dynamics of actin — a structural protein of the cell skeleton. Knockdown of *eps-8* in adult worms prevented the destabilization of actin networks in muscles of the body wall and the concomitant decrease in motility in old worms (Fig. 1b). When *eps-8* expression was reduced specifically in the muscles or neuronal cells of adult worms, the worms lived longer than normal. Concordantly, knockdown in adulthood of *mig-2*, one of the three *C. elegans* genes that encode RAC-like proteins, also extended lifespan.

In summary, Koyuncu *et al.* successfully link the age-related decrease in ubiquitination and subsequent increase in the abundance of two proteins, IFB-2 and EPS-8, to the loss of intestinal and muscular integrity — both problems well known to be associated with age in *C. elegans*[5,6]. A key feature of this study is that it bridges the gap between molecular changes that occur with ageing and their effects on tissue function and lifespan in the worm.

This work opens up several new research opportunities, yet also leaves open intriguing questions. One such question concerns the potential for leveraging these findings to explore ways of extending lifespan. Koyuncu *et al.* showed that knocking down individual genes that express proteins that tend to accumulate and aggregate with age can extend lifespan by a maximum of 21%. Given that these genes probably act independently in different tissues, reducing the expression of a combination of different genes in different tissues might result in a much stronger effect on longevity. This strategy should not necessarily be limited to the targets identified in this study.

Treatment of old worms with the DUB inhibitor PR-619 generally returned ubiquitination in old worms to levels seen in younger worms, possibly by preventing or delaying age-related accumulation of protein aggregates in multiple tissues simultaneously. However, given its broad activity, PR-619 might also interfere with crucial deubiquitination and therefore disturb cellular homeostasis in a way that is not compatible with longevity.

Another remaining pertinent question is, what causes the increase in DUB activity in old animals? The culprit could be sustained insulin/IGF-1 activity in the worms, because the authors found that its reduction was sufficient to prevent upregulation of all age-dysregulated DUBs. Moreover, insulin/IGF-1 activity has been proposed to promote senescence and age-related tissue deterioration in *C. elegans*, thus causing older individuals to die, while increasing fitness of the species at the colony level[7]. Hence, the increase in DUB activity with age might be one of the mechanisms by which the insulin/IGF-1 signalling pathway controls lifespan.

The increased ubiquitination of proteins in aged worms with reduced insulin/IGF-1 signalling compared with wild-type aged worms is consistent with previous work showing that, in the mutant worms, deubiquitination is actively repressed and proteasome activity is increased[8]. Previous work also showed that the longevity of these animals is, in part, supported by an increase in ubiquitination[9].

From a broader perspective, Koyuncu and co-workers' findings elegantly support the antagonistic-pleiotropy hypothesis[10] — an evolutionary explanation for why ageing is adaptive and therefore occurs. This hypothesis posits that genes that are beneficial for development or reproduction, but are detrimental later in life, will be selected for during evolution. The study identifies two excellent examples of such genes — *ifb-2* and *eps-8*.

A final and major question is whether or not the age-associated deubiquitination that triggers late-life protein accumulation and aggregation in worms is also seen in more-complex animals. In rats, protein ubiquitination seems to increase, rather than decrease, with age[11–13], suggesting that the dynamics of ubiquitination in worms are different from those in mammals. The distinctive ubiquitination pattern observed in *C. elegans* might relate to its peculiar 'boom-and-bust' life history that favours both a programmed early collapse of the regulation of protein levels[14] and ageing[7]. However, it is still possible that proteins for which proteasomal clearance is dysregulated with ageing have an important role in the development of age-related mammalian disorders that involve protein aggregation, such as Parkinson's disease and Alzheimer's disease[15]. Therefore, understanding how the ubiquitination dynamics for different proteins change in different species with ageing could provide insight into potential strategies for treating such disorders.

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Metabolism

How an amino acid affects a key cell-growth regulator

Tibor Vellai

Cells can tailor their growth to current conditions by sensing nutrients. The protein complex mTORC1 enables cell growth to be coordinated with the level of certain amino acids, and how it senses the amino acid leucine has now become clearer. **See p.281**

The protein mTOR is a type of enzyme called a kinase, and it functions in two distinct complexes, termed mTORC1 and mTORC2. mTORC1 boosts cell growth and proliferation in response to nutrient availability and hormone-dependent signals that promote cell division. On page 281, Chen *et al.*[1] provide insights into how mTORC1 is regulated by a specific amino acid — leucine.

mTORC1 carries out its role by regulating certain anabolic (synthetic) and catabolic (degradative) processes. These involve protein synthesis; production of the protein-synthesis machinery in the form of ribosomes; lipid synthesis; and autophagy, a degradation process mediated by organelles called lysosomes[2]. mTORC1 deficiency has been implicated in various human disorders, including cancer, neurodegeneration, metabolic diseases and muscle atrophy, as well as being linked to changes involved in the ageing process[3]. Yet despite its major physiological and clinical significance, how mTORC1 signalling is influenced by upstream regulatory factors is not fully understood.

The amino acid leucine is a potent

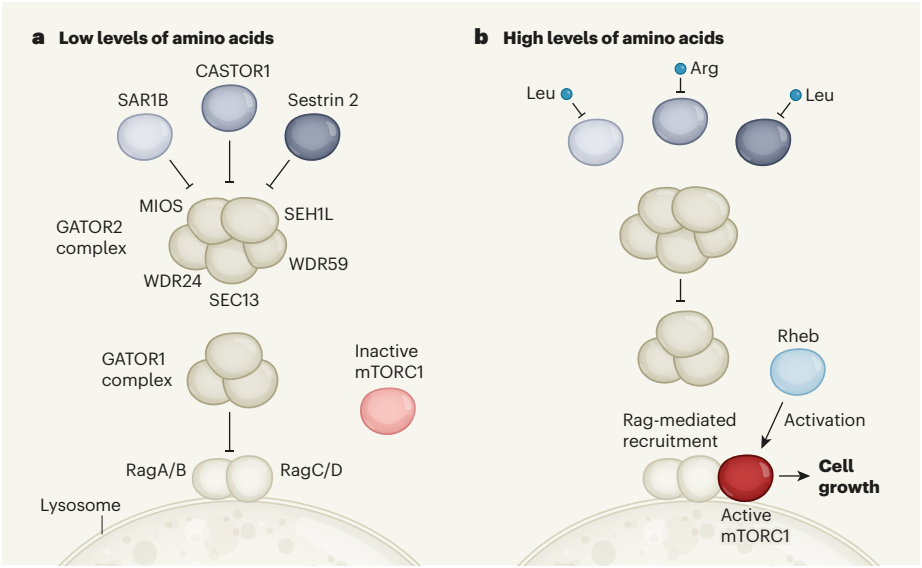

Figure 1 | A mechanism to activate the mTORC1 complex that depends on the level of specific amino acids. The complex mTORC1 regulates cell growth, and its activity is modulated by the intracellular availability of amino acids. Chen *et al.*[1] report their discovery that the protein SAR1B functions in this pathway. **a**, If amino-acid levels are low, SAR1B and two other proteins, CASTOR1 and sestrin 2, inhibit the protein complex GATOR2 (which consists of the proteins MIOS, SEH1L, WDR24, SEC13 and WDR59). SAR1B inhibits MIOS and sestrin 2 inhibits SEH1L. The complex GATOR1 (subunits not named here) inhibits the enzymes RagA, RagB, RagC and RagD, and the inactive mTORC1 complex fails to be recruited to an organelle called the lysosome. **b**, High levels of the amino acid leucine (Leu) inhibit SAR1B and sestrin 2, and high levels of arginine (Arg) inhibit CASTOR1. As a result, GATOR2 is able to inhibit GATOR1. The Rag enzymes, which are no longer inhibited, then recruit mTORC1 to the lysosome, and mTORC1 is activated by the protein Rheb (which can sense aspects of cellular function such as energy levels). This pathway can thereby integrate information about the cell to initiate growth when conditions are suitable.

stimulator of mTORC1: it blocks the inhibitory effect of the protein sestrin 2 on the GATOR2 complex that activates mTORC1 (ref. 4). Such regulation links the cellular nutrient status — the level of intracellular amino acids — to the control of cell growth (Fig. 1).

Chen and colleagues show that leucine is also sensed by another regulatory factor in mammalian cells, the protein SAR1B, which is a type of enzyme called a small GTPase. The authors report that reduced expression of the *SAR1B* gene makes cells insensitive to leucine scarcity and activates the mTORC1 pathway. Similarly to the way in which sestrin 2 functions, SAR1B interacts physically with GATOR2 when leucine is removed, affecting GATOR2's function. However, the authors show that SAR1B binds to a different GATOR2 subunit (the protein MIOS) from sestrin 2 (which binds to the SEH1L protein). When leucine levels in the cell are sufficiently high, the amino acid binds to SAR1B, causing a conformational change in the protein, which then dissociates from GATOR2. The liberated GATOR2 inhibits another protein complex, GATOR1, which leads four Rag GTPase enzymes[5] (RagA, RagB, RagC and RagD) to recruit mTORC1 to the surface of the lysosome, where the enzyme Rheb GTPase activates mTOR (Fig. 1). Thus, the inhibitory function of GATOR1 on Rag GTPases is SAR1B-dependent. Loss of SAR1B function enables mTORC1 to localize to lysosomes, even under conditions of leucine starvation.

The authors found that SAR1B and sestrin 2 detect leucine by recognizing different parts of the amino acid's structure. They report that SAR1B recognizes the amino group and side chain of leucine, whereas it was known[6] that sestrin 2 identifies leucine's amino and carboxyl groups. These two leucine sensors bind to the amino acid with different affinities: SAR1B has a higher binding affinity than does sestrin 2.

"These findings suggest that SAR1B might serve as a potent target for antitumour therapy."

These features permit mTORC1 activation to occur over two steps by sequentially prompting the dissociation of SAR1B and sestrin 2 from GATOR2 at different intracellular leucine concentrations. Furthermore, the relative levels of SAR1B and sestrin 2 in a cell can vary in different tissues. At the subcellular level, some SAR1B localizes to lysosomes, and leucine stimulates its dissociation from both GATOR2 and lysosomes. Together, the authors' evidence indicates that SAR1B is a previously unknown leucine sensor that regulates mTORC1 activity through modulation of GATOR2.

The authors report that inhibition of SAR-1 protein, the counterpart of human SAR1B in the nematode worm *Caenorhabditis elegans*, also renders the worm version of mTORC1, called *Ce*TORC1, insensitive to nutrient starvation in a GATOR2-dependent manner. When a human *SAR1B* gene is introduced into nematodes defective in SAR-1 production, *Ce*TORC1 sensitivity to nutrient restriction is restored. And, consistent with *Ce*TORC1's function in ageing[7], SAR-1 affects the lifespan of *C. elegans* under prolonged starvation. These results suggest that SAR1B's role in the control of mTORC1 is evolutionarily conserved.

Consistent with the association between mTORC1 hyperactivity and cancer[2], Chen *et al.* report that the *SAR1B* gene is frequently deleted in lung tumours called squamous cell carcinoma and adenocarcinoma. The authors also find that treatment of cultured SAR1B-deficient human cells with the mTORC1 inhibitor rapamycin significantly suppresses proliferation. Finally, eliminating SAR1B activity promotes tumour growth in mice given transplants of human tumour cells. These findings suggest that SAR1B might serve as a potent target for antitumour therapy.

Arginine was previously shown[8,9] to be another essential amino acid that activates the mTORC1 pathway (Fig. 1). This amino acid is sensed by the CASTOR1 protein, which also interacts directly with GATOR2 (refs 8, 9). Loss of CASTOR1 function or arginine abundance also leads to mTORC1 activation.

Why do these two amino acids, leucine and arginine, seem to have a specific role in controlling mTORC1 signalling, given that they are sensed[4,9] by different GATOR2 modulators? An organism's genetic code has redundancy because different nucleotide triplets (codons) can specify the same amino acid. Methionine and tryptophan are each encoded by a single codon, whereas all the other amino acids are specified by multiple codons. It is intriguing that leucine and arginine are two of the three amino acids that are determined by the highest number of codons — six in total. The relative frequency of a given amino acid in protein is affected by the number of codons that can code for it (see go.nature.com/36xqd3l)

Thus, it is possible that a system has evolved in which the amino acids that are used most frequently in proteins also function as signalling factors that determine the activity of the major orchestrator of cell growth, mTORC1. Indeed, the third amino acid that is specified by six codons is serine, and there is evidence that this amino acid is also involved in mTORC1 control[10]. The intracellular level of methionine, one of the amino acids that occurs less frequently in proteins, is sensed by the SAMTOR protein, which induces

GATOR1 to repress mTORC1 signalling[11].

Together, these data trace out a model in which the regulatory factors modulating mTORC1 activity can sense the intracellular level of specific amino acids that limit the rate of protein synthesis. Using this strategy, there would be no need for the intracellular concentration of every amino acid to be monitored in a cell; instead, only those used the most and the least frequently in protein synthesis would need to be tracked. That would enable the evolution and operation of a relatively simple, robust and economical regulatory network for maintaining a balance between protein synthesis and degradation.

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Physical chemistry

Single reaction events imaged in total darkness

Frédéric Kanoufi & Neso Sojic

Single photons emitted from individual electrochemically excited molecules in solution can now be detected. The technique can be used to image cells at nanometre resolution, without using an external light source. **See p.244**

If you want to admire the Milky Way in all its splendour, you should choose a moonless (and possibly romantic) night, away from urban light pollution. Exactly the same principle applies in microscopy, at the other end of the observational size scale: it is much easier to observe faint objects in complete darkness. On page 244, Dong *et al.*[1] report their use of this simple but powerful idea in a direct optical method for imaging single photons generated by individual chemical-reaction events in solution. The authors' method is conceptually different from the fluorescence-based approaches used in conventional single-molecule microscopy, because the excitation process that leads to photon emission is controlled by electrochemistry and chemical reactivity, and does not require irradiation with light. The authors demonstrate that their imaging method can be used for super-resolution microscopy of living cells.

Chemical reactions typically involve the collisions of individual molecules in solution. However, because measurements of reactions usually record the average parameters of ensembles of these molecular events, the characteristics of individual events are usually obscured, and the precise location and time of each reacting molecule in the solution is unknown. This issue can be partly overcome by restricting the volume of the solution, or by separating events in space and/or time, allowing each one to be detected alone. For example, high-throughput single-molecule measurements have been developed in ultrasmall containers, to detect individual biomolecules[2]. Extraordinary achievements in single-molecule studies have also have been reported using scanning tunnelling microscopy[3], nano-electrochemistry[4] and an optical technique called super-resolution localization microscopy (which achieves a resolution higher than the diffraction limit – a fundamental restriction that usually limits the resolution of optical imaging techniques)[5].

After decades of development, methods in which samples are irradiated with light have arguably become the most useful means of 'seeing' individual molecules – the 2014 Nobel Prize in Chemistry was awarded for discoveries in this field (see go.nature.com/3zs4aph). Biologists, physicists and chemists quickly adopted these revolutionary microscopy techniques, all of which use clever arrangements of light and dark – similar to the chiaroscuro technique in art. However, because these methods require the use of short, high-power laser pulses, their performance is often limited by background scattered light and photobleaching (the extinguishing of fluorescence as a result of the continuous excitation of fluorescent molecules by light). In biological applications, samples can also be damaged by the intense laser pulses, or produce their own fluorescence, obscuring the image of the fluorescent molecule of interest.

Some organisms can produce light *in situ* using biochemical reactions, rather than relying on external light sources – such bioluminescence illuminates the darkest regions of the oceans, for example. Dong and colleagues' approach to single-molecule imaging takes a

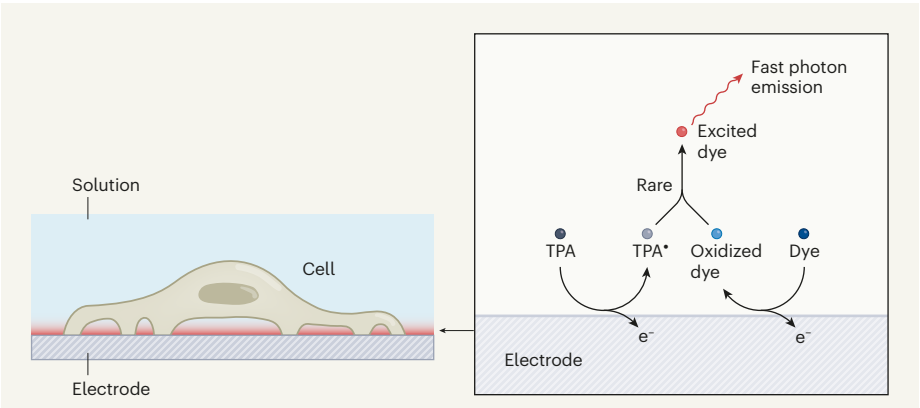

Figure 1 | A microscopy technique involving single-molecule imaging of electrochemical reactions. Dong *et al.*[1] have produced images of cells attached to the surface of electrodes using a phenomenon called electrochemiluminescence. The electrode is immersed in a solution of dye molecules (a ruthenium complex) and a co-reactant (tripropylamine, TPA). TPA is oxidized (loses an electron, e^-) at an electrode, and produces a radical ($TPA^{\bullet}$). The radicals have an extremely short lifetime, and are therefore found only at very high dilution in a thin region above the electrode surface. The radicals thus encounter dye molecules only rarely. If dye molecules are oxidized at the electrode and encounter a radical, the dye enters its excited state. The excited dye molecules almost instantaneously emit a photon, revealing the location and time of the reaction event. By imaging photons over time, an image of the electrode surface is produced. Photons are not produced at regions where the cell is attached, thus creating a negative image of the cell.