Mechanisms regulating proteostasis are involved in sympatric speciation of the blind mole rat, *Spalax galili*

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**ABSTRACT**

Genome-wide analysis demonstrates extensive genomic adaptive complexes involved in sympatric speciation between blind mole rats (*Spalax galili*) in abutting populations living in basalt and chalk soils. Among the gene ontology (GO) enrichment, musculature and metabolism stood out in basalt dwellers while nutrition and neurogenetics were highlighted in chalk residents. Measurements of mechanisms regulating protein homeostasis inspired by these GO terms suggest that at the proteomic level there is also a habitat/soil-type driven divergence with the basalt residents exhibiting higher proteasome activity whereas elevated levels of markers of autophagy are evident in the chalk inhabitants.

Sympatric speciation, the origin of new species in a free-breeding population, or contiguous populations, with gene flow, is still a very divisive subject in the field of evolution. However, natural selection can sometimes exert a stronger influence than gene flow, causing adaptation within a species in response to divergent ecological forces. Due to the abundance of sharp contrasts in climate and biota even in abutting ecological habitats, sympatric speciation may occur. One such location where in evolutionarily distinct living organisms documented instances of this mode of speciation have taken place is "Evolution Canyon," Mount Carmel, Israel. Living in this milieu, species of 5 distant taxa including bacteria, wild barley, fruit flies, beetles, and spiny mice have all exhibited this phenomenon. In the present study, genomics and protein biochemistry were examined in 2 adjacent populations of the blind mole-rat (*Spalax galili*), one the ancestral Senonian population living in chalk soils and the other residing in basalt soils.

We discovered distinct differences in protein degradation mechanisms in the leg muscle of blind mole rats from these 2 adjacent, albeit divergent, populations. The samples from the basalt-dwelling *Spalax* showed greater proteasome activity as measured by 3-fold greater chymotrypsin-like, and 2-fold greater trypsin-like and caspase-like activities. Further, the basalt dwelling animals had higher protein expression of PSMA7 (proteasome subunit \(\alpha 7\)) than did the chalk inhabitants, suggesting higher numbers of proteasome catalytic core particles. In contrast, the population living in chalk soil showed significantly higher protein levels of the autophagy protein ATG7 and of the measure of autophagic flux, based on the LC3-II/LC3-I ratio. The divergent reliance on separate mechanisms for proteolytic degradation in animals residing in different soil types might suggest that the basalt dwelling population maintains proteostasis through the more efficient removal of short-lived, damaged or misfolded proteins. The greater level of chymotrypsin-like activity vs. the other 2 types of activity could also imply more substrates that are damaged or misfolded, as this activity preferentially degrades after hydrophobic residues. In the mole rat population living in chalk soils there might be greater accumulation of aggregates, or a need to degrade longer-lived proteins or organelles, and hence the increase in autophagy.

The GO terms enriched in the genomic analyses point toward these possibilities. The chalk dwelling population, living in a nutrition-compromised environment with low food resources showed enrichment for lipid metabolism, including lipid transport, and homeostasis. In the basalt-dwelling population, enrichment of genes associated with muscle remodeling, which is dependent on proper proteasome function, could have been instigated by greater digging in the harder basalt soil (Fig. 1). Likewise, the sensory related G-protein coupled receptor genes enriched in the basalt mole rat population could be another substrate target of proteasome-mediated degradation. These genes important both in skeletal muscle repair and sensory function could have been selected to allow these animals, dwelling in the basalt soil to adapt better to their new environment.

As a byproduct of this increase in proteasome activity, removal of damaged and misfolded proteins could have...
increased (as evidenced by higher chymotrypsin-like activity over the other activities), thereby improving the quality of the proteome and the maintenance of proteostasis. This could help the cells to resist oxidative and other stressors, and in turn increase cellular health. A more detailed analysis of the ubiquitinome and other substrates degraded by the proteasome could give valuable insight to explain the mechanism behind these findings. Examination of aggresomes to also characterize autophagic substrates in the muscle tissues of chalk-dwelling mole rats could help to strengthen the speculation that lipid metabolism and neurogenesis are linked to this degradative process.

Other underground living and burrowing animals, such as the naked mole rat, also possess high levels of proteasome activity and markers of autophagy in multiple tissues. Increase in protein degradation mechanisms could be a phenotype linked to the underground milieu in which both these species have evolved. In the Spalax species, further examination of tissue from other organs could determine if these observations are specific to the muscle or systemic to the whole animal. Perhaps some tissues are more sensitive to environmental change, and could influence speciation. Moreover, it will be critical to investigate how this change in the type of stress that animals experience moving from the chalk to the basalt environment, reprograms the proteomic environment in muscle and other tissues, to enhance or suppress a particular mode of degradation. Finally, investigation of proteasome activity and autophagy in the tissues or cells from other animals that have shown sympatric speciation at Mount Carmel could determine if changes in protein degradation mechanisms universally influence evolution.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.