



Determinants of mitotic recombination

What cellular processes carry out the quality check of the DNA substrate in mitotic recombination in mammals? An interesting determinant of mitotic recombination is the human 'Bloom syndrome' gene (*BLM*) that encodes a RecQ DNA helicase. Remarkably, *BLM* was identified in revertant cells from Bloom patients by the frequent mitotic recombination between both mutant alleles, resulting in restoration of one wildtype allele¹¹. Recently, Luo and colleagues¹² showed that mitotic recombination is also dramatically enhanced in *Blm*-deficient mice. The role of *BLM* (and other RecQ DNA helicases) in recombination is still unclear, but it has been postulated that *BLM*

may influence the choice of template DNA used during recombinational repair of DNA double-strand breaks¹³.

It can readily be assumed that mismatch repair is also involved in determining substrate specificity. It has been shown that suppression of recombination between DNA molecules from different inbred *Mus m. musculus* mouse strains in mouse embryonic stem cells is released by mismatch repair-deficiency¹⁴. In these experiments, the suppressing effect on recombination in ES cells is apparent at much lower levels of genetic diversity than those observed by Shao *et al.*¹, perhaps because a different assay was used. Nevertheless the question remains: are small genetic differences between humans

sufficient to restrict mitotic recombination in various tissues, thereby reducing the chance for cancer development? □

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Asking the age-old questions

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Control of lifespan in *Caenorhabditis elegans* by the DAF-2 insulin-like signaling pathway requires *daf-16*, which encodes a member of the forkhead family of transcription factors. A new study provides evidence that DAF-2 negatively regulates DAF-16 activity by promoting its retention in the cytoplasm. But, constitutive targeting of DAF-16 to the nucleus is not sufficient to extend lifespan, revealing a new layer of complexity in the genetic control of aging in the nematode.

What is aging? Anyone can give a descriptive answer to this question, whether a young child or a centenarian. It's a biological reality that is familiar to us all. But what is aging at the molecular level and what are the cellular mechanisms that contribute to the characteristics we label aging? Which changes can be avoided, compensated for, or postponed? It is not so simple to define aging in sufficient detail to design experiments that will uncover its causes. On page 139 of this issue, however, Kui Lin and colleagues¹ report results that further unravel the processes underlying aging, using approaches that take advantage of longevity mutations, transparent bodies and transgenic lines of the nematode, *C. elegans*.

Single gene mutations can increase longevity in *C. elegans*, and a genetic pathway involved in the determination of lifespan has been identified². The genes *daf-2* and *age-1* increase adult lifespan when mutated, and it has been known for some time now that this longevity is dependent on *daf-16*. Two

major questions in the field have been whether *daf-16* is sufficient to increase lifespan and, of course, how this increase is effected. Guided by the properties of homologous vertebrate genes, Lin and colleagues investigated adult lifespan by examining the effects of green fluorescent protein (GFP)-tagged *daf-16* transgenes on the developmental (Daf) and aging (Age) phenotypes of live animals.

DAF-2 signaling and longevity

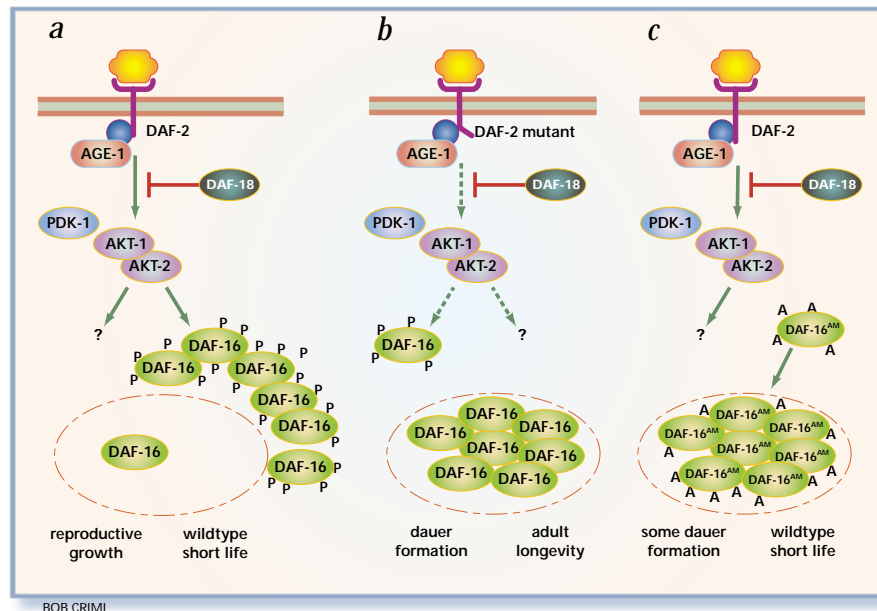
Based on sequence homologies, the genes in the *daf-2* pathway resemble those related to insulin signaling. In wildtype animals, the inferred relationships between these gene products are as follows. The insulin-like receptor tyrosine kinase DAF-2 (ref. 3) binds an insulin-like ligand and activates a phosphatidylinositol-3-OH kinase, AGE-1 (ref. 4), that generates the signal-transducing molecule phosphatidylinositol-3,4,5-trisphosphate (PIP₃). The concentration of PIP₃ is negatively affected by DAF-18, a presumed phosphatase with homology to PTEN

phosphatase (ref. 5). Subsequently, PIP₃ and PDK-1 are thought to activate the kinases encoded by *akt-1* and *akt-2* (refs. 6,7), which, in turn, have been proposed to phosphorylate DAF-16, a protein homologous to forkhead transcription factors^{8,9}, thereby rendering DAF-16 inactive. Under favorable culture conditions, this inactivation and downregulation of DAF-16 allows rapid growth to reproductive maturity and a short adult life in wild-type animals. (Fig. 1a). Unfavorable conditions experienced by young larvae, including overcrowding, limited food and high temperature, induce formation of a specialized third larval stage, the dauer larva, in part because of the lack of DAF-2 signaling¹⁰. Dauer larvae are developmentally arrested, resistant to harsh conditions and designed for long-term survival. Mutations in *daf-2* or *age-1* that inappropriately induce dauer formation are referred to as dauer-constitutive or Daf-c.

Lin *et al.*¹ increased the gene dosage of *daf-16* in wild-type animals and found that it promotes neither constitutive

**The ins and outs of DAF-2 signaling.**

A model for regulation of the subcellular localization of DAF-16 by the DAF-2 signaling pathway. This is an insulin-like signaling pathway, based on gene homologies that are described in the text. DAF-2 is a membrane-bound receptor. The dashed ovals represent the nuclear membrane. The phosphorylation state of DAF-16 is denoted by the presence or absence of "P". **a**, Under favorable growth conditions, an unidentified hormone activates the DAF-2 signaling pathway, which restricts DAF-16 to the cytoplasm and results in growth to a reproductive adult that has a mean lifespan of approximately two weeks. **b**, A *daf-2* mutant signaling pathway. The minimal inhibition by crippled DAF-2 signaling allows DAF-16 to move to the nucleus and alter transcription of genes that extend lifespan in adults to approximately four weeks. In wild-type larval development, low DAF-2 signaling results from low food availability and high dauer pheromone concentrations. The effect on the animal is the same as a *daf-2* mutation, which is to induce dauer larva formation. **c**, In this situation the DAF-16 protein (DAF-16^{AM}) has had the putative AKT phosphorylation sites changed to unphosphorylatable alanine residues, each of which is represented by "A". The wildtype DAF-2 signaling no longer restricts the altered DAF-16 to the cytoplasm. The phenotypes of these animals differ from those observed when DAF-16 is localized in the nucleus due to mutation of the *daf-2* gene as shown in **b**. The similarities or differences in transcriptional output are unknown.



dauer formation nor a *daf-2* mutant-like long lifespan, suggesting that wild-type DAF-2 might be inhibiting excess DAF-16 function. Their *daf-16::gfp* transgenes were shown to be functional because they rescued the *daf-16(mu86)*; *daf-2(e1370)* double mutant to the *Daf-c* and the *Age* phenotypes of the *daf-2(e1370)* single mutant. They proceeded to correlate the intracellular localization of DAF-16 with DAF-2 signaling. Active DAF-2 signaling prevents accumulation of DAF-16::GFP in the nucleus, as evidenced by the different subcellular localization of green fluorescence in *daf-2(+)* and *daf-2(e1370)* backgrounds (Fig. 1a,b). Furthermore, the cytoplasmic localization of DAF-16::GFP depends on its phosphorylation by AKT-1 and AKT-2; changing the putative serine and threonine phosphorylation sites to alanine (DAF-16^{AM}::GFP) results in nuclear accumulation of the green fluorescence (Fig. 1c). Despite the altered amino acids of DAF-16^{AM}, it is still functional, as shown by its rescue of the dauer-defective and non-*Age* phenotypes of the *daf-16(mu86)*; *daf-2(e1370)* double mutant to the *Daf-c* and *Age* phenotypes of *daf-2(e1370)*.

New answers, new questions

Surprisingly, the authors found virtually no effect of DAF-16^{AM}::GFP on lifespan in wild-type animals, and so, nuclear localization of DAF-16 is not sufficient for the longevity phenotype. This was unex-

pected, as the only known inhibitory effect of DAF-2 on DAF-16 is to restrict it to the cytoplasm, which leads to the short adult lifespan in wild-type animals. Hence, placement of DAF-16 in the nucleus was anticipated to increase lifespan as in *daf-2* mutants. The authors go on to conclude that there are additional, as yet unidentified, DAF-2-controlled processes that contribute to lifespan determination and dauer formation. This proposal differs from the current view that DAF-16 is the major output of the pathway. This is possible, if the originally observed suppression of the *daf-2* *Age* phenotype by mutations in *daf-16* was due in part to the sickness of the *daf-16* mutants, which might have masked an incomplete *Age* suppression. It may also be that expecting the artificial activation of DAF-16 alone to result in the doubling of adult lifespan, as do mutations in *daf-2*, reflects an oversimplified view of this pathway. Indeed, the relationship between DAF-2 signaling and lifespan extension is likely to be a complex one.

In *C. elegans*, the ablation of either certain sensory neurons or the germ line are two other means to increase lifespan that involve DAF-2 signaling¹¹. Both of these require *daf-16* for the longevity effect, and Lin *et al.* show that both treatments cause DAF-16::GFP to be localized in the nucleus. The germline-ablated animals uniquely show nuclear localization of DAF-16::GFP occurring only in young adults and predominantly restricted to the

intestinal cells. This result is intriguing because the timing coincides with oocyte production, with the yolk proteins being synthesized by the intestine and transported to the gonad¹².

Finally, the favored mechanisms for the longevity of *daf-2* or *age-1* mutants include alteration of metabolism and resistance to stress, especially to oxidative stress^{2,11}. Lin and colleagues find that heat shock causes nuclear localization of DAF-16::GFP and the effect is initiated upstream of *daf-18*. This result holds promise for future investigations into the mechanism that links stress resistance and longevity. The control of metabolism, stress resistance, and longevity by the DAF-2 signaling pathway is probably conserved across species.

Keys to a long life?

Recently, mutations in genes encoding similar insulin-like signaling proteins in *Drosophila* were shown to extend lifespan^{13,14}. Now, the behavior of the *C. elegans* DAF-16 gene product is found to be remarkably conserved with the vertebrate insulin signaling system, in both its regulation by phosphorylation and its subcellular localization. Together, these findings give hope that the identification of those aspects of aging that can be avoided, compensated for, or postponed in vertebrates will be arrived at rather more quickly by studying the conserved mechanisms in worms, as they have substantially shorter lives than vertebrates.



In particular, attention should be directed at identifying genes downstream of DAF-2 signaling that coordinately affect the rate of aging in all of the cells in the body. □

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Mitochondria: integrators in tumorigenesis?

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The mitochondrial genomes of tumor cells accumulate mutations during transformation. A new study raises questions regarding the way in which mutations accumulate and has implications for the potential role of mitochondrial function in tumor phenotype.

In the ancient history of tumor cell biology and biochemistry (that is, pre-1995), it was established that mitochondrial DNA accumulates more damage from genotoxic agents than does the nuclear genome, probably because the mitochondria have less efficient DNA repair systems than the nucleus^{1–3}. This is consistent with more recent observations that mutations appear in mitochondrial DNA of tumor cells, and can be homoplasmic – that is, present in each of the hundreds of mitochondrial genomes in a cell and in virtually all of the cells of a tumor⁴. Further, following the fusion of two tumor cell lines, the mitochondrial genome of one quickly replaced that of the other, leading to the conclusion that once a particular mitochondrial sequence becomes homoplasmic in a cell, selective growth advantage conferred by mutations in either that mitochondrial genome, or in the nuclear genome of the cell, can result in the mitochondrial alteration becoming homoplasmic in the tumor⁴.

On page 147 of this issue, a study by Hilary Collier and colleagues⁵ indicates otherwise. The authors present a mathematical model that shows the presence of a homoplasmic mitochondrial mutation in a tumor can result from random segregation of mutant genomes in the many cell generations that occur during tumor development⁵. The predictions of the model agree with experimental data documenting the incidence and nature of tumor mitochondrial mutations. It would therefore seem that there is no need to

invoke a selective advantage for a mutation in the mitochondrial genome to explain its presence. The model also predicts, in agreement with more limited data, that homoplasmic mitochondrial variations should arise with significant frequency in normal tissues derived from stem cells that have undergone a high number of cell divisions.

Models of mitochondrial mutations

The report should stimulate extensive debate regarding the validity of the model. At this point, a reasonable, if broad, speculation is that, among a high background of random mutations that provide no selective advantage, there are a smaller number of mutations that alter mitochondrial function and cell physiology in a manner that has significant effects on tumor development, or phenotype in one of several tumor-associated environments (for example, hypoxia, angiogenic insufficiency or stimulation, or shift of metabolic phenotype). This is a scenario similar to that of tumors with defects in DNA repair: thousands of mutations accumulate in the nuclear genome, the vast majority of which are functionally and physiologically silent and overlaying the few that have an impact on tumorigenesis. Unfortunately, there is still no way to identify the functionally important mutations⁶.

It is even harder to identify relevant mutations in the mitochondrial genome because we do not know how mutations in the many genomes of individual mitochondria, and among the hundreds of mitochondria per

cell, interact genetically in terms of dominant and recessive effects. Even if consideration is focused only on mutations that become essentially homoplasmic, the problem will be difficult to solve.

First, mitochondrial function is intimately linked to the expression of nuclear genes, both directly, as some mitochondrial proteins and subunits of mitochondrial complexes involved in electron transport and oxidative phosphorylation are encoded by nuclear genes, and indirectly, as mitochondrial oxidative phosphorylation is linked to many pathways of intermediary metabolism. Therefore, the extensive mutations in nuclear genes coupled with altered profiles of gene expression that characterize tumors, will potentially interact with mitochondrial mutations. It is possible that only rare combinations of coincident changes in the two genomes may be of functional significance, and indeed there is evidence that nuclear–mitochondrial gene interaction provides a selective advantage for a particular mitochondrial genome^{7,8}.

Second, given the large number of cell divisions necessary for tumor development—estimated at 600 by Collier *et al.*⁵—an alteration in mitochondrial function that is exceedingly subtle in terms of its biochemical or physiological manifestation may be all that is necessary to significantly alter the probability of tumor formation or the final clinical phenotype, including the malignant potential, of the tumor. Cell and molecular biologists are generally not comfortable working with such small phenotypic effects.