

STUDYING GENE REPRESSION USING HIGH-THROUGHPUT 3D MICROSCOPY

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Most of the eukaryotic genes are transcribed in so called transcription bursts, i.e. short periods of time when gene promoter becomes permissive allowing the production of one to dozens of transcript copies at once. Bursts are typically followed by relatively long periods of transcriptional inactivity. This feature of transcription introduces additional variability in the population of genetically identical cells (transcription noise) resulting in distribution of transcription levels more dispersed than Poisson distribution typical for constitutively expressed genes.

Polycomb group proteins have a major role in mammalian embryonic development and cell differentiation through repression of their target genes. Whether their repressive function stems from alteration (decrease) of the transcription burst size or frequency still remains poorly understood. Moreover, whether Polycomb-repressed genes are uniformly lowly expressed in the entire cell population remains unknown and has not been studied likely due to technical caveats. Namely, to accurately assess transcription level distribution of repressed genes hundreds to thousands of single cells have to be studied.

Therefore, we employed high-throughput 3D widefield microscopy in combination with single molecule fluorescence in situ hybridisation (smRNA-FISH) of repressed genes in mouse embryonic stem cells. This approach allowed us to count individual molecules of transcripts and capture relatively rare cells (1:100 cells) to obtain accurate information about single-cell variability in a population. We demonstrate that the repressed genes have higher transcription noise than actively transcribed genes. Furthermore, fitting two-state model of transcriptional regulation to single-cell transcription level distributions provided new insights into mechanistic aspects of gene repression mediated by Polycomb group proteins.

[1] G. Kar et al., “Flipping between Polycomb repressed and active transcriptional states introduces noise in gene expression”, *Nature Communications*, 8:36 (2017).