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A switch-like dynamic mechanism for the initiation of replicative senescence

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ABSTRACT

Telomeres are specialized structures protecting chromosomes against genome instability. Telomeres shorten with cell division, and replicative senescence is induced when telomeres are badly eroded. Whereas TRF2 (telomeric-repeat binding factor 2), ATM (ataxia telangiectasia mutated) and p53 have been identified involved in senescence induction, how it is triggered remains unclear. Here, we propose an integrated model associating telomere loss with senescence trigger. We characterize the dynamics of telomere shorting and the p53-centered regulatory network. We show that senescence is initiated in a switch-like manner when both the shortest telomere becomes uncapped and the TRF2-ATM-p53-Siah1 positive feedback loop is switched on. This work provides a coherent picture of senescence induction in terms of telomere shortening and p53 activation.

1. Introduction

Telomeres are specialized chromatin structures that cap the ends of linear chromosomes and protect them from deterioration, end-to-end fusion, and being recognized as damaged DNA [1]. The function of a telomere depends on its length, specific DNA structure and engagement of telomere proteins. Either end of a chromosome has repetitive nucleotide sequences (like 5′-TTAGGG-3′ in mammals). Because conventional DNA polymerases are unable to replicate the very ends of chromosomes (i.e., end-replication problem), telomeres in normal human somatic cells shorten with each round of DNA replication [2]. Telomere DNA forms a lariat structure (called t-loop) by invading of the 3′-overhang into the duplex telomeric repeat array [3]. Telomeric repeats provide binding sites for the telomere-specific protein complex, shelterin, which consists of TRF1 (telomeric-repeat binding factor 1), TRF2, POT1 (protection of telomeres protein 1), RAP1, TIN2, and TPP1 in mammals [4]. TRF1 and TRF2 directly bind the double-stranded telomere repeats, while POT1 directly binds the single-stranded 3′-overhang; they are interconnected by the other three components. Shelterin is indispensable for mediating telomere end-capping. Specifically, TRF2 can modulate t-loop formation [3] and prevent the ATM (ataxia telangiectasia mutated) kinase from initiating DNA damage responses at functional telomeres [5].

Telomeres can switch between two states: capped and uncapped, and the probability that a telomere becomes uncapped rises as its length shortens [6]. Capping is defined as preserving the physical integrity of the telomere, involving the t-loop structure and recruitment of enough shelterin complexes [4]. Uncapping is characterized by loss of 3′-overhang, telomere fusion-induced chromosome instability and activated DNA damage signaling. In telomerase-negative cells, if a telomere remains uncapped persistently, either senescence or apoptosis is triggered to prevent propagation of damaged DNA [7]. Replicative senescence is characterized by irreversible cell cycle exit with sustained metabolic activity. Apoptosis provides an efficient way to kill irreparably damaged cells.

The tumor suppressor p53 has a critical role in inducing senescence and apoptosis by regulating expression of target genes [8]. As an endogenous DNA damage, sustained telomere uncapping activates ATM and p53. Moreover, p53 is not only a downstream effector of the damage signaling, but also functions upstream to regulate TRF2. p53 can downregulate TRF2 levels through Siah1 (seven in absentia homolog 1), a p53-inducible E3 ubiquitin ligase. Siah1 targets TRF2 for ubiquitination and proteasomal degradation [9]. That is, the TRF2-ATM-p53-Siah1 positive feedback loop can amplify the cellular response initiated by uncapped telomeres [10]. Moreover, p53 isoforms, such as Δ133p53 and p53β, and its

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family members, p63 and p73, are also regulators of cellular senescence [11].

The underlying mechanism for the induction and maintenance of senescence is less well understood. Whereas much attention has focused on the dynamics of telomere shortening and its correlation with senescence induction, few studies explored the dynamics of the p53 network during the initiation of replicative senescence. It is generally assumed that senescence is triggered when the shortest telomere reaches a critical length (i.e., the Hayflick limit) [12]. But how is it determined? The cell fate is closely associated with the (un)capping status of telomeres, and it seems essential to probe how replicative senescence is coherently triggered by coupling telomere shorting with DNA damage signaling.

Motivated by the above considerations, we build a minimal model correlating telomere shortening with p53 activation to clarify the mechanism for senescence induction in human diploid fibroblasts. We characterize both the temporal evolution of telomere length and the dynamics of the TRF2-ATM-p53-Siah1 and p53-Mdm2 feedback loops. We take into account three mechanisms contributing to telomere shortening. When telomeres are critically eroded, the TRF2-ATM-p53-Siah1 loop is switched on, contributing to downregulation of TRF2. This further leads to full activation of ATM and p53. Meanwhile, the concentration of phosphorylated p53 rises from a low to high level, so that senescence is triggered. The p53 dynamics exhibit a switch-like behavior, subserving the maintenance and reinforcement of senescence.

2. Materials and methods

2.1. Modeling telomere loss

We develop a stochastic algorithm to simulate the temporal evolution of the length of each telomere in a cell (the detailed description is presented in the Supplemental Material). There are 46 chromosomes and 92 telomeres in human fibroblasts. They can be divided into three categories in terms of initial length, separately comprising 30, 30, and 32 telomeres [13]. Their initial length is assumed to obey a normal distribution with the mean of 9, 7, and 11 kb, respectively, and the variance of 1 kb. The telomeres are considered in pairs for each chromosome, i.e., whether a telomere at one end of the chromosome is shortened after cell division depends on what occurs on the telomere at the other end. Similar to [14], we take into account three mechanisms underlying telomere loss at each division, i.e., incomplete end replication [2], processing of C-rich strands by exonuclease [15], and unrepaired single-strand breaks caused by oxidative stress [16]. The contribution of each mechanism to telomere shortening is illustrated in Fig. S1A, and the estimates for telomere loss after each division are presented in Fig. S1B. Note that the minimal telomere length after each division is taken as the input to the p53 network.

2.2. p53 network

The telomere dysfunction is detected by a regulatory signaling network including TRF2, ATM and p53. We construct a minimal model to characterize how eroded telomeres activate p53, focusing on the TRF2-ATM-p53-Siah1 and p53-Mdm2 loops (Fig. 1). TRF2 is mainly localized in the nucleus. There are three forms of TRF2: $TRF2_m$, $TRF2_{db}$ and $TRF2_{df}$. $TRF2_m$ denotes TRF2 monomers. $TRF2_{db}$ refers to functional TRF2 dimers that bind the telomeric repeats to rebuild the t-loop structure, preventing ATM from autophosphorylation [17], whereas $TRF2_{df}$ represents free, unbound dimers. With telomere shortening, the number of binding sites for TRF2 on telomeres decreases gradually, and thus the amount of $TRF2_{db}$ declines. The relationship between the concentrations of $TRF2_{db}$ and $TRF2_{df}$ and the telomere length is derived in the Supplemental Method. In our model, the information about the telomere state is transmitted to the p53 signaling network via TRF2.

The binding of TRF2 dimers to telomeres inhibits the phosphorylation of ATM on Ser1981 [17], an early step in its activation. ATM exists predominantly as dimers in unstressed cells. Dysfunctional telomeres promote the conversion of ATM from dimers to inactive monomers and to active, phosphorylated monomers. Phosphorylated ATM (ATM_p) further accelerates the activation of ATM via intermolecular autophosphorylation [18]. The total level of ATM is assumed to be constant since ATM is mainly regulated post-translationally. The phosphorylation and dephosphorylation of ATM are taken as enzyme-catalyzed reactions and are assumed to follow the Michaelis–Menten kinetics [19].

Activated ATM phosphorylates p53 and Mdm2, repressing their interaction and promoting the degradation of Mdm2. Consequently, p53 is stabilized and activated [20]. Phosphorylated p53 ($p53_p$) induces the expression of *mdm2*, whereas Mdm2 targets p53 for proteasomal degradation; this negative feedback loop ensures that p53 has a very low level under unstressed conditions. $p53_p$ transactivates the expression of *siah1*, and Siah1 targets $TRF2_{df}$ for proteasome-mediated degradation [9]. The transcription of genes by p53 is characterized by Hill function, and the Hill coefficient is set to 4 given the cooperativity of the tetrameric form of p53 as a transcription factor [21]. Since we concern only the initiation of senescence, for simplicity we do not consider the downstream targets of p53 that contribute to maintenance of senescence, including p21.

2.3. Method

The details of model construction are presented in Supplemental Material. The telomere shorting with cell division is simulated stochastically, whereas the concentration of each species is represented by a state variable in deterministic rate equations ([...] represents the concentration of species throughout the text). The initial values of variables and standard parameter values are presented in Supplemental Table S1 and S2, respectively. The time step for integrating ordinary differential equations is 0.01 min. The cell is assumed to divide every 3 days.

3. Results

3.1. Dynamics of the telomere length and the p53 network

We keep track of the lengths of 92 telomeres; they shorten with cell replication. We illustrate the temporal evolution of the minimal telomere length L_m in a cell (Fig. 2A). As the number of cell divisions rises, L_m gradually decreases in a stochastic way; in this example, the cell stops dividing after the 56th division, where L_m reaches 1 kb. After that, the cell becomes senescent. This result agrees with the experimental observation [13]. We also show the distributions of telomere length among 92 telomeres at the beginning and at the end of replicative lifespan in the inset. The distribution shifts leftward and telomere lengths are more widely distributed in the senescent state. Whereas there is a large variability in telomere length, senescence is triggered when the minimum telomere length is sufficiently short and the telomere is badly eroded, as shown later.

Furthermore, we display the distribution of L_m at the end of replicative lifespan among 2000 cells (Fig. 2B). Clearly, most of L_m is located around 1 kb, whereas L_m in some cells can be much less than 1 kb. In fact, L_m is longer than 1.1 kb immediately before the senescent state, but after the replication, L_m may drop to various extents because of three mechanisms: the loss is uniformly