the safety and efficacy of the techniques in males—no matter how long it will take to collect it—would be needed. This evidence would come from experience with numerous male children followed at least through their early childhood years, as well as evidence from animal models that showed no adverse intergenerational effects when MRT was used to produce female offspring. This long-term follow-up is not unique to boys but, rather, is a feature of the necessity of monitoring the results of these initial investigations.

Should sufficiently compelling evidence of safety and efficacy (*8*) be obtained, expanding MRT to include transfer of female embryos would remain a controversial step as it would introduce a heritable genetic modification. A public discussion and international process is under way to create a shared framework to guide the circumstances of when, if ever, it would be acceptable to perform heritable genetic modification (*13, 14*).

Safeguards in the conduct of clinical investigations. Consideration of issues of safety and efficacy, and the ultimate determination about whether the agency should move forward with evaluating applications for MRT clinical investigations, rests with the FDA. The committee cautioned, however, that, with significant complexities and unknowns remaining regarding the field of mitochondrial genetics, it will be important for the scientific community and the agency to develop a thorough understanding of the state of the science related to mtDNA genetics and MRT to further inform, in an ongoing way, the benefit and risk assessment entailed in clinical investigations. Although providing guidance to the FDA about what preclinical research would need to be conducted was outside the scope of the committee's charge, the committee noted that the FDA's Advisory Committee had suggested a need for animal studies across a variety of species designed to evaluate safety over the long term. If MRT were ever to be extended to transfer of female embryos, the committee noted, "animal studies of second, and perhaps third, generations would need to be performed" (*8*).

"If shown to be effective, MRT could satisfy the desire of some women to have a genetically related child..."

The primary value to be considered in assessing the ethics of the balance of benefits and risks in clinical investigations of MRT is the minimization of risk of harm to the resulting child. For initial clinical investigations, the committee recommended, in addition to restricting transfer to male embryos, limiting clinical investigations to women who are otherwise at risk of transmitting a serious mtDNA disease (as defined above). Additional principles for all clinical investigations include attention to clinical issues specific to the technique, such as the health of the intended mother to carry a pregnancy, ensuring technical expertise of MRT investigators and centers, and attention to the science relating to addressing potential mtDNA–nDNA incompatibilities. The design of protocols should include mechanisms for standardization, maximizing data quality, data sharing, and collection of long-term information. The report also emphasizes the need to pay close attention to best practices for consent in research and special attention to communicating the novel aspects of MRT research to potential participants. Transparency and partnership with prospective parents and the general public are crucial, and public engagement is vital. ■

REFERENCES AND NOTES

1. S. B. Vafai, V. K. Mootha, *Nature* **491**, 374 (2012).
2. R. P. Saneto, M. M. Sedensky, *Neurotherapeutics* **10**, 199 (2013).
3. H. R. Elliott, D. C. Samuels, J. A. Eden, C. L. Relton, P. F. Chinnery, *Am. J. Hum. Genet.* **83**, 254 (2008).
4. G. S. Gorman *et al.*, *Ann. Neurol.* **77**, 753 (2015).
5. D. P. Wolf, N. Mitalipov, S. Mitalipov, *Trends Mol. Med.* **21**, 68 (2015).
6. U.K. Parliament, Lords discusses mitochondrial donation (29 October 2015); http://bit.ly/1KIxvfx.
7. FDA, 59th Meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee, Gaithersburg, MD, 25 February 2014 (FDA, 2014); http://1.usa.gov/1OSXrJ8.
8. National Academies of Sciences, Engineering, and Medicine, "Mitochondrial replacement techniques: Ethical, social, and policy considerations" (National Academies Press, Washington, DC, 2016).
9. M. Araki, T. Ishii, *Reprod. Biol. Endocrinol.* **12**, 108 (2014).
10. R. Isasi, E. Kleiderman, B. M. Knoppers, *Science* **351**, 337 (2016).
11. International Bioethics Committee, "Report of the IBC on updating its reflection on the human genome and human rights" (United Nations Educational, Scientific, and Cultural Organization, Paris, 2015).
12. Nuffield Council on Bioethics, *Novel Techniques for the Prevention of Mitochondrial DNA Disorders: An Ethical Review* (Nuffield Council on Bioethics, London, 2012); http://bit.ly/NuffieldPubs.
13. National Academy of Medicine, "International Summit on Human Gene-Editing: A Global Discussion," 1 to 3 December, 2015, Washington, DC (National Academies, Washington, DC, 2015); bit.ly/209qZKZ.
14. National Academies of Sciences, Engineering, and Medicine, Human Gene-Editing Initiative (2015); http://bit.ly/20w0A75.

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CELL GROWTH

(TORC)ing up purine biosynthesis

An enzyme complex coordinates metabolism and nucleotide production to fuel cell growth

By **Eric H. Ma**[1,2] ***and*** **Russell G. Jones**[1,2]

Proliferating cells must coordinate their metabolic activities to meet the bioenergetic and biosynthetic demands of anabolic growth. Rapidly growing cells—such as cancer cells—achieve this in part by rewiring their metabolic pathways to increase flux through specific biosynthetic pathways. A more complex issue is how metabolic enzymes are organized to ensure efficient processing of metabolic intermediates. On pages 728 and 733 of this issue, Ben-Sahra *et al.* (*1*) and French *et al.* (*2*), reveal how a protein complex called mammalian/mechanistic target of rapamycin complex 1 (mTORC1) orchestrates metabolism and purine nucleotide biosynthesis to promote cell proliferation.

Purine nucleotides are important metabolic intermediates for growth as they function both as energy carriers (i.e., adenosine 5′-triphosphate and guanosine 5′-triphosphate and building blocks for RNA and DNA synthesis. Whereas purine bases can be recycled through endogenous salvage pathways, de novo purine biosynthesis is increased in proliferating cells and required for optimal cell proliferation (*3*). The construction of purine nucleotides is sourced from several nutrients: The ribose sugar is derived from carbohydrates such as glucose; the purine base is constructed from the amino acids glutamine, aspartate, and glycine; and one-carbon groups are derived from the tetrahydrofolate (THF) cycle (*4*).

mTORC1 is a protein kinase complex that integrates growth signals and environmental cues to regulate diverse metabolic and growth control programs in mammalian cells (*5*). Given previous work linking mTORC1 to de novo pyrimidine biosynthesis (*6*), Ben-Sahra *et al.* used metabolic tracing techniques to assess the impact of mTORC1 activity on the production of purine nucleotides. Using both genetic and pharmacologi-

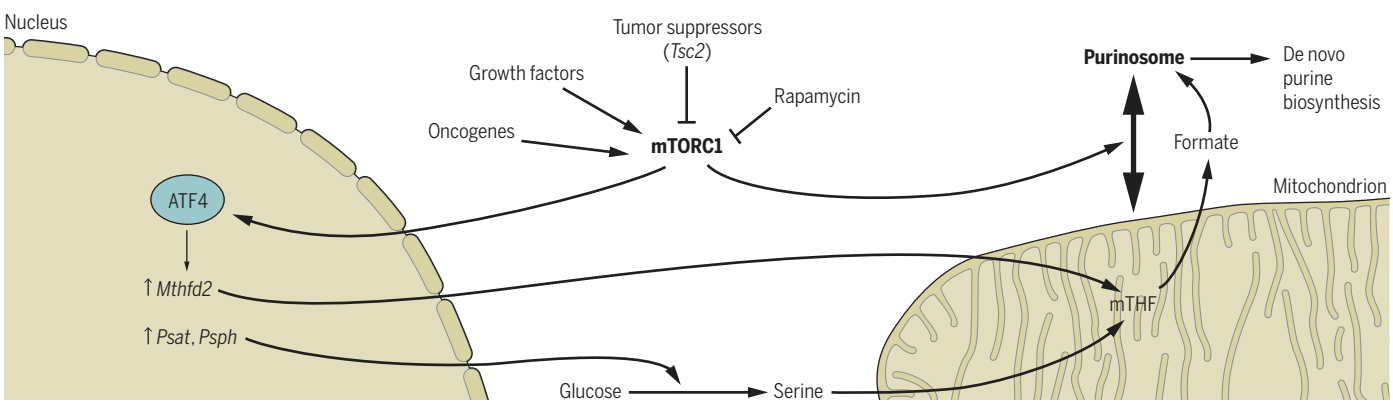
cal approaches, the authors demonstrate that mTORC1 activation enhanced de novo purine biosynthesis in human and mouse cells. mTORC1 affected purine biosynthesis at multiple levels: by regulating the expression of phosphoribosyl-pyrophosphate synthetase 2 (PRPS2), an enzyme whose function is a rate-limiting step for nucleotide biosynthesis (*7*); by enhancing nucleotide production from serine and glycine; and by increasing the expression of methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), an enzyme of the mitochondrial tetrahydrofolate (mTHF) cycle. MTHFD2 was essential for generating a pool of mitochondrial-derived formate to fuel purine biosynthesis. Silencing the expression of MTHFD2 blocked both serine and glycine incorporation (for the purine bases) into growing RNA chains and arrested cell proliferation to a similar extent as either cells treated with the mTORC1 inhibitor rapamycin or cells in which expression of the essential mTORC1 component Raptor was silenced.

protein synthesis (*5*). The authors found that mTORC1 promoted increased translation of activating transcription factor 4 (ATF4), a stress-responsive transcription factor linked to metabolic control (*8*). ATF4 drove the expression of the mitochondrial enzyme MTHFD2 as well as serine biosynthesis pathway genes, thereby stimulating purine production in response to mTORC1 signals. Ben-Sahra *et al.* thus show that by mobilizing both the mitochondrial machinery of the mTHF cycle and shunting metabolic intermediates into this pathway to fuel serine-dependent one-carbon metabolism, mTORC1 can translate growth signals into cell division.

Purine biosynthesis occurs at multienzyme complexes celled purinosomes, which form transiently in response to low purine amounts to stimulate de novo biosynthesis (*9*). This raises the question of how mTORC1-stimulated metabolic products, such as formate, are shuttled from the mitochondrion to purinosomes. French *et al.* address this issue through three-dimensional stochastic optical reconstruction microscopy (3D STORM) (*10*). The authors mapped the cellular location of purinosomes upon stimulation of de novo purine biosynthesis, and found colocalization of these enzyme complexes with mitochondria. Using an RNA interference–based screening approach, the authors identified mTOR as a regulator of epinephrine-induced purinosome formation. Furthermore, culturing cells with the mTORC1 inhibitor rapamycin blocked colocalization of purinosomes with mitochondria, but did not affect the amount of purinosomes. Although the mechanism by which mTORC1 controls purinosome-mitochondrial association remains unclear, the findings of French *et al.* indicate that mTORC1 exerts spatial and temporal control over purinosome assembly.

mTORC1 is an important regulator of cellular metabolism, influencing diverse pathways from glycolysis and mitochondrial metabolism to lipid biosynthesis (*11, 12*). The studies of French *et al.* and Ben-Sahra *et al.* provide new insight into how this protein complex coordinates metabolic reprogramming to promote cell growth and proliferation. In essence, mTORC1 directs a cellular supply chain system, channeling mitochondrial-derived metabolic intermediates to the purinosome machinery for efficient nucleotide production (see the figure). Perhaps more importantly, the studies highlight how successful metabolic remodeling involves coordination of both metabolic flux and spatial organization of organelles and enzyme complexes. This coordination promotes efficient production of metabolic intermediates essential for growth.

It remains to be seen whether mTORC1 exerts similar effects on other metabolic enzyme complexes, such as those that drive glycolysis or lipid biosynthesis, and whether this is a common mechanism of other signal transduction networks. Regardless, both studies highlight how mitochondrial function and anabolic growth are orchestrated to drive cell proliferation, which has substantial implications for many diseases, including cancer. ■



The mitochondria-purinosome shuttle. Factors that activate the mTORC1 complex lead to increased expression of the transcription factor ATF4. ATF4 promotes the expression of genes in the serine biosynthesis and mitochondrial tetrahydrofolate (mTHF) pathways that drive formate production. In parallel, mTORC1 promotes association of purinosomes with mitochondria, enabling the efficient shuttling of metabolic intermediates for de novo purine biosynthesis. *Tsc2, tuberous sclerosis complex 2*.

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REFERENCES

1. I. Ben-Sahra, *Science* **351**, 728 (2016).
2. J. B. French, *Science* **351**, 733 (2016).
3. T. Yamaoka *et al.*, *J. Biol. Chem.* **272**, 17719 (1997).
4. S. Y. Lunt, M. G. Vander Heiden, *Annu. Rev. Cell Dev. Biol.* **27**, 441 (2011).
5. M. Laplante, D. M. Sabatini, *Cell* **149**, 274 (2012).
6. I. Ben-Sahra *et al.*, *Science* **339**, 1323 (2013).
7. J. T. Cunningham, M. V. Moreno, A. Lodi, S. M. Ronen, D. Ruggero, *Cell* **157**, 1088 (2014).
8. J. Seo *et al.*, *Diabetes* **58**, 2565 (2009).
9. S. An *et al.*, *Science* **320**, 103 (2008).
10. B. Huang *et al.*, *Nat. Methods* **5**, 1047 (2008).
11. M. Morita *et al.*, *Cell Metab.* **18**, 698 (2013).
12. K. Duvel *et al.*, *Mol. Cell* **39**, 171 (2010).

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