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Chapter 18

Telomere Shortening Mechanisms

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1. Introduction

Telomeres are the terminal regions of the linear chromosomes of eukaryotes, which are composed of telomeric DNA and associated specific telomeric proteins. In most kinds of organisms, telomeric DNA is presented by a large number of repetitive, strictly defined short nucleotide sequences, such as: TTAGGG (in vertebrates), TTTAGGG (in the majority of terrestrial plants) and TTGGGG (in the ciliated infusoria Tetrahymena), etc. Although telomeric proteins differ among different groups of organisms they perform similar functions, which mainly consist of telomere length regulation and their protection against degradation (Grach, 2009). For a long time, it was considered that telomeres did not code RNA molecules and thus proteins. Subsequently, it was found that RNA is still transcribed from telomeres but that it did not encode any proteins. Further studies showed that this RNA plays an important role in telomere length regulation and chromatin reorganisation during both development and cell differentiation (Azzalin et al., 2007). In spite of the fact that telomeres do not code proteins, they also perform very important functions, the main role of which is to maintain the stability and functionality of the cellular genome. Among these are the protection of chromosomes from fusion with each other (Blackburn, 2001), participation in mitotic and meiotic chromosome segregation (Conrad et al., 1997; Dynek and Smith, 2004; Kirk et al., 1997), the stabilisation of broken chromosome ends (Pennaneach et al., 2006), their attachment to the nuclear envelope (Hediger et al., 2002; Podgornaya et al., 2000), influencing gene expression (Baur et al., 2001; Pedram et al., 2006), counting the quantity of cell divisions (Allsopp et al., 1992; Kurenova & Mason, 1997; Olovnikov, 1973), and also an original buffer function (Olovnikov, 1973). The latter consists of the protection of the mRNA coding regions of chromosomes from the end replication problem. The end replication problem consists of the impossibility of the full reproduction of the previous length of linear DNA ends on the leading telomeres during of the S-phase of the cell division cycle. It is caused by peculiarities in their structure and the functioning of the DNA replication machinery. As a result the telomeric regions of chromo-

somes in daughter cells are shortened by several tens of nucleotides at each cell division (Lingner et al., 1995). In addition to the end replication problem, the telomere repair problem can also play a role in telomere length shortening. This problem in turn can be divided into the end repair problem and the shelterin-mediated telomere repair problem. The end repair problem includes the incomplete repair of DNA ends and direct damage-mediated telomere shortening and it can occur at the extreme ends of chromosomes. The incomplete repair of DNA ends consists in the inability of a repair system to complete the repair of damage if it occurred at the extreme ends of telomeres, since repair proteins cannot work correctly on the brink of a template and, as a result, they will also be shortened. Direct damage-mediated telomere shortening is based on the fact that, in some cases, the repair of damage at the extreme ends of chromosomes cannot even begin, in contrast to the incomplete repair of DNA ends at which the repair process begins but is not fully completed. It can generally be invoked by the fact that the breaks occurring on the extreme ends of chromosomes lead to the complete separation of the terminal DNA section and as a result repair system proteins are not able to even partially restore such damage. The consequence of this - and also of the subsequent actions of nucleases, which restore previous telomere ends' configuration - is the DNA ends shortening again. The shelterin-mediated telomere repair problem consists of the inability of the proteins involved in DNA damage response to detect and repair the damage occurring at telomeres due to the fact that the telomeric proteins in combination with telomeric DNA form a special structure on the telomeres called a telomeric loop (t-loop) that directly blocks DNA damage response proteins, as well as they block various DNA repair pathways themselves that is especially actual for the uncapped telomere condition when t-loop is not yet formed. This ultimately leads to the accumulation of damage and telomere shortening, and occurs along the entire length of telomeres where there is a telosome organisation and not just at their extreme ends as is the case with the end repair problem.

Telomere shortening is closely related to the replicative potential of cells and their lifespan. Thus, in accordance with A. M. Olovnikov's telomere theory of aging, when the telomere length approaches a certain critical level the cells stop dividing and begin ageing and are exposed to apoptosis upon reaching that level (Olovnikov, 1973). This fact has been confirmed experimentally in a number of studies (Allsopp et al., 1992; Allsopp et al., 1995; Aubert & Lansdorp, 2008). Besides playing a key role in aging, telomeres are also have great significance for carcinogenesis, as some cells with shortened telomeres acquire mechanisms to bypass the aging program and gain (among other characteristics) the ability to maintain telomere length and hence to "unlimited" quantity of divisions (Desmaze et al., 2003; Londoño-Vallejo, 2008; Stewart & Weinberg, 2006). The ability to elongate telomeres in vertebrates can be realised by means of two known mechanisms. The first and the most widespread mechanism among tumours provides for the use of a special enzyme called telomerase. It is a ribonucleoprotein enzyme consisting of a catalytic subunit, a telomerase RNA molecule and several additional components. Joining in with the ends of telomeres, its catalytic subunit uses reverse transcription of RNA, which is a part of telomerase to elongate a G-rich chain of telomeric DNA, which corresponds to the 3'-end regions of the DNA. Further, a C-rich chain corresponding to 5'-end DNA is synthesised on a template of a significantly elongated G-rich chain by a regular DNA polymerase reaction. As a result, the telomere ends gain the same structure as they had prior

to the telomerase action but they become much longer in this case (Blackburn & Collins, 2011; Dong et al., 2005; Testorelli, 2003). The second mechanism, which is found in a minority of neoplasm types, is accomplished by recombination-mediated telomere replication and belongs to the alternative lengthening of telomeres mechanisms (ALT) (Grach, 2011a; Grach, 2011b; Henson et al., 2002; Muntoni & Reddel, 2005; Stewart, 2005). Besides the elongation of telomeres in tumour cells, telomerase also has a high activity in stem and germ cells, thereby providing them a high proliferative capacity (Meeker & Coffey, 1997). Meanwhile, its activity is low or absent in normal somatic cells, making their replicative capacity strictly limited (Rhyu, 1995). As for ALT, it is usually repressed in normal cells by telomeric proteins and certain other factors (Grach, 2011b).

In recent years, the study of telomeres has becomes increasingly popular among scientists who are engaged both with different branches of molecular biology as well as with the most distant problems in the whole of modern medicine. Such heightened interest in their study is first of all caused by the fact that telomeres perform very important functions in the maintenance of eukaryotic cell genome normal functionality. Besides this, telomeres define the replicative capacity of cells and play a key role in their aging and transformation processes which make these end structures an even more important subject for research. All of the above mentioned roles of telomeres depend upon their shortening and, therefore, telomere shortening mechanisms are among the key aspects of telomere biology, because the loss of chromosome functions, cell aging and degeneration are associated with the telomeric regions of chromosomes' length shortening. In this respect, the study of these mechanisms as well as the factors involved in their protection and elongation are of primary importance as long as our cumulative knowledge can help in the future in the struggle against aging, tumours and many other diseases, the treatment of which requires a high replicative capacity in the cells. Based on the great significance of the telomere shortening process, the nub of various telomere-shortening mechanisms will be considered in detail in this chapter, namely the end replication and telomere repair problems.

2. The end replication problem

2.1. Early views of the end replication problem

2.1.1. The end replication problem as a cause of telomere length shortening, which determines the replicative potential of cells

For the first time, the problem of eukaryotic linear chromosome ends' replication was proposed and described in detail in the form of the theory of marginotomy by A. M. Olovnikov in Russian in 1971 (Olovnikov, 1971). One year later, in 1972, the problem was also described by J. D. Watson, independently of Olovnikov (Watson, 1972). In 1973, the problem proposed by Olovnikov was represented in its English version (Olovnikov, 1973). At the heart of this problem, as was suggested, lies the inability of the usual DNA replication system to fully complete the replication of linear DNA ends in the process of cell division. As a result of this,

it was assumed that the telomeric regions of chromosomes are shortened by roughly tens of base pairs (bps) at each cell doubling (Olovnikov, 1973). This state of affairs should explain why normal somatic cells, having divided a number of times, stop their further reproduction, start ageing and undergo apoptosis (i.e. the causes of the Hayflick cell division limit) (Hayflick, 1965). It was therefore suggested that in all of the non-transformed somatic cells of the organism, the telomere replication mechanism is absent and as a result of which they are gradually shortened on their division. When telomeres shorten to the definite minimal length needed for their normal functioning, the cells stop their division, age and then die. In other words, it was suggested that the telomere shortening process is a kind of "counter" which determines the replicative potential of cells (Olovnikov, 1973). These suppositions have been confirmed experimentally in several studies (Allsopp et al., 1992; Allsopp et al., 1995; Levy, et al., 1992). Thus, in one such experiment it was found that cells with shortened telomeres could perform far fewer divisions than cells with long telomeres (Allsopp et al., 1992).

2.1.2. The old theoretical model of the end replication problem

We now consider the actual mechanisms of the incomplete replication of the ends of linear DNA. As is known, every human chromosome consists of two anti-parallel DNA strands, which together form a single linear double-stranded DNA molecule with two ends. When the end replication problem was described for the first time, it was still considered that according to the generally accepted DNA model its ends would also have a double-stranded structure. Proceeding from this understanding of DNA, has been formulated the old theoretical model of the end replication problem, which was based on two possible mechanisms by means of which DNA ends could not be completely replicated.

The first mechanism suggests that DNA polymerase implementing DNA synthesis only in the 5' → 3' direction should have besides the catalytic centre also the DNA binding site, which should be located in front of catalytic one and be responsible for attachment of enzyme to a parent DNA strand. As such, and during DNA replication, when a DNA polymerase approaches the very end of the template by moving in front of the DNA binding site it cannot continue synthesis and so dissociates from the DNA because it will have nothing more to bind to. As a result, the end portion of a template which is equal in length to a DNA binding centre cannot be replicated, since a DNA polymerase cannot simply bring its catalytic centre to the last nucleotides of a parent strand without being dissociated from the DNA. Thus, after an incomplete replication process of such a kind, the 3'-end of a new DNA strand should become shortened by several nucleotides when compared with the parental one (Olovnikov, 1973).

The second mechanism of the incomplete replication of DNA ends was based on the fact that a DNA polymerase is not able to begin new DNA synthesis itself but is capable only of elongating already existing oligonucleotides. Therefore, at the very beginning of replication, primase synthesises a short RNA primer of around 9-12 nucleotides long, which is subsequently elongated by a leading strand DNA polymerase. When the DNA polymerase has already synthesised a long enough DNA strand, the RNA primer is removed by RNase after that the gap is filled by the polymerase and the ends between the DNA fragments are connected by a ligase. The DNA end regions there do not form an exception and are replicated according

to the same principle. However, the problem arises with the RNA primer, which is attached to the 3'-end of the DNA and defines the 5'-end of a new strand. The end gap arising after removal of the RNA primer cannot be filled later by the DNA polymerase, as there is no free 3'-end, which it could elongate. As a result, such incomplete end replication mechanism leads, this time, to the shortening of the 5'-end of a new DNA strand (Olovnikov, 1973).

Thus, according to the old theoretical model of the end replication problem, the 5' overhang at one end and the 3' overhang at the other can be formed at both daughter DNAs arising due to the peculiarities in the functioning of the DNA polymerase system. Such single-stranded protrusions should be cut by nucleases further in order to achieve double-stranded DNA ends' structure - which, as it was supposed earlier, they had initially. Consequently, the daughter chromosome telomere ends upon completion of the replication process will have the same configuration as they had before doubling. However, at the same time they will be shortened by a certain number of nucleotides (Olovnikov, 1973). The old theoretical model of the end replication problem is depicted in Figure 1.

2.1.3. Experimental confirmation of the old theoretical model of the end replication problem

As is shown in Fig. 1, the old theoretical model of the end replication problem assumes that incomplete replication can result from two mechanisms at both DNA ends - both on leading and lagging strands, resulting in each daughter chromosome being shortened at each of it's telomeres simultaneously. Further experimental verification of these circumstances has demonstrated quite different results for the leading strand and has completely confirmed the suppositions concerning the lagging DNA strand. In the course of one piece of research into the end replication problem using the artificially-created linear DNA replication system of the SV40 virus *in vitro*, it has been clearly determined that leading strand is synthesised entirely up to the very 5'-end of the template (Ohki et al., 2001). The explanation for this is that the DNA helicase unwinds its parent DNA to the very end and thus allows the completion of the synthesis of a new DNA strand. This discovery could abruptly undermine the possibility of the existence of the first proposed incomplete DNA replication mechanism, which assumes that due to the peculiarities of the functioning of DNA polymerase, the leading DNA strand synthesis cannot be fully completed up to the very end of the template. However, it is perhaps too early to judge this.

The results of another study of the end replication problem have shown the absolutely opposite situation. In the course of experiments looking into G-rich and C-rich single-stranded DNA in human fibroblasts, researchers unexpectedly revealed that the 5'-end of the DNA leading strand template is not replicated completely in the proliferating cells. Therefore, the 5'-overhangs appears in these cells predominantly during S-phase. This information provides grounds to suppose that the replication fork can terminate before reaching the chromosome's end. The authors of this study explain this in such a way that if the last RNA primer of the lagging strand is to be created as closely as possible to the 3'-end of the template then, in this case, the complete synthesis of the leading strand up to the very end of the 5'-end of a template is possible. If priming occurs more centromerically, then incomplete DNA leading strand

replication and related to it, enhanced telomere shortening may be observed (Cimino-Reale et al., 2003).

Similar results have also been observed in one more study, although it was not carried out with nuclear DNA but rather with the linear mitochondrial DNA end regions of the yeasts (in humans, mitochondrial DNA is ring-shaped). It was found during this work that the DNA polymerase stops at a distance of approximately 110 nucleotides from the 5'-end of a template and does not continue further leading strand synthesis, thereby again leaving the 5'-overhangs. However, the authors of the research could not explain why this happens (Nosek et al., 1995).

Consequently, the results of the researches just reviewed are quite conflicting. Unlike the first investigation described, where DNA leading strand synthesis continues up to the very end of the 5'-end of the parent DNA, creating "blunt" end, in the second and in the third studies we observed the incomplete replication of the leading strand with the creation of a "sharp" end of the DNA molecule. Moreover, none of the studies describe the possibility that complete or incomplete replication of the parent DNA 5'-ends was due to the peculiarities in the function of DNA polymerase as had been suggested by Olovnikov in its first incomplete DNA ends replication mechanism. Instead they consider quite other reasons for - in one case of complete leading strand synthesis to the very end of a template, and in another instance of incomplete leading strand replication.

Therefore, this data cannot fully support or refute the possibility of the inability of DNA polymerase to complete the replication of the 5'-end through a failure to bring its catalytic site to the last nucleotides of a template. It is also very important to note that the above mentioned studies, which describe the incomplete synthesis of the DNA leading strand with the creation of a 5' overhang, in practice are almost unique in their kind. The prevailing majority of studies show that leading strand is synthesised completely up to the very end of a template (Chai et al., 2006; Lingner et al., 1995, Wright et al., 1997).

It was also shown in the experimental research reviewed by us initially that lagging strand synthesis stops within the area located at a distance of approximately 500 bps from the end of a parent strand leaving 3' overhangs there (Ohki et al., 2001). This in its turn fully supports assumptions concerning existence of the second mechanism of incomplete DNA replication described above. As too long extension of incomplete replication was found here, in this work the authors have reviewed somewhat in a new way this mechanism, performed on the lagging strand. It is known that the length of RNA primers range from 9 to 12 nucleotides, which has been described in most of the studies that we have analysed (Griep, 1995; Hao & Tan, 2002; Sfeir et al., 2005). In some cases, primers of 20-30 nucleotides in length are also mentioned (Bouche et al., 1978; Dai et al., 2009). Nonetheless, the length of an incomplete replication of a DNA lagging strand is much longer, and has been discovered to be as long as 500 nucleotides. Therefore, the authors of this research propose the following mechanisms for the incomplete replication of the DNA lagging strand. It should be noted that the first mechanism completely corresponds to that proposed in the old theoretical model of the end replication problem for DNA. As has already been noted, it is based on the removal of the end RNA primer and the further failure to fill the resulting gap with deoxyribonucleotides. As lagging strand's incomplete replication reached approximately 500 nucleotides and the RNA primer length ranges

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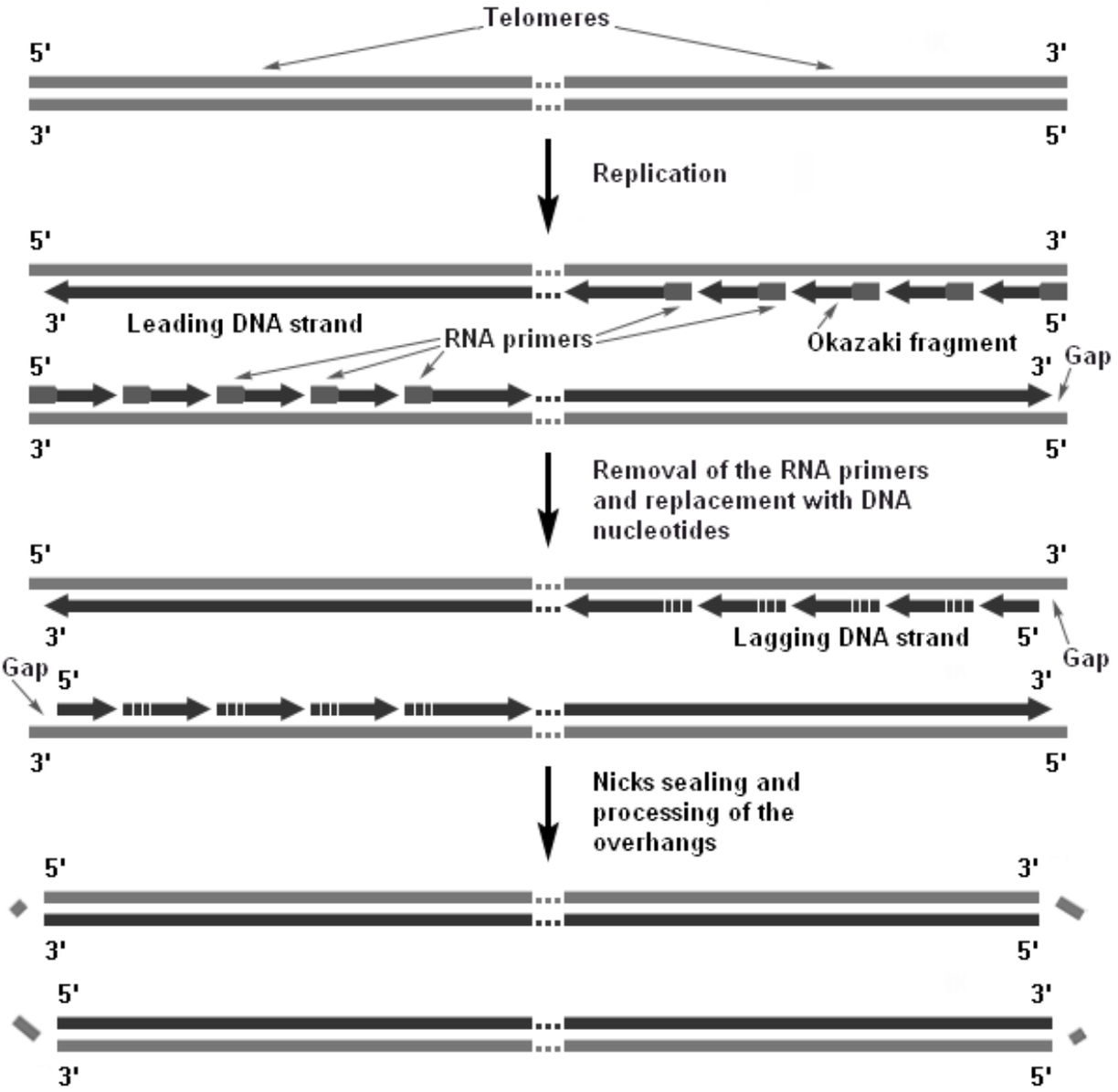

Figure 1. Schematic representation of the old theoretical model of the end replication problem, described by A. M. Olovnikov in 1971.

from 9 to 12 nucleotides, the authors of the work considered it very unlikely that this mechanism was the only one for the shortening of the 5'-end of daughter DNA. The second assumed mechanism consists of the inability of the DNA polymerase α-primase to begin lagging strand synthesis at the very end of a linear DNA molecule that can be the main cause of the end replication problem of the parent DNA's 3'-end (Ohki et al., 2001). It is also interesting that the length of Okazaki fragments, which represent the short DNA fragments with RNA primers at the 5'-end and are the key feature of the lagging strand, can range from between 100 to 500 nucleotides (Burgers, 2009; Mackenney et al., 1997; Zheng & Shen, 2011). Therefore, if such a fragment cannot be formed at the very end of the parent strand then the DNA daughter strand

after the replication process will appear to be shortened in its length. Besides this research, many other studies showing similar results with incomplete lagging strand synthesis are also known.

Thus, most of the conditions of the old theoretical model of the end replication problem initially proposed by Olovnikov were confirmed absolutely in the course of the experiments carried out. In particular, it has been confirmed that telomeres are shortened at every cell division and that, specifically, these circumstances define the replicative potential of the cells and appear to be the cause of their aging and subsequent programmed cell death. Nevertheless, it was a failure to acquire any information absolutely confirming that the specifically incomplete replication of the DNA strands and subsequent cleavage of the resulting single-stranded overhangs of the molecule by nucleases results in telomeres' ends shortening, as was supposed by the old theoretical model. Unfortunately, we also failed to find any experimental data which fully confirms the first mechanism for the incomplete replication of DNA ends, assuming that DNA polymerase is not able to completely copy the 5'-end of the DNA leading strand template since it is incapable of bringing its catalytic site to the last nucleotides of a parent strand. At the same time, the results of the studies show that the 5'-end of a template remains in some cases not completely replicated, but other reasons for this, which are not directly related to DNA polymerase are specified in these cases. Therefore, the assumption regarding incomplete replication of the DNA leading strand is basically confirmed, but it is still unclear whether DNA polymerase directly plays a key role here or whether some other factors are involved (such as the ones that have been mentioned by the authors of the studies already discussed). Given all this, the assumption concerning the second mechanism of the incomplete replication of DNA ends is completely confirmed. Thus, the 3'-ends of the parent DNA, as was confirmed by the results of the experiments and initially stated in the theory, cannot be completely replicated during the lagging strand synthesis. As long as the extension of the incomplete replication of a lagging strand was much longer than the RNA primer length, it was supposed that the reason for the incomplete formation of a lagging strand along with the end primer removal might be due to the inability to prime and create the whole Okazaki fragment at the very DNA end. Given that the incomplete synthesis of the DNA leading strand is described only in some studies, but in the overwhelming majority of works it is shown that leading strand synthesis is performed completely up to the very end of a template, and that the 3'-end of the DNA template cannot for sure be replicated completely, the old theoretical model of end replication problem was also named a problem of incomplete lagging strand synthesis.

2.2. Modern views of the end replication problem

2.2.1. The establishment of the fact that telomere ends have a single-stranded structure

In the early 1980s, the data began to appear suggesting that both ends of each chromosome need not necessarily have a double-stranded structure but that they have a single-stranded structure (i.e. they are represented by 3'-overhangs). In 1981, it was noted for the first time that the ends of the linear minichromosomes, which are present in macronuclei of such ciliates as *Oxytricha*, *Stylonychia* and *Euplotes*, possess G-rich 3'-overhangs between 12 and 16 nucleotides

long (Klobutcher et al., 1981). Later on, in 1989, similar results were also acquired for the linear extrachromosomal ribosomal DNA of ciliate *Tetrahymena* and - evolutionarily distant from it – the slim mould *Didymium* (Henderson & Blackburn, 1989). Later, in 1993, it was found that the telomeres of the yeast *Saccharomyces cerevisiae* also gain 3'-end overhangs in the late S phase of the cell cycle and which differ a little in their dimensions from the ones which were described in previous works, being formed by more than 30 nucleotides (Wellinger et al., 1993). Unlike the above-mentioned organisms, which have a constant G-overhang length, the telomeric overhangs of higher eukaryotes display variability, even among the different cells studied in one group. As has been demonstrated by the results of a great number of studies, human telomeres possess very heterogeneous 3' overhangs, ranging from very short ones 35 nucleotides long and even less, to very long ones with an extension of 500 nucleotides or more. Furthermore, such varying in their dimensions G-overhangs are observed in all types of examined cells including the telomerase-positive transformed cells, telomerase-negative normal mitotic cells and post-mitotic cells (Cimino-Reale et al., 2001; Makarov et al., 1997; McElligott & Wellinger, 1997; Stewart et al., 2003; Wright et al., 1997). All these observations allow the supposition that G-overhangs are a general feature of eukaryotic chromosome telomeres.

2.2.2. A new theoretical model of the end replication problem

Based on numerous experimental observations showing that telomere ends' structure is not double-stranded but single-stranded, J. Lingner et al. have shown that this situation considerably changes established views as to the end replication problem. In particular, they demonstrated that the second mechanism of incomplete DNA replication, based on last RNA primer removal, no longer necessarily appears to be a problem for DNA replication machinery and the cause of telomere shortening. As primer cutting all the same leads to the creation of a 3' overhang, which also existed prior to replication and which is a normal structural feature of chromosome ends, so no genetic informational loss occurs in this case. In this respect, the incomplete synthesis of the lagging strand up to the end of a template can be considered now to be the mechanism of normal single-stranded 3' overhang telomere ends' structural formation. At the same time, a problem arises with the leading strand synthesis. This is caused by the fact that in the course of replication on such telomeres, daughter chromosomes lose the 3' overhang which was present in the parent chromosome and in the absence of telomerase this will accordingly result in their shortening. Moreover, if it is not restored by this enzyme to its previous state then, and only in this case, in the next round of replication might be observed the problem of incomplete lagging strand synthesis and already associated to it DNA shortening (Lingner et al., 1995) (Fig. 2). Thus, the result of replication with the new theoretical model proposed by Lingner et al. is a formation of two daughter DNAs which have one "sharp" end with a 3' overhang forming due to lagging strand synthesis and the other "blunt" end (or a "sharp" one with a 5' overhang if we take into consideration the possibility of performing the first mechanism of the old model of the end replication problem proposed by Olovnikov) forming on the leading strand. In contrast to the earlier proposed theoretical model of the end replication problem where overhangs should be cut, now single-stranded 3'-end protrusions remain intact, forming the natural eukaryotic chromosomes ends. Given all this, if according

to a new theoretical model leading strand synthesis results in the loss of 3' overhangs and the formation of "blunt" DNA ends, but the results of many experiments show that both chromosome ends have G-overhangs, and given that incomplete lagging strand replication assumes its formation only on one end, then there should also be a mechanism creating such an overhang on leading telomeres (Fig. 2).

Lingner et al. have also proposed two possible mechanisms for previous 3' overhang formation which also guarantee that DNA shortening, due to a problem of incomplete lagging strand synthesis, can never occur. The first mechanism presupposes that after the DNA replication process the end of the newly synthesised leading strand in the "blunt" DNA end can be elongated by telomerase and as a result a "sharp" DNA end, with a previous 3' overhang, will be restored. The possible caveat of this variant is that the DNA molecule with the “blunt” end acts here as a substrate for telomerase but telomerase is able to elongate only single-stranded ends rather than double-stranded ends as was found earlier *in vitro*. Nevertheless, the possibility that telomerase access could be provided in this case by helicases, nucleases or proteins binding single-stranded DNA has been considered. The second mechanism assumes that telomerase acts before the replication process, elongating the 3' overhang. It creates a template for the gap-filling synthesis of the complementary C-strand. As a result of the elongation of the 5'-end by conventional DNA replication machinery and the subsequent removal of the RNA primer, a telomere end region acquires a 3' overhang structure again but becomes much longer. Now, when the replication process approaches its end, the overhang on the leading strand is also lost but the genetic material no longer decreases, since before replication the parent strand of the leading strand was elongated. Further, the so-formed DNA "blunt" end and, in particular, its 5'-end region are exposed to processing by nucleases resulting in the formation of a "sharp" end with a previous 3' overhang that existed prior to the elongation by the telomerase (Lingner et al., 1995).

2.2.3. Experimental evidence for the new theoretical model of the end replication problem

These mechanisms describe the different possibilities for the creation of a previous 3' overhang and opposition to telomere shortening due to an inability during leading DNA strand synthesis to create such a single-stranded protrusion. However, both of these mechanisms are based on the possibility of telomerase action. At the same time telomerase activity is either very low in most normal human somatic cells or else is not observed at all (Rhyu, 1995). In spite of this, 3' overhangs are observed at both chromosome ends in normal cells (Makarov et al., 1997). There are also the results of focused experimental studies, showing that the removal of the genes coding for telomerase components does not affect the G-overhang structure considerably and this in turn also shows that the formation of such overhangs occurs irrespective of telomerase activity (Dionn & Wellinger, 1996; Hemann & Greider, 1999; Yuan et al., 1999). Besides, it was found during another study that these overhangs are exposed to cell cycle-regulated changes independent of telomerase activity (Dai et al., 2010). At the same time, telomerase in the cells where it is present is capable of elongating the 3' overhang after it is formed and thus make it like in the previous parental telomere. On this basis, it might be supposed in principle that previous 3' overhang in cells where there is no telomerase activity cannot be restored, but at the same time in its place a new overhang, by means of a special mechanism which will be reviewed

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later, is formed which results in the telomere shortening. It is should also be mentioned that if it were forever restored to a previous state with the telomerase participation that was assumed in the above described mechanisms, it would lead to telomeres not be shortened during the course of cell doubling. This is equivalent to the acquisition of the unlimited replication potential which is observed mainly in the transformed cells. At the same time, the second mechanism reviewed presupposes that after preliminary elongation by polymerases, a DNA "blunt" end formed during replication due to the impossibility of creating a 3' overhang through leading strand synthesis is exposed to treatment by nucleases which process its C-rich strand and thus create an overhang of a specific length. Recent studies suggest that such post-replication treatment of a parent strand by nucleases, independently of whether there is telomerase in the cells or not, seems likely to be the main mechanism of 3' overhang formation in the leading telomeres (Lenain et al., 2006; van Overbeek & de Lange, 2006; Wu et al., 2010). If the parent 3' overhang before replication were to be elongated by telomerase, then the nucleases activity would further lead to previous 3' overhang formation, i.e. telomere end length does not decrease upon that and even increases, and if not, then these enzymes will create a new 3' overhang resulting in the shortening of the telomere's length. It is important to note that there are studies, showing that telomerase elongates the 3' overhangs of the leading daughter telomeres (Chai et al., 2006). Therefore, the first of the above reviewed mechanisms can be considered more realistic for previous 3' overhang restoration, especially taking into account that the DNA's "blunt" end after replication is necessarily exposed to the nuclease's influence and only after this does it become accessible for telomerase. It is also important to note that if in a case of accomplishment of the first mechanism of the old model of the end replication problem a DNA " sharp" end with a 5' overhang will be formed, the telomere ends shortening in that case would be even greater, as the incomplete synthesis of the DNA leading strand up to the end of a template and - related to this - excessive post-replication processing will take place. The latter is caused by the situation that nucleases now, in order to create a 3' overhang, will not only remove a certain number of C-strand nucleotides as a part of the double-stranded DNA, but also its single-stranded overhang. The schematic representation of the new theoretical model of the end replication problem is presented in Fig. 2.

It is clearly shown in Fig. 2 that under the new theoretical model of the end replication problem the incomplete DNA lagging strand synthesis, as a consequence of the impossibility of creating an Okazaki fragment and the removal of an end RNA primer, no longer leads to the daughter telomere's shortening but appears instead to be a kind of mechanism of their normal 3' overhanging structures' renewal. At the same time, during the synthesis of the leading strand, the DNA replication machinery is not able to recreate such an overhang on other chromosome ends as for its synthesis there is simply no template. Therefore, the leading telomeres of daughter chromosomes with respect to a parent chromosome lose their 3' overhang, which can be the cause of their further shortening. Experimental evidence for the claim that it is 3' overhang loss, which really leads to telomere shortening is derived from one study where it was found that the length of this overhang completely agrees with the chromosome end regions' shortening rate (Huffman et al., 2000). Nevertheless, there is also the data from another study showing that the G-overhang length does not correlate with the telomere-shortening rate (Keys et al., 2004). The authors of the research suggest that besides the 3' overhang loss in the course of DNA replication; the telomere-shortening rate is also influenced by damage from oxygen free radicals (Keys et al., 2004). As a result of such replication, there occurs the

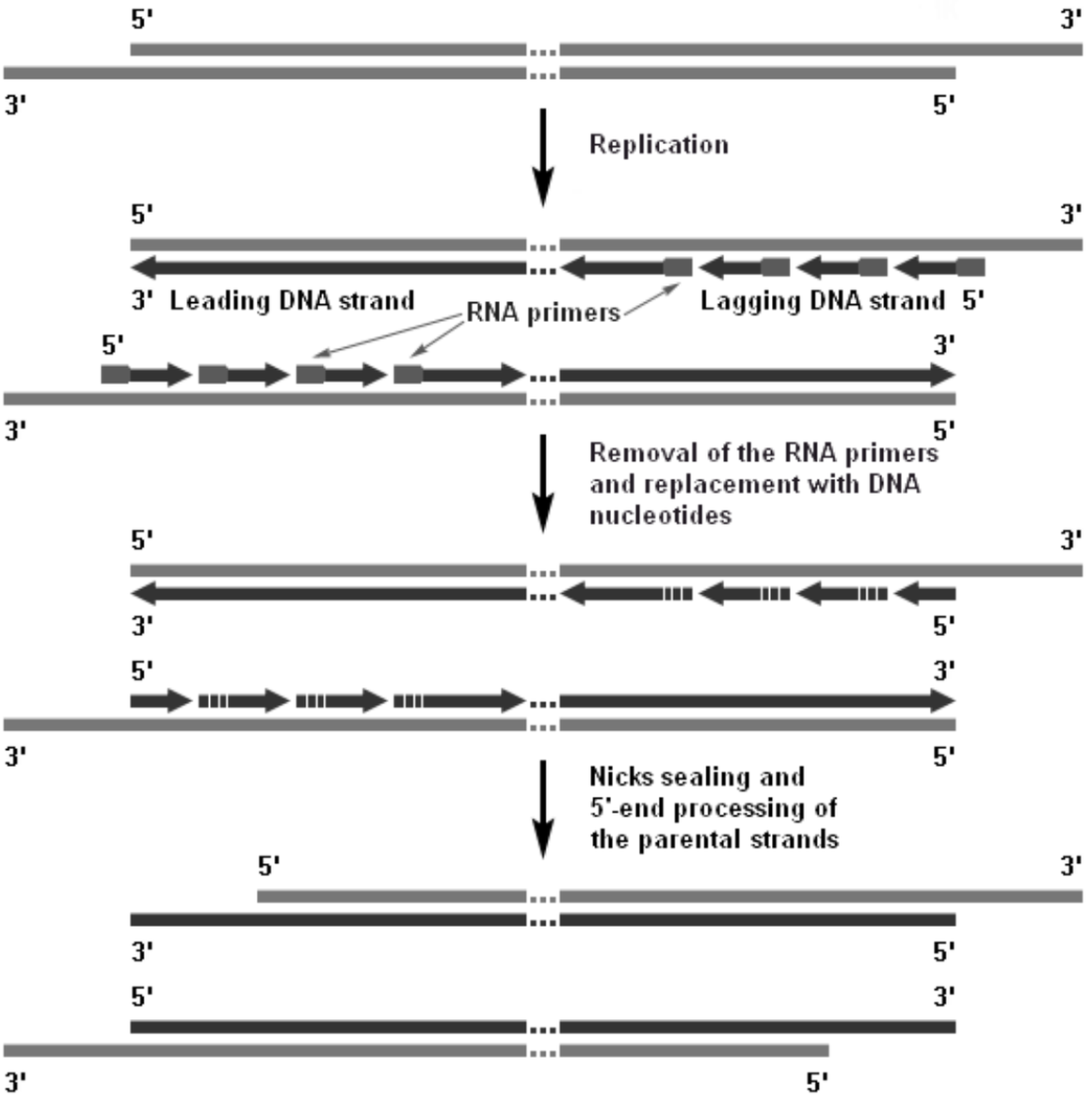

Figure 2. New theoretical model of the end replication problem.

formation of DNA daughter molecules that have one "sharp" end with a 3' overhang and the other "blunt" end. Taking into account that both chromosome ends have G-overhangs the "blunt" end on the leading telomere is further exposed to treatment by nucleases which cut its C-strand and thereby create the new 3' overhang, the length of which, will determine the rate of telomere shortening due to the end replication problem in the next replication cycle. The processing of the 5'-end of a parent strand of DNA can occur with the participation of such factors as the MRN protein complex (MRE11-RAD50-NBS1) as well as EXO1 and Apollo nucleases (Dewar & Lydall, 2010; Larrivee et al., 2004; Lenain et al., 2006; Maringele & Lydall, 2002; Tran et al., 2004; van Overbeek & de Lange, 2006; Wu et al., 2010; Zubko et al., 2004). Given this, the likely leading role is assigned to the Apollo nuclease, as RNA interference

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mediated repression of the gene encoding Apollo nuclease, leads to the loss of 3' overhangs, subsequent cell cycle arrest and programmed death (van Overbeek & de Lange, 2006). The dominant role of such overhangs on the ends of chromosomes, as has long been established in the course of experiments, consists of the formation of special structures called telomeric loops (t-loops, see below), which protect DNA ends from being recognised as double-stranded breaks by the repair system proteins and other enzymatic influences (Grach, 2009; Griffith et al., 1999; Stansel et al., 2001). This is why it is so important that the leading telomere form a new 3' overhang, even taking into account some DNA parent strand shortening. It should also be noted that if we assume - hypothetically - the possibility that chromosomes could function normally if a 3' overhang was present on only one DNA end, at the same time if in this case the parent 5'-end was never cut by nucleases, it would lead to the impossibility of telomere shortening in a considerable number of primary cells and their immortalisation without telomerase. Fortunately, it is not possible because then there would be a high probability of such cells transforming. As is shown in Fig. 2, telomere shortening as a result of one round of DNA replication happens due to the impossibility of recreating a 3' overhang on a leading daughter telomere and a subsequent undercut of a 5'-end by nucleases in creating such a new overhang. If, after these events, a telomerase does not become active and does not elongate this new 3' overhang, thereby providing the possibility of recreating a previous overhang, then the shortened 3'-ends, having passed to the next round of replication will lead to a situation such that now, on their template, even shorter 5'-ends will be created as a result of incomplete lagging strand synthesis. Therefore, it is possible to say that in such cases telomere shortening can be performed by means of incomplete lagging strand synthesis, which, it should be especially emphasised, is possible only after the previous loss of the 3' overhang or, as some have noted, after incomplete DNA leading strand synthesis, and so cannot proceed on the initial chromosomes. It is interesting to notice here that in certain scientific works, which describe the new model of the end replication problem, the 3' overhang loss is designated as a problem of incomplete leading strand synthesis. This description - as it appears to us - does not fully correspond to the actuality because, in this case, a template is replicated to the very 5'-end, unlike the variant proposed by the first mechanism of the old model of the end replication problem, where its incomplete replication with C-overhang formation can be carried out. Therefore, with regard to a single-stranded template, the leading strand synthesis here is performed completely, however the truth is that in relation to the parent double-stranded DNA with a 3' overhang on both sides it does not do so completely. Thus, irrespective of these descriptions, but in the case of absence of telomerase activity, it seems to be possible that the following chain of events under the new theoretical model of the end replication problem lead to a daughter telomeres' shortening: a 3' overhang loss on the DNA daughter strand, the processing of the 5'-end of the DNA parent strand within one round of replication, and then the incomplete replication of a shortened 3'-end of the previous DNA daughter strand in the next one.

In order to understand in more detail how incomplete DNA lagging strand synthesis and 3' overhang loss on the leading telomere is accomplished under the new theoretical model of the end replication problem, let us examine the structure of replication forks on both chromosome ends, as presented by Fig. 3.

Fig. 3 shows two replication bubbles on eukaryotic chromosome ends, each of which consists of a pair of replication forks moving in opposite directions. As is known, in most cases the initiation of replication is accomplished from a non-telomeric origin (Gilson & Geli, 2007). Later on, one fork of the replication bubble moves towards a centromere and another one towards a telomeric end. Here it is seen that helicase unwinds a double-stranded DNA molecule up to the very ends. It allows for DNA polymerases to finish leading strand synthesis completely, to the very end of a template. The double-stranded ends on the leading telomeres are formed upon that. As is clear from the figure, in such cases, the previous 3' overhang, which earlier was on the parent DNA ends, cannot be reproduced, in principle, in daughter molecules during leading strand formation as there is nothing for it to be synthesised on and, consequently, it is lost, resulting in telomere shortening. While the leading strand concerning the parent strand is synthesised completely, the lagging strand synthesis cannot be completed up to the end of the template. Earlier, it was thought that the removal of the end RNA primer is responsible for it. However, today many researchers are inclined to consider that this is not the only reason and it is also probably significantly complemented with the impossibility of creating the last Okazaki fragment. This situation is also well represented by Fig. 3. As is known, first of all the leading strand is synthesised in the motion of the unwinding of the parent DNA, and later on, after the DNA polymerase has synthesised a certain leading strand extension, it moves to a lagging strand and elongates it, thereby catching up with the first one. When such synthesis of both strands reaches the last point of unwinding - which can correspond to the 5'-end of the parent DNA - there remains a long stretch of single-stranded DNA in the form of a 3' overhang beyond its limits. Upon this, there is no more space for synthesising the leading strand in order that later on a DNA polymerase can move and fill such an overhang with a lagging strand. In this connection, the Okazaki fragment on the 3' overhang is not created and it remains non-replicated, and after the last primer removal its length increases a little more. However, as an overhang - which occurred prior to replication - is created anyway, the telomere's shortening does not happen in this case.

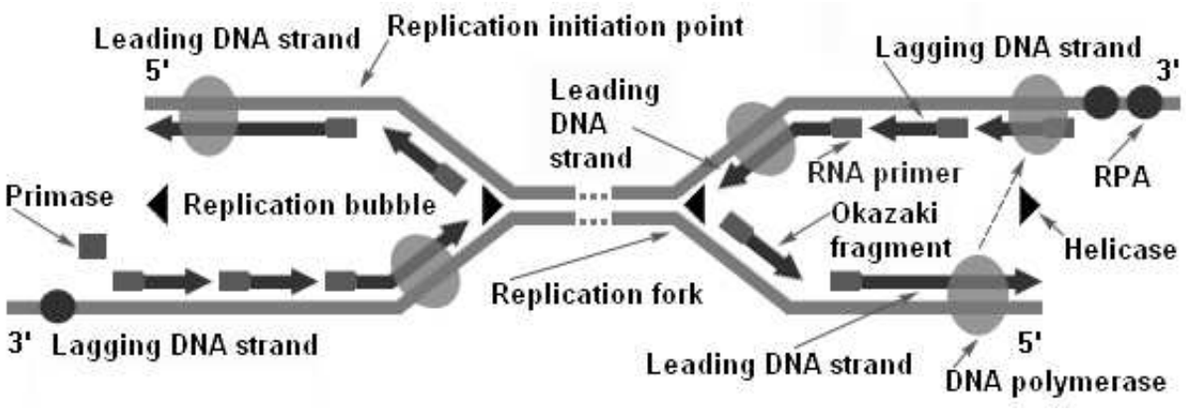

Figure 3. Telomeric replication forks.

Thereby, almost all conceptions of the new theoretical model of the end replication problem have been proved to be true in the course of the studies. It has been proved experimentally

that 3' overhang loss in particular leads to telomere shortening. Besides it has also been confirmed that a new overhang is formed due to leading telomere processing by nucleases. At the same time, the assumptions as to the point at which a telomerase itself directly restores a previous overhang were not confirmed. For all this, according to new views of the end replication problem and which have been confirmed by a number of studies, from now on it should be construed not as the lagging strand synthesis problem but rather as the DNA leading strand synthesis problem.

2.3. The conclusion of this section

Thus, summarising all of the aforesaid, the views of the end replication problem as the cause of telomere shortening have changed over a period of several decades. Initially, when everyone considered that the structure of chromosome ends was double-stranded, it was supposed that telomeres were shortened mainly due to incomplete DNA lagging strand synthesis, which leads to the formation of 3' overhangs in support of which there is much experimental data. Furthermore, some studies have demonstrated the possibility of the accomplishment of incomplete leading strand synthesis up to the very end of a template with 5' overhangs forming, but the truth is that their number is small. Such overhangs further should be undercut, which would lead to the single-step shortening of chromosomes from both ends. As most of these works nevertheless provide evidence in favour of the idea that the replication of 5'-ends was carried out completely, it was later considered that telomeres were shortened only due to the problem of incomplete lagging strand synthesis. Here, it would seem that if the telomere shortening mechanism acts only from one end of a chromosome then the its other end would never decrease in length. Actually, this is not precisely true. The matter is that, if we were to monitor two strands of any initially parent DNA then one of them - after a certain number of divisions and in case of the absence of telomerase - will be really shortened from one end and the other one would be from the opposite end. If we continuously monitor some formed daughter strands, then in the subsequent generations of the cells there will also appear chromosomes which are shortened at their own two ends. After the establishment of the important circumstance that the structure of telomere ends is not double-stranded but 3' overhanging single-stranded, the problem of incomplete lagging strand synthesis already actually ceased to be treated as being the problem, since it no longer led to telomere shortening now, and only restored a previous configuration of their ends, which is important for normal chromosome functioning. At the same time, the existence of 3' overhangs on the chromosomes' ends creates a significant problem for leading strand synthesis. It is caused by the fact that in the course of replication, the 3' overhang which is present in the parent DNA on two ends cannot be renewed in the daughter DNAs during leading strand formation because of the absence of a template for its synthesis; in this connection it will be absent at one end in one daughter molecule and on another end of another one. Such a 3' overhang loss, the further processing of the 5'-ends of leading telomeres resulting in the formation of new G-overhangs and also the subsequent incomplete lagging strand synthesis in the next generation on a template of an already shortened 3'-end, actually lead to telomere shortening. In this connection the end replication problem is inverted from the lagging strand to the leading strand. However, this does not mean that leading strand is necessarily synthesised incompletely up

to the very end of a template, and it can be reproduced fully in this case. Thus, all of the observations described above have shaped our current thinking about telomere shortening during cell division.

3. The telomere repair problem

3.1. Early ideas of the telomere repair problem — The incomplete repair of double-stranded DNA ends

The problem of the incomplete repair of the very ends of DNA was also described, first, by A. M. Olovnikov as early as 1995. As with the old model of the end replication problem it was based on the idea that telomere ends have a double-stranded structure. The two suggested mechanisms of incomplete DNA ends' repair that are actually similar to the mechanisms of incomplete replication described in the previous section were distinguished. The essence of the first one concluded that if a single-stranded break (SSB) or "nick" occurred close to a 3'-end of a DNA strand at a distance of just several nucleotides, then this damage could not in principle be repaired. It was presumably connected with the following situation. The short end oligonucleotide created by the nick could not remain hybridized to the rest of the DNA molecule resulting in the formation of a gap with a protruding 5'-end. Later on, a repair DNA polymerase should attach to a DNA molecule and, while moving along the undamaged C-rich strand, synthesizes the lost 3'-end region on its template. However, it could not be performed in this case as DNA polymerase again, as well as with respect to the replication of the very 5'-end, not able to bring its catalytic site to the last nucleotides of a template in order to reproduce them on a complementary strand. Therefore, the DNA molecule remains shortened at the 3'-end afterwards. The second suggested mechanism provided for the impossibility of damage repair if a nick happened near the 5'-end of the DNA strand. A gap formed after the separation of a DNA fragment that was too short to remain hybridized to the template, could not be filled in for another reason in this case. The chain which was subject to repair and shortened due to the single-stranded break has no 3'-end or primer to which a DNA polymerase can add nucleotides in the course of repair synthesis and, therefore, should also remain non-elongated. On this basis, in both cases of the incomplete repair of DNA ends, single-stranded overhangs are formed which should be cut further by nucleases that would subsequently lead to telomere shortening (Olovnikov, 1995a; Olovnikov, 1995b; Olovnikov, 1995c).

3.2. The end repair problem — The incomplete repair of DNA ends and direct damage-mediated telomere shortening

The discovery that telomere ends had a single-stranded 3' overhanging structure, besides changing views on the end replication problem also considerably changed conceptions of the repair of chromosome ends. Before considering in detail exactly what these changes are characterised by, it is necessary to clearly define what should be understood by an incomplete DNA ends' repair. It is suggested by A. M. Olovnikov that it can proceed in two cases. In the first case, damage resulting in the breaking off of a single-stranded DNA fragment happens

near the very end of the 3'-end of a double helix, and further DNA polymerase is not able to completely synthesise the lost region insofar as by moving along the opposite undamaged strand it cannot bring its catalytic site to the last nucleotides of the template. The second case assumes that damage with the separation of a DNA fragment happens near the 5'-end of a double-stranded molecule, and as a result DNA polymerase cannot restore the lost part again, since there is no available 3'-end as a primer to elongate. It is known that to begin polynucleotide synthesis at primer absence, as already noted, it is not able. Moreover there appears that the gap is so short that primase cannot even create an RNA primer. It is thus meant that in the first case a DNA polymerase binds to a DNA molecule and synthesizes several nucleotides, but the truth is that the last bases, which should lie opposite the very edge of the template, do not form (i.e. the damage repair process starts but does not come to its completion), and that in the second case the DNA polymerase, due to a RNA primer absence, cannot attach to a DNA double-stranded molecule and even to begin damage repair process. Based on these differences, specifically as to whether the repair process can start but does not come to its end or whether it does not start at all, we propose to look at the problem of incomplete DNA ends' repair and related to it telomere shortening somewhat in a new way. In particular, it is proposed that, under the problem of incomplete repair to consider that, the repair of damage at the end of double-stranded DNA starts but cannot finish completely because of the inability of most repair system proteins to correctly function at the very edge of a template that leads to chromosome ends shortening. It is necessary to emphasise that when noting that repair cannot be finished it is meant not only that DNA polymerase is unable to copy a template completely up to the very end in the course of repair DNA synthesis, but also that other enzymes can begin and even accomplish some stage of the repair process, however that later, due to certain reasons, repair cannot continue and so it finishes prematurely. As such, in the first case described, it is possible to say that what is actually accomplished is the incomplete repair of DNA ends. In addition, it is also proposed that if the repair of damage at a DNA end cannot start at all, again owing to the inability of repair proteins to work correctly at the edge of a template - and it will lead to chromosome telomere regions shortening, then this situation further should be designated as direct damage-mediated telomere shortening (DDMTS). The second described case can be related to this. Thus, we define two possible variants by means of which telomere shortening can be performed in a case where damage occurs at the very ends of a double-stranded DNA molecule, namely incomplete DNA ends' repair and direct damage-mediated telomere shortening.

Based on these new conceptions, let us consider once again Olovnikov's theoretical model of incomplete DNA ends' repair. As was mentioned, since it is described in the first variant that a DNA polymerase attaches to a DNA molecule, reproduces several nucleotides but subsequently cannot finish repair synthesis to the very end of a template, then in this case there occurs incomplete DNA repair. In the second variant, the enzyme cannot even attach itself to a template to begin repair - that was designated as DDMTS. It is necessary to note here that if, in the case of the first variant, the gap is very short then the DNA polymerase - even if it attaches itself to a template - will also not be able to begin repair because it will place its anchor region directly onto the very end of an undamaged DNA strand and, as a result, it will be immediately separated from the DNA molecule. This situation can already be regarded as DDMTS. At the

same time, if with the second variant the gap will be long enough for an RNA primer to be formed, and then it is possible that there will be two variants, in both cases of which there will now occur the incomplete repair of the DNA ends. If the gap will be long enough to fit just an RNA primer, then in this case it might be supposed that when a DNA polymerase attaches itself, it will not be able to synthesise nucleotides as well, but as far as primase synthesizes the primer, then it is possible to consider that repair has started and that one of its stages has finished, but also that another one is not able to begin. In the future, such a primer is removed by RNase and a gap of the same length as it was before the repair arises. In the case where the length of a gap is such that in spite of the RNA primer several nucleotides are able to fit there, then the DNA polymerase synthesises them. However, after primer removal there will be a gap anyway but which the truth is that will be smaller than before the repair. As a result, such situation should also be viewed as incomplete repair. Ultimately, it is important to note that at replicative and cell senescence stages it is known that repair systems act poorly. Therefore, if a long enough gap appears at one of the DNA ends in senescent cells, then it will probably not be even partially repaired, and as a result DDMTS will take place. Thus, if such gaps are repaired in young cells, even if incompletely, then in old ones they will be not be repaired. It is also necessary to emphasise that at one time is apparently possible to separate only terminal single-stranded DNA fragment that was less than nine nucleotides in length, since it is widely known that RNA primers 9-12 nucleotides in size are strongly hybridized to a template. For that matter, when it was mentioned that a gap can arise is longer than primer itself or else the same, it can proceed only according to several steps, i.e. a successive separation of several fragments 8 nucleotides in size or less. Thus, if a break occurs, e.g. at a distance of 9 or more nucleotides, then such a terminal oligonucleotide will not only be able to hang on a template but will also be reunited with the remaining proximal part by DNA ligase. In summary, it is also necessary to add that since it was experimentally discovered that 5'-end copying by a DNA polymerase in the course of replication is, in most cases, accomplished completely, then in such a case only the second variant of incomplete repair based on RNA primer removal so described could feasibly be carried out.

3.3. Modern views of the end repair problem

Now let us consider what exactly are characterized by the changing of conceptions of repair at the ends of chromosomes, if the telomeres have single-stranded 3' overhanging structure. They are characterised by the following circumstances. First, given such telomere ends organisation, the problem of incomplete repair can arise, as it seems to be possible, only if a single-stranded break occurs at a distance of up to approximately eight nucleotides towards the centromere from a place where the 3' overhang begins and the 5'-end of complementary strand is situated (Fig. 4). In such a case, if a DNA polymerase even manages to copy a template up to the very 5'-end in the course of the repair synthesis of the lost single-stranded DNA fragment, the previous 3' overhang would still not be able to renew, and so it can be seen that in such a situation repair has begun but cannot be finished, insofar as the damage could not be fully repaired. If we take into account that a DNA polymerase might not be able to copy the last nucleotides of a template, then in such a case if a break with a subsequent separation of a DNA fragment occurs at a very short distance (e.g. of one nucleotide) from the above

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mentioned place, then repair will not begin and it will be designated as DDMTS. Secondly, if a break occurs somewhere at the 3' overhang or near its base (i.e. at a region where the opposite C-rich strand ends), then the distal part of the overhang or its entirely will separate from the DNA molecule and will be lost, as a result of which DDMTS will be observed - as far as repair in that case cannot even begin in principle due to single-stranded DNA fragment loss and the absence of a template for synthesizing the new one (Figure 5). With both variants, the new overhang will form in the future through 5'-end processing by the nucleases. Thirdly, an incomplete repair problem of the 5'-end, which should arise due to RNA primer removal on telomeres with "blunt" ends, is no longer a problem, and hence the reason for telomere shortening in instances with "sharp" ends, because as is the case with lagging strand synthesis at DNA replication, its cutting out leads only to the formation of the previous 3' overhanging configuration of telomere ends (Figure 6). Thus, single-stranded end breaks, at the 3' overhanging telomere structure, can lead to incomplete telomere repair and further telomere shortening only if they will occur on a G-rich strand at a distance of several nucleotides in front of a place where a complementary C-strand ends. If the breaks affect the 3' overhang itself, then this will lead to DDMTS. Finally, the breaks of a C-rich strand occurring near the very 5'-ends will repair completely.

As is known, apart from single-stranded breaks, there are also such basic types of DNA damage as various nucleotide modifications, double-stranded breaks and cross-links (Sancar et al., 2004). Various nucleotide modifications (of a single one, a pair or else several) arising at DNA ends in most cases cannot lead to single-stranded breaks of the molecule in themselves. Further, they are exposed to various repair pathways, such as a direct repair (DR), a base excision repair (BER) and a nucleotide excision repair (NER) (Sancar et al., 2004). Since a direct repair is accomplished without any breakage of the phosphodiester backbone it cannot, in principle, lead to telomere shortening and, therefore, in our case, is of no particular interest. This type of reactions includes the photoreactivation of ultraviolet-induced pyrimidine dimers by a DNA photolyase enzyme, the removal of the O^6-methyl group from O^6-methylguanine (O^6MeGua) in DNA by the DNA methyltransferase enzyme, and the repair of apurinic/apyrimidinic sites through the direct insertion of bases by the insertase enzyme (Sancar et al., 2004). In addition, the repair of single-stranded DNA breaks by DNA ligase enzymes also belongs to this type but only if they do not arise at the very ends of DNA and do not lead to gap formation. Base excision repair consists of the cutting out of damaged nucleotide from a DNA strand by means of several reactions with the participation of DNA glycosylases, AP-endonuclease and phosphodiesterase, resulting in the formation of a very short gap (Fromme & Verdine, 2005; Krokan et al., 1997; Seeberg et al., 1995). This gap should be filled further by a DNA polymerase on a template of an undamaged complementary strand, after which the free ends are sealed by a ligase. If such a form of repair is carried out somewhere in the middle of the DNA molecule or near its 5'-end, then no problem will arise. However, if it proceeds at a distance of, say, 9 nucleotides from the place where the 3' overhang begins, i.e. the 9th nucleotide will be removed at that, then the end DNA fragment 8 nucleotides long up to the 5'-end of an opposite strand, together with the 3' overhang, would be lost. It will lead to gap formation, which can be filled further to form a "blunt" DNA end, but upon this, as well as in the case of a single-stranded break forming at a distance of up to 8 nucleotides and the

subsequent formation of the same gap as discussed earlier, the previous 3' overhang it will not be able to restore itself and the telomere will shorten. Thus, base excision repair imposed on the end regions of a G-strand may be lead to an incomplete DNA repair. It is also interesting to discuss the situation where such a damaged nucleotide arises within the 3' overhang itself. BER system enzymes are apparently incapable of acting on a single-stranded DNA. As a result, such damage will not be repaired, and where further the base modification can similarly lead to DNA strand breaking and 3' overhang distal part loss, then this situation should be considered as DDMTS. However, if repair enzymes all the same could cut out a damaged nucleotide, which again will lead to the separation and loss of the 3' overhang terminal fragment, and so there will be an incomplete repair in this case. As for the nucleotide excision repair, it is very similar to BER but is accomplished by other enzymes, and in this case not only one damaged nucleotide is removed but up to thirty (de Laat et al., 1999; Reardon & Sancar, 2005; Sancar et al., 2004). With NER as well as with BER, if the gap is formed on the G-strand of telomeric DNA, in such a way that 8 or less nucleotides remain up to the end of a double-stranded DNA structure in the G-strand, then again a short end fragment of a G-strand together with the 3' overhang will be separated and lost and as a result an incomplete repair and related to it telomere shortening will be observed subsequently again. In order not to repeat this, it may be said that all other situations involving NER at the end of the telomere, including whether several nucleotides on the 3' overhang will be damaged, are similar to those that have been reviewed in relation to BER.

Double-stranded breaks (DSBs) of DNA can be repaired by three mechanisms: non-homologous end joining (NHEJ), microhomology-mediated end joining (MMEJ) and homology-directed repair (HDR) (Chu, 1997; Liang et al., 2008; Lieber et al., 2003). The first mechanism consists of the direct joining of broken ends by a specialised enzyme DNA ligase IV with the participation of the protein Ku and DNA-PK, which is carried out within G0/G1 and the early S phases of the cell cycle (Lieber et al., 2003). The second mechanism does not depend on these proteins and also differs from NHEJ in that this mechanism of DSBs repair uses 5-25 base pair microhomologous sequences to align the broken strands before joining, and it is carried out within the S phase of the cell cycle (Liang et al., 2008). The third mechanism is based on homologous recombination of a damaged chromosome with a sister chromatid or homologous undamaged chromosome, and therefore the damaged chromosome is repaired on their template that is carried out within the late S and G2 phases of the cell cycle (Chu, 1997). Nevertheless, NHEJ and HDR seem to be the main mechanisms for DSB's repair. If DSB occurs near the very end of a chromosome at a distance of only several nucleotides from the place where the 3' overhang begins, then such damage will probably not be repaired since the distal double-stranded fragment of DNA would be too short for the repair enzymes to bind to it, and, in the case of NHEJ and MMEJ, connect it to the rest of a molecule. Additionally, HDR also would be ineffective in repairing such damage since the homology tract would be again too short to effectively engage the enzymes that catalyze homologous recombination. As a result, the repair of such damage will not begin and there will be observed DDMTS. In addition, it is also necessary to note that if telomere damage occurs at a great enough distance from the very end of a DNA, but still within the telomere region organized by the telosome, it is unlikely that it could be repaired by homologous recombination. This is caused by the ability of

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telomeric proteins to block recombination events for the preventing of the elongation of chromosome telomere regions through ALT (Grach, 2011b).

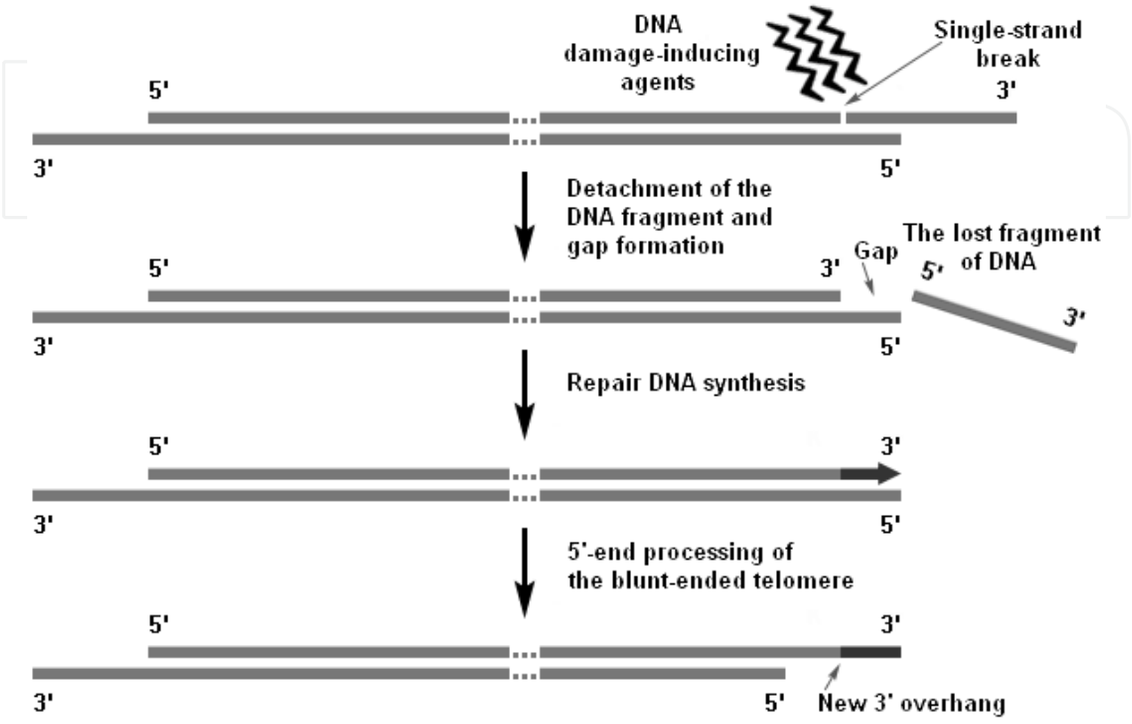

Figure 4. Incomplete double-stranded DNA end repair.

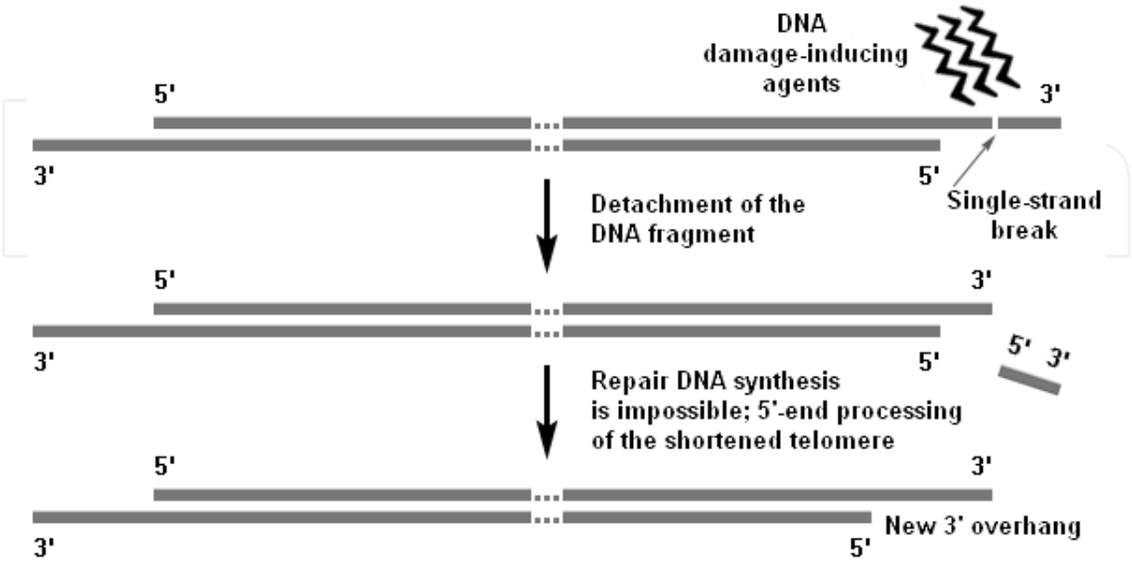

Figure 5. Direct damage-mediated telomere shortening.

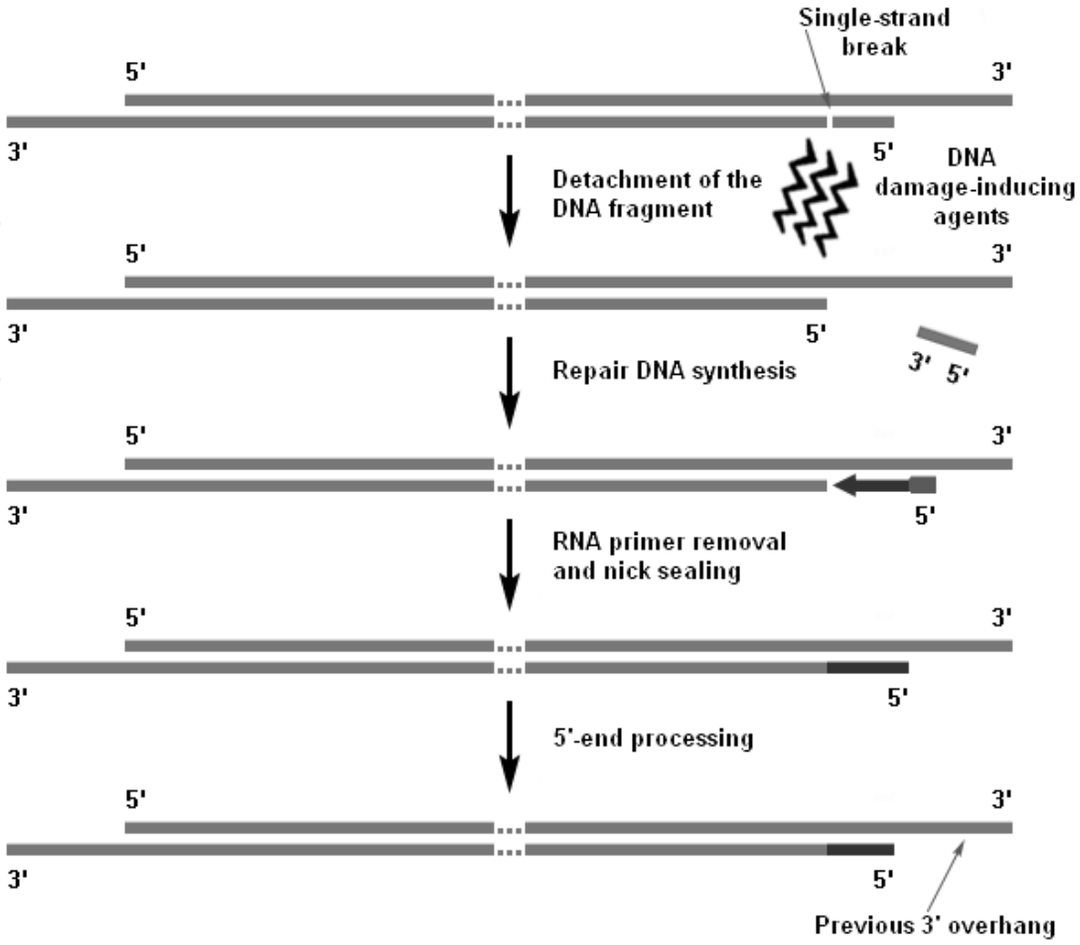

Figure 6. Complete double-stranded DNA end repair.

DNA cross-links represent connections between the nucleotide bases by covalent bonds (normally they are bound by hydrogen bonds) which can be of two types: intrastrand and interstrand (Dronkert & Kanaar, 2001). The latter ones represent a very serious problem for DNA replication machinery insofar as before a DNA polymerase replicates a parent DNA, it is at first unwound by helicase through the breaking of the hydrogen bonds between two strands. Therefore, if two complementary nucleotides are covalently linked, helicase will not be able to separate them and this will lead to the stalling of the replication fork and potential DSB formation. Two mechanisms for the repair of such damage are known which can be carried out during different phases of a cell cycle. Both of these mechanisms begin identically by forming cuts on both sides of the cross-link on one DNA strand by NER system proteins, after which there occurs a twisting of the oligonucleotide carrying the damage and gap formation. Later, such a gap will be filled; one mechanism for this is by TLS (Translesion synthesis), which makes use of DNA polymerases that are able to replicate DNA despite template damage arising before them. This is followed by another round of NER during which the second DNA strand is cleaved, and adduct removal is carried out. The second gap that is formed can than be filled by a conventional DNA polymerase on a complementary strand template and the ends are connected by DNA ligase. In the case of the other mechanism, the

filling of the first gap is carried out during the course of recombination on a homologous chromosome template within a G2 phase of a cell cycle, upon completion of which NER proceeds again. The subsequent stages of repair are the same as those for the first mechanism. If cross-links occur somewhere on the non-telomeric chromosome regions, then these two repair mechanisms can act without any problems arising. If, however, they arise too close to an end of a double-stranded DNA, in such a way that there are 8 or less nucleotides left towards the place where the 3' overhang begins on a G-strand during gap formation, then NER system activity will lead subsequently to such overhang loss and incomplete repair with telomere shortening. It could probably be restored to a previous state only in the case of the recombination-mediated DNA synthesis, which on the very ends of chromosomes, is very tightly blocked, as has already been mentioned. Every other event here is similar to those that have been described in relation to BER and NER.

Thus, we have reviewed the possible cases of the occurrence of incomplete repair and of DDMTS for various types of the damage of chromosome ends with 3' overhangs, which in their turn, should lead to the telomere shortening. At the same, not all such possible cases (as well as variations of damage and mechanisms for their repair) have been analysed but only those that seem to be the most important. Also, it should be emphasized that every possible case of incomplete repair and DDMTS, which has been assumed, can arise on uncapped linear telomere ends. If telomere ends are in a capped condition (i.e. in the form of a t-loop) then already other such cases will probably be observed, which also will lead to their shortening. Nevertheless, it seems for us that if telomere ends are organised into t-loops then the cases of incomplete repair and DDMTS characterised by telomere shortening will occur much less frequently than with linear telomeres. It should be noted that experimental data fully and directly confirming the appearance of incomplete repair or DDMTS for different described variants of damage could not be found. At the same time, there are many studies providing general information, demonstrating that various kinds of damage can occur on telomeres, which are repaired much less efficiently than those which are formed on the non-telomeric chromosome regions, and that they lead to telomere shortening (Passos et al., 2007).

3.4. The shelterin-mediated telomere repair problem

Many investigations have been performed focussing on the influence of reactive oxygen species (ROS) on the occurrence of telomere damage and associated telomere shortening. In one of the earliest studies it was found that mild hyperoxia leads to accelerated telomere shortening and inhibits the proliferation of fibroblasts which, as it was supposed at that time, could happen due to the accumulation of single-stranded DNA breaks on chromosome end regions (von Zglinicki et al., 1995). In another study, it was found that oxidative stress really leads to the accumulation of single-stranded breaks on telomeres whereupon they actually become rapidly shortened (Petersen et al., 1998). In yet another study, it was found that ultraviolet radiation combined with riboflavin induces the formation of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodG) in DNA fragments with telomere sequences that further leads to the appearance of breaks in the area of the central guanine of GGG sequences. It was also shown that under the influence of hydrogen dioxide (H_2O_2) together with Cu (II) on these

fragments DNA damage also occurred, which included the formation of 8-oxodG at the GGG sequence in the telomere sequence (5'-TTAGGG-3'), and which also led to breaks. Therefore, it was concluded that the formation of 8-oxodG in a GGG telomere sequence triplet induced by oxidative stress could play an important role in the acceleration of telomere shortening (Kawanishi & Oikawa, 2004). Along with these studies, many others showing that ROS leads to telomeric DNA damage formation and to their subsequent shortening are known (Passos & Von Zglinicki, 2006; Richter & von Zglinicki, 2007; Saretzki et al., 1999; Tchirkov & Lansdorp, 2003; Toussaint et al., 2000; von Zglinicki, 2000; von Zglinicki et al., 2000; von Zglinicki, 2002). It is also possible that besides the occurrence of single-stranded breaks on telomeres under the influence of ROS, there could also be oxidative modifications of nucleotides, which should be subject to repair by BER and NER systems, as well as double-stranded breaks (Passos et al., 2007). ROS has special importance concerning telomere damage, because unlike other mutagenic factors such as ionizing radiation, ultraviolet emanation, different chemical agents etc, ROS are constantly formed by mitochondria in a human organism during its normal metabolic activity. This is what triggered a strong interest to their study. Based on this, it may be that these other damaging agents can affect an organism and damage its telomeres in only very rare cases, while ROS continuously damages chromosomes' end regions, leading to their shortening. This situation actually should explain the fact that under normal conditions in the course of a cell's division, the telomere shortening rate considerably exceeds that which is expected only as a result of 3' overhang loss under the end replication problem (Keys et al., 2004). There are also studies which demonstrate that ROS can directly damage mitochondria themselves, and their mitochondrial DNA (mtDNA) in particular, thereby leading to their dysfunction which in turn can lead to the more intensive production of free radicals and, as a consequence, can result in even more intensive telomere damage and their shortening (Liu et al., 2002; Passos et al., 2006; Passos et al., 2007).

As was already noted, the damage occurring on telomeres is repaired less efficiently than that which originates in other genome regions (Kruk et al., 1995; Petersen et al., 1998; von Zglinicki, 2002). The reasons for such deficiencies in telomere-specific repair have not yet been completely established. At the same time, it is supposed that a basic role in the significant lessening of damage repair efficacy on telomeres belongs to the formation at their ends of the t-loops (capped telomere condition) (Passos et al., 2007). It was mentioned earlier that t-loops protect telomere ends from the activity of repair system proteins and another enzymatic influences (Grach, 2009; Griffith et al., 1999; Stansel et al., 2001). The example of the latter, incidentally, could be the telomerase attaching to the 3' overhang and its elongation. How does such repair suppression on telomeres by t-loops proceed? In order to answer this question let us first consider in detail what these t-loops represent and what actually characterises the response to DNA damage.

A t-loop represents a structure on eukaryotic chromosome ends which is formed at the bending back and subsequent insertion of a single-stranded telomeric DNA (3' overhang) into a double-stranded one (Grach, 2009). Upon this, the G-overhang forms a heteroduplex with the complementary C-strand region in double-stranded telomeric DNA, which is accompanied by the local untwisting of the latter and which leads to the formation of the so-called displacement

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loop (D-loop). The latter represents a triple-stranded structure which consists of a double-stranded DNA, formed by a 3' overhang and a C-strand of the previous double-stranded DNA, and single-stranded DNA, corresponding to the G-strand region of the previous double-stranded DNA (Fig. 7).

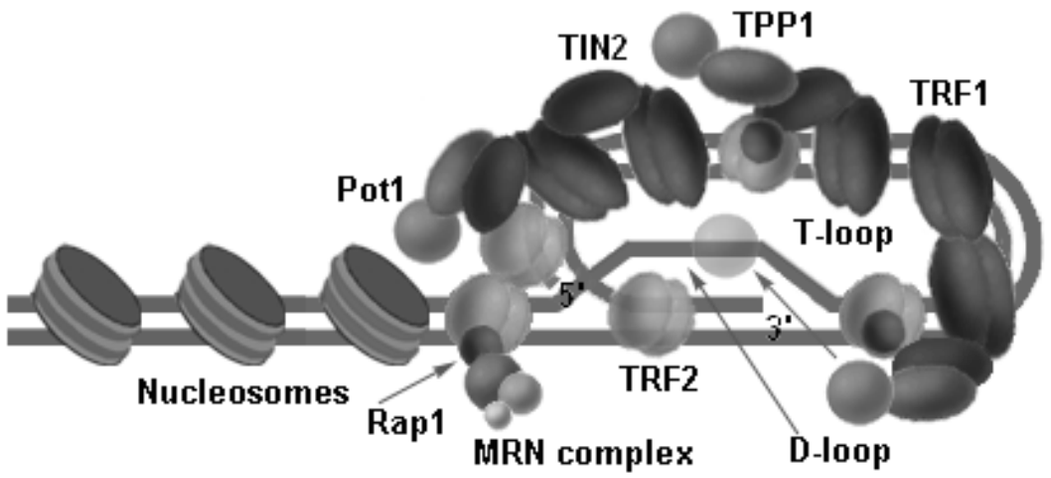

Figure 7. Structural organisation of a mammalian t-loop (Grach, 2009).

In t-loop formation, the primary role belongs to specific telomeric proteins, which are collectively referred to as the shelterin complex or telosome. Telomeric proteins differ slightly with different groups of organisms, but along with this they carry out similar functions. In mammals, shelterin includes six basic proteins, namely TRF1, TRF2, Rap1, TIN2, Pot1 and TPP1. These in turn can be divided into three groups: 1). double-stranded telomeric DNA binding proteins (TRF1 and TRF2); 2). single-stranded telomeric DNA binding proteins (Pot1); 3). proteins necessary for higher-order nucleoprotein complex formation (Rap1, TIN1 and TPP1) (Grach, 2009). The role of TRF1 function in t-loop formation it is to promote the bending back, twisting and linking of double-stranded telomeric DNA regions (Bianchi et al., 1997; Bianchi et al., 1999; Griffith et al., 1998; Griffith et al., 1999). Subsequently, TRF2 carries out the self-introduction of single-stranded telomeric DNA into a double-stranded one (Greider et al., 1999; Griffith et al., 1999; Stansel et al., 2001). Pot1 binds to single-stranded regions of telomeric DNA, which are represented only by a G-strand, and stabilises them (Baumann & Cech, 2001; Bunch et al., 2005; Churikov et al., 2006). Moreover, if a telomere end turns up in an uncapped condition, then Pot1 will cover the 3' overhang. If it turns up in a capped condition, then this protein will bind a single-stranded G-strand region in a D-loop structure. The Rap1 protein interacts with telomeres through binding with TRF2 (Li et al., 2000). It is suggested that the main functions of Rap1 are connected with its ability to recruit various repair system proteins, including Mre11/Rad50/Nbs1, Ku70/80 and PARP-1, to the telomeres (O'Connor et al., 2004). TIN2's role consists of both the binding of TRF1 proteins among themselves (Kim et al., 1999) and the binding of TRF1 with TRF2 (Ye et al., 2004). TPP1 is also necessary for the binding of TRF1 and TRF2 sub-complexes. It assists in the stabilising of TRF1-TIN2-TRF2 interaction. Besides this, TPP1 also directly binds Pot1 and regulates its activity (O'Connor et

al., 2006). Thus, TIN2 and TPP1 play a key role in the association of different telomeric proteins in a single functional complex, which participates in the formation of t-loops and the capping of telomere ends, and this provides telomeres protection from different kinds of enzymatic action.

The DNA damage response is characterised by the following circumstances. It is possible to distinguish such basic key points as DNA damage detection from repair itself. Earlier, we considered the most important repair mechanisms, and therefore we will analyse the damage detection pathways. As is known, there are two distinct DNA damage detection pathways, which can potentially be activated by a chromosome's natural ends, namely the ATM kinase pathway and the ATR kinase pathway (de Lange, 2010). The ATM kinase pathway is triggered in response to double-stranded DNA breaks (Lee & Paull, 2007). Upon this, the process by which the ATM kinase response is accomplished is still not completely clear (Lee & Paull, 2007). It is known that the Mre11/Rad50/Nbs1 complex (sensor proteins which take part in double-stranded breaks' detection) binds to DNA ends upon this and activates the ATM kinase in a combination with Tip60 HAT (Carson et al., 2003; de Lange, 2010; Williams et al., 2010). Later on, the ATM kinase phosphorylates some key proteins involved in the damage response to DNA double-strand breaks and initiates the activation of the DNA damage checkpoint, which pauses the cell cycle and allows time for a cell to repair damage before continuing its division (Lee & Paull, 2007). Upon this, Tip60 HAT, through histone acetylation, modulates repair proteins' loading and repair of double-stranded DNA breaks (Murr et al., 2006). In that case, if the damage is not resolved, the p53 protein is then activated, which triggers an apoptosis program (Polyak et al., 1997). The ATR kinase pathway is activated in response to the single-stranded DNA, and is based on the point that the abundant single-strand DNA binding protein RPA recognises and associates with single-stranded DNA, resulting in an RPA-ssDNA complex (Cimprich & Cortez, 2008; de Lange, 2010; Nam & Cortez, 2011). Further, the ATR kinase together with the ATRIP protein recognises such a single-stranded DNA coated with RPA and attached to the DNA's damage site (Cimprich & Cortez, 2008). At the same, the ATR kinase's recruitment to the RPA-ssDNA complex strongly depends on the ATRIP protein, which itself directly attaches to RPA-ssDNA, and thus binds this complex with the ATR (Zou & Elledge, 2003). The checkpoint clamp complex containing RAD9-HUS1-RAD1 (9-1-1) proteins, which take part in checkpoint activation, cell cycle arrest, and recruitment of specific DNA polymerases and other repair proteins to damaged DNA is also independently recruited to the DNA damage site by RAD17 protein (checkpoint clamp loader) (Bermudez et al., 2003; Sohn & Cho, 2009). Besides, the ATR activator TOPB1 is recruited to the DNA damage site (Choi et al., 2009). After these events, the activation of ATR by TOPBP1 and the phosphorylation of downstream targets in a signal transduction cascade proceeds, which eventually leads to checkpoint activation, cell cycle arrest and subsequent damage repair (Cimprich & Cortez, 2008). Later on, all of the events are similar to those which were considered concerning the ATM kinase pathway, i.e. if damage is completely repaired then the cell cycle is resumed and the cell will continue its division, and if not then there will occur a trigger of the apoptosis program and subsequent cell death.

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Now that we have considered what t-loops represent in themselves and what the DNA damage response is characterised by, it is possible to answer the question – how the repair of damage on telomeres is so strongly repressed. As such, it was proposed that shelterin hides the chromosome end from the ATM kinase pathway of DNA damage detection by remodelling telomeres into a closed structure – the t-loop. In a t-loop, Mre11/Rad50/Nbs1 is unlikely to recognise the telomere end as a double-stranded DNA end, which thus prevents ATM kinase activation, with subsequent cell cycle arrest and initiation of DNA damage repair (de Lange, 2009; de Lange, 2010; Griffith et al., 1999). This situation can be implemented as after the DNA replication, as well as after the double-stranded breaks occurred at telomeres. On the other hand, ATR signalling on telomeres is blocked by the shelterin Pot1 protein. It was noted earlier that, telomeres, on their own ends, contain a single-stranded DNA. This DNA at the uncapped condition of telomere ends is represented by 3' overhangs, and at the capped condition by a single-stranded region of a G-strand as a part of a D-loop. Furthermore, such single-stranded DNA can arise after a single-stranded damage and double-stranded breaks occurred at telomeres. It is potentially capable of activating the ATR kinase; however it was suggested that Pot1 binds a single-stranded telomere DNA and excludes the RPA protein from it. Later on, in the absence of this protein, such single-stranded DNA can no longer be distinguished by the ATR-ATRIP complex as damage, which prevents ATR kinase pathway activation and all subsequent events, including repair (de Lange, 2009; de Lange, 2010; Denchi & de Lange, 2007).

Along with the blocking of ATM and ATR kinase DNA damage detection pathways, shelterin can also block the DNA repair reactions by the direct blocking of repair proteins. It was mentioned earlier that the repair of double-stranded breaks can basically be performed by two pathways - NHEJ and HDR. These two pathways in turn are triggered in a manner similar to the ATM and ATR signalling pathways in various ways (de Lange, 2010). NHEJ first employs the ring-shaped Ku70/80 protein complex, which loads onto DNA ends and facilitates their further synapsis and ligation by DNA ligase IV. As such, there is the suggestion, that a t-loop in addition to the repression of the ATM signalling pathway, also - probably - effectively blocks Ku70/80 joining and thus could thwart NHEJ in its earliest steps (de Lange, 2009; de Lange, 2010; Palm & de Lange, 2008). Besides this, the possibility was discussed that additional mechanisms not involving the t-loop can be used for telomere ends' protection from NHEJ (de Lange, 2010). It is suggested that POT1 contributes in NHEJ repression, especially after DNA replication when the t-loop is not yet formed (de Lange, 2009). HDR is initiated when Rad51 (the protein playing one of the most important roles in homologous recombination, since it organizes the proteinaceous complex which is necessary for chromosomes pairing and subsequent DNA strands exchange (Babynin, 2007)) replaces RPA on a single-stranded DNA (de Lange, 2010). In this connection, for blocking such a pathway of DSB's repair at telomeres, it is enough simply to repress RPA binding. Such repression on the telomere ends of mammals is carried out with the help of the POT1 protein, the binding of which to a single-stranded telomeric DNA, as was already noted, excludes RPA (de Lange, 2010). Therefore, POT1 is probably capable not only of blocking the ATR signalling pathway, and NHEJ after DNA replication, but also HDR on telomeres (de Lange, 2010). Besides this, there is data suggesting that Ku70/80 is also capable of repressing HDR in the absence of POT1 (Celli et al., 2006). It is necessary to also note that there is experimental data showing that TRF2 overexpression

weakens the repair of single-stranded breaks on telomeres, resulting in their accelerated shortening. This suggests the possibility that the repair of such damage on telomeres, as well as the other types of single-stranded damage, is again hindered by t-loops and shelterin, in the formation of which TRF2 participates. In this connection, at increased TRF2 production, the t-loops are probably formed more intensively in this case, and damages are repaired less effectively, leading to accelerated telomere shortening (Richter et al., 2007).

Thus, shelterin can inhibit repair on telomeres by the repression of various pathways of DNA damage detection, as well as of their repair itself. T-loop formation, in particular, leads to the blocking of the access of Mre11/Rad50/Nbs1 and Ku70/80 to double-stranded DNA ends, which prevents the activation of ATM signalling pathway and NHEJ that in its turn protects DNA natural ends, but blocks the repair of double-stranded breaks at telomeres. Besides, it is possible that the t-loop sterically blocks the repair of single-stranded damage at the telomeres by its three-dimensional structure. POT1 binding to single-stranded telomeric DNA excludes RAP from it and, therefore, prevents the recognition of damage by ATR in a complex with ATRIP, and which in turn prevents ATR kinase pathway activation. POT1 also blocks the binding of the Rad51 protein to single-stranded telomeric DNA, which prevents repair through HDR. Besides this, it is supposed that POT1 - after DNA replication when the t-loop yet is not formed - takes part in NHEJ repression, which is carried out, apparently, by Ku70/80 heterodimer blocking. There is also data suggesting that telomeric proteins themselves directly repress the pathways of single-stranded damage repair as well.

All of these mechanisms for repair repression on telomeres would seem, at first sight, to be the enemies for them, since repair deficiency leads to the circumstance where damage, occurring at chromosomes' ends is badly repaired and this leads to their shortening and, ultimately leads to cell cycle arrest and apoptosis. Nevertheless, a certain amount of time is needed for this purpose, which in some cases can be a quite considerable. On the other hand, if telomeric proteins did not repress the DNA damage response at chromosomes' ends, it would result in apoptosis being triggered immediately rather than after telomeres had been shortened to a critical degree, which is caused by the following circumstances. If shelterin were be absent on distal telomere regions, or in other words if nucleosome organisation would be observed rather than telosome organisation, that probably would not distinguish them from other chromosome areas – it would lead to such a case whereby DNA natural ends would be recognised by the damage detection system as double-stranded breaks. In this connection, Mre11/Rad50/Nbs1 would activate subsequently the ATM kinase pathway. Besides this, it is known that MRN, attaching to double-stranded DNA ends and recognising them as DSBs, is also capable of performing the 5'-3' resection of such ends, thus creating 3' single-stranded tails or overhangs (Mimitou & Symington, 2009). With the absence of telosome and, in this case, of the POT1 protein in particular, this would now lead to the activation of the ATR kinase pathway. The activation of any of the DNA damage detection pathways would lead to cell cycle arrest and damage repair. The repair of such, let us say, false DSBs in the case of ATM signalling pathway would probably be carried out by the NHEJ way. Insofar as during NHEJ broken chromosome ends are directly joined, but in our hypothetical variant the natural ends of different chromosomes would be recognized as broken ends, then NHEJ in this case could lead to various

chromosomes' fusion with one another. This situation will result in genome instability, which leads to the initiation of the apoptosis program. The repair of damage by the ATR kinase pathway due to the presence of recombinogenic 3' single-stranded protrusions would possibly occur through HDR. In such a case, the fusion of different chromosomes and, later on, cell destruction through the initiation of the apoptosis program, will also take place. There is experimental data supporting all of this and showing that repression of the shelterin proteins leads to chromosomes' fusion and subsequent apoptosis. Besides this, such chromosome ends' vulnerability from homologous recombination could lead to continuous telomere elongation through the ALT which would essentially increase the probability of cell transformation. Thus, the repair suppression mechanisms on telomeres, although they lead to the accumulation of damage and shortening, at the same time protect chromosomes' ends from fusion among themselves and ALT activation. In the case of protection against chromosome fusion, it appreciably extends cells' lifespan. As for protection from ALT, thus shelterin prevents inappropriate telomere elongation and importantly the probability of cells' transformation. Therefore, the repair suppressing mechanisms on telomeres appear as friends for them.

It follows from the discussion above that the DNA damage response on telomeres, as well as damage detection and their repair pathways is potentially detrimental. This view was held for a long period of time. However, opinions changed when it was discovered that the proteins involved in the DNA damage response were present on the functional telomeres of mammals and interact with shelterin components, as previously in some way mentioned in reference to telomeric Rap1 protein, which recruits various proteins of the repair system to telomeres (Boulton & Jackson, 1996; Francia et al., 2006; Nugent et al., 1998). Moreover, further experiments have shown that they are also involved in telomere maintenance. This discovery was absolutely unexpected and somewhat paradoxical, as earlier it was thought that these proteins were the enemies for telomeres. However, it has now been discovered that this is not exactly the case, which has radically changed established views. Moreover, from now on DNA damage response proteins should be considered at the same time as equally the enemies and friends of telomeres. For the first time, such a role for DNA damage response proteins was found in yeast, where the NHEJ factor Ku is required for the maintenance of telomeres (Boulton & Jackson, 1996; Nugent et al., 1998; Polotnianka et al., 1998). In particular, in one such study it was found that in cells lacking telomerase but with functional Ku, telomere shortening slightly decelerates, i.e. it is less than in cells with repressed Ku function (Nugent et al., 1998). Further studies have shown that in addition to Ku, other proteins, involved in various DNA damage detection and their repair pathways, widely interact with telomeric proteins (Francia et al., 2006; Hsu et al., 1999; Hsu et al., 2000; Lenain et al., 2006; Palm & de Lange, 2008; Tarsounas et al., 2004; van Overbeek & de Lange, 2006; Zhu et al., 2000). Such factors in the mammals were called "shelterin accessory factors". Although they are present on telomeres transiently, at the same time they are very important for the maintenance of their normal structural organisation and functioning (Palm & de Lange, 2008).

Thus we emphasise, once again, that the main cause of poor damage repair on telomeres in comparison with other chromosome regions is the formation by their ends of t-loops, a process in which shelterin directly participates. Shelterin protects telomeres from the influence of both

different sensor proteins that carry out DNA damage detection and the repair proteins themselves. Although this in turn leads to an accumulation of damage on telomeres and their shortening, at the same time it protects chromosomes from joining with each other, which ensures the maintenance of the integrity and normal efficiency of the cell genome. While shelterin protects telomeres from the action of DNA damage response proteins, it effectively interacts with them, which is also very important for telomere maintenance. It is necessary to add to all this also that in our opinion the cause of an inefficient damage repair on telomeres - specifically on their very ends - and their subsequent shortening, besides formation by telomeric proteins of the t-loops as well as their direct inhibiting influence on the DNA repair proteins is the fact that DNA repair proteins themselves cannot act correctly on the very DNA molecule end, which was discussed in the beginning of the section and can lead to incomplete repair or DDMTS.

3.5. The conclusion of this section

In summary, Olovnikov first described the problem of repairing the damage occurring on telomeres in the early 1990s. As at that time, it was considered that telomere ends had a double-stranded structure and so the model of telomere repair problem was described according to that conception. At that time, this problem was named the incomplete DNA repair problem, under which was considered the possibility of whether damage (DNA single-stranded break) occurred at a distance of only several nucleotides from the very end of a DNA molecule, then it could not be completely repaired in the course of copying of the complementary DNA strand because of DNA polymerases' functional peculiarities. As a result of this, telomeres should be shortened. In addition, based on that model, as well as on the point that in certain cases repair can start but does not come to completion or else does not begin at all, we considered it necessary to propose – an incomplete DNA repair and damage-mediated telomere shortening, or DDMTS. Upon this, incomplete repair and DDMTS - which in several different ways can lead to telomere shortening - can be considered to be two variants of the end repair problem. Considering these proposed new conceptions, the old model of the end repair problem was revised. As the time, it was found that the structure of telomere ends is not double-stranded, as was supposed earlier, but 3' overhanging single-stranded, we considered the eventualities of incomplete repair and the occurrence of DDMTS on the very linear telomere ends in instances of single-stranded breaks and other types of damage in accordance with this model. Theoretically, it is expected that incomplete repair and DDMTS for different variants of damage should uniquely lead to telomere shortening. However, it is not known whether it can actually occur, as unfortunately we were unable to find the experimental data confirming these assumptions. At the same time, there is much general data showing that damage on telomeres occurs and that this damage is repaired less effectively than damage in other regions of the chromosome. It was demonstrated that researchers' main attention in this has been given to telomere damage by reactive oxygen species, which are constantly formed in mitochondria at normal cell vital activity. It should explain why the genuine telomere-shortening rate exceeds the one that is expected as being only as a result of end replication problem. The circumstances by which the damage on telomeres is repaired more poorly has been explained well by many researchers in terms of t-loop formation. Therefore, in order to demonstrate exactly how t-

loops repress repair, we considered what they represent in themselves and also what DNA damage response is characterised by. As a result, it was shown that t-loops, formed with the participation of the shelterin protein complex, as well as the telomeric proteins themselves, block various damage detection mechanisms and their repair pathways directly, and this actually causes telomere repair deficiency. This can be designated as the "shelterin-mediated telomere repair problem". It should be distinguished from the end repair problem, which can be carried out not along the whole telomere length but only on their ends, because of the inability of repair proteins to act on a template end. Thus, in principle, it is possible to identify two problems of repair on telomeres – the end repair problem, which is carried out near the very DNA molecule ends, and the shelterin-mediated telomere repair problem, which can affect all telomere regions where there is telosome organisation. At the same time, both of these problems can be referred to, in general, as the telomere repair problem.

4. Conclusion

In summarising all the data, it is necessary to emphasise that there exist two basic telomere-shortening mechanisms – the end replication problem and the telomere repair problem. The end replication problem, which is based on the 3' overhang loss during the course of DNA leading strand synthesis while the genetic material is doubling, has been studied in depth. The study of this problem allowed for the discovery of the telomerase enzyme and finding of the connection between telomere shortening and ageing, as well as carcinogenesis and various degenerative diseases. At the same time, many aspects of the end replication problem are still not absolutely clear. Therefore, further detailed studies of this process are necessary. The problem of telomere repair has been studied much less. Thus, there is no experimental data fully confirming that the end repair problem - which includes incomplete repair and DDMTS - can really be carried out on telomere ends and so lead to their shortening. At the same time, there is general data showing that damage on telomeres is frequently formed and repaired much less efficiently than on other chromosome regions leading to telomeric shortening. The researchers' main focus has been given to the influence of ROS on telomere damage. Given that they are constantly formed in cells by mitochondria, this is quite justifiable. At the same time, it is also necessary to study other negative factors concerning telomere damage and their shortening. Faint damage repair on telomeres is explained mainly by the formation at their ends of the t-loops, which are created with the participation of the telomeric protein complex shelterin and block different proteins involved in DNA damage response in order to prevent chromosomes' fusion with each other. This situation was referred to as the shelterin-mediated telomere repair problem. In this connection, it is necessary to study in more detail the structure of the proteins included in shelterin and their functions as well as mechanisms for t-loop formation. It is also important to study the proteins, which take part in DNA damage detection and the repair process itself. The latter should be studied in relation to both DNA damage response and their role in telomere maintenance. Thus, telomere-shortening mechanisms remain quite poorly understood and require further research.

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