Telomeres in the Chicken: Genome Stability and Chromosome Ends

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ABSTRACT Telomeres are the complex nucleoprotein structures at the termini of linear chromosomes. Telomeric DNA consists of a highly conserved hexanucleotide arranged in tandem repeats. Telomerase, a ribonucleoprotein of the reverse transcriptase family, specifies the sequence of telomeric DNA and maintains telomere array length. Numerous studies in model organisms established the significance of telomere structure and function in regulating genome stability, cellular aging, and oncogenesis. Our overall research objectives are to understand the organization of the telomere arrays in chicken in the context of the unusual organization and specialized features of this higher vertebrate genome (which include a compact genome, numerous microchromosomes, and high recombination rate) and to elucidate the role telomeres play in genome stability impacting cell function and life span. Recent studies found that the chicken genome contains three overlapping size classes of telomere arrays that differ in location and age-related stability: Class I 0.5 to 10 kb, Class II 10 to 40 kb, and Class III 40 kb to 2 Mb. Some notable features of chicken telomere biology are that the chicken genome contains ten times more telomeric DNA than the human genome and the Class III telomere arrays are the largest described for any vertebrate species. In vivo, chicken telomeres (Class II) shorten in an age-related fashion and telomerase activity is high in early stage embryos and developing organs but down-regulates during late embryogenesis or postnatally in most somatic tissues. In vitro, chicken cells down-regulate telomerase activity unless transformed. Knowledge of chicken telomere biology contributes information relevant to present and future biotechnology applications of chickens in vivo and chicken cells in vitro.

(*Key words*: chicken, telomere, development, aging, senescence)

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INTRODUCTION AND BACKGROUND

Telomere History: Cytogenetics of the Ends at the Beginning

The telomere is the "end part" of a linear chromosome (*telos* = end, *meros* = part), a term attributed to several early twentieth-century geneticists, including Muller, Haldane, and Darlington (Gall, 1995) and described by Muller (1938) as the "unipolar gene" bounded only on one side by another gene. Embedded in this term is one of the earliest cytogenetic concepts developed, in which linear chromosomes are organized into segmented parts (e.g., centromere, telomere, chromomere) that are physically (morphologically) and genetically (mechanistically) different from each other (Gall, 1995). Muller found evidence for this concept from mutation research on the cytogenetic and phenotypic alterations following radiation exposure of the fruitfly (*Drosophila melanogaster*). Barbara McClintock (1941) provided further insight regarding the specialized cytogenetic features of telomeres through her analysis of the "breakage-fusion-ring-bridge-breakage" cycle in maize (*Zea mays*), wherein damage to telomeres resulted in a chromosome with "broken" telomeres fusing to form a ring chromosome followed by formation of a "bridge structure" during anaphase, resulting ultimately in another break, which began the cycle again. Further, McClintock developed a theory of "telomere healing," whereby broken telomeres could be repaired, and the breakage-fusion-bridge cycle halted (McClintock, 1942). Based largely on observations of chromosome behavior and phenotypic traits, the research of Muller and McClintock led to the concepts that the ends of linear chromosomes, the telomeres, were essential "caps" that "seal" the chromosome and when "broken" could be "healed" in certain lineages. It took another forty years for scientists to begin to unravel the molecular basis for these early cytogenetic tenets defining the role of telomeres in regulating cell proliferation, replicative senescence, and im-

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Abbreviation Key: E = days of embryonation; FISH = fluorescence in situ hybridization; TERT = telomerase reverse transcriptase; TRF = terminal restriction fragment; TRF1/TRF2 = telomere-repeat-binding factor 1, 2; TR = telomerase RNA, UCD = University of California, Davis.

mortalization profiles of meiotic and mitotic lineages of normal, oncogenic, and cloned cells in vivo and in vitro.

Chromosome Replication: A Problem with the Ends

Watson (1972) and Olovnikov (1973) independently recognized that the ends of linear chromosomes possess an intrinsic problem. DNA replication by DNA polymerase synthesis requires initiation by a 5′ RNA primer with complementary (daughter) strand synthesis proceeding unidirectionally from the 5′ to the 3′ end. For both the leading and the lagging strand, removal of the most terminal RNA primer results in a gap at the 5′ end of each new daughter strand, thus, producing a "shortened" daughter strand. Subsequent rounds of replication lead to successive shortening of the end sequences. Olovnikov's "Theory of Marginotomy" suggested that the replicative limit of cells might be the consequence of progressive loss of telomeric DNA (Olovnikov, 1973).

Telomeres: Tandem Repeats and Protective Proteins

In 1978, Elizabeth Blackburn published the telomeric sequence of the single-cell ciliate, *Tetrahymena* (Blackburn and Gall, 1978). This ground-breaking research led to an explosion of studies showing that telomeres consist of a complex nucleoprotein structure. The features of this structure include tandem repeats of an evolutionarily conserved DNA sequence (vertebrate repeat: 5′-TTAGGG-3′/3′-AATCCC-5′) and several telomere-binding proteins (Fang and Cech, 1995; Hendersen, 1995; Stavenhagen and Zakian, 1998). Telomere array lengths are established by terminal restriction fragment (TRF) Southern blot analysis, i.e., use of restriction enzymes to cut the DNA as close to the telomere as possible. Differences in the length of adjacent DNA will affect TRF sizing; thus, cocktails of enzymes or multiple single enzyme digestions should be performed to characterize sizes. Inter- and intrachromosomal variation for telomere array size along with intercellular variation results in overlapping size classes, which resolve as a smear on Southern blots. Thus, telomere array lengths are typically reported as a range or an average size or both. For example, the telomere repeat length ranges from 300 to 400 bp in yeast (*Saccharomyces cerevisiae*) (Zakian, 1995), 50 to 200 kb in the laboratory mouse (*Mus musculus*) (Kipling and Cooke, 1990), and 5 to 25 kb in humans (average telomere size of ~20 kb in a newborn, ~5 kb in an octanagerian, due to shortening, see below, Harley et al., 1990). Telomere repeat length averages ~15 kb in cattle (*Bos taurus*) and ~18 kb in swine (*Sus scrofa*) (Kozik et al., 1998).

At the extreme end of the array, the 5′ (G-rich strand) exists as a single-stranded 3′ overhang of variable size (e.g., 50 to 100 nucleotides in mammals, Griffith et al., 1999). The current model for mammalian telomere structure proposes that the double-stranded telomeric DNA forms a large duplex loop (t-loop) such that the single-stranded 3′ overhang of the G-rich strand tucks around and back into the double-stranded DNA forming a displacement loop (D-loop) (Griffith et al., 1999). The TTAGGG repeat-binding factors, TRF1 and TRF2, along with other proteins bind to the double- and single-stranded telomeric DNA regulating access (e.g., to telomerase and repair enzymes) (Biladu et al., 1997; Broccoli et al., 1997; van Steensel and de Lange, 1997). Evidence exists both for and against organization of the telomeric DNA into a specialized nucleosome structure (Fajkus and Trifonov, 2001). Thus, in addition to providing a mechanism for chromosome end-replication (see below), telomeres through their specialized architecture protect chromosome ends from degradation and fusion (Griffith et al., 1999). Telomeres are also implicated in transcriptional silencing, meiotic chromosome pairing, meiotic and mitotic chromosome segregation, and organization of nuclear architecture (Blackburn, 1994; Dernburg et al., 1995; Shore, 1995; Zakian, 1995; Stavenhagen and Zakian, 1998).

In addition to terminally positioned telomeric DNA sequences, subtelomeric and intersitital sequences have been described in vertebrate chromosomes (Meyne et al., 1990; De La Sena et al., 1995). Internal locations of telomere sequence are believed to result from chromosome rearrangements or transposition events.

Telomerase

The mechanism by which cells and their telomeres circumvent the "end-replication problem" was solved by the discovery of telomerase (Greider and Blackburn, 1985). Telomere array length is maintained by telomerase, a specialized reverse transcriptase that extends the 3′ end of the parental DNA strand by de novo addition (6 to 26 nucleotides) of the telomere repeat sequence (Greider and Blackburn, 1989; Yu et al., 1990). Thus, telomerase creates an *extended* 3′ template for a *more complete* replication of the 5′ end of daughter strand, preventing erosion of the end sequences.

Telomerase consists of a RNA component (TR) having both a binding function as well as a template function for extension and a protein component (telomerase reverse transcriptase; TERT) with catalytic function for strand synthesis. Chen et al. (2000) compared 35 cloned TR components (including the chicken 465 bp TR gene), developing a consensus secondary structure containing four structural domains conserved in all vertebrates. TERT has been cloned in humans, mouse, yeast, ciliates, and *Arabidopsis* (Meyerson et al., 1997; Chen et al., 2000). The human TERT gene is about 35 kb, consisting of 16 small exons, several sharing a high degree of homology with reverse transcriptase motifs (Xia et al., 2000).

Telomere Theory of Aging: Mitotic Clocks and Cancer

Telomere stability has been implicated in the control of replicative senescence in human cells (Harley, 1995). The average telomere length of human germ cells is longer

than that of differentiated somatic cells. As somatic cells age in vivo or in vitro, telomere arrays shorten in a progressive manner (Harley et al., 1990); telomere shortening in humans correlates with the developmental regulation of telomerase activity. Somatic cells have low or undetectable telomerase activity (Counter et al., 1992), and thus upon successive replication cycles, telomere sequences shorten as a result of incomplete replication of the 5′ end of the daughter strand (Harley, 1995; Forsyth et al., 2002). Telomere shortening is proposed as the predominant "mitotic clock" that measures and controls the replicative life span of somatic cells. The telomere clock theory of aging states that erosion of the chromosome end triggers significant genome instability inducing cell senescence (Olovnikov, 1973; Hayflick, 1997). Numerous studies provide support for the telomere clock theory of cell aging (Harley et al., 1990; Harley, 1991; Harley, 1995; Forsyth et al., 2002). Telomere shortening is correlated with increased frequency of chromosome rearrangements (Counter et al., 1992) and p53-induced apoptosis (Karlseder et al., 1999). Of significant interest was the finding that telomerase activity resumes in the majority of immortalized cell lines and human tumors (Shay and Bacchetti, 1997) and that telomere array length stabilizes, and in some cases lengthens, in cancerous cells (Counter et al., 1992; Kim et al., 1994). Thus, telomere stabilization and abrogation of the normal telomere clock via abnormal telomerase activity (or an alternate pathway, see below) in cancerous cells may contribute to the immortalization capacity of metastatic cells (Harley et al., 1994; for a recent review, see Shay et al., 2001). Interestingly, transfection of TERT into human epithelial or fibroblast cells (Bodnar et al., 1998) has produced cell lines that are immortalized without being transformed.

Vertebrate Telomere Biology

The laboratory mouse (*Mus musculus*), in contrast to human, retains high levels of telomerase activity in adult somatic tissues and shows no evidence of telomere shortening as a regulatory mechanism of cellular senescence (Starling et al., 1990; Mantell and Greider, 1994; Prowse and Greider, 1995: Blasco et al., 1997; Bassham et al., 1998). Thus, it has been proposed that the regulation of cell proliferation and senescence, as well as immortalization and tumor formation, in animals with short life spans, may be less reliant on a genome-based mitotic clock (for review of murine data, see Forsyth et al., 2002). Interestingly, telomerase knockout mice (TR −/−) over a multigenerational time scale eventually lose significant telomere sequence (note that the lab mouse has very large telomere arrays, up to 200 kb) resulting in increased chromosome rearrangements and development of problems with lymphoproliferative cells, germ cell apoptosis, and infertility (Blasco et al., 1997; Lee et al., 1998; Niida et al., 1998; Hermann et al., 2001b). Thus, there appears to be universality to the telomere clock mechanism regulating genome stability, cell replicative life span, and thus ultimately longevity, although the timescale may differ in a species-specific manner. Table 1 reviews telomere array sizes and telomerase profiles established for a number of vertebrate species, including livestock species.

The field of telomere biology developed predominately through studies of the relatively simple model systems (e.g., yeast and ciliates), as well as the more complex human system, and the mouse model. Until recently, detailed molecular information remained relatively uncharted for the domestic chicken, which is an important biomedical and agricultural research model, a globally important food commodity, the main laboratory model for the approximately 9,000 species of birds, a significant animal system for human vaccine production, and a species with enormous potential for biotechnology applications related to human and animal therapeutics.

AVIAN TELOMERE BIOLOGY

Telomere Organization and Telomerase Activity in Chicken

Telomere Sequence Organization and Stability. Fluorescence in situ hybridization (FISH) of mitotic and meiotic chicken chromosomes showed that the chicken genome (2n = 78) contains both terminal (156 telomeres) and interstitial telomeric sequences (Nanda and Schmid 1994; Solovei et al., 1994). The termini of the macrochromosomes (pairs 1 to 8 and the Z, Ladjali-Mohammedi et al., 1999), as well as intersititial and centromere-proximal positions, contain the TTAGGG sequence. The microchromosomes (pairs 9 to 28 and the W) bear telomere repeats at the termini and interstitial regions and conspicuously, on some microchromosomes the telomere-FISH signal appears to encompass the entire chromosome (Nanda and Schmid, 1994; Figure 1).

The initial molecular analyses of telomere array lengths in chicken provided conflicting conclusions. Bloom et al. (1993) reported telomere array sizes from 250 kb to 2.2 Mb and Lejnine et al. (1995) reported chicken telomere arrays in the range of 3 to 100 kb. Venkatesan and Price (1998) reported chicken telomere arrays ranging in size from 8 to 20 kb with larger tracts reported. It was concluded that the larger tracts represented interstitial arrays or fragments poorly digested by restriction enzymes.

To clarify the molecular aspects of telomere array organization in chicken, we embarked on a series of studies to examine telomeric sequence abundance, array length, and location (Delany et al., 2000). The lengths of chicken telomere arrays were found to range from 0.5 to ~2 Mb (Figure 2). The overall percentage of TTAGGG sequence was high, 3 to 4% per diploid chicken genome; this value can be contrasted with the significantly lower value of 0.3% per human diploid cell (assessed within the same experiments). Figure 3 illustrates the disparity in genome content of the TTAGGG sequence by comparing telomere FISH hybridization signals from human and chicken metaphase and interphase cells processed in the same experiment. The diploid chicken genome, which is one-third the size of the human genome (2.5 pg vs. 6.5 pg),

TABLE 1. Cytogenetic and telomere characteristics of vertebrate animal species (in vivo)

Organism reference (maximum longevity)	2n/no. of Telomeres	Terminal telomere array sizes	Telomere shortening
Rainbow trout *Oncohynchus mykiss* Lejnine et al., 1995 (20 yr)	58–60/116–120	20 kb	Unknown
African clawed toad *Xenopus laevis* Bassham et al., 1998 (15 yr)	36/72	10–50 kb	No
Laboratory mouse *Mus musculus* Kipling and Cooke, 1990 (2 yr)	40/80	50–150 kb	No
Wild mouse *Mus spretus* Prowse and Greider, 1995 Coviello-McLaughlin and Prowse, 1997 (2 yr)	40/80	5–25 kb	Yes
Cow *Bos taurus* Kozik et al., 1998 (20 yr)	60/120	15 kb	Yes
Pig *Sus scrofa* Kozik et al., 1998 (15 yr)	38/72	18 kb	Yes
Sheep *Ovis aries* Shiels et al., 1999 (15 yr)	54/108	25 kb	Yes
Chicken *Gallus gallus* Delany et al., 2000 Taylor and Delany, 2000 (20 yr)	78/156	10 kb – 1–2 Mb	Yes

contains significantly more (10-fold) telomeric DNA sequence. This is interesting given the common explanation for the compact chicken genome, i.e., that it contains less repetitive DNA than the larger mammalian genomes. However, this theory does not apply to all classes of noncoding (e.g., telomeric) or coding repetitive sequences (e.g., rDNA, Gonzalez and Sylvester, 1995; Su and Delany, 1998; Delany and Krupkin, 1999). Interestingly, the chicken genome appears to lack degenerate telomeric sequence (Delany et al., 2000), whereas in humans, the sequence most proximal to the terminal telomere array consists of variant "degenerate" repeat sequence (Allshire et al., 1989).

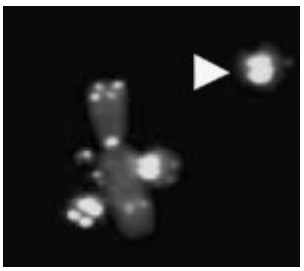

FIGURE 1. Variation in telomere sequence location and concentration in the chicken genome. The image is of several mitotic metaphase chromosomes (4′,6-diamidino-2-phyenylindole, DAPI counterstain) with terminal as well as interstitial locations of the telomeric sequence. The enormous variation in the quantity of sequence within and among chromosomes is shown by differences in the intensity of the fluorescence in situ hybridization (FISH) signal (white spots). Chromosomes were hybridized with a 42 bp TTAGGG peptide nucleic acid (PNA) oligonucleotide. White arrowhead points to a microchromosome entirely covered by the FISH telomere signal.

Based on size, chromosome location, Southern blot banding pattern, and age-related stability, three different classes of telomeric DNA arrays were distinguished in chicken (designated I, II, III, Figure 2, see also Delany et al., 2000). The Class I arrays span 0.5 to 8/10 kb, exhibit a Southern blot pattern of discrete bands and do not exhibit age-related telomere shortening. Resistance to digestion by Bal 31 exonuclease indicated the Class I arrays are located within the chromosomes (interstitial) rather than terminally. The Class II arrays range from 10 kb to 35/40 kb, exhibit the characteristic "smeared" Southern blot banding pattern indicating overlapping telomere array sizes and show evidence of age-related shortening (Delany et al., 2000; Taylor and Delany, 2000). The Class III arrays range in size from 40 kb to ~2 Mb (depending on the individual, the upper range may be ~800 kb), and exhibit discrete Southern blot banding patterns (Figure 2) that are hypervariable (i.e., highly polymorphic patterns

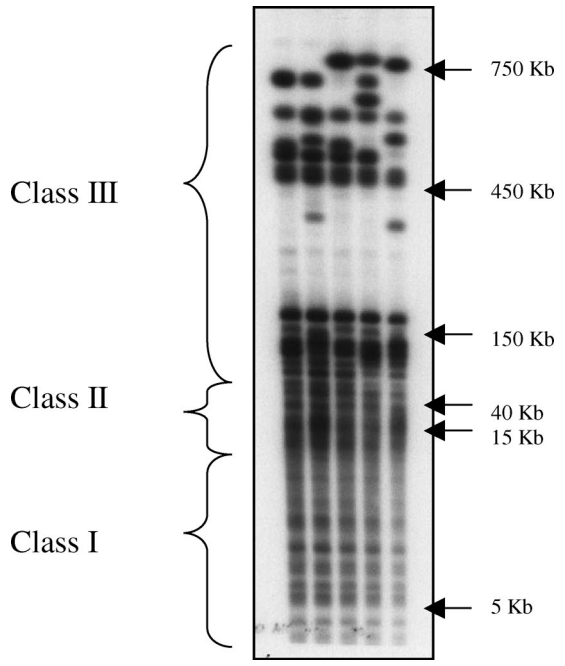

FIGURE 2. Hypervariability in the ultralong telomere arrays (Class III, >40 kb to 1 to 2 Mb) of University of California, Davis (UCD) inbred line UCD-003 (Single Comb White Leghorn). Cells were embedded in agarose plugs. DNA was extracted and digested (*Hae*III) in situ. DNA was size-separated by pulse-field gel electrophoresis, Southern blotted, and hybridized using a telomeric sequence probe $(TTAGGG)_7$. Each lane contains DNA from a different individual (all males). Note that no two individuals share the identical pattern for the Class III arrays (unexpected as UCD-003 is a highly inbred line, ≥99%), whereas the patterns are identical for the Class I arrays (0.5 to 10 kb interstitial fragments).

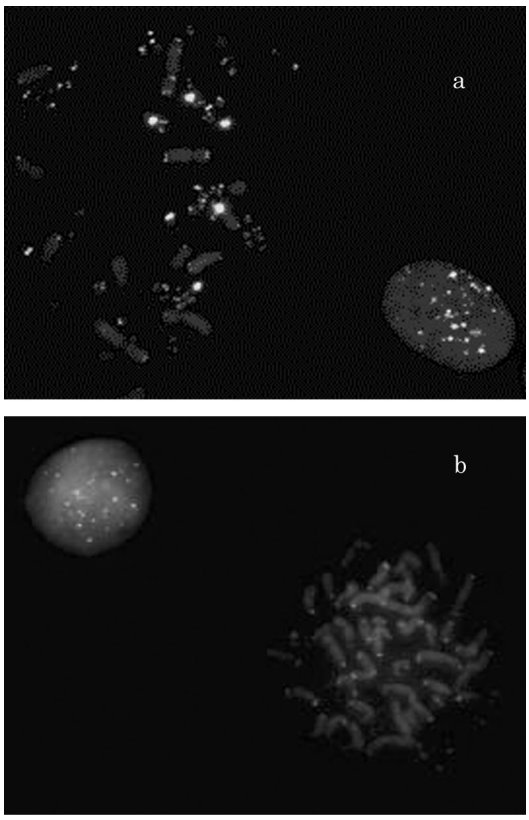

FIGURE 3. Telomere arrays of chicken and human chromosomes: the chicken genome contains more telomere sequence than the human genome. Chicken (a) and human (b) metaphase chromosomes and interphase cells hybridized with a telomeric sequence-peptide nucleic acid (PNA)-fluorescein probe. Human and chicken slide preparations were processed, and images were captured using the same parameters. Qualitatively, the telomere-positive fluorescent signals (white spots) from chicken cells and chromosomes have greater intensity than those of human (4′,6 diamidino-2-phenylindole, DAPI counterstain).

observed between individuals within inbred lines). These ultralong arrays are the largest reported for any vertebrate species to date. Class III arrays were rapidly digested by Bal 31 exonuclease indicating a terminal location.

Comparisons of erythrocyte (somatic) vs. sperm (germ) comparisons provided a minimum telomere shortening estimate for the Class II arrays of 160 bp per cell division (Delany et al., 2000); germ (gonadal) vs. somatic tissue comparisons (organ-derived cells from embryo and adult stages were compared) indicated ~600 bp lost per year (Taylor and Delany, 2000). Thus, a chicken that achieves its maximum expected life span (20 yr) could realize significant shortening, i.e., 12 kb in a proliferating cell population lacking telomerase. Many birds live considerably longer than poultry possessing maximum life span expectancies of six to seven decades (Holmes and Austad, 1995); a similar rate of erosion as observed in chickens could result in telomere sequence losses of 36 kb for a bird living 60 yr if a cell lineage continues dividing throughout the life span in the absence of telomerase. Haussman and Vleck (2002) report telomere shortening in zebrafinches on the order of 500 bp per year; erythrocytes of different-aged birds were examined and the older birds exhibited shorter telomeres than younger birds. This result may indicate telomere shortening in the "aging" hematopoietic stem cell population or accelerated shortening, or both as the erythrocyte lineage develops in older birds. Terminally differentiated erythrocytes are between 6 to 12 divisions downstream from a myeloid progenitor stem cell. Since erythrocytes have a finite life span in circulation and are replenished/replaced continuously, the circulating erythrocytes from an older bird are not necessarily older than those of a younger bird.

Telomere array profiles of 18 different bird species and subspecies were studied (Delany et al., 2000). In order to resolve the Class III arrays, it is essential that the DNA extraction proceed from cells embedded in agarose, otherwise the mechanical manipulation involved with standard DNA extraction and purification shears the DNA to fragments of ~50 to 100 kb even before restriction enzyme digestion (Figure 1, Delany et al., 2000). By using this method, the three size classes of arrays, including the ultralong Class III arrays were found to different degrees in most of the birds examined including turkey, pheasant, hawk, kestrel, zebra finch, and crane. Two species in the survey did not exhibit the ultralong arrays, the American Bald Eagle and Northern Goshawk. Interestingly, these two species were the only representatives in the sample group having karyotypes with small numbers of microchromosomes (four pair) (Renzoni and Vegni-Talluri, 1966;

Tagaki and Sasaki, 1974; De Boer, 1976). We are currently studying the hypothesis that the ultralong Class III arrays map specifically to the microchromosomes, perhaps serving a protective function, for these small genetic elements (estimated size 7 to 23 Mb, Bloom et al., 1993) to prevent premature negative effects of erosion on the michrochromosomes. Recently, Hermann et al. (2001a) provided evidence that the shortest telomere in the system is the critical signal for telomere dysfunction limiting cell survival. Figure 3 illustrates that the most intense telomere FISH hybridization signals appear on a defined subset of microchromosomes; preliminary data suggest that there is a set number of such chromosomes in mitotic figures and that the number corresponds to the number of ultralong TRF bands observed on Southern blots (Delany, unpublished data).

Telomerase Activity of Cells In Vivo and In Vitro. Telomerase activity is developmentally regulated in chicken tissues in vivo (Taylor and Delany, 2000) showing a similar profile to that seen in human and unlike that described for the lab mouse (constitutive expression in somatic tissues, Forsyth et al., 2002). High levels of telomerase activity were found in early stage chicken embryos (preblastula through neurula). From days of embryonation (E) E5 to E10 all organs surveyed showed high levels of telomerase activity. Subsequently, telomerase activity down-regulates in a temporal and tissue-specific manner for most somatic tissues. Activity patterns fell into three general categories: embryonic down-regulation (brain, liver, heart, muscle) from strong or moderate levels at E10 to trace/absent levels at E17 with no evidence of activity posthatch (birds up to 5 yr of age were studied); postnatal down-regulation (lung, kidney); constitutive activity in "renewable" tissue types (immune organs, e.g., bursa, thymus, spleen; intestine; organs/cells of the reproductive system, including undifferentiated gonad, testis, small white follicles, oviduct). Embryo-restricted tissues (e.g., chorioallantoic membrane, mesonephros, female right gonad) exhibited a high degree of interindividual variation but generally exhibited telomerase down-regulation during embryogenesis. In general, the telomerase activity correlated with the proliferative potential of the tissue. At 5 wk posthatch (35 d of age, an age that market broilers reach), there was moderate telomerase activity in lung and kidney and strong activity in intestine, spleen, bursa, thymus, gonads; at 18 mo (an age that recycled layers reach), there was low activity in kidney and high activity in intestine and spleen. Male and female gonad/reproductive tissues were not tested at 18 mo but were presumed high as telomerase activity was high in these tissues even in birds 4 to 5 yr of age.

Class II array lengths were compared in embryo (E10) and adult (5 yr) stages from the same tissues assayed for telomerase activity. The telomere arrays of the somatic tissues in the embryo were similar in size to that of the embryonic germ tissue, whereas in the adult the somatic tissues arrays were shorter, exhibiting an average decrease in size of 3.2 kb; this value was similar to the difference between the somatic tissues of embryos compared to the adult (Taylor and Delany, 2000). Notably, telomere shortening was also detected in telomerase positive adult tissues (kidney, intestine, spleen), a result also reported for some human tissues (Hiyama et al., 1996). One explanation for this result is that the telomerase activity derives from a minor pool of stem cells in the organ (the assay is sensitive enough to detect telomerase in one cell), whereas the array length profiles derive from a larger pool of cells lacking telomerase (differentiated cells and tissue components). The telomerase activity profile of the spleen indicated down-regulation of activity in the late embryo with reemergence of activity corresponding to the time frame for infiltration of lymphocytes (~20 days of incubation). Alternatively, discordant telomerase activity and telomere length profiles (i.e., shortened telomeres in samples with telomerase activity) may be a function of the control of telomere length exerted by telomere binding proteins that regulate accessibility to telomerase (Lustig et al., 1990; van Steensel and de Lange, 1997). TRF2 is implicated in "hiding" telomeres from checkpoint proteins (p53) that detect DNA damage (Karlseder et al., 1999). TRF2 has been cloned and characterized in chicken (Konrad et al., 1999), both embryos (E12 to 15) and DT40 cells express TRF2 mRNA, but expression was greater in the embryos.

The telomerase activity profiles of chicken cells in vitro exhibit the same general pattern reported for human cells in vitro; absent or down-regulated activity in nontransformed primary cells (chicken embryo fibroblasts: Venkatasen and Price, 1998; Swanberg et al., 2002) and moderate to high activity in transformed cells (Pain et al., 1996; Falchetti et al., 1999; Swanberg et al., 2002; Swanberg and Delany, unpublished data). Stage X (Eyal-Giladi and Kochav staging system) embryo blastodermal cell cultures were reported to have high activity, which could be abrogated upon exposure to retinoic acid (Pain et al., 1996), whereas Swanberg et al. (2002) found evidence for telomerase activity down-regulation upon long-term culture of blastodermal cells. Telomerase activity was detected in short-term posthatch turkey muscle satellite cells cultured in vitro, and both large-colony and small-colony forming types exhibited telomerase activity (Mozdziak et al., 2000). Quail embryo myoblasts and chicken neuroretina and fibroblast cells were telomerase-negative but became telomerase positive upon retroviral transformation correlating with *v-myc* expression (Falchetti et al., 1999).

When considering the role of genome stability in defining health and longevity of cell populations in vivo, tissue-specific consideration of stress may be an important factor. Interestingly, hemodynamic stress appears to be involved in accelerated rates of telomere erosion and replicative senescence in human vascular tissue impacting vascular function (Chang and Harley, 1995). Animals under strong selection pressure for rapid tissue growth and high rates of reproduction (such as poultry production stocks) may experience physiological stresses that could impact the stability of the chromosome ends through mechanisms not yet understood (e.g., affecting telomere binding proteins).

Telomeres and Recombination. Evidence has emerged indicating a role for telomeres seemingly opposite to their role in maintaining genome stability. Telomere sequences at chromosome termini as well as at interstitial locations appear to function as "hot spots" for recombination (Cooke et al., 1985; Hastie and Allshire, 1989). Results from a number of investigations provide evidence for a role of both interstitial and terminal telomeric sequences in somatic (mitotic) and germ lineage-specific (meiotic) recombination (Starling et al., 1990; Kipling and Cooke, 1990; Alvarez et al., 1993; Murnane and Yu, 1993; Bouffler et al., 1996; Kipling et al., 1996; McEachern and Blackburn, 1996; Preston, 1997; Riboni et al., 1997; Wintle et al., 1997; Zijlmans et al., 1997; Day et al., 1998; Bailey et al., 1999; Dunham et al., 2000; Cornfoth and Eberle, 2001).

Our initial study of telomere array organization (see Figure 1 in Delany et al., 2000) included analysis of several individuals from the University of California, Davis (UCD)-001 line, estimated to be about ≥80% inbred (Red Jungle fowl, *Gallus gallus gallus*). Different individuals exhibited different banding patterns, an unexpected result from an inbred line. Specifically, the ultralong Class III arrays exhibited a highly polymorphic pattern banding pattern, whereas the Class I (interstitial) arrays exhibited the identical pattern. (Note that the overlapping smear of Class II arrays makes it difficult to establish the heterogeneity/homogeneity of these arrays, but our quantitative analyses suggest the best array length comparisons (for shortening) are conducted by comparing the Class II arrays within an individual (e.g., germline vs. somatic tissues) rather than between, suggesting these arrays are also variable between individuals of an inbred line). Recently, three highly inbred lines (99%) were studied for their Class III array patterns (UCD-003, -077, and 342, Pisenti et al., 2001, for genetic line descriptions) (Delany, unpublished data). Hypervariability in the banding patterns of the ultralong arrays between individuals within each line was observed (Figure 2). Studies are underway to examine the inheritance patterns of the ultralong telomere arrays in meiotic and mitotic lineages to evaluate the hypothesis that there exists a telomere-driven mechanism to enhance recombination.

An interesting line of inquiry for poultry geneticists relates to the seeming "flexibility" of the chicken genome; for example, in industry stocks, selection progress has continued in populations essentially closed for over 50 yr. Use of large populations and the combinatorial association of the many linkage groups surely contributes (i.e., 38 linkage groups plus the sex chromosomes) to the generation of variation available for selection. It is of value to consider whether other genetic mechanisms are "enhanced" and contribute to the creation of new genetic variation, e.g., higher mutation rates or enhanced recombination to shuffle chromosomal "haplotypes." The chicken genome has been described as having a higher recombination rate than the mammalian (latter estimated at ~1,000 kb/cM), and recent data indicates that the microchromosomes have a higher recombination rate (150 to 250 kb/cM) than the macrochromosomes (396 kb/cM) (Schmid et al., 2000). Specific recombination hotspots have been reported for at least two microchromosomes, that is, genes are segregating independently that are known by physical mapping to be on the same chromosome (Schmid et al., 2000).

SUMMARY

Chicken Telomere Array Organization and Telomerase Function

The compact chicken genome contains a large amount of telomeric DNA (3 to 4%), ten times that found in the human genome but does not appear to contain degenerate telomeric DNA (Delany et al., 2000). Three classes of telomeric DNA arrays (designated I, II, III) have been identified differing in length, Southern blot banding pattern, chromosomal location, and age-related stability. The Class III arrays are the longest reported for any vertebrate species to date. Telomerase activity in vivo follows a pattern of high activity early in embryogenesis followed by down-regulation during the latter one-third of the incubation period or postnatally for most somatic tissues. Renewable tissues such as reproductive and immune organs retain high levels of telomerase activity even in adults of 4 to 5 yr of age. Telomere shortening occurs in somatic tissues in vivo and correlates with advanced developmental age (Taylor and Delany, 2000). Telomerase profiles in vitro indicate an absence of activity in primary cells, down-regulation in blastodermal cells, and presence of telomerase activity in transformed cells (Swanberg et al., 2002).

Telomerology, Biotechnology, and Poultry: Of Artificial Chromosomes, Immortalization, and Clones

Knowledge of avian telomere organization and telomerase function in poultry has current and future utility for applied developments in several areas, including the construction of artificial chromosomes for in vivo and in vitro applications (by de novo creation or telomere-seeding and truncation), modification of cellular aging and oncogenesis in vivo or senescence/immortalization profiles in vitro by transfection of telomerase gene components or through modulation of telomere binding proteins. The development of a series of immortalized non-transformed chicken cell lines having greater life span potential than primary cells by circumvention of telomere-driven instability would be of great value. Future uses of the somatic cells of the chicken for cloning purposes should integrate the knowledge that telomeres shorten in chicken cells both in vitro and in vivo. The potential for restoration of telomere lengths during embryogenesis (perhaps equivalent to embryonic "healing" of broken ends, McClintock, 1942) will require careful examination in avian cloning, whether the cells are derived from adult somatic nuclei (in vivo or vitro origin)

or cultured embryonic blastodermal stem cells, as it is clear that species differences exist in the resetting of telomere lengths/clocks, e.g., shortened telomeres reported in sheep (Dolly) but not in cows (Shiels et al., 1999; Lanza et al., 2000). Knowledge of telomere array characteristics and regulation contributes information relevant to present and future biotechnology applications of chickens and their cells.

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