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Mini Review

SIRT1 regulates the ribosomal DNA locus: Epigenetic candles twinkle longevity in the Christmas tree

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ABSTRACT

Ribosomal RNA (rRNA) genes arrange themselves in a tandem pattern in nucleolus and during the transcription of rRNA genes, the elongating nascent rRNA transcripts create a structure called Christmas tree. rRNA genes in the rDNA locus can be either active or silent depending on the epigenetic regulation of the chromatin structure. Yeast Sir2 (silent information regulator 2) protein containing complexes can repress the recombination in the rDNA locus and subsequently extend the replicative lifespan of the budding yeast. The mammalian rDNA locus is also under the epigenetic regulation by protein complexes, such as NoRC (nucleolar remodeling complex) and eNoSC (energy-dependent nucleolar silencing complex), involving histone deacetylases and methyltransferases. SIRT1, a NAD^+-dependent histone deacetylase, is the key component in the eNoSC complex and hence energetic changes can regulate the activation of eNoSC complex and in this way mediate the epigenetic silencing of rRNA gene expression. The eNoSC complex links SIRT1-induced longevity regulation to the metabolic rate theory of aging.

Epigenetic control of transcription in Christmas tree

Ribosomal RNA (rRNA) genes are arranged in tandem arrays which form clusters known as nucleolus organizer regions (NOR) [1,2]. Mammalian genomes contain several hundred rRNA genes in the ribosomal DNA (rDNA) locus which are transcribed by RNA polymerase I (Pol I). rRNA synthesis and processing take place in the nucleolus which is the site of ribosome biogenesis. Studies on the molecular organization of active rRNA genes in the nucleolar architecture have revealed structures which resemble Christmas trees [3–5]. During the transcription of rRNA genes, elongating nascent pre-rRNAs radiate away from the trunk, forming a gradient of increasing length which can be viewed as the branches of a Christmas tree. One nucleolus can contain a number of Christmas trees at the same time with active sites of rRNA synthesis. Ribosome biogenesis is closely dependent on the metabolic activity of the cell [6]. In growing cells, rRNA synthesis represents the main part of total transcriptional activity, whereas in senescent cells it is greatly reduced.

Several research approaches have revealed that only a subset of rRNA genes is active at any given time [6–8]. Active and silent rRNA genes are distributed randomly in the rDNA clusters. One exception to this is nucleolar dominance, where there is coordinated silencing of the clusters of rRNA genes which are inherited from one of the parents [6,7]. Nucleolar dominance appears in many genetic hybrids where the rRNA genes of one parent dominate over the other and they assemble nucleolar structures. It seems that it is the regulation of the Pol I transactivation which controls the transcription of the rRNA genes [6–9]. In particular, epigenetic regulation seems to be the major mechanism which directs the activity of rRNA genes in the rDNA clusters. There are both active and silenced NORs depending on the distinct chromatin structure of rDNA. Epigenetic mechanisms such as DNA methylation and histone modification determine whether the chromatin structure of certain rDNA locus is open or closed for Pol I transactivation [6–9]. The methylation of CpG domains within the upstream promoters of rRNA genes is the common mechanism to silence mammalian rRNA genes but the budding yeast *Saccharomyces cerevisiae* lacks this mechanism [6]. Furthermore, histone code with acetylation, methylation and ubiquitination determines the accessibility of the chromatin structure to permit rRNA gene transcription [9,10]. The transcription and silencing of rRNA genes are closely controlled by several forms of epigenetic regulation. In addition, epigenetic mechanisms also regulate the recombination in rDNA locus. Interestingly, the epigenetic regulation via different silencing complexes seems to link the metabolic regulation of the cell to the aging processes of the organism (see below).

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Sir2 complexes in yeast rDNA locus

SIR (silent information regulator) proteins are well-characterized inducers of repressed chromatin structure in budding yeast [11]. Sir2 protein is a NAD^+-dependent protein deacetylase [12] and it is the functional component in epigenetic complexes which mainly silence the transcription of three genomic loci in *S. cerevisiae*: rDNA locus, telomeres, and mating loci [13]. Sir2 has several homologs in different species, e.g., seven human SIRTs. Sir2 types of enzymes comprise the class III histone deacetylases (HDACs) and are called Sirtuins [14,15]. Recent studies have demonstrated that Sirtuins target a wide array of acetylated proteins, not only histones, and they are involved in multitude of vital cellular functions, such as metabolic regulation and maintenance of cell survival [14–16].

In budding yeast, Sir2 can form different silencing complexes, either with other SIR proteins or with other types of accessory proteins, such as with components of the RENT (regulator of nucleolar silencing and telophase exit) complex [13,17,18]. Diverse Sir2 complexes regulate each of the rDNA locus, telomeres and mating loci [13]. In the rDNA locus, Sir2 complexes can silence the transcription of rRNA genes but in addition, they can suppress the homologous recombination in rDNA locus [17,18]. Accessory proteins, e.g., condensin and cohesin are involved in the silencing complexes against recombination. Huang et al. [17] have identified the protein partners participating in the formation of cohesin-associated clamp complexes which restrict unequal crossover of rDNA sister-chromatids. In addition to Sir2 complexes, several Sir2-independent complexes epigenetically regulate the transcription and recombination in the rDNA locus. ISWI (initiation switch) and Set1 (Su(var) 3–9, Enhancer of zeste, and Trithorax) complexes also regulate chromatin remodeling in rDNA locus [19,20].

In 1997, rDNA research entered the field of aging when Sinclair and Guarente [21] demonstrated that the recombination in yeast rDNA locus and the subsequent accumulation of extrachromosomal rDNA circles (ERCs) in old cells were responsible for the aging phenotype of budding yeast. Later studies revealed that Sir2p could repress recombination in rDNA locus and promote yeast longevity [22]. Kobayashi et al. [23] observed that Sir2 protein prevented unequal sister-chromatid recombination by enhancing the formation of cohesin clamps. It seems that Sir2 protein regulates the recombination between rDNA tandem repeats and hence it can protect rDNA against ERC formation and the aging process. Further progress in the field revealed that though ERCs do not appear in species other than budding yeast, Sir2 could still extend replicative lifespan. Studies by Valter Longo and his group [24,25] have demonstrated that Sir2 has opposite effects on aging in replicative and chronological models of yeast aging. It seems that Sir2 can block the longevity extension induced by caloric restriction or by Sch9 mutation in yeast [24]. This discrepancy could be due to the dual function of Sir2 complexes in budding yeast, i.e., they can either prevent recombination of rDNA or inhibit transcription of rRNA genes. The mammalian Sir2 analog, SIRT1, can limit rRNA transcription in eNoSC complex [[26], see below].

NoRC complexes regulate mammalian rDNA locus

The best known of the epigenetic regulators of mammalian rDNA locus is the NoRC complex, a nucleolar remodeling complex [6,9]. NoRC complex is an ATP-dependent chromatin remodeling complex which is composed of SNF2 type of ATPase and TIP5 (TTF-I-interacting protein 5) but the complex can interact with DNA methyltransferases (DNMT) and Sin3 corepressor complex containing histone deacetylases HDAC1 and HDAC2 but not SIRT1 [9]. The NoRC complex is a potent epigenetic repressor which silences rDNA repeats via DNA methylation and histone deacetylation. Non-coding RNA transcripts originating from the intergenic rDNA spacer (IGS) interact with TIP5 and potentiate the transcriptional silencing mediated by NoRC complex [27]. DNA methylation induced by NoRC complex impairs the binding of upstream binding factor (UBF) to the promoter region of rRNA genes and inhibits the activation of Pol I and the transcription of rRNA genes. Furthermore, the interaction of NoRC complex with Sin3 attracts HDACs and HMTs (histone methyltransferases) to the complex which subsequently can deacetylate and methylate three histone lysines, H3K9, H3K20 and H3K27. Histone methylation leads to heterochromatin formation which in turn, stabilizes nucleolar structure [9].

The balance between active and silent clusters of rRNA genes is regulated by the binding of CBS (Cockayne syndrome B protein) and TIP5 to TTF-1 protein. TIP5 forms the silencing NoRC complex (see above) but CBS, SNF-like ATPase, in collaboration with G9a, histone methyltransferase methylating H3K9, is a powerful activator of rRNA gene transcription [9,28]. Truncation of the CBS protein represses the RNA elongation by Pol I but does not impair rRNA transcription [29]. Defects in CBS function lead to the genetic disorder called Cockayne syndrome (CS). CS is a well-characterized progeroid syndrome linked to DNA damage [29]. It seems that CBS regulates rDNA stability rather than rRNA transcription (Fig. 1).

SIRT1 controls eNoSC complex of mammalian rDNA

Recently, Murayama et al. [26] revealed a novel mammalian rDNA complex called eNoSC (energy-dependent nucleolar silencing complex) which acts in conjunction with SIRT1, instead of HDAC1 and HDAC2 present in NoRC complex. This rDNA silencing complex is able to sense the energy status of cell, since SIRT1 activation is dependent on the level of NAD^+ [12]. The NAD^+/NADH balance rapidly increases when the availability of energy is reduced. The activation of the eNoSC complex represses rRNA transcription and ribosome biogenesis which is the most energy-consuming process undertaken by cells. Switching off the rRNA synthesis is an effective way of saving energy in an attempt to maintain cellular homeostasis during acute stress. The eNoSC complex is targeted to rDNA locus via nucleomethylin protein [26]. The activation of SIRT1 deacetylates histone H3 which subsequently, induces the dimethylation of H3 at Lys9 by SUV39H1 and the formation of silent chromatin in the rDNA locus. The eNoSC complex provides a

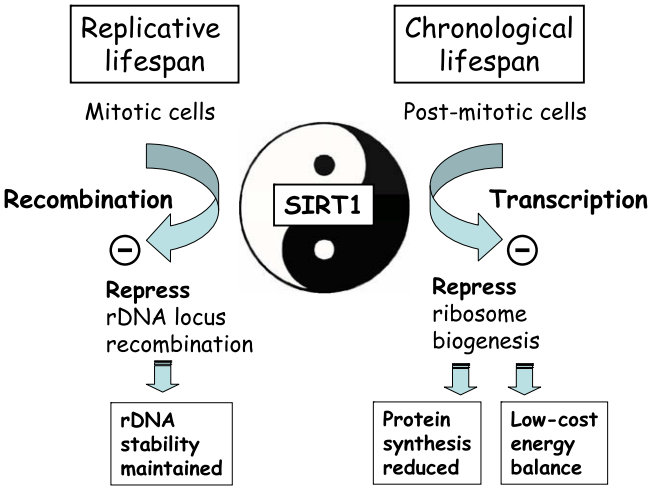

Fig. 1. A schematic presentation depicting the yin-yang role of SIRT1 in the regulation of replicative and organismal aging via epigenetic complexes which can repress either rDNA locus recombination or rRNA gene transcription.

novel regulatory link between cellular energy balance and the epigenetic state of rDNA locus. Silencing of rRNA gene transcription promotes the restoration of energy balance and the suppression of recombination increases the stability of the rDNA locus (Fig. 1).

Epigenetic longevity control in rDNA locus

There is convincing evidence that the transcription of rRNA genes is epigenetically regulated by silencing complexes containing histone deacetylases and methyltransferases [6–9]. The formation of the closed chromatin structure also prevents homologous recombination by increasing the stability of the rDNA locus. In budding yeast, the stability of the rDNA locus is the major determinant of yeast longevity [12,13]. Mammalian aging seems to differ from that of budding yeast although there are common elements. In fact, the role of replicative senescence seems to be a minor mechanism in the regulation of mammalian organismal lifespan [30,31].

Interestingly, the epigenetic control of the eNoSC complex involving SIRT1 activation in rDNA regulation integrates several aging theories (i) rate-of-living metabolic theory [32,33], (ii) epigenetic chromatin regulation theory [34,35] and (iii) hormesis theory [36]. It has been known almost for a century that the metabolic rate of animals correlates with their maximum lifespan, i.e., the more active the metabolic rate, the shorter the lifespan [see Ref. [33]]. This observation was later explained by the free radical theory, formulated by Harman [37], which postulated that high oxygen consumption produces oxygen radicals, i.e., oxidative stress is the driving force in the aging process. The work of Murayama et al. [26] indicated that the intracellular energy status can regulate rRNA gene transcription via SIRT1 activation and rDNA silencing which repress ribosome biogenesis and subsequently protein synthesis (Fig. 1). These energy-driven events resemble those occurring during caloric restriction (CR) which is the most common way to extend the lifespan in organisms from yeasts to mammals [38,39]. The role of Sirtuins in the lifespan extension mediated by CR is still under debate [40] but SIRT1-eNoSC provides a novel regulation pathway for mammalian aging since it reduces the metabolic rate (Fig. 1). It is known that also TOR (target of rapamycin) signaling can repress ribosome biogenesis as well as protein synthesis and degradation during CR [see Ref. [38]]. Moreover, it seems that the eNoSC complex and epigenetic repression of rDNA locus could be involved in hormetic adaptation [see Refs. [36,41]] by saving energy and shifting metabolic rate to a lower level as a way of increasing stress resistance and extending the chronological lifespan (Fig. 1).

It seems plausible that epigenetic SIRT1-eNoSC complex is involved in the regulation of organismal lifespan but the role of SIRT1 in replicative lifespan seems to be more complicated. A common observation shows that SIRT1 expression is upregulated in proliferating cancer cells [42] and in primary cells with high rates of mitotic activity [43]. Sasaki et al. [43] demonstrated that the level of SIRT1 protein is significantly reduced with serial cell passage in culture, i.e., in replicative senescence. Immortalization of senescent cells restored the high level of SIRT1 protein [43]. These observations indicate that the eNoSC complex is not the major regulator in replicative senescence. Somatic mutations accumulate during aging which may be due to the increase in reactive oxygen production and the decline in DNA repair capacity. In particular, DNA replication stress induces genome instability which can evoke cellular senescence [44]. Furthermore, the genes responsible for premature aging syndromes, e.g., Werner syndrome, are involved in DNA repair and the defects in these genes seriously increase genomic instability [45].

In view of the role of SIR proteins as stability factors of the rDNA locus in the aging of budding yeast, it seems that protection against recombination of the rDNA locus could be an important factor also in mammalian replicative senescence. Interestingly, the methylation of rRNA genes is clearly accelerated in Werner syndrome cells as well as in replicatively senesced cells [46]. Teleologically, sirtuins might have the same role in mammals as they have in yeast which could explain the changes in SIRT1 expression associated with replicative senescence [43]. It seems that SIRT1 could be a yin-yang type of anti-aging factor: via repression of rRNA gene transcription it improves the chronological longevity of post-mitotic cells but conversely, via repression of rDNA recombination it supports replication by maintaining rDNA stability (Fig. 1). Ultimately, replicative senescence can be postponed but this means mitotic activity and cancerous growth, a well-known rival to the aging process.

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