Xenohormetic, hormetic & cytostatic selective forces drive the evolution of longevity regulation mechanisms within ecosystems

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Aging can be delayed by …

- **Single-gene mutations in a limited number of "master" genes regulating longevity**
- **Caloric restriction (CR) or dietary restriction (DR)**

Anti-aging compounds:

➔ **Resveratrol (yeast, worms, flies, mice)**

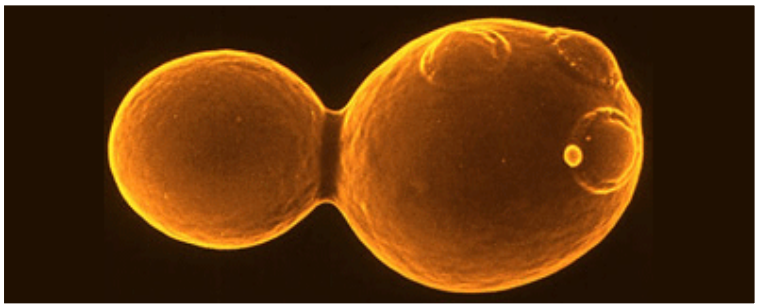
➔ **Spermidine (yeast, worms, flies, human immune cells)**

➔ **Rapamycin (yeast, mice)**

➔ **Caffeine (yeast)**

- **Longevity signaling pathways & their modulation by dietary & pharmacological interventions are <u>evolutionarily conserved</u>**

Thus …

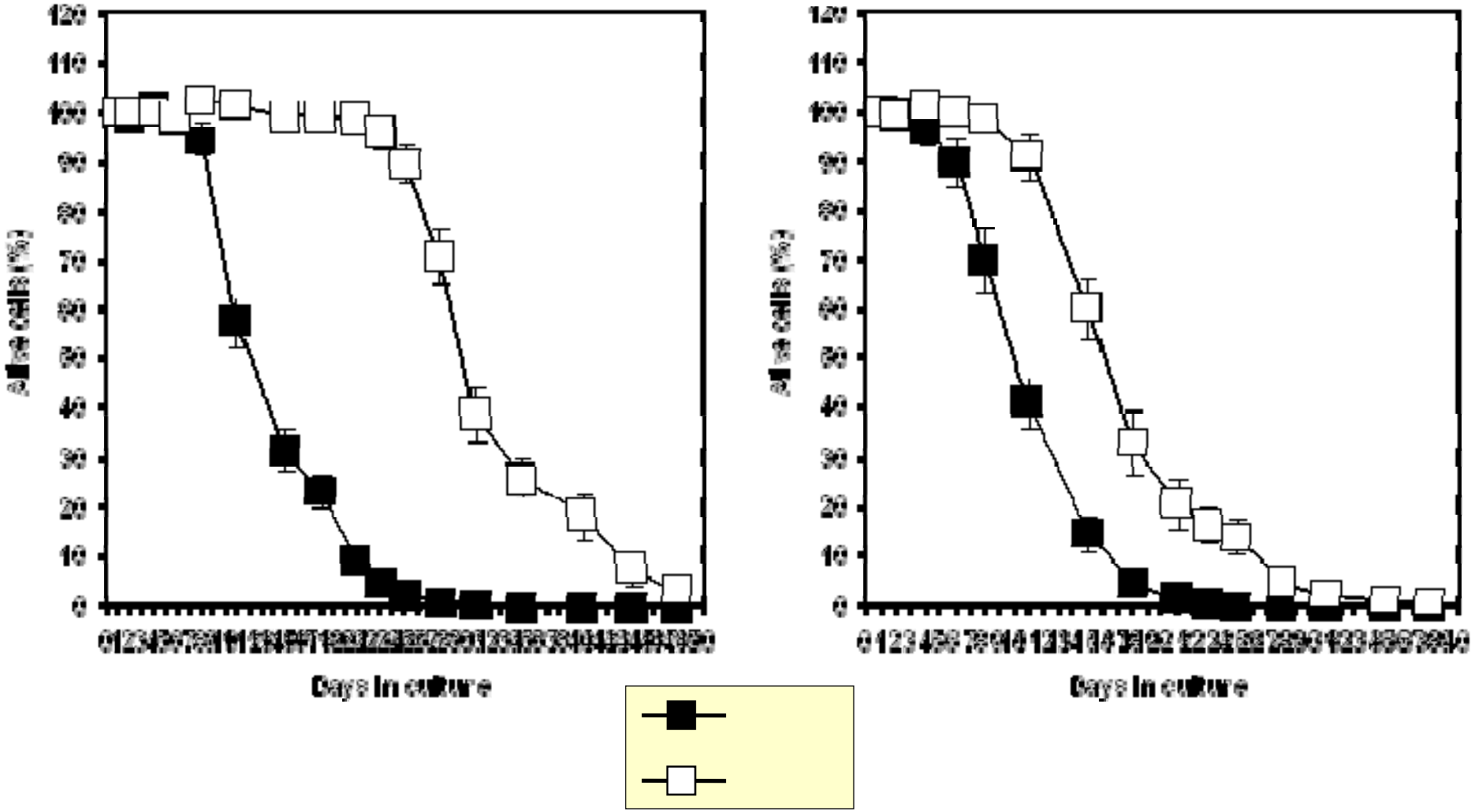


- **The baker's yeast is a <u>valuable model</u> for unveiling mechanisms of cellular aging in multicellular eukaryotes**

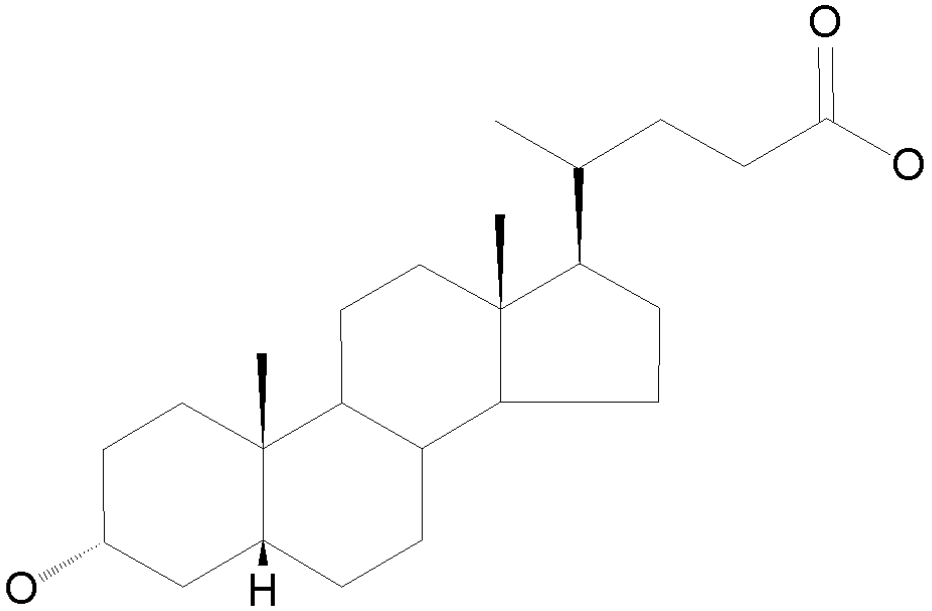
- **We identified 24 novel compounds that greatly extend yeast longevity & belong to 5 chemical groups**

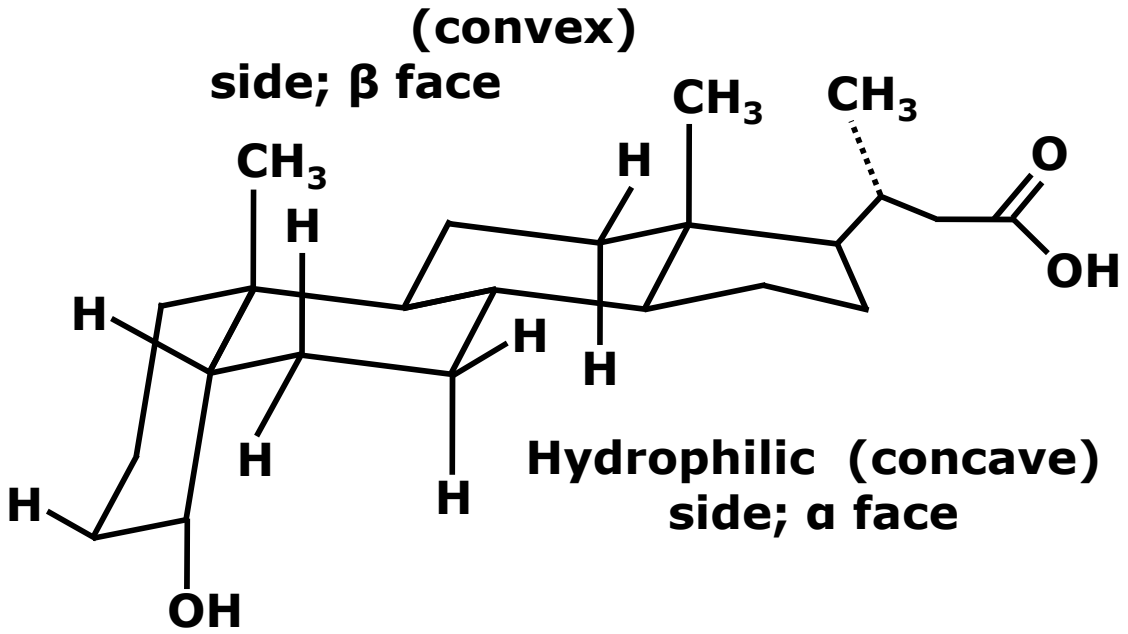
- **All these compounds are structurally & functionally distinct from currently known anti-aging compounds, namely resveratrol, spermidine, rapamycin & caffeine**

- **Lithocholic acid (LCA) is one of these anti-aging compounds extending yeast chronological life span under caloric restriction (CR) conditions to a higher degree than that under non-CR conditions**



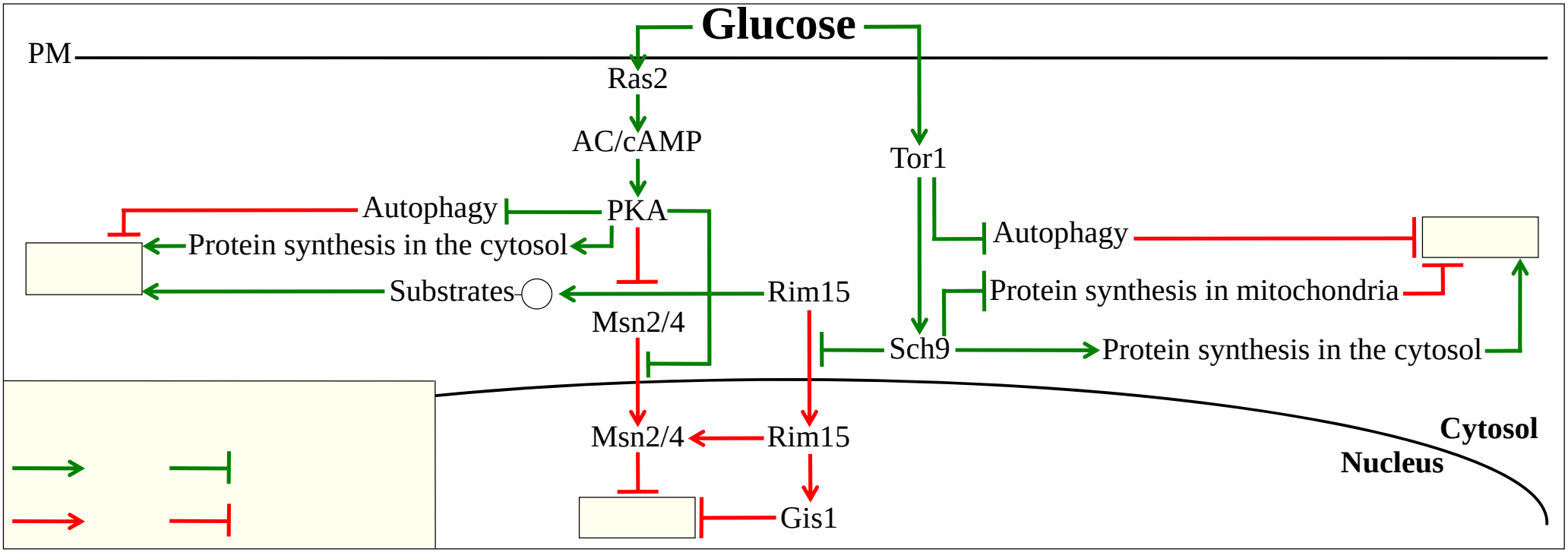
Lithocholic acid (LCA)





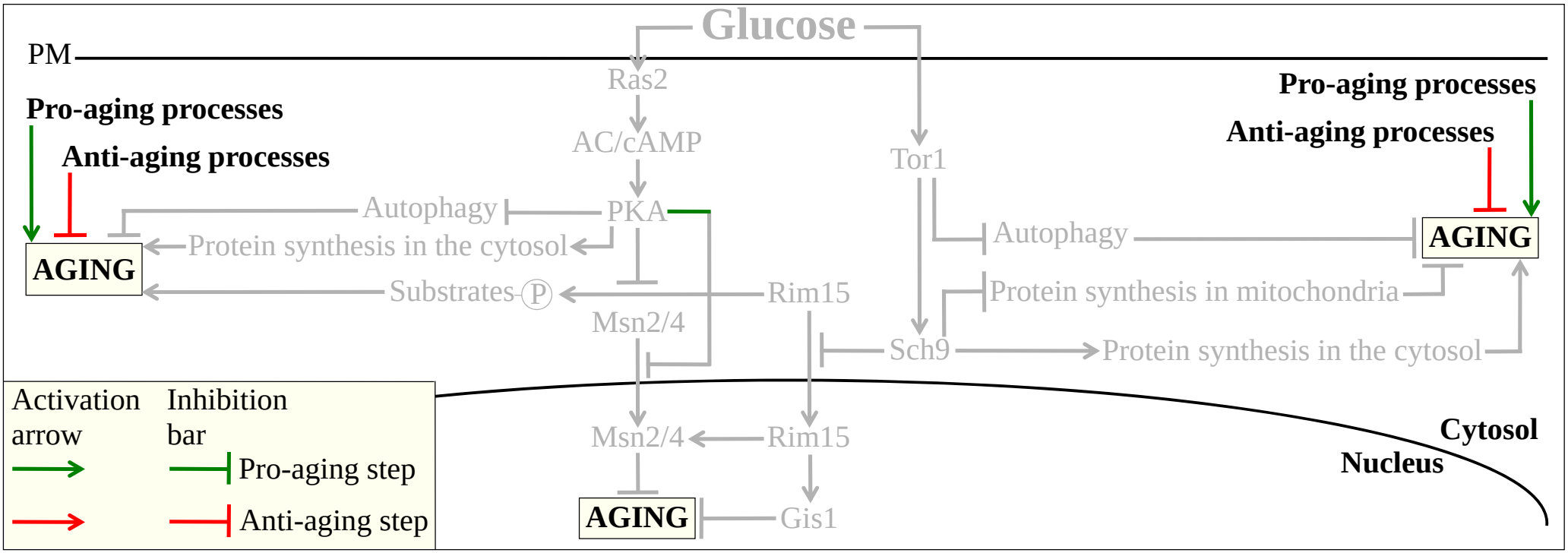
- **The TOR & cAMP/PKA longevity signaling pathways are "<u>adaptable</u>" by nature …**

- **They regulate longevity <u>only in response to certain changes</u> in the organismal & intracellular nutrient & energy status (*e.g.*, <u>calorie availability</u>)**

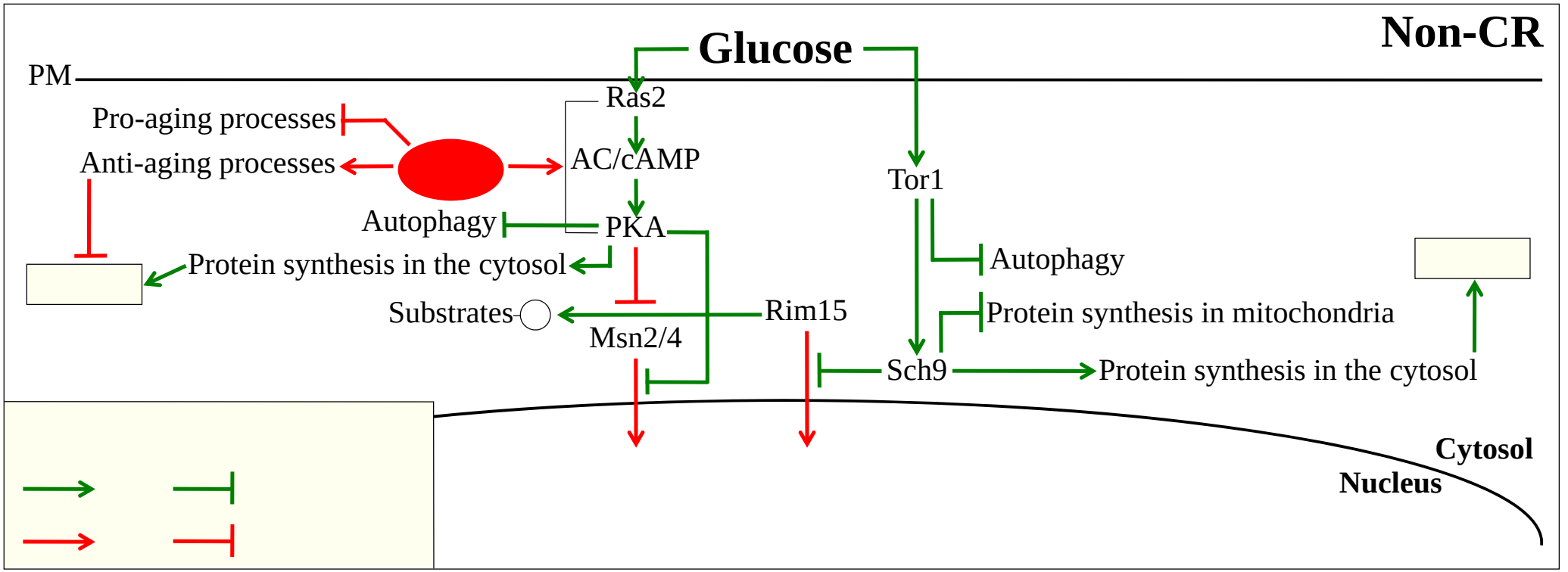


- **However, we found that some longevity regulation pathways are "constitutive" or "housekeeping" by nature …**

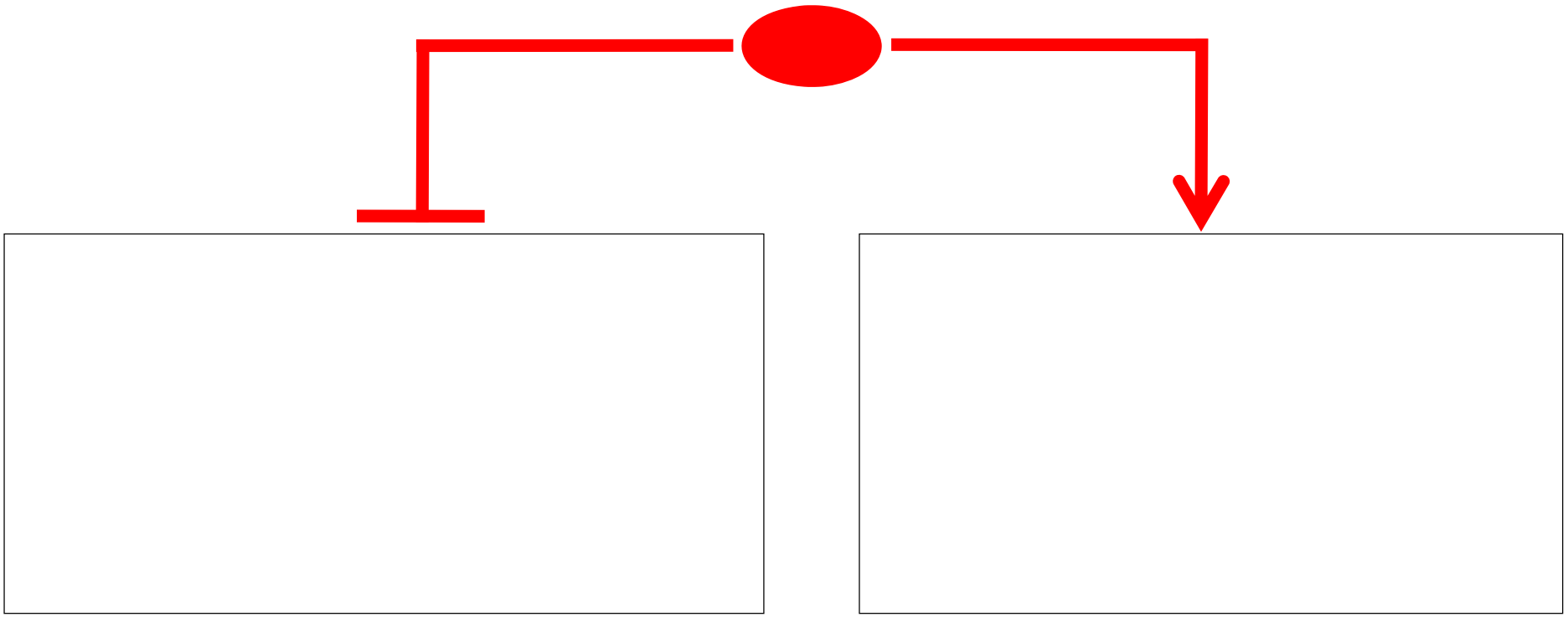
- **They regulate longevity irrespective of the organismal & intracellular nutrient & energy status & do not overlap with the adaptable pathways**



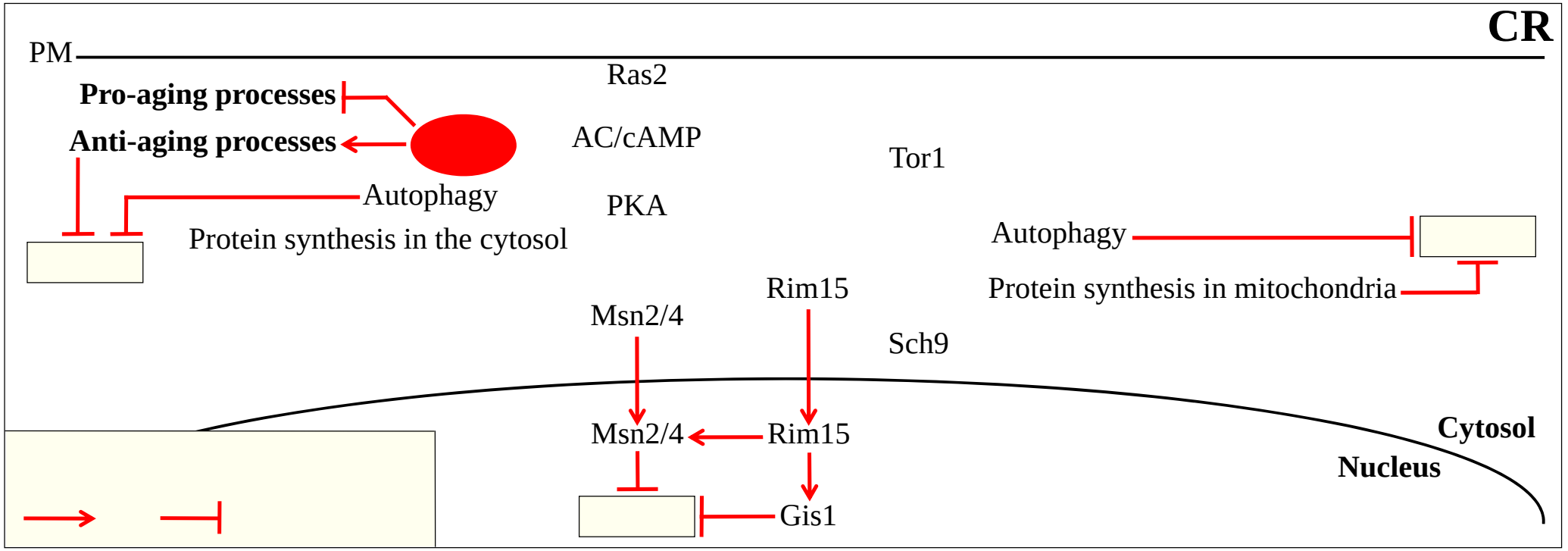
❑ Under non-CR conditions, LCA targets "housekeeping" longevity assurance processes & the "adaptable" cAMP/PKA longevity pathway



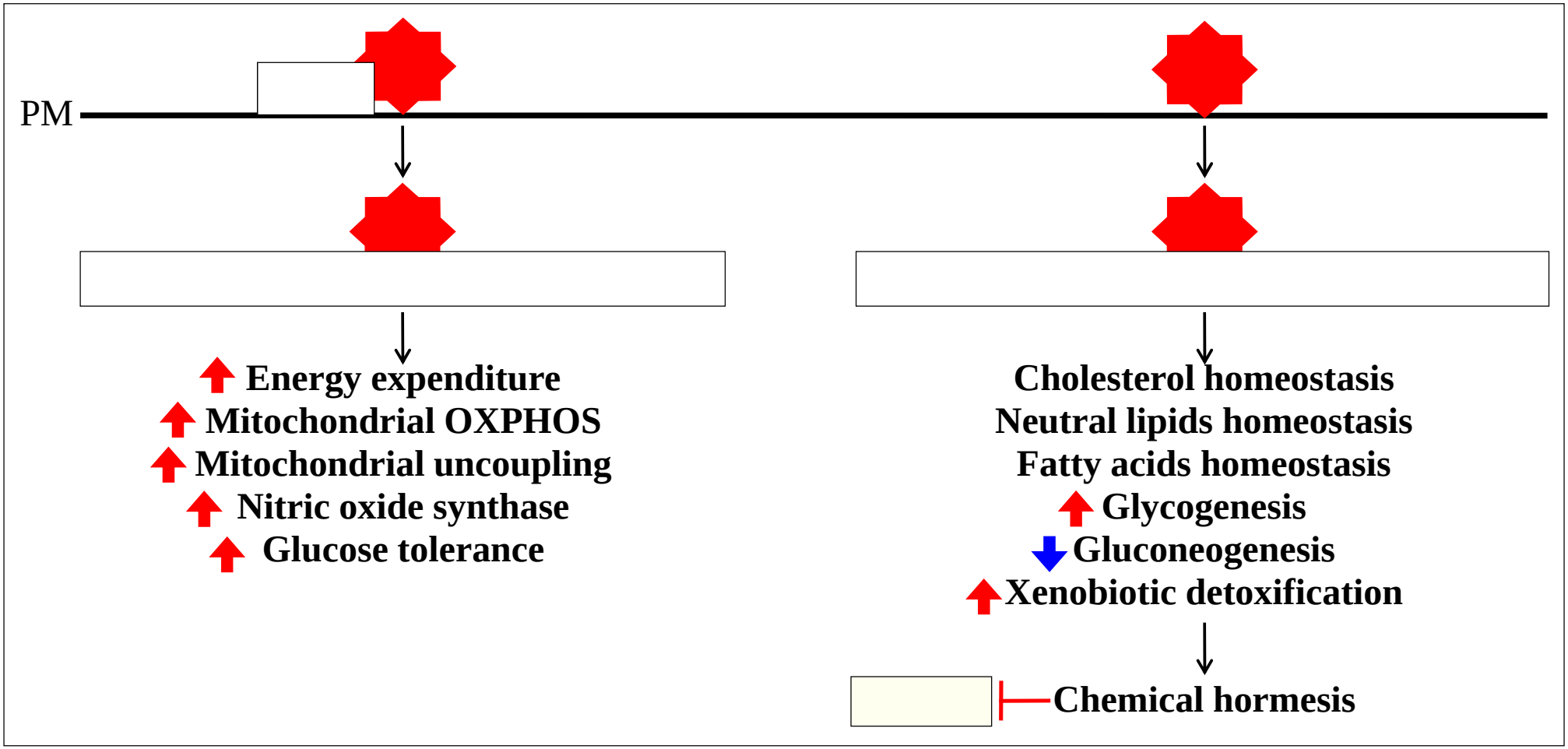
- **We found that LCA modulates the following "housekeeping" longevity assurance processes**



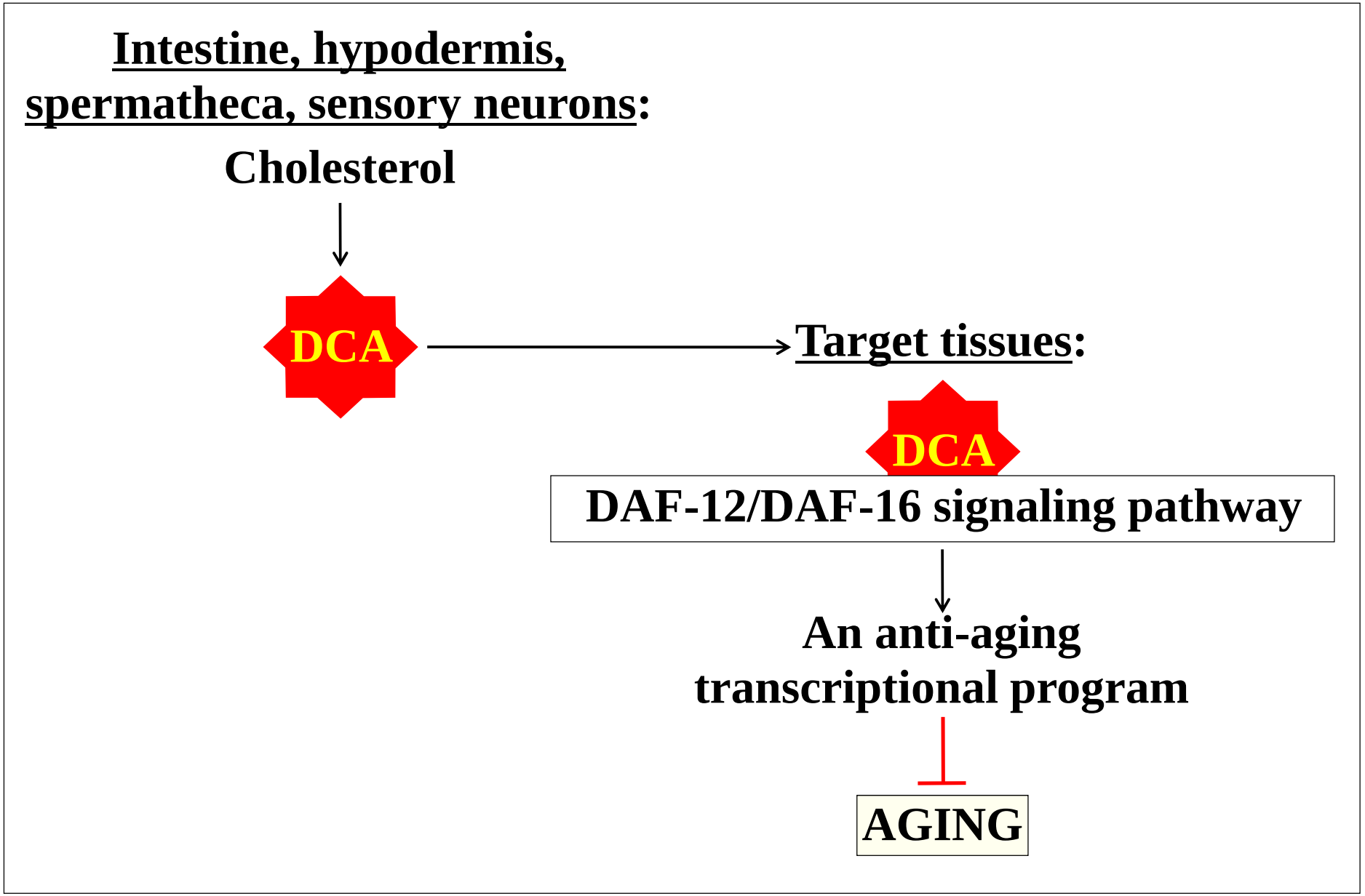
❑ Under CR conditions, LCA targets only "housekeeping" longevity assurance processes



❑ Bile acids (BA) are beneficial to health & longevity in mammals



❑ Bile acid-like dafachronic acids (DCA) extend longevity in worms



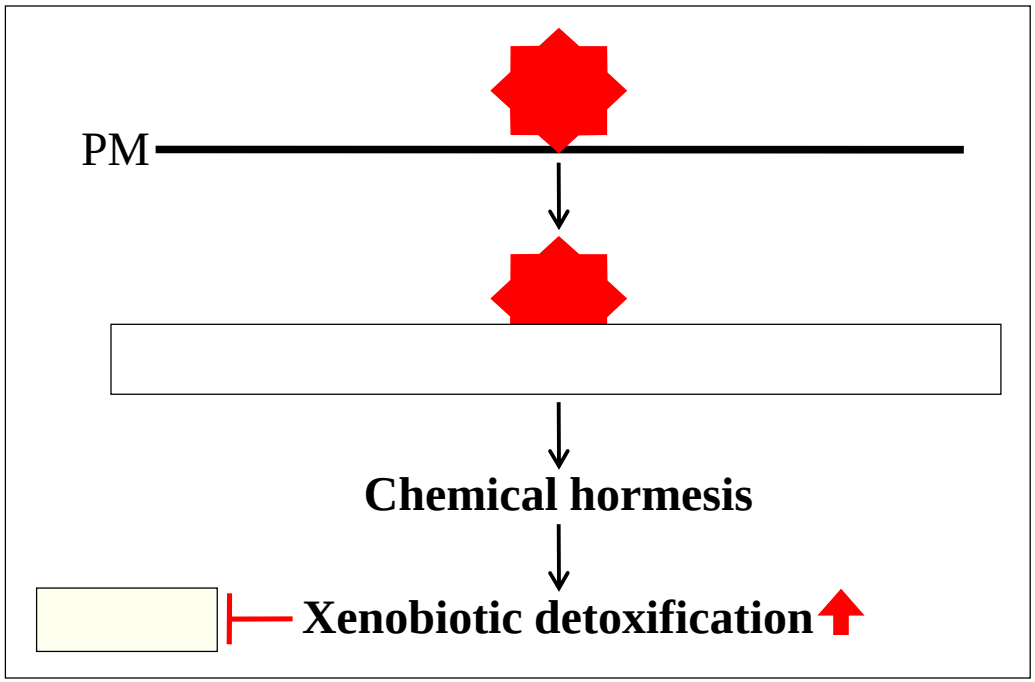
Important observations:

- **The levels of bile acids are elevated in the long-lived Ghrhr$^{lit/lit}$ mice**

- **Cholic acid, a bile acid, administered to food of wild-type mice activates transcription of numerous xenobiotic detoxification genes**

Therefore, it has been proposed that ...

- **By promoting chemical hormesis in mammals, bile acids - mildly toxic molecules with detergent-like properties - may extend their longevity by acting as endobiotic regulators of aging**



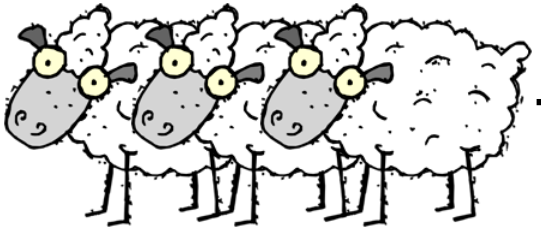
Importantly …

- **Yeast do <u>not</u> synthesize LCA or any other bile acid found in mammals**

Therefore, we hypothesize that …

- **Bile acids released into the environment by mammals may act as <u>interspecies chemical signals extending yeast longevity within ecosystems</u>**

In our hypothesis ...



Mammals

BA

⊘ A gene allele that enables a cell to respond to BA by undergoing specific life-extending changes to its physiology

Yeast

This yeast species will <u>live longer</u> than other yeast species within the ecosystem

∵

This yeast species has <u>increased chances of survival</u>

In our hypothesis …

- **Bile acids released into the environment by mammals extend longevity of yeast species & other organisms that can sense these compounds**

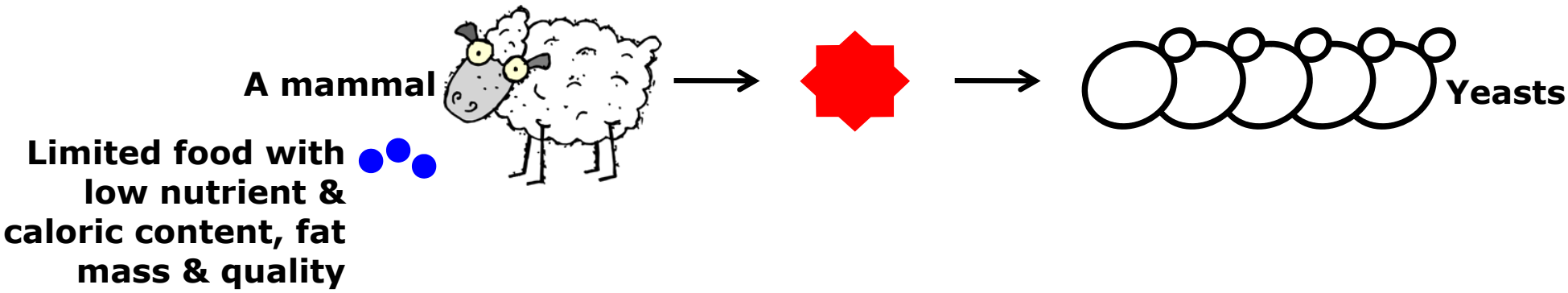
Thereby …

- **Increasing their chances of survival & creating selective force aimed @ maintaining the ability of organisms composing the ecosystem to respond to bile acids by undergoing specific life-extending changes to their physiology**

In our hypothesis …

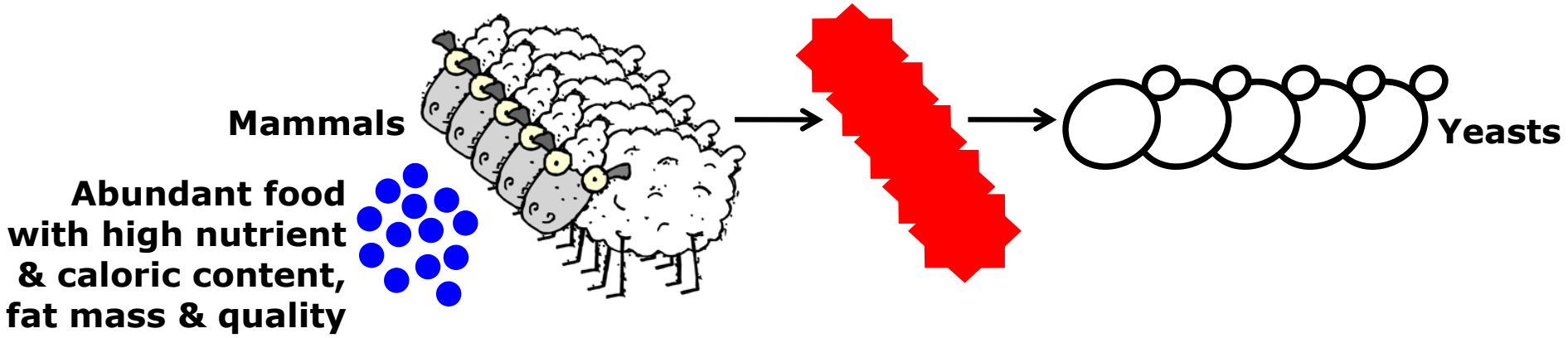
- **The evolution of longevity regulation mechanisms in yeast species & other organisms composing an ecosystem is driven by their ability to undergo specific life-extending physiological changes in response to bile acids & other mildly toxic, hormetic compounds that are permanently or transiently released to the ecosystem by mammals**

- **Yeast are <u>permanently</u> exposed to BA due to their fecal loss by mammals**



- **Thus, in yeast exposed to BA released by mammals, BA modulate <u>HOUSEKEEPING</u> longevity assurance pathways that …**
- **Regulate yeast longevity <u>irrespective</u> of the number of mammals or their food supply**
- **Do <u>not</u> overlap with the <u>adaptable</u> TOR and cAMP/PKA longevity pathways**

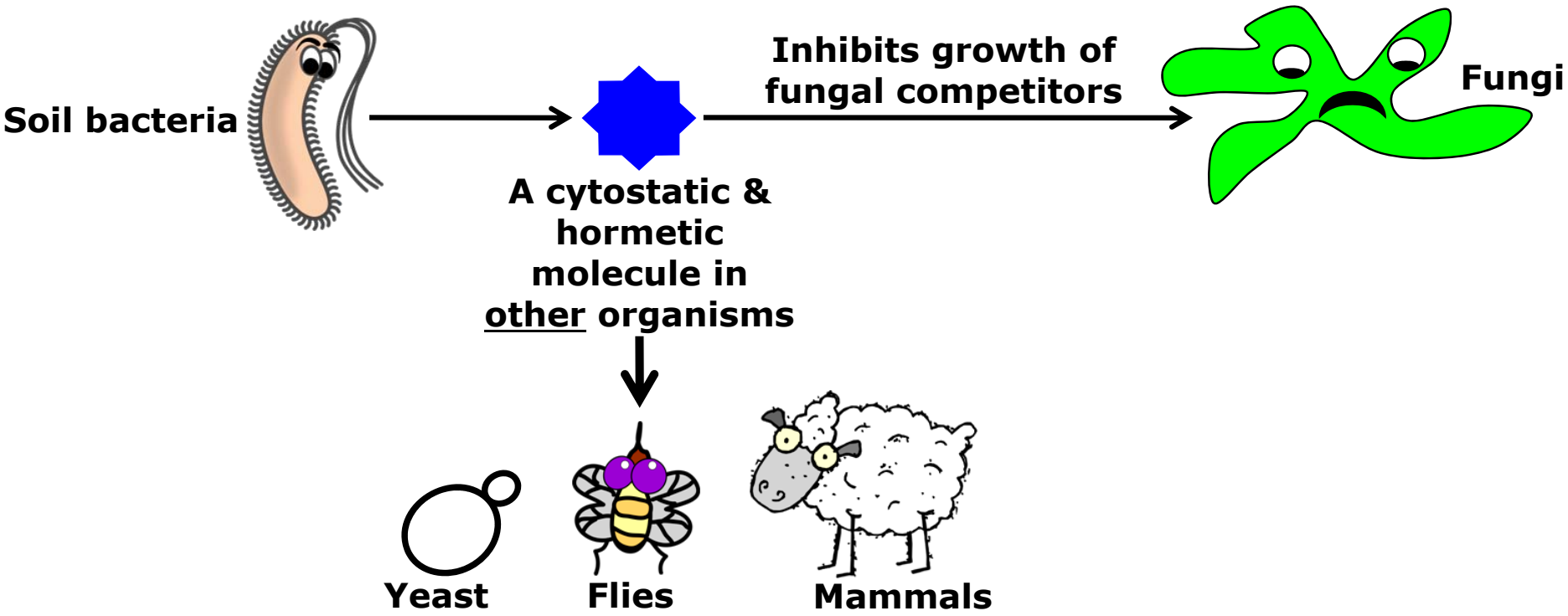
- **The quantity of BA released into the environment by mammals could vary due to changes in the density of mammalian population & abundance of food & its quality**



- **Thus, in addition to the ability of yeast to respond to the permanently available exogenous pool of BA by modulating housekeeping longevity pathways …**

- **Yeast may have also evolved the ability to sense the environmental status-dependent variations of BA abundance by modulating the ADAPTABLE TOR & cAMP/PKA longevity pathways**

- **Another anti-aging compound, called rapamycin (RAP), may also act as an interspecies chemical signal modulating longevity at the ecosystemic level**

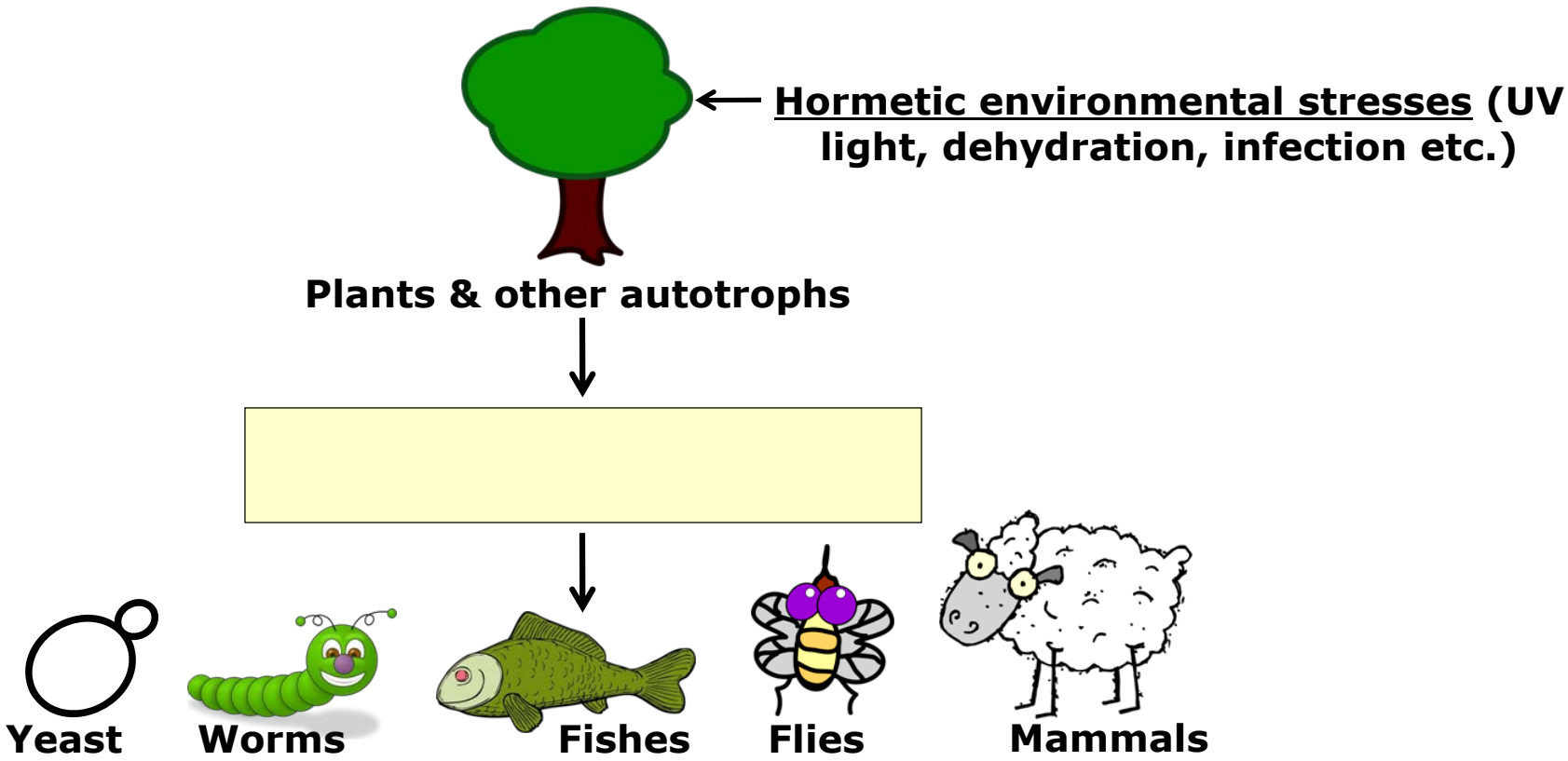


- **RAP extends longevity in yeast, fruit flies & mice by inhibiting TOR, a nutrient-sensory protein kinase that operates as a master negative regulator of the key adaptable longevity pathway**

Therefore, we hypothesize that ...

- **The ability of yeast, fruit flies & mice to sense RAP produced by soil bacteria & then to respond by undergoing certain life-extending changes to their physiology may increase their chances of survival, thereby creating selective force for maintaining such ability**

❑ "Xenohormetic" hypothesis (Howitz & Sinclair)



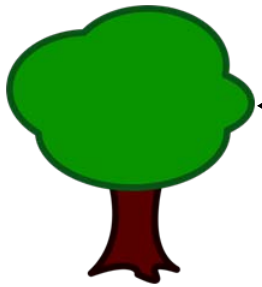
❑ **Extend longevity of yeast, worms, fishes, flies & mammals by:**

❑ **Modulating the key enzymes of stress-response pathways governing longevity-related processes**

❑ **Inhibiting the pro-aging TOR signaling pathway (*i.e.*, exhibiting a cytostatic effect)**

We propose a unified hypothesis of the xenohormetic, hormetic & cytostatic selective forces driving the evolution of longevity regulation mechanisms @ the ecosystemic level

Xenohormetic, hormetic & cytostatic selective forces may drive the evolution of longevity regulation mechanisms within an ecosystem

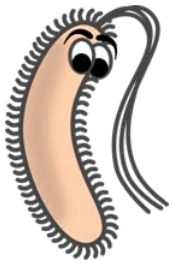


Environmental stresses

Plants & other autotrophs

Transiently produce

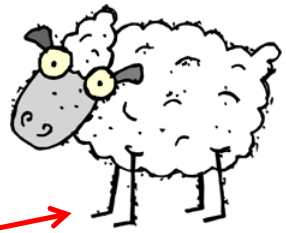
Xenohormetic phytochemicals (cytostatic but not hormetic molecules in other organisms): resveratrol, caffeine & others



Soil bacteria

Permanently produce

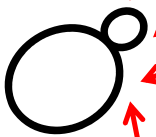
Macrocyclic lactone (a hormetic & a cytostatic molecule in other organisms): rapamycin



Mammals

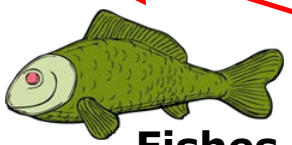
Permanently produce

Bile acids (hormetic molecules in other organisms): LCA



Yeast

Worms



Fishes

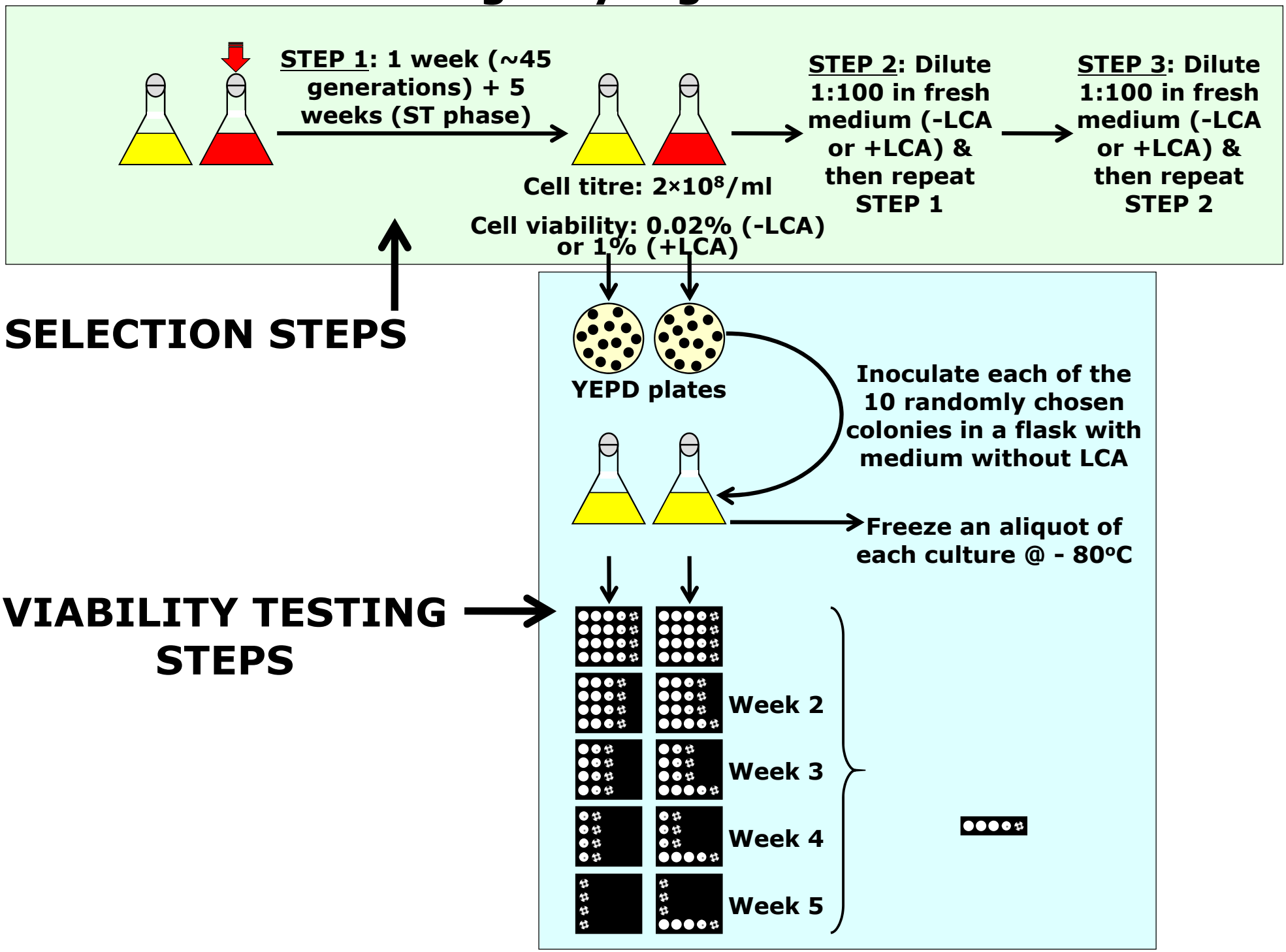
Flies

Permanently produce

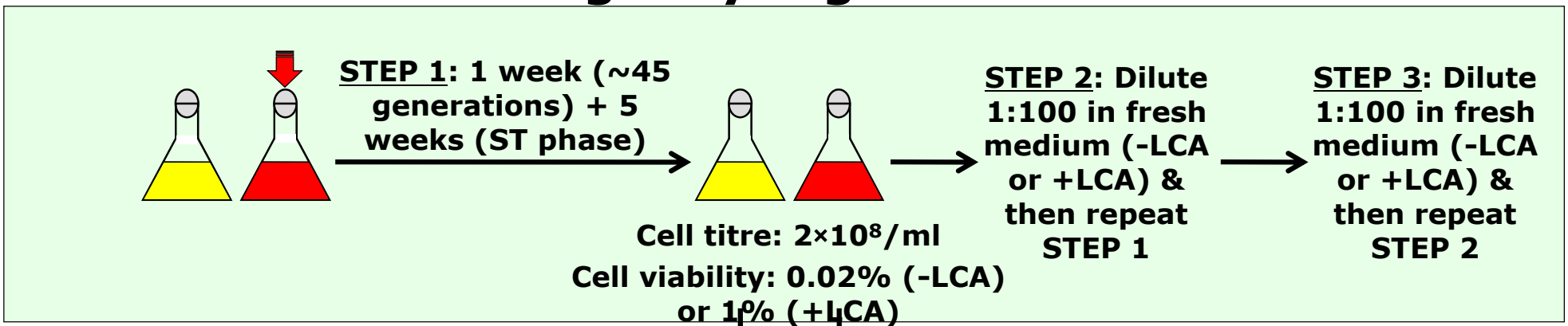
Bile acid-like compounds (hormetic molecules in other organisms): DCA

- **To test the validity of our hypothesis of the xenohormetic, hormetic & cytostatic selective forces driving the evolution of longevity regulation mechanisms within an ecosystem, we carried out the LCA-driven multistep selection of long-lived yeast species**

❑ A 3-step process of the LCA-driven experimental evolution of longevity regulation mechanisms

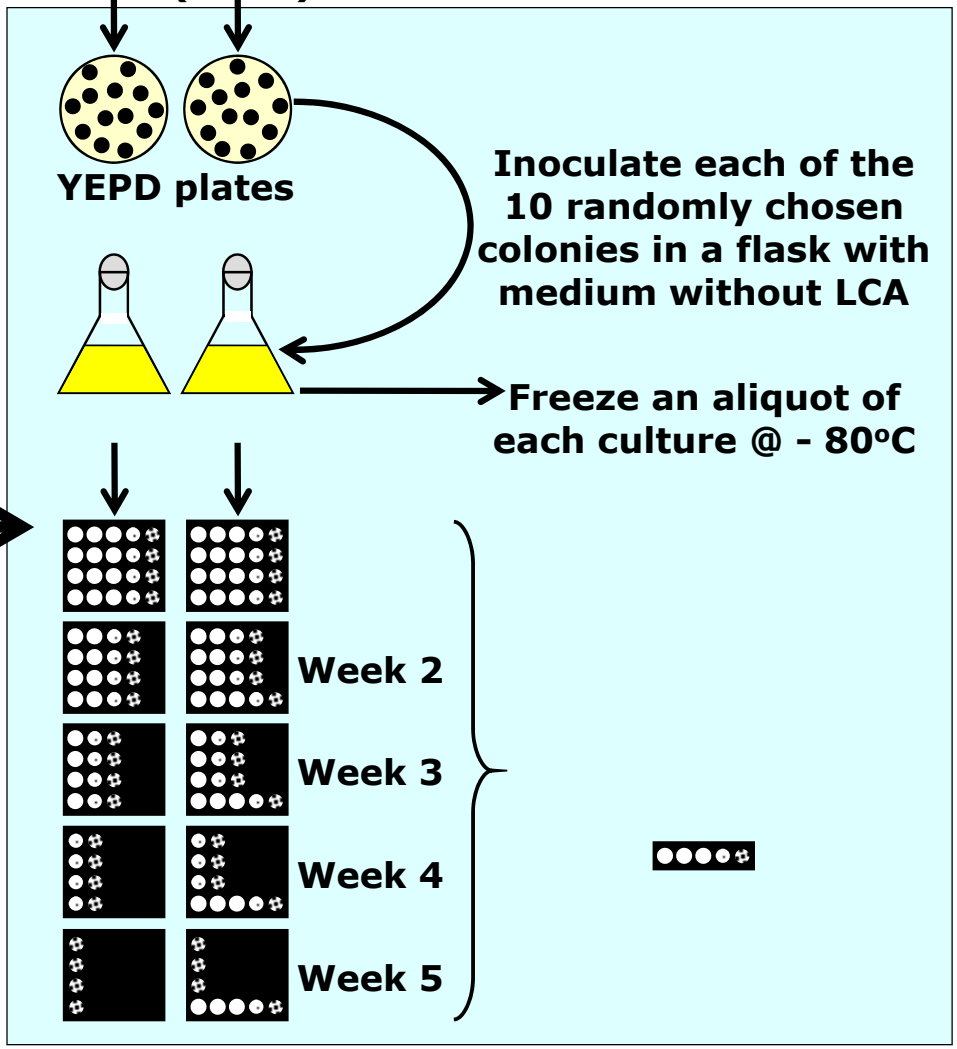


❑ A 3-step process of the LCA-driven experimental evolution of longevity regulation mechanisms

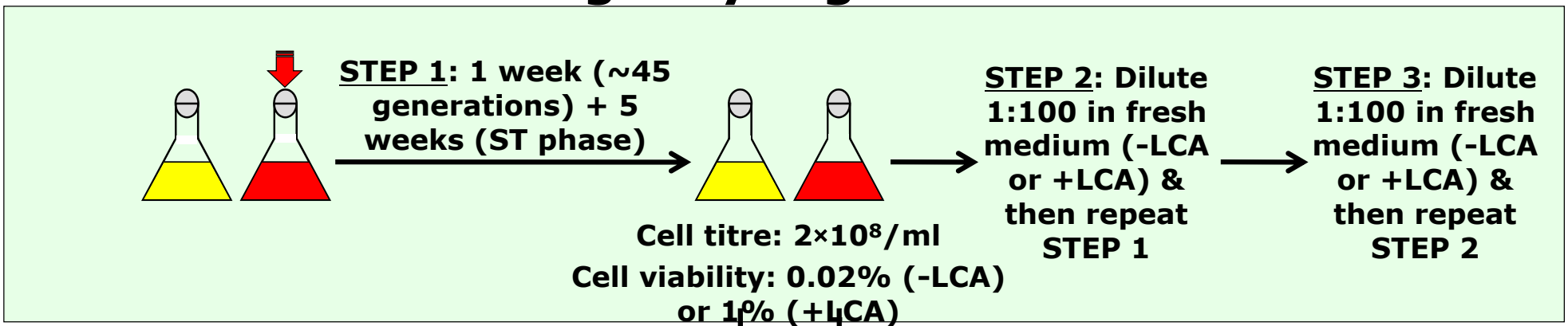


The number of cell generations in each of the selection steps prior to entry into a non-proliferative state (*i.e.*, stationary phase [ST] of senescence):

(2 x 10^8 cells/ml) : (10^5 cells/ml) = 2,000 ~ 45 generations

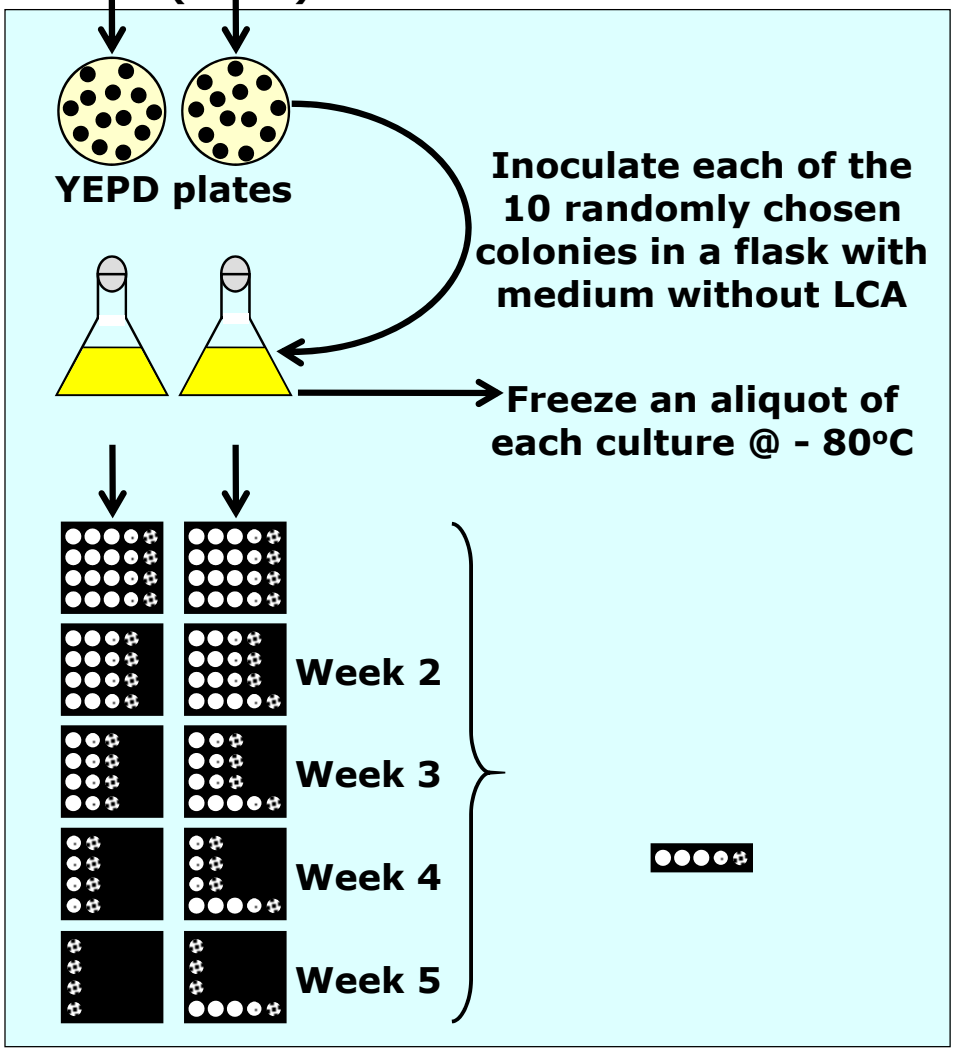


❑ A 3-step process of the LCA-driven experimental evolution of longevity regulation mechanisms

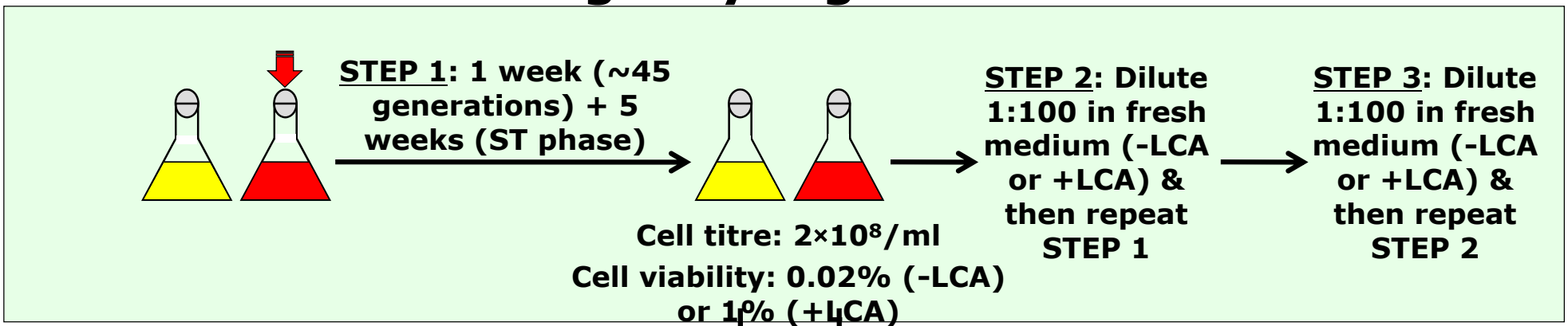


Enrichment factor for long-lived mutants by the end of each of the 5-weeks selection steps (in LCA-treated samples):

100% viable cells (start) : 1% viable long-lived cells (end) = 10^2



A 3-step process of the LCA-driven experimental evolution of longevity regulation mechanisms



Concentrations of LCA used during each of the selection steps:

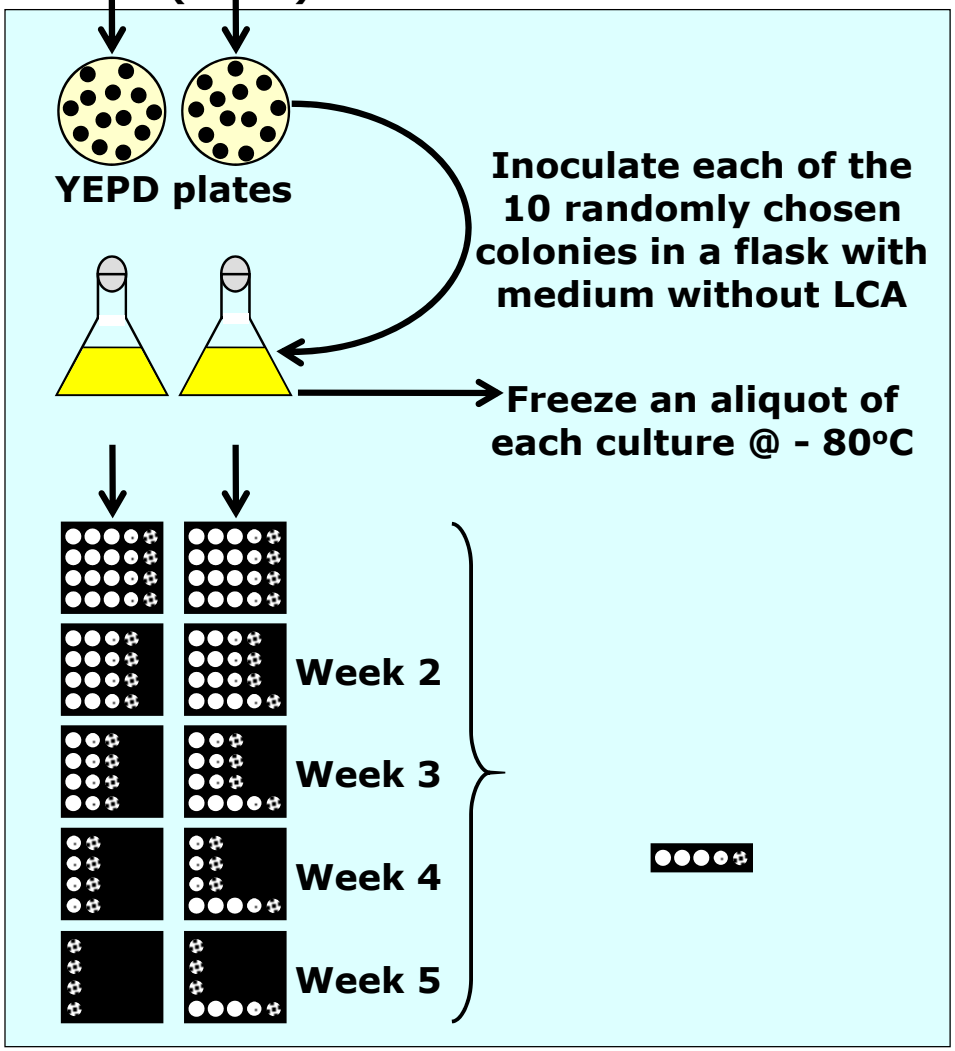
Sample 1: no LCA

Sample 2: 5 µM LCA

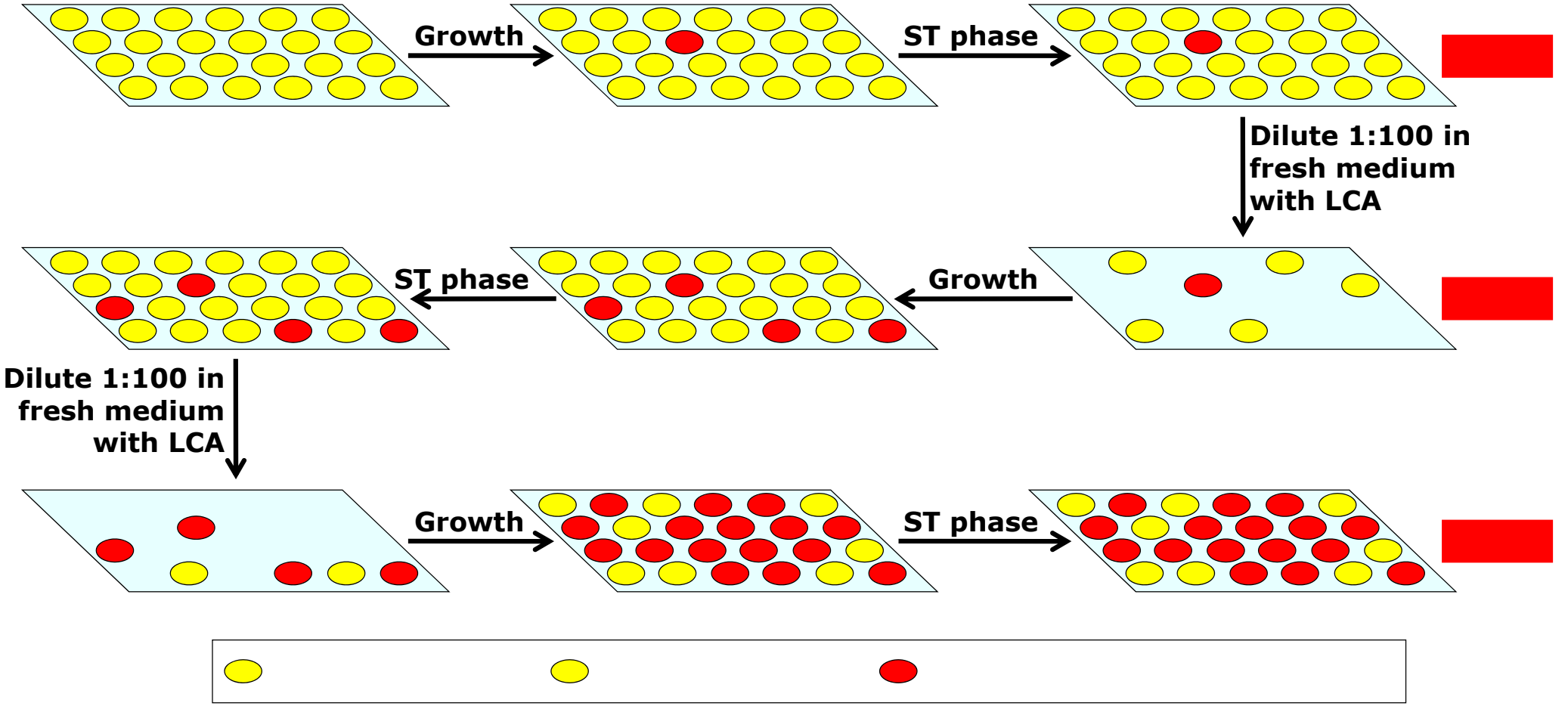
Sample 3: 50 µM LCA

Sample 4: 250 µM LCA

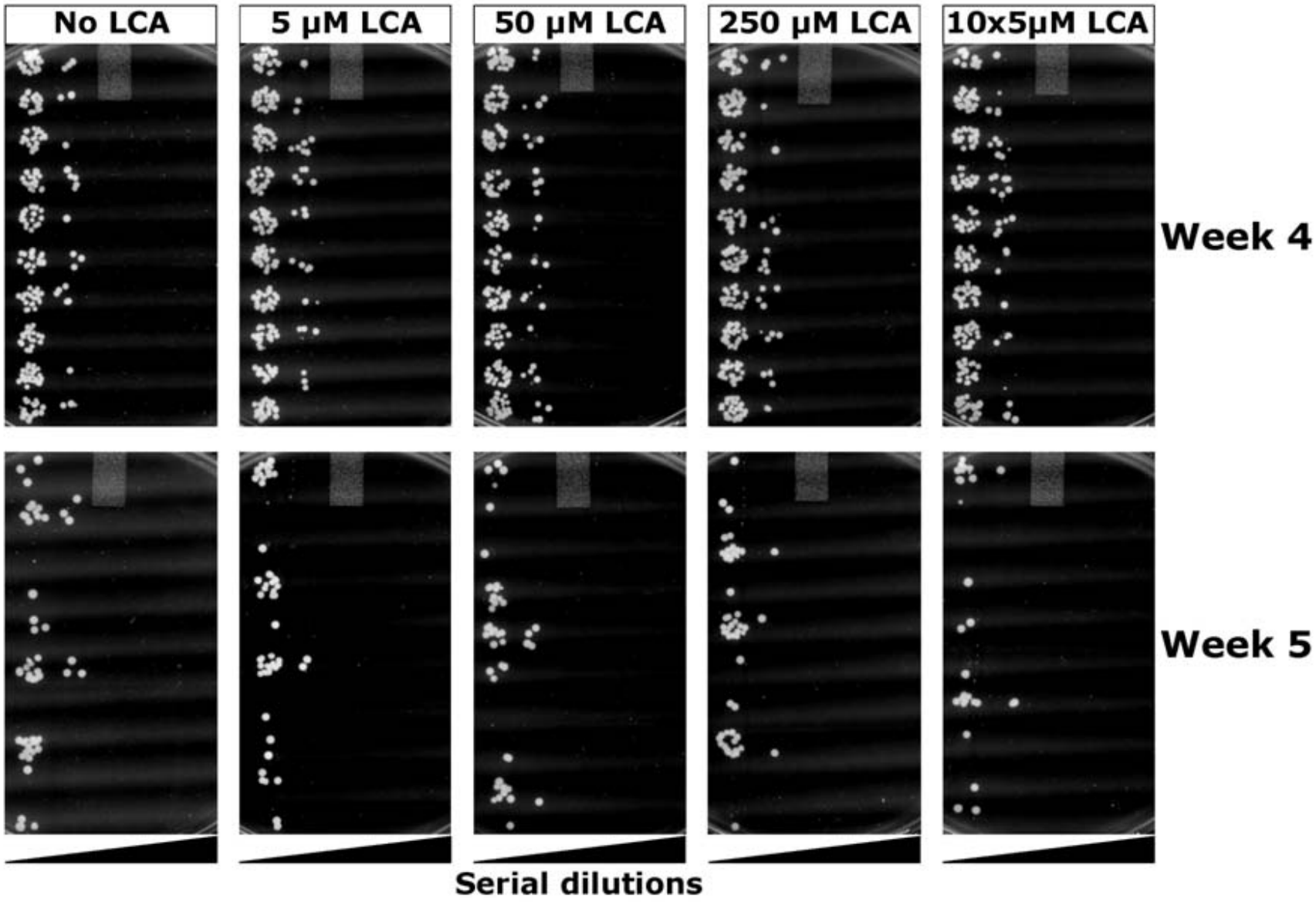
Sample 5: 10 doses x 5 µM LCA added every 3-4 days



- **The fraction of long-lived mutants in a population of yeast is increased by the end of each of the 3 steps of the LCA-driven experimental evolution**

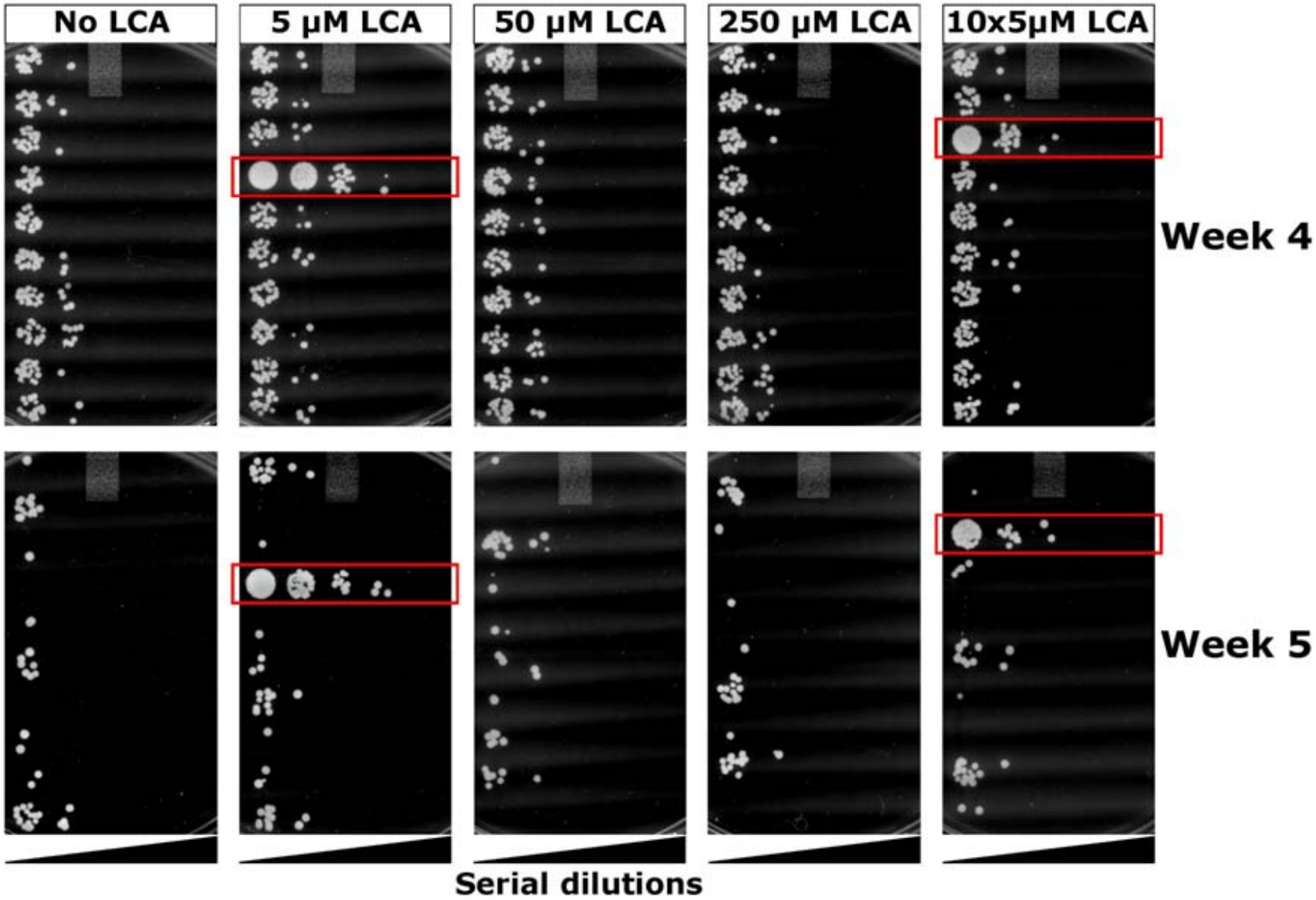


❑ The 1st step of the LA-driven experimental evolution of longevity regulation mechanisms in yeast



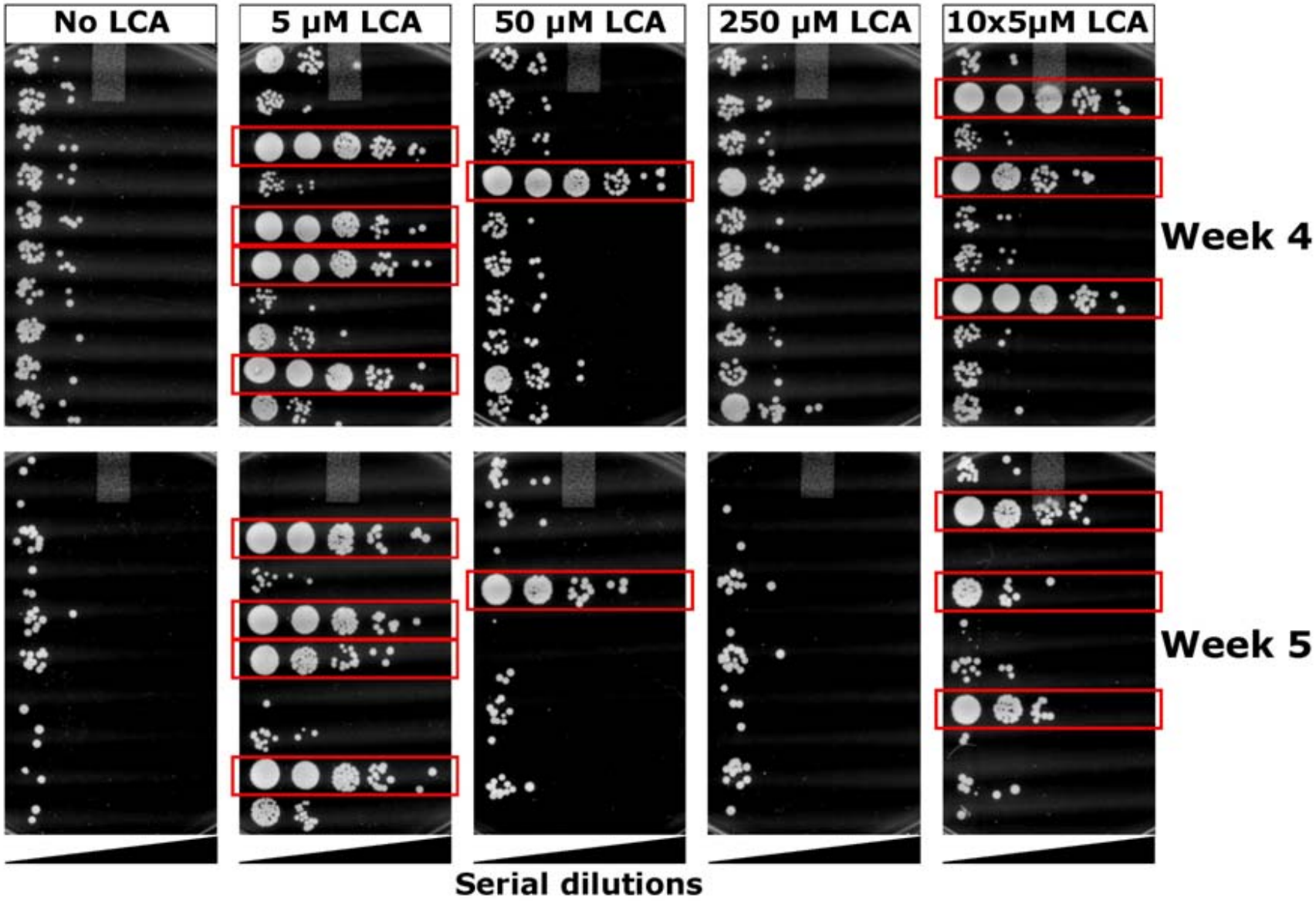
☯ **<u>No</u> long-lived mutants have been found!**

❑ The 2nd step of the LA-driven experimental evolution of longevity regulation mechanisms in yeast



Long-lived mutants!

The 3rd step of the LA-driven experimental evolution of longevity regulation mechanisms in yeast



Long-lived mutants!

Conclusions:

- **A long-term exposure of wild-type yeast to LCA under laboratory conditions results in selection of yeast species that live longer in the absence of LCA than their ancestor**

- **The order of different LCA concentrations ranked by the efficiency with which they cause the appearance of long-lived species (frequencies of such appearance are shown):**

- **5 μM LCA (~ 4 x 10^8/generation) > 10 doses x 5 μM LCA (~ 3 x 10^8/generation) > 50 μM LCA (~ 1 x 10^8/generation) > 250 μM LCA (no long-lived species found)**

- **Because the lowest used concentration of LCA results in the highest frequency of long-lived species appearance, it is unlikely that the life-extending mutations they carry are due to mutagenic action of LCA**

Future perspectives:

- **What genes are affected by mutations responsible for the extended longevity of selected long-lived yeast species?**

- **How these mutations influence the "housekeeping" longevity-related processes modulated by LCA in chronologically aging yeast?**

- **Do these mutations affect the growth rate of yeast in media with or without LCA?**

- **Will selected long-lived yeast species be able to maintain their ability to live longer than wild-type yeast if they undergo several successive passages in medium without LCA? [Is there selective pressure aimed at maintaining of an "optimal" rather than a "maximal" chronological life span of yeast?]**

- **If mixed with an equal number of wild-type yeast cells, will selected long-lived yeast species out-grow and/or out-live them in medium without LCA or the opposite will happen?**

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Faculty of Arts and Science

CIHR IRSC
Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

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