Molecular Cell

CellPress

Previews

Tracking the folding of RNA at its birth

Jinsong Zhang[1,2,3,4,5,6] and Qiangfeng Cliff Zhang[1,2,3,4,5,6,*]
[1]State Key Laboratory of Membrane Biology, Tsinghua University, Beijing 100084, China
[2]MOE Key Laboratory of Bioinformatics, Tsinghua University, Beijing 100084, China
[3]Center for Synthetic and Systems Biology, Tsinghua University, Beijing 100084, China
[4]Beijing Advanced Innovation Center for Structural Biology & Frontier Research Center for Biological Structure, Tsinghua University, Beijing 100084, China
[5]School of Life Sciences, Tsinghua University, Beijing 100084, China
[6]Tsinghua-Peking Center for Life Sciences, Tsinghua University, Beijing 100084, China
*Correspondence: qczhang@tsinghua.edu.cn
https://doi.org/10.1016/j.molcel.2025.03.022

In this issue of *Molecular Cell*, Schärfen et al.[1] describe an advanced RNA structure-probing technology called CoSTseq that enables transcriptome-wide detection of nascent RNA base pairing during transcription in living yeast cells.

RNA molecules are fundamental to cellular processes, with their functions intricately linked to their three-dimensional structures. Misfolded RNAs are implicated in diseases ranging from neurodegenerative disorders to cancers, underscoring the importance of understanding RNA folding mechanisms. It is widely accepted that RNA begins to fold as it is being synthesized, a process known as co-transcriptional folding. While its role in regulating transcription and translation is well documented in bacteria,[2] only a few cases have been reported in eukaryotes, such as the formation of the stem-loop structures in nascent histone RNAs that facilitates 3′ end cleavage.[3] Technical challenges have hindered high-resolution studies of co-transcriptional folding in eukaryotes. In this issue of *Molecular Cell*, Schärfen et al.[1] describe CoSTseq (co-transcriptional structure tracking), a breakthrough technology that enables transcriptome-wide mapping of RNA base pairing during transcription in live yeast cells.

Historically, co-transcriptional folding has been studied via chemical/enzymatic probing and single-molecule Förster resonance energy transfer. Early eukaryotic studies focused on Pol II-associated RNAs using chemical/enzymatic probing.[4,5] However, in those studies, the nascent RNAs were treated as a pool, lacking the connection between base-pairing status and polymerase position. Incarnato et al.[6] pioneered Structural Probing of Elongating Transcripts (SPET-seq) to profile nascent RNA folding, albeit in the bacterium *E. coli*, by exploiting nucleoid-associated RNA 3′ ends as readouts of polymerase position. However, compared to prokaryotes, the increased complexity in the composition of eukaryotic nuclear RNA and regulation of eukaryotic transcription and the resulting lower abundance of nascent RNAs collectively present challenges for high-resolution analysis of co-transcriptional folding in eukaryotes.

Yu et al.[7] partially addressed this by adapting SPET-seq to eukaryotes, enriching nascent RNA through chromatin fractionation and 3′-OH ligation (Figure 1A). Their approach exploited the fact that degraded RNA fragments, marked by terminal phosphates, cannot undergo ligation, effectively excluding them from the enriched nascent RNA pool. Schärfen et al. now advance the field with CoSTseq, which combines biotin-NTP transcriptional run-on and dimethyl sulfate (DMS) probing (Figure 1B). The approach hinges on biotinylated CTP incorporation, which serves dual roles: enabling selective purification of nascent RNAs by capturing biotin-tagged molecules and defining their 3′ ends through transcriptional run-on. Unlike SPET-seq's reliance on reverse transcription truncations, CoSTseq encodes chemical modifications as mutations during reverse transcription, reducing sequencing depth requirements. Schärfen et al. used CoSTseq to track the global base-pairing status of nascent RNA after transcription of both Pol I and Pol II. They observed that base pairings initiate about 16 nucleotides from the transcription site, aligning with structural models[8] showing template DNA base pairing and physical constraints in the polymerase exit channel.

Schärfen et al. first apply CoSTseq to study ribosomal RNA (rRNA), as the high coverage of CoSTseq enables a fine-resolution analysis of rRNA co-transcriptional folding. The functionalized ribosome required correct assembly during its biogenesis, which is coordinated by co-transcriptional folding of rRNA and cooperative associations with proteins, such as assembly factors, chaperones, helicases, and so on.[9] Schärfen et al. revealed that only ∼23% of rRNA nucleotides achieve their mature structures near Pol I, while many others form transient structures requiring rearrangements. Quantitative analysis uncovered distinct co-transcriptional folding trajectories and parameters (Figure 1C). Notably, Schärfen et al. identified the helicase Dbp7 as a global modulator of rRNA folding, acting co-transcriptionally across pre-rRNA transcripts (Figure 1D)—consistent with its known role in pre-60S rRNA processing.[10] These findings illuminate how rRNA folding cooperates with protein factors during ribosome assembly, a process ripe for further exploration.

In contrast to rRNA, Schärfen et al. found that pre-mRNAs exhibited minimal structural divergence between nascent and mature states, corroborated by reanalysis of human *in vivo* click selective 2-hydroxyl acylation and profiling experiment data[5] and SPET-seq observations

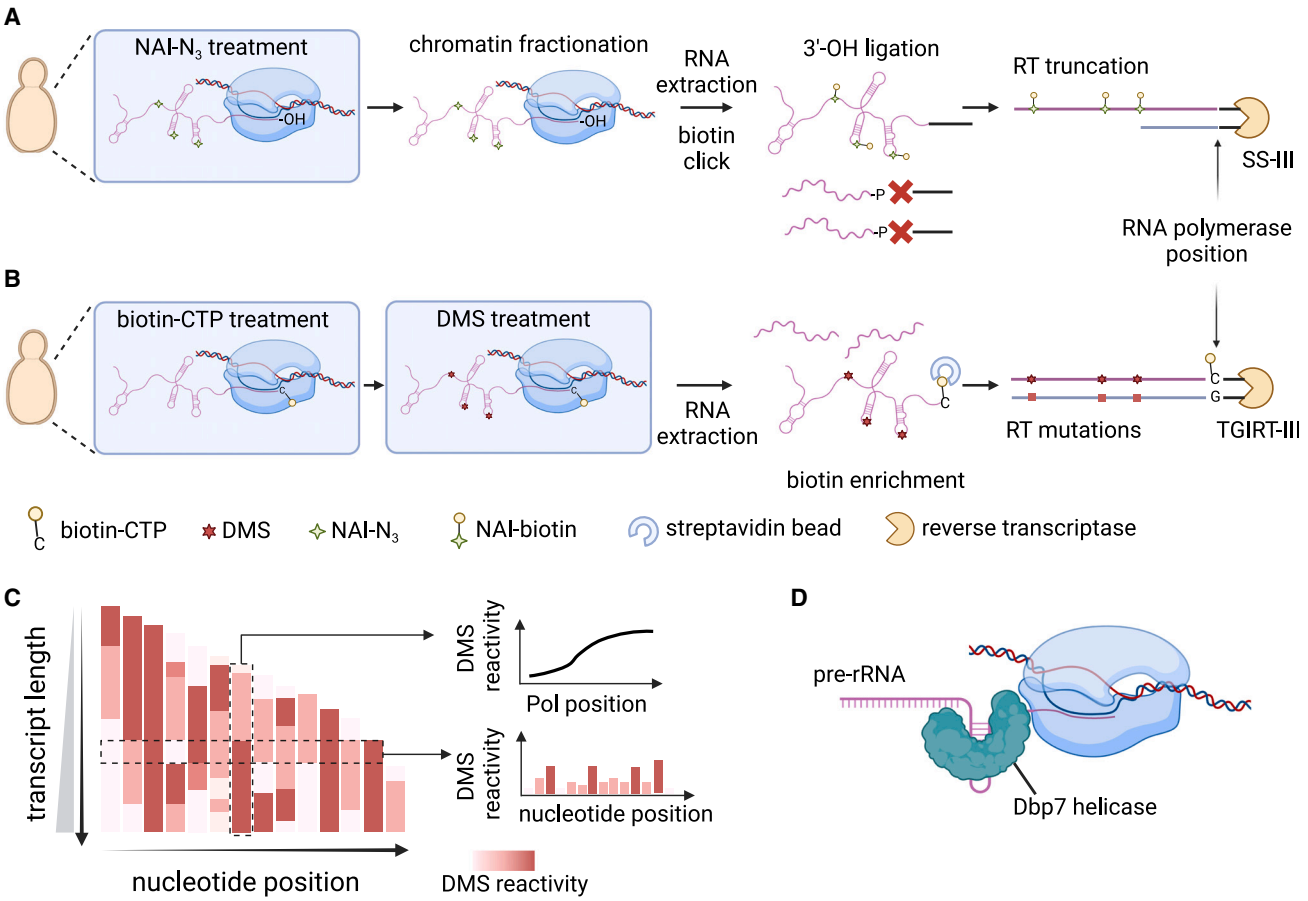


Figure 1. CoSTseq enables transcriptome-wide mapping of co-transcriptional RNA folding in eukaryotes

(A) Schematic workflow of adapting SPET-seq for eukaryotes. Cells are treated with 2-(azidomethyl)nicotinic acid imidazolide (NAI-N_3) to modify unpaired bases in nascent RNA. The modified RNA is then enriched by isolating the chromatin fraction through cellular fractionation, followed by 3′ adaptor ligation. NAI-N_3 modifications are biotinalyted via click chemistry. The biotin is used for downstream enrichment of modified RNA but not for nascent RNA. Finally, the structural signals are read out through SuperScript-III (SS-III) reverse transcription (RT) truncations on the modified nucleotides.

(B) Schematic workflow of CoSTseq. Cells are first treated with biotinylated CTP, which is incorporated into nascent RNA and halts transcription and marks the RNA's 3′ end. The cells were then treated with DMS, which modifies unpaired A and C bases. Notably, this design allows the enrichment of biotinylated nascent RNA via streptavidin pulldown. Finally, the structural signals are read out through TGIRT-III (a thermostable reverse transcriptase) RT mutations on the modified nucleotides, with an adaptor added through template switching.

(C) Co-transcriptional folding matrix of a nascent RNA. Each row represents the DMS reactivity profile of nascent RNA at a specific RNA polymerase position, indicated along the y axis of the matrix. Each column represents the DMS reactivity trajectories for a specific nucleotide, indicated along the x axis of the matrix, starting from the position where the nucleotide was added to the nascent RNA.

(D) Helicase Dbp7 functions as a global modulator of rRNA folding during transcription.

The figure was created in https://BioRender.com.

of fewer structural transitions in mRNAs.[7] This difference might be explained by the fact that mRNAs undergo similar refolding upon exiting the ribosome during translation. A previous study showed that lower transcription elongation rates promote overall structure formation of nascent mRNA in human cells.[4] However, no significant changes in the overall structure of nascent mRNA were detected by CoSTseq upon depletion of Pol II elongation factors in yeast cells. This may be due to the rapid folding of nascent RNA and faster splicing of introns in yeast. Alternatively, the lack of detectable changes could be due to the analysis primarily focusing on exonic regions, potentially overlooking structural alterations in introns.

Schärfen et al.'s work establishes CoSTseq as a powerful tool for illuminating eukaryotic RNA folding dynamics, opening new avenues to explore transcriptional regulation at unprecedented resolution. Future refinements, such as targeted sequencing strategies or enhanced enrichment approaches, could further broaden its utility across diverse RNA classes. Integrating long-read sequencing platforms may also extend its capacity to map RNA structural transitions across full transcriptional landscapes. Excitingly, CoSTseq paves the way for probing structural heterogeneity within individual transcripts, offering insights into real-time folding diversity during synthesis. As the method evolves, it holds immense promise for decoding disease-associated RNA folding aberrations in conditions like cancer and neurodegeneration, bridging molecular mechanisms to therapeutic discovery. By unraveling

how RNA-binding proteins and helicases choreograph co-transcriptional folding, this technology could inspire novel strategies to harness RNA structure for precision medicine, accelerating the development of next-generation RNA therapeutics.

ACKNOWLEDGMENTS

This work was supported by the National Key Research and Development Project of China (grant no. 2022YFF1203100 to Q.C.Z.), the National Natural Science Foundation of China (grants no. 32230018 and 32125007 to Q.C.Z.).

DECLARATION OF INTERESTS

Q.C.Z. is a member of *Molecular Cell*'s advisory board.

REFERENCES

1. Schärfen, L., Vock, I.W., Simon, M.D., and Neugebauer, K.M. (2025). Rapid folding of nascent RNA regulates eukaryotic RNA biogenesis. Mol. Cell *85*, 1561–1574.e5. Published online March 25, 2025. https://doi.org/10.1016/j.molcel.2025.02.025.

2. Shine, M., Gordon, J., Schärfen, L., Zigackova, D., Herzel, L., and Neugebauer, K.M. (2024). Co-transcriptional gene regulation in eukaryotes and prokaryotes. Nat. Rev. Mol. Cell Biol. *25*, 534–554.

3. Saldi, T., Fong, N., and Bentley, D.L. (2018). Transcription elongation rate affects nascent histone pre-mRNA folding and 3' end processing. Genes Dev. *32*, 297–308.

4. Saldi, T., Riemondy, K., Erickson, B., and Bentley, D.L. (2021). Alternative RNA structures formed during transcription depend on elongation rate and modify RNA processing. Mol. Cell *81*, 1789–1801.e5.

5. Sun, L., Fazal, F.M., Li, P., Broughton, J.P., Lee, B., Tang, L., Huang, W., Kool, E.T., Chang, H.Y., and Zhang, Q.C. (2019). RNA structure maps across mammalian cellular compartments. Nat. Struct. Mol. Biol. *26*, 322–330.

6. Incarnato, D., Morandi, E., Anselmi, F., Simon, L.M., Basile, G., and Oliviero, S. (2017). In vivo probing of nascent RNA structures reveals principles of cotranscriptional folding. Nucleic Acids Res. *45*, 9716–9725.

7. Yu, G., Liu, Y., Li, Z., Deng, S., Wu, Z., Zhang, X., Chen, W., Yang, J., Chen, X., and Yang, J.R. (2023). Genome-wide probing of eukaryotic nascent RNA structure elucidates cotranscriptional folding and its antimutagenic effect. Nat. Commun. *14*, 5853.

8. Hein, P.P., Kolb, K.E., Windgassen, T., Bellecourt, M.J., Darst, S.A., Mooney, R.A., and Landick, R. (2014). RNA polymerase pausing and nascent-RNA structure formation are linked through clamp-domain movement. Nat. Struct. Mol. Biol. *21*, 794–802.

9. Rodgers, M.L., and Woodson, S.A. (2021). A roadmap for rRNA folding and assembly during transcription. Trends Biochem. Sci. *46*, 889–901.

10. Aquino, G.R.R., Hackert, P., Krogh, N., Pan, K.T., Jaafar, M., Henras, A.K., Nielsen, H., Urlaub, H., Bohnsack, K.E., and Bohnsack, M.T. (2021). The RNA helicase Dbp7 promotes domain V/VI compaction and stabilization of inter-domain interactions during early 60S assembly. Nat. Commun. *12*, 6152.

Unraveling the proton-sensing mechanisms of G protein-coupled receptors: Insights from cryogenic electron microscopy studies

Chenyang Xue[1,2] **and Zhongmin Liu**[1,2,*]
[1]Department of Immunology and Microbiology, School of Life Sciences, Southern University of Science and Technology, Shenzhen, Guangdong, China
[2]Institute for Biological Electron Microscopy, Southern University of Science and Technology, Shenzhen, Guangdong, China
*Correspondence: liuzm@sustech.edu.cn
https://doi.org/10.1016/j.molcel.2025.03.020

In this issue, Guo et al.[1] and Chen et al.[2] resolved the cryo-EM structures of G protein-coupled receptor (GPR) 4 and GPR68, unveiling that histidine protonation initiates conformational rearrangements and dictates G protein coupling bias, illuminating pH-sensing mechanisms.

Protons are commonly found in aqueous solutions as H_3O^+, which is reflected in pH values. Proton-sensing G protein-coupled receptors (GPCRs)—including GPR4, GPR65, and GPR68—are pivotal regulators of extracellular pH homeostasis, orchestrating biological processes ranging from immune surveillance to tumor microenvironment dynamics.[3–6] Unlike canonical GPCRs that require steric bulk-mediated ligand-receptor interactions for orthosteric or allosteric activation, these pH-sensitive receptors employ protons themselves as primary ligands.[7] This unique activation mechanism bypasses the structural complementarity constraints inherent to classical GPCR signaling, instead relying on protonation-induced conformational changes within conserved extracellular sensor domains.[8,9] Although recent studies have begun to unravel this paradigm—Howard et al. identified a network of titratable residues spanning from extracellular loops to the receptor core as essential for activation,[8] and Wen et al.[9] and Ma et al.[10] revealed both conserved and species-specific proton-sensing adaptations in GPR4—critical knowledge gaps persist. The precise molecular logic governing proton perception, signal transduction fidelity, and G protein coupling selectivity has remained enigmatic, underscoring