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Making Christmas trees under duress, or how cells regulate the production of ribosomal RNA

Some of the most enduring images for a molecular biologist are electron microscopy micrographs of the so-called "Christmas trees", famously first observed by Oscar Miller from newt oocytes in 1969. These intriguing structures (Fig. 1A) capture the birth of multiple ribosomal RNA (rRNA) transcripts emanating from rDNA genes along chromatin. In humans, mice and other mammals, there are between 200-300 tandem rDNA gene copies, present in megabase arrays distributed over 5 different chromosomes in the genome. We need so many gene copies because the rRNAs that are processed out of the long RNA transcripts, the branches of the beautiful "Christmas trees", are the building blocks of ribosomes, the molecular machines that synthesise every single protein that makes cells and sustains life. Ribosome subunit synthesis takes place in a dedicated compartment of the nucleus, the nucleolus.

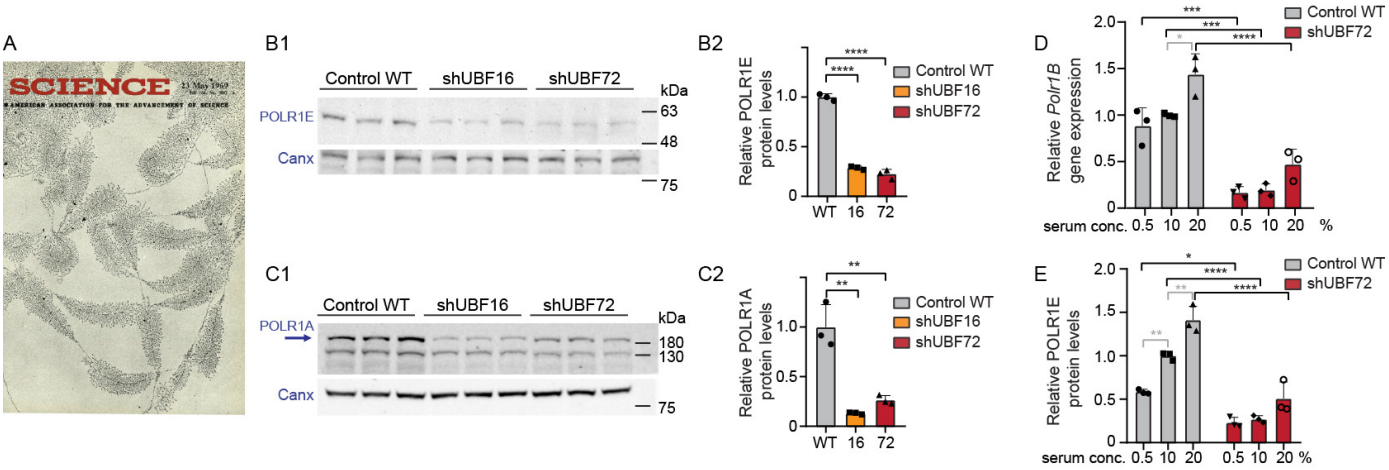

Fig. 1. Christmas trees and modulation of Pol1 levels upon UBF depletion. (A) Cover of Science magazine from May 1969 illustrating the now famous "Christmas trees" from the article by Miller and Beatty. (B1-C2) Analysis of protein levels of RNA Pol1 subunits POLR1E (B1-B2) and POLR1A (C1-C2) by Western blot and their quantification shows profound decreases in both shUBF16 and shUBF72 silenced cells, relative to wild-type (WT) cells. (D-E) Modulation of growth conditions (three different serum concentrations in culture medium at 0.5, 10, 20%), reveals a serum-dependency of Polr1E mRNA levels (D) and protein levels (E) in both WT and shUBF cells.

In the last few decades, molecular biologists worked painstakingly to tease out the cellular manual of how these rRNA transcripts are made and how their production is responsive to extracellular growth signaling (for example nutrient availability) and also internal cues (for example the cell cycle) to precisely control the availability of ribosomes capable of supporting cellular growth. This is important, not only because rRNA/ribosome synthesis is one of the most fundamental processes of life but also because mistakes in this process account for some serious human genetic syndromes, including the so-called ribosomopathies. Through these studies, a number of critical transcription factors, that help initiate transcription of rRNA, and also chromatin remodeling factors, that enable the acquisition of a transcription-competent structure of rDNA chromatin, have been identified. Among them, the pivotal role of master transcription factor UBF was delineated, specifically its interactions with the rRNA transcriptional machinery and its role in the non-

nucleosomal conformation of rDNA. However, one difficulty has been that UBF gene deletion is embryonically lethal very early on. In our work, we made stable cell lines, derived from NIH 3T3 mouse fibroblasts, partially silenced for UBF via shRNA-mediated lentiviral transduction. Thus, these new tools give the opportunity to study rRNA transcription under prolonged, viable, reduced UBF levels that may reveal longer-term adaptive, rather than acute responses.

We find that UBF-silenced cells (shUBF cells) exhibited UBF levels that could be compared with a haploinsufficiency status, known to be viable in mouse. They display cell cycle G1 delay, reduced synthesis of 47S rRNA precursor and 28S rRNA at baseline and serum-challenged conditions. Growth-related mTOR signaling is downregulated in these cells, with the fractions of active phospho-S6 Kinase and pEIF4E translation initiation factor reduced, similar to phosphorylated cell cycle regulator retinoblastoma, pRB, acting as a positive regulator of UBF availability/rRNA transcription. We find the proportion of transcription-competent pUBF (pSer484) responsive to extracellular cues. Fractional UBF occupancy on the rDNA unit is decreased in shUBF. Expression of major factors involved in different aspects of rRNA transcription, namely preinitiation complex proteins TAF1A-C, TBP and RRN3, termination factor TTF1, chromatin regulator CCTF, cyclin D, important for UBF Ser484 phosphorylation, and also master transcription factor C-MYC is severely downregulated by UBF depletion. Surprisingly, we identified reduction of polymerase 1 (Pol1) gene expression and protein levels under UBF silencing (Fig. 1B1-C2); we also identified regulation of RNA POL1, responsive to serum availability both in UBF-depleted cells (Fig. 1D-E) and also in wild-type, unmanipulated cells of different origin in human and mouse. Thus, it would appear that modulation of the polymerase itself is a regulatory mechanism generally applicable to cells. This is a novel finding because typically transcriptional regulation is considered either through modulation of polymerase binding at initiation or modulation of the transcription elongation rate. Modulation of RNA POL1 intracellular concentration at the transcriptional level, as shown here, is a less recognized and rarely discussed mode of regulation of rRNA synthesis that may deserve better attention, suggesting that regulation of rRNA transcription may not be restricted to modulation of POL1 promoter binding/elongation rate.

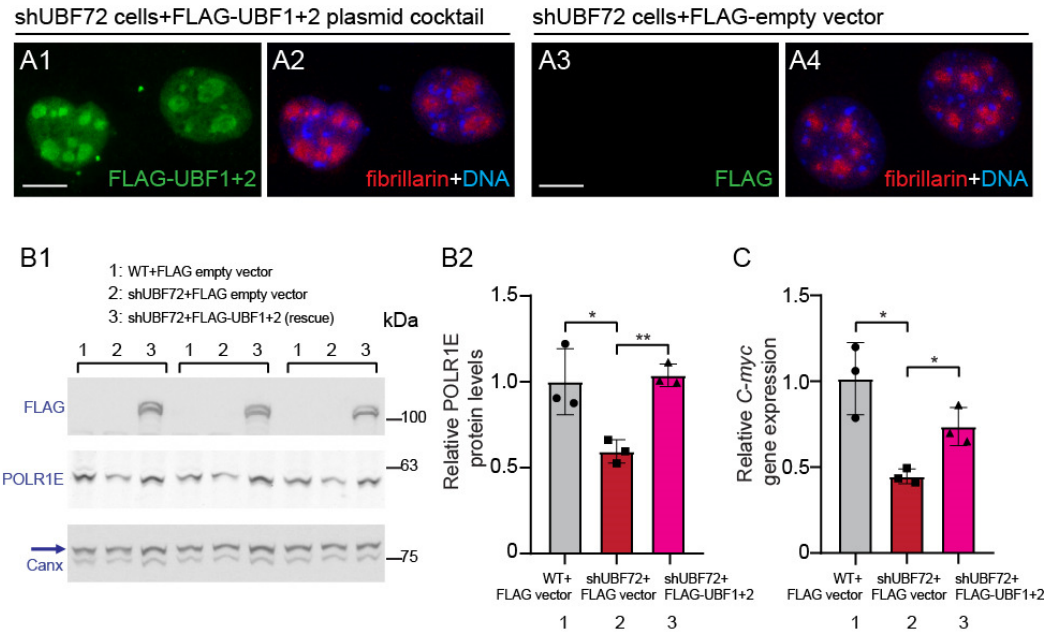

Fig. 2. Rescue of upstream UBF silencing phenotypes. (A1-A4) Correct nucleolar localization in shUBF72 cells, transiently transfected with a plasmid cocktail expressing silencing-resistant, synonymous triple mutant FLAG-tagged UBF1 and UBF2 in rescue experiments. Scale bars 10 μm. (B1-B2) Comparison by Western blot of POLR1E protein levels (middle panel) in 3 independent rescue experiments (B1) and its quantification (B2). (C) Quantification of C-myc gene expression in rescue experiments in the same set up.

The specificity of the wide-ranging impact of UBF depletion on all the factors of the rRNA transcriptional machinery was confirmed by recapitulation in an additional another stable cell line, constructed with a different silencing targeting sequence, and by rescue experiments. Specifically, transient transfection of shUBF cells with a plasmid mix expressing silencing-resistant, synonymous triple mutant FLAG-tagged UBF fully restored the protein levels of Pol1 and substantially rescued expression of *C-myc*, compared with silenced cells transfected in parallel with an empty FLAG vector (Fig. 2).

Overall, this work reveals that UBF depletion has a critical downstream and upstream impact on the whole network orchestrating rRNA transcription in mammalian cells and in co-ordination with growth signaling. In other words, “Christmas tree-making” is downsizeable to match the restrictive growth conditions that cells may encounter (or made to face by targeted UBF depletion) and this is a mechanism that affects simultaneously the entire Pol1 transcription network and, presumably by feedback loops, the upstream signaling as well.

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Publication

Transcription factor UBF depletion in mouse cells results in downregulation of both downstream and upstream elements of the rRNA transcription network
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