SULFUR METABOLISM

GSH hoards all the cysteine—what a slimy thing to do

Nutrient availability dictates cell differentiation and transition through the *Dictyostelium discoideum* life cycle. Kelly et al. reveal that the increase in reactive oxygen species associated with nutrient limitation coincides with a sequestration of available cysteine in glutathione, thus limiting sulfur-dependent mitochondrial respiration and promoting aggregation into the differentiated spore form.

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Fluctuations in nutrient availability have profound effects on biological systems. Yet, understanding the physiological responses to such stresses is hindered by the complexity of multicellular organisms with diverse cell types. Model systems can provide great insight into fundamentally conserved processes such as metabolism. The early eukaryotic slime mould *Dictyostelium discoideum* is a particularly useful model system for these studies, because it bridges the evolutionary gap between unicellular and multicellular organisms. Under nutrient-replete conditions, *D. discoideum* exists in a unicellular state, whereas nutrient limitation promotes its aggregation into a multicellular and differentiated spore form[1]. Reactive oxygen species (ROS) production has been implicated in this process[1]; however, the mechanism of this regulation has not been fully elucidated. As understanding of the multifaceted signalling functions of ROS expands[2], further exploration of the diverse effects of these reactive intermediates on cell fate, fidelity and function is vital.

In an article in *Nature*, Kelly et al. uncover a novel mechanism through which ROS elicits rewiring of sulfur metabolism in *D. discoideum* in response to nutrient limitation. After starvation, available cysteine is preferentially diverted into glutathione (GSH) synthesis at the expense of other cysteine-dependent processes, including biosynthesis of [Fe–S] clusters[3], thus leading to a loss of mitochondrial respiratory function that promotes multicellular aggregation into the differentiated spore form (Fig. 1). Through a comprehensive assessment of mitochondrial function, the authors revealed that starved cells exhibited decreased oxygen consumption, compromised electron transport chain (ETC) function, an ATP deficit and a dissipation of mitochondrial membrane potential relative to that in nutrient-replete cells. Pharmacological inhibition of the ETC accelerated *D. discoideum* aggregation, thus indicating that starvation-induced mitochondrial respiratory dysfunction has a causal role in promoting the aggregation phenotype.

Although nutrient stress, such as glucose starvation, can elicit mitochondrial dysfunction[4], the starvation response in *D. discoideum* was not affected by glucose availability. Interestingly, amino acid supplementation prevented aggregation in a nitrogen-independent manner, because ammonia had no effect on aggregation under starvation conditions. Instead, amino acids prevented aggregation by replenishing sulfur, because cysteine was the only amino acid able to affect aggregation on its own. Furthermore, whereas the intracellular levels of other amino acids were maintained under starvation conditions, cysteine

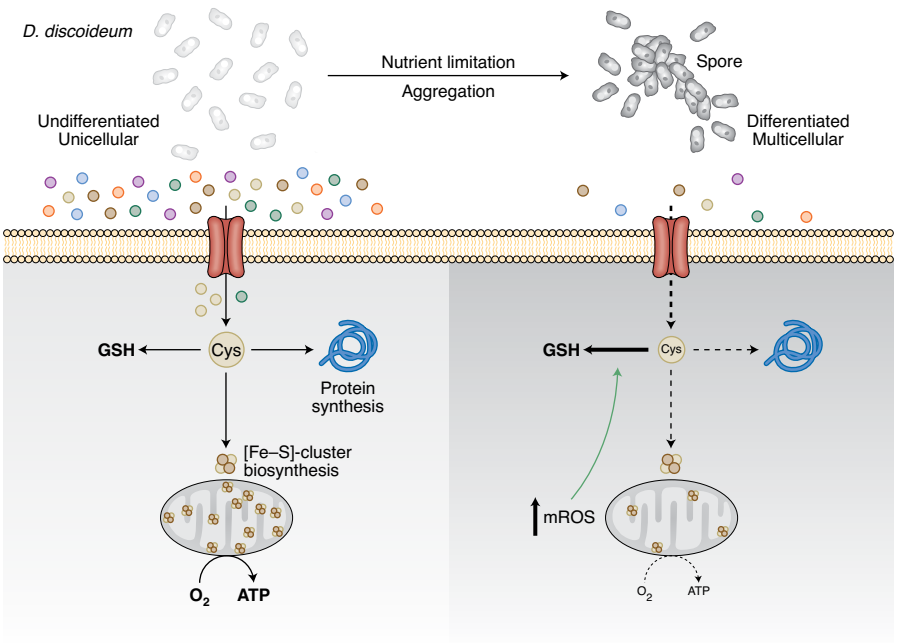

Fig. 1 | Rewiring of sulfur metabolism under nutrient limitation inhibits mitochondrial function and promotes *D. discoideum* aggregation. Under nutrient-replete conditions, cysteine availability is sufficient to support the diverse cellular processes that require sulfur in *D. discoideum*. Severe nutrient limitation promotes metabolic rewiring that prioritizes the use of cysteine for GSH synthesis over [Fe–S]-cluster biosynthesis, thus leading to the loss of mitochondrial respiratory function, and consequently precipitating the cellular differentiation and aggregation of *D. discoideum* cells into its multicellular spore form. mROS, mitochondrial ROS.

levels diminished over time. In addition, starved *D. discoideum* cells demonstrated an increased capacity for cysteine uptake, thus further suggesting an enhanced demand for cysteine.

Cysteine supports several vital cellular functions, primarily the generation of the tripeptide antioxidant GSH[5]. Stable isotope tracing revealed that starved cells engaged in enhanced GSH synthesis, which coincided with an increase in ROS. GSH supplementation attenuated aggregation, but, unexpectedly, so did inhibition of GSH synthesis, thus suggesting that sequestration of cysteine for GSH synthesis, rather than an alternative effect of ROS, promotes aggregation. Therefore, Kelly et al. assessed whether enhanced GSH metabolism under starvation conditions might limit other cysteine-dependent processes. They found severe deficiencies in protein synthesis, possibly as a direct result of a decreased capacity for cysteine incorporation into nascent protein or the inhibition of tRNA thiolation, a cysteine-dependent process that facilitates translation. More strikingly, the authors observed a significant loss of [Fe–S]-protein function.

[Fe–S] proteins have diverse and critical biological functions mediated by their resident [Fe–S] clusters, which are redox cofactors that derive their namesake sulfur from cysteine[6]. Mitochondrial metabolism is particularly dependent on the biosynthesis of [Fe–S] clusters[6], which are highly sensitive to oxidative inactivation by ROS[7]. The authors found that the activity of [Fe–S]-dependent components of the ETC as well as the tricarboxylic-acid-cycle enzyme m-aconitase were compromised under starvation conditions. Critically, cysteine supplementation attenuated the loss of these activities under starvation conditions in *D. discoideum*, despite an inability of cysteine supplementation to attenuate mitochondrial ROS production. This finding suggests that the loss of [Fe–S]-protein activity is specifically linked to the lack of sulfur and restricted cluster synthesis, rather than a loss of cluster integrity due to oxidation. To this end, short interfering RNA–mediated silencing of cysteine desulfurase (NFS1), which releases the cysteine-derived sulfur for [Fe–S]-cluster biosynthesis, inhibited the effect of cysteine supplementation on aggregation in starved *D. discoideum* cells.

Collectively, Kelly et al. demonstrate that nutrient limitation rapidly induces oxidative stress in vegetative unicellular *D. discoideum*, thereby necessitating increased GSH synthesis, which in turn depletes cellular cysteine to an extent that inhibits other cysteine-dependent pathways. Mitochondrial respiratory function is consequently compromised because of a lack of [Fe–S]-cluster biosynthesis, thus promoting cell differentiation and aggregation into the multicellular spore form of *D. discoideum*. These findings are exciting, because they broaden understanding of the eukaryotic response to nutrient stress, particularly in sulfur-restricted conditions. Relative to carbon and nitrogen, sulfur is underappreciated for its importance in biology, and these results highlight the need for robust investigation into sulfur metabolism. In particular, these findings highlight the need to understand the prioritization and partitioning of intracellular cysteine under nutrient-replete and nutrient-starved conditions, because cysteine metabolism is compartmentalized in *D. discoideum*, as in other eukaryotes, such as mammals. GSH synthesis occurs in the cytosol, whereas [Fe–S]-cluster biosynthesis is initiated in the mitochondria, and there is a cysteine requirement for translation in both compartments.

These findings are also intriguing in light of the profound interest in manipulating cysteine availability as an anticancer therapy[8]. In mammalian cells, prolonged cysteine deprivation can elicit ferroptosis, an iron-mediated form of cell death marked by lipid peroxidation. This process is impeded by GSH-dependent detoxification of lipid peroxides[9]. However, in certain malignant contexts, this protective mechanism can be overcome by persistent mitochondrial ROS production and eventual mitochondrial dysfunction[9], which may arise in a similar manner to that described by Kelly et al.[3]. In contrast, recent evidence indicates that GSH synthesis is not prioritized in lung cancer cells, which die under cysteine starvation[10]. These findings highlight the need to compare and contrast *D. discoideum* with mammalian systems. In addition, as the complex roles of ROS in determining the fate of cells in multicellular organisms[2] are beginning to be understood, this study suggests that further exploration of the role of cysteine partitioning in this process is warranted. ❐

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Competing interests

The authors declare no competing interests.