gests that activating leukemia stem cells from quiescence prior to chemotherapy may result in more efficient elimination of these cancer-repopulating cells (Saito et al., 2010). An unexpected benefit of such a prestimulation strategy may be that normal hematopoietic stem cells activated from quiescence would simultaneously be protected from accumulating long-term DNA damage. However, as shown by Milyavsky and colleagues, stem cell escape from acute damage, particularly if it involves a decrease in p53 activity, may lead to long-term deleterious effects on stem cell fitness and repopulating ability. The interplay between the response to acute injury and long-term fitness needs to be more fully understood and will require both laboratory models and the thoughtful correlative study of stem cells from patients receiving genotoxic chemotherapy. Understanding these events may point the way to methods for preserving short-term tissue reconstitution while maintaining long-term cell and genomic integrity.

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Mitochondrial Matrix Reloaded with RNA

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Although mitochondrial biogenesis requires the import of specific RNAs, the pathways and cellular machineries involved are only poorly understood. Wang et al. (2010) now find that polynucleotide phosphorylase in the intermembrane space of mammalian mitochondria facilitates import of several RNAs into the mitochondrial matrix.

Mitochondria, the power plants of the eukaryotic cell, are bound by two membranes and contain 1000–1500 different proteins and tens of RNAs. Most of the genes that encode mitochondrial proteins are found in the nuclear genome and thus are translated in the cytosol and then imported into mitochondria. The pathways and machineries required for protein import into mitochondria have been extensively studied and are highly conserved among fungi, plants, and mammals (Endo and Yamano 2009; Chacinska et al., 2009). The mitochondrial matrix also contains several kinds of noncoding RNAs that are also imported from the cytosol. However, in contrast to protein translocation, the mechanisms that mediate import of RNAs into mitochondria remain enigmatic (Salinas et al., 2008; Lithgow and Schneider, 2010). In this issue of *Cell*, Wang et al. (2010) shed light on this question, revealing that polynucleotide phosphorylase (PNPase) is a much sought after component of the RNA import apparatus in mammalian cells.

PNPases comprise an evolutionally conserved enzyme family (found in bacteria, plants, flies, and mammals but not in yeast) that has 3′→5′ exoribonuclease and RNA-polymerase activities (Chen et al., 2007). Although bacterial PNPases are cytosolic, eukaryotic PNPases are mainly localized in mitochondria or chloroplasts. Prior work has established how PNPases get to the intermembrane space (IMS). After crossing the mitochondrial outer membrane via the translocase of outer mitochondrial membrane 40 (TOM40) complex, the PNPase precursor engages with the translocase of the inner membrane 23 (TIM23) complex (Figure 1) (Chen et al., 2006; Rainey et al., 2006). After the PNPase presequence is removed by matrix processing peptidase (MPP), an AAA protease Yme1 in the inner membrane pulls PNPase into the IMS, where PNPase assembles into a trimeric complex (Figure 1).

Wang et al. now assess the function of mammalian PNPase by tissue-specific disruption of the *PNPase* gene in mouse hepatocytes. They find that mitochondria from hepatocytes deficient in PNPase display defects in oxidative phosphorylation (OXPHOS), the major ATP-generating metabolic pathway of respiration. This defect is shown to arise from the failure in the processing of polycistronic mitochondrial mRNAs encoding the

subunits of the OXPHOS complexes. But how does PNPase in the IMS affect the processing of RNAs in the matrix?

A hint to the answer came from the observation that the RNA component of mammalian mitochondrial RNase P is markedly decreased by PNPase depletion. Given that RNase P mediates processing of mitochondrial transfer RNAs (tRNAs), which are encoded between open reading frames for OXPHOS components, failure in tRNA excision also impairs maturation of the OXPHOS subunit transcripts. In addition, expression of human PNPase in yeast mitochondria, which do not possess PNPase, enhances the import efficiency of heterologous human *RNase P* RNA, 5S ribosomal RNA, and the RNA component of MRP, a ribonucleoprotein that mediates mitochondrial DNA replication. Similar PNPase-dependent import in vitro is confirmed with mitochondria isolated from PNPase-deficient mouse liver or embryonic fibroblasts. These findings suggest that PNPase in the IMS is a component of the RNA import system in human mitochondria.

What is the mechanism of RNA import that is facilitated by PNPase? In yeast and plant mitochondria, substrate tRNAs are recognized by the mitochondrial surface components including the import receptor Tom20, and then move through the Tom40 channel or voltage-dependent anion channel (VDAC). This movement probably involves several mechanisms, including a piggyback mechanism in which the RNA substrate is associated with an appropriate escort protein (Salinas et al., 2008; Lithgow and Schneider, 2010). Although no receptor or translocation channel for RNA import is known for mammalian mitochondria, the present study reveals that PNPase binds to substrate RNAs with a specific stem-loop structure. When grafted onto other nonsubstrate RNAs, this stem-loop can direct the RNA into yeast mitochondria containing human PNPase. Therefore, after crossing the outer membrane by an unknown mechanism, substrate RNAs with a specific stem-loop structure are recognized by PNPase, which subsequently allows the transfer of a subset of RNAs to downstream components of the import pathway. The binding of PNPase to substrate RNAs may also contribute to the unidirectional translocation of RNAs across the outer membrane via a trapping mechanism or by preventing their backward movement to the cytosol.

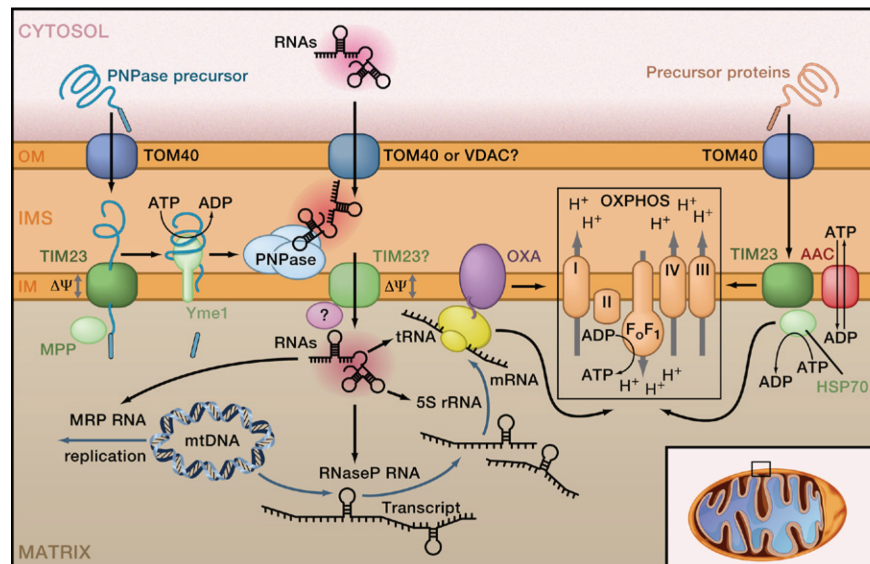

Figure 1. PNPase Facilitates the Import of RNAs into the Mitochondrial Matrix
The polynucleotide phosphorylase (PNPase) precursor is imported into the intermembrane space with the aid of the translocase of outer mitochondrial membrane 40 (TOM40) complex, the translocase of the inner mitochondrial membrane (TIM23) complex, matrix processing peptidase (MPP), and AAA protease Yme1. The trimeric PNPase promotes the import of RNAs from the cytosol into the matrix. Mitochondrial mRNAs are processed in the matrix and their translation products are assembled into the oxidative phosphorylation (OXPHOS) complexes I–IV. The OXA complex integrates proteins from the matrix into the inner membrane. OM, outer membrane; IM, inner membrane. Heat shock protein 70 (Hsp70) is shown as an import motor for presequence-containing proteins. AAC, ADP-ATP carrier; $\Delta\Psi$, membrane potential.

The identity of the import channel in the inner membrane is unknown, although Wang et al. find that translocation of *RNase P* RNA into yeast mitochondria with human PNPase requires the membrane potential. This suggests that the membrane potential may help remove bound RNAs from PNPase or facilitate unidirectional translocation of RNAs across the inner membrane (Figure 1). Translocation of RNAs through the inner membrane import channel may also require an additional protein, such as mitochondrial heat shock protein 70, as an import motor for presequence-containing mitochondrial proteins. Although many of the components of the yeast and human RNA import pathway remain to be identified, the efficient in vitro system developed by Wang et al. should accelerate the pace of discovery.

The study by Wang et al. casts a spotlight on mitochondrial RNA import, with mammalian PNPase taking its rightful place center stage. Will the unfolding drama of RNA translocation in mitochondria reveal common principles or expose a diversity of organism specific mechanisms?

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