in Fig. 1, together with a comparison of the derived protein sequence of human PARP with bovine PARP. Close agreement was observed with 17 largely conservative differences, demonstrating the successful cloning of human PARP. In addition, complete agreement was found for the nucleotide sequence at the 3′ end of NZ2 with the published nucleotide sequence of human α2(XI) in the same region [2]. This result therefore establishes that PARP is derived from the amino-terminal non-collagenous (NC3) domain of the α2(XI) chain.

With the sequence of the amino terminus of the α2(XI) chain (Fig. 1) and the complete sequences of the α1(XI) and α1(V) chains now available [9,10–12], it is possible to recognize several different domains and a model labeling these structures is shown in Fig. 2. In the same manner as several other collagens, the domains are numbered from the 3′ end so that the major collagenous domain is designated COL1. A second shorter collagenous domain is labeled COL2 and the NC3 domain is further subdivided into four subdomains. In several previous studies homologies between the sequence of PARP and the α1(XI),α1(V),α1(IX),α1(XII), and α1(XIV) chains were noted suggesting a common ancestral origin for this structure [1,3,4].

The NC3-PARP subdomain is followed by the NC3-variable region (NC3-VR) which is much shorter for α2(XI) than for α1(XI) and α1(V) (Fig. 2). Previous comparison of this region for the α1(XI) and α1(V) chains showed very little homology [12], and this region

←

Fig. 1. The nucleotide sequence and deduced amino sequence of PCR-derived clones NZ1 and NZ2 which bridge from PARP to the COL1 domain of α2(XI). Sites 1 and 2 are the location of degenerate primers used initially to obtain clone NZ1. Sites 3 and 4 represent the location of primers used to obtain clone NZ2 which bridges from PARP to α2(XI). The collagenous domains COL1 and COL2 are shown by boxed sequences between which is the NC2 domain. The carboxyl-terminus of PARP is designated by, and differences between the amino acid sequence of bovine and human PARP are shown on the third line for this sub-domain. The sequence from * to ● represents the variable region of the NC3 domain (VR-NC3) and the short sequence after ● to COL2 represents the constant region of NC3 (CR-NC3). The conserved crosslinking site (KGHR) in COL1 is indicated by ▲. The start of the published nucleotide sequence of the human α2(XI) chain is indicated by ↑.

for α2(XI) did not appear closely related to either the α1(XI) or α1(V) sequences. The remaining NC3-constant region (NC3-CR), COL2, NC2 and COL1 domains all showed a high degree of homology between the α2(XI), α1(XI) and α1(V) chains.

## 4. DISCUSSION

The successful cloning of human PARP and its origin from the α2(XI) chain allow a comparison to be made with other chains of the type V/type XI family. The results show close homologies with the α2(XI) and α1(V) chains but marked differences from the α2(V) chain as noted previously [13]. However, at one subdomain (called NC3-VR) marked differences were observed for the α2(XI), α1(XI) and α1(V) chains both in length and in deduced amino acid sequence. This domain is, however, acidic for all three chains and does not contain cysteine residues. It appears that the NC3 domain may form an extended structure that cannot be visualized after rotary shadowing [14]. With the availability of the amino-terminal sequences of the α1(XI) and α2(XI) chains, it will now be possible to analyze the processing events that occur before the final tissue forms of these chains are achieved [14].

*Acknowledgements:* This study was supported by Research Grants AR 30481 and HD 20691 from the National Institutes of Health. The human tissues utilized in this study were obtained through the UAB Cooperative Human Tissue Network which is funded by the National Cancer Institute.

## REFERENCES

[1] Neame, P.J., Young, C.N. and Treep, J.T. (1990) J. Biol. Chem. 265, 20401-20408.
[2] Kimura, T., Cheah, K.S.E., Chan, S.D.H., Lui, V.C.H., Mattei, M-G., van der Rest, M., Ono, K., Solomon, E., Ninomiya, Y. and Olsen, B.R. (1989) J. Biol. Chem. 264, 13910–13916.
[3] Wälchli, C., Trueb, J., Kessler, B., Winterhalter, K.H. and Trueb, B. (1993) Eur. J. Biochem. 212, 483–490.
[4] Bork, P. (1992) FEBS Lett. 307, 49–54.
[5] Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[6] Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[7] Brewton, R.G., Ouspenskaia, M.V., van der Rest, M. and Mayne, R. (1992) Eur. J. Biochem. 205, 443–449.
[8] Knoth, K., Roberds, S., Poteet, C. and Tamkun, M. (1988) Nucleic Acids Res. 16, 10932.
[9] Yoshioka, H. and Ramirez, F. (1990) J. Biol. Chem. 265, 6423–6426.
[10] Bernard, M., Yoshioka, H., Rodriquez, E., van der Rest, M., Kimura, T., Ninomiya, Y., Olsen, B.R. and Ramirez, F. (1988) J. Biol. Chem. 263, 17159–17166.
[11] Takahara, K., Sato, Y., Okazawa, K., Okamoto, N., Noda, A., Yaoi, Y. and Kato, I. (1991) J. Biol. Chem. 266, 13124–13129.
[12] Greenspan, D.S., Cheng, W. and Hoffman, G.G. (1991) J. Biol. Chem. 266, 24727-24733.
[13] Petit, B., Freyria, A-M., van der Rest, M. and Herbage, D. (1992) in: Biological Regulation of Chondrocytes (Adolphe, M. Ed.) pp. 33–84, CRC Press, Boca Raton.
[14] Thom, J.R. and Morris, N.P. (1991) J. Biol. Chem. 266, 7262–7269.