heterochromatin at telomeres, thus protecting against premature aging[16].

These intriguing studies have provided new answers regarding the regulatory mechanisms controlling silencing at pericentromeric repeats. However, they also raise numerous new questions: how are pericentric transcripts toxic to the cell? Do they compromise chromosome segregation merely by physically disrupting compacted chromatin? Do these aberrant transcripts activate a signaling event that triggers senescence? What are the SIRT6-dependent effectors that compact and maintain silencing in this HP1-independent mechanism? What determines the specificity of SIRT6 for H3K9, H3K56 and H3K18 in these different chromatin landscapes? Those questions await future answers, yet it is clear that the presence of SIRT6 in different cellular neighborhoods provides protection against undesired genomic abnormalities.

COMPETING FINANCIAL INTERESTS
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Step back for seminal translation

Shu-Bing Qian

Translation elongation entails a one-codon movement of the mRNA–tRNA complex along the mRNA and is catalyzed by the forward translocase EF-G. The structurally related back-translocase EF4 catalyzes movement in the opposite direction when the ribosome stalls, but its physiological role in mammals had been unknown. Genetic ablation of EF4 in mice is now found to cause testis-specific mitochondrial deficiency and impaired spermatogenesis.

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Translation can be divided mechanistically into four stages: initiation, elongation, termination and recycling. In bacteria, the elongation phase is mediated by the highly conserved factors EF-Tu and EF-G, which deliver amino acid–charged tRNA to the ribosome A site and catalyze ribosomal translocation, respectively[1]. The stepwise, codon-by-codon progression of the ribosome along the mRNA from the 5′ to the 3′ end is primarily driven by translocation[2]. It is conceivable that translocation not only controls the overall speed of protein synthesis but also influences the quality of translational products.

The discovery of the elongation factor EF4 has added a new twist to the understanding of translocation. Structurally related to EF-G, EF4 catalyzes back-translocation, causing ribosome movement in a backward direction[3]. A lack of EF4 has been shown to decrease translation fidelity in bacteria under stress conditions[4], thus suggesting that translocation is error prone. Recent structural studies of EF4 in complex with prokaryotic ribosomes have provided much-needed molecular insights into the dynamic process of translocation[5,6].

EF4 has been highly conserved throughout evolution and can be found in prokaryotes, mitochondria and chloroplasts. Oddly, no EF4 orthologs exist in the cytoplasm of eukaryotes. Further, deletion of EF4 from several species has yielded no evident phenotypes under normal growth conditions. Debate has swirled around the biological importance of EF4 in spite of its high conservation. This controversy may be settled now, if not completely. In this issue, Qin and colleagues[7] characterize the physiological role of EF4 in mouse models. Surprisingly, the authors have found that genetic ablation of this back-translocase from either the whole body or germ cells gives rise to identical phenotypes of testis-specific mitochondrial dysfunction. As a consequence, severely impaired spermatogenesis leads to male sterility.

In mammals, the nuclear-encoded EF4 gene (*Guf1*) bears a mitochondrial targeting signal, and Qin and colleagues[7] have demonstrated that EF4 protein indeed resides in mitochondria. Given the evolutionary origin of mitochondria from bacteria, the function of EF4 as a back-translocase may be expected to be preserved in mitochondria. Therefore, the observed mitochondrial deficiency in the absence of EF4 is not entirely surprising. In fact, EF4 deletion had a more striking effect in spermatocyte mitochondria than in bacteria. Morphologically, the mitochondrial inner membrane disappears, as revealed by transmission electron microscopy, primarily because of disrupted assembly of oxidative phosphorylation complexes, which are required for mitochondrial respiration. As a result, apoptosis prevails during spermatogenesis, and the sperm concentration in the testes of EF4-knockout (KO) mice is approximately one-tenth that of wild-type mice.

How could the lack of back-translocation lead to such a severe phenotype in mitochondria? The answer is two-fold. First, in the absence of EF4, overall mitochondrial protein synthesis is nearly doubled, regardless of the tissue type. This result is in line with the role of EF4 in counteracting forward translocation. Second, despite accelerated translation, mitochondrial proteins exhibit a much shorter half-life in EF4-KO spermatocytes. The underlying mechanism has two distinct but interrelated parts. The first lies in the poor quality of translational products during rapid elongation. The second lies in the shortage of cytoplasmic proteins needed to assemble multisubunit oxidative phosphorylation complexes in the mitochondria. This protein-synthesis

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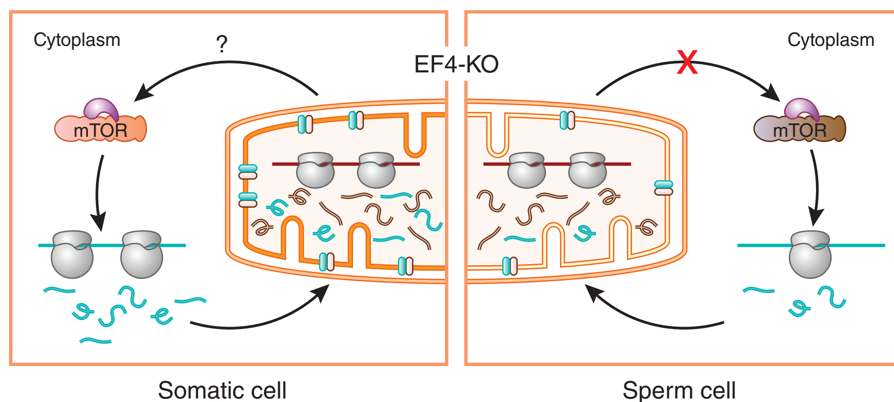

Figure 1 Distinct cross-talk between the mitochondrial and cytoplasmic translation machinery in somatic and sperm cells. The elongation factor EF4 catalyzes back-translocation in bacteria. In mitochondria, lack of EF4 enhances mitochondrial protein synthesis (dark red). In somatic cells, the increased mitochondrial protein load is sensed by mTORC1, which promotes cytoplasmic protein synthesis (turquoise), thereby maintaining the assembly of multisubunit oxidative phosphorylation complexes in the mitochondria. In sperm cells, however, mTORC1 signaling is generally suppressed during maturation, and the insufficient cytoplasmic protein synthesis leads to disrupted mitochondrial protein homeostasis. Therefore, EF4 is essential for sperm maturation and male fertility.

imbalance potentially explains both why the absence of EF4 causes a mitochondrial phenotype more severe than the bacterial phenotype and why the effect is testis specific, as discussed below.

Since the endosymbiotic event that originated mitochondria, nearly 1,000 genes encoding mitochondrial proteins have migrated to the nucleus, leaving only 13 protein-coding genes in the mitochondrial genome[8]. Thus, mitochondria must import many nuclear-encoded proteins to assemble multisubunit complexes for oxidative phosphorylation, the main source of cellular ATP. The involvement of both mitochondrial and nuclear genomes in complex assembly presents unique coordination challenges. Therefore, cells have developed several sensing systems to monitor the quality and quantity of proteins synthesized in different compartments. For instance, the mitochondrial unfolded-protein response elicits a transcriptional response in the nucleus[9]. Accumulation of mitochondrial precursor proteins in the cytoplasm triggers a unique cellular response by decreasing protein synthesis and promoting protein degradation[10,11]. When mitochondrial protein synthesis is accelerated, as it is in the absence of EF4, cytoplasmic protein synthesis must catch up to maintain the protein balance.

The true surprise reported by Qin and colleagues[7] is the increased mammalian target of rapamycin complex I (mTORC1) signaling in somatic tissues lacking EF4 (**Fig. 1**). mTORC1 is an evolutionarily conserved serine/threonine kinase that senses extracellular signals as well as the intracellular energy status[12]. mTORC1 controls cap-dependent mRNA translation via downstream targets such as the eIF4E-binding protein (4EBP) and p70 S6 kinase (S6K)[13]. Indeed, both targets show increased phosphorylation in heart tissue from EF4-KO mice. A more abundant polysome fraction is also present in this somatic tissue, thus further indicating enhanced protein synthesis. Interestingly, inhibiting mTORC1 signaling with rapamycin treatment leads to mild heart failure in EF4-KO mice. Despite the strong evidence supporting cross-talk between the mitochondrial and cytoplasmic translation machinery, exactly how mTORC1 senses the translational status in mitochondria remains to be understood. Nevertheless, this intercompartmental connection provides support for the notion that somatic cells rapidly adapt to alterations in mitochondria via translational regulation.

What is particularly interesting is that the feedback communication between the mitochondrial and cytoplasmic translation machinery does not seem to occur in the testes (**Fig. 1**). Without enhanced mTORC1 signaling in spermatogenic cells lacking EF4, the supply of nuclear-encoded mitochondrial proteins becomes limiting. Because excess proteins synthesized in the mitochondria cannot be assembled into electron-transport-chain complexes, these surplus proteins undergo rapid degradation. In support of this notion, EF4 deletion mainly affects the integrity of complex IV but not complex II, whose subunits are entirely encoded by nuclear genes. Although it is still unclear why spermatocytes lack the sensing system used by somatic cells, Qin and colleagues[7] propose some ideas. One possibility is suppressed mTORC1 signaling during sperm maturation, a tightly programmed developmental process[14]. Although many details remain to be determined, this unique feature explains the testis-specific phenotype in mouse models lacking EF4.

Evolution has used many sensing systems to balance protein synthesis across various cell types, during distinct developmental stages, under normal or stress conditions or between different compartments within the same cell. A growing body of evidence has suggested the constant communication of translation machinery between the mitochondria and cytoplasm[15]. The exciting study by Qin and colleagues[7] adds further credence to the idea that translational homeostasis is essential for cell survival. Deciphering the details of this sensing system and identifying regulatory molecules hold therapeutic and diagnostic potential for a wide spectrum of human diseases.

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