proteins often occurs in the presence of specific chaperones, the probability of native proteins undergoing domain swapping for folding is low. Specific chaperones often ensure that client proteins remain in functionally relevant folded states, inhibiting the formation of low-energy intermediates observed in vitro. However, this report by Assar et al. (2016) provides a clear glimpse of how a specific bonding interaction and protein concentration can influence the stability of protein folding intermediates.

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Tunable Liaisons: eEF-2K, CaM, and Calcium

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In this issue of *Structure*, Lee et al. (2016) perform NMR analysis of calmodulin (CaM) binding to the eukaryotic elongation factor 2 kinase (eEF-2K). They find that eEF-2K interacts mainly with the C lobe of CaM in a Ca^{2+}-tunable manner, revealing a high level of control in cellular homeostasis.

Protein synthesis is tightly regulated to maintain cellular homeostasis. This task is achieved for the three main phases of protein synthesis: initiation, elongation, and termination (Merrick, 2010). Elongation factors play a fundamental role in these processes, as they regulate the growth and translocation of polypeptide chains during protein synthesis. Specifically, eukaryotic elongation factor 2 kinase (eEF-2K) slows down the elongation phase by inhibiting eukaryotic elongation factor 2 (eEF-2) through phosphorylation at Thr56 (Kenney et al., 2014; Ryazanov, 1987).

In addition to controlling protein synthesis, recent studies suggest that eEF-2K may have additional functions because it protects certain cancer cells against nutrient starvation and plays a role in both learning and memory (Kenney et al., 2014). Furthermore, aberrant control of protein synthesis by eEF-2K leads to diseases such as cancer, Alzheimer's, depression, and heart disease (Li et al., 2005; Meric-Bernstam et al., 2012; Monteggia et al., 2013; Usui et al., 2013). Given these diverse and essential roles, it is not surprising that eEF-2K activity is carefully regulated. Indeed, Ca^{2+} concentration and calmodulin (CaM) binding keep the activity of eEF-2K within a tight physiological window. In particular, the recognition of eEF-2K by Ca^{2+}-loaded CaM (Ca^{2+}-CaM) represents the first stage of the two-step mechanism by which eEF-2K is activated in cells. It has been hypothesized that this two-step mechanism may be linked to the dynamic conformational change occurring upon CaM binding to eEF-2K; namely, binding of Ca^{2+}-CaM was proposed to induce a dramatic conformational change in the catalytic domain of eEF-2K that would lead to auto-phosphorylation of Thr348. The latter would be followed by a second conformational transition of the regulatory loop initiated by the binding of the phosphorylated Thr348 to a key allosteric pocket (Tavares et al., 2014).

In this issue of *Structure*, Lee et al. (2016) shed light on this allosteric regulation, elucidating the structural basis for this crucial recognition of CaM. The authors use NMR spectroscopy coupled to thermocalorimetry to obtain atomic-level resolution of the structural dynamics of the complex between CaM and the CaM-binding domain region of eEF-2K (eEF-$2K_{CBD}$; residues 74–100). They show that eEF-$2K_{CBD}$ interacts with either Ca^{2+}-free or Ca^{2+}-loaded CaM through CaM's C lobe. The N lobe of CaM interacts only very weakly with eEF-$2K_{CBD}$ and only when the Ca^{2+} sites of CaM are fully occupied. Remarkably, even at elevated Ca^{2+} concentrations, the C lobe sites of CaM are Ca^{2+}-free when bound to eEF-$2K_{CBD}$, yet CaM still adopts a Ca^{2+}-bound like structure in its positioning of the EF hand motifs. Unlike most CaM-dependent kinases, Ca^{2+} appears to increase the affinity of CaM toward eEF-2K, yet it is not the condition sine qua non for eEF-2K activation. These findings also suggest that CaM's binding partners may modulate its affinity for Ca^{2+}, as eEF-$2K_{CBD}$ binding reduces the C lobe's affinity toward Ca^{2+}. In the large array of targets that CaM binds and regulates, this mechanism likely provides another layer of tunable

control, allowing CaM-dependent kinases to be activated at a particular Ca^{2+} concentration and cellular location.

How does CaM recognize the kinase? The solution structure of the Ca^{2+}-CaM•eEF-$2K_{CBD}$ complex reported by Lee et al. (2016) provides a detailed mapping of the interactions between the two proteins. Specifically, the authors demonstrate an antiparallel 1-5-8 binding mode of eEF-$2K_{CBD}$ with the C lobe of CaM, contrary to the predicted 1-5-8-14 mode (Hoeflich and Ikura, 2002), suggesting a dynamic interplay between the two proteins. Their nuclear spin relaxation experiments also emphasize significant interlobe dynamics, which agree with the minimal number of interactions found between the N lobe of CaM and eEF-$2K_{CBD}$, underscoring the importance of the flexibility of the complex in its allosteric regulation. Their study also highlights the importance of W85 of the kinase, a conserved residue that inserts deep in the hydrophobic pocket of CaM's C lobe, which is fundamental in the activation of the full-length protein. W85 emerges as a key hydrophobic anchor for the binding of eEF-2K to CaM that is required for activation. In cell mutagenesis of W85 to a polar residue (e.g., serine) hampers autophosphorylation of eEF-2K even under conditions that would otherwise enhance its activity, such as starvation, oxidative stress, and enhanced Ca^{2+} influx.

Overall, Lee et al. (2016) offer a framework for deciphering eEF-2K activation during protein synthesis. Further studies are necessary to investigate how the interactions determined in this work contribute to the two-step activation of eEF-2K. At the same time, this work will have a broader impact in our understanding of how CaM functions as a structural sensor to orchestrate spatiotemporal regulation of CaM-dependent kinases.

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