Decelerating ageing and biological clocks by autophagy

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Ageing is the most important risk factor for the majority of human pathologies. We propose the concept that the advancement of the hallmarks of ageing is dictated by several distinct biological clocks that can be decelerated by the induction of autophagy. This 'time dilation' delays the time-dependent manifestation of multiple diseases.

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Chronological time is measured as a physical constant, whereas biological time is a less rigid concept that can be temporarily halted, for instance, by hibernation, cryo-preservation, formation of spores or the dauer diapause. Thus, biological model systems can age at a variable pace, as a result of the desynchronization of chronological and biological clocks. Distinct intrinsic (for example, genetic) or environmental (for example, nutritional) fac- tors may accelerate or decelerate the biological processes that cause the progressive decadence and inexorable col- lapse of any living system[1]. Autophagy is one of the most important cytoplasmic recycling mechanisms, which delays ageing by acting directly on biological clocks.

Undoubtedly, time is the most important risk factor for the manifestation of all major human pathologies in several major epidemiological categories, including neoplastic, cardiovascular and neurodegenerative dis- eases. It is a close-to-trivial reality that all these maladies tend to manifest themselves as we age. Even genetically determined ailments such as cystic fibrosis and Wilson disease, the two most frequent monogenetic diseases in humans, develop over time, and hereditary predispo- sitions for cancer, arteriosclerosis, cardiac arrhythmia and neurological diseases materialize with age, although precociously with respect to the general population. Nonetheless, in biomedical research, time is usually per- ceived — and *in fine* neglected — as a 'non-modifiable' risk factor, a vision that must change if 'dilation' of chronological ageing by deceleration of biological clocks is to be considered as a valid therapeutic strategy.

At the cellular level, the hallmarks of ageing comprise genomic instability, telomere attrition, epigenetic altera- tions, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication[1]. All these hallmarks represent biological clocks, although with rather distinct characteristics. Genomic instabi- lity becomes irreversible and transmissible as altered nuclear and mitochondrial genomes divide; it ticks like a mechanical clock in which each genomic replication constitutes a unit of measurement, the amplitude of which can be influenced by the level of external damage and internal

repair. Telomere attrition is a count-down mechanism in which somatic cells lose telomere repeats each time they divide until a critical threshold is reached, unless telomerase maintenance mechanisms are acti- vated. Epigenetic alterations more accurately reflect bio- logical ageing than chronological ageing, which has led to the notion of an 'epigenetic clock' that can be decoded by measuring chemical modification of DNA and his- tones using 'omics' approaches. Loss of proteostasis, in particular the ageing-related insufficiency in autophagy (see below), and deregulated nutrient sensing, may be profoundly influenced by circadian clocks, given that the metabolic responses to both caloric intake and auto- phagy undergo profound daily oscillations. Progressive mitochondrial dysfunction, accumulation of senescent cells and ever-increasing stem cell exhaustion may be considered as hourglasses that do not follow simple internal cues (such as duplications of the genome), or external cues (such as circadian oscillations), but instead reflect an irreversible accumulation of dysfunctional units (for example, mitochondria or cells), the build- up of which may be counteracted by their elimination or replacement[1]. One of the multiple manifestations of altered intercellular communication is the deteriora- tion of circadian rhythms, which tend to lose amplitude and precision; this observation provides yet another important link between ageing and biological clocks[2].

Reducing temperature in the environment can pro- long the chronological lifespan of poikilothermic model organisms (animals whose body temperature varies con- siderably), and so does caloric restriction (CR; defined as reduced caloric intake without malnutrition). CR decreases energy expenditure and causes a sizeable decrease in body temperature, even in warm-blooded animals. This observation has been interpreted as ageing being the result of an increase in entropy that intrin- sically results from the concatenation of biochemical reactions. However, conflicting with this hypothesis, it has been found that intermediate fasting (that is, regu- latory periods of fasting followed by ad libitum feeding) and administration of CR mimetics (CRMs, which are nutritional or pharmacological agents that mimic the age-delaying effects of CR[3]) increase healthspan and lifespan without reducing total energy expenditure.

One of the common consequences of CR and admin- istration of CRMs is the induction of macroautophagy (herein referred to as autophagy)[1,3–5]. This lysosomal degradation pathway, which is one of the most impor- tant effectors of proteostasis[1], plays a crucial role in cellu- lar physiology, including adaptation to metabolic stress, removal of deleterious cargo (such as protein aggregates, damaged organelles, including dysfunctional mitochon- dria, and intracellular pathogens), cellular recycling during differentiation and development, and preven- tion of genomic damage[5]. Generally, these and other functions protect against ageing and against numer- ous diseases, including cancer, cardiovascular disorder and neurodegeneration, as well as infections[4,5], Genetic inhibition of autophagy (by ablation or inactivation of essential autophagy-related (ATG) genes) abolishes the lifespan-extending effects of CR and CRMs[3]. Old age, obesity (one of the most important ageing accel- erators) and a series of genetically determined diseases, are coupled to inefficient autophagy, locking the organ- ism into an inevitable accelerating spiral towards deca- dence[1]. Conversely, genetic manipulations designed to increase autophagy, such as overexpression of *Atg5*

orgain-of-function mutation in *Bcln1* (the mammalian orthologue of yeast *ATG6*), are sufficient to extend the healthspan and longevity of mice[4,5].

What, then, are the effects of CR or CRM-induced autophagy on the biological clocks that dictate the advancement of ageing? In our opinion, there are four major effects of autophagy on age-related processes. First, the autophagy-mediated increase in cytoplasmic turnover antagonizes the degeneration of organelles and macromolecular complexes. Second, autophagy may improve the mechanisms of energy homeostasis, thus facilitating the maintenance of genomic and epigenomic stability and improving the acuity of nutrient sensing. Third, autophagy has marked anti-inflammatory and immune response-improving effects, thus ameliorating system homeostasis. And fourth, thus far neglected, its effects might be related to circadian clocks. There is increasing evidence in favour of a bidirectional relation- ship between circadian clocks and autophagy[2]. While circadian rhythms modulate autophagic flux, they are themselves influenced by autophagy. The molecular sensors that trigger autophagy in response to CR, which include AMPK (responsive to a reduction in energy charge reflected by increased AMP and decreased ATP levels), mTOR complex 1 (mTORC1) (responsive to depletion of free amino acids), SIRT1 (responsive to enhanced NAD levels) and EP300 (responsive to dimin- ishing acetyl-CoA), also regulate circadian clocks[2]. As a consequence, autophagy is rhythmically induced in a circadian clock-dependent manner, while autophagy induction influences the circadian clock. For example, inhibition of mTORC1, which activates autophagy, slows down the circadian clock, whereas activation of mTORC1 accelerates it, accentuating the period and amplitude of clock oscillations both in vitro and in vivo[2]. Admittedly, the multiple pathways through which autophagy can reduce ageing require further in-depth exploration. However, irrespective of the mechanistic uncertainties, the concept that even for humans age- ing is not an unalterable process is clearly emerging. Although we remain sceptical with respect to the pos- sibility of reversing the arrow of time (and hence reju- venating old organisms), it appears increasingly clear that biological clocks can be decelerated to dilate time and delay the ageing process. Lifestyle factors (such as CR, nutritional uptake of CRMs and exercise), as well as autophagy-stimulating drugs (including pharmaco- logical CRMs) might be taken advantage of to combat ageing and any type of age-related, time-dependent dis- ease. Thus, the goal of time-dilating interventions should be to break the link between chronological and biologi- cal time, to delay (and possibly suppress) the manifes- tation of ageing-related diseases to extend healthspan. It remains to be determined whether distinct autophagy- inducing strategies might be advantageously combined and whether autophagy stimulation is the sole strategy for obtaining such a broad effect.

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Competing interests
G.K. is a scientific cofounder for Samsara Therapeutics. The other author declares no conflict of interest.