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Addendum

Autophagy and Insulin/TOR Signaling in *Caenorhabditis elegans pcm-1* Protein Repair Mutants

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Addendum to:

The L-Isoaspartyl-O-Methyltransferase in Caenorhabditis elegans *Larval Longevity and Autophagy*

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ABSTRACT

Biological responses due to nutrient deprivation in the nematode *Caenorhabditis elegans*, including L1 diapause and autophagy during dauer formation, can be mediated through the linked DAF-2/insulin/IGF receptor and target-of-rapamycin (TOR) kinase pathways. Here we discuss how altered insulin/TOR signaling may underlie the previously reported phenotypes of worms with a null mutation in the *pcm-1* gene that results in reduced autophagy during dauer formation and decreased L1 arrest survival. PCM-1 encodes a protein repair methyltransferase and mutants of the encoding *pcm-1* gene are incapable of converting spontaneously damaged l-isoaspartyl residues in cellular proteins to normal forms by this pathway. We speculate that PCM-1 may function either directly or indirectly as an inhibitor of insulin/TOR signaling, perhaps in a role to balance autophagy with alternative protein degradation pathways that may be more specific for recognizing age-damaged proteins.

AUTOPHAGY AND SURVIVAL IN ARRESTED LARVAE

When a *C. elegans* egg hatches, the new L1 larvae must process environmental cues to determine if it will be able to progress in development to the adult form. If an L1 larvae comes into an environment with no food, development will stop and the larvae can survive for a period of weeks to await more favorable conditions.[1] However, if there is a limited food supply and pheromone present, the hatchling can develop into an alternate third larval stage known as the dauer larvae. Dauer larvae do not eat nor defecate, store large amounts of fat in their intestine, and live for a period of months whereas an adult nematode can only live for a few weeks.[2] If environmental conditions improve, both arrested L1 and dauer larvae may exit their diapauses and resume reproductive development.

It had previously been determined that *pcm-1*-mutant *C. elegans,* animals deficient in the highly conserved PCM-1 protein L-isoaspartyl-*O*-methyltransferase that repairs one of the most common forms of protein damage, the unfavorable formation of L-isoaspartyl residues, contain the following phenotypes: they are selected against in long-term, competitive population studies, they have a reduced dauer life span, and they have defects in dauer formation.[3,4] Interestingly, no differences in the accumulation of damaged proteins has been detected in *pcm-1* mutants compared to wild-type animals, suggesting that enhanced proteolytic pathways may be involved that minimize the accumulation of altered protein species even in the absence of PCM-1 catalyzed-repair.[5]

Recently, we have shown that the L-isoaspartyl-*O*-methyltransferase in *C. elegans* plays a role in the survival of L1 larvae and the ability to perform autophagy in dauer larvae.[6] *pcm-1* mutations decrease arrested-L1 larval survival under starvation but have no effect on L1 recovery when food is present.[6] These data suggest that the *pcm-1*-mutant L1 larvae are not defective in utilizing added nutrients but are defective in recycling their aged proteins in proteolytic processes. We then found that the *pcm-1* mutation inhibits normal levels of autophagy during dauer entry.[6] We also showed a pheromone-induced autophagosomal accumulation in wild-type L2d larvae, which increases with time as the L2d larvae approach the dauer molt. The massive cellular remodeling that occurs prior to the dauer molt may thus be dependent in part on autophagy. Since this process recycles nutrient supplies to increase survival,[7] autophagy may be important for the formation of proper dauer larvae, and for survival during both a dauer and L1 larvae diapause. The demonstration of a novel link between the protein-repair methyltransferase and autophagy[6] complements a recent study that concluded that autophagy is required for longevity.[8]

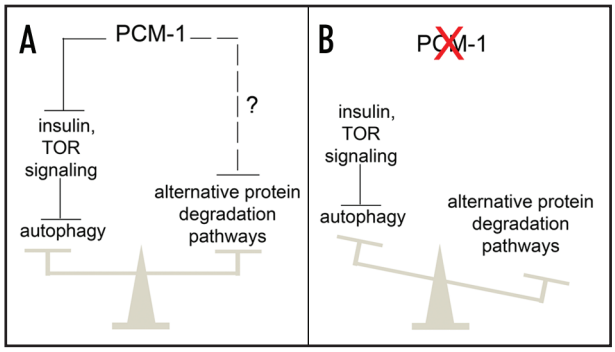

Figure 1. PCM-1 may help maintain a homeostatic balance for the metabolism of damaged proteins in *C. elegans*. In wild-type worms (A), the presence of the protein repair enzyme PCM-1 inhibits both insulin/TOR signaling, allowing for autophagy to occur in concert with alternative protein degradation pathways that may be specialized for the removal of proteins with spontaneous age-damage. In a *pcm-1* mutant lacking the repair methyltransferase (B), the balance of the two processes is shifted to decrease autophagy and increase the activity of the alternative protein degradation pathways to prevent the accumulation of damaged proteins no longer repaired by PCM-1.

Table 1 **Comparison of *pcm-1* and *daf-2* mutant phenotypes**

	Physiology in Comparison to Wild Type	
Condition	***pcm-1* mutant**	***daf-2* mutant**
L1 Starvation Lifespan	reduced[6]	increased[1]
Dauer Formation	daf-d[4]	daf-c[18]
Dauer Fat Storage	reduced[a]	increased[17]
Dauer Formation Autophagy	reduced[6]	increased[16]
Adult Lifespan	normal[3,4]	increased[18]

[a]Gomez TA, Clarke SG, unpublished results.

THE BALANCE BETWEEN PROTEIN DEGRADATION AND REPAIR

pcm-1 mutants do not show an increase in damaged l-isoaspartyl residue accumulation relative to wild-type worms, either in adults,[5] dauer larvae[5] or L1 larvae (Gomez TA, Lowenson JD, Clarke SG, unpublished). These results suggest that *pcm-1* mutants compensate for the lack of repair by upregulating other pathways of protein turnover.[5] Thus, it appears paradoxical that the *pcm-1* mutation results in defects in autophagy.[6] However, there are multiple pathways for protein degradation in cells[9-13] and it is possible that under conditions where specific types of damaged proteins accumulate, specific types of proteolytic systems are activated. Hence, the lack of protein repair in the *pcm-1* mutant may cause the animal to try to compensate for this loss by activating non-autophagosomal pathways of protein degradation. Interestingly, in the *C. elegans* model of polyglutamine expansion disease, autophagy and the proteasome work in conjunction to clear the cell of polyQ repeats.[14]

In the case of the *pcm-1 C. elegans* mutant, the cellular imbalance between autophagy and other forms of cellular clearance may not be detrimental, at least in adults as it does not cause distinguishable phenotypes. However, under stress, the homeostatic balance between the cellular cleansing mechanisms may be more critical.

PCM-1 HAS AN INHIBITORY EFFECT ON INSULIN SIGNALING?

We have been interested in the possibility that the PCM-1 protein repair methyltransferase may directly or indirectly inhibit insulin/IGF/TOR signaling. In knockout mice lacking the repair enzyme (*Pcmt1*$^{-/-}$), there is a marked increase in insulin receptors and activated downstream components.[15] If a similar situation occurs in *C. elegans*, such activation would be expected to inhibit autophagy (as has been observed)[6] because inactivating *daf-2* mutants show increased autophagy (Fig. 1).[16] It is also noteworthy that the phenotypes of *pcm-1* mutants are the opposites of those found in *daf-2* mutants (Table 1). We speculate that the PCM-1 repair methyltransferase may inhibit insulin signaling to ensure an appropriate balance between general protein degradation mechanisms such as autophagy, and perhaps more specialized proteolytic processes that may only be activated when repair is not possible (Fig. 1).

It appears plausible that *pcm-1* mutants fare more poorly under stressful conditions due to improper levels of insulin signaling. Low insulin signaling has been attributed to life span extension in *C. elegans* and the dauer formation genes/insulin signaling genes are important in autophagy and the regulation of aging.[8,16-18] Additionally, a recent study shows that DAF-16/FOXO is required for proper L1 arrest and starvation survival.[19] Without *pcm-1*, insulin signaling may be too high and possibly out of balance with other cellular processes, causing compromised survival.

PROTEIN REPAIR, INSULIN/IGF AND PROTEOLYTIC PATHWAYS IN OTHER ANIMALS

Mice deficient in the l-isoaspartyl protein repair methyltransferase can accumulate damaged proteins, but this accumulation becomes limited by increased proteolytic mechanisms.[20] It will be very interesting to see how the situation in mammals may parallel that found in worms. In mice, there is a clear activation of the PI 3-kinase/Akt kinase pathway in repair-deficient animals;[15] such activation has been suggested by the phenotypes of *pcm-1* mutant worms but has not been biochemically confirmed. In bovine aortic endothelial cells, links between the PI 3-kinase/Akt kinase pathway, proteasomal activity, and repair methyltransferase activity have been reported.[21] Finally, it is interesting that overexpression of the repair methyltransferase in flies leads to an extended life span,[22] a result that is consistent with the attenuation of the PI 3-kinase/Akt kinase pathway. Further work is clearly called for to examine the extent of the physiological conservation of protein repair and degradation balance mechanisms in worms, flies, and mice.

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