Human HtrA, an Evolutionarily Conserved Serine Protease Identified as a Differentially Expressed Gene Product in Osteoarthritic Cartilage*

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The human homologue of the Escherichia coli htrA gene product was identified by the differential display analysis of transcripts expressed in osteoarthritic cartilage. This transcript was identified previously as being repressed in SV40-transformed fibroblasts (Zumbrunn, J., and Trueb, B. (1996) FEBS Lett. 398, 187–192). Levels of HtrA mRNA were elevated ~7-fold in cartilage from individuals with osteoarthritis compared with nonarthritic controls. Differential expression of human HtrA protein was confirmed by an immunoblot analysis of cartilage extracts. Human HtrA protein expressed in heterologous systems was secreted and exhibited endopeptidolytic activity, including autocatalytic cleavage. Conversion by mutagenesis of the putative active site serine 328 to alanine eliminated the enzymatic activity. Serine 328 was also found to be required for the formation of a stable complex with α1-antitrypsin. We have determined that the HtrA gene is highly conserved among mammalian species: the amino acid sequences encoded by HtrA cDNA clones from cow, rabbit, and guinea pig are 98% identical to human. In E. coli, a functional htrA gene product is required for cell survival after heat shock or oxidative stress; its role appears to be the degradation of denatured proteins. We propose that mammalian HtrA, with the addition of a new functionality during evolution, i.e. a mac25 homology domain, plays an important role in cell growth regulation.

Osteoarthritis (OA), the most prevalent form of degenerative joint disease, involves chondrocyte loss and the breakdown of extracellular matrix components, leading to cartilage degeneration and the eventual deterioration of joint function (1). From a therapeutic perspective, it is important to understand the molecular events triggering the onset of OA and the biochemical pathways responsible for the disease progression that appear to be influenced by a complexity of environmental and genetic factors (2–4). Chondrocytes, the exclusive cell type in cartilage, maintain the integrity of the collagen/proteoglycan network by responding to a variety of stresses, including the normal mechanical load as well as abnormal trauma and injury (5). The cellular response to stress stimuli occurs through the regulation of a myriad of signal transduction pathways, leading to alterations in gene expression.

Analysis of differential gene expression using various molecular biological techniques has been increasingly applied to investigate complex biological phenomena (6). As an approach to study the molecular pathobiology of OA, we have used a mRNA differential display (7) to screen for differences in gene expression between osteoarthritic and nonarthritic cartilage. A major advantage of this methodology is the capability to amplify minute amounts of transcripts and rapidly determine their nucleotide sequences. We have identified over 120 transcripts that appear to be differentially expressed in OA cartilage, only 29 of which correspond to known proteins.

In this report, we describe the identification and characterization of one transcript ( provisionally named ORF480) and its translation product, both of which are expressed at elevated levels in OA cartilage. The nucleotide sequence of ORF480 is identical to a recently described transformation-sensitive cDNA isolated from human fibroblasts (8). ORF480 codes for a protein with distinct domains of homology to human mac25 (9) and to a bacterial serine protease (HtrA) that is critical for the cellular response to thermal and oxidative stress (10, 11). This report provides the first evidence that the ORF480-encoded protein (human HtrA) exhibits autocatalytic cleavage as well as endopeptidolytic activity against an exogenous substrate, β-casein. When incubated in the presence of serum, HtrA protein binds to and forms a stable complex with α1-antitrypsin. In addition, we have determined that the sequence of the HtrA-related domain of ORF480 is highly conserved among mammalian species. These findings open new avenues of investigation toward an understanding of the biological function(s) of mammalian HtrA.

**EXPERIMENTAL PROCEDURES**

*MATERIALS—Total RNA from OA and nonarthritic human cartilage was isolated according to published methods (12) and supplied to our laboratories by Drs. I. Patel and A. Amin (Hospital for Joint Diseases, New York University Medical School, New York, NY). Independent biochemical analyses of the isolated cartilage* as well as the differential mRNA expression of type II and type III collagens (see "Results") were consistent with the indicated pathological state of the samples used in this study.

mRNA Differential Display and RT-PCR Product Identification—First-strand cDNA was synthesized from 0.2 μg of total RNA with each of the three anchored oligodeoxynucleotide acid primers from GenHunter Corp. The reaction (20 μl) was carried out at 37 °C for 60 min. For PCR amplification, 1 μl of the cDNA served as the template in a 10-μl reaction mix containing 10 mM Tris-HCl (pH 8.4), 1.5 mM MgCl2,
50 mm KC1, 0.001% gelatin, 2 μM deoxyadenosine triphosphates, 0.2 μM 5’-terminal primer (AP-49 or AP-58 from the RNA image kits obtained from GenHunter Corp.), 2 μM of the same anchored primer used in the cDNA synthesis, 5 μl of [α-32P]dATP (2000 Ci/mmol; DuPont New England Nuclear), and 2.5 units of AmpliTaq DNA polymerase (Perkin-Elmer) were used for each 25 μl reaction. The PCR conditions were as follows, excluding the labeled nucleotide. PCR products were resolved on a 1.5% agarose gel, excised, and ligated into cloning vector DH5α (Life Technologies, Inc.). DNA sequences were determined for at least three independent clones of each fragment using Dye Terminator Cycle Sequencing on an ABI PRISM 377 DNA sequencing system (Perkin-Elmer).

Semiquantitative RT-PCR—First-strand cDNA was synthesized from total RNA isolated from OA and nonarthritic cartilage. 200 ng of total RNA and 10 pmol of primer T30VN (where V = A, C, and G, and N = A, C, G, and T) were mixed in a 6-μl volume, heated to 72 °C for 3 min, followed by 3 min of cool on ice. Primers used for reverse transcription of each primer set to verify the absence of contamination.

First-strand cDNA was synthesized utilizing the pFASTBAC1/ORF480 donor plasmid and the BAC-TO-BAC Baculovirus Expression System (Life Technologies, Inc.). Optimal infection conditions were determined by varying the multiplicity of infection and time course. Expression of the secreted ORF480 protein in both heterologous systems was confirmed by the immunoblot blot analysis of culture supernatants using anti-HtrA antisera as described above. For immunoblot analyses to discern the presence of high molecular weight complexes of HtrA protein (Fig. 6), we used anti-HtrA antisera that had been depleted of anti-albumin cross-reactivity by affinity chromatography. To monitor the protease activity of ORF480-encoded protein, 75 μl of culture medium were incubated with 50 μl of casein (Sigma) in 50 mM Tris-HCl (pH 7.5) for 1 h at 37 °C, and the reaction products were analyzed by SDS-polyacrylamide gel electrophoresis using either standard 12% or 7.5–20% gradient gels (Bio-Rad Laboratories).

**Heterologous Expression of ORF480—**Human embryonic kidney cells, 293 (American Type Culture Collection), were maintained in Minimal Essential Medium (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies, Inc.) and 1× antibiotic-antimycotic solution (Life Technologies, Inc.) at 37 °C in a humidified CO2 incubator. Cells were stably transfected with the pcDNA3-ORF480 expression vector using the ProFection Mammalian Transfection System (Promega), incorporating [32P]methionine. The reaction proteins were separated on a 10% SDS-polyacrylamide gel. At the end of the run, the gel was dried under a vacuum at 80 °C and analyzed on a PhosphorImager (Molecular Dynamics).
Cloning Mammalian Homologues of HtrA—Cow HtrA was isolated from a lung cDNA phage library (CLONTECH) by hybridization screening using the human cDNA as a probe. HtrA cDNA fragments were isolated by PCR from rabbit and guinea pig liver cDNA using primers designed from the human coding sequence corresponding to regions of maximum amino acid sequence identity with E. coli htrA. The DNA sequences of the derived clones were determined as described above. Additional PCR primers were then designed and used for 5’ and 3’ RACE to obtain extended cDNA clones.

RESULTS

Identification, Cloning, and Sequence Analysis of ORF480 cDNA—In the course of screening for differences in gene expression between osteoarthritic and nonarthritic cartilage by mRNA differential display, two PCR fragments, designated 49A50 and 58A5, were identified using different arbitrary primers (Fig. 1). Sequence analysis of 49A50 and 58A5 demonstrated that the two cloned PCR products correspond to the 3’ end of the same mRNA (data not shown). Using the 5’ RACE technique (13), a ~1.4-kb PCR fragment corresponding to 49A50/58A5 was isolated and cloned from OA cartilage-derived cDNA. The DNA sequences of two independent clones derived from RACE products C05 and C13 revealed overlapping open reading frames of 337 and 328 codons, respectively. Because no ATG initiation codon was evident in the 5’ sequences of clones C05 and C13, and Northern blot data indicated a mRNA size of over 2 kb (data not shown), it was evident that these cartilage-derived cDNA clones did not represent the entire transcript. At this point in the study, a BLAST (14) search of the GenBank and EST databases identified two 3’ EST sequences (GenBank accession numbers W47107 and W67176) and a 2036-bp cDNA entry (GenBank accession number D87258). The translated sequence of GenBank entry D87258 contains an open reading frame of 480 amino acids (ORF480). The DNA sequence of clone C05 was identical to bases 477–1646 of D87258, except for a 1-bp difference (a probable PCR-generated error). Thus, the cDNA clones isolated from OA cartilage and the D87258 sequence cloned from osteoarthritis cartilage represent the same gene product. RT-PCR analysis indicated that the mRNA for ORF480 is expressed in human placenta and in normal human dermal fibroblasts (data not shown). Overlapping PCR-generated fragments corresponding to the entire ORF480 were isolated from cDNA derived from fibroblast RNA (see “Experimental Procedures”). The DNA sequence of the fibroblast-derived ORF480 cDNA was determined to be identical to that of GenBank entry D87258. An alignment of the cDNA clones described in this report in relation to those identified from searching the databases is illustrated in Fig. 2. Also shown in Fig. 2 are the deduced protein domains within ORF480 as described below.

During the course of the work described here, Zumbrunn and Trueb (8) reported the isolation of the same cDNA from human fibroblast; the expression of the corresponding mRNA was repressed in SV40-transformed cells. Their analysis of the sequence encoded by the cDNA revealed a putative signal sequence (codons 1–22), an IGF-binding protein 3 homology domain (codons 1–140), a Kazal-type inhibitor motif (codons 97–155), and the major domain of homology to E. coli HtrA (codons 140–480). In addition, a recent survey of proteins containing the so-called PDZ domains (15) includes bacterial HtrA as well as the human HtrA homologue. The location of the PDZ domain within ORF480 (codons 372–466) is indicated schematically in Fig. 2.

Our analysis of the ORF480 sequence, although in general agreement, differed somewhat from that of Zumbrunn and Trueb due to differences in searching methods and the scope of the databases used. Using BLASTP to search the GenBank protein sequence data base, we found human mac25, a presumed member of the IGF-binding protein family (9), to have the highest degree of homology to the amino-terminal domain of ORF480 (Fig. 3A). For instance, the p value for the mac25 alignment with ORF480 residues 107–158 is 1.4 × 10−23, compared with p = 3.8 × 10−8 for insulin-related growth factor-binding protein 3. Interestingly, unlike IGF-binding proteins, the sequence of mac25 within the region homologous to ORF480 contains a conserved Kazal-type serine protease inhibitor motif (Fig. 3). Moreover, as pointed out by Kato et al. (16), mac25 is more closely related to follistatin than to insulin-related growth factor-binding protein, and we find that the sequence of follistatin also contains the Kazal-type inhibitor motif (Fig. 3).

Differential Expression of ORF480 mRNA and HtrA Protein in OA Cartilage—To verify the initial differential display results and quantify the relative difference in the mRNA levels of human HtrA (ORF480) in OA cartilage compared with nonarthritic cartilage, oligonucleotide primer pairs specific for a 3’ segment of ORF480 cDNA were used for semiquantitative RT-PCR (Fig. 4A). The results of this analysis, using expression levels of β-actin and Hsp60 for comparison, indicate that HtrA mRNA is present at levels that are ~7-fold higher in OA cartilage than in nonarthritic controls. Also included in Fig. 4A are data for both type II (COL2A) and type III (COL3A) collagen mRNA, which were also identified in our differential display screening, that show that levels of these transcripts are significantly elevated in the OA-derived samples used in these studies, a finding that is consistent with previous reports of induced collagen synthesis in remodeling OA cartilage (17). To confirm the differential expression of human HtrA protein
Fig. 3. ORF480 contains a Kazal-type inhibitor motif within the mac25 homology domain. A, the amino acid sequence alignment of ORF480 and mac25 is shown; identical (vertical line) and similar (:) residues are indicated. The Kazal-type inhibitor motif is outlined by a rectangle. B, multiple sequence alignment of the conserved Kazal-type inhibitor motif within ORF480 (shaded sequence), human and murine mac25, agrin, and several known serine protease inhibitors: elastase inhibitor from H. medicinalis (36), protease inhibitor from crayfish (37), and human trypsin inhibitor C (38).

Fig. 4. Elevated levels of ORF480-encoded HtrA mRNA and protein in human OA cartilage. A, comparison of mRNA expression in OA versus nonarthritic (NA) cartilage by semiquantitative RT-PCR. A composite image is shown from a representative experiment; PCR products were resolved by polyacrylamide gel electrophoresis followed by staining with SYBR®-Green (Molecular Probes). Band intensities were quantified using a PhosphorImager™ and ImageQuant software (Molecular Dynamics). *, the mean ratio of expression (normalized to actin) in OA cartilage to that in nonarthritic cartilage from multiple (n) experiments using at least three samples from each is indicated. COL3A and COL2A are abbreviations for procollagen type I and II, respectively. B, detection of ORF480-encoded HtrA protein in high-salt extracts of human OA and nonarthritic cartilage by immunoblot analysis (see "Experimental Procedures"). The anti-HtrA antibody recognizes two cross-reactivity with serum albumin. A supernatant sample from cultured 293 cells transfected with pcDNA3-ORF480 was included as a positive control (+) in the immunoblot analysis.

in OA cartilage, high-salt extracts of OA and control cartilage samples were tested by immunoblot analysis using an anti-HtrA protein detected in OA versus nonarthritic cartilage extracts, results that are consistent with the analysis of the mRNA levels.

Expression and Proteolytic Activity of ORF480-encoded Protein—A cDNA segment containing the entire coding region of ORF480 was inserted into expression vector pcDNA3 (see "Experimental Procedures" and Fig. 2). This construct directed the expression of the expected size protein (M, ~50,000) in an in vitro transcription/translation system (Fig. 5A, lanes 1 and 2). Lower molecular weight products (M, ~40–45,000) were also evident in the gel electrophoresis pattern, a pattern that was remarkably similar to that observed for E. coli HtrA (11, 18). Converting the conserved active site serine 328 in ORF480 to alanine by site-directed mutagenesis resulted in the elimination of the lower molecular weight proteins (Fig. 5A, lanes 3 and 4). This result, which was previously shown for E. coli HtrA (18), demonstrates that the translation product of ORF480, the human HtrA protein, is a serine protease with autocatalytic activity.

When ORF480 is expressed in baculovirus-infected Sf9 cells (Fig. 5B), the primary translation product (M, ~50,000) is detected by immunoblot analysis using anti-HtrA antisera in the culture medium by 41 h postinfection. A distinct set of lower molecular weight proteins is evident in the immunoblot (Fig. 5B), which is consistent with the autocatalytic activity of the ORF480-encoded protein (HtrA) expressed in the cell-free expression system described above. As discussed below, a high molecular weight protein is observed by immunoblot analysis in the samples taken at 65 and 73 h postinfection. Incubation of the ORF480-encoded protein (HtrA) with β-casein results in the generation of specific proteolytic cleavage products of this substrate (Fig. 5B, bottom panel).

To examine the expression of ORF480 in mammalian cells, cultures of 293 cells were transfected with pcDNA3/ORF480, resulting in the synthesis and secretion of the M, ~50,000 HtrA protein as determined by immunoblot analysis (Fig. 5C, top panel). The media from different isolated clones derived from the transfected culture contain endoprotease activity against β-casein (Fig. 5C, bottom panel), resulting in at least four distinct fragments of the substrate, which is similar to the pattern observed in the experiment using baculovirus-expressed HtrA shown in Fig. 5B. The level of proteolytic activity in the samples from each clone (Fig. 5C, bottom panel) correlates with the relative amount of immunoreactive HtrA protein observed in the immunoblot (Fig. 5C, top panel). The S328A
FIG. 5. Expression of ORF480 cDNA and functional analysis of its encoded protein, HtrA. A, in vitro transcription/translation of ORF480 and ORF480-S328A cDNAs yields the expected Mr ~50,000 translation product (arrow) resolved by SDS-polyacrylamide gel electrophoresis. Note the presence of three lower molecular weight protein bands in lanes 1 and 2 (duplicate samples of ORF480) that are absent in lanes 3 and 4 (duplicate samples of the S328A mutant). B, human HtrA protein expressed in baculovirus-infected Sf9 cells is secreted (top panel; immunoblot analysis) and exhibits proteolytic activity against β-casein (bottom panel). Proteins were resolved by SDS-polyacrylamide gel electrophoresis (12% polyacrylamide). Postinfection incubation times (in hours) for each sample are indicated. A set of immunoreactive degradation products of HtrA protein are framed by the bracket (top panel). A stable high molecular weight form of HtrA is labeled with an asterisk. Specific HtrA-generated cleavage products of β-casein are indicated by arrows (bottom panel). C, culture supernatants from clonal isolates of 293 cells transfected with pcDNA3/ORF480 were analyzed by immunoblot analysis (top panel) and proteolytic activity against β-casein (bottom panel). Proteins were resolved by SDS-polyacrylamide gel electrophoresis (4–20% gradient gel). Lanes 1 and 2 are negative controls; lanes 3–7 are from independent clones expressing decreasing levels of HtrA protein. Specific HtrA-generated cleavage products of β-casein are indicated by arrows. D, HtrA protein containing the S328A mutation lacks proteolytic activity against β-casein. Culture supernatants of 293 cells transfected with ORF480 and ORF480-S328A cDNAs incubated for the times indicated (in hours) were tested for proteolytic activity using albumin and casein. Postinfection incubation times (in hours) for each sample are indicated. A set of immunoreactive degradation products of HtrA protein are framed by the bracket (top panel). A stable high molecular weight form of HtrA is labeled with an asterisk. Specific HtrA-generated cleavage products of β-casein are indicated by arrows (bottom panel).
40% identical to bacterial HtrA serine protease. The evidence presented here demonstrates the in vitro proteolytic activity of the ORF480-encoded protein, which we conclude is the human homologue of bacterial HtrA.

In bacteria, HtrA is a critical component of the universal cellular response to stress, which is characterized by the induction of a set of so-called heat shock proteins (19). In addition to temperature elevation, heat shock proteins are induced by oxidative stress (20), viral (phage) infection (21), and intracellular expression of aberrant proteins (22). A functional htrA (high temperature requirement) gene is indispensable for the bacterial cell to survive heat shock (11). HtrA is identical to DegP.
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from the nature of the various proteins to which it is related, will guide future experimental approaches to reveal the (patho-)physiological role of mammalian HtrA.

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REFERENCES

1. Howell, D. S. (1984) in Osteoarthritis Diagnosis and Management (Moskowitz, R. W., Howell, D. S., Goldberg, V. M., and Mankin, H. J., eds) pp. 129–146, Sanders, Philadelphia.

2. Felson, D. T. (1990) Rheum. Dis. Clin. N. Am. 16, 499–512

3. McAulindon, T., and Dieppe, P. (1990) Br. J. Rheumatol. 29, 471–473

4. Odds, C. V. (1996) Am. J. Med. 100, 10s–15s

5. Muir, H. (1995) Biosci. Rep. 15, 1039–1048

6. Wan, J. S., Sharp, S. J., Poirier, G. M.-C., Wagaman, P. C., Chambers, J., Pyati, J., Hem, Y.-L., Galindo, J. E., Huvos, A., Peterson, P. A., Jackson, M. R., and Erlander, M. G. (1996) Nat. Biotechnol. 14, 1685–1691

7. Liang, P., and Pardee, A. (1992) Science 257, 967–971

8. Zumbrunn, J., and Trueb, B. (1996) FEBS Lett. 398, 187–192

9. Swisshelm, K., Ryan, K., Tsai, H., and Sager, R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4472–4477

10. Lipinski, B., Sharma, S., and Georgopoulos, C. (1988) Nucleic Acids Res. 16, 1053–1060

11. Lipinski, B., Zielzie, M., and Georgopoulos, C. (1990) J. Cell Biol. 172, 1369–1379

12. Amin, A. R., Attur, M., Patel, R. N., Thakker, G. D., Marshall, P. J., Rediske, J., Stuchin, S. A., Patel, I. R., and Abramson, S. B. (1997) J. Clin. Invest. 99, 1231–1237

13. Frohman, M. A. (1993) Methods Enzymol. 218, 340–358

14. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410

15. Ponting, C. P. (1997) Protein Sci. 6, 464–468

16. Kato, M. V., Sato, H., Tsukada, T., Ikawa, Y., Aizawa, S., and Nagayoshi, M. (1996) Oncogene 12, 1361–1364

17. Aigner, T., Berling, W., Stoss, H., Weseloh, G., and von der Mark, K. (1993) J. Clin. Invest. 91, 829–837

18. Skorko-Glonek, J., Wawrzynow, A., Krzewski, K., Kurpierz, K., and Lipinski, B. (1995) Gene (Amst.) 163, 47–52

19. Neidhardt, F. C., VanBeneloge, R., and Vaughn, V. (1984) Annu. Rev. Genet. 18, 255–329

20. Christman, M. F., Morgan, R. W., Jacobson, F. S., and Ames, B. N. (1985) Cell 41, 753–762

21. Bahl, H., Echols, H., Strauss, D. B., Court, D., Croll, J., and Georgopoulos, C. P. (1987) Genes Dev. 1, 57–64

22. Goff, S. A., and Goldberg, A. L. (1985) Cell 41, 587–595

23. Streatch, K. J., Johnson, K., and Beckwith, J. (1989) J. Bacteriol. 171, 2689–2696

24. Goldberg, A. L., Swamy, K. H. S., Chung, C. H., and Larimore, F. S. (1982) Methods Enzymol. 80, 660–702

25. Laskowska, E., Kuczynska-Wisnisk, D., Skorko-Glonek, J., and Taylor, A. (1996) Mol. Microbiol. 22, 555–571

26. Kolomar, H., Weller, P. R., and Sauer, R. T. (1996) J. Bacteriol. 178, 5925–5929

27. Walton, E. A., Upfold, L. I., Stephens, R. W., Ghosh, P., and Taylor, T. K. F. (1981) Semin. Arthritis Rheum. 1, 73–74

28. Petraglia, F. (1997) Placenta 18, 3–8

29. Patthy, L., and Nikolics, K. (1993) Trends Neurosci. 16, 76–81

30. Biroc, S. L., Payan, D. G., and Fisher, J. M. (1993) Dev. Brain Res. 75, 119–129

31. Shimasaki, S., Koga, M., Esch, F., Cooksey, K., Mercado, M., Koba, A., Ueno, N., Ying, S.-Y., Ling, N., and Guillemin, R. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4218–4222

32. Rupp, F., Oncel, T., Linal, M., Peterson, K., Francke, U., and Scheller, R. J. (1992) J. Neurosci. 12, 3535–3544

33. Tschesche, H., Kolkenbrock, H., and Bode, W. (1987) Biol. Chem. Hoppe-Seyler 368, 1297–1304

34. Friedrich, T., Kroger, B., Bialojan, S., Lemaire, H. G., Hoffen, H. W., Reuschenbach, P., Otte, M., and Dodt, J. (1993) J. Biol. Chem. 268, 16216–16222

35. Laskowska, M., Kato, I., Ardwel, W., Cook, J., Denton, A., Empie, M. W., Kuhl, W. J., Park, S. J., Parks, K., Schatzley, B. L., Schoenberger, O. L., Tashiro, M., Vichot, G., Whaley, H. E., Wieczorek, A., and Wieczorek, M. (1987) Biochemistry 26, 202–210

36. Söderhäll, K.,寺田, P., Slomiany, B., Stark, J., and Söderhäll, K. (1994) Biochem. Biophys. Res. Commun. 208, 99–104

37. Kikuchi, N., Nagata, K., Yosihara, K., and Ogawa, M. J. (1985) J. Biochem. (Tokyo) 98, 687–694

38. Brenner, S. (1988) Nature 334, 528–530

6. R. Ghai, personal communication.

7. M. Klein and S.-I. Hu, unpublished observations.
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