Single molecule imaging of histone-regulated TBP binding to DNA in Zebrafish genome activation

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Cellular tasks such as transcription rely on stochastic interactions of biomolecules. Thus, single molecule methods are beneficial in revealing the kinetic and structural underpinnings of these tasks, and consequently single molecule tracking has been developed for live cells and organisms. Yet, following individual fluorescently labeled biomolecules throughout the development of an organisms is still challenging. Here, we establish single molecule tracking of genetically encoded fluorescent proteins in live developing Zebrafish embryos using reflected light-sheet microscopy. In addition, we design a novel time-lapse acquisition scheme allowing for fast quantification of biomolecular interaction kinetics. We use our methods to monitor and quantify the interactions between the general transcription factor TBP and DNA during embryo development. We find that TBP interacts with DNA at all developmental stages, but binding to DNA changes from transient to stable within the first twelve cell division cycles. This kinetic transition temporally coincides with the delayed onset of transcription in the Zebrafish embryo. Our single molecule experiments reveal a potential mechanism of an important step in Zebrafish embryo development.