Long-noncoding RNAs contribute to clonal heterogeneity by modulating transcription factor recruitment

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Mechanisms through which long intergenic noncoding RNAs (ncRNAs) exert regulatory effects on eukaryotic biological processes remain largely elusive. Most studies of these phenomena rely on methods that measure average behaviors in cell populations, lacking resolution to observe the effects of ncRNA transcription on gene expression in a single cell. Here, we combine quantitative single-molecule RNA FISH experiments with yeast genetics and computational modeling to gain mechanistic insights into the regulation of the Saccharomyces cerevisiae protein-coding gene *FLO11* by two intergenic ncRNAs, *ICR1* and *PWR1*. Direct detection of *FLO11* mRNA and these ncRNAs in thousands of individual cells revealed alternative expression states and provides evidence that *ICR1* and *PWR1* contribute to *FLO11*'s variegated transcription, resulting in Flo11-dependent phenotypic heterogeneity in clonal cell populations by modulating recruitment of key transcription factors to the *FLO11* promoter.

***Keywords* — Single cells, long non-coding RNA, RNA FISH, flocculation, *FLO11*, *PWR1*, *ICR1*, yeast.**

I. BACKGROUND

THE sequencing of genomes from many different organisms has shown that a large fraction of the genome does not code for proteins alone but also does code for ncRNA molecules. Genome-wide studies have identified many long ncRNA, yet very little is known about the function of these ncRNA. We are investigating the transcriptional interference of two, long ncRNA strands (*PWR1* and *ICR1*) in the regulation of an epigenetic switch (*FLO11*) in single *Saccharomyces cerevisiae* yeast cells. Single-cell experiments have the advantage that the gene expression of multiple non-coding and coding RNA molecules can be directly compared with each other in the same cell, without averaging over many individual cells, leading to novel mechanistic insights into gene regulation.

II. RESULTS

We observe bimodal expression of *FLO11* at the transcript level in single cells. This bi-modality results in a cellular phenotype in which cells expressing low levels of mRNA transcripts are unable to flocculate. This bi-modal expression and the cellular phenotype are modulated by two ncRNA (ICR1 and PWR1). By using two colors RNA FISH experiment in single cells, positive regulation of the *PWR1* ncRNA and negative regulation of the ICR1 ncRNA on the *FLO11* gene was observed. The two ncRNA change the fraction of cells expressing *FLO11* transcripts but not the mean *FLO11* expression level. This change in the fraction of cells expressing *FLO11* transcripts is correlated with localization of key transcription factors to the *FLO11* promoter.

III. CONCLUSION

Our single cell data demonstrate that the non-coding RNAs modulate the localization of key transcription factors, which influence the occurrence of downstream events that lead to active or silenced *FLO11* transcription. Furthermore these long non-coding RNA are detected in the nucleus and the cytoplasm resolving a long-standing question to which extend this class of RNA molecules are exported out of the nucleus. The combination of quantitative single-molecule RNA experiments in single cells with yeast genetics- and single-molecule-based modeling is a major step towards a detailed understanding of the function of long non-coding RNA in gene regulation in single cells and will lead to a better mechanistic understanding of this class of RNA molecules ranging from yeast to humans.

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