Specificity in transcription factor clustering is governed by DNA binding, while IDRs lower threshold concentration in a sequence-aspecific manner

Transcription factors (TFs) cluster in the nucleus. This is important in the context of transcription regulation. It is unclear, however, what regulates TF clustering. Most TFs have a DNA binding domain (DBD) as well as intrinsically disordered regions (IDRs). The current model in the field explains clustering via binding of TFs to DNA followed by recruitment of additional factors via multivalent interactions (Sabari et al, 2018; Boija et al, 2018; Shrinivas et al, 2019). This model, however, would predict that all TFs would cluster together via IDR mediated interactions. This is not the case as different TFs form distinct clusters and do not all overlap. Here, we ask how TFs cluster in the nucleus and what dictates the specificity of TF clusters *in vivo*. We used the zebrafish embryo to visualize the clustering of three TFs with an important role in early development: Nanog, Sox19b and Pou5f3. We show that these TFs form different numbers of clusters in the nucleus and that they are DNA seeded. Focusing on Nanog, we find that the number of clusters scales with the level of Nanog in the nucleus, suggesting that clustering is sensitive to the strength of binding sites. Also using Nanog, we show that *de novo* clustering requires the DBD and at least one of the IDRs. Contrary to the existing model, we find that the DBD is also required to integrate into a pre-existing cluster and that the IDRs alone are insufficient for this integration. Collectively, these results suggest that every TF molecule that is part of a cluster needs to have both the DBD and at least one IDR. Finally, to dissect whether IDRs play a sequence-specific or non-specific role, we generated a chimera that has a Nanog DBD and Sox19b IDRs. This chimera clusters like full-length Nanog and can activate Nanog target genes. This suggests that the IDRs between the two TFs are interchangeable and required only to provide multivalent interactions. Taken together, we find that the specificity in transcription factor clustering is governed by DNA binding, while IDRs lower threshold concentration in a sequence-aspecific manner.