Real-time transcription initiation by *E.coli* RNA polymerase *in vitro* and *in vivo*

Anne Plochowietz[1], Diego Duchi Llumigusin[1], Pawel Zawadzki[2], Afaf H El-Sagheer[3], Tom Brown[3], Achillefs N Kapanidis[1]

[1]Clarendon Laboratory, Biological Physics Research Group, Department of Physics, University of Oxford, Parks Road, Oxford, OX1 3PU, United Kingdom

[2]current address: Microbiology unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, United Kingdom

[3]Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Mansfield Road, Oxford, OX1 3TA, United Kingdom

Transcription initiation is the most important step in gene regulation and is orchestrated by RNA polymerase (RNAP). However, initial transcription mechanisms have remained unclear mainly due to the presence of transient intermediates and heterogeneity. To characterize such mechanisms, we used an *in vitro* real-time FRET assay on immobilized transcription complexes for minute-long observations of DNA scrunching and unscrunching. We characterized the kinetics of abortive initiation (by following single cycles of abortive synthesis) and promoter escape, and identified functionally important heterogeneity. We observed, for the first time, extensive RNAP pausing (lasting for ~15 s) and backtracking during initiation; such behaviors may play regulatory roles.

We also studied initial transcription *in vivo* using electroporation to internalize doubly labeled promoter-DNA fragments into live *E.coli* and track them using TIRF microscopy. We observed low-FRET species of 0.18±0.05 (corresponding to duplex DNA) and fluctuations to higher FRET-states, which we attribute to RNAP promoter binding, open complex formation and initial transcription. Specifically, we see FRET of 0.35±0.10 (linked to initiation pausing), and 0.85±0.06 (linked to promoter escape); both levels are absent in non-promoter DNA. Our work reveals the detailed kinetics of initial transcription *in vitro* and offers the first such observations in living cells.