A gradual path to mortality

Many of the features associated with senescence appear steadily over time before cells stop dividing.

NA YANG AND PAYEL SEN

In 1961, Hayflick and Moorhead discovered that human fibroblast cells cultured in the laboratory could only divide a limited number of times, after which they stopped multiplying but remained metabolically active (Hayflick and Moorhead, 1961). It did not matter how the cells were cultured – whether they were repeatedly transferred, or ‘passaged’, into a fresh environment, or if their growth was interrupted by episodes of freezing – the number of divisions they could make was always finite. This state was termed replicative senescence and was found to occur in a range of cell types.

Further research revealed that senescence is caused by the shortening of caps, or ‘telomeres’, on the end of chromosomes (Bodnar et al., 1998). Every time a cell divides, its telomeres shrink until they reach a critical length that stops the cell from multiplying. Once senescent, cells display unique features such as expressing certain proteins, releasing inflammatory molecules, and loosening tightly packed regions of DNA known as heterochromatin (Hernandez-Segura et al., 2018). New evidence showed that senescence is induced by cell stress as well as successive divisions, and that the number of senescent cells increases as tissues age (van Deursen, 2014).

Despite almost 60 years of research, many questions still remain about senescence; for instance, what happens to cells as they transition into the senescent state? How does their metabolism change during this shift, and do they take on a new cell identity? Now, in eLife, David Botstein, David Hendrickson and colleagues from Calico Life Sciences – including Michelle Chan as first author – report the results of experiments that exquisitely profile the roadmap cells take on their path to senescence (Chan et al., 2022).

The team used a new experimental design to survey the entire genome and repertoire of RNAs, proteins, and metabolites present in fibroblasts cultured in the laboratory. These patterns were traced over time as the cells grew until they stopped dividing. Chan et al. then used a range of control conditions to pinpoint which changes were specific to replicative senescence. This included repeating the experiment on cells growing at a similar density to senescent cultures, cells that had only been passaged a few times (and therefore unlikely to be senescent), cells that never become senescent, and cells made senescent by radiation-induced stress.

To begin with, Chan et al. measured the level of unique RNAs in single cells to investigate how the genes that fibroblasts expressed changed over time. The data revealed that RNAs known to be expressed in fully senescent cells progressively accumulate throughout the cell cycle. This suggests that senescent cells in vivo may be slowly amassing these features, but not yet expressing the classic biomarkers associated with the end-point of senescence, such as the beta-galactosidase enzyme. This may explain why previous studies found less than 20% of cells in...
The findings of Chan et al. suggest that cells gradually acquire a number of changes on the path to replicative senescence: they express different genes, rewire their metabolic reactions and take on a new identity similar to mesenchymal cells (Figure 1). Previous studies have shown that removing senescent cells can increase the health- and life-span of mice (Di Micco et al., 2021). Therefore, interventions that target these early changes could help improve the wellbeing of individuals by stopping the cascade of events that lead to replicative senescence.

**Na Yang** is in the Laboratory of Genetics and Genomics, National Institute on Aging, National Institutes of Health, Baltimore, United States

**Payel Sen** is in the Laboratory of Genetics and Genomics, National Institute on Aging, National Institutes of Health, Baltimore, United States

[Payel Sen](mailto:payel.sen@nih.gov)

[http://orcid.org/0000-0003-2809-0901](http://orcid.org/0000-0003-2809-0901)

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**References**

Biran A, Zada L, Abou Karam P, Vadai E, Roitman L, Ovadya Y, Porat Z, Krizhanovsky V. 2017. Quantitative...
identification of senescent cells in aging and disease. *Aging Cell* **16**:661–671. DOI: https://doi.org/10.1111/acel.12592, PMID: 28455874

Bodnar AG, Ouellette M, Frolikis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE. 1998. Extension of life-span by introduction of telomerase into normal human cells. *Science* **279**:349–352. DOI: https://doi.org/10.1126/science.279.5349.349, PMID: 9454332

Chan M, Yuan H, Soifer I, Maile TM, Wang RY, Ireland A, O’Brien JJ, Goudeau J, Chan LJG, Vijay T, Freund A, Kenyon C, Bennett BD, McAllister FE, Kelley DR, Roy M, Cohen RL, Levinson AD, Botstein D, Hendrickson DG. 2022. Novel insights from a multiomics dissection of the Hayflick limit. *eLife* **11**:e70283. DOI: https://doi.org/10.7554/eLife.70283, PMID: 35119359

Di Micco R, Krizhanovsky V, Baker D, d’Adda di Fagagna F. 2021. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. *Nature Reviews Molecular Cell Biology* **22**:75–95. DOI: https://doi.org/10.1038/s41580-020-00314-w, PMID: 33328614

Hayflick L, Moorhead PS. 1961. The serial cultivation of human diploid cell strains. *Experimental Cell Research* **25**:585–621. DOI: https://doi.org/10.1016/0014-4827(61)90192-6, PMID: 13905658

Hernandez-Segura A, Nehme J, Demaria M. 2018. Hallmarks of cellular senescence. *Trends in Cell Biology* **28**:436–453. DOI: https://doi.org/10.1016/j.tcb.2018.02.001, PMID: 29477613

Idda ML, McClusky WG, Lodde V, Munk R, Abdelmohsen K, Rossi M, Gorospe M. 2020. Survey of senescent cell markers with age in human tissues. *Aging* **12**:4052–4066. DOI: https://doi.org/10.18632/aging.102903, PMID: 32160592

van Deursen JM. 2014. The role of senescent cells in ageing. *Nature* **509**:439–446. DOI: https://doi.org/10.1038/nature13193, PMID: 24848057

Wang C, Zhu X, Feng W, Yu Y, Jeong K, Guo W, Lu Y, Mills GB. 2016. Verteporfin inhibits YAP function through up-regulating 14-3-3sigma sequestering YAP in the cytoplasm. *American Journal of Cancer Research* **6**:27–37 PMID: 27073720.

Yang N, Sen P. 2018. The senescent cell epigenome. *Aging* **10**:3590–3609. DOI: https://doi.org/10.18632/aging.101617, PMID: 30391936