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ORIGINAL PAPER

Characterization of the telomere regions of scleractinian coral, *Acropora surculosa*

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Abstract Terminal ends of vertebrate chromosomes are protected by tandem repeats of the sequence (TTAGGG). First thought to be vertebrate specific, $(TTAGGG)_n$ has recently been identified in several aquatic invertebrates including sea urchin (*Strongylocentrotus purpuratus*), bay scallop (*Argopecten irradians*), and wedgeshell clam (*Donax trunculus*). We analyzed genomic DNA from scleractinian corals, *Acropora surculosa, Favia pallida, Leptoria phrygia*, and *Goniastrea retiformis* to determine the telomere sequence. Southern blot analysis suggests the presence of the vertebrate telomere repeats in all four species. Treatment of *A. surculosa* sperm DNA with *Bal31* exonuclease revealed progressive shortening of the DNA fragments positive for the $(TTAGGG)_{22}$ sequence, supporting location of the repeats at the chromosome ends. The presence of the vertebrate telomere repeats in corals is evidence that the $(TTAGGG)_n$ sequence is highly conserved among a divergent group of vertebrate and invertebrate species. Corals are members of the Lower Metazoans, the group of organisms that span the gap between the fungi and higher metazoans. Corals are the most basal organism reported to have the $(TTAGGG)_n$ sequence to date, which suggests that the vertebrate telomere sequence may be much older than previously thought and that corals may share a number of genes with their higher relatives.

Keywords *Acropora* · Anthozoa · Cnidaria · Coral · Marine invertebrate · Scleractinia · Telomere · Telomeric DNA

Abbreviations

DNA	Deoxyribonucleic Acid
SSC	Salt Sodium Citrate
EST	Expressed Sequence Tag
bp	Basepair
kb	kilobase
SDS	Sodium Dodecyl Sulfate
EDTA	Ethylenediamine Tetraacetic Acid

Introduction

DNA-protein complexes known as telomeres protect ends of linear eukaryotic chromosomes. These specialized regions are essential to the maintenance of chromosomal integrity and stability by preventing end-to-end fusions and degradation of chromosome ends (reviewed in Zakian 1995). Telomeres also appear to be involved in chromosome positioning and segregation during mitosis (reviewed

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in Zakian 1995). Loss of one or more telomeres leads to the interruption of cell growth (Bennett et al. 1993; Sandell 1993).

The DNA portion of the telomere consists of double-stranded tandem repeats of a short nucleotide sequence (5–26 bp). The telomere repeat may be uniform such as $(TTAGGG)_n$ found in vertebrates (Meyne et al. 1989), or $(TTTAGGG)_n$ as found in nearly all plants (Cox et al. 1993; Fuchs et al. 1995; Pich et al. 1996). Invertebrates, fungi, slime molds, and protozoa exhibit a variety of sequences from those identical to the vertebrate repeats (i.e., *Cladosporium* fungi) (Coleman et al. 1993) to the irregular repeat (TG_{1-3}) in *Saccharomyces* yeasts (Shampay et al. 1984) and the complex repeat $(ACACCAAGAAGTTAGACATCCGT)_n$ in *Candida* fungi (Muller et al. 1991; McEachern and Hicks 1993). Most insect orders may be classified into those that have $(TTAGG)_n$ repeats and those that do not, with the exception of the Coleoptera, which are heterogeneous (Okazaki et al. 1993; Meyne and Imai 1995; Sahara et al. 1999). Terrestrial worm, *Ascaris lumbricoides*, has telomere repeats of $(TTAGGC)_n$ (Muller et al. 1991). The number of repeats also varies from as short as 36 bp in *Oxytricha fallax* (Pluta et al. 1982) up to 150 kb in *Mus musculus* (Kipling and Cooke 1990; Starling et al. 1990; Zijlmans et al. 1997).

Limited information is available for the telomeric regions of marine invertebrates. Of those organisms analyzed to date, genomic DNA from a species of sea urchin (*Strongylocentrotus purpuratus*) (Lejnine et al. 1995), ragworm (*Platynereis dumerilii*) (Jha et al. 1995), keel worm (*Pomatoceros lamarcki*) (Jha et al. 1995), pacific oyster (*Crassostrea gigas*) (Guo and Allen 1997), bay scallop (*Argopecten irradians*) (Estabrooks 1999), neogastropod (*Fasciolaria lignaria*) (Vitturi et al. 2000), blue mussel (*Mytilus galloprovincialis*) (Plohl et al. 2002), and sea cucumber (*Holothuria tubulosa*) (Plohl et al. 2002) show strong homology to probes of the vertebrate sequence $(TTAGGG)_n$. Recently, the telomere sequence of the wedgeshell clam, *Donax trunculus*, was cloned and found to be identical to the vertebrate sequence (Plohl et al. 2002).

Phylum Cnidaria contains the simplest animals with tissue level organization, *e.g.* jellyfish, hydra and corals. Corals are thought to be the most basal class of the Cnidarians, making them an important metazoan ancestor (Bridge et al. 1992, 1995; Odorico and Miller 1997). Here we show evidence that the telomere regions of the scleractinian corals, *Acropora surculosa, Favia pallida, Leptoria phrygia*, and *Goniastrea retiformis* contain repeats of the vertebrate telomere sequence $(TTAGGG)_n$.

Materials and methods

Specimens

Sperm from *A. surculosa, F. pallida, L. phrygia*, and *G. retiformis* were collected during spawning in outdoor seawater tanks at the University of Guam Marine Laboratories, Mangilao, Guam. Spawning corals were isolated in plastic buckets filled with seawater and floating gamete bundles, upon release, were collected with plastic pipets. Eggs were removed and the sperm collected by centrifugation at 1600 *g* for 30 min at room temperature.

Purification of Genomic DNA

High molecular weight DNA was isolated from the sperm using a modified DNeasy Tissue Kit protocol (Qiagen, Valencia, CA). Briefly, the sperm pellets were resuspended in 10 ml Buffer 1 (150 mM NaCl, 10 mM EDTA pH 8). Sperm were repelleted and all but 1 ml of the buffer was removed. The sperm pellet was resuspended by vortexing and 300 µl of Buffer 2 (100 mM Tris HCl pH 8, 10 mM EDTA pH 8, 500 mM NaCl, 1% SDS, 2% 2-mercaptoethanol) and 200 µg Proteinase K (Qiagen) were added. The suspension was incubated for 2 h at 55°C with occasional inversion to mix. An additional 80 µg of Proteinase K was added and the suspension was returned to 55°C for 2 h. At the end of the incubation, 400 µl Buffer AL (Qiagen) and 400 µl 100% ethanol were added and the suspension was mixed by inversion. The suspension was loaded onto the DNeasy spin column and washed with Buffers AW1 and AW2 as directed by the manufacturer. Genomic DNA was eluted in 400 µl Buffer AE and subsequently concentrated by standard ethanol precipitation.

Southern hybridization

Sperm DNA (5 µg) from *A. surculosa, F. pallida, L. phrygia*, and *G. retiformis* was digested to completion with *Hind*III (New England Biolabs, Beverly, MA). Digested DNA was electrophoresed on a 0.7% agarose gel and transferred onto Hybond N membrane (Amersham Biosciences, Piscataway, NJ) by alkaline blotting in 0.4 M NaOH following partial depurination. DNA probes $(TTAGGG)_{22}$ (Plohl et al. 2002), $(TTAGG)_3$, $(TTAGGC)_3$, and $(TTAGGGG)_3$ were labeled with [α-^{32}P]dCTP (Perkin Elmer Life Sciences, Boston, MA) by random priming with Ready-To-Go labeling beads (Amersham Biosciences). Labeled probes were passed through Quick Spin columns (Roche Diagnostics Corporation, Indianapolis, IN) to remove unincorporated nucleotides. Following purification,

the probes were scanned with a Geiger-Müller detector to verify the presence of radiolabeled probe. Hybridizations were performed for 3 h at 42°C (probes $(TTAGG)_3$, $(TTAGGC)_3$ and $(TTAGGGG)_3$) or 65°C ($(TTAGGG)_{22}$) in Rapid-hyb buffer (Amersham Biosciences). Membranes were washed under normal stringency conditions: 2×15 min in 5× SSC, 0.5% SDS at RT; 2×15 min in 1× SSC, 0.5% SDS at 37°C; 2×15 min in 0.1× SSC, 1% SDS at 37°C. Blots hybridized with the $(TTAGGG)_{22}$ probe were washed in additional high stringency washes: 2×15 min in 0.1× SSC, 1% SDS at 65°C. Blots were exposed to x-ray film at −70°C.

Bal31 exonuclease and *Hind*III digestion

Sperm DNA (20 µg) from *A. surculosa* was digested with 10 U *Bal31* exonuclease (New England Biolabs) in the supplied buffer for 0, 10, 20, 30, 40, and 50 min at 30°C. Digestions were stopped with the addition of EDTA to 50 mM followed by incubation at 75°C for 10 min. Samples were ethanol precipitated, resuspended in sterile water and digested with *Hind*III. Digests were electrophoresed on a 0.7% agarose gel, transferred to Hybond N membrane, and hybridized with the $(TTAGGG)_{22}$ probe, as described above.

Dot-blot quantitation

Graded amounts of *A. surculosa* sperm DNA and the recombinant $(TTAGGG)_{22}$ clone were spotted onto Hybond N membrane and probed with the [α-^{32}P] labeled $(TTAGGG)_{22}$ fragment. The membrane was hybridized and washed under the conditions described above. Spot intensities were quantified with a VersaDoc Imaging System (BioRad, Hercules, CA) and Quantity One software.

Results

Detection of the telomeric repeat sequence in scleractinian corals

The radiolabeled telomere probes $(TTAGGG)_{22}$ (Plohl et al. 2002), $(TTAGG)_3$, $(TTAGGC)_3$, and $(TTAGGGG)_3$, were hybridized to *Hind*III-digested DNA from *A. surculosa, F. pallida, L. phrygia*, and *G. retiformis* (Figs. 1–4). All four coral species showed strong positive signals when hybridized with the wedgeshell clam probe, $(TTAGGG)_{22}$ (Fig. 1). The strongest region of hybridization in *A. surculosa* fell below 4 kb in length while *F. pallida, L. phrygia* and *G. retiformis* showed strongest hybridization around 9 kb. The $(TTAGGG)_{22}$ signals remained strong through multiple high stringency washes suggesting the coral telomere sequence in each coral species analyzed is highly similar if not identical to that of the wedgeshell clam. Hybridization with the probes $(TTAGGC)_3$ (Fig. 2), $(TTAGG)_3$ (Fig. 3), and $(TTAGGGG)_3$ (Fig. 4) resulted in weak signals that were lost with medium stringency washes.

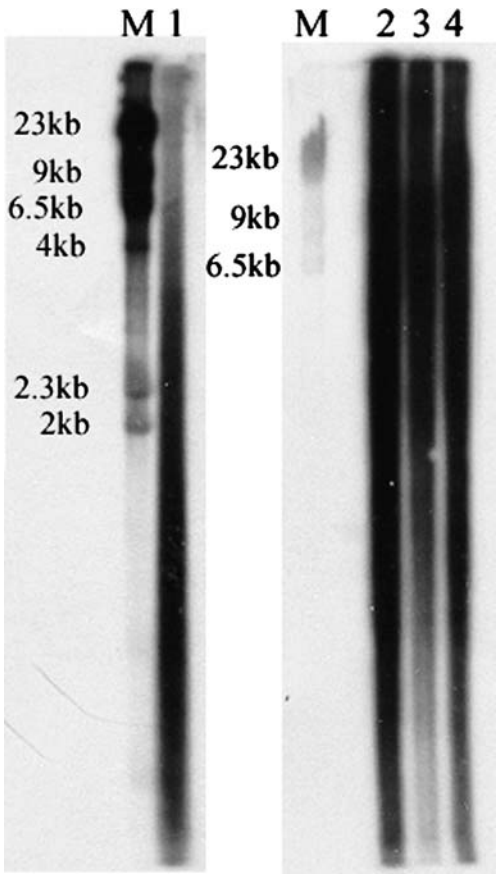

Fig. 1 Southern hybridization of *Hind*III-digested *Acropora surculosa* (Lane 1), *Favia pallida* (Lane 2), *Leptoria phrygia* (Lane 3), and *Goniastrea retiformis* (Lane 4) sperm DNA with radiolabeled $(TTAGGG)_{22}$ probe. M=*Hind*III-digested lambda DNA marker

Bal31 exonuclease analysis and terminal location of *A. surculosa* telomere repeats

To determine if the *A. surculosa* telomere repeats are located at the chromosome ends, *A. surculosa* sperm DNA was digested with *Bal31* exonuclease. *Bal31* progressively removes nucleotides inward from the chromosome ends. If the sequence of interest is located at or near the end of the chromosomes, a decrease in the size and strength of the signal obtained by Southern blot analysis will be observed. Prior to Bal31 digestion, the $(TTAGGG)_{22}$ probe hybridized to a broad band of DNA approximately 500 bp-9 kb in size (Fig. 5, lane 1). After 10 min of digestion with *Bal31* (Fig. 5, lane 2), the intensity of the hybridization signal as well as the size of the telomere band had decreased. After

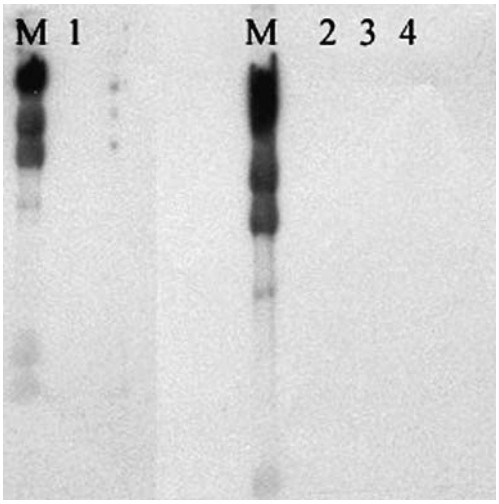

Fig. 2 Southern hybridization of *Hind*III-digested *A. surculosa* (Lane 1), *F. pallida* (Lane 2), *L. phrygia* (Lane 3), and *G. retiformis* (Lane 4) sperm DNA with radiolabeled $(TTAGGC)_3$ probe. M=*Hind*III-digested lambda DNA marker

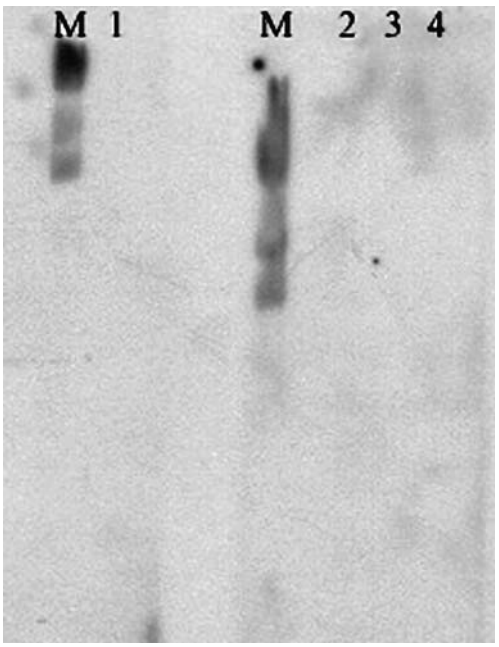

Fig. 3 Southern hybridization of *Hind*III-digested *A. surculosa* (Lane 1), *F. pallida* (Lane 2), *L. phrygia* (Lane 3), and *G. retiformis* (Lane 4) sperm DNA with radiolabeled $(TTAGG)_3$ probe. M = *Hind*III-digested lambda DNA marker

30 min of *Bal31* digestion (Fig. 5, lane 4), the telomere band had dramatically decreased in size and intensity.

Relative amount of (TTAGGG) in *A. surculosa* coral genome

In order to approximate the relative abundance of telomeric sequence in the *A. surculosa* genome, increasing amounts

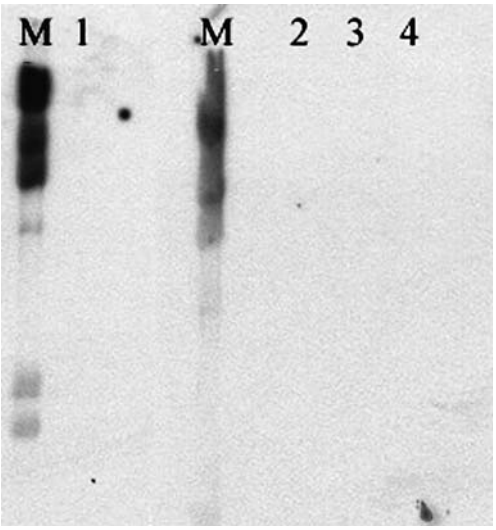

Fig. 4 Southern hybridization of *Hind*III-digested *A. surculosa* (Lane 1), *F. pallida* (Lane 2), *L. phrygia* (Lane 3), and *G. retiformis* (Lane 4) sperm DNA with radiolabeled $(TTAGGGG)_3$ probe. M=*Hind*III-digested lambda DNA marker

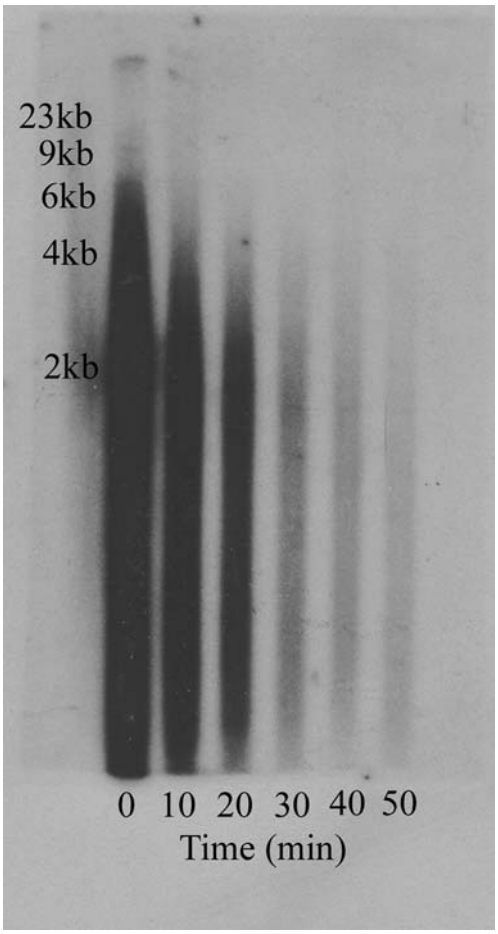

Fig. 5 Southern hybridization of *Bal31*/*Hind*III-digested sperm DNA with radiolabeled $(TTAGGG)_{22}$ probe

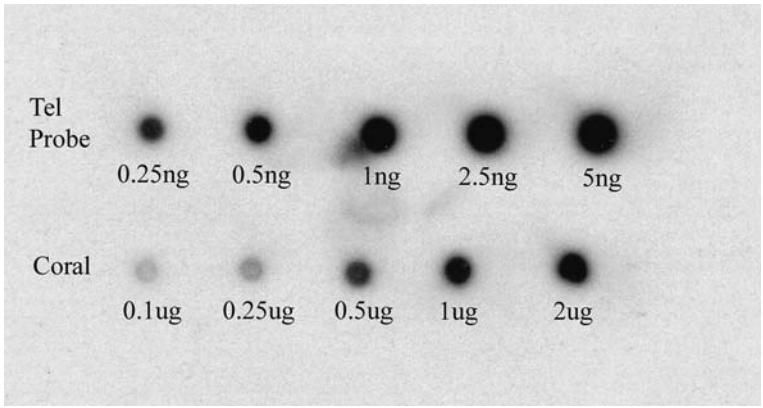

Fig. 6 Southern hybridization of graded amounts of *A. surculosa* sperm DNA and the recombinant $(TTAGGG)_{22}$ clone with the radiolabeled $(TTAGGG)_{22}$ insert probe

Table 1 Densitometry values for dot blot intensity[a]

Tel Probe[b]	0.48 (0.25 ng)	1.24 (0.50 ng)	1.79 (1 ng)	3.66 (2.5 ng)	3.94 (5 ng)
Coral[b]	0.77 (0.10 μg)	1.40 (0.25 μg)	2.73 (0.50 μg)	4.81 (1.0 μg)	6.21 (2.0 μg)

[a] Values represent the mean of two independent experiments and have been adjusted for background

[b] The known amounts of DNA placed on the blot are given in parentheses

of the recombinant clone containing the $(TTAGGG)_{22}$ insert along with graded amounts of *A. surculosa* sperm DNA were dot-blotted. Hybridization with the ^{32}P-labeled $(TTAGGG)_{22}$ insert produced signals of varying intensity (Fig. 6) that were quantified by densitometry (Table 1).

The amount of $(TTAGGG)_{22}$ insert was determined to be 5% of the total construct (cloning vector + insert) based on the sizes of the insert and the vector. Using this percentage, we determined the amount of repeats in the 0.5 ng Tel probe as 0.025 ng. The densitometry values for the remaining samples were normalized to the 0.5 ng Tel Probe. The relative amount of telomere repeats in the coral sperm DNA was calculated by multiplying the densitometry values by the amount of repeats in the known Tel Probe sample and dividing by the total amount of coral sperm DNA of that dot. Based on two independent experiments, the abundance of the telomeric repeats in *A. surculosa* is approximately 0.01% (SD=0.005%) of the total sperm DNA.

Following the calculations by Plohl et al. (2002), we estimated the number of copies of the $(TTAGGG)_n$ repeat and the length of the *A. surculosa* telomeres. The haploid DNA complement (C-value) for scleractinian coral, *Siderastrea stellata*, is 1.14 pg (Gregory 2005) or 1.11×10^9 bp. Assuming that *A. surculosa* has the same value, this is equivalent to approximately 16,500 copies of the repeat. Most species of *Acropora*, including *A. surculosa*, have 14 haploid chromosomes (Kenyon 1997). Therefore each chromosome has approximately 1100 (TTAGGG) repeats or 590 repeats per end. The latter value is equal to 3.5 kb of repeat DNA on each chromosome end, which agrees with our results from Southern blotting *Hind*III-digested *A. surculosa* sperm DNA. The broad range of positive hybridization suggests variation between the lengths of the repeats on individual chromosomes as well as intraspecies variation between sperm.

Conclusions

We present evidence to suggest that the telomere regions of the scleractinian corals, *A. surculosa*, *F. pallida, L. phrygia*, and *G. retiformis*, contain repeats of the vertebrate telomere sequence $(TTAGGG)_n$. Southern blot analysis revealed strong hybridization of the $(TTAGGG)_{22}$ probe to sperm DNA from all four coral species, a clear indication for the presence of $(TTAGGG)_n$ repeats in coral telomeres. Probes of telomere repeats $(TTAGG)_3$, $(TTAGGC)_3$, and $(TTAGGGG)_3$, only weakly hybridized to the coral DNA providing additional evidence that the coral telomere regions are composed of the vertebrate sequence.

Similar hybridization results were reported from the telomere analysis of the bay scallop, *A. irradians* (Estabrooks 1999). *Rsa*I and *Hinf*I digested-genomic DNA from the bay scallop hybridized strongly to the vertebrate-type repeat, $(TTAGGG)_3$, but failed to hybridize to probes for $(TTAGGC)_3$, $(TTAGG)_3$, and $(TTGGGG)_3$. The strongest bands were approximately 500 bp and 23 kb in length with additional banding revealed as the amount of DNA increased. The wedgeshell clam had positive bands for the $(TTAGGG)_{22}$ probe between 500–1000 bp and 10–20 kb (Plohl et al. 2002). In addition, the blue mussel *M. galloprovincialis* and sea cucumber *H. tubulosa* hybridized to the $(TTAGGG)_{22}$ probe with positive banding between 500 bp-23 kb and 9–23 kb, respectively.

The preferential decrease in size and signal intensity following *Bal*31 exonuclease digestion suggests that the telomere sequences of *A. surculosa* are located at the ends of the coral chromosomes. These results are consistent with those reported for the bay scallop (Estrabrooks 1999), wedgeshell clam, and sea cucumber (Plohl et al. 2002).

There are reports of telomere sequence probes hybridizing to internal regions of invertebrate chromosomes in addition to the chromosome termini (Nomoto et al. 2001; Plohl et al. 2002). These observations are most likely due to the existence of highly similar sequences, often located in the centromeric regions, or are the result of chromosome fusion events. Internal regions would be protected from digestion by *Bal*31 and would therefore be visible on blots through most if not all of the digestion periods, as illustrated with the digestion of blue mussel DNA (Plohl et al. 2002).

The phyla Cnidaria (corals and jellyfish), Porifera (sponges), Ctenophora (comb jellies), and Placozoa (*Trichoplax adhaerens*) are collectively known as the Lower Metazoans. Commonly thought of as the sister taxa to higher animals, this group of organisms forms the crucial bridge spanning the evolutionary gap between the fungi and higher animals.

Cnidarians are divided into four classes: Cubozoa (box jellies), Hydrozoa (hydra), Scyphozoa (jellies), and Anthozoa (corals). Of the four classes, Anthozoans are the only group to have circular mitochondrial genomes (Bridge et al. 1992), a characteristic of metazoans. The remaining classes have linear mitochondrial DNA, which are believed to be derivations of the circular form. This finding suggests that anthozoans are the most basal class of cnidarians and therefore represent an important step in metazoan evolution. Further evidence to support anthozoans as the most basal class is found in studies of the 18S ribosomal DNA sequences, mitochondrial 16S ribosomal DNA sequences and morphological characters (Bridge et al. 1995), and in the analysis of the 23S-like ribosomal DNA structure (Odorico and Miller 1997).

Genetic studies of the coral genome have provided additional data to support corals as a key step in metazoan evolution. The integrin beta subunit from scleractinian coral, *Acropora millepora*, is reported to have a remarkable 45% identity to the human sequence (Brower et al. 1997). Homologs for several metazoan developmental genes including *dpp/BMP2/4* (Hayward et al. 2002; Hwang et al. 2003), *Pax* (Catmull et al. 1998; Miller et al. 2000), *Hox* (Hayward et al. 2001), and *snail* (Hayward et al. 2004) have been reported. The identification of a coral homolog of *snail* is particularly interesting since the gene product is associated with epithelial-mesenchyme transition or morphogenetic movements in triploblastic organisms and corals are diploblastic. Recently, the gene for a plasma-membrane calcium pump (PMCA), which is responsible for long-term regulation of resting intracellular calcium levels, was cloned from the coral *Stylophora pistillata* (Zoccolo et al. 2004). Sequence comparison revealed that the coral PMCA was more closely related to the vertebrate sequence than to *Caenorhabditis elegans*. Lastly, a study comparing expressed sequence tags (ESTs) from the coral *Acropora millepora* to similar sequences from *Drosophila melanogaster, C. elegans*, and *Homo sapiens* found that *A. millepora* had stronger sequence similarities with human than with *D. melanogaster* or *C. elegans* (Kortschak et al. 2003). Genes involved in the analysis included transcription factors and metabolic proteins thought to be vertebrate specific. The authors suggest that gene loss may have occurred during the evolution of *D. melanogaster, C. elegans* and other invertebrates while genes thought to have emerged with the rise of vertebrates may have originated in a metazoan ancestor, specifically corals.

Identification of the vertebrate telomere repeat sequence in a number of divergent species including invertebrates, suggests that the origin of telomeres, and perhaps the associated maintenance protein, telomerase, is quite ancient. Plohl et al. (2002) put forth the idea that $(TTAGGG)_n$ may actually be the consensus sequence for telomere repeats. Variations of this sequence, such as the insect sequence $(TTAGG)_n$ (Okazaki et al., 1993; Meyne and Imai 1995), the *Tetrahymena thermaphila* $(TTGGGG)_n$ (Blackburn and Chiou 1981), and the *Ascaris lumbricoides* $(TTAGGC)_n$ (Muller et al. 1991) may represent evolutionary changes as these organisms branched from the eukaryotic tree. As previously mentioned, *D. melanogaster* and *C. elegans* appear to have lost several genes during evolution that corals continue to share with humans and other higher metazoans.

Cnidarians diverged from their bilateral sister group approximately 700 million years ago. Here we report the identification of the vertebrate telomere sequence $(TTAGGG)_n$ in four scleractinian corals. Presence of this repeat in corals suggests that the sequence is more ancient than previously thought and indicates that corals may represent an essential step in metazoan evolution.

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References

Bennett CB, Lewis AL, Baldwin KK, Resnick MA (1993) Lethality induced by a single site-specific double-strand break in a dispensable yeast plasmid. Proc Natl Acad Sci USA 90:5613–5617

Blackburn EH, Chiou S (1981) Non-nucleosomal packaging of a tandemly repeated DNA sequence at termini of extrachromosomal DNA coding for rRNA in Tetrahymena. Proc Natl Acad Sci USA 78:2263–2267

Bridge D, Cunningham CW, DeSalle R, Buss LW (1995) Class-level relationships in the phylum Cnidaria: Molecular and morphological evidence. Mol Biol Evol 12:679–689

Bridge D, Cunningham CW, Schierwater B, DeSalle R, Buss LW (1992) Class-level relationships in the phylum Cnidaria: evidence from mitochondrial genome structure. Proc Natl Acad Sci USA 89:8750–8753

Brower DL, Brower SM, Hayward DC, Ball EE (1997) Molecular evolution of integrins: Genes encoding integrin β subunits from a coral and a sponge. Proc Natl Acad Sci USA 94:9182–9187

Catmull J, Hayward DC, McIntyre NE, Reece-Hoyes JS, Mastro R, Callaerts P, Ball EE, Miller DJ 1998 *Pax-6* origins—implication from the structure of two coral *Pax* genes. Dev. Genes Evol 208:352–356

Coleman MJ, McHale MT, Arnau J, Watson A, Oliver RP (1993) Cloning and characterization of telomeric DNA from Cladosporium fulvum. Gene 132:67–73

Cox AV, Bennett ST, Parokonny AS, Kenton A, Callimassia MA, Bennett MD (1993) Comparison of plant telomere locations using a PCR-generated synthetic probe. Ann Botany 72:239–247

Estabrooks SL (1999) The telomeres of the bay scallop, *Argopecten irradians* (Lamarck). Shellfish Res 18:401–404

Fuchs J, Brandes A, Schubert I (1995) Telomere sequence localization and karyotype evolution in higher plants. Plant Syst Evol 196:227–241

Gregory TR (2005) Animal Genome Size Database. http://www.genomesize.com

Guo X, Allen SK Jr (1997) Fluorescence in situ hybridization of vertebrate telomere sequence to chromosome ends of the pacific oyster, *Crassostrea gigas thunberg*. Jour Shellfish Res 16:87–89

Hayward DC, Catmull J, Reece-Hoyes JS, Berghammer H, Dodd H, Hann SJ, Miller DJ, Ball EE (2001) Gene structure and larval expression of *cnox-2Am* from the coral *Acropora millepora*. Dev Genes Evol 211:10–19

Hayward DC, Miller DJ, Ball EE (2004) *snail* expression during embryonic development of the coral *Acropora*: blurring the diploblast/triploblast divide. Dev Genes Evol 214:257–260

Hayward DC, Samuel G, Pontynen PC, Catmull J, Saint R, Miller DJ, Ball EE (2002) Localized expression of a *dpp/BMP2/4* ortholog in a coral embryo. Proc Natl Acad Sci USA 99:8106–8111

Hwang S-PL, Chen CA, Peng M-Y, Chen C-P (2003) Evolutionary conservation of the bone morphogenetic protein 2/4 gene between diploblastic and triploblastic metazoans. Zool Stud 42:227–234

Jha AN, Dominguez I, Balajee AS, Hutchinson TH, Dixon DR, Natarajan AT (1995) Localization of a vertebrate telomeric sequence in the chromosomes of two marine worms (phylum Annelida: class polychaeta). Chromosome Res 3:507–508

Kenyon JC (1997) Models of reticulate evolution in the coral genus *Acropora* based on chromosome numbers: parallels with plants. Evolution 51:756–767

Kipling D, Cooke HJ (1990) Hypervariable ultra-long telomeres in mice. Nature 347:400–402

Kortschak RD, Samuel G, Saint R, Miller DJ (2003) EST analysis of the Cnidarian *Acropora millepora* reveals extensive gene loss and rapid sequence divergence in the model invertebrates. Curr Biol 13:2190–2195

Lejnine S, Makarov VL, Langmore JP (1995) Conserved nucleoprotein structure at the ends of vertebrate and invertebrate chromosomes. Proc Natl Acad Sci USA 92:2393–2397

McEachern MJ, Hicks JB (1993) Unusually large telomeric repeats in the yeast *Candida albicans*. Mol Cell Biol 13:551–560

Meyne J, Ratliff RL, Mayzis RK (1989) Conservation of the human telomere sequence $(TTAGGG)_n$ among vertebrates. Proc Natl Acad Sci USA 86:7049–7053

Meyne JH, Imai HT (1995) FISH analysis of the telomere sequences of bulldog ants (Myrmecia:Formicidae). Chromosoma 104:14–18

Miller DJ, Hayward DC, Reece-Hoyes JS, Scholten I, Catmull J, Gehring WJ, Callaerts P, Larsen JE, Ball EE (2000) *Pax* gene diversity in the basal cnidarian *Acropora millepora* (Cnidaria, Anthozoa): Implications for the evolution of the *Pax* gene family. Proc Natl Acad Sci USA 97:4475–4480

Muller F, Wicky C, Spicker A, Tobler H (1991) New telomere formation after developmentally regulated chromosomal breakage during the process of chromatin dimunition in *Ascaris lumbricoides*. Cell 67:815–822

Nomoto Y, Hirai M, Ueshima R (2001) Cloning of molluscan telomere DNA with $(TTAGGG)_n$ repeat and its chromosomal location in the freshwater snail *Biwameiania habei*. Zool Sci 18:417–422

Odorico DM, Miller DJ (1997) Internal and external relationships of the Cnidaria: Implications of primary and predicted secondary structure of the 5′-end of the 23S-like rDNA. Proc Roy Soc London B 264:77–82

Okazaki S, Tsuchida K, Mackawa H, Fujiwara H (1993) Identification of a pentanucleotide telomere sequence $(TTAGG)_n$ in the silkworm *Bombyx mori* and in other insects. Mol Cell Biol 13:1424–1432

Pich U, Fuchs J, Schubert I (1996) How do *Alliaceae* stabilize their chromosome ends in the absence of TTTAGGG sequence? Chromosome Res 4: 207–213

Plohl M, Prats E, Martinez-Lage A, Gonzalez-Tizon A, Mendez J, Cornudella L (2002) Telomeric localization of the vertebrate-type hexamer repeat $(TTAGGG)_n$, in the wedgeshell clam *Donax trunculus* and other marine invertebrate genomes. Jour Biol Chem 277:19839–19846

Pluta AF, Kaine BP, Spear BB (1982) The terminal organization of macronuclear DNA in *Oxytricha fallax*. Nuc Acids Res 10:8145–8154

Sahara KF, Marec F, Traut W (1999) TTAGG telomeric repeats in chromosomes of some insects and other arthropods. Chromosome Res 7:449–460

Sandell LL, Zakian VA (1993) Loss of a yeast telomere: arrest, recovery, and chromosome loss. Cell 75:729–739

Shampay J, Szostak JW, Blackburn EH (1984) DNA sequences of telomeres maintained in yeast. Nature 310:154–157

Starling JA, Maule J, Hastie ND, Allshire RC (1990) Extensive telomere repeat arrays in mouse are hypervariable. Nuc Acids Res 18:6881–6888

Vitturi R, Colomba MS, Gianguzza P, Pirrone AM (2000) Chromosomal location of ribosomal DNA (rDNA), $(GATA)_n$, and $(TTAGGG)_n$ telomeric repeats in the neogastropod *Fasciolaria lignaria* (Mollusca:Prosobranchia). Genetica 108:253–257

Zakian VA (1995) Telomeres: Beginning to understand the end. Science 270:1601–1606

Zijlmans JM, Martens UM, Poon SS, Raap AK, Tanke HJ, Ward RK, Lansdorp PM (1997) Telomeres in the mouse have large inter-chromosomal variations in the number of T_2AG_3 repeats. Proc Natl Acad Sci USA 94:7423–7428

Zoccola D, Tambutté E, Kulhanek E, Puverel S, Scimeca J-C, Allemand D, Tambutté S (2004) Molecular cloning and localization of a PMCA P-type calcium ATPase from the coral *Stylophora pistillata*. Biochim Biophys Acta 1663:117–126