Finding models for the process of human aging

Cycles of Life and Death

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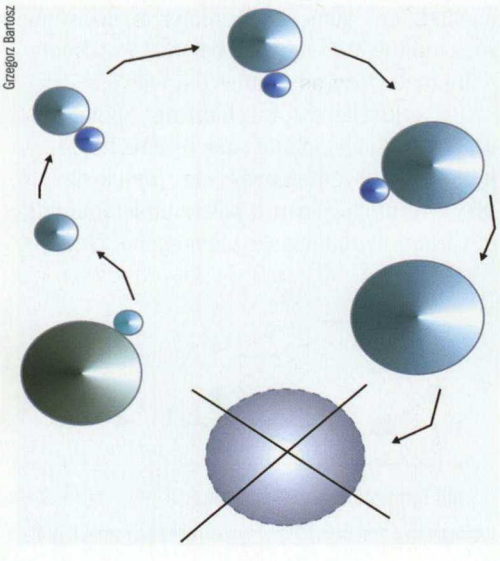
Identifying the genes governing longevity in yeast could lay the groundwork for studying analogous genes in humans. Will yeast prove to be an excellent model organism for gerontology research?

Why do our bodies age? Is the process inevitable, or can it be halted or even reversed? Such questions have intrigued mankind for millennia, but it was not until the late 19th century that aging became a field of scientific research. These basic questions proved difficult to answer. One of the obstacles encountered by the study of aging - the field known as gerontology, focused mainly on aging in humans - is the long lifespan of the object of observation, meaning we humans ourselves. The aging process can be studied by comparing people of different ages. Some studies look at individuals who reach a ripe old age, hoping to identify what factors contribute to their longevity. Yet the limits of that methodological approach are obvious: it compares individuals who lived under different conditions, with different dietary customs and food availability. Significantly more valuable and clear-cut findings could be derived from studying a whole group of individuals from birth to death. However, such research would require cooperation among several generations of researchers, and moreover even planning such a study would be unrealistic. Within the timeframe of 50 or 100 years, not only research methodology but also our level of understanding of the processes occurring within cells and organisms will change to such an extent that the assumptions on which the project is based would soon prove archaic and it would chiefly serve as a museum piece for future generations.

Model organisms

Gerontology can find a way around those difficulties by changing its object of research and studying aging in model organisms. Such an approach assumes that the mechanisms of aging are similar in different organisms, and that the findings will also be applicable to humans.

The most obvious "substitute" organisms might seem to be laboratory rodents, whose lives are significantly shorter than those of humans (around 3 years). Unfortunately, that is not short enough: several years is in fact quite a long time given the pace of advancement in contemporary biology. That problem is exacerbated by the issue of funding: gerontological research with a large number of rodents conducted for their entire life span (which we moreover hope to be as long as possible) is quite simply very costly. For these reasons, studies use organisms that are more distant from humans, yet offer more convenient models - danio fish (*Danio rerio*), fruit flies (*Drosophila melanogaster*), or roundworms (*Caenorhabditis elegans*).



Grzegorz Bartosz

The life cycle of *Saccharomyces cerevisiae* yeast cells begins from the stage of a newly created bud. Each new cell then produces cells of the next generation, constantly increasing its own volume until it ultimately becomes incapable of further budding and dies

Gert-Jan Kappert, www.sxc.hu

Some studies focus on those individuals who reach a ripe old age, with the aim of identifying what factors may contribute to their longevity. Unfortunately, this research methodology is not perfect

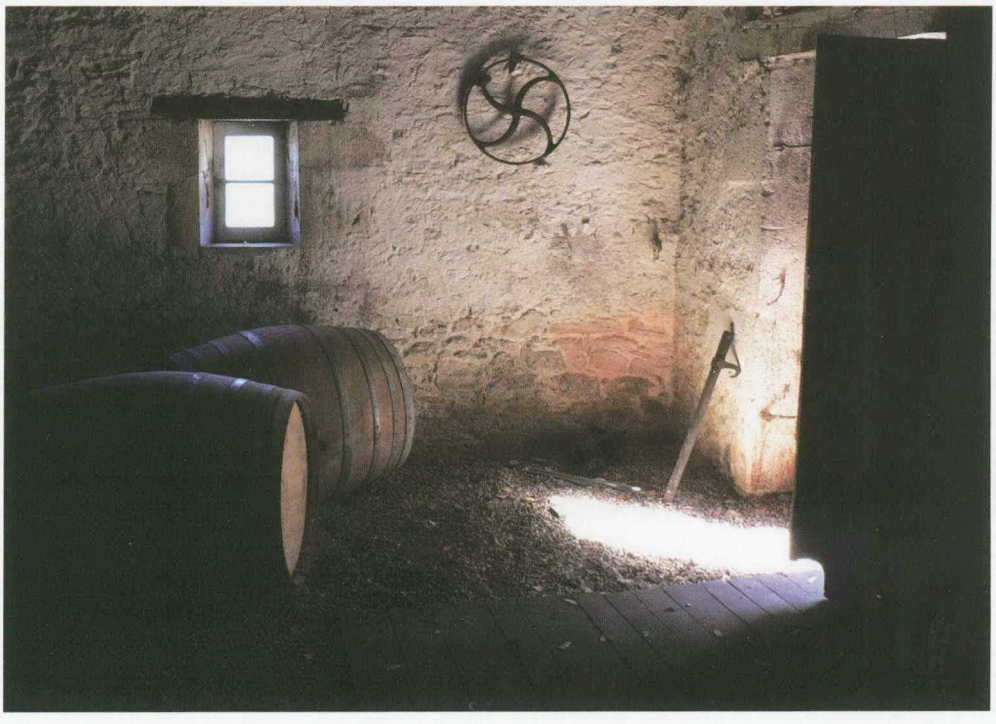
Recently, another very simple model organism has gained popularity: baker's yeast (*Saccharomyces cerevisiae*). At first glance, the notion of using yeast as an object of study to help unlock the mechanisms of aging in humans may seem completely incomprehensible. Yeast is a single-cell organism that gives the impression of being immortal. Anyone who has encountered the practical uses of yeast - for baking cakes or making homemade wine - knows that yeast cells are easy to obtain and when the right food is present they begin to reproduce quickly. That opinion was until recently shared by microbiologists, who breed yeast cells taken from frozen cultures, in a procedure that can be repeated indefinitely. How can any sort of counterpart to human aging be found here?

Yeast instead of mice?

Baker's yeast reproduces through a process known as budding: a daughter cell grows out of a mother cell, then separates and begins its independent life. Budding involves an even division of the genome, but an uneven division of cytoplasm between mother and daughter cells. Half a century ago, painstaking microscope observations of individual budding yeast cells discovered that the capacity of *S. cerevisiae* cells to form new buds is in fact limited. A given cell may produce several dozen buds, but it then becomes incapable of budding further and dies after a certain period of time. Every cell in a yeast population begins its life cycle as a newly formed bud and starts to bud itself after reaching a certain minimal size, to ultimately reach the limit of its budding capacity following a certain number of divisions. The curve followed by the fraction of cells within a population capable of further budding is very similar to the survival rate of human populations. The functional abilities of yeast cells therefore change over time, and this meets the definition of aging. It is known as replicative aging, where by the parameter determining the "biological age" of a cell is the number of divisions it has performed. At the same time, a *Sacharomyces* population as a whole can still be considered immortal in a certain sense. This trait limiting a given cell's ability to continue to divide it is not shared with its new offspring cells. Under optimal laboratory conditions yeast cell populations will continue to divide continually (as long as the right conditions are present). As a result, a given population will consist mainly of young buds and cells which have so far performed one or two divisions. The fraction of cells reaching the limit of their division ability is slim and does not affect the fate of the population as a whole.

Mothers and daughters

Why is it that each budding cycle reduces the mother cell's capacity for further division? Yeast cells are surrounded by a cell wall, which has to be broken down in the spot where a new bud forms. After the daughter cell breaks off, the resulting hole in the wall is filled in with a material containing chitin, leaving behind a division



John Evans, www.sxc.hu

Anyone who has encountered the practical uses of yeast – for baking cakes or making homemade wine – knows that yeast cells are easy to obtain and when the right food is present they begin to reproduce quickly. Such replication can be continued indefinitely

scar. Another bud cannot form in the same location, so the cell wall becoming covered with such scars was thought to cause the cap in division capability. Yet that hypothesis proved false. Researchers then postulated an uneven division of a certain "aging factor" between mother and daughter. Some researchers are inclined to identify it with circular extra-chromosomal fragments of rDNA, the number of which increases with each successive cell division, and which – by binding transcription factors – can presumably hamper and ultimately prevent the further functioning of the cell. However, situations have been observed in which the accumulation of extra-chromosomal rDNA fragments is not significant, yet yeast cells still exhibit replicative aging. Another intriguing observation has involved the uneven division of oxidatively damaged proteins between mother and daughter cells. Reactive forms of oxygen arise in every cell, causing damage to proteins. During budding a substantial majority of oxidatively damaged proteins remains within the mother cell, in a poorly-understood mechanism involving the cytoskeleton and chaperone proteins.

Aside from replicative aging, yeasts may also age chronologically. When a population exhausts the available nutritional ingredients, cells cease to divide and gradually die. The percentage of living cells then drops over time. Many researchers feel that while replicative aging corresponds to the aging of our body's cells that are capable of division, chronological aging may be a model for the aging of non-dividing cells (such as muscle cells or neurons).

Ideal for research?

If we assume that the main mechanisms of aging – like the genetic code and metabolic pathways – are underlyingly the same in all organisms, in yeast we have an organism in which this basic life cycle runs full circle within the course of only a day or two. Moreover, it has a simple genome (some 6,000 genes) of a well-known structure, it is easy and cheap to breed, genetic manipulations are easy to perform on it, and experiments do not raise objections of an ethical nature. The most interesting aspect of gerontological research seems to be identifying the genes which regulate lifespan and the retention of functional fitness. Yeast may be an ideal object for such screening studies, and then for more detailed studies encompassing the entire genome. Identifying the genes responsible for longevity in yeast could lay the groundwork for studying the impact of orthologous genes in higher organisms, including humans. It is of course

likely that genes not found in yeast are significant lifespan determiners in humans, but research with yeast should enable us to evaluate the role played by a significant portion of the genome. Notably, yeast studies have drawn attention to the possible impact of sirtuins (histone deacetylases) on organism lifespans. Resveratrol, a compound thought to be responsible for the favorable health impact of red wine, extends the replicative lifespan of yeast cells by simulating sirtuin activity.

Pinch of salt

But perhaps the study of yeast as a model organism in gerontological research will yield information solely about the aging... of yeast itself. A research team led by Prof. Tomasz Biliński, with whom the present author has the pleasure of working, has shown that related strains of yeast differing in terms of the presence or absence of certain significant enzymes providing antioxidative protection, and therefore in terms of the number of cell divisions they can perform, cease to divide after reaching the same final cell volume. Perhaps, therefore, no "aging factor" actually exists, and the mechanism which limits cell division may simply be that the mother cell achieves its maximum size. If there is no "aging factor" in yeast other than growth in cell volume, screening studies looking for genes which influence the "replicative longevity" will mainly end up identifying size-regulating genes that limit cells of excessive dimensions. Analogous genes could also have an impact on lifespan in mammals, but that impact should be expected to be only indirect.

Mortal after all

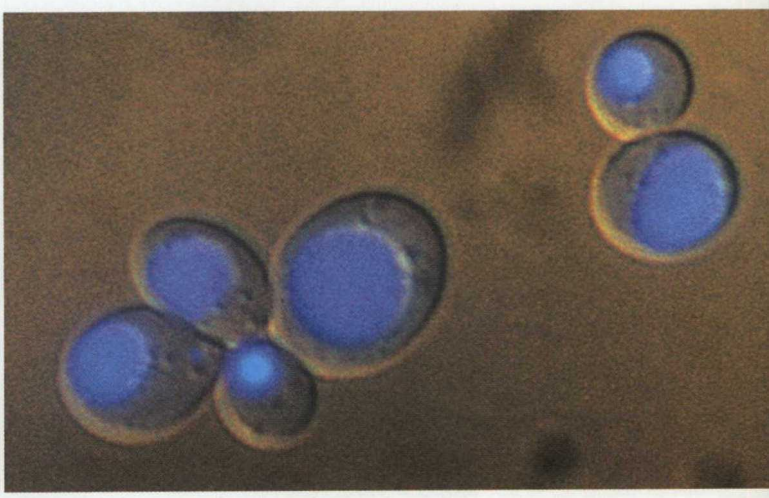
Until recently, symmetrically dividing single cell organisms were considered immortal. The cells of *S. cerevisiae* were, alongside the paramecium (if it reproduces vegetatively) and the asymetrically dividing bacteria *Caulobacter crescentus*, thought to be the only mortal exceptions. Microbiologists used to joke that the dream of every bacteria cell... is to become two bacteria cells. If there is no dead body, there has been no death. The development of bacterial populations could therefore be graphed as a tree of dichotomously branching segments. This seemingly obvious picture was challenged three years ago, when automatic microscope registration of divisions occurring in more than 30,000 *Escherichia coli* bacteria cells showed that the offspring of a single cell do not in fact show identical paces of cell division. If we mark one pole of the cell, it can be seen to be inherited by only one of the two offspring cells. The cell wall of the other is created entirely via *de novo* synthesis, as are other elements, including the DNA. Microscope observations of a huge number of cells derived from a single bacteria cell indicated that those which inherited the "old pole" divide more slowly than those which do not contain any "old" component parts. So do single-cell organisms really divide fully symmetrically? If not, the development of bacteria populations should be described as a set of life cycles each of finite length, just like in the case of yeast.

If single-cell microorganisms are indeed also fated to grow old, our understanding of the living world will have to be revised yet again. It is hard to say whether this modification will bring us closer to understanding the mechanisms involved in human aging or whether it will have any practical significance – but the same could be said for many important scientific discoveries in the past. ■

Further reading:

Zadrag R., Bartosz G., Bilinski T. (2005). Replicative aging of the yeast does not require DNA replication. *Biochem Biophys Res Commun, 333 (1)*, 138-41.

Steinkraus K.A., Kaeberlein M., Kennedy B.K. (2008). Replicative aging in yeast: the means to the end. *Annu Rev Cell Dev Biol., 24*, 29-54.



Invitrogen/Molecular Probes, www.probes.invitrogen.com

Images of *Saccharomyces cerevisiae* yeast cells. The curves mapping the retention of budding ability within a yeast cell population are similar to curves mapping the survival rate in human populations, and the two phenomena can be described by the same equation