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Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes

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Abstract. The intrachromosomal distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric repeat was determined for 100 vertebrate species. The most common non-telomeric location of this sequence was in the pericentric regions of chromosomes. A variety of species showed relatively large amounts of this sequence present within regions of constitutive heterochromatin. We discuss possible relationships between the non-telomeric distribution of the $(TTAGGG)_n$ sequence and the process of karyotype evolution, during which these sites may provide potential new telomeres.

Introduction

Eukaryotic genomes are extremely complex and highly variable in DNA content and organization, sometimes even among closely related species. The amount and arrangement of DNA in genomes can be altered by any of several mechanisms. Small regions of DNA can be deleted, duplicated, mutated, or translocated. Chromosomes can undergo structural (e.g., translocation, deletion, duplication) or numerical (e.g., fission, fusion, ploidy shift) changes. Fixation of these types of alterations can create genetic barriers contributing to reduced fertility in heterozygotes and possible speciation. As our understanding of the organization and evolution of eukaryotic genomes increases, we become better equipped to address specific questions concerning the relationships among changes in DNA, karyotype evolution, and speciation.

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One set of questions pertains to the maintenance of functional telomeres during karyotypic evolution. Can new telomeres be added de novo? Is a genome limited to a basic number of telomeres that can be changed only by increases in ploidy? Or, is there some mechanism of amplification, addition, and/or rearrangement of telomeric repeat sequences whereby increases and decreases in chromosome number can occur while functional telomeres are maintained on all chromosomal termini?

The isolation of the repetitive DNA sequence located at the extreme termini of vertebrate chromosomes (Moyzis et al. 1988; Wurster-Hill et al. 1989; Meyne et al. 1989) allows examination of these questions. This sequence, $(TTAGGG)_n$, was originally isolated from a human repetitive DNA library by high stringency screening with rodent repetitive DNA (Moyzis et al. 1988). During initial studies of other vertebrates, it was observed that, in addition to telomeric sites, relatively large blocks of this repeat sequence were also present at non-telomeric sites in some species. The genomes of most eukaryotes contain many families of repetitive DNA sequences. Many of these repeats occur in the form of tandem arrays localized near centromeres or telomeres or, less frequently, at interstitial regions or whole chromosome arms. The $(TTAGGG)_n$ repeat is known to be a component of the satellite DNA of some mammalian species (Southern 1970; Fry and Salser 1977; Fanning 1987; Arnason et al. 1988). In this paper we describe the variability in amount and distribution of the $(TTAGGG)_n$ sequence in the chromosomes of a diverse group of vertebrate species ranging from fish to human. The variation in chromosomal distribution patterns of major, non-telomeric sites of this sequence among species points to an intriguing evolutionary history that may be related to the mechanisms of karyotypic evolution during speciation (White 1978; Baker and Bickman 1986).

Materials and methods

The source of cells used for metaphase preparations differed with the species studied. In some cases, lymphocyte or fibroblast cultures were grown under conditions optimized for the species of origin. For other species, direct bone marrow preparations were collected, usually from animals treated with yeast extract to stimulate cell division. In most cases, mitotic blocks were applied before harvest. Air-dried slides were made from standard methanol:acetic acid (3:1) fixed preparations.

In situ hybridization to metaphase chromosomes utilized the biotin-labeled deoxynucleotide oligomers $(GGGTTA)_7$ and $(TAACCC)_7$, as described previously (Moyzis et al. 1988). After RNase treatment (100 µg/ml in 2 × SSC, pH 7), the slides were denatured in 70% formamide in 2 × SSC at 70° C for 2 min (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate). The denatured slides were hybridized overnight with 0.4 µg/ml probe DNA in 30% formamide, 2 × SSC, and 500 µg/ml *Escherichia coli* carrier DNA. All slides were given five 2 min rinses in 2 × SSC at 42° C. Slides from at least one species of each order also were washed in 0.2 × SSC, and the temperature was gradually raised to 50° C for 5 min (Meyne et al. 1989). This "melt" wash step was added to increase the stringency of hybridization to confirm the presence of the $(TTAGGG)_n$ sequence. The hybridized probe was detected by fluorescein isothiocyanate (FITC)-labeled avidin and a single round of anti-avidin antibody to amplify the signal. Fluorescence microscopy and photography were with a Zeiss Axioplan and Kodacolor 400 film, respectively.

Results

We evaluated at least one species of as many orders of vertebrates as possible to ascertain the distribution of the major non-telomeric sites of the $(TTAGGG)_n$ repeat sequence. As expected, the availability of metaphase chromosome preparations was the limiting factor in determining taxonomic diversity of the species studied. We were, however, able to obtain chromosomes from 100 species representing 14 Orders of mammals, 5 Orders of birds, 2 Orders of reptiles, 1 Order of amphibians, and 2 Orders of fishes. The intrachromosomal location of tandem repeats of the $(TTAGGG)_n$ sequence was determined by in situ hybridization of biotinylated oligomers of this sequence followed by fluorescence detection of the hybridized DNA. The termini of the chromosomes of each of the 100 species hybridized with the telomere probe, strongly suggesting that the $(TTAGGG)_n$ sequence is the functional vertebrate telomere (Meyne et al. 1989). Not all telomeres are clearly labeled in each metaphase. Some telomeres have only faint hybridization signals, but the intensity of label appears to be random. The fluorescent label is at the very end of prometaphase chromosomes, but, as the chromosomes condense, counterstained chromosomal DNA can often be seen beyond the labeled site. It is not known if this is a function of chromosomal condensation or the result of technical manipulation.

The presence of at least one non-telomeric block of this sequence was also detected in about half of these species. The other species had a telomere-only pattern, although the size of the telomeric blocks varied among chromosomes and among species. These data are summarized in the following sections. Only the major, readily visible and consistent sites of $(TTAGGG)_n$ are reported here. Some species may have short stretches of this sequence that will be detected by analysis of larger numbers of cells or multiple amplifications of the fluorescent signal. It should also be noted that apparent solid blocks of fluorescence do not necessarily mean that only tandem repeats of the $(TTAGGG)_n$ sequence are present in that chromosomal region. Amplification of the fluorescent signal can bridge interspersed sites of hybridization. Species are grouped by Class and Order but are not arranged phylogenetically.

Class: Mammalia

Order: Marsupialia. Only one marsupial, Bennett's wallaby, *Macropus rufogriseus*, was studied. Bands of the telomeric sequence were present at the margins of the centric heterochromatin, and essentially all of the long arm of the Y chromosome was covered with hybridization signal (Fig. 1a). The wallaby chromosomes were quite susceptible to adverse effects of the denaturation process.

Order: Insectivora. Three moles, *Condylura cristata, Scapanus latimanus,* and *Scalopus aquaticus*, each showed different patterns of hybridization. *C. cristata*, the star-nosed mole, had several pairs of chromosomes with bands of telomeric sequence at both margins of the centric heterochromatin (Fig. 1b). *S. latimanus* (broad-footed mole) showed wide bands of signal at one terminus of several chromosomes, and one pair of chromosomes frequently had a centric band. *S. aquaticus* (Eastern mole) had a standard telomere-only pattern with slightly larger, brighter sites of hybridization at one terminus of three pairs of chromosomes. These sites may be nucleolus organizer regions (NORs). The size of the chromosomes, difficulty in obtaining good spreads from bone marrow preparations, and susceptibility to overdenaturation limited the number of metaphases examined for these species of moles.

Order: Edentata. The chromosomes of the giant anteater, *Myrmecophaga tridactyla*, had very prominent terminal hybridization sites (Fig. 1c). In addition, several chromosomes had centric or pericentric bands. Precise identification of the centromere was not always possible after the chromosomes had been denatured and hybridized.

Order: Chiroptera. The chromosomes of several species of bat investigated, *Macrotus waterhousii, Macrotus californicus* (California leaf-nosed bat), *Monophyllus redmani, Molossus molossus,* and *Pteronotus macleayii*, had a telomere-only pattern of hybridization. *Artibeus jamaicensis* had four pairs of chromosomes with centric blocks of the $(TTAGGG)_n$ sequence. *Carollia perspicillata* (Seba's fruit bat) had eight pairs of chromosomes with centric blocks and there was a light band of signal above the secondary constriction (NOR) of the X chromosome

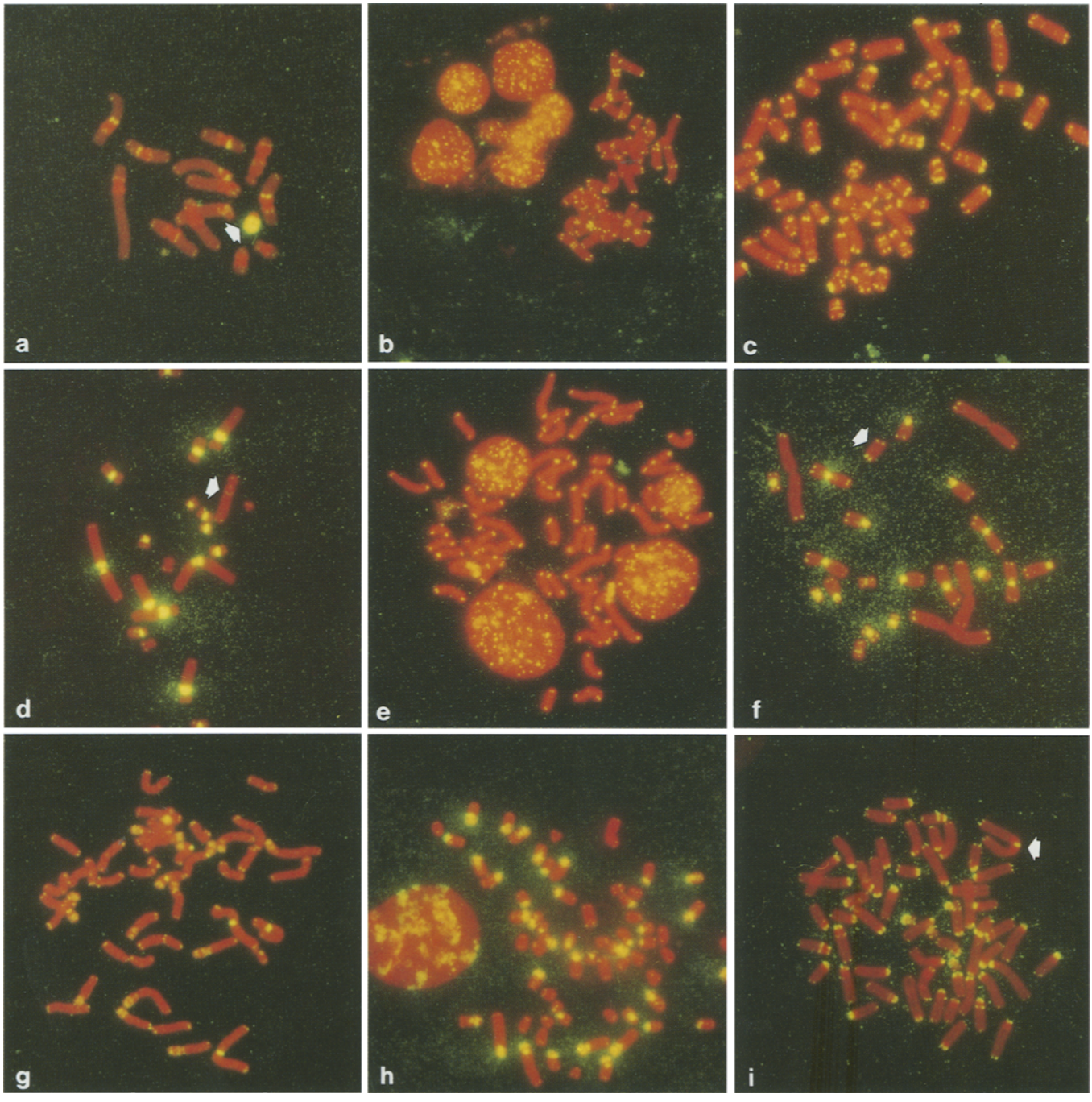

Fig. 1 a–i. Metaphase chromosomes of nine species of mammals after in situ hybridization with biotin-labeled $(GGGTTA)_7 \cdot (TTACCC)_7$ oligomers. Chromosomes were counterstained with propidium iodide (*red*) after detection of the sites of hybridization with fluorescein-labeled avidin (*yellow*). The photos were taken to optimize detection of non-telomeric hybridization sites, so the telomeric signals are not always visible in the metaphases shown. **a** Bennett's wallaby, the *arrow* indicates the intense signal over the long arm of the Y chromosome; **b** star-nosed mole; **c** giant anteater; **d** Seba's fruit bat, the *arrow* indicates the faint signal at the secondary constriction of the X chromosome; **e** *Acomys spinosissimus*; **f** Chinese hamster, the *arrow* indicates a putative fission product chromosome; **g** Syrian or golden hamster, note the band-like pattern in the short arms; **h** Guinea pig; **i** *Sigmodon mascotensis*, note the interstitial bands below the larger pericentric blocks of hybridization signal in some chromosomes. The *arrow* indicates a metacentric chromosome resulting from a Robertsonian fusion

(Fig. 1 d). The fluorescent signal of this region is faint and difficult to detect on photographs. This species was studied before the melt wash conditions were utilized, so it is not known if this region is highly conserved or divergent. Both *Eumops glaucinus* (Wagner's mastiff bat) and a *Chiroderma* species had large centric blocks on more than half of the chromosomes, but better metaphase spreads are required for exact counts.

Order: Rodentia. Of the 27 species of rodents studied, the following had a telomere-only pattern: *Mus musculus, Mus poschiavinus* (tobacco mouse), *Peromyscus boylii* (brush mouse), *Peromyscus leucopus* (white-footed mouse), *Reithrodontomys fulvescens, Reithrodontomys montanus, Aethomys chrysophilus, Uromys caudimaculatus, Microtus agrestis* (European field vole), and *Rattus norvegicus* (Norway rat). Species with predominantly telomere-only patterns and a single additional site were *Rattus rattus* (Fisher 344; one interstitial band on chromosome 2; Moyzis et al. 1988), *Peromyscus eremicus* (cactus mouse; a light signal near the centromere of a medium-sized submetacentric), *Sigmodon fulviventer* (tawny-bellied cotton rat; centric band in one small metacentric), and *Acomys spinosissimus* (a large block at the end of the longest pair of chromosomes; Fig. 1e). *Aethomys namaquensis* had two pairs of chromosomes with centric bands. *Mus dunni* had two pairs of large submetacentrics (putative fusion chromosomes) and two to three pairs of small chromosomes with small centric blocks of $(TTAGGG)_n$ sequence. Other species with three or more sites of hybridization signal, usually in large centric blocks or on the short arms of submetacentric chromosomes include *Reithrodontomys megalotis* (Western harvest mouse), *Reithrodontomys sumichrasti, Cricetulus griseus* (Chinese hamster; Fig. 1f), *Mesocricetus auratus* (golden or Syrian hamster; Fig. 1g), *Microtus montanus* (montane vole), *Cavia porcellus* (Guinea pig; Southern 1970; Fig. 1h), *Meriones libycus, Meriones meridianus, Sigmodon hispidus* (hispid cotton rat), and *Sigmodon mascotensis* (Fig. 1i). The chromosomes of the Mexican ground squirrel, *Spermophilus mexicanus* (Fig. 2a), had a pattern more like the wallaby (Fig. 1a) and star-nosed mole (Fig. 1b), i.e., some chromosomes had bands of the telomeric repeat at the margins of the centric heterochromatin. The patterns of hybridization of $(TTAGGG)_n$ and the relationship between its distribution and karyotype evolution of rodents will be discussed in greater detail in a forthcoming paper.

Order: Carnivora. The 12 carnivores studied showed less variability than the rodents. Sequential G-banding and in situ hybridization were used to identify individual chromosomes of some species. *Ailuropoda melanoleuca* (giant panda), *Mellivora capensis* (ratel) and *Fossa fossa* (fanaloka) had a telomere-only pattern. *Prionodon linsang* (linsang) had an interstitial band in the short arm of chromosome A1. (For carnivore chromosome identifications see Wurster-Hill and Gray 1973, 1975; Wurster-Hill and Centerwall 1982.) *Panthera pardus* (leopard) had a centric signal in chromosome A1 (Fig. 2b), whereas *Felis pardalis* (ocelot) had centric signal in two pairs, A1 and C3. *Melogale* sp. (ferret badger) had centric signals in three *pairs* of chromosomes identified as A1 and carnivore chromosomes nos. 21 and 106. *Mustela frenata* (long-tailed weasel) had one pair with a centric band and several pairs with one terminus with a wider band than the average telomeric signal. The patterns of *Vulpes vulpes*, (red fox) *Urocyon cinereoargenteus*, (grey fox), *Nyctereutes procyonoides procyonoides*, (Chinese raccoon dog), and *Nyctereutes procyonoides viverrinus*, (Japanese raccoon dog) have been presented in detail elsewhere (Wurster-Hill et al. 1989). The B chromosomes of the raccoon dogs had signal along their length. Part of this signal was removed by the melt wash conditions (see Materials and methods), indicating that some of the sequences in these B chromosomes have diverged. It is interesting to note that the raccoon dogs are the only species studied where the B chromosomes have any pattern other than telomere-only.

Order: Lagomorpha. The swamp rabbit, *Sylvilagus aquaticus*, had very large blocks of $(TTAGGG)_n$ on all chromosomes (Fig. 2c). A comparison of the relative amount of this sequence in the swamp rabbit and the Guinea pig (Fig. 1h) indicates it is probably also a major component of the satellite DNA in this rabbit species. The lagomorphs will be the subject of future study.

Order: Cetacea. The fin whale, *Balaenoptera physalus*, showed blocks of hybridization signal on the short arms of several submetacentrics and over the distal half of the long arm of the X chromosome (Fig. 2d). The $(TTAGGG)_n$ sequence has been reported as a component of the satellite DNA of this genus (Arnason et al. 1988).

Order: Pinnipedia. The Pacific harbor seal, *Phoca vitulina*, had a telomere-only pattern.

Order: Proboscidea. Chromosomes of both the African and Indian elephants, *Loxodonta africana* and *Elephas maximus*, were hybridized. Fairly large centric blocks were present on most chromosomes. The African elephant preparation was from a male. Essentially all of the long arm of the Y chromosome showed intense hybridization signal (Fig. 2e).

Order: Hyracoidea. The rock hyrax, *Procavia capensis* had a telomere-only pattern.

Order: Perissodactyla. Equus caballus (domestic horse) and *Tapirus pinchague* (mountain tapir) had a telomere-only pattern. *Diceros bicornis* (black rhinoceros) had several chromosomes with a moderate amount of signal on the short arm.

Order: Artiodactyla. Cervus timorensis (timor deer) had a centric band in the largest pair of chromosomes. Speke's gazelle, *Gazella spekei*, had centric blocks in most of the large (biarmed) chromosomes and a small interstitial band in the largest chromosome. The Addra gazelle, *Gazella dama ruficollis*, had a similar distribution, but the centric blocks were much larger. *Capra hircus* (goat), *Ovis aries* (sheep) and *Okapia johnstoni* (okapi) had telomere-only patterns. *Muntiacus muntjak* (Indian muntjac) and *Cervus albirostris* (white-lipped deer) also had a telomere-only pattern, but the signals were quite small, and cells from other individuals of these species should be examined.

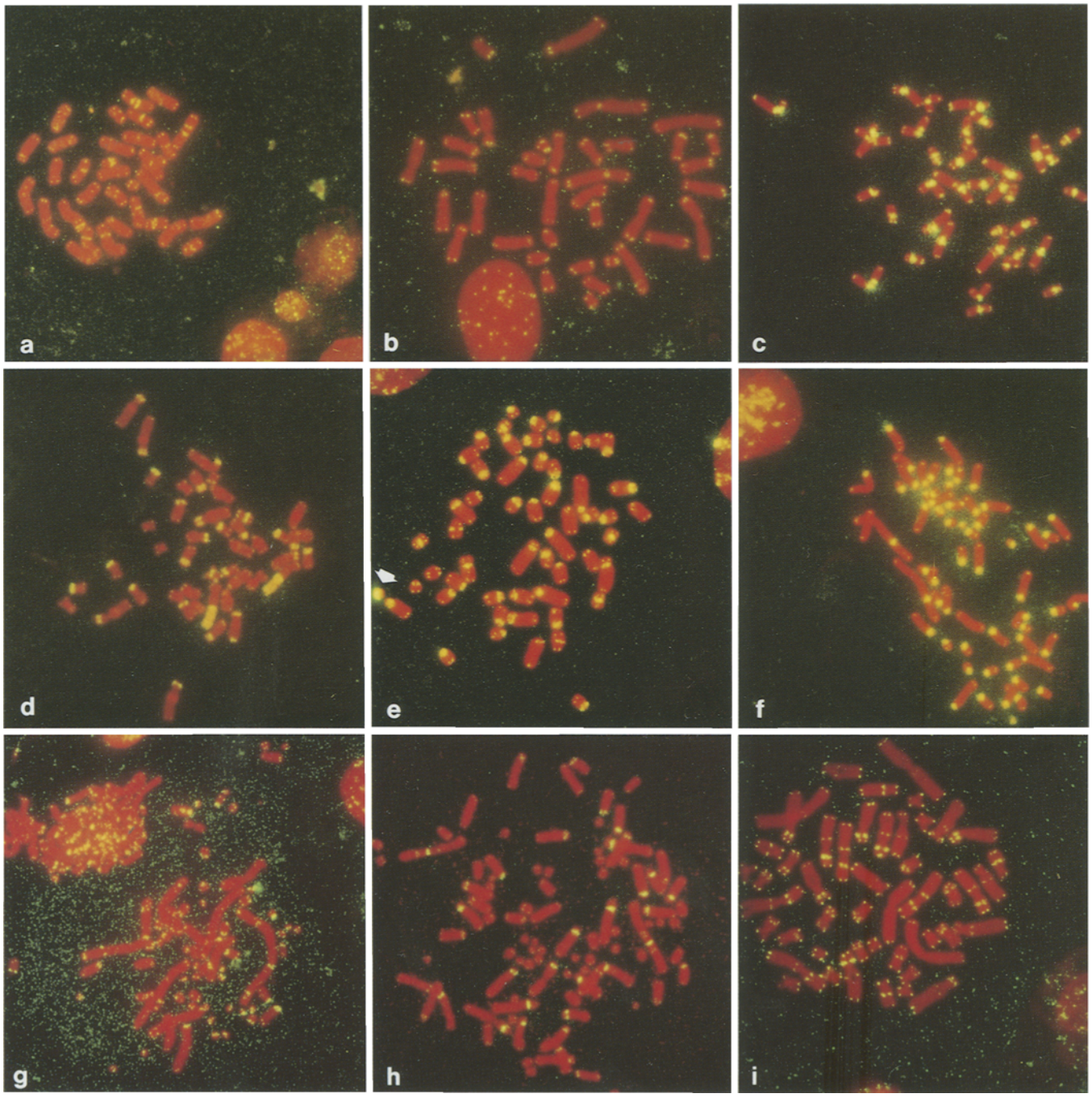

Fig. 2a–i. Metaphase chromosomes of nine species of vertebrates after in situ hybridization (see legend to Fig. 1). **a** Mexican ground squirrel, note bands on either side of centric heterochromatin; **b** leopard; **c** swamp rabbit; **d** fin whale; **e** African elephant, the *arrow* indicates the intense signal over the long arm of the Y chromosome; **f** lemur; **g** domestic chicken; **h** six-lined race-runner; **i** gray tree frog. Note the interstitial bands in some chromosomes of the last three species (**g–i**)

Order: Primates. Homo sapiens, Gorilla gorilla, Pongo pygmaeus, Pan troglodytes, Pan paniscus (pygmy chimp), *Microcebus murinus* (mouse lemur), and *Perodicticus potto* (potto) all had a telomere-only pattern. The black lemur, *Lemur macaco rufus*, had a block of this sequence at the centromere of the large submetacentric pair and blocks at one end of all but one pair of the acrocentrics (Fig. 2f).

Class: Aves

Orders: Columbiformes, Galliformes, Falconiformes, Passeriformes, and Ciconiiformes. The distributions of $(TTAGGG)_n$ given below for seven bird species are all tentative due to various difficulties with the preparations used. *Gallus gallus domesticus* (chicken; Fig. 2g) and *Vireo bellii* (Bell's vireo; Meyne et al. 1989) had very similar

patterns, with a few narrow centric and interstitial bands in the larger chromosomes. *Passer domesticus* (house sparrow), *Cyanocitta cristata* (blue jay), and *Leptoptilas javaniius* (lesser adjutant stork) had a telomere-only pattern. *Buteo jamaicensis* (red-tailed hawk) appeared to have two to three chromosomes with centric bands. *Scardafella inca* (Inca dove) had large blocks of $(TTAGGG)_n$ on all of the smaller chromosomes and a submetacentric with a centric band.

Class: Reptilia

Orders: Sauria and Serpentia. Cnemidophorus sexlineatus (six-lined racerunner; Fig. 2h) and *Cnemidophorus gularis* (Texas spotted whiptail) had similar patterns with centric blocks on most of the chromosomes and bands on one or both sides of the centromere of the largest chromosomes. *Sceloporus olivaceus* (Texas spiny lizard) and *Cophosaurus texanus* (greater earless lizard) had interstial bands of $(TTAGGG)_n$ on at least three pairs of large chromosomes. *Phrynosoma cornutum* (Texas horned lizard) appeared to have a telomere-only pattern. Better metaphase preparations are necessary to confirm the patterns of the latter two species. *Crotalus* sp. (rattlesnake) had a telomere-only pattern (Meyne et al. 1989). This preparation came from a spontaneously transformed aneuploid culture. Direct preparations from a rattlesnake species should be used to confirm this result.

Class: Amphibia

Order: Salientia. Bufo woodhousei fowleri (Fowler's toad) and *Bufo terrestris* (Southern toad) had a telomere-only pattern. *Xenopus laevis* (African clawed frog) had one pair of chromosomes with an interstitial band (from fibroblast culture; Meyne et al. 1989). *Hyla versicolor* (common gray tree frog) has several chromosomes with interstitial bands (Fig. 2i). *Hyla squirella* (squirrel tree frog) and *Hyla chrysoscelis* (Cope's gray tree frog) also have interstitial bands, but in fewer chromosomes than *H. versicolor*.

Class: Pisces

Orders: Cypriniformes and Salmoniformes. Gambusia affinis (mosquito fish), *Eigenmannia virescens* (glass knife fish), and *Eigenmannia* sp. (Toledo et al. 1988) all had telomere-only patterns (Meyne et al. 1989).

Discussion

Telomeres are important molecular entities required for accurate replication and stability of chromosomal ends. Data shown in this and other papers support the hypothesis that the $(TTAGGG)_n$ repeat is the functional vertebrate telomere (Moyzis et al. 1988; Meyne et al. 1989; Riethman et al. 1989). Of the species studied in the present investigation, 55 had, in addition to the telomeres, at least one non-telomeric site of the $(TTAGGG)_n$ sequence, and 44 of these had more than three additional non-telomeric sites. In several of these species, the in situ hybridization pattern indicated that enough of this sequence is present to represent a major component of the repetitive (satellite) DNA of the species (Southern 1970; Fry and Salser 1977; Fanning 1987; Arnason et al. 1988). It is interesting to note that the telomeric sequence was observed at the termini of all chromosomes of all species studied, including B chromosomes and microchromosomes. Although detailed molecular studies will be required to determine whether the $(TTAGGG)_n$ sequence is at the extreme terminus of each telomere of each chromosome, the in situ data indicate the likelihood that none of the species studied have true telocentric chromosomes, as described by Darlington (1939).

Why are variable amounts of the telomeric sequence present at non-telomeric sites in so many species? The origin of these non-telomeric sites of $(TTAGGG)_n$ is unknown. The most obvious mechanisms would be either (1) fusion of chromosomes of ancestral species at the telomeres with subsequent amplification of retained stretches of telomeric sequence, or (2) amplification of short stretches of tandem $(TTAGGG)_n$ sequence present in ancestral karyotypes as latent telomeres. As such, they may merely represent selfish or junk DNA (Doolittle and Sapienza 1980; Orgel and Crick 1980). The in situ hybridization method used in this study would probably not detect very short (<1 kb) stretches of $(TTAGGG)_n$, and true primitive vertebrate karyotypes, in the evolutionary sense, are no longer available for study. Thus, we have no way to determine the exact origin or purpose of the non-telomeric $(TTAGGG)_n$ sequence. Regardless of the mechanism of origin, we present the hypothesis that the rearrangement and amplification of this sequence within vertebrate genomes allows greater flexibility for karyotype changes, by providing alternate sites for telomere formation. Obviously, the chromosomal evolution process also depends upon availability of centromeres, but more information on the molecular composition of this chromosomal component is required to speculate on its changes during karyotype evolution.

The majority of the non-telomeric sites of $(TTAGGG)_n$ are within, or at the margins of, blocks of constitutive heterochromatin identified by C-banding. The amount and chromosomal location of constitutive heterochromatin within a genome does not, however, appear to be an accurate predictor for presence or absence of non-telomeric $(TTAGGG)_n$, even among closely related species. The predominant location of both constitutive heterochromatin and non-telomeric $(TTAGGG)_n$ is in the pericentric regions of chromosomes. As fission and fusion are among the most common mechanisms by which karyotypic changes are thought to occur, pericentric regions are logical sites for potential new telomeres. For example, vertebrate cells are likely to contain telomere terminal transferase activity for replication of chromosomal termini similar to that found in lower eu-

karyotes (Greider and Blackburn 1985). Fission or other breaks in regions of $(TTAGGG)_n$ repeats would provide a template for completion of a "new" telomere at the break site. At present there is no evidence that the telomerase system will identify any sequence other than known telomeric repeats (Greider and Blackburn 1987). Therefore, chromosome breaks in general would not be expected to provide templates for telomerase activity and the broken ends would remain unstable until rejoined with broken ends of fragments containing functional telomeres. The presence and/or amplification of this sequence at chromosomal fusion sites may also allow for maintenance of intranuclear structure. The telomeric region of chromosomes is assumed to be associated with the nuclear membrane. If this attachment is essential for intranuclear structure and chromosomal interaction within the nucleus, retention of this sequence at tandem or centric fusion sites may allow maintenance of the association with the nuclear membrane as shown in Figure 3. Maintenance or loss of this repeat sequence at fusion sites may, therefore, affect intranuclear position and interaction of chromosomes during interphase.

Identification of individual chromosomes of some carnivore species by G-banding before in situ hybridization allowed more detailed analysis of association of non-telomeric $(TTAGGG)_n$ and karyotype evolution for this order. The A1 chromosome of linsang, considered to be homologous to felid A1, has an interstitial short arm band, whereas the A1 in each of the other carnivores studied that possess an A1 chromosome (leopard, ocelot and ferret badger) shows a centric signal. The long arm of A1 is considered to be a very primitive carnivore chromosome with the short arm being a more recent addition through a fusion process (Wurster-Hill and Gray 1975). The interstitial band in the short arm of A1 of the linsang may represent an interstitial inversion that occurred subsequent to the original fusion event. The C3 chromosome in ocelot is the tandem fusion product of the F2 and F3 chromosomes in other felids (Wurster-Hill and Gray 1973), showing, as in the A1, centric signal where fusion has occurred.

This study included species for which G-banding studies have indicated that a series of end-to-end fusions was involved in karyotype evolution such as Indian muntjac (Shi et al. 1980) and *Sigmodon* spp. (Elder and Hsu 1988). Interstitial bands of $(TTAGGG)_n$ were not found at these tandem fusion sites. Perhaps the telomeric repeats are lost during some types of telomere-telomere fusions. Alternately, short regions of telomeric sequence may be retained but be too small to detect. Data obtained for chromosomes resulting from fission and fusion occurring in cells in culture, however, have illustrated the viability of certain types of chromosome rearrangement in some species. Figure 1f shows a cell from a spontaneously transformed cell line from a female Chinese hamster embryo, CCHE/27 (Ray et al. 1986), that has lost one X chromosome and has a "new" chromosome with the G-band pattern of 3*q*. The 3*q* chromosome has been stable for many passages and is most likely the result of a fission event. Some of the

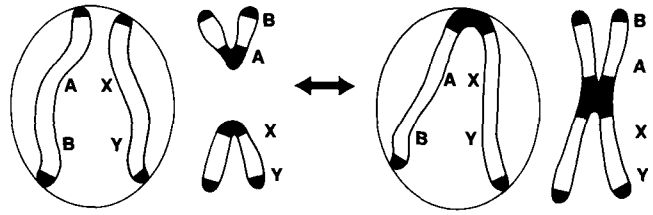

Fig. 3. Diagram illustrating a possible role for pericentric blocks of the $(TTAGGG)_n$ sequence during karyotype evolution. Fission of a chromosome with a pericentric block of this sequence or Robertsonian fusion with retention of this sequence within the pericentric region could preserve attachment sites to the nuclear membrane, thereby retaining relative position of the chromosome(s) within the nucleus. Fusion without retention of $(TTAGGG)_n$ at sites of previous telomeres may allow changes in intranuclear interaction/association of chromosomal regions. Fission of a chromosome within a region of $(TTAGGG)_n$ repeats provides a template for telomere terminal transferase activity to replicate "new" telomeres at break sites ending in this sequence

$(TTAGGG)_n$ sequence from the pericentric region of the pre-fission chromosome was retained to act as a telomere for the fission product. Stable fission product chromosomes have been reported previously in Chinese hamster cell cultures (Kato et al. 1973). Figure 1i shows a cell from a *Sigmodon mascotensis* culture with a spontaneous fusion chromosome. The fusion product chromosome apparently has retained the $(TTAGGG)_n$ sequence from the telomeres within the pericentric region. Only about 20% of the metaphase cells in this culture contained the fusion chromosome, indicating this event was fairly recent. It seems unlikely, therefore, that the pericentric $(TTAGGG)_n$ would have been amplified.

It is tempting to speculate that the presence of non-telomeric $(TTAGGG)_n$ is related to the evolutionary status of the species. Three of the orders studied, Chiroptera, Rodentia, and Primates, included species ranging from the primitive to the advanced within the Order. The more primitive species or those determined to have "primitive" karyotypes (Baker and Bickham 1980) have the $(TTAGGG)_n$ sequences only at the telomeres. Species that are intermediate or considered to be evolving have non-telomeric sites of the sequence. The amplification of this sequence, especially in the pericentric regions of chromosomes, allows a greater latitude for sequential fission and fusion of chromosomes during karyotype evolution. Fusions are frequently accompanied by a reduction in heterochromatin at the fusion junctions (Elder and Hsu 1988). Since organisms can obviously function without non-telomeric blocks of this sequence, their loss during karyotype change would not be detrimental. Thus, the highly evolved species may have little or no non-telomeric $(TTAGGG)_n$, as the amplified regions may be lost during extensive chromosome arrangement. Not all highly evolved species will have lost all of their non-telomeric sequences. Thus primitive species would generally have telomere-only patterns. Evolving species would have several non-telomeric sites. Highly evolved species may have either non-telomeric sites or a telomere-only pattern.

This hypothesis is based on data from a small percentage of the total number of extant vertebrate species. Further studies are necessary to confirm or refute the concepts presented here. Regardless of the outcome, the distribution of $(TTAGGG)_n$ sequence in vertebrate species should prove to be an important and interesting piece of the molecular evolution puzzle.

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References

Arnason U, Allderdice PW, Lien J, Widegren B (1988) Highly repetitive DNA in the baleen whale genera Balaenoptera and Megaptera. J Mol Evol 27:217–221

Baker RJ, Bickham JW (1980) Karyotypic evolution in bats: Evidence of extensive and conservative chromosomal evolution in closely related taxa. Syst Zool 21:239–251

Baker RJ, Bickham JW (1986) Speciation by monobrachial centric fusions. Proc Natl Acad Sci USA 83:8245–8248

Darlington CD (1939) Misdivision and the genetics of the centromere. J Genet 37:341–364

Doolittle WF, Sapienza C (1980) Selfish genes, the phenotype paradigm and genome evolution. Nature 284:601–603

Elder FFB, Hsu TC (1988) Tandem fusion in the evolution of mammalian chromosomes. In: Daniel A (ed) The cytogenetics of mammalian autosomal rearrangements. Alan R. Liss, New York, pp 481–506

Fanning TG (1987) Origin and evolution of a major feline satellite DNA. J Mol Biol 197:627–634

Fry K, Salser W (1977) Nucleotide sequences of HS-α satellite DNA from kangaroo rat Dipodomys ordi and characterization of similar sequences in other rodents. Cell 12:1069–1084

Greider EW, Blackburn EH (1985) Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 43:405–413

Greider EW, Blackburn EH (1987) The telomore terminal transferase of tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. Cell 51:887–898

Kato H, Sagai T, Yosida TH (1973) Stable telocentric chromosomes produced by centric fission in Chinese hamster cells in vitro. Chromosoma 40:183–192

Meyne J, Ratliff RL, Moyzis RK (1989) Conservation of the human telomere sequence $(TTAGGG)_n$ among vertebrates. Proc Natl Acad Sci USA 89:7049–7053

Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, Meyne J, Ratliff RL, Wu JR (1988) A highly conserved repetitive DNA sequence, $(TTAGGG)_n$ present at the telomeres of human chromosomes. Proc Natl Acad Sci USA 85:6622–6626

Orgel LE, Crick FHC (1980) Selfish DNA: the ultimate parasite. Nature 284:604–607

Ray FA, Bartholdi MF, Kraemer PM, Cram LS (1986) Spontaneous in vitro neoplastic evolution: Recurrent chromosome changes of newly immortalized Chinese hamster cells. Cancer Genet Cytogenet 21:35–51

Riethman HC, Moyzis RK, Meyne J, Burke DT, Olsen MV (1989) Cloning human telomeric DNA fragments in Saccharomyces cerevisiae using a yeast artificial-chromosome vector. Proc Natl Acad Sci USA 86:6240–6244

Shi L, Ye Y, Duan X (1980) Comparative cytogenetic studies on the red muntjac, Chinese muntjac, and their F_1 hybrids. Cytogenet Cell Genet 26:22–27

Southern EM (1970) Base sequence and evolution of Guinea pig α-satellite DNA. Nature 227:794–798

Toledo LFA, Viegas-Pequignot E, Foresti F, Filho SAT, Dutrillaux B (1988) BrdU replication patterns demonstrating chromosome homologies in two fish species, genus Eigenmannia. Cytogenet Cell Genet 48:117–120

White MJD (1978) Modes of speciation. Freeman, San Francisco

Wurster-Hill DH, Centerwall WR (1982) The interrelationships of chromosome banding patterns in canids, mustelids, hyena, and felids. Cytogenet Cell Genet 34:178–192

Wurster-Hill DH, Gray CW (1973) Giemsa banding patterns in the chromosomes of twelve species of cats (Felidae). Cytogenet Cell Genet 12:377–397

Wurster-Hill DH, Gray CW (1975) The interrelationships of chromosome banding patterns in procyonids, viverrids, and felids. Cytogenet Cell Genet 15:306–331

Wurster-Hill DH, Ward OG, Davis BH, Park JP, Moyzis RK, Meyne J (1989) Fragile sites, telomeric DNA sequences, B chromosomes and DNA content in raccoon dogs, Nyctereutes procyonoides, with comparative notes on foxes, coyote, wolf and raccoon. Cytogenet Cell Genet 49:278–281