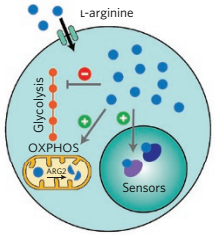
IMMUNOLOGY

T cells get L-ARGed

Cell **167**, 829–842 (2016)



ELSEVIER

T cells undergo metabolic changes at various stages of maturation and activation, including an increased utilization of glycolysis upon activation from a quiescent state. In order to define critical molecular players involved in T cell metabolism changes, Geiger *et al.* performed proteomic and metabolomics analyses of activated T cells to find 2,824 proteins whose expression levels differed from those in quiescent T cells, as well as 49 metabolites that significantly increased and 14 metabolites that decreased in abundance upon activation. Several of these 14 metabolites belong to the same metabolic pathway, so the authors decided to focus on one, L-arginine (L-Arg). Experiments in which the authors added excess L-Arg to T cells or inhibited its degradation indicated that L-Arg can be internalized and can cause metabolic profile switches from glycolysis to oxidative phosphorylation, accompanied by increases in relevant enzymes. In addition, L-Arg limits T cell differentiation while promoting T cell survival; the latter phenomenon also occurs *in vivo* in mice, accompanied by increased antitumor activity. Further proteomic analysis of samples from donors found three transcriptional regulators, BAZ1B, PSIP1 and TSN, that were implicated in knockout experiments as mediating the L-Arg effect on T cell survival. These results suggest that L-Arg sensors act to transcriptionally regulate metabolic pathways that control T cell survival and differentiation. *MB*

EPIGENOMICS

Massive ATAC

Nat. Methods http://dx.doi.org/10.1038/nmeth.4031 (2016)

Before DNA transcription, replication and repair can occur in eukaryotes, chromatin must transition from a tightly packed, condensed state to a more 'open' state. Chen *et al.* modified a previously published method that can identify and sequence these 'accessible' regions of DNA so that they are labeled with a fluorescent tag. The new approach—termed 'ATAC-see'—can be used to visualize the locations of all open chromatin in a fixed or living cell. The authors first used DAPI, which stains tightly packed DNA, and ATAC-see to show that the spatial organization and accessibility of genomic DNA varies across different human cell types. They then obtained ATAC-see images of neutrophils, which can sacrifice themselves to kill bacteria via an unusual type of programmed cell death called NETosis. ATAC-see revealed that the chromatin in cells undergoing NETosis associates with lamina-associated domains at the nuclear periphery, where it disassembles into mononucleosomes and eventually disassociates into free histones and DNA. The authors also showed that ATAC-see could be combined with flow cytometry to sort cells that appeared to be at the same step in the cell cycle into distinct subpopulations, suggesting that this method could be used to uncover the molecular basis of cellular heterogeneity. *JMF*

RNA STRUCTURE

Know when to fold 'em

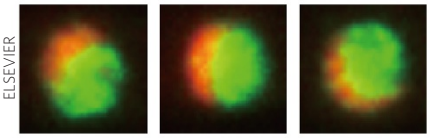
Science http://dx.doi.org/10.1126/science.aaf5371 (2016)

In vitro, some G-rich RNA sequences can fold into four-stranded structures called RNA G-quadruplexes (RG4s), which are stable in the presence of K^+ ions. Although a number of techniques have hinted at the presence of RG4s in cells, it is unclear whether the folding observed *in vitro* reflects folding in cells. To test this, Guo and Bartel developed a method to measure the folding states of endogenous RNA transcripts at a global level by coupling methylation of guanine residues with the capacity of RG4s to stall reverse transcriptase, and then deep sequencing the resulting complementary DNA fragments. They verified *in vitro* that folded RG4 structures produced truncated transcripts, whereas unfolded RG4 structures produced longer transcripts. Surprisingly, analysis in mouse embryonic stem cells and yeast revealed that most of the RG4 regions were unfolded, despite the high intracellular of K^+ in these cells, and RG4 constructs ectopically introduced into either cell type were similarly unfolded. In contrast, analysis of the *Escherichia coli* transcriptome revealed far fewer RG4 structures, suggesting that RG4-forming sequences were eliminated during bacterial evolution. Consistent with this idea, ectopically introduced RG4 constructs were folded in *E. coli* but also disrupted growth and translation. Future studies will focus on determining the molecular mechanism of the machinery that unfolds RG4s in eukaryotic cells. *GM*

PROTEIN SYNTHESIS

Breaking down the NSA1

Cell **167**, 512–524 (2016)



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Ribosomal proteins are organized into 60S and 40S subunits, which require protein assembly in the nucleolus. In particular, the maturation and nuclear export of the 60S subunit requires the removal of the ribosome factors such as Rsa4 and the Ytm1–Erb1–Nop7 complex at distinct times by the AAA^+ ATPase Midasin (Rea1 in *Saccharomyces cerevisiae* and Mdn1 in *Schizosaccharomyces pombe*). However, it remains unclear which assembly steps require Mdn1 activity. Kawashima *et al.* identified *mdn1* mutations in the yeast S. *pombe* that confer resistance and sensitivity to ribozinoindole-1 (Rbin-1), a compound toxic to yeast through an unidentified target. These mutations were clustered near the AAA domains, and complementation analysis revealed that four of the ATPase sites are required for Mdn1 activity. Rbin-1 directly blocked the ATPase activity of recombinant Mdn1, while a Mdn1 protein variant containing a resistance mutation was unresponsive. Rbin-1 blocked nuclear export of the pre-60S subunit, which led to the accumulation of these particles in nucleoli, phenocopying the *mdn1* null mutations. Kawashima *et al.* identified additional requirements for Mdn1 in regulating the assembly of the ribosomal particles Nsa1. Interestingly, the effects of Rbin-1 are rapidly reversible by compound washout, providing a useful tool to disrupt ribosomal assembly with efficient temporal resolution. *GM*

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