Novel Longevity Regulator Cgi121: Paradox in Aging Mechanism

By SONG Jianlan (Staff Reporter)

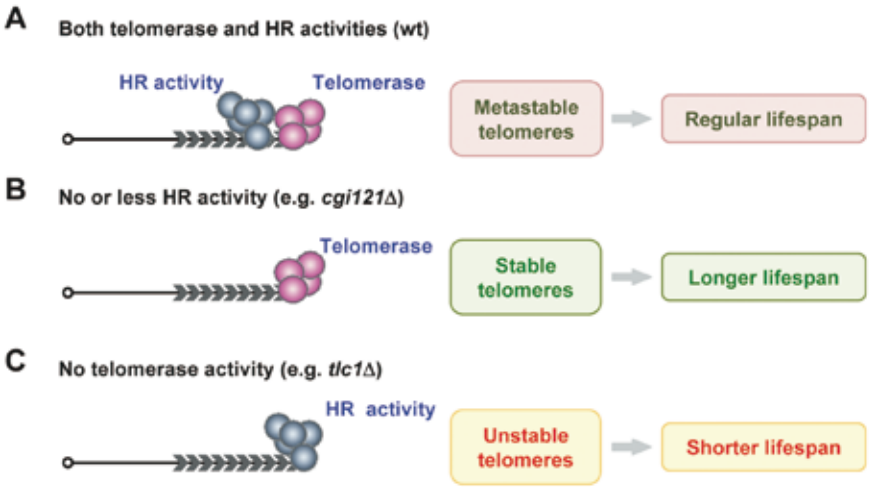
A group of scientists at the Shanghai Institutes for Biological Sciences (SIBS), CAS lately revealed a paradox in longevity regulation, which has long baffled scientists. They discovered in a species of budding yeast that a protein called "Cgi121" works as a molecular switch capable of activating an aging-accelerating mechanism on the telomere, the "cap" on either end of the chromosome. Paradoxically, this mechanism has been known as a DNA-repairing pathway and believed to be beneficial for cellular longevity, if occurs somewhere else than the telomere. This unexpected dark side might indicate that aging/longevity regulation is even more complicated than thought.

The group, led by Prof. ZHOU Jinqiu at the Shanghai Institute of Biochemistry and Cell Biology (SIBCB) under SIBS, reported online in the *PLOS Genetics* on March 30 successful identification of the long-sought-after novel aging regulator Cgi121, and articulated the detailed mechanism of regulation. According to the authors, this regulator facilitates homologous recombination (HR, a kind of genetic recombination in which nucleotide sequences are exchanged between two similar or identical molecules of DNA), even in the presence of telomerase, and surprisingly promotes premature aging.

The dual role of HR throws more clouds over aging mechanism, of which little is understood so far, though known to be sophistically regulated in organisms. At the cellular level, aging involves a network of intertwining pathways including genomic instability, shortening of telomeres, and epigenetic alterations, whose exact roles and interplaying are far from clear. Now with the emergence of the novel regulator Cgi121 and the complicated regulation as it revealed, telomere, whose length believed to set the upper limit for cellular lifespan and hence a well-known target for research on longevity regulation, comes back into spotlight in a subtle shade.

Mysterious Triangle of Aging

A protagonist in the complicated regulation system of aging, telomere remained obscure until the 1970s, when



The SIBCB team reveals in their latest research that protein Cgi121, a subunit in the KEOPS complex can trigger homologous recombination (HR) on the telomere and accelerate aging, a paradox in aging/longevity regulation. (By courtesy of Prof. ZHOU)

biologists found out that this seemingly useless "cap" featuring simple repeating sequence, turned out to be a hero in DNA replication. It was then finally realized that the last bit of the chromosome body would be missed in the process of replication due to the intrinsic order of replication programmed in the genome and the linear structure of the DNA strand. In the absence of telomerase, which can feed the strand with extra base pairs, the sequence of the telomere will be cut off a little bit every time the DNA replicates; and once it is shortened to a critical length, the cell will lose the ability to reproduce itself (undergo cell cycle arrest) and become senescent. In this case, due to the finite length of the telomere, a given cell can only generate a limited number of daughters before entering mitotic arrest. This phenomenon is termed "replicative aging", and the replicative lifespan is accordingly defined as the number of daughter cells a virgin mother cell can generate.

In the complicated landscapes of aging research, it is a well-accepted hypothesis that the telomere length counts the number of times a cell can proliferate, and consequently the replicative lifespan of the host. "Telomere-shortening triggered cellular senescence is one of the generally accepted pathways in the field," introduces ZHOU, who has been engaged in research in this field since the early 2000s.

Therefore it is of critical importance for eukaryotes to maintain the integrity and length of their telomeres. Capable of replenishing the sequence of the telomere by feeding it with base pairs, telomerase is the primary means for telomere maintenance and lengthening, employed by the vast majority of eukaryotic species. Its exact role in longevity regulation, however, still eludes biologists, as its concentration in the cell might not directly correlate to the lifespan of the host. Nor does the telomere length directly correlate to the lifespan of the cell, either. Research has found that in some organisms, for example a seabird species, shortened lifespan did not result in shortened telomere, or vice versa. This hints on the complexity of aging and longevity, and also suggests existence of alternative mechanisms to secure telomere integrity.

DNA recombination pathways are among the maintaining mechanisms other than telomerase. Some species can use both telomerase and recombination to maintain the integrity and length of their telomeres, including the budding yeast *Saccharomyces cerevisiae*, which is employed by the SIBCB group as a model organism. Due to this trait, a small percentage of the yeast cells can survive even if telomerase is depleted: they can use HR to maintain and lengthen their telomeres as a backup pathway, and hence can continue on to generate daughter cells. The telomere length elongated via recombination, however, does not result in extended lifespan; instead, it accelerates the aging process of the yeast, and leads to a lifespan significantly shorter than that of normal cells, as demonstrated by ZHOU's group in their previous research published in the *PLOS Genetics* in 2009.

If this does not look confusing enough, HR's role as a pathway to repair serious DNA damages can make this paradox even more surprising. According to a theory, aging could be seen as the accumulated effect from DNA damages, including serious ones like double strand break (DSB), in which both strands of the chromosome are broken. According to this theory, DNA damages occur naturally and frequently in the nuclei and accumulate if remained unrepaired; and aging could be seen as the consequence from the accumulated lesions.

Misfortune in Disguise of Angel?

Therefore it is understood that genome instability is a hallmark, and also a causal factor of cellular aging. The group also observed that failure in DNA repair, like mutation of the genes involved in DSB repair of the yeast could greatly reduce the replicative lifespan of the organism and increase cell dysfunction, cellular apoptosis or senescence. Furthermore, unlike the controversial role of telomere length in longevity regulation, a more consistent correlation between genome instability and the finite lifespans of organisms from yeast to humans has been established in literature. Given that HR can protect the cell from genome instability, naturally it is anticipated to assume a positive role in longevity.

"As failure to repair DNA lesions could result in cell cycle arrest and therefore cell death, homologous recombination is generally believed to be essential for cell viability," explains Prof. ZHOU. However, his group unveiled the dark side of the moon: recombination can backfire once occurs at a wrong place.

What has made the protector of both telomeres and the stability of main-body chromosome harmful for longevity? How does the telomere invite misfortune in the disguise of an angel? Where gets wrong? These remained unclear until more detail of the regulation mechanism emerged.

Crossroad of DNA Repair

To study the relationships between the intrinsic cellular traits and aging, biologists have employed various model organisms, from single-cell species like budding yeasts to multicellular species like fly, fishes, mouse and monkey. As mentioned above, the budding yeast *Saccharomyces cerevisiae* employed by the group as a model organism can use either telomerase or HR to replicate its telomeres,

and maintain their integrity and length. This offers the researchers opportunities to examine which pathway is more preferable in preserving the integrity of telomeres.

In their earlier efforts, the researchers used telomerase-deficient cells (also called survivors, as they survived depletion of telomerase) of *Saccharomyces cerevisiae*, which employed homologous recombination to replicate their telomeres, to rule out the influence of telomerase. They observed that these telomerase-deficient cells, though successfully maintained chromosomal integrity, suffered a replicative lifespan significantly shorter than that of telomerase-proficient cells, showing a sign of accelerated aging. Given that reintroduction of telomerase immediately restored the replicative lifespan, the authors suggested that telomerase might be a telomere maintenance pathway superior to HR in sustaining yeast replicative lifespan.

"Organisms could have developed HR in adversity as a rescue to elongate the telomere, 'struggling' desperately to survive," suggests Prof. ZHOU.

But how come the cell could allow HR to occur on the telomere despite the preference for telomerase? Who are the players and how they interplay to regulate the process together? More importantly, why telomere HR, which elongates the length of the telomere and is hence expected to correspondingly elongate the replicative lifespan, could rather shorten the replicative lifespan?? Now with the latest exploration the group not only confirmed their discovery in 2009, but also further unveiled a competing mechanism in the regulation system, in which the unique structure of the telomere misleads the HR.

Lethal Resemblance

Structurally the telomere resembles to DSB, but generally it is well protected with a specialized architecture consisting of repetitive guanine-rich DNA bound by telomere-specific proteins, and hence frees itself from being repaired – by mistake – as a DSB. It could be confused with a DSB by mistake, however, due to some intrinsic traits, as explained by the authors in their latest paper. For example its repetitive sequences could be favourable substrates for HR; moreover, quite a few proteins or protein complexes involved in DNA repair pathways can also bind and function at telomeres.

Therefore, the authors suggested, this resemblance makes it possible for HR to occur on the telomere; meanwhile the resemblance misleads the involved regulators to pass through a competing mechanism and target at the telomere.

"Thus it is not surprising, but rather logical, to see that HR is able to function as a back-up system for telomere replication when telomerase pathway fails," the authors proposed.

Searching for the Regulator

But who are involved in the regulation? How do they conspire to regulate the accelerated aging process? The group carried on in a search for the regulators.

In September 2011, the group updated their progress and verified that a tail-module of yeast Mediator complex is indispensable for telomere heterochromatin maintenance. They performed genome-wide screenings and succeeded in identifying several components of the transcriptional regulator involved in telomere length regulation and telomere heterochromatin maintenance. In 2013, as a result from their new round of genetic screening, the group first reported the identification of several regulators in telomere recombination, including the conserved KEOPS complex, and meanwhile verified that Cgi121, a subunit of the KEOPS complex, is required for efficient telomere recombination. Also, they uncovered new functional roles for 32 genes that affect telomeric DNA recombination.

At last in their latest work, they confirmed their previous discoveries and further identified the culprit – Cgi121, one of the five proteins in the "KEOPS complex". The group focused their attention to this subunit and found out a special trait of the Cgi121 subunit, *e.g.* it is indispensable for both telomere length regulation and recombination, but not required for tRNA modification, therefore manipulation of its functioning on the telomere would not interfere in the normal homologous recombination in the body of the DNA strands. With this understanding they were able to dissect the functions of KEOPS complex in telomere recombination from those in tRNA modification. Finally they demonstrated that Cgi121 works as a positive regulator in telomere recombination: it could facilitate HR on the telomere, and inactivation of it could result in weakened telomere recombination and promote cell longevity.

Paradox of Recombination

Still, it seems quite confusing that HR, capable of lengthening the telomere, could actually backfire and shorten the replicative lifespan. Based on their observations the researchers reasoned that the long-telomere length *per se* in the telomerase-deficient cells is not associated with the accelerated cellular aging. "Most likely, the survivors aged prematurely in a telomere-length independent manner," they proposed.

In an effort to understand the potential mechanism underlying this paradox, the group addressed the effect of

telomere recombination on replicative aging. They chose again telomerase-deficient cells to examine the latter's lifespans. The cells that use recombination to replicate telomeres appeared much shorter lifespan than telomerase-proficient cells.

Consistent with their discovery in 2009, their further experiments confirmed that reactivation of telomerase in the survivors inhibits telomere recombination and at least partially restores cellular lifespan. Analysis of the telomere structure of the telomerase-negative cells revealed signs of genome instability, and based on this the group gave their explanation in terms of why telomere HR could reduce the cellular longevity.

HR activities occurring on the telomere, the authors concluded, can elicit genome instability and pose a negative effect on cellular longevity. Wherever there is no or less HR activity on the telomeres, telomerase is not (or less) "harassed" and hence can maintain better genome stability on the telomere, resulting in lengthened lifespan.

"The HR-mediated repair activity tangles or competes with telomerase activity on telomeres, leading to false-alarms which make the cells undergo aging. Thus, the balance of both activities results in metastable telomeres and normal lifespan," the authors concluded.

"Disruption of Cgi121 promotes telomere stability by inhibition of telomere recombination, without compromising the essential function of recombination on maintaining the integrity and stability of the whole genome, therefore leading to extended lifespan," emphasize ZHOU and Dr. PENG Jing, corresponding author and first author of the latest *PLOS Genetics* paper.

Longevity and Beyond

When asked to what extent the newly revealed regulation is relevant to human beings, ZHOU warns of the large difference between yeast and mammalian cells and the complicated landscapes of research in the field of aging/longevity regulation.

"Most adult cells in mammalians do not have telomerase activity. Moreover, unlike the robust HR efficiency in yeast, HR frequency in mammalian cells is low, too. It remains elusive whether telomere HR activity is important in these cells. It will be extremely hard to detect HR activity in them because of the low frequency, plus other technical difficulties in mammal systems," he introduces.

Despite the above-mentioned facts, however, understanding longevity regulation in model organisms provides fundamental basis for further explorations on longevity in multicellular organisms and mammalians. On the other hand, the crucial importance of telomere and recombination goes beyond longevity, as they also play some roles in cancer as well as other serious diseases, ZHOU emphasizes.

In mammalians, stem cells, germ line cells and some of somatic cells have robust telomerase activity, and are able to keep telomeres at certain length, he explains. In contrast, most of the somatic cells do not have telomerase activity, and their telomeres gradually shorten with each cell division. Once the telomeres reach the critical short, these somatic cells will undergo senescence. In tumor cells, however, things are greatly different. Tumor cells cannot survive unless their telomeres are maintained at a long enough length. Therefore in the tumor, integrity of telomeres has to be maintained. About 85% of the tumor cells use telomerase to elongate their telomeres, and 15% of the tumor use recombination pathway to replicate telomeres.

"For example, approximately 25% of all *in vitro* simian virus 40 (SV40) large T-antigen immortalized human fibroblast cell lines use the recombination pathway," introduces ZHOU: "and it is of the same case in about 7% tumor-derived cell lines or human tumor samples."

"Indeed, about 15% of cancer cells do not have telomerase activity, but are still able to maintain certain telomere length," he continues: "This suggests that they employ the HR pathway to replicate telomere, and to maintain cell viability; therefore furthered understanding of telomere and HR might contribute to cancer-related research."

For more information please refer to:

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