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VISIONS AND PERSPECTIVES

Telomeres and telomerase in basal Metazoa

I Udroiu, V Russo, T Persichini, M Colasanti, A Sgura

Department of Science, University ROMA TRE, Rome, Italy

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Abstract

Telomeres are protein-bound tandemly repeated simple DNA sequences placed at the chromosome ends, their role being essential for maintaining genome integrity. Severe telomere damage can trigger potential carcinogenic events such as chromosome fusions, whilst telomere shortening results (at least in mammals) in a protective mechanism known as 'replicative senescence'. In most Metazoa, telomere shortening is avoided by the ribonucleoprotein telomerase. In this brief overview, we focused on the evolutionary conservation of telomeres and telomerase in basal Metazoa (Ctenophora, Porifera, Placozoa, Cnidaria). In all these taxa, telomerase seems to be active and telomeres show the canonical TTAGGG sequence. Presence of telomerase activity and absence of telomere shortening is in accordance with the lack of senescence seen in basal Metazoa, although a clear correspondence between the “demographic senescence” and “replicative senescence” remains to be elucidated. Nonetheless, basal Metazoa can be useful as model organisms in studies on the evolution of telomere biology, Evo-Devo investigations on the control of telomerase activity, the emergence of senescence, as well as telomere-independent effects of telomerase.

Key Words: basal Metazoa; senescence; telomerase; telomeres

Introduction

Telomeres are specialized structures at the ends of eukaryotic chromosomes, consisting of protein-bound tandemly repeated simple DNA sequences (Armstrong and Tomita, 2017). With a few exceptions, including plant and insect species, telomeric DNA sequences is usually rich in guanine and is a repeat of six bases. The sequence is TTAGGG/CCCTAA in all vertebrates underlining that is highly conserved to protect genome (Armstrong and Tomita, 2017).

Telomeres fulfill unique and essential functions in genome integrity. In general, non-telomeric double-strand DNA breaks (DSBs) are not tolerated and are rapidly repaired. This scenario, however, does not apply to telomeres, thanks to the association of telomeric DNA with specialized proteins whose role is to organize the linear chromosome end into a stable structure (T-loop) that is not recognized by the cell as a chromosome break. In mammals, the T-loop is held together by seven known proteins, the most notable ones being TRF1, TRF2, POT1, TIN1, and TIN2, collectively referred to the Shelterin complex (Blackburn, 2000; Armstrong and Tomita, 2017) (see also Fig. 1).

This structure is required to maintain chromosome physiology, and loss of telomeric DNA or mutations of telomere-binding proteins triggers a series of events including chromosomal fusions (Fig. 2) and genomic instability that ultimately compromise cell proliferative capacity and/or viability (Blackburn, 2000; DePinho, 2000; Berardinelli *et al.*, 2010; Armstrong and Tomita, 2017).

All chromosomes loss a small amount of telomeric DNA every time a somatic cell divides because of the noted “end replication problem”. To avoid continuous sequence loss from the telomeres in dividing cells, special mechanisms have evolved. In most eukaryotic organisms, the solution to the problem involves a ribonuclear complex, called telomerase that consists of an enzymatic part (transcriptase, TERT) and a RNA component (TR). This last functions like a template for the *de novo* synthesis of telomeric DNA sequences. However, telomerase activity varies across taxa and differs in mortal and immortal cells (Armstrong and Tomita, 2017). It is well established that an active telomerase is required for unlimited growth; in fact, it is repressed in the majority of human somatic cells while its activity is higher in immortal cell lines, germline cells, stem cells, activated lymphocytes,

Corresponding author:
Marco Colasanti
Department of Science
University ROMA TRE
Viale G. Marconi 446, 00146, Rome, Italy
E-mail: marco.colasanti@uniroma3.it

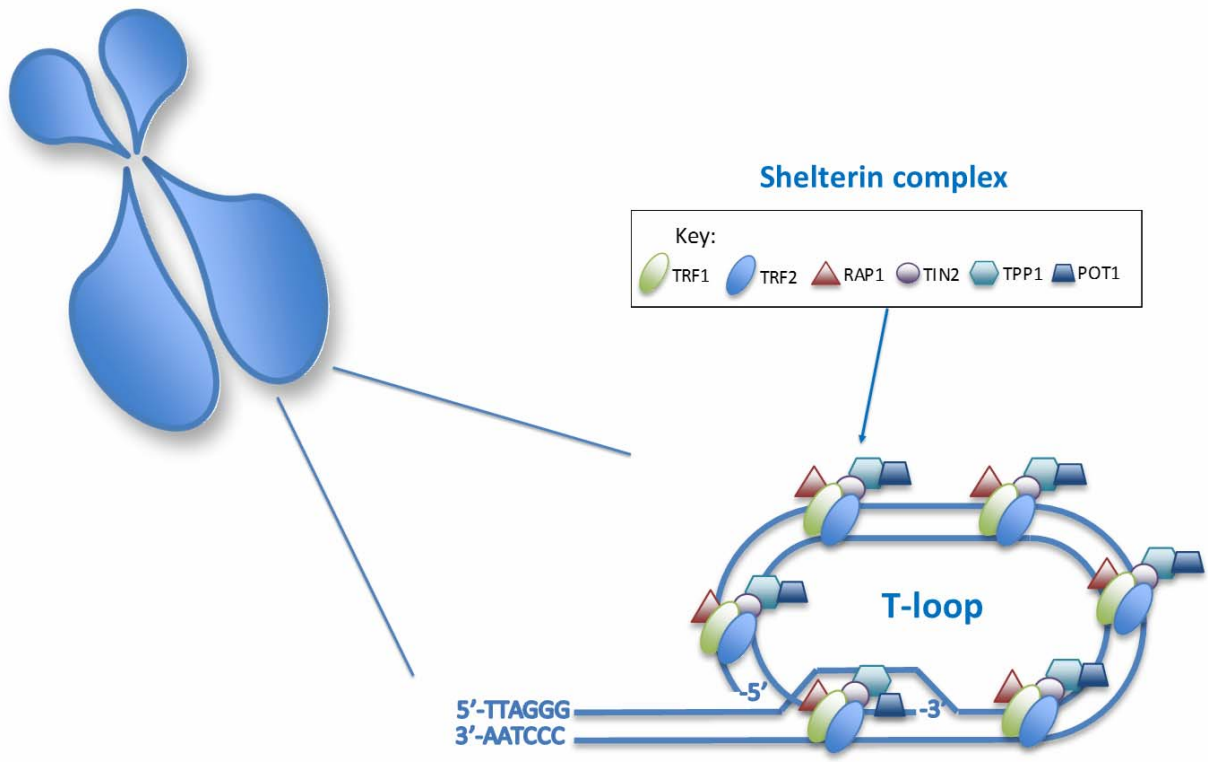

Fig. 1 Representation of a closed (capped) telomere. In mammals, specialized proteins, collectively referred to the Shelterin complex, organize the linear chromosome end into a stable structure (T-loop) [modified from (Berardinelli *et al.*, 2016)].

and most of the tumor cells analyzed (Schmitt *et al.*, 1994; Bolzan *et al.*, 2000). Note that loss of telomerase enzymatic function leads to progressive telomere shortening over time, eventually resulting in the disappearance of detectable telomeric DNA. Loss of chromosomal end capping has consequences in a wide range of cellular processes, including senescence, apoptosis, and carcinogenesis (Harley *et al.*, 1992; Morin, 1996) (Fig. 3).

Telomeres and telomerase in senescence

Telomere shortening progresses with each cell cycle due to the "end replication problem" up to reach a critically telomere length that induce cell cycle arrest and activation of senescence profile that contribute to tumor suppressor mechanism. Though, sometimes cells lose the ability to senesce because, for example, of mutation in p53 protein.

Telomere shortening may initiate chromosomal instability through end-to-end fusion of unprotected chromosomes. *In vitro*, cells with extensive chromosomal instability succumb to crisis or M2 stage of replicative senescence, which is characterized by wide-spread cell death. However, a rare human cell can escape M2 by reactivating or up-regulating telomerase activity, which will result in indefinite cell proliferation, typical of tumor cells (Armstrong and Tomita, 2017). Despite many factors have been involved in cell transformation, up to today telomerase activity seems to be the principal responsible of unlimited proliferation of tumor cells confirmed by the knowledge that most tumor cells express telomerase. More rarely, a cell may engage an alternative to telomerase for maintaining telomeres (Bryan *et al.*, 1997) that appears to involve DNA recombination between the telomere sequences. Nevertheless, activation of a telomere-maintenance mechanism does not seem to be enough to confer a cancerous phenotype, even when combined with *p53* inactivation, which represses the senescence response. Therefore, it appears plausible that other changes must occur at the cellular level for initiation of carcinogenesis process.

Telomeres and telomerase in basal Metazoa

Telomeric DNA of eukaryotes is formed by a long array of short tandem repeats. The TTAGGG sequence is conserved in almost all Metazoa (Fig. 4) and, since this motif is shared also by their unicellular sister-group Choanoflagellata (Fairclough *et al.*, 2013), it can be inferred that it represents the ancestral telomeric repeat. Other patterns present among Metazoa are likely to have appeared later during evolution. This should be the case of the TTAGGC motif in nematodes, as their sister-group - Chaetognatha - displays the standard metazoan sequence. Similarly, we believe that the TTAGG sequence found in most Arthropoda represents an apomorphic character, since their sister-group Onychophora still shows the common TTAGGG

motif. Finally, it must be added that - among Arthropoda - Diptera shows telomeres composed of transposable elements, which are added to chromosome ends by transposition, an alternative mechanism of telomere maintenance present in this taxon that does not show telomerase (Frydrychova *et al.*, 2004).

Telomere length has been studied in some basal metazoan species. Southern hybridization of *Rsa*I/*Hin*fI-digested genomic DNA showed that telomere lengths are between 0.5 and 20 kb in *Leucosolenia*, *Sycon* (Porifera) and *Pleurobrachia pileus* (Ctenophora) (Traut *et al.*, 2007). Among Cnidaria, using the same technique, telomeres measure 0.5 - 10 kb in *Chrysaora hysoscella*, 1 - 20 kb in *Cyanea lamarcki*, 4 - 20 kb in *Hydra vulgaris*, 2-25 kb in *Nematostella vectensis* (Traut *et al.*, 2007) and 3.5 kb in *Acropora surculosa* (Sinclair *et al.*, 2007). Using telomere restriction fraction (TRF), lengths are 8.9 kb in *Acropora digitifera* (Tsuta *et al.*, 2014), 21.0 kb in *Acropora millepora* and 19.2 kb in both *Agaricia fragilis* and *Madracis auretenra* (Zielke and Bodnar, 2010). Performing the single telomere length assay (STELA), telomere length in *Cassiopea andromeda* is valued 1 - 2 kb (Ojimi and Hidaka, 2010). Finally, in *Galaxea fascicularis* telomere length measured with STELA was 4 kb and 15.6 kb with TRF (Tsuta and Hidaka, 2013).

As we can see, there is a great variability in telomere length measures, most probably due to differences in protocols and technique employed. Nonetheless, all this data fall in the same order of size below 25 kb, similar to other taxa like nematodes

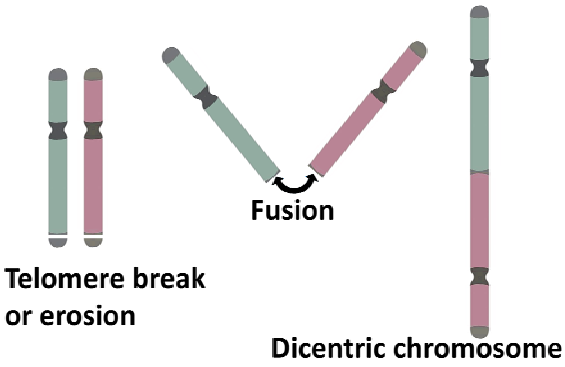

Fig. 2 Mechanism of telomere fusion. Break or replicative erosion leave telomeres uncapped, leading to end-to-end fusion, resulting in a dicentrici chromosome.

(Wicky *et al.*, 1996), platyhelminths (Koroleva *et al.*, 2013), non-dipteran insects (Vitkova *et al.*, 2005) and fish (Ocalewicz, 2013). A much greater variability, instead, is present among tetrapods, reaching the maximum in endotherms, with telomeres lengths ranging from 5 to hundreds of kb. This is thought to be the result of different evolutionary pressures and linked to telomere-shortening senescence, lifespan and body mass (Gomes *et al.*, 2011).

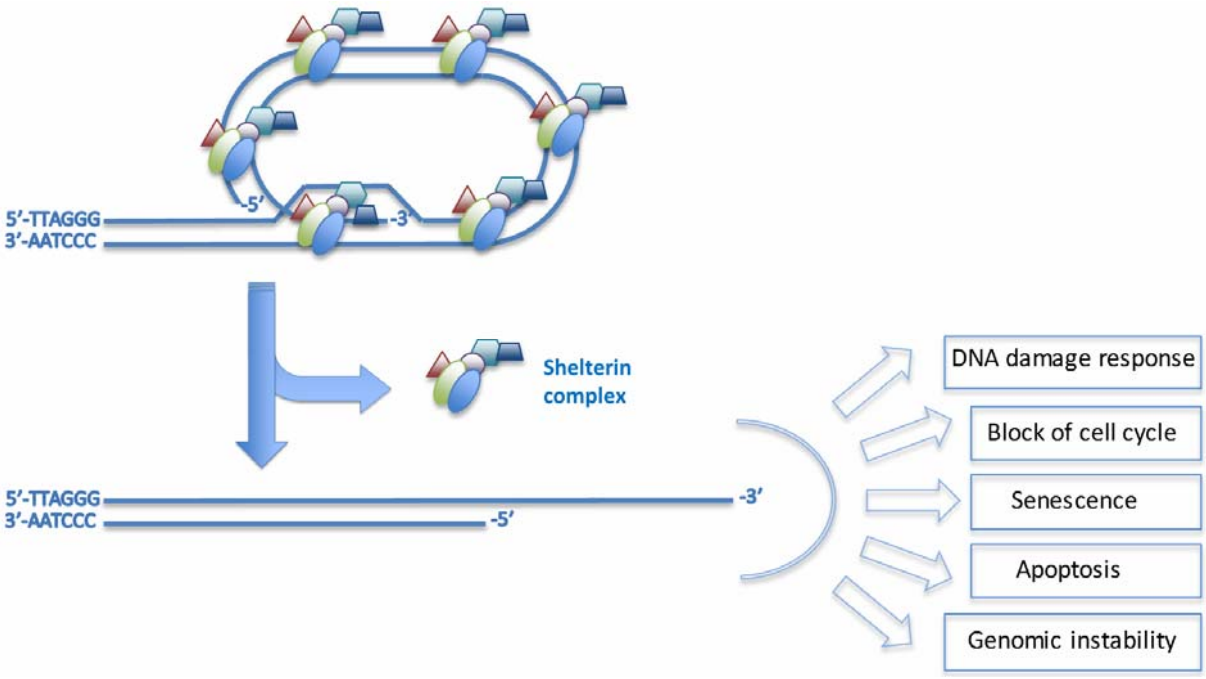

Fig. 3 Telomeric dysfunction/shortening. Extensive telomeric erosion or telomere dysfunction activate several biological processes including activation of DNA damage response, block of cell cycle, apoptosis and senescence [modified from (Berardinelli *et al.*, 2016)].

A few studies investigated the presence of telomerase in basal Metazoa (Table 1). Applying the telomeric repeat amplification protocol (TRAP) assay, telomerase activity has been detected in *Cassiopea* sp. (Ojimi *et al.*, 2009), *Galaxea fascicularis* (Nakamichi *et al.*, 2012), *Madracis auretenra* and *M. decactis* (Zielke and Bodnar, 2010). Traut *et al.* (2007), performing the same assay, found positive results in *Pleurobrachia pileus* and *Aurelia aurita*, but did not find telomerase activity in *Chrysaora hysoscella*, *Cyanea lamarcki*, *Hydra vulgaris*, *Nematostella vectensis*, *Trichoplax adhaerens*, *Leucosolenia* sp., *Sycon* sp. and *Suberites domuncula*. However, it must be underlined that the same authors stated "this may be merely due to technical reasons". In fact, another study employing the TRAP assay on *Suberites domuncula* found positive results (Koziol *et al.*, 1998). Moreover, the gene encoding telomerase reverse transcriptase (TERT) is present (Table 1) in *Trichoplax adhaerens* (Robertson, 2009b), *Hydra vulgaris* and *Nematostella vectensis* (Steele *et al.*, 2011). From all these evidences, and from the detection of the TERT gene also in Choanoflagellata (Robertson, 2009a), we deduce that telomerase is present in all basal Metazoa.

Some of the studies on Cnidaria cited above investigated also possible differences in telomere length and telomerase activity in the different tissues and/or life stages of the studied species. In *Cassiopea andromeda*, longer telomeres were found within cells of the bell region of the medusa compared to those of polyps, asexual propagules, or other regions of the medusa (Ojimi and Hidaka, 2010), but no differences in telomerase activity was found between polyps and the bell region of the medusa (Ojimi *et al.*, 2009). In *Galaxea fascicularis*, no differences in telomerase activity were found between somatic and gonad-containing tissues (Nakamichi *et al.*, 2012); moreover, no differences in telomere length of its developmental stages (sperm, planula larvae and polyps) were detected (Tsuta and Hidaka, 2013). On the other hand, differences were observed in the mean telomere lengths during development (sperm > planulae > polyps) of *Acropora digitifera* (Tsuta *et al.*, 2014). Finally, another study indicated that sperm of *Ctenactis echinata* has longer telomeres than that of somatic tissues and no relationship between telomere length and the weight/age of individuals (Ojimi *et al.*, 2012); however, in that work a great variability was present both among replicates and among individuals.

However, in recent years it has become apparent that telomerase (*e.g.*, its protein subunit TERT) exerts functions that are relevant to cell proliferation but unrelated to telomere maintenance, as shown in mammal models. Among the growing list of telomere-independent functions of TERT/telomerase is the ability of TERT to amplify signaling by the Wnt pathway, by serving as a cofactor of the β-catenin/LEF transcription factor complex (Park *et al.*, 2009). Other ascribed telomere-independent effects include demonstrable enhancement of cell proliferation and/or resistance to apoptosis (Kang *et al.*, 2004), involvement in DNA-damage repair (Masutomi *et al.*, 2005), and

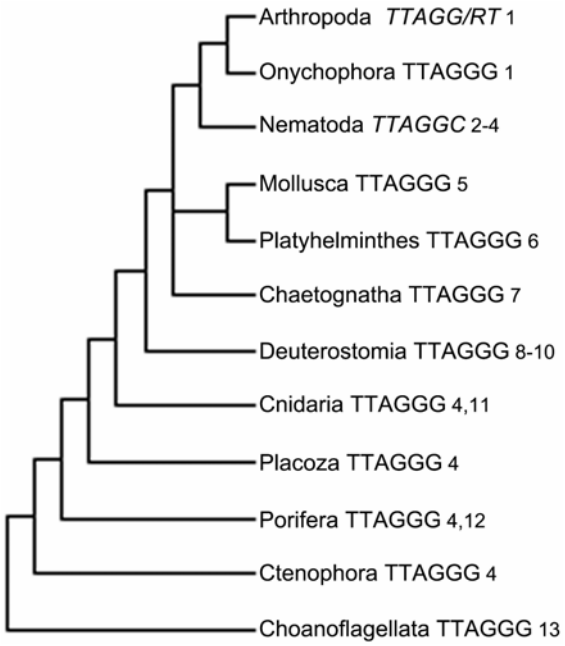

Fig. 4 Phylogenetic tree showing telomere motifs in Metazoa. Tree after Dunn et al. (2014). Length of branches is arbitrary. RT: retrotransposons. References: 1: (Vitkova *et al.*, 2005); 2: (Wicky *et al.*, 1996); 3: (Niedermaier and Moritz, 2000); 4: (Traut *et al.*, 2007); 5: (Nomoto *et al.*, 2001); 6: (Bombarova *et al.*, 2009); 7: (Barthelemy *et al.*, 2008); 8: (Meyne *et al.*, 1989); 9: (Castro and Holland, 2002); 10: (Li *et al.*, 2007); 11: (Sinclair *et al.*, 2007); 12: (Sakai *et al.*, 2007); 13: (Fairclough *et al.*, 2013).

RNA-dependent RNA polymerase function (Maida *et al.*, 2009). Consistent with these broader roles, TERT can be found associated with chromatin at multiple sites along the chromosomes, not just at the telomeres (Masutomi *et al.*, 2005, Park *et al.*, 2009). Hence, telomere maintenance is proving to be the most prominent of a diverse series of functions to which TERT contributes. However, the contributions of these additional functions of telomerase in basal Metazoa remain to be elucidated.

Are telomeres and telomerase involved in basal Metazoa senescence?

Presence of telomerase activity and absence of telomere shortening is in accordance with the lack of senescence seen in basal Metazoa (Finch, 1990). However, the issue about aging in coral reefs - which is seen at a colonial level (Rinkevich and Loya, 1986) - is open, as it is the possibility that this is linked or not to telomere shortening.

In mammals, telomerase is differentially regulated in different tissues. This regulation also varies throughout development (Table 2). At early stages, telomerase is highly expressed. Later on, it is downregulated in a tissue-specific manner (Forsyth *et al.*, 2002). This downregulation reaches

Table 1 Telomerase in basal Metazoa

Phylum	Species	TRAP assay	TERT gene
Porifera	*Suberites domuncula*	+ (1) - (2)	?
	Leucosolenia sp.	- (2)	?
	Sycon sp.	- (2)	?
Placozoa	*Trichoplax adhaerens*	- (2)	+ (3)
Ctenophora	*Pleurobrachia pileus*	+ (2)	?
Cnidaria	*Aurelia aurita*	+ (2)	?
	Cassiopea sp.	+ (4)	?
	Chrysaora hysoscella	- (2)	?
	Cyanea lamarcki	- (2)	?
	Galaxea fascicularis	+ (5)	?
	Hydra vulgaris	- (2)	+ (6)
	Madracis auretenra	+ (7)	?
	Madracis decactis	+ (7)	?
	Nematostella vectensis	- (2)	+ (6)

References: 1: (Koziol *et al.*, 1998); 2: (Traut *et al.*, 2007); 3: (Robertson, 2009b); 4: (Ojimi *et al.*, 2009); 5: (Nakamichi *et al.*, 2012); 6: (Steele *et al.*, 2011); 7: (Zielke and Bodnar, 2010).

complete silencing in many species (like humans), where almost all adult tissues are telomerase-negative (with the notable exceptions of germ and hematopoietic cells). We can infer that in basal Metazoa, as well as in ectotherm chordates, different levels of telomerase activity in the various tissues and/or developmental stages are due to different proliferating rates, while in endotherms an additional level of control exists, in the form of active silencing of telomerase, probably through alternative splicing.

A correlation between telomere shortening and cellular senescence was proposed many years ago and is currently widely accepted (reviewed in (Collado *et al.*, 2007). Senescence is defined as irreversible growth arrest due to reduced number of cellular divisions. Hayflick and Moorhead (1961) were the first to describe this limitative replicative potential; later others hypothesized that this mechanism was genetically defined and proposed that telomere shortening was correlated to cellular senescence (Hastie *et al.*, 1990; Rodier and Campisi, 2011). When telomeres reach a critical length, the cells arrest proliferation and acquire enlarged morphology expressing senescence-associated gene like the β-galactosidase and p16. This response has been called "replicative senescence" (Harley *et al.*, 1992) and attribute to telomere function the role of "molecular clock" (Kirkwood, 2011) that measure cell proliferative history. Many works report that some human telomerase negative cells can be immortalized by introduction of hTERT, while someone require also inactivation of p16, calling in question telomere role in replicative senescence (Kiyono *et al.*, 1998; Dickson *et al.*, 2000; Calado and Young, 2012).

As seen above, studies on telomeres and telomere biology in basal Metazoa are quite scarce. Moreover, the issue about aging in some of these species remains opens: in fact, the studies that indicated the onset of "senescence" in *Hydra oligactis* (Yoshida *et al.*, 2006) and coral reefs *Stylophora pistillata* (Rinkevich and Loya, 1986) evidenced an age-related increase of the mortality rate, thus at a population level. Differently, biological senescence is usually intended (in vertebrates, for example) as a phenomenon due to (or at least correlated with) cellular senescence. Cellular (or Replicative) senescence is the phenomenon by which normal cells cease to divide, and it is the result of several biological mechanisms among which a preponderant role has the telomere shortening that ultimately triggers a DNA damage response. It would be useful and interesting to investigate if the "demographic senescence", noticed by the above-mentioned authors, is characterized by the appearance of the classical endpoints of cellular senescence (such as SA- β-galactosidase) and, more specifically, if it involves telomerase biology and telomere shortening (as demonstrated in mammals).

Finally, with the aim to better understand the role of telomere both in senescence and in cell proliferation, further studies on the T-loop and/or Shelterin complex in basal Metazoa should be performed, given the few available data.

Concluding remark

In conclusion, we can infer that all basal Metazoa display telomerase activity, as evidenced by our survey of the literature. This seems to point

Table 2 Telomerase activity in different species

	Telomerase Activity			
	High	**Medium**	**Low**	**Absent**
Basal Metazoa [(1)]	Whole organism			
Fish [(2)] **Amphibian** [(3)]	Germ cells	Somatic cells		
Human fetus [(4)]	Germ cells, Stem cells	Forming tissues		
Human adult [(4)]	Germ cells	Hematopoietic cells	Dividing cells	Non-dividing cells

References: 1: (Ojimi *et al.*, 2009; Nakamichi *et al.*, 2012); 2: (Lau *et al.*, 2008); 3: (Bousman *et al.*, 2003); 4: (Forsyth *et al.*, 2002).

to an absence of telomere shortening and, consequently, an absence of replicative senescence in these taxa. Unfortunately, the lack of a detailed molecular characterization of cellular senescence in basal Metazoa is one major limitation in the field. Future experiments, using both known and possibly new markers, are one of the crucial steps to understand if replicative senescence is really absent in all these taxa and when it first emerged in evolution.

However, basal Metazoa can be useful as model organisms not only in studies on emergence of senescence but also in Evo-Devo investigations on the control of telomerase activity and telomere biology in other cellular processes, such as carcinogenesis. In fact, an increasing number of evidence indicates that telomere dysfunction (*e.g.*, shortening) is responsible of chromosome instability (Diede and Gottschling, 1999; Rudolph *et al*., 2001; Coluzzi *et al*., 2014), the latter playing an important role in cancer evolution by increasing cellular changes accumulation (Bailey and Murnane, 2006). Interestingly, it has been reported that naturally occurring tumor formation can be observed also in the basal metazoan *Hydra* (Domazet-Lošo *et al*., 2014). Cellular and molecular properties of *Hydra*'s tumor cells appear similar to some cancer hallmarks described in vertebrates. Therefore, although evidence is still scarce and further experiments need to be performed, the possibility that telomere and telomerase may be involved also in the evolutionary origin of the molecular mechanisms of carcinogenesis is very intriguing.

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