Minireview

Antagonizing Methuselah to extend life span

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Abstract

A recent report describes the identification through the use of *in vitro* selection of a peptide that antagonizes Methuselah signaling in *Drosophila in vitro* and extends fly life span *in vivo*.

Like their biblical namesake, *methuselah*^{1} (*mth*^{1}) mutant *Drosophila melanogaster* significantly outlive their wild-type peers. Aging, observed on a population level as a time-dependent increase in the probability of death, is delayed in homozygous *mth*^{1}/*mth*^{1} flies, resulting in an approximately 35% increase in average and maximum life span of males at 29°C, the basis on which *mth* was identified and named [1]. Almost a decade after the first report of the *mth*^{1} mutant, the function of the *mth* gene product and the mechanism by which it extends life span are still under intensive investigation, with the most recent study, by Ja and co-workers [2], reporting an antagonist of *mth* function that extends life span.

The longevity phenotype of *mth*^{1} has been observed to depend on the sex of the flies, the temperature at which the experiment is conducted [3], food source and mating status [4], and is greatest in males at 29°C on a highly nutritious medium. Life-span extension is accompanied by a reduction in reproductive output, sensitivity to cold [3] and increased resistance to a superoxide-generating drug (paraquat), heat stress or starvation [1,4]. A clue to the cellular role of *mth* comes from observations that *mth* controls synaptic efficacy at neuromuscular junctions [5]. Furthermore, *mth* plays an essential role in the fly, as null alleles are not viable [1]. How these phenotypes are connected, as well as the nature of their interaction with the environment, remain unclear. To answer these questions, an insight into the molecular and cellular functioning of the Methuselah protein (Mth) is required.

mth encodes a G-protein coupled receptor (GPCR) [1]. GPCRs comprise a large family of integral membrane proteins that respond to extracellular cues by activating intracellular signaling pathways through the regulation of heterotrimeric G proteins. GPCRs have a common protein architecture, with an amino-terminal extracellular domain, which is held to be mainly responsible for ligand binding, followed by seven transmembrane domains, linked by three extra- and three intracellular loops, and an intracellular carboxy-terminal domain [6]. The native ligand of Mth, the product of the *stunted* (*sun*) gene, has been identified and *sun* mutants also display an extension of life span [7]. The signaling pathway within which Mth functions has not yet been elucidated.

mth is a part of a larger puzzle, as it is now clear that alterations in many genes encoding signal transduction proteins result in longevity. Indeed, it appears that several interacting signal-transduction pathways, including insulin/insulin-like growth factor, target of rapamycin (TOR) and Jun N-terminal kinase (JNK) signaling pathways, control animal physiology in such a way that modulating them can extend life span [8-11]. Mutations in the components of these pathways in model organisms such as *D. melanogaster* are being used intensively to probe the biology of aging. Unfortunately, there is often a wide gap between the wealth of knowledge of the genetics of the pathways and their biochemical functioning within a given model organism. In their recent publication in *Nature Chemical Biology*, Ja *et al.* [2] make a significant contribution to bridging that gap for *Drosophila* Mth.

Creating a Methuselah antagonist

Ja *et al*. [2] set off to uncover artificial ligands for Mth, peptides that would interact with its ectodomain and hence might modulate the activity of the receptor, using an *in vitro* selection method developed previously [12]. The authors created a DNA library encoding random peptides of 27 amino acids. The library was transcribed *in vitro* and the resulting mRNAs fused to a peptide acceptor at their 3' ends. During the subsequent *in vitro* translation of the messages, the ribosomes were cheated into covalently attaching the nascent peptide onto its cognate mRNA. Hence, the chimeric molecules in the library contained both the functional part (the peptide) that allowed selection based on its capacity to bind the purified Mth ectodomain, and the informational part (mRNA) that allowed for amplification of the selected peptides. The authors started with an initial library containing approximately 10^{13} peptides and performed eight rounds of enrichment and amplification. To reduce the occurrence of 'sticky' peptides that may be nonspecifically retained on the Mth-binding column, the last four rounds included pre-clearing of peptides and specific elution with free Mth.

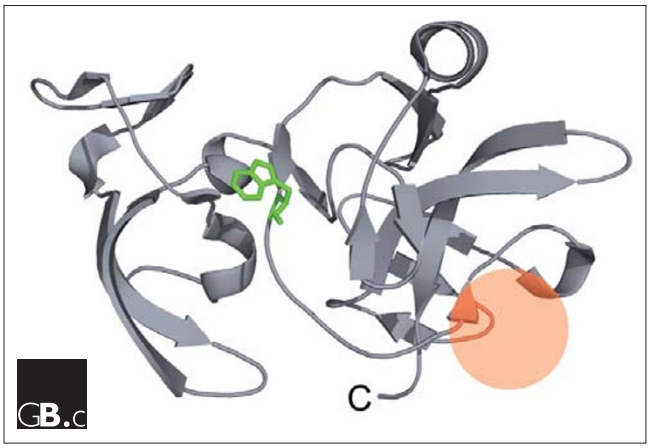

Figure 1
The location of RWR peptide binding to the Mth ectodomain. The Mth ectodomain structure [13] was visualized with PyMOL (PyMOL Molecular Graphics System). The tryptophan (W) residue previously thought to be positioned at the ligand-binding site is indicated in green, and the region near the carboxyl terminus to which the RWR peptide was located by Ja *et al*. [2] is indicated by an orange circle.

After the last round of amplification, Ja *et al*. [2] recovered ten unique peptides with high binding affinities for the Mth ecodomain (K_d values as low as 18 nM). Interestingly, all the peptides contained a simple (R/P)XXWXXR motif and, by mutation of the tryptophan (W) or the initial arginine (R) in one of the peptides, the authors demonstrated that this motif is required for binding. The simplicity of the motif precluded any meaningful identification of *Drosophila* proteins as potential native ligands.

The peptides competed with each other for Mth-ectodomain binding, indicating that they bind at the same site. The authors obtained the crystal structure of the Mth ectodomain bound to an RWR peptide, which revealed the binding to occur near the carboxyl terminus of the ectodomain (Figure 1), at a site different from the ligand-binding site previously proposed on the basis of the structure of the Mth ectodomain alone [13]. The RWR peptides competed with the amino-terminal portion of Sun (N-Sun), indicating that the native ligand may also bind the carboxy-terminal part of the ectodomain. Because the competition may have occurred through allosteric interactions between two separate sites, further work is required to establish the binding site of the native ligand. Experiments in cell culture showed that an RWR peptide can bind the whole Mth receptor presented on the cell surface and act as an antagonist of Mth activation by its native ligand, the latter revealed by a reduction in N-Sun-induced Mth-dependent calcium mobilization.

As well as providing an important insight into the molecular functioning of the receptor, the identification of a peptide antagonist of Mth demonstrates that peptides with biologically relevant activity towards GPCRs, an important class of drug targets [14], can be obtained by *in vitro* selection of mRNA-peptide fusions. Furthermore, a multitude of orphan GPCRs are present in animal genomes [15] and the elucidation of their function will be aided by an unbiased identification of modulators of their activity. Most importantly for aging research, Ja and co-workers [2] have provided us with a biochemically characterized tool for the examination of the *in vivo* function of Mth.

Methuselah antagonist and life span

The authors initiated the examination of the effects of the Mth antagonist *in vivo*. They expressed an RWR peptide under the control of the GAL4 transcription factor that was in turn expressed from the *daughterless* promoter. This ubiquitous expression of the antagonist led to extension of life span of males at 29°C and 25°C. The result indicates that the RWR peptide can antagonize the activity of Mth *in vivo*. Indeed, mutations in the RWR motif, critical for binding to Mth *in vitro*, abolished the life span extension. It is still possible that the peptide may act through another GPCR, however, as the specificity of the peptide for Mth among *Drosophila* GPCRs, including 12 Mth paralogs, is unknown, making it important to examine the genetic interactions between *mth* and its agonist and antagonist. It will also be reassuring to see that the effect of the antagonist on life span is robust to placing the transgenes into a standard genetic background.

The authors carried out the life-span experiment under conditions in which the effect of modulating Mth activity should be most pronounced. Because the effect of *mth*[1]

appears to depend on environmental conditions, mating status and the sex of the flies, it will be interesting to expand on the initial findings of Ja and co-workers [2], including the examination of the effects of the antagonist in different genetic backgrounds. Indeed, the antagonist may prove useful in establishing mechanistic links between the environmental conditions and *mth*[1] phenotypes, as well as the connections between different phenotypes.

In the system the authors used, the peptide was not targeted for excretion, while the receptor binding site is extracellular. This would imply that the peptide engages the receptor before extracellular presentation of the ectodomain. If this is the case, the antagonist will only affect the Mth in the cell that expresses it: it will be acting autonomously. It will be interesting to know if the secreted version of the peptide can act in a cell-nonautonomous manner. And conversely, the system that is described by Ja *et al.* [2] may provide a tool for the examination of physiological outcomes of tissue-restricted Mth inhibition.

At least 30% of currently available drugs act on GPCRs [14]. Ja and co-workers [2] demonstrated that a targeted design of an artificial modulator of GPCR activity could extend life span. Although Mth has no homologs in humans, is it likely that a modulator of a signaling pathway could be used as a drug to delay aging? It will probably not be as simple as that. Signaling pathways that affect aging in model organisms have pleiotropic effects. Many of these would be undesirable in humans, including the reduced reproductive output in *mth*[1] flies, or the diabetic phenotypes in long-lived flies with reduced production of insulin-like peptides [16]. Furthermore, *mth*[1] is an interesting example of the fact that a longer life span need not mean a healthier old age; the delay in aging, observed in the *mth*[1] mutants on a population level, appears to be uncoupled from a delay in age-related functional decline in olfaction and motor activity [17,18]. Much further work is required to gain a detailed understanding of the molecular mechanisms that underlie ageing, and the reagents developed by Ja and co-workers [2] will help just that.

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References

1. Lin YJ, Seroude L, Benzer S: **Extended life-span and stress resistance in the *Drosophila* mutant *methuselah*.** *Science* 1998, **282:** 943-946.
2. Ja WW, West AP Jr, Delker SL, Bjorkman PJ, Benzer S, Roberts RW: **Extension of *Drosophila melanogaster* life span with a GPCR peptide inhibitor.** *Nat Chem Biol* 2007, **3:**415-419.
3. Mockett RJ, Sohal RS: **Temperature-dependent trade-offs between longevity and fertility in the *Drosophila* mutant, *methuselah*.** *Exp Gerontol* 2006, **41:**566-573.
4. Baldal EA, Baktawar W, Brakefield PM, Zwaan BJ: ***Methuselah* life history in a variety of conditions, implications for the use of mutants in longevity research.** *Exp Gerontol* 2006, **41:**1126-1135.
5. Song W, Ranjan R, Dawson-Scully K, Bronk P, Marin L, Seroude L, Lin YJ, Nie Z, Atwood HL, Benzer S, *et al.*: **Presynaptic regulation of neurotransmission in *Drosophila* by the G protein-coupled receptor *methuselah*.** *Neuron* 2002, **36:**105-119.
6. Harmar AJ: **Family-B G-protein-coupled receptors.** *Genome Biol* 2001, **2:**reviews3013.1-3013.10.
7. Cvejic S, Zhu Z, Felice SJ, Berman Y, Huang XY: **The endogenous ligand Stunted of the GPCR Methuselah extends lifespan in *Drosophila*.** *Nat Cell Biol* 2004, **6:**540-546.
8. Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS: **A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function.** *Science* 2001, **292:**107-110.
9. Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, Leevers SJ, Partridge L: **Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein.** *Science* 2001, **292:**104-106.
10. Kapahi P, Zid BM, Harper T, Koslover D, Sapin V, Benzer S: **Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway.** *Curr Biol* 2004, **14:**885-890.
11. Wang MC, Bohmann D, Jasper H: **JNK signaling confers tolerance to oxidative stress and extends lifespan in *Drosophila*.** *Dev Cell* 2003, **5:**811-816.
12. Roberts RW, Szostak JW: **RNA-peptide fusions for the in vitro selection of peptides and proteins.** *Proc Natl Acad Sci USA* 1997, **94:**12297-12302.
13. West AP Jr, Llamas LL, Snow PM, Benzer S, Bjorkman PJ: **Crystal structure of the ectodomain of Methuselah, a *Drosophila* G protein-coupled receptor associated with extended lifespan.** *Proc Natl Acad Sci USA* 2001, **98:**3744-3749.
14. Wise A, Gearing K, Rees S: **Target validation of G-protein coupled receptors.** *Drug Discov Today* 2002, **7:**235-246.
15. Howard AD, McAllister G, Feighner SD, Liu Q, Nargund RP, Van der Ploeg LH, Patchett AA: **Orphan G-protein-coupled receptors and natural ligand discovery.** *Trends Pharmacol Sci* 2001, **22:**132-140.
16. Broughton SJ, Piper MD, Ikeya T, Bass TM, Jacobson J, Driege Y, Martinez P, Hafen E, Withers DJ, Leevers SJ, *et al.*: **Longer lifespan, altered metabolism, and stress resistance in *Drosophila* from ablation of cells making insulin-like ligands.** *Proc Natl Acad Sci USA* 2005, **102:**3105-3110.
17. Cook-Wiens E, Grotewiel MS: **Dissociation between functional senescence and oxidative stress resistance in *Drosophila*.** *Exp Gerontol* 2002, **37:**1347-1357.
18. Petrosyan A, Hsieh IH, Saberi K: **Age-dependent stability of sensorimotor functions in the life-extended *Drosophila* mutant *methuselah*.** *Behav Genet* 2007, **37:**585-594.