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Review

Telomere biology of the chicken: A model for aging research

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ABSTRACT

Division-dependent telomere shortening correlating with age triggers senescence on a cellular level and telomere dysfunction can facilitate oncogenesis. Therefore, the study of telomere biology is critical to the understanding of aging and cancer. The domestic chicken, a classic model for the study of developmental biology, possesses a telomere genome with highly conserved aspects and distinctive features which make it uniquely suited for the study of telomere maintenance mechanisms, their function and dysfunction. The purpose of this review is to highlight the chicken as a model for aging research, specifically as a model for telomere and telomerase research, and to increase its utility as such by describing developments in the study of chicken telomeres and telomerase in the context of related research in human and mouse.

Contents

1. Introduction

Telomeres, the nucleoprotein "caps" protecting the ends of linear chromosomes, are maintained by telomerase. Division-dependent telomere shortening correlating with age triggers senescence on a cellular level and telomere dysfunction can facilitate oncogenesis. Therefore, the study of telomere biology is critical to the understanding of aging and cancer. The mouse has been used extensively as a model for

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human telomere biology. However, there are fundamental differences between mouse and human cells which make the mouse less than optimal as a paradigm for the study of telomeres and telomerase. Other model systems should be characterized, refined and promoted to facilitate new developments in aging research. The chicken, a classic model for the study of developmental biology, possesses a telomere genome with highly conserved aspects and distinctive features which make it uniquely suited for the study of telomere maintenance mechanisms, their function and dysfunction. The benefits of the chicken telomere genome as a model are enhanced by the existence of chicken cell lines and genetic lines which exhibit unique variations in the distribution and heterogeneity of telomeric DNA arrays as well as diversity in telomerase activity profiles and expression of telomerase components. The purpose of this review is to highlight the chicken as a model for aging research, specifically as a model for telomere and telomerase research, and to increase its utility as such by describing developments in the study of chicken telomeres and telomerase in the context of related advances in human and mouse.

2. Biogerontology of the chicken

In general, avian life expectancy appears to correlate with body mass and many birds have higher maximum life spans than mammals (Holmes and Austad, 1995; Partridge and Barton, 1993). However, tremendous variability in longevity is observed, with both exceptionally long- and exceptionally short-lived species reported (scarlet macaw, over ninety years and common quail, up to eight years) (Etchepare, 1990; Puigcerver et al., 1992). The lifespan of the domestic chicken (*Gallus gallus domesticus*) and other Gallinaceous species is considered to be less than twenty years. In comparison, maximum human longevity is approximately 120 years and lifespan of the mouse rarely exceeds 6 years (Holmes and Austad, 1995). Laboratory birds have been used to model a range of diseases and conditions of aging including cardiovascular disease, hyperlipidemia, reproductive senescence, cancer and declining bone density (Holmes and Austad, 1995 and references therein). In recent years, the domestic chicken has emerged as a versatile model of aspects of telomere biology relevant to aging and cancer.

3. The chicken genome: telomeres, macros, micros and other features

The chicken genome contains 1.25 pg of DNA per haploid cell, about one-third the size of human and mouse genomes (Gregory, 2010). The karyotype of the chicken displays a number of unique characteristics, including three major chromosome size groups: five pairs of macrochromosomes (GGA 1–5), five pairs of intermediate chromosomes (GGA 6–10) and 28 microchromosome pairs (GGA 11–38). In addition to the autosomes, birds possess two sex chromosomes (Z and W) with the female as the heterogametic sex (ICGSC, 2004). The draft sequence of the chicken genome confirmed its lack of retrotransposed pseudogenes, microsatellites, LINES and SINES relative to the genomes of other higher vertebrates (ICGSC, 2004; Primmer et al., 1997). Surprisingly, the amount of telomeric sequence is 10-fold greater in chicken than in mammals (Delany et al., 2000).

4. Heterogeneity of telomere length in the chicken

Using fluorescence *in situ* hybridization (FISH), Nanda et al. (2002) studied the chromosomal distribution of the highly conserved telomere repeat sequence, $(TTAGGG)_n$, in 16 bird species, including chicken. Avian telomeric repeat arrays are divided into three classes based upon size and/or location (Delany et al., 2000). Class I arrays, interstitial telomeric DNA arrays ranging from 0.5 to 10 kb in length, are highly variable among species suggesting that genomic fusions and rearrangements involving these arrays occurred during avian radiation (Nanda et al., 2002). The Class I arrays present in chicken display a distinctive profile by which several macrochromosomes can easily be distinguished (Fig. 1). Class II arrays range from 10 to 40 kb, are terminally located and exhibit division-dependent telomere shortening. Class III arrays or mega-telomeres, which are also terminal arrays, range from 200 kb to 4 Mb (Delany et al., 2000; Delany et al., 2007; O'Hare and Delany, 2009; Rodrigue et al., 2005). In comparison, terminal telomeric array length ranges from 10 to 15 kb in human and from 30 kb to greater than 200 kb in the laboratory mouse (de Lange et al., 1990; Kipling and Cooke, 1990; O'Hare and Delany, 2009; Rodrigue et al., 2005). For a comparison of aspects of telomere biology in human, mouse and chicken, including the size of telomeric DNA arrays, see Table 1.

The existence of mega-telomeres in chicken has been shown by molecular (Southern blot) and cytogenetic (FISH) techniques (Delany et al., 2000, 2007; Nanda et al., 2002; O'Hare and Delany, 2009; Rodrigue et al., 2005). The length and distribution of mega-telomeres are highly variable among avian species, between individuals (even in highly inbred chicken lines), and among genotypes (Delany et al., 2000, 2007; O'Hare and Delany, 2009; Rodrigue et al., 2005). In one highly inbred line, mega-telomeres were mapped to four autosomes, GGA 9, 16, and 28 (with the fourth locus unknown) and the W sex chromosome (Delany et al., 2007; O'Hare and Delany, 2009; Rodrigue et al., 2005). A telomeric ideogram or graphic of the telomeric karyotype (Fig. 1), displays the in-common mega-telomeres across genetic lines and the genetic-line-specific mega-telomeres. Figs. 2 and 3 illustrate overall telomeric profiles and genotypic variability among chicken genetic lines and cell lines which are frequently used in research: UCD 001, UCD 003, DF-1 and DT40 (Delany et al., 2007; O'Hare and Delany, 2009; Rodrigue et al., 2005; Swanberg and Delany, 2003). Interestingly, the greatest amount of total telomeric DNA, 17%, was found in DF-1 (a non-transformed, immortalized, telomerase-negative chicken embryo fibroblast [CEF] cell line), compared to 5% in UCD 001 (an inbred chicken line) and 1.2% in DT40 (a chicken B-cell lymphoma cell line) (O'Hare and Delany, 2009). The reason for this variability in the amount of telomeric DNA is unknown, although differing recombination patterns and the presence or absence of telomerase are likely contributing factors.

An inheritance study in chicken revealed unexpected hypervariability and heterogeneity with non-Mendelian segregation of mega-telomeres in inbred line siblings (Rodrigue et al., 2005). Arrays seen in the parents disappeared in the offspring and conversely novel arrays were frequently generated in the progeny, a phenomenon which was also reported in the laboratory mouse and suggests a high rate of recombination, the generation of new somatic and germline length variants and the possibility of sister-chromatid exchange events occurring at the telomeres (Delany et al., 2007; Rodrigue et al., 2005; Starling et al., 1990).

5. Telomerase activity in proliferating cells and division-dependent telomere shortening

Telomerase activity is observed during early development in both chicken and human, continues to be maintained in renewable tissues and stem cells requiring multiple rounds of cell division, and diminishes in most somatic tissues shortly after embryogenesis (Forsyth et al., 2002; Kim et al., 1994; Swanberg and Delany, 2003, Swanberg et al., 2004; Taylor and Delany, 2000; Wright et al., 1996). Telomerase activity is also present in most transformed cells examined in both human and chicken (Kim et al., 1994; Swanberg and Delany, 2003, 2005, 2006 and references therein). In contrast, the laboratory mouse displays constitutive telomerase expression throughout the lifespan in both somatic and renewable tissues and is more prone to oncogenesis than either human or chicken (Blasco et al., 1997; Forsyth et al., 2002; Sherr and DePinho, 2000; Wright and Shay, 2000).

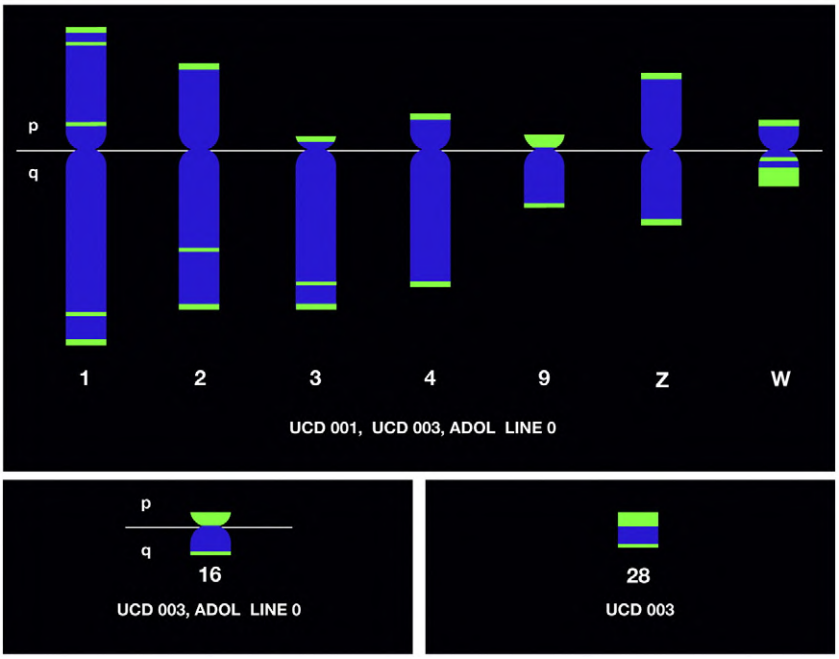

Fig. 1. Telomeric sequence ideogram of chicken chromosomes. The telomeric sequence profile of chicken genomes derived from the following lines is displayed: UCD 001 (*Gallus gallus*; Red Jungle Fowl inbred line, $F>0.9$), UCD 003 (*Gallus domesticus*; Single Comb White Leghorn highly inbred line, $F>0.99$) and ADOL Line 0 (Single Comb White Leghorn, closed line). The interstitial telomeric arrays, which appear to be uniform across these and other lines, are located on GGA 1 (three arrays), GGA 2, GGA 3 and GGA W. In this Fig. are four macrochromosomes (GGA 1–4), one intermediate chromosome (GGA 9), two microchromosomes (GGA 16, 28), and both sex chromosomes (GGA Z and W) (Delany et al., 2007; Nanda et al., 2002; O'Hare and Delany, 2009). In-common mega-telomeres are located on GGA 9p and Wq, whereas GGA 16p and GGA 28 mega-telomeres are present in some but not all chicken genomes. The centromere position and mega-telomere location (p or q arm) for GGA 28 is unknown. The GGA W mega-telomeres observed in the genomes of UCD 001 and UCD 003 are 4 and 2.8 Mb in size, respectively. The mega-telomere on GGA 9 in the UCD 001 genome is 1.2 Mb. Blue = chromosomal DNA; green = telomeric sequence. Ideogram adapted from Ladjali-Mohammedi et al., 1999. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Division-dependent telomere shortening, correlating with age, occurs in chicken somatic tissues *in vivo* (Delany et al., 2003; Taylor and Delany, 2000), in non-immortalized, non-transformed chicken cells *in vitro* (Swanberg and Delany, 2003, Swanberg et al., 2004; Venkatesan and Price, 1998) and in non-immortalized, non-transformed human cells (Harley et al., 1990). However, with the exception of the telomerase-deficient mouse ($TR^{-/-}$ or $TERC^{-/-}$ mouse), laboratory mice and other rodents generally do not exhibit age-related or division-dependent telomere shortening (Blasco et al., 1997; Forsyth et al., 2002; Samper et al., 2001; Wright and Shay, 2000) and at senescence mouse cells retain long telomeres (Parrinello et al., 2003).

Table 1
Comparative telomere biology in human, mouse and chicken.

	Human	Mouse	Chicken
Telomere shortening	Yes[a,b]	No (Yes in $TR^{-/-}$ mice[i])	Yes[p,q,r]
Telomerase activity in			
Somatic cells	No[b]	Yes[j,k]	No[p,r]
Transformed cells	Yes[b,c]	Yes[j]	Yes[p,s]
Embryonic cells	Yes[d]	Yes[l]	Yes[k,t,u]
Germ cells	Yes[d]	Yes[m]	Yes[r,s,v]
Terminal telomere length	10–15 kb[e]	20–150 kb[n]	8 kb–4 Mb[w,x]
TERT gene identified	Yes[f,g] (GeneID: 7015)	Yes[l] (GeneID: 21752)	Yes[y] (GeneID: 420972)
TR gene identified	Yes[h] (GeneID: 7012)	Yes[o] (GeneID: 21748)	Yes[z] (GeneID: 379037)

[a]Harley et al. (1990), [b]Kim et al. (1994),[c]Shay and Bacchetti (1997), [d]Wright et al. (1996), [e]de Lange et al. (1990), [f]Nakamura et al. (1997), [g]Kilian et al. (1997), [h]Feng et al. (1995), [i]Blasco et al. (1997), [j]Chadeneau et al. (1995), [k]Pain et al. (1996), [l]Greenberg et al. (1998), [m]Lee et al. (1998), [n]Kipling and Cooke (1990), [o]Blasco et al. (1995), [p]Swanberg and Delany (2003), [q]Venkatesan and Price (1998), [r]Taylor and Delany (2000), [s]O'Hare and Delany (2005), [t]Swanberg et al. (2004), [u]Swanberg and Delany (2005), [v]van de Lavoir et al. (2006), [w]O'Hare and Delany (2009), [x]Delany et al. (2000), [y]Delany and Daniels (2004), [z]Delany and Daniels (2003).

6. Telomere structure and telomere-associated proteins

Human, mouse, and chicken telomeres form closed chromatin loops or t-loops that arise from the invasion of a 3′ overhang on the G-rich strand of the telomere into the double-stranded region of the TTAGGG tract (Griffith et al., 1999; Nikitina and Woodcock, 2004; Wei and Price, 2004). The t-loop, and its associated D-loop formed as a result of the strand invasion, may protect telomere ends from cellular DNA damage repair pathways (Griffith et al., 1999). Proteins involved in the protection of telomeres include the shelterin complex composed of TRF1, TRF2, and POT1 (which recognize TTAGGG sequence repeats) and TIN2, TPP1, and RAP1 (which do not directly bind DNA) (de Lange, 2005 and references therein). Shelterin enables telomeres to elude DNA damage response pathways and may possess activity capable of remodeling telomeric chromatin (de Lange, 2005). Four shelterin proteins have been identified in chicken (De Rycker et al., 2003; Konrad et al., 1999; Tan et al., 2003; Wei and Price, 2004; Table 2). The fact that the two remaining chicken shelterin proteins have not yet been identified is likely due to gaps which remain in the draft sequence of the chicken genome (ICGSC, 2004). Additional proteins are known to function at the telomeres. In human, tankyrase 1 and possibly tankyrase 2 destabilize binding of TRF1, enhancing access to the telomere by telomerase (Cook et al., 2002; De Boeck et al., 2009). Tankyrase is highly conserved and chicken tankyrases 1 and 2, both of which bind TRF1, have been identified (De Rycker et al., 2003). A variety of other proteins, including DNA repair factors such as replication protein A (RPA), Rad54, the Rad51 paralogs and BRCA1, all of which are found in chicken, may also act at the telomeres (Bezzubova et al., 1993a,b; De Boeck et al., 2009; Orelli et al., 2001; Salas et al., 2006).

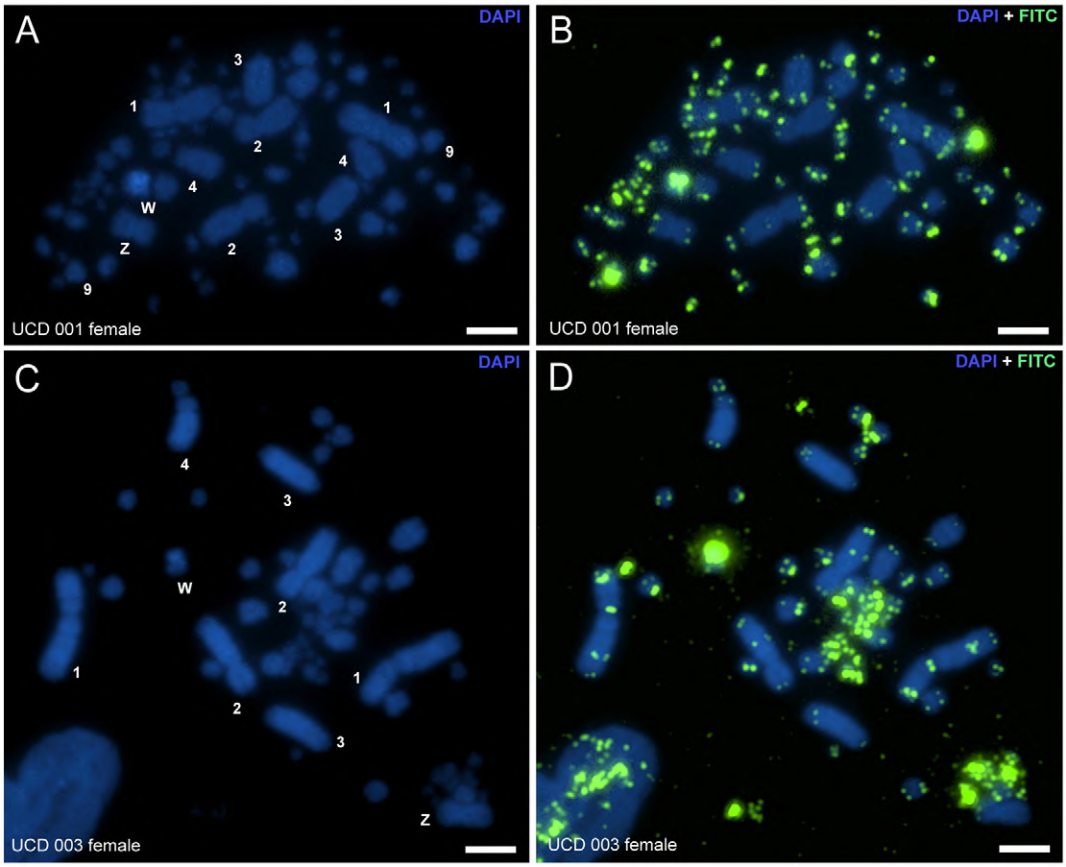

Fig. 2. Cytogenetic profile of telomeric sequence in chicken genetic lines. (A) UCD 001(female) cell with DAPI-stained chromosomes; GGA 1–4, 9, Z and W are identified. (C) UCD 003 (female) cell, with DAPI-stained chromosomes; GGA 1–4, Z and W are identified. (B, D) DAPI+FITC images show telomeric DNA (FITC) including mega-telomeres on GGA 9 and W in UCD 001 (O'Hare and Delany, 2009) and GGA 9, 16 and W in UCD 003 (Delany et al., 2007). One GGA 4 chromosome does not appear in the field displayed for UCD 003. DAPI (blue) = chromosomal DNA and FITC (green) = telomeric sequence. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Examination of transcriptional levels of several shelterin complex proteins in pluripotent, differentiated and transformed chicken cells revealed differences of expression consistent with the proliferative potential of each cell type (Swanberg and Delany, 2005). Higher expression of *TRF1* and *TRF2* and greater ratios of *TRF2*:*TRF1* were observed in the pluripotent gastrula and DT40, both of which are telomerase-positive cell types requiring maintenance of telomere stability after multiple rounds of cell division. In contrast, five of six CEF cultures (differentiated, telomerase-negative, senescing cells with shortening telomeres) showed decreased *TRF1* expression and

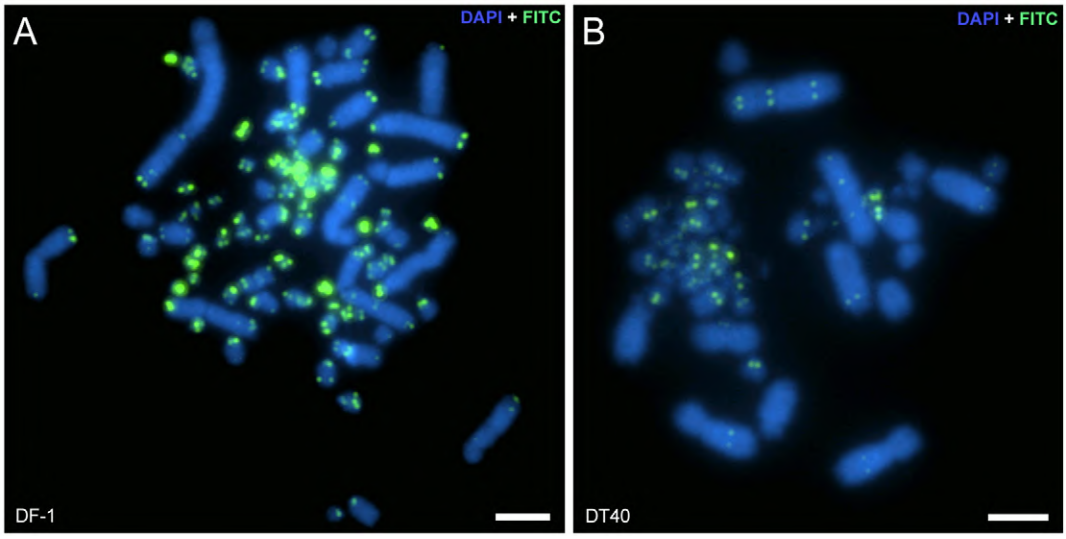

Fig. 3. Cytogenetic profile of telomeric sequence in chicken cell lines. (A) DF-1, an immortalized CEF cell line. (B) DT40, a transformed B-cell lymphoma cell line. DF-1 displays more telomeric sequence, including mega-telomeres, than DT40. DAPI (blue) = chromosomal DNA and FITC (green) = telomeric sequence. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Shelterin components identified in chicken.

Gene	Gene ID	Accession	Function	Locus
cTRF1	378896	AY237359	Binds ds[a] telomeric DNA	GGA 2
cTRF2	395598	AJ133783	Binds ds telomeric DNA	GGA 11
cPOT1	404538	AY555718	Binds ss[b] telomeric DNA	GGA 1
cRAP1	395122	AY083908	Telomere-associated protein	GGA 7

[a] Double-stranded.
[b] Single-stranded.

unchanged *TRF2* expression. In the same study, tankyrase 2, which binds TRF1, was up-regulated in DT40 cells compared to a majority of CEF cultures and the gastrula embryo (Swanberg and Delany, 2005).

Recently, telomeric repeat-containing RNA (TERRA), a large non-coding RNA containing UUAGGG repeats, was identified as a component of telomeric heterochromatin. While its function is not fully understood, TERRA appears to be transcribed in a developmentally-regulated manner by DNA-dependent RNA polymerase II, to accumulate at the telomeres and, in concert with shelterin proteins, may contribute to the regulation of telomere length (Luke and Lingner, 2009 and references therein). The discovery of TERRA in mammals was foreshadowed by the work of Solovei et al. (1994) who observed the transcription of telomeric DNA in chicken lampbrush chromosomes.

7. Telomerase reverse transcriptase (TERT) and its variants

The core telomerase enzyme contains a catalytic protein subunit, telomerase reverse transcriptase or TERT, and an RNA subunit, *TR* (or *TERC*), with a template for the addition of telomere repeat sequences (Delany and Daniels, 2004). The highly conserved structure of vertebrate *TERT* includes seven reverse transcriptase (RT) motifs: 1, 2, A, B′, C, D, and E as well as the telomerase-specific "T" motif (Lingner et al., 1997; Nakamura et al., 1997). Chicken *TERT* contains all of these motifs as well as unique features including an additional 15 amino acid residues in region c-I, an additional 27 amino acids in region v-IV and a longer flexible linker than other vertebrate TERTs examined (Delany and Daniels, 2004). In addition to conserved coding regions, the 5′ flanking sequence of *cTERT* contains conserved 5′ regulatory regions (Delany and Daniels, 2004). Chicken *TERT* is located within chromosome band 2q21 (Delany and Daniels, 2004).

As in other vertebrates, regulation of telomerase activity in chicken occurs through a variety of mechanisms, including alternative splicing of *TERT* (Chang and Delany, 2006; Kilian et al., 1997). Nineteen *cTERT* splice variants have been identified in the telomerase-specific (T) and reverse transcriptase (RT) regions (Table 3). Several of these variants resemble but are not identical to human splice variants, with regions within the same motifs disrupted in each species. For example, *cTERT* variants include a deletion of exon five, similar to the human α variant in that it disrupts the A motif (chicken variant 4), and a deletion of *cTERT* exon eight (found in chicken variants 9, 11, 12, 13 and 14) which, like the human β deletion, disrupts the B′ motif (Chang and Delany, 2006) (Table 3).

The relatively large insertion into a region between conserved telomerase RT motifs A and B, called the finger domain, distinguishes other reverse transcriptases from TERT. In addition to the splice variants mentioned above, in-frame and out-of-frame insertions into this finger domain were observed in chicken. In human, mutations of certain conserved residues in the finger domain insertion or IFD affect TERT repeat addition processivity, the ability of telomerase to repetitively add repeats without dissociating from its template (Lue et al., 2003). IFD mutations have been linked to human disease including idiopathic pulmonary fibrosis, aplastic anemia and autosomal recessive dyskeratosis congenita (Liang et al., 2006; Marrone et al., 2007; Mitchell et al., 1999; Tsakiri et al., 2007; Yamaguchi et al., 2005). At least one of the IFD variants in chicken, V7, is a potentially productive in-frame variant which was found in the chicken gastrula embryo, embryonic day (E) 7.5 liver, adult liver, CEFs and DT40 cells (Chang and Delany, 2006). No disease-associated phenotype connected with V7 has been reported. For an excellent summary of *TERT* and *TERC* (*TR*) variants which are associated with human disease, see the Telomerase Database-Diseases (Podlevsky et al., 2008; http://telomerase.asu.edu/diseases.html).

Table 3
Motifs or regions important to telomerase function are disrupted by cTERT splice variants.

cTERT motif/region[a]	Variant(s)[b]
v-IV	V1 (ins), V3 (del, PTC)
T	V2 (ins, PTC), V3 (del, PTC)
2	V4 (del), V5 (del)
A	V4 (del)
IFD	V6–V14 (various ins, del, PTC)
B′	V9 (del), V11–V14 (various ins, del, PTC)
C	V15 (ins, PTC), V16 (various ins, PTC)
D	V17–V19 (various ins, PTC)

IFD = insertion in finger domain of *TERT*, see Lue et al. (2003).
ins = insertion.
del = deletion.
PTC = premature stop codon.
[a] For specifics of *cTERT* motifs and regions see Delany and Daniels (2004).
[b] For specifics of variants see Chang and Delany (2006).

8. Telomerase RNA (*TR* or *TERC*)

Alignment of thirty-five vertebrate *TR* sequences has revealed eight conserved sequence motifs (CRs) with 90% or greater identity, including CR1 which contains the template region for the addition of telomere repeats (Chen et al., 2000). The consensus sequence for the template region is: 5′-CUAACCCU-3′, which includes two base pairs at the 3′ end (CU) used for alignment of the template region to its complementary DNA strand (Chen et al., 2000; Gavory et al., 2002). Some vertebrates, including chicken, have "extra" alignment nucleotides adding further stability to the association of telomerase with telomeric DNA thus improving processivity (Gavory et al., 2002). In a comparison of human and chicken *TR* 5′ regions SP1, GR, c-myb, ER and CCAAT binding motifs were located. The relative positions of these motifs within *hTR* and *cTR* were in close proximity. However, chicken possessed a greater number of the Sp1, ER and CCAAT motifs than human. The 5′ Sp1 sites, in particular, were 3-fold more frequent in chicken compared to either human or mouse (Delany and Daniels, 2003). A syntenic block of five GGA 9q loci, including *cTR*, is found on the q arm of human chromosome 3 (HSA3q) (Delany and Daniels, 2003).

In their analysis of the cytogenetic and genomic organization of chicken *TR*, Delany and Daniels (2003) also noted other conserved 5′ and 3′ regulatory elements and elaborated upon the 5′ and 3′ sequence homology between *cTR* and the viral TR (*vTR*) sequences within the Marek's disease herpesvirus (MDV) genome previously described (Fragnet et al., 2003). Recently, a study that examined host–genome interactions established that the MDV virus is integrated into chicken chromosomes at the telomeres in late-stage MDV tumors. On average, there are 4–5 integration locations per tumor, with a range of 0 to 9 integrations (Delany et al., 2008; Robinson et al., 2009). For two excellent reviews describing Marek's disease as well as the relationship between the MDV virus and telomerase, see Artandi (2006) and Osterrieder et al. (2006).

9. TERT and TR expression in chicken

In addition to telomere shortening and telomerase activity, expression of telomerase holoenzyme components has been studied

in chicken cells. An ontological study of *cTR* and *cTERT* transcript expression examined these telomerase components in embryonic day (E) 0 to E19 embryos and two year old adults. The expression level was highest at the earliest embryonic ages, E0 and E1. The expression patterns of *cTERT* and *cTR* correlated developmentally with telomerase activity in embryonic and adult tissues. The observation that little or no *cTR* was transcribed in telomerase-negative tissues suggested that *cTR* could be the regulating component of telomerase (O'Hare and Delany, 2005). Other experiments examined telomerase gene expression in chicken cells and cell lines (Swanberg and Delany, 2005; Swanberg et al., 2004). DT40 cells displayed the highest expression levels of *cTR* and *cTERT,* with high levels also observed in chicken embryonic stem cells (cES) and the lowest levels of *cTR* and *cTERT* observed in CEFs (Swanberg and Delany, 2005; Swanberg et al., 2004).

10. Alternative Lengthening of Telomeres (ALT)

Telomerase-negative mammalian somatic cells display progressive telomere shortening as they approach senescence. If senescence pathways are evaded, cells continue to divide until telomere erosion induces fusion of chromosomes and/or cell death (Cesare and Reddel, 2008). Occasionally, a population of cells may escape the senescence barrier, become immortalized and re-establish telomere length homeostasis through the up-regulation of telomerase or activation of a telomerase-independent mechanism for telomere maintenance called Alternative Lengthening of Telomeres or ALT (Cesare and Reddel, 2008). Hallmarks of ALT cells include the presence of long telomeres, telomeres that are heterogeneous in length, extrachromosomal telomeric repeats, ALT-associated promyelocytic leukemia nuclear bodies (APBs), and a high rate of telomeric recombination (Cesare and Reddel, 2008). ALT has been documented in human cells (Cesare and Reddel, 2008; Dunham et al., 2000; Muntoni and Reddel, 2005) and in cells of a telomerase-deficient mouse (Chang et al., 2003) but not, thus far, in chicken. Chicken terminal telomeric arrays are heterogeneous in length and hypervariable between individuals within an inbred line suggesting recombination (Delany et al., 2000; Rodrigue et al., 2005). Further, DF-1, a telomerase-negative, immortalized chicken cell line, displays long, heterogeneous telomeres (O'Hare and Delany, 2009). These observations suggest that mechanisms consistent with ALT may be operating in some chicken systems.

11. Reconstitution of telomerase in avian cells

Telomerase activity has been reconstituted in human cells (Bodnar et al., 1998) and the cells of late generation telomerase-deficient mice (Samper et al., 2001). Attempts to reconstitute chicken telomerase or a chicken/human telomerase hybrid in chicken cells or a heterologous system such as the knockout murine $TR^{-/-}$ cell line produced mixed results. While the *hTERT*-transfected CEFs were shown to express the *hTERT* gene, no functional telomerase was produced nor were telomeres rescued from the expected pattern of telomere shortening (Michailidis et al., 2005).

The observation that little or no *cTR* was transcribed in telomerase-negative tissues suggested that *cTR* might be the regulating component in the assembly of telomerase (O'Hare and Delany, 2005). Robb and Delany set out to test the hypothesis that cTERT expression is constitutive and regulation of c*TR* expression the driving force regulating telomerase activation in chicken (unpublished results, Robb and Delany). In a series of experiments, a variety of promoter and vector constructs, with *cTR* fragment length altered to include or exclude potential regulatory regions, were used to transfect *cTR* into telomerase-negative CEFs. In most cases TR transcript expression was seen 48 h post-transfection along with the endogenous *cTERT* transcript. However, telomerase activity was not consistently detected in any of the transfected cells compared to telomerase activity observed in positive control extracts from DT40 and HeLa cells. It is possible that an additional factor such as dyskerin, which has been identified as a component of active telomerase in human cells (Mitchell et al., 1999) and is found in the chicken genome (GeneID: 422196), was not present in sufficient concentrations to support reconstitution of telomerase in the cell system used (CEFs). However, recent expression profiling indicates that dyskerin is expressed in CEFs (O'Hare and Delany, in press; and manuscript in preparation) and thus lack of dyskerin is unlikely to be the basis for the inability to reconstitute telomerase in the experiments described.

Functionality of a viral form of TR (*vTR*) which exhibits 88% sequence identity with chicken *TR*, was analyzed in the knockout mouse $TR^{-/-}$ cell line (Fragnet et al., 2003). This study reported that telomerase activity was reconstituted by the interaction of mouse *TR* (*mTR*), *hTR*, *vTR* or *cTR* with the endogenous mouse telomerase reverse transcriptase (Fragnet et al., 2003). In a later study, Fragnet et al. (2005) reconstituted telomerase activity in a rabbit reticulocyte lysate system by assembly of cTERT and *vTR* or *cTR*. Robb and Delany (unpublished results) also introduced *vTR* with results similar to those seen with *cTR* introduction. In spite of the progress made in reconstitution of telomerase, the immortalization of chicken cells by transduction or transfection of *cTR*, *cTERT* or both *cTR* and *cTERT* has not been accomplished. It remains to be seen whether reconstitution of telomerase by addition of exogenous c*TERT* and/or c*TR* can fully restore the function of endogenous telomerase in chicken.

12. Conclusions

Understanding the role telomeres and telomerase play in human aging and age-related diseases such as cancer, requires the development and utilization of animal models. The laboratory mouse is an organism commonly used as a model for human biology. However, fundamental differences exist between mouse and human telomere biology including constitutive telomerase in mouse but not human somatic cells, the lack of telomere-length-triggered cellular senescence in mouse and the elevated susceptibility to immortalization of mouse cells. Development of a mouse telomerase knockout model, the $TR^{-/-}$ mouse, has ameliorated this divide somewhat. However, it is clear that in order to promote progress in our understanding of the fundamental mechanisms underpinning cellular senescence and oncogenesis we must expand the array of animal models available to the research community. Advances in our understanding of chicken telomere biology have been accumulating making the chicken a viable choice for the study of telomere-based mechanisms relevant to human biology.

A wide-ranging set of resources and tools for the study of telomere biology is currently available in the chicken. The structure and classification of chicken telomeric arrays have been studied. Chicken genotypes with discrete telomere-related phenotypes have been characterized by cytogenetic and molecular techniques. The status of telomerase expression and activity has been examined in chicken stem and primordial germ cells as well as transformed, non-transformed, immortalized and non-immortalized cell types, resulting in the discovery that telomerase expression profiles in chicken resemble human telomerase profiles. The genes and regulatory sequences for *cTERT* and *cTR* have been well characterized and *cTERT* splice variants have been described. Proteins relevant to the maintenance of telomeres have been identified or are identifiable due to the availability of chicken genome sequence data. Transcription of RNA from chicken telomeres has been observed. All of the accumulated research strongly supports the conclusion that vertebrate telomere biology is highly conserved and the chicken is an excellent model for the study of telomeres and telomerase. While not a perfect proxy for human telomere biology, the chicken has matured as a model organism and is well positioned to be exploited as a vehicle for future discovery and innovation in telomere and telomerase research.

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