The precise contribution of regulatory regions of the genome to gene expression is largely uncharacterized. One method of decoding such regions has been to identify sequence elements within that contribute to observed expression levels, such as transcription factor binding sites (TFBSs). Yet, it is still unknown how different regulatory architectures, such as variable TFBS placement, number and affinity, relate to gene expression. Here, we developed a massively parallel reporter assay (MPRA) that determines cellular expression of large pools of designed regulatory elements to isolate the regulatory constraints for a single TFBS, the c-AMP response element (CRE). Overall, we find a combination of CRE number and binding strength explains most expression in our assay followed by CRE position and surrounding sequence content. We find our multi-copy, episomal MPRA follows similar expression trends for most of these features when compared to a single-copy, genomically-integrated MPRA. Overall, we show how a high-definition analysis of TFBS features can elucidate how such features combine to shape TFBS activity in regulatory elements.

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Spire forms filament bridges to build a dynamic actin mesh in vitro

The eggs of mammals, fruit flies, and likely other species, are filled with a network of actin filaments referred to as a mesh. How Spire and Capu collaborate to construct the mesh in the fly is poorly understood. In this work, Spire’s in vivo localization on vesicles is mimicked by in vitro conjugation to microspheres. Using multi-color TIRF microscopy, I find that Spire-decorated beads are sufficient to produce dense networks of actin filaments and appear to serve as junctions connecting them. This system offers a simple but powerful tool to decipher the complex mechanism of actin assembly by Spire and Capu, their mammalian homologues, and potentially other actin assembly pairs.