COMMENTARY

Metabolic role for yeast DJ-1 superfamily proteins

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The DJ-1 superfamily comprises a mysterious assortment of proteins whose functions have been surprisingly difficult to elucidate. These diverse proteins are present in most organisms and are often associated with stress response; however, the biochemical mechanisms of only a few have been clearly established. Interest in this superfamily has been driven primarily by the involvement of human DJ-1 in rare forms of heritable parkinsonism (1). In addition, human DJ-1 is a weak oncogene (2) and has also been implicated in ischemia-reperfusion injury (3), making it an appealing target for understanding multiple diseases at the molecular level. Consequently, several model systems have been used to study the function of DJ-1 and its homologs, with an emerging consensus that animal DJ-1 is protective against oxidative stress and mitochondrial dysfunction by modulating the activities of multiple cytoprotective pathways (4). However, the details of DJ-1's mechanism are actively debated, even after years of study. *Saccharomyces cerevisiae* has four homologs of DJ-1 (Hsp31, Hsp32, Hsp33, and Hsp34), the latter three of which are ∼99% identical to each other at the amino acid level. Therefore, this venerable model system presents some peculiar technical challenges to the study of DJ-1 superfamily proteins. The comprehensive study by Miller-Fleming et al., reported in PNAS, addresses these complications and finds that the *S. cerevisiae* Hsp31 minifamily plays a key role in the transition of proliferating yeast cells through diauxic shift then into a nonproliferative state known as stationary phase (5). The authors report that yeast Hsp31 family proteins are candidate upstream modulators of target of rapamycin complex 1 (TORC1), an important kinase that regulates nutrient sensing and cell proliferation and has been implicated in the function of human DJ-1 as well. Miller-Fleming et al. provide both a wealth of new data and a set of testable hypotheses that promise to drive the study of yeast Hsp31 proteins in the future.

The Prior Understanding of Hsp31-Like Proteins

Despite sharing a conserved core fold and some key residues, DJ-1 superfamily proteins differ widely in structure, oligomerization, and function. Consequently, the DJ-1 superfamily can be divided into multiple clades with distinct presumed functions (6, 7). Some of these proteins are degradative cysteine proteases, whereas others are transcription factors, isocyanide hydratases, glyoxalases, and chaperones. However, most of these proteins are of unknown function. The Hsp31 proteins compose one clade of the DJ-1 superfamily and are abundant in bacteria and fungi but rare in animals. *Escherichia coli* Hsp31 and *S. cerevisiae* Hsp31 (23% amino acid sequence identity) share similar structures and a conserved set of catalytic residues centered on a cysteine that is present in nearly all DJ-1 superfamily proteins (8, 9). *E. coli* Hsp31 is a validated chaperone (10) and has recently been shown to also be glyoxalase III, a glutathione-independent glyoxalase that detoxifies methylglyoxal produced by central metabolism (11). Fungal Hsp31 proteins from *Candida albicans* and *S. cerevisiae* have also been shown to be glutathione-independent glyoxalases (12), although there are presently no published reports of chaperone activity for yeast Hsp31. Both yeast and bacterial Hsp31 proteins are strongly expressed as cells enter stationary phase, a nonproliferative state characterized by global shifts in metabolism and stress response that conspire to maintain cell viability in nutrient-depleted environments. A prior study has shown that the Hsp31 knockout in *S. cerevisiae* is more sensitive to oxidative stress (13), although studies of an *E. coli* Hsp31 knockout have not found a strong oxidative stress phenotype (14). Therefore, fungal and bacterial Hsp31 exhibit considerable but not complete functional overlap, consistent with their membership in a single clade of the DJ-1 superfamily.

Miller-Fleming et al. have identified robust phenotypes and cellular roles for the yeast Hsp31-like proteins.

Given the interest in DJ-1 superfamily proteins, why have there been so few prior studies about the function of DJ-1 superfamily members in *S. cerevisiae*? As the premier model system for lower eukaryotes, *S. cerevisiae* would reasonably have been expected to be the first model organism to have its DJ-1 superfamily members studied closely. One possible reason is that *S. cerevisiae* contains only Hsp31-like members of the DJ-1 superfamily but not closer homologs of the human protein, which belong to another clade. A second and compelling technical reason is that three of the four DJ-1 homologs in *S. cerevisiae* (Hsp32, Hsp33, Hsp34) are over 99% identical to each other, and thus are difficult to knock out individually. Work on these proteins has also been hampered by a related concern about functional compensation of the remaining Hsp31-like genes in single knockout strains. Miller-Fleming et al. (5) successfully obtained three of the four knockouts (Hsp34 remains problematic) and then characterized each using microarray transcriptomics. The authors found that the subset of transcripts perturbed in all three knockouts encode proteins involved in translation, temperature stimulus response, oxidative stress response, and carbon metabolism. These processes are profoundly altered as cells exhaust glucose in the medium during diauxic shift and prepare for nonproliferative survival in stationary phase. Moreover, transcription of the *Hsp31*-like genes is also greatly elevated during diauxic shift and stationary phase, suggesting a direct connection between the function of the Hsp1-like proteins and metabolic reprogramming that occurs during entry into stationary phase.

Author contributions: M.A.W. wrote the paper.

The author declares no conflict of interest.

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Hsp31 is Important for Viability in Stationary

The transition to stationary phase is also accompanied by global changes in yeast physiology, including thickening of the cell wall, up-regulation of autophagy, and enhanced oxidative stress defense. Yeast lacking Hsp31-like proteins are impaired in all of these processes, demonstrating their importance in diauxic shift and stationary phase (5). Furthermore, Hsp31 knockouts do not survive as long as wild-type cells once in stationary phase, measured using the chronological lifespan of the cell. Of these phenomena, the connection between yeast Hsp31 and oxidative stress is especially intriguing, because animal DJ-1 also protects against oxidative stress and suppresses the generation of reactive oxygen species (ROS) (15, 16). Diauxic shift in yeast involves a transition from fermentative metabolism driven by glycolysis to respiratory metabolism driven by oxidative phosphorylation, thereby elevating levels of mitochondrially derived ROS. Presumably, defects in the diauxic shift resulting from the absence of Hsp31 result in improper management of ROS produced by oxidative phosphorylation, thereby causing oxidative stress. Importantly, this switch from fermentative to oxidative metabolism only occurs in certain yeasts (e.g., *S. cerevisiae*) that preferentially ferment glucose in an aerobic atmosphere; this phenomenon is called the Crabtree effect. Therefore, the details of the connection between the Hsp31-like proteins and ROS generation are potentially specific to Crabtree-positive yeasts, possibly explaining why a similar phenotype is not observed for the prokaryotic Hsp31 knockout. However, the general connection between DJ-1 superfamily proteins and mitochondrial function is consistent with much of the published literature on animal DJ-1.

A critical cellular strategy for survival in stationary phase is autophagy, in which the cell adopts a more miserly approach to its molecular constituents by recycling them via targeted lysosomal degradation. Autophagy is tightly integrated with nutrient status by signaling through the protein complex TORC1, which contains the serine-threonine protein kinase TOR. TORC1 kinase activity is repressed by nutrient limitation, leading to decreased phosphorylation of a downstream effector Atg13 and a corresponding increase in autophagy. Therefore, negative regulators of TORC1 would be expected to be more active during stationary phase to increase autophagy to levels necessary for sustained viability. Miller-Fleming et al. (5) find that the Hsp31-like proteins are strongly up-regulated by nutrient limitation and repress the phosphorylation of Atg13, indicating a decrease in TORC1 activity. A direct connection between Hsp31 and TORC1 is suggested by the colocalization of Hsp31 and the Kog1 subunit of TORC1 in RNA-rich punctate structures, called P-bodies and stress granules. The presumed purpose of these inclusions is to sequester RNA from active translation, which may conserve resources during stationary phase. In total, these findings place Hsp31-like proteins and TORC1 in the same places in the cell and demonstrate a strong negative correlation between Hsp31 levels and TORC1 activity, providing a mechanistic model that links Hsp31 and the cellular response to diauxic shift and stationary phase.

Directions for Future Work

Miller-Fleming et al. (5) have elucidated several new aspects of Hsp31 function, but important questions remain to be answered. First, how do Hsp31 proteins regulate nutrient sensing and TORC1 activity at the molecular level? A physical interaction between the Hsp31-like proteins and TORC1 is suggested by this work, but more direct detection of this presumed binding event is an important goal for future studies. Second, does the methylglyoxalase activity of Hsp31-like proteins have any impact on their role in diauxic shift and stationary phase? As methylglyoxal is produced by the spontaneous dephosphorylation of triose phosphate sugars, the glyoxalase activities of the Hsp31-like proteins could provide a connection between glycolytic carbon flux and Hsp31's protective activity in stationary phase. Third, and most importantly, what does the previously unidentified role of the Hsp31-like proteins in stationary phase tell us about the function of animal DJ-1 proteins? This question will be the most challenging to address; however, the authors point out that human DJ-1 has been previously implicated in autophagy (17–19) and RNA binding (20), hinting at a possible functional kinship between these proteins. Intriguingly, yeast cells in stationary phase face many of the same challenges as postmitotic cells, such as neurons, and thus it is reasonable to speculate that maintenance of viability in quiescence could be a tie that binds animal and yeast DJ-1 proteins. Now that Miller-Fleming et al. (5) have identified robust phenotypes and cellular roles for the yeast Hsp31-like proteins, the full power of the *S. cerevisiae* model system can be deployed to answer lingering questions about DJ-1 and its many homologs.

ACKNOWLEDGMENTS. This work is supported by National Institutes of Health Grant GM092999 (to M.A.W.).

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