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Review

Coevolution of telomerase activity and body mass in mammals: From mice to beavers

Vera Gorbunova *, Andrei Seluanov

Department of Biology, University of Rochester, Rochester, NY 14627, USA

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ABSTRACT

Telomerase is repressed in the majority of human somatic tissues. As a result human somatic cells undergo replicative senescence, which plays an important role in suppressing tumorigenesis, and at the same time contributes to the process of aging. Repression of somatic telomerase activity is not a universal phenomenon among mammals. Mice, for example, express telomerase in somatic tissues, and mouse cells are immortal when cultured at physiological oxygen concentration. What is the status of telomerase in other animals, beyond human and laboratory mouse, and why do some species evolve repression of telomerase activity while others do not? Here we discuss the data on telomere biology in various mammalian species, and a recent study of telomerase activity in a large collection of wild rodent species, which showed that telomerase activity coevolves with body mass, but not lifespan. Large rodents repress telomerase activity, while small rodents maintain high levels of telomerase activity in somatic cells. We discuss a model that large body mass presents an increased cancer risk, which drives the evolution of telomerase suppression and replicative senescence.

1. Introduction

1.1. Telomeres and telomerase

Linear chromosomes present a challenge for genome maintenance systems, since chromosome ends need to be distinguished from DNA breaks. An unrepaired DNA break is deleterious for the cell, but if chromosome ends are recognized as a break and are fused together, the result will be gross genomic rearrangements. To solve this problem, chromosome ends are comprised of specialized repetitive sequences called telomeres (reviewed in Chan and Blackburn, 2004). Telomeres are bound by multiple telomere binding proteins (Smogorzewska and de Lange, 2004), and the very end of the chromosome folds back and invades the double-strand region forming the so-called T-loop, which "hides" the free DNA end (Griffith et al., 1999; Stansel et al., 2001; de Lange, 2002). Telomeric DNA consists of tandem repeats of a simple, often G-rich, sequence (Moyzis, 1991; Chan and Blackburn, 2004). In vertebrates, telomeric sequences are highly conserved, and are comprised of several kilobases (kb) of TTAGGG sequences (Moyzis, 1991).

During replication of linear chromosomes regular DNA polymerases are unable to completely replicate chromosome ends, the so called "end-replication problem" (Olovnikov, 1973). The leading strands are synthesized completely, while lagging strand synthesis leaves nascent DNAs incomplete at their 5′ ends. The end replication problem arises due to the inability for the most distal RNA primer to be replaced by DNA, and to inefficient initiation of DNA synthesis by α-primase from the very end of linear DNA (Ohki et al., 2001). To prevent progressive telomere shortening most organisms use the enzyme telomerase (Chan and Blackburn, 2004). Telomerase is a ribonucleoprotein reverse transcriptase, which uses its own RNA molecule as a template to synthesize telomeric DNA at the ends of chromosomes (Chan and Blackburn, 2004).

1.2. Replicative senescence

Telomerase is uniformly expressed in unicellular organisms such as yeast (Vega et al., 2003) and ciliates (Blackburn, 1997). Multicellular organisms, however, only require telomerase in the germline if the telomeres in somatic cells are sufficiently long to allow for enough cell division to occur for development and cell renewal for the duration of animal lifespan.

In humans, telomerase is expressed in early embryos, but later becomes progressively shut off (Bekaert et al., 2004). The majority of normal human somatic cells have no detectable telomerase activity, and their telomeres shorten with every cell division. The

* Corresponding author at: Department of Biology, University of Rochester, 213 Hutchison Hall, Rochester, NY 14627, USA. Tel.: +1 585 275 7740; fax: +1 585 275 2070.
E-mail address: vera.gorbunova@rochester.edu (V. Gorbunova).

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exceptions are immune cells during clonal expansion and stem cells that express a very low level of telomerase activity. Human fibroblasts do not divide indefinitely in culture, and after approximately 60 population doublings (PDs) enter a growth-arrested state termed replicative senescence (Hayflick, 1965). Replicative senescence is triggered by telomere shortening, and irreversible growth arrest is induced when telomeres reach a critical length (Harley et al., 1990; Bodnar et al., 1998) and trigger DNA damage signal (d'Adda di Fagagna et al., 2003; Takai et al., 2003; Herbig et al., 2004).

Replicative senescence is believed to evolve as an adaptive mechanism to protect the organism from uncontrolled cell proliferation and cancer (Campisi, 2001). Tumor cells acquire the ability for uncontrolled proliferation, and telomerase is activated in 90% of human tumors (Kim et al., 1994). Besides its role in telomere maintenance, telomerase has other less well-understood functions in promoting cell proliferation. Telomerase was shown to promote cell growth, transformation, and protects cells from apoptosis (Chang and DePinho, 2002; Gorbunova et al., 2002; Stewart et al., 2002; Gorbunova and Seluanov, 2003). Thus, the repression of telomerase activity in somatic cells provides anticancer effect on multiple levels.

Tumor suppression achieved by the repression of somatic telomerase activity comes at a price. By restricting cell proliferation replicative senescence may slow down wound healing, or dampen immune response. Furthermore, since the force of natural selection declines with age and becomes non-existent in post-reproductive age, replicative senescence has evolved to protect young organisms from cancer, but is deleterious to aged organisms. Senescent cells with shortened telomeres accumulate in aging tissues (Dimri et al., 1995; Campisi, 2005; Jeyapalan et al., 2007). Since senescent cells have altered gene expression, their accumulation in tissues may contribute to age-related deterioration of tissue structure and function (Krtolica et al., 2001; Campisi, 2005).

Replicative senescence was first described in human cells (Hayflick, 1965), and is not a universal phenomenon even among mammals. Mice, for example, being a staple model species for cancer and aging research, differ from humans dramatically in their telomere biology (Wright and Shay, 2000; Davis and Kipling, 2005). Mice express telomerase in multiple somatic tissues (Prowse and Greider, 1995), and mouse cells do not show senescence when cultured at a physiological oxygen concentration (3%) (Parrinello et al., 2003; Itahana et al., 2004). If mouse cells are grown at atmospheric oxygen concentration (20%), which provides an additional oxidative stress to the cells, cell growth slows after 12–15 PDs, but within a few days immortal clones emerge and continue to grow (Parrinello et al., 2003; Itahana et al., 2004). Notably, human fibroblasts enter irreversible growth arrest even if grown in low oxygen, and once senescence is established human cells do not spontaneously immortalize.

The difference between human and mouse telomere biology raises intriguing questions. What is the status of telomerase in other species, beyond humans and mice? What determines whether some species evolve telomerase suppression, while others do not?

2. Coevolution of telomerase repression and body size in rodents, the role of cancer selection

The strikingly different telomere biology of humans and mice could be explained by their differences in lifespan and body mass (Wright and Shay, 2000; Forsyth et al., 2002). Mice are short-lived and in the wild die mostly by predation, thus they do not need efficient anticancer mechanisms. Notably, up to 90% of captive mice die of cancer (Lipman et al., 2004). In contrast, humans are long-lived, and therefore evolved genetic systems that set strict limits on cell proliferation (Wright and Shay, 2000). Similarly, telomerase activity may coevolve with body mass. Humans are larger than mice, and their bodies contain many more cells. Since malignant transformation may occur in any single cell, humans would require more efficient anticancer mechanisms.

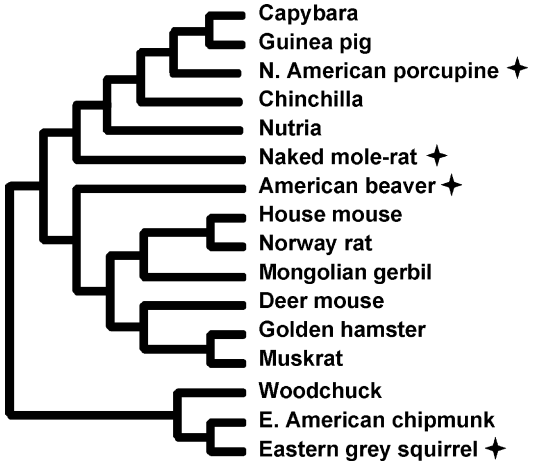

Fig. 1. Rodent phylogeny. The tree topology is based on molecular phylogenies inferred from Martin et al. (2000), Michaux et al. (2001), Murphy et al. (2001), Montgelard et al. (2002), Adkins et al. (2003), Steppan et al. (2004). Stars indicate the species with lifespan longer than 20 years.

We set out to test whether telomerase activity coevolves with lifespan or body mass by analyzing telomere biology in a collection of rodent species (Seluanov et al., 2007). Order Rodentia is ideal for this kind of comparative analysis since it contains species with highly diverse lifespans and body masses: from short-lived mice and rats, to grey squirrel, beaver, porcupine, and naked mole-rat that live over 20 years (AnAgeDatabase; Buffenstein and Jarvis, 2002; Weigl, 2005); and from a 20 g deer mouse to 55,000 g capybara (AnAgeDatabase; Nowak, 1999). The Order possesses another characteristics that are very important for an evolutionary study; these long-lived species belong to different phylogenetic branches (Fig. 1), indicating that long lifespan has evolved at least four times in rodents (Austad, 2005).

Telomerase activity was measured in several individuals from 15 rodent species (Fig. 1) (Seluanov et al., 2007). From each animal, seven tissues (heart, liver, spleen, kidney, skin, lung, and testes for males) were analyzed by TRAP assay. Most rodents showed high telomerase activity in multiple somatic tissues. Surprisingly, high telomerase activity was found in the longest living rodents: naked mole-rat and grey squirrel. The two species almost completely lacking telomerase activity were the beaver and the capybara, which are the largest rodents.

We then calculated a total telomerase activity index for each species and statistically examined its correlation with body mass and lifespan. Telomerase activity in the testes was not included in the index, but served as a positive control. The analysis revealed significant negative correlation between telomerase activity and body mass, but no correlation between telomerase activity and lifespan (Fig. 2). Namely, larger species have stronger repression of somatic telomerase activity. In order to draw conclusions concerning coevolution of traits, it is essential to correct the species data for phylogenetic non-independence (Felsenstein, 1985). The correlation between telomerase activity and body mass remained significant following phylogenetic correction, and also after correction for possible correlation between body mass and lifespan (Seluanov et al., 2007). Thus reduced telomerase activity appears to have evolved in larger but not in longer lived species (Seluanov et al., 2007).

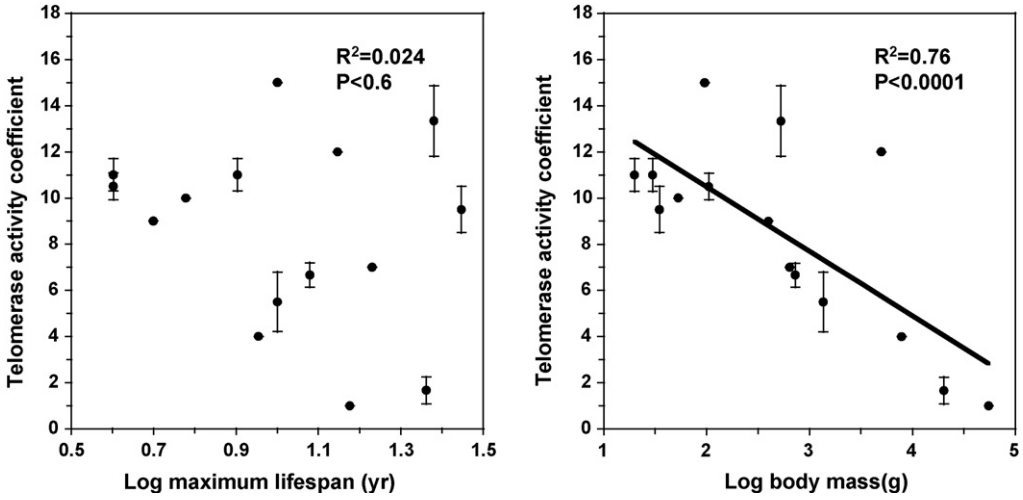

Fig. 2. Correlation between telomerase activity, lifespan, and body mass. R^2 and *P*-values are shown for phylogenetically independent contrasts.

We also measured telomere length in these rodents (Seluanov et al., 2007). The large rodents, beaver and capybara, had "human-like" mean telomeres length (18 kb in capybara and 10 kb in beaver), which suggests that these species may experience replicative senescence. Most other species had long telomeres ranging in size from 30 to 70 kb. Statistical analysis showed that telomere length was not significantly related to either body mass or lifespan (Seluanov et al., 2007). Previous studies showed that telomere length can be highly variable in closely related species of mice or even within the same species (Kipling and Cooke, 1990; Hemann and Greider, 2000), which further indicates that telomere length evolves independently of either body mass or lifespan.

We propose the following model to explain correlation between telomerase activity and body mass (Fig. 3). Evolutionary increases in body mass increase the risk of cancer, as larger bodies contain more cells, and each cell may potentially become cancerous. An average beaver is 2000 times larger than a mouse, thus it may be expected to have 2000 times greater the risk of developing a malignancy. Although we have little data on cancer rates in beavers and other large rodents, the fact that they are doing well as a species, and live considerably longer than mice, suggests they evolved efficient mechanisms to mitigate the cancer risk. We propose that the repression of telomerase activity in somatic tissues is such an adaptive tumor-suppressor mechanism that evolved with an increase in animal body mass (Seluanov et al., 2007).

The idea that a selection imposed by cancer may drive adaptive evolution of tumor-suppressor mechanisms has been proposed earlier (Graham, 1983; Nunney, 1999; Leroi et al., 2003). It was predicted that larger animals should have more tumor-suppressor genes, and their cells may require more steps for tumor formation (Nunney, 1999; Leroi et al., 2003). The data obtained on rodents provides the first experimental support for this theory. In addition to telomerase suppression, other tumor-suppressor mechanisms may evolve with body mass as well (Promislow, 1994).

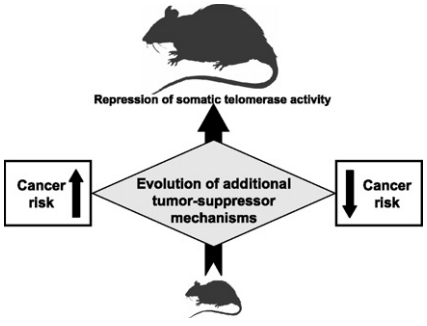

Fig. 3. Model explaining coevolution of telomerase activity and body mass. Evolutionary increases in body mass lead to increased cancer risk. To counteract this risk large species evolve additional tumor-suppressor mechanisms such as the repression of telomerase activity in somatic cells.

The proposed relationship between body mass, cancer risk, and evolution of tumor-suppressor mechanisms (Seluanov et al., 2007) applies to between-species comparisons where increased cancer risk conferred by increase in body mass is counteracted by additional tumor suppressors arising during million years of evolution. In contrast, larger individuals within the same species are likely to experience greater cancer incidence. For example, there are reports of a positive correlation between body weight and tumor incidence in mice and rats (Gries and Young, 1982; Anisimov et al., 2004a,b).

Longer lifespan would also be expected to increase the life-time cancer risk. It is intriguing that no correlation was detected between telomerase activity and lifespan (Seluanov et al., 2007). Two explanations may be offered. Body mass may confer a much greater cancer risk than lifespan, or alternatively, different risk factors may be associated with distinct sets of anti-tumor adaptations. Large body mass drives the evolution of replicative senescence, while long lifespan may be associated with other adaptations, such as more precise DNA repair.

3. Telomere biology across mammalian species

3.1. Primates

Telomere biology has been studied in several primate species, and a relatively extensive body of data is available about telomeres in our closest relatives. The tissue distribution of telomerase activity in several species of Macaques (rhesus monkey (*Macaca mulatta*), Japanese monkey (*Macaca fuscata*), and crab-eating monkey (*Macaca fascicularis*)) is similar to humans. Telomerase is repressed in most somatic tissues; low activity is detected in spleen, thymus, and digestive tract, with high activity found in testes (Kakuo et al., 1999; Gardner et al., 2007). Primate telomeres have been reported to be 15–23 kb, which is somewhat longer than in human cells (Kakuo et al., 1999). However, more recent studies reported telomere lengths from 4 to 16 kb in a few New World primates: spider monkey (*Ateles geoffroyi*), and squirrel monkey (*Saimiri scuireus*), and in several Old World primates: rhesus

monkey (*Macaca mulatta*), orangutan (*Pongo pygmaeus*), and pigmy chimpanzee (*Pan paniscus*) (Steinert et al., 2002), which are similar to human (Moyzis et al., 1988). The difference may be explained by a longer subtelomeric region in non-human primates (Gardner et al., 2007).

Telomere shortening was shown to occur *in vivo* in proliferative tissues of aged crab-eating monkey, pig-tailed macaque (*Macaca nemestrina*), chimpanzee, and baboon (*Papio hamadryas*) (Feng et al., 1998; Shibata et al., 1999; Lee et al., 2002; Gardner et al., 2007). Tissues of aging baboons accumulate senescent cells that display senescence-associated DNA damage foci colocalizing with telomeres (Herbig et al., 2006; Jeyapalan et al., 2007), indicating that replicatively senescent cells accumulate in aged primate tissues. Anthropoid fibroblasts display cellular senescence in culture and do not spontaneously immortalize (Steinert et al., 2002), which is again similar to human cells. In contrast, fibroblasts from the prosimian ring-tailed lemur (*Lemur catta*) stop dividing at PD 80, but after a period of several days single-cell colonies emerge and continued to grow (Steinert et al., 2002). Interestingly, escape from senescence in lemur fibroblasts was not accompanied by telomerase activation, but was explained by fusion between the chromosomes with critically short telomeres and other chromosomes (Steinert et al., 2002).

From this data, it can be concluded that repression of telomerase activity and replicative senescence is evolutionarily conserved among anthropoid primates, and arose in a common ancestor of anthropoids around the time of separation between anthropoid and lemurs. An alternative possibility is that repression of telomerase activity evolved independently in primate lineages due to their large body mass. Notably, the primates which showed repression of telomerase activity and the stringent senescent arrest, are large animals. Body mass ranges from 64,000 g in orangutan to 8000 g in macaques. The ring-tailed lemur, which showed a somewhat relaxed senescence arrest, is the smallest of examined species with an average adult body mass of 2500 g. In this respect, it would be interesting to examine telomerase activity in the tissues of marmoset (*Callithrix jacchus)*, the smallest anthropoid with average adult body mass of 300 g.

3.2. Ungulates

Among ungulates, the best-studied species are farm animals, including cow, sheep, pig, and horse. No telomerase activity was detected in cow, sheep, horse, deer *Muntiacus* (muntjac) fibroblasts, and various sheep, or equine somatic tissues (Thomas et al., 2000; Cui et al., 2002; Zou et al., 2002; Argyle et al., 2003; Hartmann and Scherthan, 2005). Telomere length in cow, sheep, horse and donkey is similar and ranges from 7 to 23 kb (Kozik et al., 1998; Argyle et al., 2003; Davis et al., 2005; Jeon et al., 2005). Cow, sheep, and horse fibroblasts have a finite lifespan in culture, after which the cells enter senescence (Hornsby et al., 1986; Argyle et al., 2003; Davis et al., 2005). Thus telomere biology in ungulates is similar to human. Cow, sheep, and horses are all rather large animals and repression of telomerase activity in these species supports the model of coevolution of telomerase activity and body mass.

Telomere biology in pigs seem to be an exception, as pig telomerase activity behaves similar to mouse and rat rather than other large animals. Pig telomeres have lengths similar to other ungulates, ranging in size from 10 to 30 kb (Fradiani et al., 2004; Jeon et al., 2005). However, two reports indicate that multiple pig somatic tissues such as lymph nodes, lung, kidney, and small intestine retain significant levels of telomerase activity (Wong et al., 2003; Fradiani et al., 2004). During *in vitro* culture pig fibroblasts enter a period of slow growth associated with appearance of senescent markers, after which immortal cells emerge and resume rapid growth (Oh et al., 2007). Interestingly, the analysis of normal fibroblasts from Sinclair swine showed no telomerase activity (Pathak et al., 2000), suggesting that telomerase regulation in pigs may differ by genetic background.

3.3. Carnivores

Among carnivores, telomere biology has only been studied in domestic cats and dogs. The majority of normal cat tissues do not express telomerase activity (Cadile et al., 2001; McKevitt et al., 2003). TERT mRNA has been detected in the testis, digestive tract, spleen, pancreas, bone marrow, and lymph nodes, while no expression was observed in the liver, adrenal gland, urinary bladder, and lung (Yazawa et al., 2003). Cat telomeres range from 5 to 26 kb, and telomere shortening was observed with age *in vivo* (Brummendorf et al., 2002; McKevitt et al., 2003).

Telomere biology in dogs is similar to that in cats. Normal dog somatic tissues show little or no telomerase activity, and high activity is found in testis (Yazawa et al., 1999; Nasir et al., 2001). Normal dog fibroblasts are telomerase negative (Yazawa et al., 2003). Mean telomere length in dogs is in the range of 12–23 kb (Nasir et al., 2001), and telomere shortening was observed with age *in vivo* in some breeds and in cultured fibroblasts (Nasir et al., 2001; McKevitt et al., 2002). There is a strong association between neoplasia and telomerase activity in dogs (Nasir, 2007), and an increase in telomerase activity or TERT expression was found in other pathological conditions such as in the failing heart (Leri et al., 2001), and in cataractous lens (Colitz et al., 1999). Overall, cats and dogs seem to regulate telomerase activity in a manner similar to humans, and are likely to utilize replicative senescence as an anticancer mechanism.

3.4. Lagomorphs

Telomere biology has been analyzed in skin fibroblasts of four species of rabbits (order Lagomorpha): European white rabbit (*Orytolagus cuniculus*), black-tailed jack rabbit (*Lepus californicus*), swamp rabbit (*Sylvilagus aquaticus*), and North American pika (*Ochotona princeps*) (Forsyth et al., 2005). All species displayed long and heterogenous telomeres ranging from 2 to 50 kb (Forsyth et al., 2005). None of the cell lines underwent growth arrest in culture after at least 60 PDs in a reduced oxygen environment. Interestingly, telomerase activity was not detected in cultures from European white, black-tailed jackrabbit and swamp rabbits, and it was concluded that extended culture was possible due to their extremely long telomeres. Since rabbits lack telomerase activity and certain telomere shortening occurred in culture, it is possible that the cells would have undergone replicative senescence if cells have been cultured for a longer period. As senescence occurring beyond 60 PDs would be unlikely to be an efficient tumor-suppressor, rabbits may represent an intermediate stage with regard to the evolution of replicative senescence. Cells from only one species, pika, displayed telomerase activity (Forsyth et al., 2005). Pika is a smaller animal (100 g body mass relative to 4000 g for jackrabbit). The finding of telomerase activity in pika while not in their larger relatives supports the model of coevolution of telomerase activity and body mass.

4. Summary and future directions

Repression of telomerase activity coevolves with increased body mass in rodents. This can be explained by repression of telomerase activity being an adaptation that evolved to mitigate the increased risk of cancer conferred by large body size. Is coevolution of telomerase activity and body mass specific to rodents or is it a general phenomenon across mammals? Telomere biology in mammals studied so far supports the model of coevolution of telomerase activity and body mass (Table 1). A

Table 1
Summary of the data available on telomerase activity and the presence or absence replicative senescence in mammalian species

Species, common name	Adult body mass (g)	Maximum lifespan (year)	Telomerase activity in normal somatic tissues[a]	Replicative senescence
Human	70,000	122	Restricted	Yes
Macaque species	7,000–8,800	30–40	Restricted	Yes
Spider monkey	8,000	47	Restricted	Yes
Squirrel monkey	925	30	Fibroblasts negative[b]	Yes
Orangutan	65,000	59	Restricted	Yes
Chimpanzee	45,000	74	Restricted	Yes
Baboon	18,000	38	Restricted	Yes
Ring-tailed lemur	2,555	37	Fibroblasts negative[b]	Yes/No[c]
Cow	750,000	20	Restricted	Yes
Sheep	70,000	20	Restricted	Yes
Pig	90,000	22	Not restricted	Yes/No[c]
Horse	500,000	57	Restricted	Yes
Cat	3,900	30	Restricted	
Dog	40,000	24	Restricted	
Jack rabbit	4,000	12	Fibroblasts negative[b]	No
Swamp rabbit	1,160	7	Fibroblasts negative[b]	No
Pika	100	7	Not restricted	No
American beaver	20,250	24	Restricted	
Capybara	55,000	15	Restricted	
Guinea pig	728	10	Not restricted	
Chinchilla	642	17	Not restricted	
Nutria	7,850	9	Somewhat restricted	
Naked mole-rat	35	28	Not restricted	
House mouse	30	4	Not restricted	No
Norway rat	400	5	Not restricted	No
Mongolian gerbil	53	6	Not restricted	
Deer mouse	20	8	Not restricted	
Golden hamster	105	4	Not restricted	
Muskrat	1,362	10	Not restricted	
Woodchuck	5,000	14	Not restricted	
E. American chipmunk	96	10	Not restricted	
Eastern grey squirrel	533	24	Not restricted	

For references please see text.

[a] Restricted, indicates that telomerase activity is undetectable in multiple somatic tissues. Not restricted, indicates that telomerase activity is detected in the majority of somatic tissues.

[b] Skin fibroblasts have no detectable telomerase activity. There is no information on other tissues.

[c] High rate of spontaneous immortalization.

general trend seems to be that animals with a body mass smaller than 2 kg exhibit telomerase activity in somatic tissues and do not use replicative senescence (Table 1). This group includes small rodents of both short and long-lived species, and the North American pika (Wright and Shay, 2000; Forsyth et al., 2005; Seluanov et al., 2007). Animals with a body mass of 2–5 kg may have a somewhat intermediate status, and may not express telomerase in fibroblasts, or undergo strict replicative senescence. Such "intermediate" telomere regulation was shown for rabbits and the ring-tailed lemur (Steinert et al., 2002; Forsyth et al., 2005). Finally, animals larger than 5 kg are likely to have strict regulation of telomerase activity and display replicative senescence. This was observed for large primates, cows, sheep, equines, cats, and dogs (Thomas et al., 2000; Cadile et al., 2001; Cui et al., 2002; Steinert et al., 2002; Zou et al., 2002; Argyle et al., 2003; McKevitt et al., 2003; Hartmann and Scherthan, 2005; Nasir, 2007).

Although, the general trend supports the model, many more species need to be examined to test the generality of the coevolution of telomerase repression and body mass. Today, we know surprisingly little about the telomere biology of species outside a usual laboratory bestiary. However, exceptions to this rule are likely to be found, one such exception being a pig, a large animal with "mouse-like" telomere biology. Other surprises and unusual telomere maintenance mechanisms may still be found, and understanding the evolution of telomerase regulation will ultimately extend our understanding of telomere biology in humans, and may open new avenues to treat human cancer, a disease in which telomerase regulation ultimately fails.

Many new questions arise from these studies. Small rodents express high levels of somatic telomerase activity, and have long telomeres (Seluanov et al., 2007), making them unlikely to use replicative senescence as an anticancer mechanism. Among these rodents are some extremely long-lived species such as grey squirrel and naked mole-rat that live over 20 years (Buffenstein and Jarvis, 2002). How do these species live their lifespan and not succumb to cancer at the age of 2 years, which is typical for mice and rats? Learning more about the mechanisms that prevent cancer in these species with active telomerase may help fight cancer in humans, where tumors often arise from telomerase positive stem cells. Even more puzzling are the giant mammals, such as whales. If body size is such an important risk factor for tumorigenesis, we can only guess how many additional tumor-suppressor pathways are required to ensure the cancer-free development of a blue whale.

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