

O-28.2 Invited speaker

Spatial organisation of bacterial transcription via phase separation of transcription factors

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Transcription is a key process that allows bacteria to respond to their environment and tune their growth. Transcription is often highly organised, as exemplified by the formation of large RNA polymerase (RNAP) clusters during fast growth in *E. coli* and *B. subtilis*; past work suggested that such structures are phase-separated condensates which maximise ribosomal RNA transcription. We recently showed that these structures contain clusters of universal transcription factor NusG. To understand the organisation and physical nature of bacterial transcriptional clusters, we performed in vivo time-lapse imaging of clusters in different growth conditions. Tracking clusters revealed that NusG clusters have similar mobilities to DNA, suggesting they are chromosome-anchored. Surprisingly, substantial NusG clustering persisted even during slow growth, in contrast to the loss of RNAP clustering. The persistence of NusG clustering could be due to its re-allocation to other processes and indicates that NusG forms or facilitates the weak protein-protein interactions that form phase-separated condensates; indeed, we showed that NusG forms phase-separated condensates in vitro, supporting that NusG is the key phase-separating protein in the in vivo condensates. Our work advances our understanding of how phase separation can tune transcription and bacterial physiology.