Ribosomal DNA Replication Affects Aging in Yeast

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VA Morris

In the quest to both define and defy the aging process, researchers seek to understand the molecular mechanisms underlying the aging of cells. Aging, and in turn lifespan, are controlled by both genetics and environmental factors. By studying lifespan in model organisms, such as yeast and fruit flies, researchers have identified some of the molecular players and environmental factors that increase longevity. Calorie restriction has been shown to extend lifespan in multiple organisms, but the mechanisms have not been fully elucidated. Comparing the genetics of two yeast strains, a new study by the Bedalov lab in the Clinical Research Division uncovered how ribosomal DNA replication influences lifespan in yeast and how calorie restriction feeds into this pathway.

Graduate student Elizabeth Kwan and colleagues in Dr. Antonio Bedalov's laboratory mapped regions of the genome, or quantitative trait loci (QTL), that extend replicative lifespan in budding yeast. Replicative lifespan is the number of daughter cells a mother cell can produce before it eventually dies, typically 20 to 30. Aging of mitotic cells, such as stem cells and epithelial cells, is thought to be similar to aging in yeast and replicative lifespan, a measure of the latter, is a convenient and quantitative marker of this process. The authors crossed a vineyard strain and a laboratory strain of yeast, monitored the daughter cells for extended lifespan, and mapped genomic regions by linkage analysis for those that control lifespan. A QTL was identified in a region of DNA that encodes ribosomal RNA, which is required for protein synthesis and growth. The vineyard strain-specific rDNA region increased lifespan in the laboratory strain by 41%.

The involvement of genes that control recombination at the rDNA locus, such as *SIR2* and *FOB1*, have previously implicated the rDNA locus in longevity control. Sir2 binds to rDNA and influences the production of extrachromosomal rDNA circles (ERCs) that accumulate in mother cells and limit lifespan. Fob1 binds to the rDNA replication fork and prevents transcription machinery from colliding with DNA replication machinery. However, the identified rDNA locus extended lifespan in the absence of *SIR2*, *FOB1* or ERCs accumulation, suggesting the vineyard rDNA extended lifespan though a different mechanism.

To understand how the rDNA locus conferred increased longevity, the researchers determined the vineyard strain rDNA had 40% fewer rDNA repeats than the laboratory strain and found that this

reduced size is caused by a single-nucleotide polymorphism (SNP) that reduced activity of replication origins in rDNA (see figure for model).

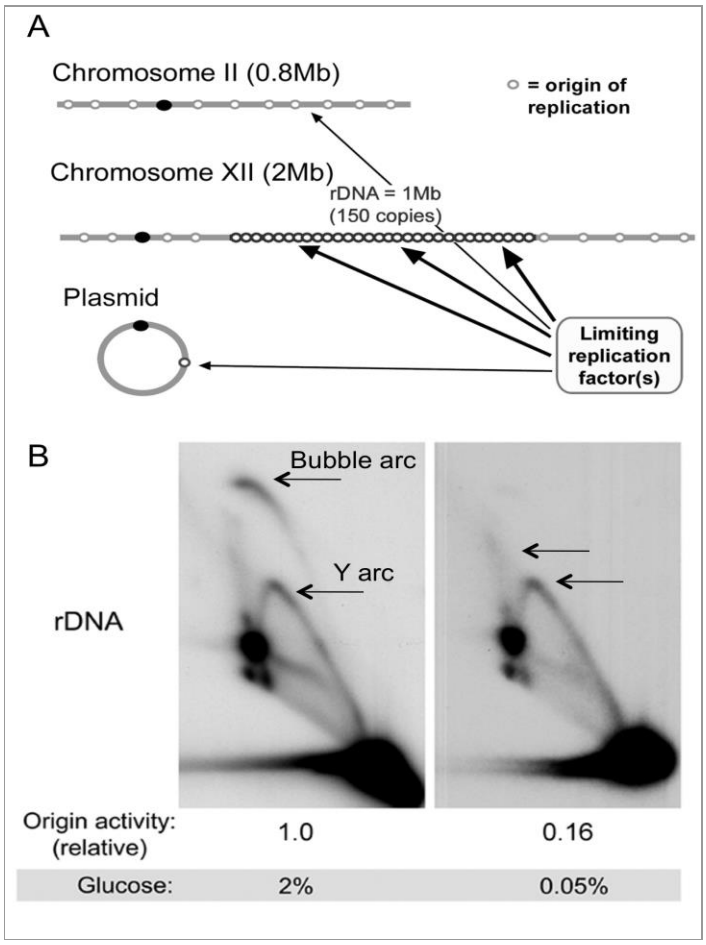
According to Dr. Bedalov, "Our study suggests that excessive firing of origins of DNA replication at the rDNA and the resulting increased rDNA array size, titrates DNA replication resources from the rest of the genome which compromises the ability of old cells to complete replication causing replicative senescence." In fact, the weaker rDNA locus increased viability in yeast strains with a temperature-sensitive mutation in *ORC2*, a subunit of the origin recognition complex that recruits DNA replication machinery, presumably by freeing up limited machinery for genome-wide replication.

Decreased calorie consumption increases lifespan in numerous organisms through conserved nutrient-sensing kinase pathways. Previous research determined that fewer calories reduce ribosome biogenesis and rRNA transcription in cells. Strikingly, the current study revealed a reduction in rDNA origin activity when the cells were grown on low glucose concentrations. In the laboratory strain, reduced glucose decreased rDNA origin activity by 60% using two-dimensional (2D) gel analysis of endogenous rDNA replication intermediates (see figure). The vineyard-specific rDNA SNP further reduced rDNA origin activity to 80% in low glucose conditions. Dr. Bedalov surmises "calorie restriction, which, we show, represses origin firing at the rDNA seems to restore the balance between the replication of rDNA and replication of the rest of the genome which could be the core mechanism by which calorie restriction extends replicative lifespan in organisms from yeast to mammals." The authors propose that reduced calorie consumption signals the cell to decrease rRNA transcription, allowing the rDNA to be populated with nucleosomes, which in turn control replication origin selection and firing.

Combined with previous QTL and genetic screens (Kwan *et al.,* 2011), the current study demonstrates the value of using model organisms such as yeast to define the molecular players that control longevity. Aging is a complex process, and this study uncovers a crucial mechanism of how both genetics and an environmental factor can converge on a single mechanism to control lifespan. The relevance of this finding in higher organisms will be determined in future studies.

Kwan EX, Foss EJ, Tsuchiyama S, Alvino GM, Kruglyak L, Kaeberlein M, Raghuraman MK, Brewer BJ, Kennedy BK, Bedalov A. 2013. A Natural Polymorphism in rDNA Replication Origins Links Origin Activation with Calorie Restriction and Lifespan. *PLoS Genetics* 9:e1003329.

Also see: Kwan EX, Foss E, Kruglyak L, Bedalov A. 2011 Natural polymorphism in BUL2 links cellular amino acid availability with chronological aging and telomere maintenance in yeast. *PLoS Genetics* 7:e1002250



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Calorie restriction decreases rDNA origin activity in yeast. (A) A proposed model on the effect of the rDNA locus on chromosomal origin firing. The rDNA locus competes for limiting replication factors with weaker plasmid and genomic origins of replication (for example, origins on chromosome II). (B) Two-dimensional (2D) gel electrophoresis resolves replication intermediates and determines relative origin activities by comparing the abundance of rDNA intermediates in the bubble arc to those in the Y arc. Origin activity at the rDNA locus is decreased in yeast grown on high-glucose (2%) media compared to calorie-restricted (0.05%) media.