be carried out in robotically controlled batch reactors integrated with liquid handlers, purification and analytical systems[6]. And a robotic system that uses conventional laboratory apparatus, such as round-bottomed flasks, and which uses a standardized approach (the 'chemputer') to translate chemical-synthesis methods into physical operations, was reported last year[7].

Such automated systems ensure reproducibility, because a given instruction set for a synthesis will be carried out in exactly the same way on an identical system at another location, assuming that the input materials are of the same quality. Furthermore, these systems make it feasible for potentially dangerous compounds, such as potent pharmaceuticals or radioactively labelled compounds used for medical diagnostics, to be made without exposure to humans. Automated systems could also be used to generate reaction data – such as the reagents used, yields and conditions – for machine learning in organic chemistry. However, this will require further miniaturization of existing systems, which are currently too large, and therefore too slow to produce sufficient data (equivalent to tens of thousands of experiments) in a useful time frame (weeks) to enable machine learning. Modules for analysing reactions will also need to be incorporated into flow systems to produce such data.

Chatterjee and co-workers' radial synthesizer and other automated platforms go a long way towards eliminating tedious manual operations from chemical synthesis. Nevertheless, challenges remain, particularly in the handling of solids – whether solid reagents or solids formed during reactions. Solids can be suspended in slurries by stirring reaction vessels, but the transfer of slurries through tubes (or even more problematically, through valves) leads to clogging.

Another problem is how to seamlessly integrate purification, isolation and analytical procedures. Robotic systems and the radial synthesizer offer opportunities to develop hybrid platforms that integrate the best elements of flow and batch technologies with purification and analytical methods. This would enable automated synthesis that incorporates state-of-the-art reactions and can produce more-complex molecular structures than have been achieved so far. As the hardware matures, the emphasis will shift to developing the control and artificial-intelligence infrastructure necessary to generate and implement chemical syntheses[5] – freeing chemists from carrying out routine procedures, so that they can focus on discovering new chemical reactions.

Klavs F. Jensen is in the Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.
e-mail: kfjensen@mit.edu

1. Chatterjee, S., Guidi, M., Seeberger, P. H. & Gilmore, K. *Nature* **579**, 379–384 (2020).
2. Bogdan, A. R. & Dombrowski, A. W. *J. Med. Chem.* **62**, 6422–6468 (2019).
3. Adamo, A. *et al. Science* **352**, 61–67 (2016).
4. Bedard, A.-C. *et al. Science* **361**, 1220–1225 (2018).
5. Coley, C. W. *et al. Science* **365**, eaax1566 (2019).
6. Godfrey, A. G., Masquelin, T. & Hemmerle, H. *Drug Discov. Today* **18**, 795–802 (2013).
7. Steiner, S. *et al. Science* **363**, eaav2211 (2019).

Cell biology

Mitochondrial distress call moves to the cytosol

Bradford P. Tremblay & Cole M. Haynes

Cellular stress can result in dysfunction and disease, and mechanisms exist to combat this. Previously unknown steps have been uncovered in a pathway that signals when mitochondrial organelles are dysfunctional. **See p.427 & p.433**

Organelles called mitochondria are responsible for storing energy derived from the food that we eat, in the form of molecules called ATP. Although a mitochondrion has its own genome, 99% of this organelle's proteins[1] are encoded by nuclear genes and imported from the cytosol (the liquid part of the cytoplasm) into the mitochondrion. To function effectively, this process requires coordination and communication, and it must be able to respond to any mitochondrial dysfunction that might occur. Environmental toxins[2] and disease-causing agents[3], as well as diverse age-associated conditions, including Alzheimer's disease[4] and Parkinson's disease[5], are linked to mitochondrial dysfunction.

"The authors identified metabolites that were consistently released from all dying cells."

Now, Guo *et al.*[6] (page 427) and Fessler *et al.*[7] (page 433) report a previously unknown mechanism that is used by mitochondria to send a signal of their dysfunction to the cytosol and nucleus, enabling the cell to adapt to mitochondrial stress.

Studies of the nematode worm *Caenorhabditis elegans* indicate that coordination between the nucleus and mitochondria during stress is regulated by a combination of remodelling of chromatin (the complex of DNA and protein in the nucleus) and activity of a transcription-factor protein that responds to mitochondrial dysfunction[8,9]. Mammalian studies paint a different picture and implicate a process called the integrated stress response (ISR), which causes an overall reduction in protein production but an increase in the production of several transcription factors. The ISR is activated in response to diverse cellular stresses, including those that don't involve mitochondria. In 2002, researchers discovered[10] that mitochondrial perturbations drive the synthesis of a component of the ISR – a transcription factor called CHOP – and induce the expression of two types of mitochondrial protein that aid the ISR. These are chaperones, which aid protein folding, and proteases, which are enzymes that cleave proteins. One enduring mystery has been whether mitochondrial dysfunction also directly regulates kinase enzymes in the cytosol that are needed for the ISR, and that act by adding a phosphate group to proteins.

The ISR is regulated by such phosphorylation of the protein eIF2α (Fig. 1), which is involved in initiating the translation of messenger RNA during protein synthesis. eIF2α phosphorylation is mediated by four kinases – GCN2, PERK, PKR and HRI – that each phosphorylate eIF2α in response to different stressors. GCN2 is stimulated by depletion of amino acids; PERK responds to the presence of unfolded proteins in an organelle called the endoplasmic reticulum; PKR acts when double-stranded RNA accumulates in the cytoplasm during viral infection; and HRI is enlisted when the molecule haem is depleted[11,12]. The phosphorylation of eIF2α results in a reduction of total protein synthesis, but promotes production of the transcription factors ATF4, ATF5 and CHOP. These transcription factors harbour regulatory elements in their mRNA that facilitate translation when eIF2α is phosphorylated[11,13].

To understand how mitochondrial stress triggers an ISR, Guo *et al.* and Fessler *et al.* took similar experimental approaches using

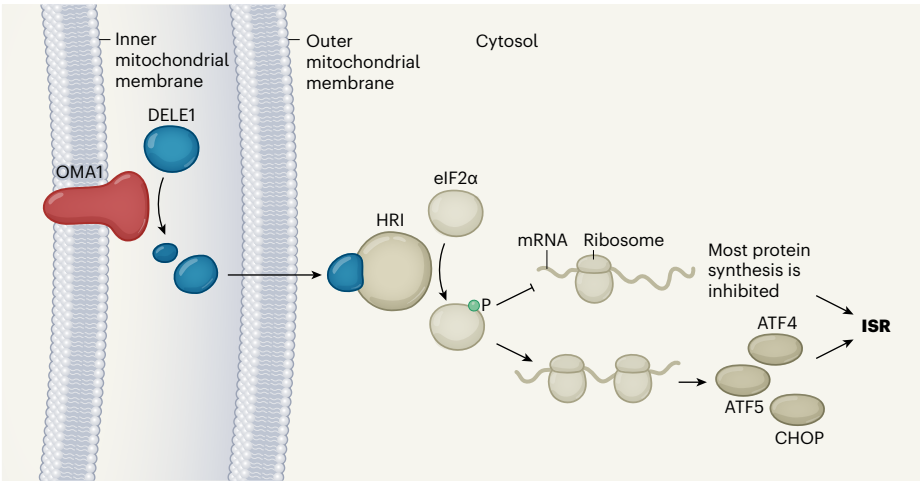

Figure 1 | A mitochondrial signal triggers a response that combats stress. When organelles called mitochondria become dysfunctional in mammalian cells, this can activate a pathway called the integrated stress response (ISR). Guo *et al.*[6] and Fessler *et al.*[7] have identified some previously unknown steps in this process. The authors report that mitochondrial dysfunction caused the protein OMA1, which is located on the inner mitochondrial membrane, to cleave the protein DELE1, a fragment of which enters the cytosol and binds to the enzyme HRI, activating it. HRI adds a phosphate group (P) to the protein eIF2α, and this phosphorylation slows the synthesis of most cellular proteins from messenger RNA (mRNA), mediated by the ribosome complex. This decrease in protein synthesis is one hallmark of the ISR. However, the production of the transcription factors ATF4, ATF5 and CHOP increases[11,13] (Guo *et al.* and Fessler *et al.* did not study ATF5). These transcription factors are required to initiate the ISR.

mammalian cells grown *in vitro*. Fessler and colleagues studied cells engineered to express a fluorescent version of CHOP that could be used to monitor the induction of an ISR. The authors induced random mutations in cells, and so identified genes that encode proteins needed to trigger the ISR. Guo and co-workers used cells engineered to express a fluorescent version of ATF4, and applied the gene-editing tool CRISPR to interfere with gene expression. Both teams identified genes that, when inhibited, altered the production of CHOP or ATF4 in their respective systems. One gene that both groups focused on encodes HRI. The authors discovered that mitochondrial dysfunction caused HRI to phosphorylate eIF2α even when haem was plentiful, which was surprising, given that HRI activation had been thought to depend on haem depletion[11,12]. The result revealed a previously unsuspected form of haem-independent regulation of HRI.

The two teams also identified another gene implicated in triggering an ISR — one that encodes the protease OMA1. OMA1 is located on the inner of the two mitochondrial membranes that surround the organelle, and is activated by a change in the electrical charge (depolarization) on the mitochondrial membrane that occurs during dysfunction[14].

The hunt was on to find a protein that is cleaved by OMA1 to activate HRI and the ISR. One gene of interest identified by Guo *et al.* and Fessler *et al.* encodes DELE1, a little-studied protein that resides in the space between the two mitochondrial membranes and is associated with the inner membrane. Inhibition of DELE1 prevented the phosphorylation of eIF2α in response to mitochondrial stress, as did inhibition of OMA1. These results are consistent with a model in which the two proteins function upstream of HRI activation.

Both groups report that mitochondrial dysfunction causes a fragment of DELE1 to accumulate in the cytosol through an OMA1-dependent process. The studies reveal that the portion of cleaved DELE1 that enters the cytosol binds to HRI and activates it. Consistent with this model, both groups demonstrate that expression of this cleaved form of DELE1 in the cytosol is enough to stimulate HRI and increase CHOP and ATF4 expression in the absence of mitochondrial dysfunction.

These studies clearly establish a previously missing link between mitochondrial dysfunction and the ISR. However, the consequences of ISR activation in response to mitochondrial dysfunction are still not fully understood. Diverse forms of cellular stress activate an ISR, raising the question of whether the ISR is tailored downstream of eIF2α phosphorylation to match the specific stress conditions. And how the ISR protects the cell during mitochondrial dysfunction isn't clear. Is the reduction in overall protein synthesis the main protective function, or is protection mainly mediated through the action of the transcription factors associated with the ISR? It would be interesting to learn whether ATF4, CHOP or ATF5 are regulated by a post-translational modification in response to mitochondrial dysfunction, because this might offer a way to tailor their action to the type of stress that initiates the ISR. In these conditions, cells lacking components of ISR signalling fared better than did cells that had such signalling components.

Findings by Guo, Fessler and their respective colleagues suggest that activation of the ISR can be protective or maladaptive, depending on the mitochondrial perturbation involved. Guo *et al.* report that DELE1 and HRI promote the survival of cells in which the mitochondrial protease LONP1, which degrades damaged proteins in mitochondria, was impaired. And some unknown aspect of the ISR can be detrimental to cells. This was observed, for example, when Guo *et al.* treated cells with oligomycin, a molecule that inhibits the mitochondrial enzyme ATP synthase (which helps to make ATP), and when Fessler *et al.* treated cells with carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), which depolarizes the inner mitochondrial membrane.

Both studies used cultured mammalian cells that mainly rely for their energy production on a metabolic pathway called glycolysis, which occurs in the cytosol, rather than depending on energy production from mitochondria. It will therefore be interesting to know whether this newly discovered pathway acts in certain mammalian tissues, such as muscle and nerves, that are particularly dependent on energy produced from mitochondria rather than by glycolysis, and whether this pathway is involved in the diverse diseases that can result from mitochondrial dysfunction. Such investigations should further illuminate how cells monitor and regulate mitochondria, and the ways in which these systems might fail during ageing and disease.

Bradford P. Tremblay and **Cole M. Haynes** are in the Department of Molecular, Cell and Cancer Biology, University of Massachusetts Medical School, Worcester, Massachusetts 01605, USA.
e-mail: cole.haynes@umassmed.edu

1. Dudek, J., Rehling, P. & van der Laan, M. *Biochim. Biophys. Acta* **1833**, 274–285 (2013).
2. Moullan, N. *et al. Cell Rep.* **10**, 1681-1691 (2015).
3. Pellegrino, M. W. *et al. Nature* **516**, 414–417 (2014).
4. Sorrentino, V. *et al. Nature* **552**, 187–193 (2017).
5. Pickrell, A. M. & Youle, R. J. *Neuron* **85**, 257–273 (2015).
6. Guo, X. *et al. Nature* **579**, 427–432 (2020).
7. Fessler, E. *et al. Nature* **579**, 433–437 (2020).
8. Nargund, A. M., Pellegrino, M. W., Fiorese, C. J., Baker, B. M. & Haynes, C. M. *Science* **337**, 587–590 (2012).
9. Tian, Y. *et al. Cell* **165**, 1197–1208 (2016).
10. Zhao, Q. *et al. EMBO J.* **21**, 4411–4419 (2002).
11. Pakos-Zebrucka, K. *et al. EMBO Rep.* **17**, 1374–1395 (2016).
12. Hunt, T., Vanderhoff, G. & London, I. M. *J. Mol. Biol.* **66**, 471–481 (1972).
13. Quirós, P. M. *et al. J. Cell Biol.* **216**, 2027–2045 (2017).
14. Zhang, K., Li, H. & Song, Z. *EMBO Rep.* **15**, 576–585 (2014).

This article was published online on 4 March 2020.