Photonics reveal Cycline T1-guided P-TEFb fate

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Transcription is a tightly regulated biological process involving a bulk of protein regulators revolving around the RNA polymerase II (RNAPII). Despite the progress in the identification and the characterization these regulators, the coordination and the dynamics of diffusion and assembling of these regulators with RNAPII in living cells remains largely ignored. We thus focus on the release of RNA polymerase II (RNAPII) elongation phase by the positive transcription elongation factor b (P-TEFb), a cyclin-dependent kinase complex. This latter has been well biochemically characterized but investigations about its biophysical behavior are poorly documented.

Although P-TEFb is assumed to diffuse throughout the nucleus, the molecular processes ruling both its diffusion and its binding to RNA POL II are mainly unknown. Because RNAPII is a supra-molecular complex, it can be expected that its steric effect restricted the accessibility of fluorescent protein to allow FRET. With molecular biology strategies, we thus developed new tools improving FRET occurrence. We confirmed that the binding of P-TEFb to the DNA-RNA POL II complex is actually dependent of the histine-rich region of the unconfirmed C-terminus of Cyclin T1. This confirms that the loss of anisotropic diffusion of P-TEFb¹, as reported recently by SPT microscopy, is induced by the loss of interaction between Cyclin T1 and RNAPII. Finally, by the mean of molecular biology tools and FCS analysis, we demonstrated that Cyclin T1 is the diffusion-limiting partner in P-TEFb, whatever in its active or inactive form. Nevertheless, we found that the interaction of Cyclin T1 with RNAPII does not modify the diffusion mode, suggesting that compact diffusion observed by STP is not directly related to the sub-diffusion behaviour of P-TEFb.

In conclusion, our data show in single living cells, that while Cdk9 is the active component of the functional P-TEFb complex, Cyclin T1 is the rating-limiting partner. Besides, we demonstrate that sub-diffusion of P-TEFb, while due of Cyclin T1, is independent of RNAPII and then requires other interactions.


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