TRANSCRIPTION

No proper rest in mitosis

Mitotic chromosome condensation has been thought to interfere with chromatin-based processes, such as transcription. For this reason, it is thought that the genome is transcriptionally silenced during mitosis and that transcription is reactivated at mitotic exit. Zaret and colleagues now show that mitotic cells maintain low levels of transcription and that rates of transcription increase as cells progress through, and as they exit, mitosis.

Previous studies indicated the presence of elongating RNA polymerase II in prometaphase-arrested cells, suggesting that transcription occurs in mitotic cells. To investigate whether mitotic cells are transcriptionally active, the authors used a cell-permeable uridine analogue, 5-ethynyluridine, which is incorporated into newly synthesized transcripts. This analogue was coupled to biotin, enabling easy pulldown using streptavidin-coated beads, followed by RNA sequencing. A considerable number of transcripts (8,074 transcripts, corresponding to 3,689 different genes) were detected in cells arrested in prometaphase following nocodazole treatment. Mitotic expression of selected transcripts was confirmed by PCR. Moreover, incorporation of fluorescent UTP was visualized on the arms of condensed chromosomes, indicating active RNA synthesis. Collectively, these data provided strong evidence that transcription persists in mitosis. In general, in mitotic cells, transcription was considerably reduced (fivefold on average) in comparison with asynchronously growing cells. However, levels of 789 mitotic transcripts (corresponding to 484 genes) were higher in prometaphase-arrested cells than in asynchronous cells; gene ontology assignment revealed that these transcripts were linked to the composition of the extracellular space and cell–matrix interactions, protein metabolism and transcription.

> mitotic cells maintain low levels of transcription

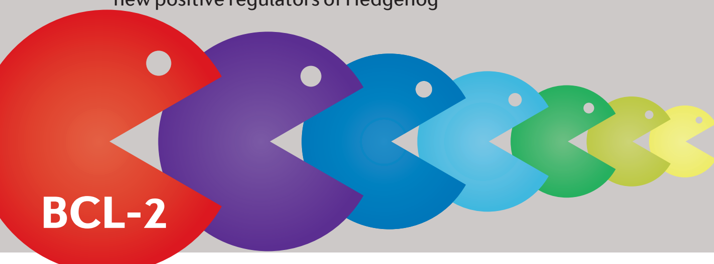
It has been previously shown that transcription is reactivated at mitotic exit, with a transcriptional burst occurring ~90 min after release of cells from the prometaphase block. However, in the present study the levels of 927 transcripts were found to increase already 40 min after nocodazole washout. Gene ontology analysis revealed that these early-activated genes primarily function in intracellular organization (prominently in the formation of membrane compartments) and in translation. A second wave of transcription activation was observed between 80 and 105 min after release from mitotic arrest, and these genes related to cell adhesions. Genes activated in a third wave (at 165–300 min, around the time of entry into G1 phase)

CELL DEATH

BCL-2 proteins feed their own expression

Apoptosis is tuned by a fine balance between the activity of pro-apoptotic and pro-survival (anti-apoptotic) proteins of the BCL-2 family. Wu *et al.* now show that the upregulation of pro-survival BCL-2 proteins — a hallmark of many cancers — can be driven by a feedforward loop, whereby pro-survival BCL-2 proteins promote the activity of GLI transcription factors, thereby bolstering their own expression.

GLI transcription factors are effectors of the Hedgehog signalling pathway, which has important roles in development and can promote cancer when overactive. Screening for new positive regulators of Hedgehog signalling, the authors identified the pro-survival BCL-2 protein MCL1 as an activator of GLI transcription.



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GLI transcription factors are directly inhibited by suppressor of fused homologue (SUFU). SUFU itself is regulated by phosphorylation, which promotes the SUFU–GLI interaction. Notably, *Mcl1*-knockout murine fibroblasts showed increased levels of total and phosphorylated SUFU, which suggests that MCL1 negatively regulates SUFU. Accordingly, the gain or loss of MCL1 inhibited or promoted SUFU–GLI interactions, respectively.

MCL1 co-immunoprecipitated with SUFU, which indicates that the proteins interact. These interactions depended on the presence of a BCL-2 core in MCL1 — a region conserved among pro-survival BCL-2 proteins that recognizes BH3 domains in anti-apoptotic BCL-2 proteins, thereby mediating their protein–protein interactions — and a previously unrecognized BH3 domain in SUFU. Notably, other pro-survival BCL-2 proteins, BCL-2 and BCL-X_L, also interacted with SUFU. Thus, pro-survival BCL-2 proteins recognize the BH3 domain in the GLI inhibitor SUFU, and, by binding to SUFU, they interfere with the inhibitory interaction between SUFU and GLI.

Depletion of pro-survival BCL-2 proteins decreased the transcription activity of GLI, both *in vitro* and in mouse liver tissue. By contrast, when overexpressed, pro-survival BCL-2 proteins promoted GLI-mediated transcription; this involved increased expression of genes that encode the pro-survival BCL-2 proteins themselves, which have been previously identified as GLI targets. This indicated that pro-survival BCL-2 proteins can establish a positive feedback loop, whereby they enhance the transcription activity of GLI to promote their own expression.

BH3 mimetics — small molecules that target the BCL-2 core of pro-survival BCL-2 proteins — inhibit

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were linked to the cell cycle and DNA replication. Interestingly, the increase in cell type-specific transcripts was generally observed at later timepoints (towards mitotic exit), suggesting that genes regulating basic cell functions and cell growth are prioritized over genes with more specialized functions.

Overall, low-level transcription persists in mitosis and high-level transcription is gradually reactivated during progression through mitosis and into G1. The biological significance of mitotic transcription remains to be determined, although it is plausible that the maintenance of gene expression during mitosis supports daughter cell integration into the surrounding extracellular space following division and primes them for the robust gene expression necessary for their growth and basic functions.

Paulina Strzyz

ORIGINAL ARTICLE Palozola, K. C. *et al.* Mitotic transcription and waves of gene reactivation during mitotic exit. *Science* http://dx.doi.org/10.1126/science.aal4671 (2017)

> pro-survival BCL-2 proteins can establish a positive feedback loop

the interactions of pro-survival and pro-apoptotic BCL-2 proteins. Wu *et al.* demonstrated that BH3 mimetics also interfered with the interactions of pro-survival BCL-2 proteins with SUFU, resulting in increased SUFU levels and phosphorylation. Importantly, the application of BH3 mimetics reduced the levels of pro-survival BCL-2 proteins and restricted the growth of tumours induced by overactive Hedgehog signalling.

Overall, pro-survival BCL-2 proteins can reinforce their anti-apoptotic effects by driving their own expression through the Hedgehog effector GLI. This finding sheds new light on the complexity of apoptosis regulation and on the mechanisms of action of BH3 mimetics and their utility for cancer therapy.

Paulina Strzyz

ORIGINAL ARTICLE Wu, X. *et al.* Extra-mitochondrial prosurvival BCL-2 proteins regulate gene transcription by inhibiting the SUFU tumour suppressor. *Nat. Cell Biol.* **19**, 1226–1236 (2017)

FURTHER READING Czabotar, P. E. *et al.* Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nat. Rev. Mol. Cell Biol.* **15**, 49–63 (2014)

Journal club

THE DISCOVERY OF CATALYTIC RNA

In 1978, Tom Cech started his lab at the University of Colorado and the project he set out on was to study the processing of ribosomal RNA (rRNA) in the ciliated protozoa, *Tetrahymena*. *Tetrahymena* was chosen because its rRNA genes are amplified about 10,000 times, so it is likely to provide a ready source of rRNA precursors. Of note is that the 26S rRNA gene in *Tetrahymena* is interrupted by an intron of 400 nucleotides.

A nuclear extract was developed to produce radioactive precursors (Zaug and Cech, 1980). A distinct 9S RNA molecule was evident in the gels and this turned out to be the 400-nucleotide intron. The appearance of this internal sequence indicated that RNA splicing was occurring in the nuclear extracts.

> The *in vitro* transcribed RNA still underwent the self-splicing reaction

The obvious next thing was to characterize the splicing of the intron. To produce a ^{32}P-labelled RNA substrate, labelled RNA was phenol extracted from nuclei and characterized by gel electrophoresis. In a wonderfully understated paragraph the discovery of RNA catalysis is reported (Cech *et al.*, 1981):

"We hoped to use the unspliced pre-rRNA … as a substrate to assay for splicing activity in nuclear extracts. This approach proved to be impossible, however, because excision of the intervening sequence occurred when the isolated RNA was incubated in transcription cocktail at normal salt concentration in the absence of a nuclear extract."

Papers rarely describe the actual circumstance of a discovery. In this case Art Zaug had carried out an *in vitro* splicing assay with a control lane containing no extract. The splicing reaction still took place. Exhaustive efforts to remove any protein still bound to the RNA after phenol extraction did not affect the reaction but this was not considered definite proof of the absence of a protein.

In a later paper, however, Kruger *et al.* (1982) described the cloning of a portion of the *Tetrahymena* rRNA gene containing the intron into an *Escherichia coli* plasmid. The *in vitro* transcribed RNA still underwent the self-splicing reaction, thereby proving that no protein is necessary.

Quite remarkably, Cech *et al.* (1981) also reported the correct mechanism of the reaction. GTP was found to be a required co-factor in the reaction, in which it forms a standard 3′–5′ linkage with the 5′ end of the intron, suggesting that the 3′-OH of GTP initiates the reaction by attacking the phosphodiester bond at the 5′ splice site.

In addition to the revolutionary implications this result has for biochemistry, there are two additional points to be made. First, the paper by Cech *et al.* (1981) is science at its best. A bound protein catalyst would have been interesting but not revolutionary. Everything possible to prove or disprove the bound protein hypothesis was done. Second, that RNA can be a catalyst has profound implications for the origin of life. Protein enzymes might have appeared but they could not replicate, so there must have been a ribozyme that could catalyse its own replication. Such ribozymes were soon discovered (Doudna and Sjostak, 1989).

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