

POSTER PRESENTATION

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# Pausing as a mechanism of nucleosome recovery

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## Background

Nucleosome survival during transcription is important for the maintenance of chromatin integrity, gene regulation, cell survival, and aging. In according to the studies *in vitro* and *in vivo*, Pol II pauses at positions inside of a nucleosome and nucleosome survives after Pol II transcription through chromatin [1]. A key intermediate,  $\emptyset$ -loop (EC+49), formed at +45 region inside of nucleosome and Pol II pausing at +45 nucleosomal region positively correlated to nucleosome survival [2,3]. A putative electrostatic interaction between Pol II and histone octamer has also been predicted to stabilize the  $\emptyset$ -loop intermediate by structural modeling [3]. In this study, we will provide more evidences to link those characteristics together.

## Materials and methods

Protein purification [3][4]

Computational structure modeling [3]

Transcription assay, Nucleosome fate and DNase I footprinting [3,5]

## Results

In structural modeling, three negatively charged surfaces (regions 1, 2, 3) were identified on Rpb1, a large subunit of yeast Pol II, and plausibly interacted to histone octamer, especially H2B N-tail region, by the electrostatic force. Results of transcriptions by *Thermus thermophilus* (*T.th.*) and *Thermus aquaticus* (*T.aq.*) RNAPs which contained less net negative charges at all three regions showed lower +45 pausing, no  $\emptyset$ -loop formation and nucleosome displacement. Finally, lower nucleosomal barrier was also shown during the transcription by mutated yeast Pol II which contains less negatively charged at region 2 of Rpb1.

## Conclusions

Collectively, we demonstrated the positive correlations of higher negative net charge of the interacting region of RNAP, the stronger +45 barrier, more efficient nucleosome survival and more efficient  $\emptyset$ -loop intermediate formation during transcription.

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