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Enhancers, phase separation and the RNA Polymerase II transfer model.

Authors: Katie Gelder, Daniel Bose*

Affiliations:

Molecular and Cellular Biology, School of Biosciences, Sheffield Institute For Nucleic Acids, The University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN, UK

*Corresponding author: Daniel Bose, e-mail: d.bose@sheffield.ac.uk

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The phenomenon of phase separation, the formation of highly concentrated regions of proteins and nucleic acids that have no separating membrane, is now recognised as an important mechanism for regulating gene expression through enhancer function. The “RNA Polymerase II (PolII) transfer model” was proposed as a mechanism for transcriptional activation at enhancers (for a definition of terms, please see box 1) [1]. The model specifically addresses how enhancers increase gene expression via phase separation, relying on transient transcription at enhancers to facilitate early release of transcription complexes. The model postulated that elongation-competent transcription complexes are assembled at enhancers during transcription of enhancer RNAs (eRNAs). These complexes are subsequently released in a dephosphorylated form and sequestered to the promoter via condensation, providing a model for initiation of gene transcription through phase separation at enhancers [1]. Although intriguing, at the time the model lacked supporting data. Here, we evaluate the PolII transfer model as a mechanism for enhancer condensate gene expression, in light of recent advances.

Condensates in enhancer function

Enhancers are transcription factor (TF) binding hubs, where TFs recruit coactivator proteins that are donated to promoters to increase the transcription of target genes. Over the last decade, phase separation at enhancers has been increasingly recognised as an important regulator of gene expression. Proteins commonly found at enhancers, such as the coactivator p300, can co-condense with multiple transcription factors, promoting activation of its catalytic activity and regulating transcription bursting kinetics, independently of p300’s catalytic activity [2]. The transfer model suggests that bursting is related to the formation of transient enhancer/promoter contacts through condensation, so the observed link between enhancer condensation and bursting appears consistent with the model.

Integrator

The PolII transfer model specifically addresses how transcription of eRNAs facilitates the early release of PolII complexes assembled at enhancers, transferring them to promoters via phase separation. eRNA transcription is terminated by the Integrator complex, which prevents PolII

progressing into productive elongation through a combination of protein phosphatase and endonuclease activities [3, 4]. When the integrator endonuclease subunit *INTS11* was depleted in murine erythroleukemia (MEL) cells, an accumulation of PolII was observed at the Locus control region (LCR) of β-globin and decreased recruitment to the promoter of β-globin, highlighting the importance of Integrator in PolII release and transfer [3]. Similar observations were apparent upon acute, degron-mediated depletion of *INTS11* in mouse embryonic stem cells (mESCs), where depletion of INTS11 caused a build-up of PolII at promoters and accumulation of short RNA transcripts, including eRNAs [4]. In this study, transferred PolII was shown to be predominantly unphosphorylated, with the phosphatase subunit of Integrator being transferred along with PolII [4]. Consequently, no alteration of PolII phosphorylation was observed at promoters [4], leaving PolII unphosphorylated and providing further support for the PolII transfer model.

eRNAs

eRNAs were also suggested to have a more active role in β-globin gene expression. When transcribed, they stay present at the gene locus and upon their depletion a reduction in gene expression was observed, as well as an accumulation of PolII at the LCR [3]. A similar mechanism has been suggested to activate gene expression at promoters. Paraspeckle promoting protein PSPC1, (an RNA binding protein found in paraspeckles, a type of condensate) was shown to bind RNA at sites of transcription initiation, contributing to recruitment of PolII. The production of short, negatively charged RNAs by basal transcription caused the premature release of PolII before it could be phosphorylated [5]. Condensates composed of these RNAs and other proteins facilitate the transfer of these complexes to promoters so that transcription can occur [5]. This evidence suggests the PolII transfer model may occur between promoters as well as between enhancer-promoter pairs and indicates a potential role of eRNAs in early release of PolII before phosphorylation can occur.

Conclusion

Recently published studies broadly support the PolII transfer model as a mechanism for increasing gene expression at enhancers through phase separation. Overall, this revised model shows that transcription of enhancers produces eRNAs, which are released upon early transcription termination via the Integrator complex. Phase separated condensates consisting of coactivators and eRNAs maintain proximity between enhancers and promoters, facilitating donation of released PolII complexes to the promoter, promoting gene expression.

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Box 1: Enhancers, Super enhancers and LCRs

Enhancers: non-coding genetic *cis*-regulatory elements which regulate gene expression from kilobases or megabses away from the transcription start site independently of orientation.

Super enhancers (SEs): extended or multiple enhancers associated with particularly high levels of the Mediator complex and PolII, characterised by high accessibility such as sensitivity to DNase I treatment, also known as hypersensitive sites.

Locus Control Regions (LCRs): Super enhancers containing several hypersensitive sites which work cohesively together to regulate gene expression independently of position in transgenic mice.

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