> Hundreds of knockouts significantly affected cell growth, including protein variant, start overlap, uORF and lncRNA CDS knockouts

Two examined uORF-encoded microproteins had different localization and function compared with their downstream-encoded proteins. Interestingly, five uORF-encoded microproteins formed complexes with their downstream-encoded proteins, thereby exhibiting translation-independent interactions between uORFs and main ORFs. Where examined, the knockout phenotypes of the microprotein and of the main-CDS protein were functionally linked.

This study provides strong evidence for the functionality of microproteins and reveals the extent to which 'non-coding' RNAs contribute to the proteome. Furthermore, the production of multiple uORF-encoded microproteins challenges the exclusivity of monocistronic RNAs in eukaryotes.

Eytan Zlotorynski

ORIGINAL ARTICLE Chen, J. et al. Pervasive functional translation of noncanonical human open reading frames. *Science* **367**, 1140–1146 (2020)
RELATED ARTICLE Couso, J.-P. & Patraquim, P. Classification and function of small open reading frames. *Nat. Rev. Mol. Cell Biol.* **18**, 575–589 (2017)

were accompanied by an increase in the production of reactive oxygen species (ROS) and a reduction in cell proliferation, suggesting that METTL4 is required for normal mitochondrial function and cell growth. Conversely, cells cultured in conditions of hypoxia gradually increased 6mA levels to up to 12 6mA bases per mtDNA molecule, an effect that was mediated by increased METTL4 expression driven by the transcription factor hypoxia-inducible factor 1α. These data suggest that mitochondrial stress represses mtDNA transcription by increasing mtDNA 6mA modification through the activity of METTL4.

Taken together, these results show that 6mA is enriched in human mtDNA and represses mitochondrial gene expression by inhibiting the binding and activity of TFAM. This repression can be enhanced in response to mitochondrial stress, potentially as a mechanism for limiting the release of harmful ROS.

Joseph Willson

ORIGINAL ARTICLE Hao, Z. et al. N^6-deoxyadenosine methylation in mammalian mitochondrial DNA. *Mol. Cell* https://doi.org/10.1016/j.molcel.2020.02.018 (2020)

Journal Club

WHEN CELLULAR FORCES BECAME VISIBLE

The history of science features many examples of fields that flourished when a key chemical or physical quantity became measurable. In recent years this has been the case for mechanobiology, the field that studies how physical forces impact cellular form, fate and function. The idea that forces can influence cell behaviour is as old as the laws of mechanics. However, the question of how much force a cell exerts to move, divide, change shape or sense its microenvironment only became experimentally accessible relatively recently, long after biochemists had learnt how to quantify key properties of genes and proteins.

> the question of how much force a cell exerts to move, divide, change shape or sense its microenvironment only became experimentally accessible relatively recently

Unlike time or length, a force cannot be directly measured; it can only be inferred from the movement (deformation) that it causes on a material of known properties. In the simplest macroscopic scenario, force is quantified as the extent to which it can deform a spring of known stiffness. The problem of how to translate this simple concept to the microscopic living world was solved by Harris, Wild and Stopak in 1980. The authors reasoned that the forces that cells exert on their underlying substrate, called tractions, could be measured if the substrate was made deformable. The first difficulty was, of course, to synthesize a substrate soft enough that a single cell was able to deform it to a measurable extent. They found a solution that, in retrospect, seems remarkably simple. They deposited a drop of a viscous silicone fluid on a coverslip and then exposed it to a flame. When the exposure was brief, only the outermost layer of the fluid polymerized, giving rise to a thin biocompatible substrate floating on a viscous polymeric fluid.

As cells spread on such deformable matrices, multiple wrinkles developed in the substrates under and around the area covered by the cell. From the shape of the wrinkles, visualized through light microscopy, the authors concluded that cells pull their substrate centripetally in the plane of their lower membrane, "much as if the bottom of the cell were occupied by an invisible tractor tread of some kind". By comparing the wrinkling fields generated by cells with those generated by a calibrated pipette, Harris et al. provided a quantitative estimation of the traction exerted by single fibroblasts. This estimation was confirmed 20 years later by the first fully quantitative implementations of traction microscopy (Dembo & Wang, 1999; Butler et al., 2002).

The measurements of cell-generated force fields by Harris et al. marks the beginning of the era of quantitative cell mechanics. In a visionary statement, the authors wrote that "it would be unlike evolution not to make use of these fields to guide morphogenesis". Today we know that force fields generated by cells not only guide the main morphogenetic functions, but also govern the onset and progression of some of the most devastating diseases.

Xavier Trepat
ICREA and CIBER-BBN at the Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST), Barcelona, Spain.
e-mail: xtrepat@ibecbarcelona.eu

The author declares no competing interests.

ORIGINAL ARTICLE Harris, A. K., Wild, P. & Stopak, D. Silicone rubber substrata: a new wrinkle in the study of cell locomotion. *Science* **208**, 177–179 (1980)
RELATED ARTICLES Dembo, M. & Wang, Y. L. Stresses at the cell-to-substrate interface during locomotion of fibroblasts. *Biophys. J.* **76**, 2307–2316 (1999) | Butler, J. P. et al. Traction fields, moments, and strain energy that cells exert on their surroundings. *Am. J. Physiol. Cell Physiol.* **282**, C595–C605 (2002)