The heat shock factor HSF1 juggles protein quality control and metabolic regulation

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Transcriptional regulators often act as central hubs to integrate multiple nutrient and stress signals. In this issue, Qiao et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201607091) demonstrate how heat shock factor 1 (HSF1) uncouples metabolic control from proteostatic regulation and unveils HSF1 as a critical transcriptional regulator of NAD+ metabolism.

The ability to respond and adapt to environmental and cellular challenges is a key evolutionary driver for organisms across all biological domains. Consequently, complex organisms, like most eukaryotes, are endowed with exquisite molecular mechanisms to sense environmental and cellular stress, allowing them to build proper protective and adaptive responses. This is not an easy task, as challenges seldom come one at a time; but rather, interconnect. For example, thermal stress promotes protein folding defects and proteostatic stress, which, in turn, requires substantial cellular energy to be resolved, leading to increased energy demands. Organisms not only have to develop highly specialized stress-sensing mechanisms and defense pathways to overcome them but also require a hierarchical coordination of different stress response mechanisms. Hence, several proteins and enzymes act as stress response hubs, or "master regulators," integrating information on the time, frequency, and amplitude of different stresses to mount an orchestrated defense.

The response to acute challenges often involves the activation of specialized transcriptional regulators that rewire gene expression, switching on genes aimed to alleviate the stress while shutting down transcriptional paths that are not needed for the immediate survival of the cell. For example, temperatures above 42°C lead to the activation of the heat shock response in most, if not all, mammals (Anckar and Sistonen, 2011). This response is coordinated by diverse evolutionarily conserved transcription factors, among which the heat shock factor 1 (HSF1) is the most well-known. HSF1 activation leads to the transcriptional up-regulation of multiple genes encoding heat shock proteins, which act as molecular chaperones preventing and resolving protein folding defects (Anckar and Sistonen, 2011). During the last decade, it has become clear that HSF1 is necessary in response to changes in temperature or proteostatic stress and also has multiple vital physiological functions and transcriptional targets beyond heat shock proteins (Anckar and Sistonen, 2011). Recently, HSF1 has also been linked to metabolic control, as nutrient deprivation reduces HSF1 DNA binding activity through the direct phosphorylation of HSF1 at Ser^{121} by the energy sensor AMP-activated protein kinase (AMPK; Dai et al., 2015). In this issue, Qiao et al. further delved into the mechanisms by which HSF1 conjugates the responses to proteostatic and energy stresses.

Qiao et al. (2017) started by determining how HSF1-deficient mice display a marked drop in cellular ATP and NAD+ levels. Because cellular ATP production is coupled to cellular NAD+ levels, the authors speculated that NAD+ deficits could be at the root of the ATP loss. In line with that, they observed that the livers from HSF1-deficient mice showed lower levels of nicotinamide phosphoribosyltransferase (Nampt), a key enzyme in the maintenance of intracellular NAD+ levels, by salvaging NAD+ from nicotinamide (Cantó et al., 2015). Qiao et al. (2017) explain this phenomenon by showing via chromatin immunoprecipitation quantitative PCR assays that HSF1 directly binds to the *Nampt* promoter. Interestingly, this binding to the *Nampt* promoter was higher when glucose availability was limited in cultured hepatocytes, as well as in fasted livers, as compared with the refed state. In contrast, they found that the binding of HSF1 to the *Hsp70* promoter was higher in the refed condition, showing an opposite regulation to that of the *Nampt* promoter and suggesting that nutrient availability signals disentangle the metabolic transcriptional actions of HSF1 from its canonical ones related to the heat shock response.

During the last decade, NAD+ has been recognized not only as a redox cofactor but also as an important degradation substrate for multiple enzymes. The sirtuin family of deac(et)ylase enzymes are critical metabolic regulators that use NAD+ as a cosubstrate. The activity of some of its members, such as SIRT1 and SIRT3, are rate-limited by low intracellular NAD+ levels (Cantó et al., 2015). Therefore, Qiao et al. (2017) tested whether sirtuin enzymes may be affected by the changes in NAD+ levels in HSF1-deficient livers. Along this concept, they found that SIRT1 and SIRT3 targets displayed a marked hyperacetylation upon HSF1 deficiency, suggesting that the enzymes are less active in HSF1-deficient cells. SIRT1 and SIRT3 are known to play a critical role in the maintenance of mitochondrial function, and, consistently, Qiao et al. (2017) observed that HSF1 deficiency, either via gene ablation or knockdown, led to decreased mitochondrial number. Interestingly, mitochondria were rounder and bigger in the livers of HSF1-deficient mice, either in the fed or the fasted state. To

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explain this observation, one possibility is that HSF1-deficient livers accumulate damaged mitochondria. Mitochondrial fission is a primordial event for the effective clearance of altered mitochondria through mitophagy (Toyama et al., 2016). Qiao et al. (2017) assessed the activation of the fission regulator Drp1 via phosphorylation analyses of Ser^{622}, which were suggestive of impaired fission rates upon HSF1 deficiency, explaining the accumulation of big, dysfunctional mitochondria. An alternative explanation is that the higher fusion rates might be adaptive to maximize energy output in situations of NAD^+ crisis. Nevertheless, the accumulation of dysfunctional mitochondria leads to impaired ATP synthesis capacity. In line with this analysis, the researchers documented impaired metabolic processes with high-energy demands, such as hepatic glucose production and protein translation in HSF1 knockout mice.

If all the phenotypes of HSF1-deficient livers derive from an NAD^+ crisis, it should be expected that recovering NAD^+ levels would reverse the phenotypes of HSF1-null hepatocytes. To test this hypothesis, Qiao et al. (2017) performed a series of elegant experiments using Nampt-independent NAD^+ precursors, such as nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN). The treatment with either NR or NMN was enough to fully rescue intracellular NAD^+ levels, mitochondrial respiration, and ATP levels in HSF1-deficient hepatocytes. Further, NR also improved hepatic glucose production capacity in HSF knockout mice, based on pyruvate tolerance tests. These experiments certified the relevance of NAD^+ as the central element driving the metabolic decline caused by HSF1 deficiency. Overall, the work of Qiao et al. (2017) shows that HSF1 depletion affects the transcriptional regulation of Nampt levels. Lower amounts of Nampt lead to lower amounts of NAD^+, which in turn dampen sirtuin activity and thereby mitochondrial biogenesis and oxidative capacity (Fig. 1).

It seems intuitive to think that critical processes in the cell, such as proteostatic responses and cellular bioenergetics, have to be exquisitely interconnected to adjust cellular energy demands and production. This can be achieved by using similar molecular nodes, such as HSF1, and making them juggle their different functions in a single, parallel, or opposite fashion depending on the upstream stress signals as well as on downstream effectors. Worm models have been very useful to understand how separated gene sets are affected by HSF1. Ectopic expression of HSF1 not only confers resistance to protein aggregation and thermal stress but also enhances lifespan in *Caenorhabditis elegans* (Hsu et al., 2003). Interestingly, overexpression of a HSF1 protein lacking 84 amino acids in the C-terminal side increases lifespan and thermotolerance without affecting the transcriptional regulation of chaperone proteins (Baird et al., 2014). Hence, the C-terminal end of the worm HSF1 protein might act as a critical platform for signaling enzymes or transcriptional coregulators triggering HSF1-mediated expression of molecular chaperones. Further, a recent model of HSF1 overexpression in the worm nervous system has allowed complete separation of the mechanisms by which HSF1 regulates thermotolerance and aging (Douglas et al., 2015). Importantly, the longevity effects of HSF1 in worms were attributed to the impact of HSF1 on *daf-16*, an evolutionarily conserved regulator of the metabolic response to fasting in worms and mammals (Douglas et al., 2015). An important future line of research is elucidating how HSF1 integrates stress and metabolic signals and whether the mechanisms described in worms are conserved in mammals. Given the influence of the fasting response on HSF1, it seems logical that energy-stress mechanisms should influence its function. Indeed, some findings already support this option: It has been described that SIRT1, generally activated by nutrient stress, prompts a more sustained HSF1 transcriptional activity response, probably through the direct deacetylation of Lys^{80} (Westerheide et al., 2009). Hence, fasting-induced SIRT1 activation in the liver could influence an increase in Nampt expression via HSF1. HSF1 could help NAD^+-consuming enzymes to ensure NAD^+ supply via the transcriptional activation of salvaging pathways. It has also been described that HSF1 knockdown leads to a transcriptional profile that overlaps with that of the metabolic stressor metformin (Dai et al., 2015). Interestingly, metformin inhibits the respiratory complex I, also leading to partial respiratory dysfunction, as now reported in HSF1 knockout livers by Qiao et al. (2017). The decrease in AMP/ATP ratio triggered by metformin is enough to activate AMPK, which in turn phosphorylates HSF1 and inhibits HSF1 DNA binding on heat shock response elements (Dai et al., 2015). It is puzzling that AMPK is a well-known activator of hepatic mitochondrial biogenesis, which contradicts the observed detrimental effects on mitochondrial function triggered by reduced HSF1 activity in hepatocytes. Certainly, HSF1 is unlikely to be the sole mediator in the regulation of mitochondrial biogenesis by AMPK. However, the results from Qiao et al. (2017) open another possibility, as their comparison of

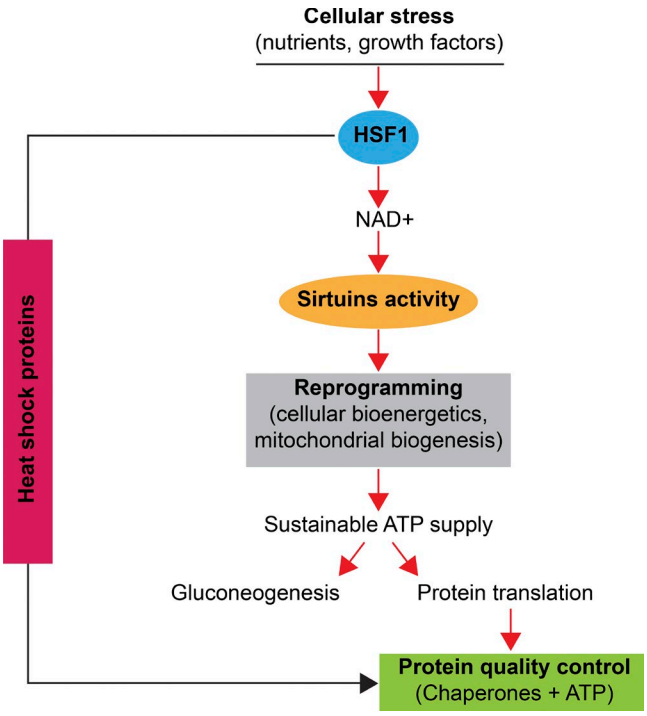

Figure 1. **Model of HSF1 function in oxidative metabolism.** Based on the analysis of HSF1-deficient animals and cells, the work of Qiao et al. (2017) suggests that, under nutrient stress conditions, hepatic HSF1 activation stimulates the expression of Nampt at the transcriptional level. Elevated levels of Nampt lead to elevated levels of NAD^+, which contribute to increase sirtuin activity in the cell. As regulators of mitochondrial function, sirtuins reprogram the cell to increase energetic efficiency and maximize ATP regeneration, thereby ensuring energy supply to maintain key biological processes, such as protein translation and gluconeogenesis in the liver. Figure adapted from Qiao et al. (2017).

the binding of HSF1 to the Nampt and the Hsp70 promoters indicates that the repression of HSF1 DNA binding to heat shock elements does not necessarily imply that the DNA binding to other genes is blunted. Indeed, phosphorylation of transcriptional regulators by AMPK does not always act as an on/off switch, but rather fine-tunes their activity toward specific gene sets by, for example, allowing or obstructing interaction with other transcriptional regulators. AMPK activation is also linked to higher *Nampt* expression (Fulco et al., 2008); however, the role of HSF1 in this process is not yet known.

Altogether, the involvement of HSF1 in the maintenance of NAD^+ levels makes it a central metabolic node and adds another layer of complexity to the multiple and distinct actions of HSF1 beyond proteostatic responses. It will be interesting to evaluate the different molecular cues and posttranscriptional modifications that drive HSF1 toward particular gene sets and their hierarchical nature. In this sense, it would be important to understand the impact of HSF1 gain-of-function on mammalian health and lifespan. As with all good scientific findings, these new observations on HSF1 give new life to its roles in mammals and leave a wealth of tasty questions to open up the appetite of avid researchers on the field.

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References

Anckar, J., and L. Sistonen. 2011. Regulation of HSF1 function in the heat stress response: Implications in aging and disease. *Annu. Rev. Biochem.* 80:1089–1115. http://dx.doi.org/10.1146/annurev-biochem-060809-095203

Baird, N.A., P.M. Douglas, M.S. Simic, A.R. Grant, J.J. Moresco, S.C. Wolff, J.R. Yates III, G. Manning, and A. Dillin. 2014. HSF-1–mediated cytoskeletal integrity determines thermotolerance and life span. *Science.* 346:360–363. http://dx.doi.org/10.1126/science.1253168

Cantó, C., K.J. Menzies, and J. Auwerx. 2015. NAD^+ metabolism and the control of energy homeostasis: A balancing act between mitochondria and the nucleus. *Cell Metab.* 22:31–53. http://dx.doi.org/10.1016/j.cmet.2015.05.023

Dai, S., Z. Tang, J. Cao, W. Zhou, H. Li, S. Sampson, and C. Dai. 2015. Suppression of the HSF1-mediated proteotoxic stress response by the metabolic stress sensor AMPK. *EMBO J.* 34:275–293. http://dx.doi.org/10.15252/embj.201489062

Douglas, P.M., N.A. Baird, M.S. Simic, S. Uhlein, M.A. McCormick, S.C. Wolff, B.K. Kennedy, and A. Dillin. 2015. Heterotypic signals from neural HSF-1 separate thermotolerance from longevity. *Cell Reports.* 12:1196–1204. http://dx.doi.org/10.1016/j.celrep.2015.07.026

Fulco, M., Y. Cen, P. Zhao, E.P. Hoffman, M.W. McBurney, A.A. Sauve, and V. Sartorelli. 2008. Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. *Dev. Cell.* 14:661–673. http://dx.doi.org/10.1016/j.devcel.2008.02.004

Hsu, A.L., C.T. Murphy, and C. Kenyon. 2003. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science.* 300:1142–1145. http://dx.doi.org/10.1126/science.1083701

Qiao, A., X. Jin, J. Pang, D. Moskophidis, and N.F. Mivechi. 2017. The transcriptional regulator of the chaperone response HSF1 controls hepatic bioenergetics and protein homeostasis. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201607091

Toyama, E.Q., S. Herzig, J. Courchet, T.L. Lewis Jr., O.C. Losón, K. Hellberg, N.P. Young, H. Chen, F. Polleux, D.C. Chan, and R.J. Shaw. 2016. AMP-activated protein kinase mediates mitochondrial fission in response to energy stress. *Science.* 351:275–281. http://dx.doi.org/10.1126/science.aab4138

Westerheide, S.D., J. Anckar, S.M. Stevens Jr., L. Sistonen, and R.I. Morimoto. 2009. Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. *Science.* 323:1063–1066. http://dx.doi.org/10.1126/science.1165946