JCB Journal of Cell Biology

SPOTLIGHT

Nucleolar condensates: A cellular machinery necessary for T cell activation

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Naive T cells must shift from a state of quiescence to an active metabolic state. To do this, T cells must ramp up their production of ribosomes. In this issue, Zhou et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202201096) identify DDB1 and Cul4-associated factor 13 (DCAF13) as a T cell activation–induced nucleolar protein that functions to enhance ribosome biosynthesis. DCAF13 binds to nucleophosmin 1 (NPM1) to form a biomolecular condensate that functions, in part, by recruiting the endonuclease UTP23 into the nucleolus.

The immune system generates billions of different T lymphocytes during the early years of an individual's life. The diversity of the T cell repertoire allows the immune system to respond to a wide variety of pathogens that could be encountered in a lifetime. Because the large majority of these T cells will never recognize their specific antigen, naive T cells are kept in a metabolically quiescent state until they are activated. Once activated, they require 12–18 h to prepare for multiple cell divisions and to initiate an effector program. During activation, T cells prepare for significant protein needs in part by synthesizing new ribosomes. In this issue of *JCB*, Zhou et al. (1) characterize the role of the nucleolar protein DCAF13 (DDB1 and CUL4 Associated Factor 13) during T cell activation and define its role in accelerating ribosomal maturation via its ability to phase separate with nucleophosmin 1 (NPM1; Fig. 1).

Ribosomes, molecular machines composed of RNAs and proteins, are assembled in the nucleolus, a membrane-less organelle in the nucleus. The nucleolus contains enzymatic RNAs that catalyze protein synthesis and proteins that function to organize the ribosomal RNAs (rRNA). A single rRNA precursor (18S) is transcribed at the center of the nucleolus and then transported through two concentric outer zones where the rRNA is modified to become the rRNAs that make up the large and small subunit of ribosomes. This process involves splicing and RNA folding and is mediated by endonucleases and exonucleases contained in the nucleolus. Remarkably, work in the last decade have shown that the components of the nucleolus can spontaneously assemble to form the nucleolar multilayered structure due to the different immiscibilities of the RNPs based on the principles of liquid–liquid phase separation (2). The organization of the nucleolus ensures ribosomal assembly by recruiting and organizing the assembly machinery.

Over the last decade, there has been an explosion in interest in the principles that underlie the formation of membrane-less organelles also referred to as biomolecular condensates. The nucleolus is one of the best examples of a biomolecular condensate and perhaps the best studied (reviewed in 3). The principles underlying biomolecular condensate organization likely evolved during the pre-cellular era dominated by RNA and ribonucleoprotein complexes to organize growing numbers of metabolic pathways. Our current understanding suggests that biomolecular condensates form from intrinsically disordered regions of proteins via electrostatic and hydrophobic forces and follow the principles of liquid–liquid phase separation (reviewed in 4, 5).

Zhou et al. used the activation of naive T cells as a model to study the dynamic changes that occur in the nucleolus during cell growth (1). They began by showing that during the activation of naive T cells, nucleoli increase in number and size, as well as changing shape. Beginning with a set of 872 nucleolar proteins expressed constitutively in a T cell line (6), they identified 52 proteins that are upregulated during T cell activation (7) and focused on DCAF13. DCAF13 is a known nucleolar protein, originally cloned as an adaptor for the Cullen Ring-finger Ubiquitin ligase 4 (8) and is known to play a role in oocyte development and is upregulated in a variety of cancers. One of its functions is to regulate the processing of the 18S rRNA (9, 10).

To study its role in T cells, Zhou et al. (1) generated a T cell specific DCAF13 KO mouse (9). While TCR development in the thymus appeared grossly normal, T cell numbers in the periphery decreased. The activation of T cells as assessed by the phosphorylation of signaling molecules appeared to be normal, but the ability of T cells to proliferate was significantly depressed. Consistent with a defect in ribosome biogenesis, nucleoli did not expand in size and number after T cell activation, and this was associated with

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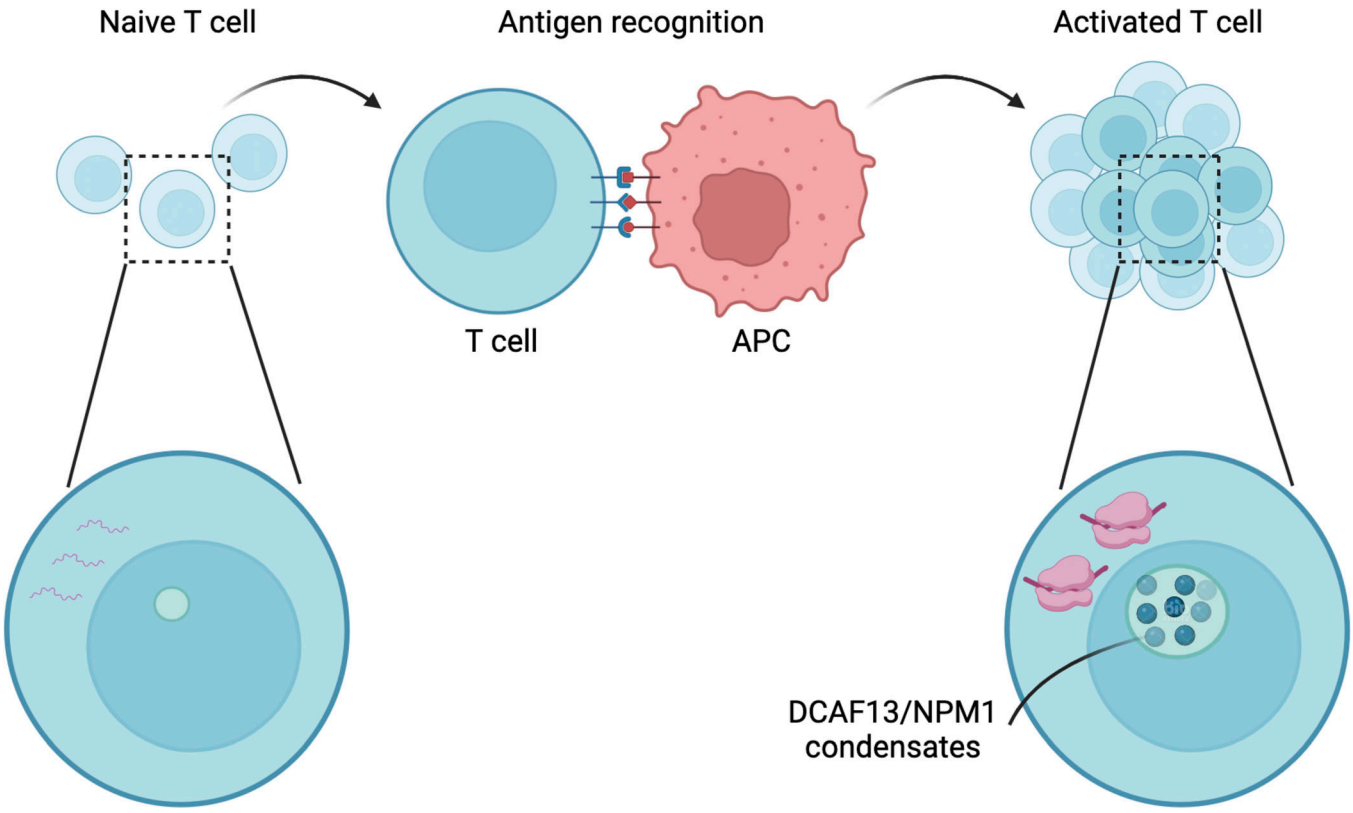

Figure 1. **Nucleolar DCAF13 promotes NPM1 phase separation, enhancing recruitment of UTP23 endonucleases to facilitate ribosome maturation.** Upon antigen stimulation, DCAF13 protein levels rise in the nucleolus. DCAF13/NPM1 condensates recruit UTP23 to facilitate 18S rRNA maturation and ribosomal biogenesis.

decreased 18S rRNA processing, decreased polyribosome formation, and decreased global protein synthesis. The role of DCAF13 was not specific to T lymphocytes. B cells, NK cells, dendritic cells, and monocytes were also significantly decreased in numbers in the spleen when DCAF13 was knocked out. Whether this was due to a developmental effect, whether DCAF13 is required for survival in the periphery, and whether DCAF13 is upregulated during cell activation was not explored.

To probe the role of DCAF13 in ribosomal biogenesis, they performed mass spectrometry on DCAF13 immunoprecipitates. They identified many nucleolar proteins that are associated with the 40S ribosomal subunit, including NPM1, a well-studied and abundant nucleolar protein. NPM1 binds to rRNAs and is required for ribosomal biogenesis (11). NPM1 is an oligomer, and when mixed with rRNAs and certain nuclear proteins in vitro, it can phase separate into biomolecular condensates (12).

Zhou et al. (1) mapped the interaction between DCAF13 and NPM1 and showed that DCAF13 interacted with NPM1 via its 30 residues at the C-terminal. They then tested its role in regulating NPM1 in the nucleolus. A critical feature of biomolecular condensates is their dynamic equilibrium with proteins outside of the condensates. Overexpression of DCAF13-GFP slowed the recovery of NPM1 in the nucleolus after photobleaching, suggesting that DCAF13 binding to NPM1 altered the fluidity of the condensate. In vitro, they found that addition of DCAF13 enhanced NPM1/rRNA condensates. One interpretation of these results is that nucleolar DCAF13 serves as a scaffold that stabilizes molecular interactions, allowing the nucleolus to expand when cells are actively growing. NPM1 is concentrated in the outside layer of the nucleolus where final processing of rRNA and ribosomal assembly occurs (2). Since the final processing step of rRNA is mediated by the endonuclease, UTP23 (13), and because DCAF13 T cells lack the 18S rRNA generated by UTP23, Zhou et al. (1) found that DCAF13/NPM1 condensates recruit UTP23.

Altogether, the work from Zhou et al. (1) suggests that when protein synthesis needs are increased, such as during T cell activation, DCAF13 upregulation promotes the biogenesis of ribosomes by increasing the size and number of nucleoli. Whether this mechanism also occurs in tumors that upregulate NPM1 was not explored in this study. While it is largely assumed that biomolecular condensate formation is specific, our understanding of the "molecular grammar" that governs protein sequence specificity is still in a nascent stage (14). While Zhou et al. (1) show that strings of positively charged residues in DCAF13 are important for its ability to form condensates, the molecular features of these charged residues was not explored in detail. Given the nebulous quality of interactions within biomolecular condensates, it will be important to better understand how condensate specificity is achieved. Nonetheless, the work shown here highlights the remarkable ability of condensates to self-assemble and dynamics to meet changing demands of protein synthesis.

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