

Deconstructing Dietary Restriction: A Case for Systems Approaches in Aging

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Dietary restriction is a robust and conserved intervention to slow aging and extend lifespan. In this issue of *Cell Metabolism*, Hou et al. (2016) use a systems biology approach in *C. elegans* to uncover key molecular nodes underlying the transcriptomic response to dietary restriction and predict novel regulators of lifespan.

Over the past few decades, many environmental and genetic perturbations that dramatically alter lifespan have been discovered (López-Otín et al., 2013), but perhaps none are as well known as dietary restriction (DR). By limiting dietary intake or inducing cycles of fasting and refeeding, model organisms as diverse as yeast, worms, flies, and mice exhibit improved healthspan and increased lifespan (Fontana and Partridge, 2015). While the efficacy of DR on lifespan extension in primates is still controversial, DR tends to delay the onset of age-related diseases in monkeys, suggesting that DR remains a promising aging intervention. The ability of DR to extend lifespan might be linked to the evolutionary benefit of keeping an organism youthful during times of famine and allow it to still reproduce (and pass on its genes) when abundance returns.

DR's ability to influence aging across species is remarkably conserved, yet the mechanisms underlying its effects on lifespan have only been partially elucidated (Greer and Brunet, 2009; Mair and Dillin, 2008). The combination of high-throughput and engineering approaches has started to show potential to uncover previously unknown mechanisms of lifespan extension (Sagi and Kim, 2012; McCormick et al., 2015). However, a systems biology approach has never been used to deconstruct and reconstruct the genetic mechanisms underlying DR. Here, Hou et al. take such an approach in the nematode worm *C. elegans* to uncover the molecular network underlying DR, revealing specific genetic modules responsible for lifespan extension by DR.

To study the genetic mechanisms underlying this longevity intervention, the au-

thors subjected *C. elegans* to DR (a 1% dilution of their bacterial food source, akin to Greer and Brunet, 2009) and intermittent fasting (IF, 2-day cycles of feeding/fasting Honjoh et al., 2009) and collected a time series of gene expression profiles over the course of lifespan. Having identified the genes that change in response to DR/IF, the authors use an unbiased clustering algorithm to compare these changes to gene expression data from 73 other genetic perturbations to identify which genes could best recapitulate the gene expression changes in response to DR/IF. The authors identified three main genetic modules, each previously implicated in the aging process, which account for most of the genome-wide transcriptional changes induced by DR/IF: the mechanistic target of rapamycin (mTOR) node, the AMP-dependent protein kinase (AMPK) node, and the insulin/insulin-like growth factor signaling (IIS)/Forkhead box O (FOXO) node (López-Otín et al., 2013). While other pathways can also mediate DR-dependent longevity (Mair and Dillin, 2008), it is striking to note that each of the three modules that emerged from this systems approach has known central roles in energy metabolism. When the authors mutated the genes in these nodes and created double and triple mutants, the mutants' gene expression profiles increasingly resembled worms under DR/IF (Figure 1), suggesting that these modules may cooperate synergistically to mediate DR. Interestingly, the mTOR and IIS modules have previously been reported to synergistically extend lifespan in an AMPK-dependent manner (Chen et al., 2013), supporting the authors' prediction

and implying that DR creates a long-lived molecular signature that can be replicated by perturbing specific metabolic nodes.

To study the dynamics of these modules, Hou et al. created a simplified network of node interactions from curating the literature and investigated the feedback loop dynamics of perturbing node combinations. These *in silico* experiments predicted that mutating multiple nodes should lead to a greater lifespan extension than any one single node by itself and that modulating IIS levels would have the single greatest effect on lifespan. To test the prediction that the molecular nodes would progressively recapitulate DR, Hou et al. performed lifespan assays on double and triple mutants fed a normal diet or subjected to DR. Strikingly, these lifespan experiments validate their *in silico* prediction: as more nodes are perturbed, lifespan extension by DR progressively diminishes, and DR is unable to increase the lifespan of the mutant affecting all three modules (mTOR, AMPK, and IIS). Beyond lifespan extension, DR also increases stress resistance and motility in late life. One would expect DR to be unable to further increase stress resistance and motility in the triple mutant, and this is exactly what the authors observe. Along with evidence that perturbing these three modules results in a gene expression profile that resembles DR, these data suggest an underlying role of the trimodule predicted by this unbiased clustering approach in mediating both the lifespan increase and healthspan benefits of DR.

To further test the utility of their unbiased systems approach for generating

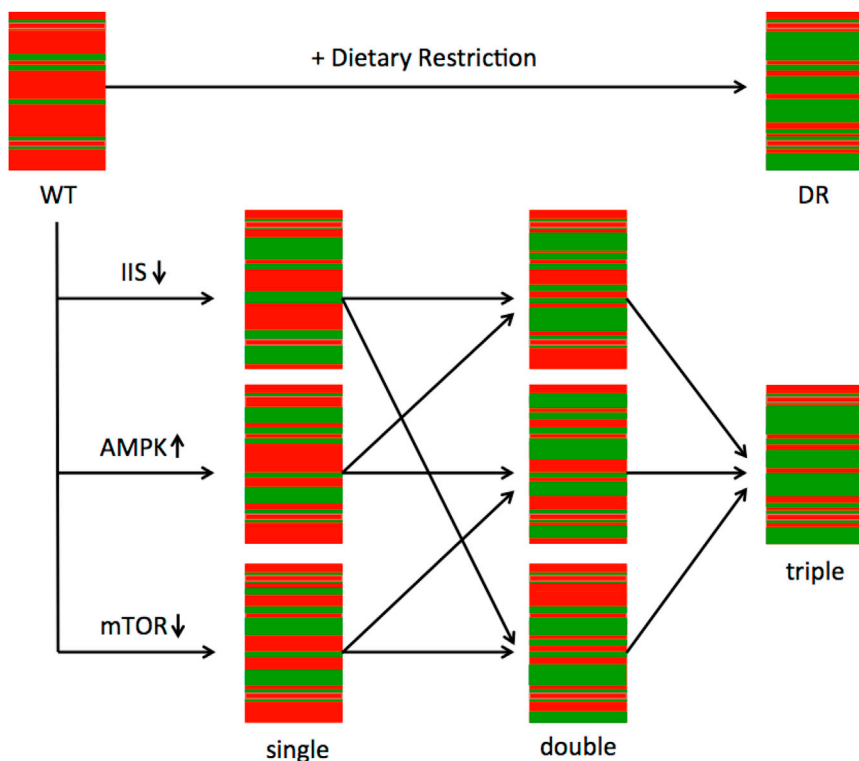


Figure 1. Perturbing Multiple Metabolic Nodes Recapitulates Gene Expression Changes of DR

By using microarrays, Hou et al. measured how the gene expression profile of *C. elegans* was affected by DR regimens as well as mutations in the three metabolic nodes predicted by their unbiased systems approach: IGF-1/insulin-like growth factor signaling (IIS), AMP-dependent protein kinase (AMPK), and mechanistic target of rapamycin (mTOR). By genetically creating pairwise combinations to create double and triple mutants in *C. elegans*, the authors recapitulated the majority of the transcriptomic changes induced by DR and showed that the predicted modules cooperate synergistically to mediate DR.

new hypotheses, the authors selected other potential regulators of aging and DR that emerged from their clustering algorithm. Knocking down *gas-1*, a mitochondrial complex I protein, extends lifespan and makes worms partially refractory to DR, implicating for the first time the mitochondrial function of *gas-1* downstream of DR. Additionally, the gene *xbp-1*, a known regulator of the unfolded protein response (UPR), which has been previously implicated in aging (Taylor and Dillin, 2013), emerged as a potential component of the AMPK module. Indeed, by performing epistatic lifespan experiments, the authors confirmed that *xbp-1* is necessary for lifespan extension by AMPK overexpression. These experiments demonstrate the power of a systems biology approach in the study of aging and its ability to generate novel hypotheses for lifespan regulation.

As the three modules (AMPK, mTOR, and IIS/FOXO) are involved in metabolic

regulation, these findings raise the question: does progressively perturbing these nodes lead to the same metabolic state as worms subject to DR/IF? Systems level interrogation of metabolomics throughout lifespan should help understand how genetic rewiring in response to environmental stimuli is connected to the differential use of metabolic pathways. Given the variety of regimens used to trigger DR, and their contextual dependence on overlapping and nonoverlapping genetic pathways (Greer and Brunet, 2009), it will be interesting to determine if the modules identified by the authors are general to the full spectrum of DR interventions and, if not, what their overlap with other pathways might be. These modules are highly conserved in evolution and can be modulated by small molecule compounds, for example, rapamycin (an mTOR inhibitor) and metformin (an AMPK activator). Determining whether they mimic DR in mammals and coop-

erate synergistically in mammalian longevity will be a key step toward identifying therapeutic interventions that could harness the benefits of DR. Finally, it will also be important to understand the temporal and spatial contributions of these pathways in mediating DR throughout lifespan. Are they acting together in the same tissue? Or are some modules acting cell nonautonomously as “sensors” in the brain while others act as “effectors” in metabolic tissues, such as muscle?

Given the growing accessibility of computational tools for analysis, there is an increasing potential for systems biology to probe biological systems in an unbiased manner. With a phenotype as multifaceted as aging, systems approaches can reveal previously unknown relationships between known longevity regulators and even implicate novel pathways in the aging process. Not long ago, aging was thought to be an immutable process, and the field has probably only begun to scratch the surface of the entire set of networks responsible for longevity. Leveraging systems biology approaches to enhance targeted genetic research should accelerate the understanding and deconvolution of the complexity of the aging process.

REFERENCES

- Chen, D., Li, P.W.L., Goldstein, B.A., Cai, W., Thomas, E.L., Chen, F., Hubbard, A.E., Melov, S., and Kapahi, P. (2013). *Cell Rep.* 5, 1600–1610.
- Fontana, L., and Partridge, L. (2015). *Cell* 161, 106–118.
- Greer, E.L., and Brunet, A. (2009). *Aging Cell* 8, 113–127.
- Honjoh, S., Yamamoto, T., Uno, M., and Nishida, E. (2009). *Nature* 457, 726–730.
- Hou, L., Wang, D., Chen, D., Liu, Y., Zhang, Y., Cheng, H., Xu, C., Sun, N., McDermott, J., Mair, W.B., and Han, J.-D.J. (2016). *Cell Metab* 23, this issue, 529–540.
- López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G. (2013). *Cell* 153, 1194–1217.
- Mair, W., and Dillin, A. (2008). *Annu. Rev. Biochem.* 77, 727–754.
- McCormick, M.A., Delaney, J.R., Tsuchiya, M., Tsuchiyama, S., Shemorry, A., Sim, S., Chou, A.C., Ahmed, U., Carr, D., Murakami, C.J., et al. (2015). *Cell Metab.* 22, 895–906.
- Sagi, D., and Kim, S.K. (2012). *PLoS Genet.* 8, e1002780.
- Taylor, R.C., and Dillin, A. (2013). *Cell* 153, 1435–1447.