Review

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Telomere Regulation by Metabolism Reprogramming

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Abstract: Telomeres – special DNA-protein structures at the ends of linear eukaryotic chromosomes define the proliferation potential of cells. Extremely short telomeres promote DNA damage response and cell death to eliminate cells potentially accumulated mutations after multiple divisions. However, telomere elongation is associated with increased proliferative potential of special types of cells, such as stem and germ cells, permanently, and is activated temporally in processes of activation of immune response, regeneration processes. Activation of mechanisms of telomere lengthening coupled with increased proliferation and with requirements of cells in energy and building resourses. To obtain necessary nutrients cells stimulate metabolism by switching of oxidative phosphorylation program to glycolysis. In this review we focused on the interconnection of metabolism program and telomere lengthening mechanisms known for the situation of the programmed activation of proliferation such as germ cells maturation, early embryonic development program and immune response activation. Here, we propose that it is possible to reprogram metabolism in order to regulate the telomere length, proliferative activity of cells that may be important for the development of approaches to regeneration, immune response modulation and cancer therapy and further investigations in this area are necessary to improve the understanding and manipulating of molecular mechanisms used for regulation of proliferation.

Keywords: telomere; development; ALT; telomerase; metabolism; glycolysis; OXPHOS

1. Introduction

Cell proliferation depends on the availability of intracellular resources and is tightly coordinated with metabolism. To divide cell should accumulate proteins and lipids and replicate the DNA in order to produce daughter cell. Eukaryotic cells could not divide endlessly; the end-replication problem restricts cellular proliferation capacity [1,2]. Telomeres – repetitive sequences located at the end of linear chromosomes provide the defense of genetic information from loss during the replication process and discrimination from internal double-stranded breaks. However, telomeres shorten in each cellular division and once they reach critical length, cells should die [3,4]. This mechanism helps a multicellular organism get rid of cells that may have gathered mutations due to multiple replication rounds. Telomeres shorten and this limits the number of times human cells can divide. This is usually enough for regular cells, but not for special cells like stem and germ cells [5,6]. Tissue regeneration, immune response, and cancer transformation all require increased cell growth. This growth is supported by the lengthening of telomeres and activation of anabolism [7–9].

Mainly telomerase – a specialized reverse transcriptase – is activated and elongates telomeres in vertebrates under conditions supporting the increased proliferation [10,11]. The alternative

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mechanism (ALT-mechanism) through a break-induced replication of telomeric DNA supports telomeres lengthening in some stages of embryonic development and in telomerase-negative cancer cells [12]. The goal of this review to summarize and discuss the information of mechanisms of telomere elongation and its regulation coupling with cellular metabolism program which are more investigated for early development process.

2. Telomeres: structure and regulation

The very ends of linear eukaryotic chromosomes are organized in special DNA-protein structures called telomeres. Telomeres protects linear eukaryotic chromosomes from the loss of genetic information during replication and false recognition as DNA breakage sites. Mammalian telomeric DNA are composed from long double-stranded regions of TTAGGG (Figure1) repeats several kilobases long which is flanked by a 3′-end single stranded G-overhang [13–15]. The length of telomeres shortens in every cell division and serves as molecular clock of the proliferative lifespan of cells. Critically short telomeres activate mechanisms of cellular senescence promoting cell death [16,17]. Telomeres may be elongated by telomerase [18] or through an alternative mechanism based on homologous recombination [19]. The initial telomere length of somatic cells is derived from germline cells [20]. Stem cells need to preserve telomere length so they can regenerate effectively. Cancer cells also use mechanisms to maintain telomere length, which allows them to keep dividing for longer periods. Telomere length is important for the lifespan of cells and organisms. If it is not properly regulated, it can lead to issues with regeneration, premature aging, and cancer progression.

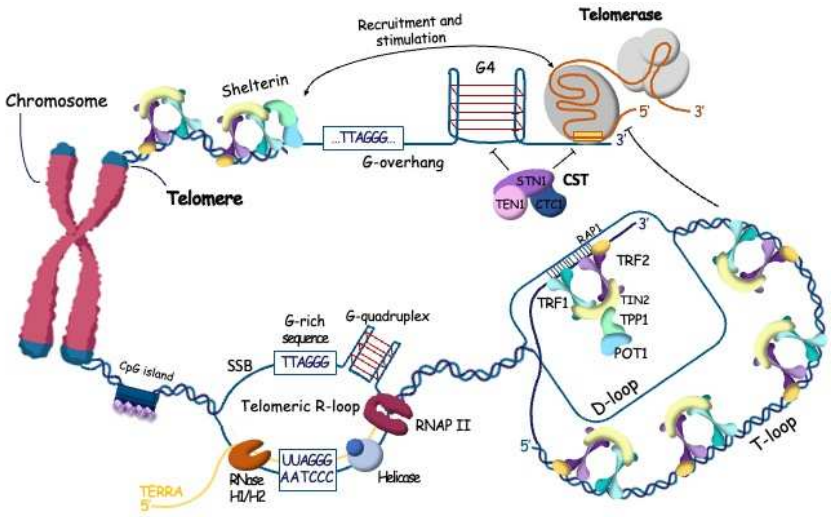

Figure 1. Human telomeric chromatin structure. Telomeric chromatin is organized by shelterin complex composed by TRF1, TRF2, TIN2, TPP1, POT1 and RAP1. To prevent the degradation and reparation of chromosome ends T-loop and D-loop protect telomeres. G-rich 3′-overhangs form G-quadruplex structures which stimulate the telomerase to synthesize the telomeric repeats and prevent the synthesis of C-rich strand by CST complex. Transcription of subtelomeric regions produces TERRA RNA which promotes R-loop formation stabilized by G-quadruplex structures at the opposite strand.

Shelterin complex associates with mammalian telomeres and regulates various aspects of telomere function [21]. Shelterin organizes telomeric regions in different DNA-protein structures (Figure 1), such as telomeric loops (T-loops), facilitating the invasion of ssDNA telomeric 3′-overhang into double-stranded region, regulates recruitment of telomerase for elongation of 3′-end and CTC1-STN1-TEN1 complex (CST) following by telomeric C-strand fill-in by pol a-primase to generate dsDNA telomeric region. Shelterin contains six protein subunits: telomeric repeat binding proteins 1 and 2 (TRF1 and TRF2), TERF1-interacting nuclear factor 2 (TIN2), protection of telomeres protein 1

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(POT1), POT1 and TIN2-interacting protein (TPP1) and repressor/activator site-binding protein (RAP1) [22–25].

TRF1 and TRF2 are homodimers that bind telomeric dsDNA through C-terminal Myb DNA-binding domains. N-terminal TRFH-domains of TRF1 and TRF2 interact with TRFH-binding domain of TIN2 [26–30]. Acidic TRFH-domain of TRF1 recruits poly(ADP-ribose) polymerase tankyrase 1 to inhibit the condensation of telomeric DNA [31]. The TRFH domain of TRF2 is involved in protecting and regulating T-loops [32], Holliday junctions [33], and G4-quadruplexes [34,35]. It also interacts with histones [36] and inhibits DNA double-strand break repair alongside RAP1 [37–39].

Heterodimers of POT1-TPP1 recruits to TIN2 through association of TIN2 binding domain of TPP1 with TIN2N (TRFH-like) domain of TIN2 [40,41]. POT1 prefers to bind ssDNA and the addition of TPP1 increases the affinity of POT1 for DNA tenfold. Despite the preference of POT1 and POT1-TPP1 heterodimer for the ssDNA and for capping the 3′-overhang they also bind internal telomeric ssDNA. POT1 interaction with DNA is mediated through two oligonucleotide- or oligosaccharide-binding structural (OB-fold) domains. The OB3 and Holliday junction resolvase-like (HJR) domains of POT1 facilitate interaction with TPP1. The HJR-domain of POT1 may be involved in regulation of organization of T-loop which is proposed to form Holliday junction structures at telomeres [38,42,43].

Shelterin contains 6 subunits, however, in the context of telomeres it demonstrates highly dynamic complex composition. The most abundant complex contains two TRF2 subunits, two RAP1 and one TIN2 subunit. A lot of shelterin complexes consists of two TRF2, two TRF1, two RAP1 and one TIN2 subunits and the minor fraction of shelterin contains all six subunits including TPP1-POT1 heterodimer. Indeed, weak affinity of interactions between shelterin components allows adjusting the composition of the complex to the context of DNA structures and other conditions [44–48].

Shelterin caps the ends of chromosomes and organizes the T-loops structures but the long double stranded region of telomeric DNA is packed with nucleosomes. The telomeric nucleosomes are less stable and more dynamic structures with unwrapping of DNA ends compared to canonical nucleosomes [49–51]. Human telomeric chromatin is enriched in histone H3 trimethylated at Lys9 (H3K9me3), the mark of constitutive heterochromatin, which is coexists in telomeres with H3K27me3, mark of facultative heterochromatin. Taken together we can overview telomeric structure at two different scales: higher-order chromatin architecture that regulates telomeres accessibility and more compacted structure of telomeric ends affecting the telomere replication specific mechanisms (Figure 1,2).

Besides T-loops telomeric DNA is involved in formation of another two noncanonical structures: G-rich chain forms G-quadruplexes and transcription of telomeric regions stimulates formation of R-loops (Figure 1). For a long time, telomeric region of chromosome was possessed transcriptionally silent. However, recently the transcription resulting in synthesis of long non-coding RNA named TElomere Repeat containing RNA (TERRA) was reported [52,53]. RNA polymerase II starts from promoters in the subtelomeric region and synthesizes G-rich heterogeneous in length (from 100 nts to more than 9000 nts in mammals) RNA consisting of 5′-UUAGGG-3′ repeats in direction to the end of chromosomes. It is known that highly transcribed regions, and regions where RNA polymerase may be paused, are prone to form R-loops as a three-stranded nucleic acid structure containing RNA:DNA hybrid and a displaced single-stranded DNA [54]. R-loops formation is closely associated with transcription and function in a variety of physiological processes [55]. The formation of R-loops by TERRA and telomeric DNA was proposed at the moment when transcription of telomeres was uncovered and confirmed later. Telomeres contain all features promoting R-loop formation, such as G-rich single-stranded DNA forms a G4-quadruplex, repeated sequence. Increased level of TERRA correlates with accumulation of R-loops at telomeres and it was demonstrated that R-loops level is higher at critically short telomeres [56,57]. Indeed, TERRA R-loops are stabilized at critically short telomeres, interferes with replication that causes replication stress and double-stranded breaks induction [58]. Activated DNA damage response promotes homology-directed repair to elongate telomeres. Interesting, that R-loops may be formed by TERRA not only co-transcriptionally. Their appearance is regulated by homologous recombination protein RAD51 which promotes invasion of TERRA molecules in telomeric dsDNA independently of transcription [59].

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The accumulation of R-loops promotes the formation of other noncanonical structures. G-rich strand which is released from dsDNA in the process of R-loops formation forms G4-quadruplex structures that is stimulated by RAD51AP1 binding with R-loops structures [60]. Interesting, that G4 structures prevent dsDNA formation after degradation of TERRA that promotes G-rich strand invasion to form telomeric D-loops that facilitate HDR. Moreover, G4-quadruplex structures are formed by G-rich single stranded 3′-overhang that is generated by telomerase. POT1 shelterin protein is able to disrupt G4 formation regulating by this way the telomere structure and telomerase binding [61,62]. Surprisingly, G4 quadruplex at telomeric 3′-overhang may add telomerase processivity [63,64]. Additionally, structural data show that telomerase has a specific space for binding G4-quadruplexes [65]. The RNA template of telomerase can directly interact with G4-structures, which helps in disrupting them when telomerase moves [66].

Taken together, the diversity of different structures formed by telomeric DNA together and with the assistance of the majority of proteins allows adapting the structure of each telomere to the environment and it is possible to conclude that there are no two identical telomeres in the cell.

3. Mechanisms of telomere length maintenance

In majority cells of vertebrate telomeres shorten in each division cycle because of the end-replication problem and nuclease action. However, telomeres must be maintained in cells with increased proliferation potential, such as stem cells, cancer cells. At the beginning of embryonic development, telomeres elongated very efficiently to obtain the length necessary for many divisions before birth and throughout the life of the organism. There are two basic mechanisms of elongation of telomeres known to be used in cells: elongation of 3′-overhang by telomerase followed by filling the C-rich by DNA polymerase a and alternative mechanism (ALT) based on homology recombination.

3.1. Telomere lengthening by telomerase

Telomerase reverse transcriptase elongates the 3′-overhang of telomeric DNA copying template region of telomerase RNA component [10,11]. Telomeric 3′-overhang is used as primers base pairing of which with 3′-end of template region of TR initiates telomerase catalytic cycle (Figures 1 and 2). RNA-protein interaction inside the telomerase complex causes the 5'-end of the template region to loop out. The formation of this loop facilitates to control the position of the 3′-OH group of telomeric end [67,68]. Binding of DNA-RNA duplex and dNTPs is necessary to initiate conformation changes in active center resulting in elongation-competent structure. After formation of closed active center telomerase adds nucleotides to the 3′-end of primer until it reaches the 5′ boundary of the template region [69]. During synthesis of telomeric repeats 5′ looped out part of template region stretches out and occurs in active site of telomerase displacing 3′ template region and flanking RNA [70]. Displacing the template 3' end destroys this part of the DNA-RNA duplex and helps open the active site for complete strand separation and template release. However, newly synthesized telomeric repeat could retain in TERT while the template translocates. During template translocation its 3′ end may form duplex with 3′ end of DNA which should be captured in active site of telomerase and new telomeric repeat will be added [71]. Alternatively, template RNA and DNA can separate from telomerase. The active site needs to be reestablished for TERT to start a new catalytic cycle.

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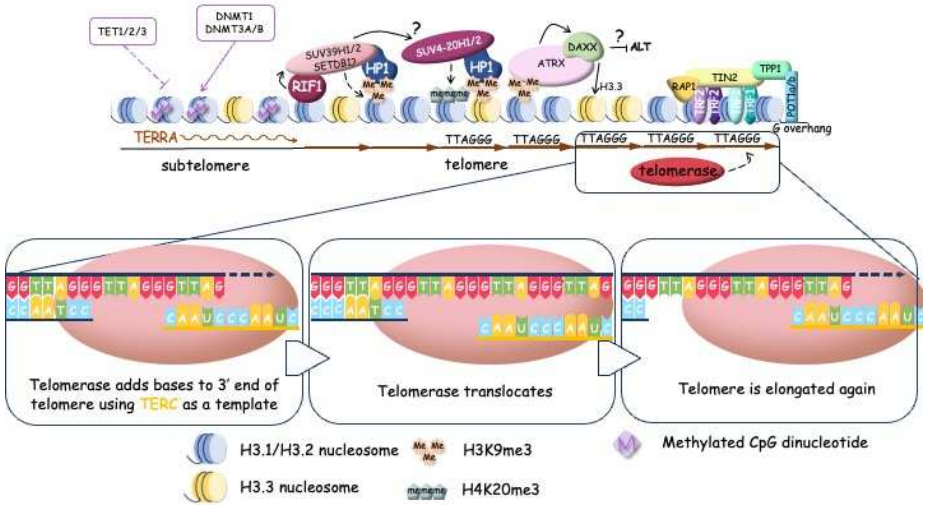

Figure 2. Scheme of telomerase action at telomeres. Telomeric chromatin is organized in closed state. Subtelomere DNA is methylated at CpG dinucleotides. Histones are characterized with heterochromatin modifications (H3K9me3 and H4K20me3). ATRX/DAXX complex stimulates the accumulation of H3.3 nucleosomes and prevents the homological recombination of telomeric regions. Shelterin complex through TPP1 loads telomerase to the very end of telomere and stimulates telomeric repeats synthesis at processive manner when enzyme translocates along the telomere in order to add more telomeric repeats after single repeat synthesis.

The interaction with telomere is the first and main step of the catalytic cycle of telomerase. Dynamic structure of telomere and shelterin play an important role in regulating telomeres accessibility for telomerase and telomerase activity. Shelterin can protect telomeres by stimulating T-loops formation and/or end-capping the telomeric 3'-overhang [22]. Alternatively, it can recruit telomerase to telomere and stimulate telomerase processivity in multiple telomere repeats adding [72–74]. The TEL-patch (TPP1 glutamate (E) and leucine (L)-rich patch) of the OB-fold of TPP1 directly interacts with the TEN (telomerase essential N-terminal) domain of TERT during the S-phase of cell cycle after genome replication. The process of this interaction is highly dynamic and multiple tentative interactions occur before stable contact will be stabilized through base pairing of telomeric end with template region of telomerase RNA [75,76]. TPP1 should help telomerase designate the telomeric tail and exclude binding with internal regions of telomeres. It was demonstrated that POT1 localizations to telomeres is regulated by its association with TPP1 [40,77]. It was proposed that a preliminary formed TPP1-POT1 heterodimer could transiently interact with TIN2-TRF1-TRF2 complexes. Sliding and hopping along telomeric dsDNA is possibly due to low affinity binding between these two complexes in context of dsDNA. Finally, the TPP1-POT1 complex finds the ssDNA-dsDNA junction where stable protein DNA complex forms, in which TPP1-POT1 interacts with ssDNA and TRF1, TRF2 are bound to the dsDNA. The localization of TPP1-POT1 at the junction of ssDNA-dsDNA triggers the activation of telomerase at the 3' end [25].

Interestingly, POT1 has controversial action in order to regulate telomerase attraction. When it is bound at the internal regions of ssDNA overhang it stimulates telomerase association and activity, however, in case of the POT1 interaction with the very end of telomere it will block telomerase binding and inhibit telomere lengthening [78]. Moreover, POT1 in coordination with TERRA and hnRNPA1 [79] removes from telomeric ssDNA replication protein A (RPA) which inhibits telomerase activity [80]. POT1 binding is necessary to protect telomeric ssDNA [81,82] and its association with TPP1 stimulates telomerase processivity [83].

Telomere extension by telomerase is terminated by heterotrimeric CTC1-STN1-TEN1 (CST) complex [84] (Figure 1). CST is an RPA-like ssDNA-binding protein with specificity to short (<30 nucleotides) G-rich sequences [85,86]. It prefers to interact with ssDNA-dsDNA junctions, specialized structures at DNA replication and breakage sites [87,88]. It can also bind non-telomeric sequences within longer ssDNA (>30 nucleotides). Structural data revealed one telomeric ssDNA OB-domain

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and five additional OB-domains without sequence specificity [89]. CST also interacts and unfolds G4 DNA structures, helping to restore stalled replication forks at telomeres. Besides action at stalled replication fork at telomeres and genome-wide CST inhibits telomere 3'overhang elongation by telomerase [90]. The binding and unfolding of G4 DNA structures by CST at newly synthesized G-rich strand may lead to the prevention of telomerase translocation [65]. CST is recruited to the telomeres by TPP1 during the late S/G2 phase of the cell cycle, where it competes with telomerase [84]. However, the mechanism of CST action at telomeres in telomerase regulating manner is not clear. It is proposed that CST unfolds G4 DNA structures and inhibits telomerase translocation, however, TPP1-POT1 can passively disrupt G4 with opposite regulatory effect on telomerase activity [62]. It was proposed that TPP1-POT1 and CST work at different stages of telomerase catalytic cycle [25]. TPP1-POT1 stimulates the interaction of telomerase with 3'overhang and CST acts when telomerase should translocate along newly synthesized telomeric DNA. However, CST terminates elongation of telomeres by telomerase and recruits pol a-primase to fill in telomeric C-strand [84]. Pol a-primase demonstrates two activities: it synthesizes RNA primer, which is used for DNA polymerization when switching of action from RNA to DNA synthesis occurs. CST stimulates both activity of pol a-primase, but details of his action are still unclear [91–93].

3.2. ALTernative mechanism of telomere lengthening

In most cases, telomeres are elongated by telomerase expression which should be reactivated [21] by mutations in promoter of TERT gene or by genomic rearrangement upstream of TERT promoter [94–96]. However, TERT may be permanently repressed in some tumours and in order to survive cells activate DNA repair pathways, particularly homologous recombination (HR) to maintain telomere length by the ALT mechanism (Figure 3) [19]. The ALT pathway is associated with inactivation of a-thalassemia/mental retardation syndrome, X-linked (ATRX) and death domain-associated protein (DAXX) [97]. ATRX and DAXX are the components of a multifunctional chromatin-remodelling histone chaperone complex that is responsible for the replication-independent localization of histone H3.3 at telomeres and pericentromeric chromatin [98–100]. ATRX interacts with DNA methyltransferase 1 (DNMT1). Mutations of lysine 27 (K27M) in histones H3.1 and H3.3 have been identified in some cases of glioblastomas [101]. Moreover, mutations in isocitrate dehydrogenase (IDH1) [102], which regulates the production of a-ketoglutarate that is required for the enzymatic demethylation of histones and DNA were found in ALT-tumours (low-grade astrocytoma and multiform glioblastoma) [103]. All mutations which are associated with ALT-mechanism of telomeres maintenance promote considerable remodification and expansion of telomeric chromatin which becomes acceptable for HDR mechanisms.

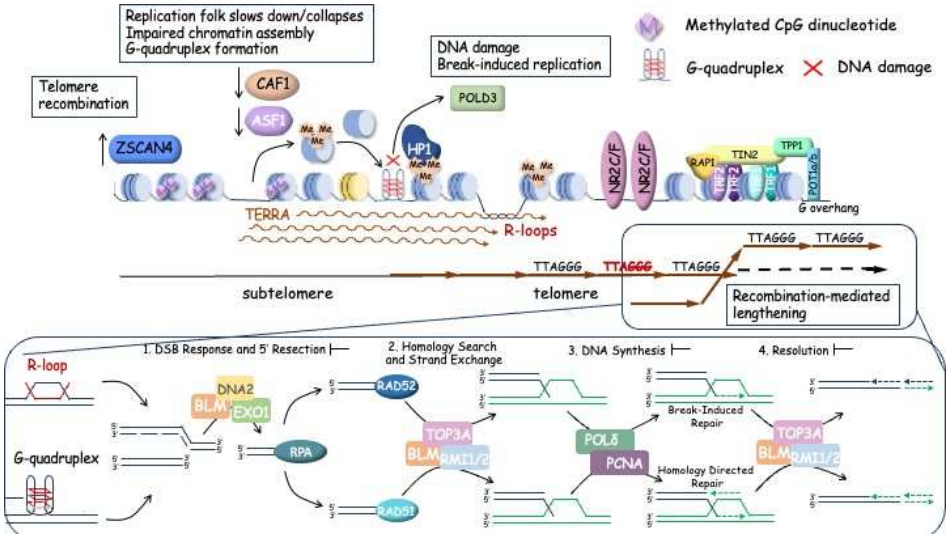

Figure 3. ALTernative mechanism of telomere maintenance. Absence of ATRX at telomeres promotes recombination at telomeric chromatin. Replication fork stalling, DNA-damage and alternative

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structures of telomercic DNA (G-quadruplexes and R-loops) at telomeres also involved in activation of ALT.

Alternative lengthening of telomeres is carried out by proteins which participate in homology recombination mechanism. Telomeric DNA synthesis in ALT-cells involves both intra- and inter-telomeric recombination and replication. ALT-cells are characterized with several hallmarks at telomeres: specialized ALT-associated promyelocytic leukemia foci (APBs), heterogeneous telomere length, abundant extrachromosomal telomeric sequences (ECTS) such as C-circles, and high-level of telomere sister chromatid exchange (T-SCE) [12,104,105]. APBs are unique specific to ALT-cells nuclear structures containing the PML-protein and telomeric DNA. In APBs clusters of telomeres and shelterin associate with recombinase RAD51, ssDNA-binding replication protein (RPA) [106], DNA resection MRE11-RAD50-NBS1 (MRN) complex [107], Bloom helicase (BLM) [108] and other HR accessory factors such as FANCM, BRCA1 [109], BRCA2, RAD51AP1 [110] and RAD52 [104]. There are two distinct types of ALT-mechanism of telomere lengthening were considered: RAD51-dependent and -independent [12].

The structural features of telomeric GC-rich sequences with G4 structures and R-loops formed with the help of TERRA hamper the replication process of these regions and leads to increased level of replication fork stalling [111]. DNA damage sensor kinase, ataxia-telangiectasia and Rad3-related (ATR) detects replisome collisions and recruits the RPA to single-stranded telomeric DNA [112]. Stalled replication forks are subsequently repaired and restarted by remodeling enzymes such as ATP-dependent strand annealing helicase SMARCAL1 [113], and/or DNA-dependent ATPase/translocase subunit of Fanconi anemia core complex (FANCM) [109,114,115]. FANCM also resolves aberrant R-loops which may be accumulated due to transcription replication conflicts at telomeric regions [116]. Hybrid RNA-DNA duplexes between TERRA and telomeric DNA are regulated by RNaseH with DNA/RNA-helicases, including RTEL1, FANCM, PIF1 [58,109,114,116,117]. SMARCAL1 and FANCM activity is important for replication fork reversal and repair. Depletion or disruption of SMARCAL1 and FANCM result in increased number and size of APBs [113–115,118].

After stabilization and repair replication fork may be restored by RAD51-dependent HR. RAD51 forms the presynaptic filaments at ssDNA and probes for identical telomeric sequences as a template for error-free repair [119]. Search of homologous template by RAD51 is coregulated and stimulated by BRCA1, BRCA2, RAD51AP1 and SMC5/6 heterodimer [110,119,120]. Surprisingly, meiotic-specific HOP2-MND1 heterodimer is involved in regulation of HR at telomeric in ALT-cells [119]. The alignment of homologous DNA strands can be stimulated by nuclear F-actin filaments. This allows the presynaptic filament to invade and form a synaptic complex, displacing the DNA strand in the D-loop [121]. Loading of terminal 3′-hydroxyl group promoted by PCNA and RFC to prime and initiate semi-conservative replication by Polh which is replaced by Pold for more processive synthesis [122]. Effective telomeric DNA synthesis requires the effective branch migration and resolving of D-loops and Holliday junctions which are stimulated by translocase activity of FANCM and unwinding and resolving of decatenates activity of BLM-TOP3A-RMI1/2 (BTR) complex [123–125]. The activity of BTR complex is necessary to suppress t-SCE events that preserves the original orientation of telomeric DNA strands [126].

In case when stalled replication fork is not reversed BLM helicase facilitates EXO1-DNA2 dependent resection of DNA in RAD51-independent HDR pathway [127]. RAD52 may be recruited to stabilize these HR intermediates and restrict resection, as suggested by studies [128,129]. RAD52 facilitates intrachromosomal pairing of telomeric DNA sequences of proximal sister chromatids and D-loop formation which are necessary to initiate break-induced replication (BIR)-related DNA synthesis at ALT telomeres [12,130]. These mechanisms occur during G2 and M phases of the cell cycle in contrast with RAD51-dependent mechanism which is realized during the S phase.

If stalled replication forks are not resolved by the HR or HDR pathways prior to entering mitosis, they should be eliminated. SLX1-SLX4, MUS81-EME1, and XPF-ERCC1 (SMX) complex creates double-strand breaks. PCNA-RFC-Pold can utilize these breaks for DNA synthesis [131]. RAD52 stimulates mitotic DNA synthesis (MiDaS) at chromosome arms and telomeres [132–134] which is

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initiated by SLX4 and SLX4IPand results in unprocessive abortive telomere synthesis, telomere cleavage and genome instability [130].

4. Metabolism

4.1. An overview of glucose metabolism

In somatic cells, telomeres shorten with each cellular division because of end-replication problem when endless RNA-primer is degraded and gap cannot be filled with the canonical mechanism working with intrachromosomal gaps. Nuclease processing further shortens telomeres. Critically short telomeres lose their protective properties and are discriminated as double stranded breaks. Cells activate senescence program and induce chromothripsis and polyploidization [135–137] and after crisis should be eliminated by authophagy [138]. Cancer cells activate telomere maintenance mechanisms to achieve replicative immortality.

Telomere maintenance mechanisms are not only activated during cancer transformation, but also during certain physiological stages and processes that require an increase in cell proliferation rate [139,140]. Proliferation rate activation requires the increased level of nutrients for synthesis of components necessary for building new cells. Moreover, it is becoming clear that metabolic pathways can also play modulatory or instructive roles in the regulation of cellular programs, which can be summarized as metabolic signaling functions [141,142].

The majority of cells in normal physiological status use the Oxidative Phosphorylation (OxPhos) as main metabolic program. However, in cases of increased proliferation rate and deficiency of oxygen OxPhos is switched to the glycolysis program, which generates less molecules of ATP per round in comparison with OxPhos, however, the turning rate is increased and moreover during glycolysis cell obtains other compounds necessary for synthesis of nucleotides, aminoacids and lipids (Figure 4) [141–143]. Intermediate metabolites affect downstream biochemical reactions and protein modifications, such as protein acetylation, glycosylation, and methylation [144].

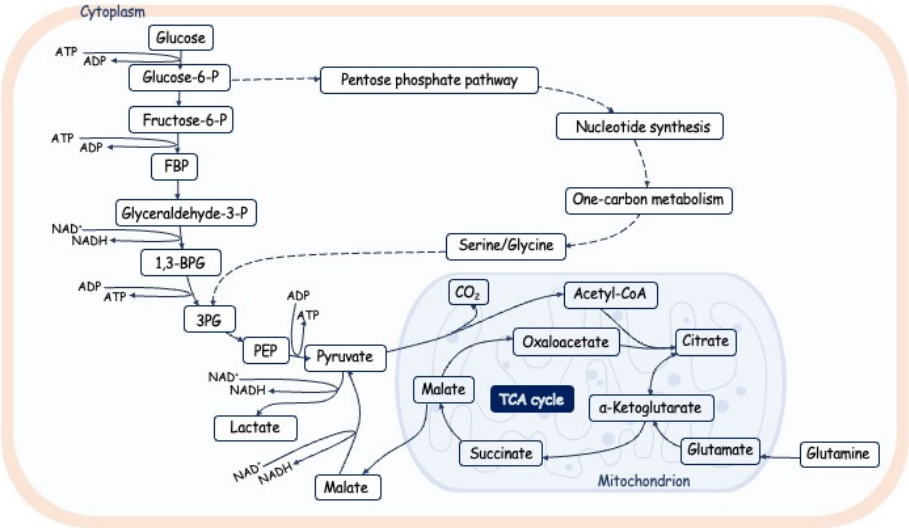

Figure 4. An overview of glucose metabolism. Glucose is catabolized in a series of enzymatic reactions yielding pyruvate. Pyruvate is converted in lactate or transported in mitochondria and metabolized into acetyl-CoA, fueling TCA cycle. FBP-fructose biphosphate, 1,3-BPG-1,3-biphosphoglycerate, 3PG-3-phosphoglycerate, PEP-phosphoenolpyruvate. Glycolysis also fuels pentose phosphate pathway, which produces nucleotides, aminoacids and fatty acids.

It is well known the regulation of metabolism in time and space, at the intercellular and tissue level, subcellular level. The metabolism is regulated during cell cycle progression. Glycolysis is activated during the G1/S transition, while mitochondrial respiration increases during the G2/M transition [145–148]. The reduced OXPHOS activity at the stage of DNA replication should minimize

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the risk of oxidative damage of DNA by ROS produced by mitochondria. Moreover, acetyl-CoA the main substrate for energy production by mitochondria, is also used for the acetylation of histones promoting the epigenetic regulation of gene expression and its level should be enhanced during DNA synthesis and epigenetic marks establish [149]. The epigenetic state of chromatin is also regulated by the level of other metabolic intermediates. The level of a-ketoglutarate is important for the maintain the methylation of DNA and histones regulating the expression activity of the genome [150].

It is interesting that the cell cycle phase when telomerase elongates telomeres is characterized by glycolytic metabolism program and, moreover, cells with increased proliferation rate where telomeres should be elongated also reduce mitochondrial OXPHOS activity and enhance the glycolysis to obtain resources necessary for synthesis of compounds for new cells.

The influence of short telomeres on the metabolism was reviewed several years ago [151]. Here we want to summarize and discuss the role of metabolism in activation of mechanisms of telomere lengthening. Cancer cells activate glycolysis to get the nutrients needed for making new cells. In transformation and cancer progression, the cells' environment changes. For example, it was demonstrated that at the first steps of tumour growth cells are in the conditions of hypoxia, but further development promotes the vascular growth and level of oxygenation reverses to the normal. Moreover, cancer cells activate the glycolysis by the mechanism known as Warburg effect, but OxPhos mechanism is hyperactivated by tumour cells during cancer progression [152]. Cancer cells multiply faster because of changes in certain mechanisms that lengthen telomeres. These changes can be caused by mutations in promoter regions [94–96] or a decrease in repair processes. This leads to the activation of a mechanism called ALT, which is based on a type of DNA recombination [19].

This review aims to discuss how telomere lengthening is regulated during processes like development and immune response activation. In these cases, the activation of telomere lengthening should be transitional and switched off after a short period of telomere elongation. We will focus on the mechanisms of switching the metabolism program and telomere lengthening mechanisms during development and T-cell activation because both these topics are investigated much better than the other examples of short-term activation of telomere lengthening coupled with increased proliferation.

4.2. Metabolism and telomere lengthening during early development and in T-cell activation

Mammalian organisms start to develop from fertilization, where two specialized cells, the spermatozoa and the oocyte, fuse together to form a totipotent 1-cell zygote. Further dividing coupled with differentiation of obtained new cells give rise the whole embryo and extra-embryonic tissues, such as a placenta. Successful development of new organism requires proper reorganization of chromatin restricted in time with the coordination of stage of development. Proper telomere length is crucial for organizing the genome of eukaryotic organisms. It enables cells to divide multiple times during development. During development, both mechanisms of telomere lengthening are used at different stages.

Gametogenesis represents a pivotal phase preceding embryonic development, wherein germline cells, having colonized the gonadal tissue, engage in successive processes of active proliferation and meiotic division, leading to substantial cellular specialization. In the field of telomere biology, a prevailing consensus asserts that germ cells prevent the attrition of telomeres through the active expression of telomerase, and then, as embryonic development ensues, telomeres undergo gradual reduction with each cycle of DNA replication [20]. Nonetheless, there exists a significant disparity in telomerase activity between oogenesis and spermatogenesis. Sperm cells go through a maturation process that increases their telomerase activity. This leads to the sperm cells having long telomeres when they are fully matured [153]. It was observed that late-generations of mice lacking telomerase RNA (mTR-/-) have unfunctional germ cells. Male germ cells, which require strong telomerase activity for their efficient maintenance, are depleted in these animals. The majority of research findings have consistently indicated that men experiencing idiopathic infertility tend to exhibit shorter telomere lengths in comparison to their fertile counterparts [154]. Telomere length is positively correlated with sperm count, motility, and the ratio of high-quality and transferable embryos [155]. However, it is negatively associated with sperm DNA fragmentation [156,157].

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Abundant evidence underscores the significant role of reactive oxygen species (ROS)-mediated sperm damage as a primary contributing factor to infertility among patients [158]. Sperm, due to their relatively limited antioxidant defense mechanisms, are particularly vulnerable to elevated oxidative stress levels. Oxidative stress can be risky for sperm DNA and RNA, and can also affect the integrity of telomeres and telomerase activity. This can lead to shorter telomeres [159]. As counterintuitive as it might appear, several studies have demonstrated an increase in telomerase activity and telomere length in human spermatozoa with advancing age [160]. Furthermore, the significance of this issue is heightened by findings from a mouse study which demonstrated that spermatozoa telomeres serve as a template for telomerase-independent telomere lengthening, leading to persistent differences in telomere length after birth [161]. Consequently, contemporary research places considerable emphasis on telomere length as a highly promising marker for evaluating male reproductive biology [162].

In contrast to the general trend of "greater potency equating longer telomeres," the female germline in many mammalian species stands as an exception. Notably, telomeres within mouse and human oocytes are among the shortest observed throughout the body, and they exhibit a low to absent level of telomerase activity [20,163]. The divergence in telomerase activity between oogenesis and spermatogenesis manifests with varying degrees across distinct mammalian species. A noteworthy illustration can be found in the Tasmanian devil and other marsupials, where an exceptional degree of telomere length dimorphism in gametes has been elucidated. This phenomenon involves a distinctive pattern: all telomeres in spermatozoa undergo hyperelongation, whereas those in oocytes experience hypershortening [164].

Nonetheless, in the case of mice, humans, and many other mammals, oocyte telomere length is delicately balanced and serves as an indicator of their quality. Telomerase activity measurements conducted through in vitro TRAP assays have unveiled a distinctive pattern: a peak in pre-ovulation oocytes followed by a subsequent decline in mature oocytes [165,166]. The resultant telomere length is directly associated with the quality of the oocyte and, in turn, influences its developmental potential. Oocytes with shortened telomeres resulting from telomerase-null mice exhibit a striking pattern of failure, occurring during both fertilization and the early cleavage embryonic stages [167]. This observation supports the hypothesis that low telomerase activity may serve as a selective mechanism, favoring the successful fertilization of egg cells that have already attained adequate telomere length during oogenesis [168]. This is predominantly because telomere attrition substantially promotes genomic instability through mechanisms like non-homologous end joining, ultimately resulting in conditions such as aneuploidy, mosaicism, and the emergence of copy number variants [137]. The shortening of telomeres in telomerase-null mice has been linked to several detrimental outcomes in oocyte development. This includes the formation of abnormal meiotic spindles [167], the arrest and fragmentation of embryos [169], a decrease in chiasmata and synapsis [170], and ultimately, infertility. Furthermore, oocyte telomere length has been associated with various challenges in in vitro fertilization (IVF) cycles, such as failed IVF cycles, embryo fragmentation [169], and blastocyst aneuploidy [171]. These findings underscore the critical role of telomere length in oocyte health and reproductive success. The diminishing ovarian reserve that occurs in tandem with advancing female age leads to a concomitant telomere depletion, which holds a central role in the process of oocyte aging. This is underscored by the observation of telomere shortening in oocytes obtained from females of reproductive age. The shortening of telomeres in oocytes could have served as an evolutionary selective mechanism actual during historical periods when the risks associated with grand-multiparity and advanced maternal age led to pronounced maternal mortality rates [172]. The age-related telomere shortening is primarily attributed to the enduring negative consequences of reduced telomerase activity and heightened exposure to reactive oxygen species (ROS) [173]. Research findings have demonstrated that the application of antioxidants enhances the quality of oocytes obtained from older females, including an increase in both telomerase activity and telomere length [174].

The process of oogenesis occurs in close collaboration with somatic follicular cells, specifically granulosa cells. These cells not only provide support for oocyte maturation but also play a crucial role in the synthesis of estrogen. During the period of follicular growth, granulosa cells exhibit robust

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proliferative activity and possess elevated levels of telomerase activity [175–178]. The length of telomeres in granulosa cells plays a pivotal role in regulating the normal progression of folliculogenesis and overall ovarian function. Reducing telomerase activity in granulosa cells has been associated with an elevated rate of apoptosis and an increase in the number of atretic (degenerating) follicles [176,178]. In granulosa cells, the activity of telomerase and the length of telomeres are regulated by estrogen levels [179,180]. High concentrations of estradiol-17β have been shown to significantly increase the telomere length of granulosa cells cultured in vitro [181]. Conversely, the withdrawal of estrogen consistently results in reduced telomerase activity, which may lead to telomere shortening in granulosa cells, subsequently contributing to follicular atresia [178,182]. Short telomere length and the absence of telomerase activity in the granulosa cells of women have been associated with occult ovarian insufficiency [183]. Indeed, short telomere length has been reported in young patients who exhibit a low ovarian response to hormonal stimulation [184]. This observation highlights the potential significance of telomere length as a predictor or indicator of ovarian responsiveness and reproductive health in individuals of reproductive age. During ovulation, a subset of the follicular cells that envelop the oocyte accompanies it as part of the cumulus-oocyte complex. These cumulus cells can be conveniently obtained alongside oocytes, presenting significant diagnostic potential when employing assisted reproductive technologies. The relative telomere length tends to be greater in cumulus cells derived from good-quality embryos as compared to cumulus cells associated with embryos of lesser quality [185]. This finding highlights the utility of assessing telomere length in cumulus cells as an effective means of evaluating embryo quality in the context of assisted reproduction.

The early stages of mammalian development involve a dynamic regulation of telomere length in embryonic cells, as observed in various mammalian species, including mice, rats, cows, and humans (Figure 5). Telomere length tends to increase during preimplantation development, reaching its peak at the blastocyst stage [186,187]. Remarkably, this elongation process occurs even in the absence of telomerase activity during the early cleavage stages, spanning from 2-4 cells up to the morula stage. Telomeres undergo elongation through an alternative lengthening mechanism (ALT-like mechanism) during these early developmental stages. The recombination mediated telomere lengthening at the early cleavage stage is driven by the telomeric chromatin reorganization due to H3K9 demethylation by KDM4 and Zscan4. The maintenance of Zscan4 activity in early embryos and 2-cell-like embryonic stem cells is facilitated by Dcaf11 (Ddb1- and Cul4-associated factor 11) [188]. Moreover, the transcription of TERRA is activated during early cleavage stages confirming the open conformation of telomeric chromatin. TERRA accumulation at telomeres promotes the ATRX recruitment followed by attraction of HP1 and compactization of the telomeres at the morula stage, accompanied by inhibition of ALT-like mechanism telomere elongation and activation of telomerase to provide the telomere lengthening at blastocyst stage [189].

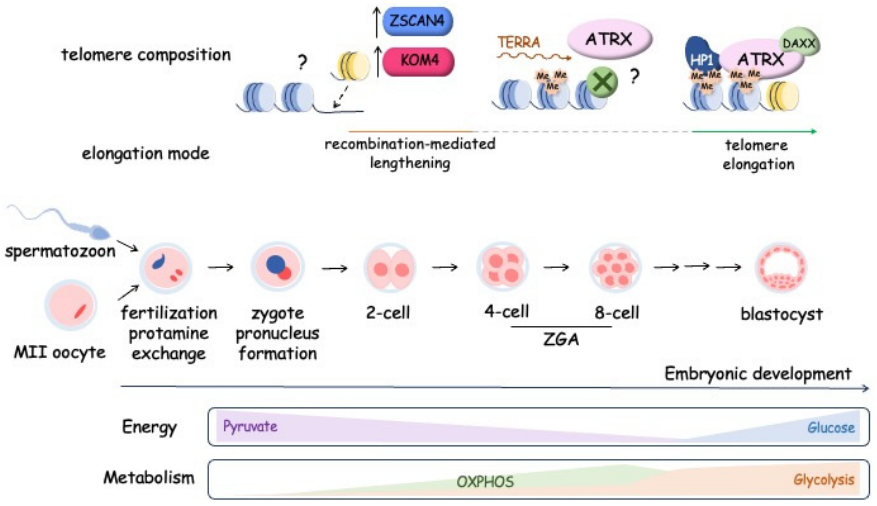

Figure 5. Telomere dynamics and metabolic program preferencies during mouse embryo preimplantation development. At first stage after fertilization extensive chromatin remodeling occurs

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with a histone-to-protamine replacement, formation of the two parental pronuclei and major zygotic genome activation (ZGA) at the two-cell stage, accompanied with elongation of telomeres by a recombination-mediated mechanism that is telomerase-independent and with activation of OxPhos metabolism program. Then, at the stage from the morula-blastocyst transition telomerase is activated. This process is characterized by switching metabolism program from OxPhos to glycolysis. In mouse zygotes, at the morula stage, ATRX is clearly targeted to telomeres. The expression of H3K9 lysine demethylases from the *Kdm4* family and *Zscan4* favors telomere elongation by recombination in mouse and human zygotes.

Interestingly, that zygotic gene activation, which occurs at the early cleavage stage, is supported by the regulation of metabolic program. During the early cleavage stage of development, the mitochondrial enzymes responsible for the production of acetyl-CoA and aKG are transiently localized to the nucleus where they impact the epigenetic histone modification to promote the chromatin opening. It was demonstrated that mitochondrial pyruvate dehydrogenase complex is phosphorylated and inhibited in cleavage-stage embryos that results in decreased activity of respiratory chain and OxPhos, but nuclear PDH remains unphosphorylated and active that influences on the epigenetic regulation of genome expression. Pyruvate metabolism supports development of embryo upon fertilization and glucose catabolism starts to be activated at the eight-cell stage and should be associated with inhibition of ALT-like telomere lengthening and activation of telomerase which elongates telomeres up to blastocyst stage and is inactivated during cell differentiation [190].

At the blastocyst stage, the maximum telomere length is observed, which exceeds that observed both at earlier stages of preimplantation development and during subsequent embryogenesis [191]. Telomerase activity emerged as a crucial factor driving telomere elongation during the transition from morula to blastocyst. Analysis of telomere length in 8-cell, morula, and blastocyst-stage embryos obtained from mTERC−/− or mTERC+/+ mouse models revealed that blastocysts from mTERC+/+ mice displayed significantly longer telomeres compared to those at the 8-cell and morula stages. Conversely, blastocysts from mTERC−/− mice did not exhibit this telomere elongation [163,192]. The blastocyst comprises two main cell types: the trophectoderm (TE) covering the outer surface of the embryo and the inner cell mass (ICM) situated inside of the blastocyst. While the ICM forms embryonic structures, the TE has more limited potential and contributes to the development of extraembryonic membranes. Interestingly, despite these distinctions in cell fate, telomere length exhibits only minor variations between the cells of the inner cell mass and the trophoblast [193]. Telomere elongation during the blastocyst stage significantly influences telomere length during subsequent stages of development. In the comparison of telomerase activity in bovine embryos undergoing development in vivo, in vitro, as well as after in vitro fertilization (IVF), and parthenogenetic activation, researchers noted only minor quantitative differences [186]. However, a recent study involving children born through assisted reproductive technology (ART) revealed that those born following a blastocyst transplant procedure exhibited shorter telomeres in their white blood cells by the age of one year [194]. Furthermore, research conducted in mice has shown that in vitro culture of mouse embryos suppresses telomerase activity during the early blastocyst stage, which subsequently leads to telomere shortening [194]. The intricate and dynamic regulation of telomere length during early development renders this mechanism susceptible to various negative influences, both external and internal. For instance, studies have demonstrated that a high-fat diet and obesity in female mice can lead to reduced telomerase activity and telomere shortening in oocytes and early embryos [195].

Embryonic stem cells (ESC) obtained from the inner cell mass (ICM) of a blastocyst have longer telomeres than mouse embryonic fibroblasts (MEF) of the same genetic background, which are typically obtained at embryonic day 13.5. It confirms that telomeres shorten after the blastocyst stage during cell differentiation to promote embryo development. Generation of induced pluripotent cells (iPSCs) from MEFs is accompanied by the telomere elongation up to length similar to the telomeres in ESCs. Moreover, telomeres are elongated in MEFs during cultivation and at early passages (P5) their length is comparable with the telomeres in ICM of blastocyst, however, telomeres length

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increased up to 24 passage and maintained constant for a long time. It was demonstrated the decreased localization of heterochromatin markers H3K9me3 and H4K20me3 at telomeric regions of ESCs and in iPSCs that could activate the mechanisms of telomere lengthening [191].

Interestingly, correlation between metabolism program, telomere length and ability to differentiate was demonstrated for human embryonic stem cells (hESC) recently. It was determined that the expression level of genes involved in OxPhos metabolic pathway is increased in hESCs with short telomeres, however, the expression level of genes related to glycolysis metabolism is up-regulated in cells with long telomeres [196]. Moreover, pluripotent ESCs was characterized with high glycolysis activity, but primed and differentiated cells demonstrate the increased OxPhos activity [197].

The switching between different metabolic programs in T cells during their development and maintaining is well studied. Quiescent T cells use catabolic pathways such as OxPhos, which provide efficient robust energy output, while activation of T cells is supported with anabolic glycolysis pathway, which provide nutrients necessary for protein production and cell division. Activation of T cells requires the increased proliferation which is accompanied by telomerase activation and telomere lengthening [140,198]. Naïve and memory T cells maintain their energy levels by relying on OxPhos, as their energy demand is relatively low during homeostasis. Upon activation, T cells proliferate at an incredibly rate and differentiate into effector cells. Early upregulation of glycolysis during T cells activation is supported with activation of PDH kinase 1, which phosphorylates and inactivates PDH. The inhibition of PDH drives engagement of aerobic glycolysis [199].

Switching between metabolism programs is regulated by different aspects. Activation of OxPhos requires an increased level of healthy mitochondria, which biogenesis is stimulated by transcription factors (Nrf1 and PPARg coactivator-1a) which drive expression of nuclear encoded mitochondrial genes [200].

Anabolic growth of cells is regulated by genes which expression is activated by mammalian target of rapamycin (mTOR), a serine/threonine kinase that integrates a multiple of extra- and intracellular signals and promotes glycolysis, growth and proliferation [201,202]. mTOR is regulated by a sensor of AMP/ATP ratio AMPK which drives catabolic metabolism when energy stores are depleted. It stimulates mitochondria biogenesis and inhibits mTOR activity. Both mTOR and AMPK form an axis of reciprocal regulation of catabolic and anabolic pathways [203–205]. Interestingly, that hTERP protein encoded in precursor of human telomerase RNA component is involved in regulation of autophagy and cell proliferation through AMPK pathway and stimulates autophagy and proliferation [206,207]. Autophagy is activated when glycolysis is inhibited, so we could hypothesize that the switching between biogenesis pathway of primary transcript of telomerase RNA gene [208] may be regulated by intracellular signals metabolism reprogramming in order to modulate the telomerase activity in accordance with cell requirement in proliferation rate.

5. Conclusions

Cell proliferation is important for organism development, regeneration, immune system function, cancer progression, and more. Cell division relies on energy, nutrition, metabolism regulation, genome integrity, and the maintenance of telomere length and structure. In this review we performed the analysis of mechanisms of metabolism and telomere maintenance in processes of activation of proliferation. We focused on different models that have experimental data on homogeneous cellular populations. These models have well-studied metabolism reprogramming and information on telomere elongation. This review provides the evidence that it is possible to reprogram metabolism in order to regulate the telomere length, proliferative activity of cells that may be important for the development of approaches to regeneration, immune response modulation and cancer therapy and further investigations in this area are necessary to improve the understanding and manipulating of molecular mechanisms used for regulation of proliferation.

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