Mapping the network regulating transcription factor activity and dynamics by TF-FRAP

P-02.5-01

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Cells respond to their environment via an intricate cellular signaling network. Insight into this network regulating cell fate is important for controlling stem cell differentiation, understanding disease pathology and defining better regenerative medicine strategies. Changes in cell fate are characterized by changes in gene transcription. These changes are dictated by changes in (master) transcription factor (TF) activity. SOX9 is the master TF of cartilage development. However, its dysfunction is associated with diseases, such as cancer, osteoarthritis (OA), fibrosis, sclerosis, etc. Here, we present a new method to directly monitor changes in TF activity. We developed Transcription Factor – Fluorescence Recovery After Photobleaching (TF-FRAP) to measure SOX9 dynamics and activity in primary human chondrocytes (hPCs) to understand its role in OA pathology. We found that changes in SOX9 dynamics as measured by TF-FRAP correlated to its transcriptional activity. Higher DNA binding and longer residence time of SOX9 on DNA increased its target gene expression levels and vice versa. SOX9 dynamics studies on hPCs showed that its residence time and DNA binding is significantly lower in OA as compared to healthy hPCs. We cross-validated TF-FRAP data with ChIP-qPCR and quantified gene expression changes with RT-qPCR. Moreover, TF-FRAP also identified subpopulations of cells within a donor, based on distinct dynamic rates of SOX9. Distinct and diffused SOX9 nuclear localization patterns were observed in the healthy and OA hPCs respectively. Distinct nuclear localization patterns correlated to higher DNA binding rate and longer residence times in healthy hPCs as compared to OA hPCs. Our data indicate a differential response of SOX9, depending on the disease state of the hPCs. This may have implications for treatment strategies that aim to restore SOX9 function. We show for the first time that our TF-FRAP method enables monitoring TF activity in real-time in primary cells.