Telomerase gene expression in the chicken: Telomerase RNA (TR) and reverse transcriptase (TERT) transcript profiles are tissue-specific and correlate with telomerase activity

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Abstract

Telomerase is the specialized enzyme which replicates the telomeres, thus maintaining the integrity of the chromosome ends; in absence of enzyme activity telomere lengths decrease, ultimately impacting genome stability. In this study, we examined the mRNA expression of both enzyme components, the RNA template (TR) and catalytic subunit (TERT) during growth and development of the chicken to better understand mechanisms which regulate telomerase activity in vertebrates. Quantitative real-time PCR was used to establish transcript profiles for six ages ranging from pre-blastula to two-year old adults. Organ-specific profiles were established for brain, heart, liver, intestine, spleen and gonad. The pre-blastula and gastrula stages exhibited very high transcript levels of both telomerase components; organs from the embryos and adult showed transcript levels either similar or down-regulated relative to the early differentiation embryo stages. Organs which are known to become negative for telomerase activity between the embryo and adult stages (brain, heart, liver) exhibited down-regulation of TR and either no change or an increase in TERT transcripts. Whereas, organs which maintain high telomerase activity even in adults (intestine, spleen, gonad), generally exhibited up-regulation of transcripts for both components. However, there were some tissue-specific differences between telomerase-positive tissues. These results show that TERT and TR transcript levels correlate with telomerase activity profiles and suggest that TR is the rate-limiting component in telomerase-negative tissues.

Introduction

Telomeres play an important role in maintaining genome stability and their status governs cellular replicative lifespan. The role of the telomere in controlling cell lifespan is primarily a function of age-related telomere erosion due to telomerase activity down-regulation. Telomerase is the dedicated ribonucleoprotein enzyme which maintains telomere length by solving the “end-replication problem” (Cong et al. 2002; Chan and Blackburn 2004). Telomerase consists of two components, an RNA (TR), which con-

tains the template for copying telomeric DNA repeats (Greider and Blackburn 1989; Feng et al. 1995) and a reverse transcriptase (TERT), which catalyzes the addition of the nucleotides to the ends of the chromosome (Lingner et al. 1997). When telomerase is active, telomeres are maintained at a length which prevents the end-DNA from recognition as damaged with the ensuing alert of damage-response pathways; thus a primary function of a healthy telomere is to act as a cap for linear chromosomes. In human and chicken cells, telomerase activity down-regulation and telomere shortening correlate with somatic cell

aging *in vitro* and *in vivo* (Sharma et al. 1995; Taylor and Delany 2000; Forsyth et al. 2002; Delany et al. 2003; Swanberg and Delany 2003; Swanberg et al. 2004).

In chicken somatic tissues such as brain, heart and liver, telomerase activity exhibits embryonic or post-natal down-regulation. However, telomerase activity remains high in proliferative tissues (intestine), reproductive organs (gonad), and tissues with large numbers of stem cells (spleen) (Taylor and Delany 2000). *In vivo* telomere erosion rate estimates are 160 bp/cell division for the hematopoietic lineage and 600 bp/yr for somatic tissue cells; *in vitro* rates for chicken embryo fibroblasts are *ca.* 60 bp/population doubling (Delany et al. 2000; Taylor and Delany 2000; Swanberg and Delany 2003). Similar telomerase activity profiles are found in human cells and tissues (Ulaner and Giudice 1997); erosion rates of 15–30 bp/yr *in vivo* and 30–100 bp/population doubling *in vitro* are reported (Harley et al. 1990; Lindsey et al. 1991; Allsopp et al. 1992; Counter et al. 1992; Levy et al. 1992; Allsopp et al. 1995).

It is important to understand the mechanisms regulating telomerase activity because telomerase status is linked to aging and oncogenesis (Urquidi et al. 2000). Telomerase is down-regulated in aging cells and up-regulated in most transformed cells. Since TERT and TR are required for telomerase activity, knowledge of their gene expression patterns by transcript profiling of normal tissues and cells will provide insight regarding telomerase regulation during aging and dysregulation during oncogenesis. Gene transcription is a primary genetic mechanism and a likely control point for regulation of telomerase activity. TR is a direct transcript (DNA to RNA) and in human cells is reported to be constitutively expressed, whereas TERT is suggested to be the rate-limiting component for telomerase activity (reviewed in Poole et al. 2001).

The chicken is an important higher vertebrate experimental model which shares key features of telomere biology in common with human (Forsyth et al. 2002; Delany et al. 2003) making it an excellent model system for the study of telomerase regulation. Interestingly, recent *in vitro* studies suggest that telomerase component gene expression in chicken (Swanberg et al. 2004; Swanberg and Delany 2005) may not correspond to the simple TERT-based regulatory model developed for human (Ducrest et al. 2002). To date, no information is

available regarding the *in vivo* expression of chicken TERT and TR. Therefore, the objective of this study was to establish transcript profiles of the telomerase components in the domestic chicken, *Gallus domesticus*, from early differentiation to tissues from the embryo and adult for the purpose of determining the genetic mechanisms controlling telomerase activity.

Materials and methods

Animals

A Single Comb White Leghorn inbred line ($F > 0.99$), UCD 003, was used for the study (Abplanalp 1992; Pisenti et al. 1999). Females were artificially inseminated and eggs collected each week, formaldehyde fumigated, and stored at 13°C. Eggs were stored for less than two weeks and then incubated at 37.5°C and 55% relative humidity to collect embryos at different days of embryogenesis (E): E0 (no incubation), E1, E7, E14, and E19. Embryos ranged in developmental status from pre-blastula stage X (Eyal-Giladi and Kochav 1976) to pre-hatching stage 45 (Hamburger and Hamilton 1951). Organ samples were also collected from adult males at two years of age.

Sample collection

The E0 and E1 samples each consisted of a pool of five embryos and three pooled samples per age were used. Brain, heart, liver, and intestine were collected at E7, E14, and E19. Gonad and spleen were collected at E14 and E19. All six organs were collected from the adults. Organs from three individuals per age were collected singly and stored in RNAlater[®] (Ambion, Austin TX) at –20°C prior to RNA isolation.

RNA isolation

The RNAqueous[®]-4PCR Kit (Ambion, Austin TX) was used to isolate RNA from the embryonic samples whereas for adult, the mirVana[™] miRNA Isolation Kit (Ambion, Austin TX) was used. All RNA samples were divided into two aliquots. One aliquot was stored directly at –70°C for archiving;

the other was DNased with 1 μ l RNase-free DNase I (2 U/ μ l) at 37°C for 60 min, treated with 5 μ l DNase Inactivation Reagent (Ambion, Austin TX) and stored at -70°C prior to cDNA synthesis.

cDNA synthesis

The DNased-RNA samples were transcribed to cDNA with the iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules CA) in a 40 μ l reaction, using 20 μ l of RNA. After first strand cDNA synthesis using a blend of oligo(dT) and random hexamer primers provided by the kits, all samples were diluted with 60 μ l of DEPC-treated water and stored at -20°C.

Quantitative PCR (qPCR)

Normalizing and target genes

Three genes were evaluated for selection of the normalizing housekeeping gene including ribosomal protein *L10* (GenBank: CB271063), transferrin receptor (GenBank: X55348.1) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*, GenBank: CB018343). *GAPDH* was selected for use because it exhibited a consistently high transcript level, *i.e.*, the threshold cycle (C_t) values averaged 17.1 ± 1.9 for a subset of representative samples (E0, E1, and E14 organs). *GAPDH* is a widely used normalizing gene and was used in prior chicken telomerase studies (Swanberg et al. 2004; Swanberg and Delany 2005). The target genes for this study were telomerase RNA (*TR*, GenBank: AY312571, Delany and Daniels 2003) and telomerase reverse transcriptase (*TERT*, GenBank: AY502592, Delany and Daniels 2004).

Real-time qPCR conditions

All qPCR reactions were run in 96-well plates using iTaq™ Supermix (Bio-Rad, Hercules, CA). Each reaction contained 480 nM of each primer (forward and reverse), 80 nM of the probe, and 5 μ l diluted cDNA, in a final volume of 12 μ l. The samples were amplified in an MJ Research Chromo 4™ Detector (Bio-Rad, Hercules CA). Fluorescence was collected during each plate read immediately following annealing and the C_t values were determined based on a

threshold of 0.04, with a baseline over cycles 3–15. Amplification conditions were as follows: 95°C for 10 min followed by 40 cycles of 95°C for 15 s, 60°C for 1 min, and fluorescence detection. Reactions were conducted in triplicate per individual (for each gene) and the average value was used for the analysis calculations (see below).

Primers and probes

The primers and probes (5'-3') were as follows: *TR* forward CTCCGCTGTGCCTAACCCTAAT, reverse TCGCCCGCTGAAAGTCAG, probe AATTGATGGTGCTGTCGCCGCG; *TERT* forward TCAGAGCGAAGTCATCACAAGAAT, reverse TGGCAAAACTCTGAAGTGACAAC, probe ATGGATACTCCTTGCTGGATGAGAA; and *GAPDH* forward GGGTCTTATGACCACTGTCCAT, reverse TGGACGCTGGATGATGTT, probe AGACGGTGGATGGCCCCTCTGG.

Sequence verification

To confirm the specificity of the primers for the target genes (*TERT* and *TR*) and normalizing gene (*GAPDH*), the amplicons from traditional PCR (*TERT*) or qPCR (*TR* and *GAPDH*) reactions using cDNA template (E14 brain was used for *TERT* and E0 was used for *TR* and *GAPDH*) were sequence-verified. Amplified products were purified using QIAquick® PCR Purification Kit (Qiagen, Valencia, CA) and the resulting purified amplicons were single-pass sequenced with forward primers on ABI 3730 DNA sequencers at Davis Sequencing (Davis CA). The *TERT* amplicon was 78 nucleotides (nt), and exhibited an exact alignment of 70 nt with *TERT* (GenBank: AY502592) between nt 369 and 439. The *TR* amplicon was 53 nt, and exhibited an exact alignment of 50 nt with *TR* (GenBank: AY312571) between nt 670 and 720. The *GAPDH* amplicon was 80 nt, and exhibited an exact alignment of 64 nt with *GAPDH* (GenBank: CB018343) between nt 54 and 118.

Quantitation analysis

Relative quantification using the comparative C_t method was used to establish fold differences be-

tween samples (Leutenegger et al. 2000; Livak and Schmittgen 2001). Data values were first normalized (target gene C_t –normalizing gene $C_t = \Delta C_t$) on an individual sample basis, then these normalized C_t values were averaged prior to the calibration calculation (e.g., average E7 brain ΔC_t –average E0 $\Delta C_t = \Delta \Delta C_t$). The final value ($1.5^{-\Delta \Delta C_t}$) represents the fold difference between each sample and the calibrator (E0 value in the example above). In this study, 1.5 instead of 2 was used in the final calculation ($1.5^{-\Delta \Delta C_t}$ rather than $2^{-\Delta \Delta C_t}$). Using 2 assumes 100% amplification efficiency and PCRs rarely yield such results (Ramakers et al. 2003). The Chromo 4 detection software, Opticon Monitor (version 3.1, Bio-Rad, Hercules CA) calculated the amplification efficiency for each reaction; from these individual efficiencies, the overall average efficiency (all samples, all genes) was determined to be 50%.

Two sets of comparisons were calculated. For the first set, the E0 transcript values of *TERT* and *TR* were used as the calibrators to compare all tissue results, across all ages. The E0 embryonic stage exhibits high telomerase activity (Taylor and Delany 2000) and represents a very early differentiation state, a pre-blastula embryo of ~40,000 cells (stage X). The

second set of comparisons examined organ-specific changes over time from the embryo, as organs are being established, to the adult. The calibrator for the organ-specific comparisons was the earliest developmental stage sampled for each organ, E7 for brain, heart, liver, intestine and E14 for gonad and spleen.

The difference between the calibrator and other samples is reported as a fold difference, in Table 1 and Figure 1, increase or decrease indicated by up or down arrows, respectively. The fold-difference value (up or down) is reported only when the value was larger than a 2-fold difference from the respective calibrator in each comparison.

Statistical analysis

Analysis of variance (ANOVA) and Tukey's pairwise comparisons were conducted using Minitab Release 13 (Minitab Inc., State College PA). Normalized C_t values were used for the statistical analysis ($\alpha = 0.05$). Some samples were represented only by an n of 1 due to processing problems (e.g., E14 heart and E19 intestine) or because no transcript was detected in some of the individuals (e.g., *TR* from adult heart and

Table 1. Transcript profiles of telomerase genes (*TERT* and *TR*) exhibit either down-regulation or stable expression in a tissue-specific manner following the early differentiation stages.

Tissue	Target gene expression profiles over time							
	<i>TERT</i>				<i>TR</i>			
	E7	E14	E19	Adult	E7	E14	E19	Adult
Brain	↔	↔	3 × ↓	↔	↔	4 × ↓	13 × ↓*	15 × ↓
Heart	5 × ↓*	(3 × ↓)	25 × ↓*	11 × ↓*	4 × ↓	(4 × ↓)	9 × ↓	(12 × ↓)
Liver	9 × ↓*	9 × ↓*	15 × ↓*	3 × ↓	4 × ↓	↔	15 × ↓*	(3 × ↓)
Intestine	↔	↔	(↔)	↔	3 × ↓	↔	(↔)	↔
Gonad	ns	3 × ↓	↔	↔	ns	↔	5 × ↓	8 × ↓
Spleen	ns	6 × ↓*	3 × ↓	↔	ns	↔	↔	↔
ANOVA <i>P</i> -value	0.000	0.001	0.000	0.000	0.085	0.065	0.002	0.008

E = days of embryogenesis; *TERT* = telomerase reverse transcriptase; *TR* = telomerase RNA.

Values in the table indicate fold differences in transcript levels as compared to the E0 levels for *TERT* and *TR*, which were set to 1 (see Methods).

Transcript levels at E1 were not significantly different than at E0 (see Results).

The E7 and E14 embryos are in the organogenesis and growth phases; E19 embryos are in the pre-hatching phase (hatching at E21/E22).

↔ = ≤2-fold difference in gene expression; ↑ = fold increase; ↓ = fold decrease.

* = indicates a statistically significant difference, as compared to the E0 calibrator.

The statistical analyses (ANOVA and Tukey's pairwise comparisons, $\alpha < 0.05$) were performed on normalized C_t values (see Methods).

Values in parentheses are so indicated because only a single sample was analyzed and these were not included in the statistical analysis.

ns = no sample collected.

Figure 1. Ontological expression patterns of telomerase genes (*TERT* and *TR*) in organ systems from embryo to adult. The y axis indicates fold-differences compared to the calibrator set to 1 (E7 for brain, heart, liver, intestine; E14 for gonad and spleen) and the units are on a log scale. Those values which have a fold ↑ (increase) or fold ↓ (decrease) are based on a threshold of greater than 2-fold different. Samples with differences less than the 2-fold threshold are equal to the origin point of 1. Statistically significant differences are marked with '*'. The statistical analyses (ANOVA and Tukey's pairwise comparisons, $\alpha < 0.05$) were performed on normalized C_t values (see Methods). Values which had an n of 1 were not included in statistical analyses; those were E14 heart *TR* and *TERT*, E19 intestine *TR* and *TERT*, adult heart *TR*, and adult liver *TR*. *E* = days of embryogenesis, *TERT* = telomerase reverse transcriptase, *TR* = telomerase RNA.

adult liver) and although these values are reported, they were not included in the statistical analysis.

Results

Comparison one: Early embryo transcript levels versus all samples

The *TERT* and *TR* transcript levels detected in the pre-blastula stage embryo (E0) were used as the comparison calibrator for all other samples. Fold differences from these comparisons are presented in Table 1. Both the *TERT* and *TR* transcript values detected at E1 were similar to those found at E0 (less than a two-fold difference and not statistically different). Twenty-two tissue systems were examined (brain, heart, liver, and intestine at four ages plus gonad and spleen at three ages). Relative to the E0 values, the *TERT* transcript levels were similar in 10 instances and down-regulated in 12 instances; *TR* transcript levels were similar in nine instances and were down-regulated in 13. In none of the 22 instances was up-regulation observed relative to the E0 values. In 13 instances the *TERT* and *TR* profiles were in the same direction, *i.e.*, both genes were similar or both genes showed a lower value compared to the E0 values; thus, in nine instances *TERT* and *TR* profiles differed from each other in terms of direction (e.g., one gene transcript value stayed the same, the other decreased). Those differences found to be statistically significant are indicated in Table 1.

Values three- to four-fold different did not show pairwise statistical significance using Tukey's multiple comparison correction method whereas the values equal to or above a five-fold difference generally showed statistical significance. However, exceptions to this were those cases wherein inter-individual variation was large, even though the direction of the change was always the same. For example, the 15-fold lower value for adult brain *TR* as compared to E0 was not found to be significantly different by the pairwise comparison even though the ANOVA result was significant, see Table 1. When the adult brain *TR* values for the three individuals were calculated separately, they were 7-, 15-, and 30-fold lower.

Brain *TERT* levels were similar at E7, E14, and in the adult but three-fold lower at E19, as compared to E0. Both heart and liver exhibited down-regulation of *TERT* in all embryonic ages and adult, with E19 heart exhibiting the largest overall down-regulation of 25-fold. Intestine *TERT* levels were similar at all ages compared to E0. The gonadal *TERT* transcript profile was generally similar to E0, however, a three-fold lower value was observed at E14, while the spleen *TERT* level was six-fold lower at E14, three-fold lower at E19, but in the adult was similar to E0.

Brain *TR* levels were down-regulated at each age after E7, which was similar to E0, with down-regulation as large as 15-fold in adult. *TR* levels in heart were down-regulated at all ages ranging from four-fold to 12-fold compared to E0. Note that the 12-fold down-regulation in the adult is from one sample

which exhibited a very high C_t value (38.5) while no TR transcript was detectable in the other two individuals. Liver TR was down-regulated four-fold at E7, similar at E14, and down-regulated at E19 (15-fold) and in adult (three-fold). Similar to the adult heart result, the three-fold down-regulation is based on an n of 1 for adult liver due to lack of detectable transcript in the second individual (and a processing loss with the third sample). Intestine TR levels were three-fold lower at E7 but at E14, E19, and in adult were similar to the E0 transcript level. At E14, the gonadal TR level was similar to E0 but then was five- and eight-fold lower at E19 and in adult, respectively. Transcript levels of TR in spleen were similar to those at E0, for all ages analyzed.

Comparison two: Organ-specific transcript levels

The earliest (E7 or E14) developmental age examined was used as the calibration set point (set to 1) for organ-specific comparisons during ontogeny, see Figure 1.

Brain TERT levels remained the same from E7 to adult; heart TERT levels remained the same between E7, E14, and adult, but were five-fold lower at E19. Liver TERT levels were similar among E7, E14, and E19, and then exhibited a slight increase (three-fold) in adult. TERT levels from the intestine showed no change among the embryonic ages, but exhibited a four-fold increase in adult. Gonadal TERT levels showed three- and six-fold increases at E19 and in adult compared to the E14 value, respectively. Spleen TERT levels were similar between E14 and E19 and then exhibited a 17-fold higher value in the adult.

Brain TR levels were found to decrease three-fold between E7 and E14, and were nine- and 10-fold lower at E19 and in adult, respectively. Heart TR levels showed no change from E7 to E14 and E19, but were three-fold lower in adult (and transcripts were not detectable in 2 of 3 adult individuals sampled). Liver TR levels stayed at the same level between E7 and E14, then decreased four-fold at E19, but in the adult the same level was observed as found at E7 (although in one of two adult individuals, there was no detectable TR transcript). Intestine TR values generally increased as development progressed, with higher levels observed at E14 and in adult than in E19, which was similar to E7. Gonadal TR values were four-fold lower at E19 and six-fold lower in

adult. Spleen TR values showed a nine-fold increase in adult TR relative to the E14 value.

Discussion

The chicken has long been a premier model organism for developmental biology (Stern 2005). Genetic and genomic resources such as specialized lines, linkage maps, BAC-physical maps, EST collections (Crittenden et al. 1993; Pisenti et al. 1999; Groenen et al. 2000; Lee et al. 2003; Delany 2004; Hubbard et al. 2005) and the draft sequence (ICGSC 2004) provide the tools essential for further advances using this experimental model system. The chicken system offers comparative biology versatility and several specific attributes as a model for human aging (Swanberg and Delany 2006) not the least of which is its unique telomere biology similarities with human (Forsyth et al. 2002; Delany et al. 2003). Although telomerase activity patterns are well-established within both chicken and human systems (Kim et al. 1994; Wright et al. 1996; Ulaner and Giudice 1997; Mozdziak et al. 2000; Taylor and Delany 2000; Forsyth et al. 2002; Swanberg and Delany 2003), less is known regarding gene transcription and regulation of TERT and TR with the added complication that regulatory controls may be tissue and cell-specific (see reviews of Poole et al. 2001; Cong et al. 2002; Ducrest et al. 2002; Mergny et al. 2002; Liu et al. 2004). Here we report the first extensive ontological study of *in vivo* expression of the telomerase components, *TERT* and *TR*, in a vertebrate organism. Prior developmental analyses using human tissues are limited due to sampling constraints and lack employment of quantitative assays (Ulaner et al. 1998).

Transcript profiles were determined spanning five embryonic ages beginning at the earliest accessible stage (pre-blastula) through to pre-hatching, and in the adult at two years of age. One of the most surprising results was that in no case were TERT or TR up-regulated as compared to the transcripts detected in the pre-blastula (40,000 cell embryo, stage prior to hypoblast formation) and gastrula (formation of endo-, meso- and ectoderm). Thus, from this study and others (Swanberg et al. 2004; Swanberg and Delany 2005) the composite view is that the levels of *TERT* and *TR* in the early stages of development are often higher than observed in other non-transformed

cell systems. The one cell system with higher levels of TERT and TR, relative to the early differentiating embryo, is the DT40 transformed B-cell line which exhibited four-fold higher levels of both transcripts as compared to the gastrula stage (Swanberg and Delany 2005); as well, levels were higher in DT40 compared to chicken embryonic stem cells (Swanberg et al. 2004). Thus, dysregulation of telomerase component gene transcription is a feature of transformation in chicken.

The ages and tissues selected for analysis were chosen to complement profiles previously established for telomerase enzyme activity (Taylor and Delany 2000). Brain, heart, and liver are organs which show high enzyme activity during the initial stages of embryo organogenesis and growth (E5 to E10) but then exhibit a pattern of decreased activity during embryonic development and are telomerase-negative by E20. These tissues remain telomerase-negative in adults. Intestine and gonad are telomerase-positive (high activity) at all embryonic ages, post-hatch and in adults. Spleen exhibits an interesting enzyme activity profile in that initially the tissue is positive (E5 to E14), then the tissue exhibits a decrease in activity to a negative state (E17), followed by an increase just prior to hatching (E20). Spleen is a telomerase-positive tissue in adult. The initial telomerase activity

profile down-regulation at E17 was interpreted to reflect the somatic tissue, whereas the activity increase by E20, was interpreted to be due to the contribution of infiltrating lymphocyte stem cells involved in the process of establishing the spleen germinal centers (Szenberg 1976).

In Table 2, the organ-specific telomerase activity profiles from embryo to adult are compared to the corresponding organ-specific transcript profiles for TERT and TR. TR transcripts exhibit down-regulation in brain, heart, and liver (which show developmental down-regulation of activity and no activity in adult). Brain and heart TERT levels do not change, whereas liver TERT levels were found to up-regulate (all comparisons are relative to E7 stage). These results suggest that TR is the rate-limiting molecule for telomerase activity, *i.e.*, that regardless of TERT levels similar to or higher than the respective telomerase-positive organ level at E7, telomerase activity is down-regulated. This result is in contrast to that reported for human wherein TERT is considered the rate-limiting molecule responsible for controlling telomerase activity (Liu et al. 2004 and references there in). One key piece of information regarding the role for TERT in controlling telomerase activity in human is that telomerase negative cells become telomerase positive upon transfection with TERT (Bodnar et al. 1998). Similar experiments have yet to be conducted with chicken cells although a recent report (Michailidis et al. 2005) shows that telomerase activity in chicken embryo fibroblasts does not resume following transfection with human TERT. Dysregulation of TR in human cancer has been reported (Avilion et al. 1996) and suggests that although perhaps constitutively expressed, TR dysregulation may play a role in human oncogenesis.

In the telomerase-positive organ systems, intestine, gonad and spleen, TERT levels were elevated relative to the corresponding initial age (E7 or E14). And interestingly in these tissues the TR levels in intestine and spleen show up-regulation. These data suggest transcription of both genes may be essential in these organs for maintenance of high enzyme activity levels post-natally; further experimental manipulation is necessary to define the relative contribution of each component. The results for the gonad, TERT up-regulated but TR down-regulated (although telomerase activity remains high in adult), suggest there is an organ-specific difference in regulation even

Table 2. Telomerase component transcript profiles and telomerase activity during ontogeny.

	Changes in profiles from embryo to adult			
	Telomerase activity ^a		Transcript profile ^b	
	Embryo	Adult	TERT	TR
Brain	+	—	↔	↓
Heart	+	—	↔	↓
Liver	+	—	↑	↔/↓ ^c
Intestine	+	+	↑	↑
Gonad	+	+	↑	↓
Spleen	+	+	↑	↑

^aData from Taylor and Delany (2000). Embryo = E7 for brain, heart, liver, intestine and E14 for gonad, spleen. + = positive telomerase activity; — = negative telomerase activity.

^bTranscript profile pattern between E7 (brain, heart, liver, intestine) or E14 (gonad, spleen) and adult (this study); ↔ = no change; ↑ = increase; ↓ = decrease, in transcript level between embryo and adult. TERT = telomerase reverse transcriptase; TR = telomerase RNA.

^cTwo individuals evaluated, one exhibited detectable transcript (similar to E7 value) and one individual had no detectable transcript.

among telomerase-positive organs, wherein TR is not as critical for the gonad to maintain telomerase activity. Splice variant patterns in chicken telomerase-positive and negative cells suggests that there is a role for alternative splice variants (involving the reverse transcriptase motifs of TERT) in regulating expression (Chang and Delany 2005, and in prep). Regulation of TERT by alternative splicing is suggested in human cells and tissues (Ulaner et al. 1998; Ulaner et al. 2001; Krams et al. 2001; Villa et al. 2001) and could involve dominant-negative forms (Colgin et al. 2000; Yi et al. 2000). Our ontological analysis of TERT transcript levels (given the primer and probe locations used for qPCR) does not discriminate between full-length and variant forms. The telomerase enzyme is an oligomer with two molecules each, of TR and TERT (Beattie et al. 2001; Wenz et al. 2001; Arai et al. 2002). An interesting consideration is that TR is a direct transcript whereas TERT message is translated. In human, the TERT mRNA exhibits a half-life of 2–3 hr while TR mRNA has a half-life ranging from 4–32 days and interestingly, the half-life increases with higher telomerase activity and presence of TERT (Yi et al. 1999, 2001). Telomerase has a reported half life of ~24 hr (Holt et al. 1997). Thus, the ratio of telomerase components, mRNA as well as enzyme half-lives, along with control by telomere-binding proteins controlling telomere access, are all of consideration for telomerase activity, regulation and functionality in normal and transformed cells.

In summary, here we show that TERT and TR levels are highest in early differentiating embryos as compared to later time points and tissues of the embryo and adult. Telomerase gene profiles are tissue-specific and have a general pattern correlating with telomerase activity. TR is not a constitutively expressed gene as in some tissues no transcript was detected. In telomerase-negative tissues TR may be the rate-limiting molecule as TERT levels are stable or are up-regulated. In two of three telomerase positive tissues both TERT and TR were up-regulated, but in one (gonad), TR levels down-regulated, suggesting an organ-specific difference even among telomerase-positive tissues. These results provide context and perspective for comparative telomere biology and baseline information for studies of the role for the telomerase pathway in aging and transformation in an important vertebrate model.

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