Novel Phosphorylation of Eef2 Inhibits Its Protein Synthesis Activity

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Protein synthesis is an energy consuming process that must be regulated in times of cellular stress when energy levels become depleted. Translation initiation and elongation are two phases of protein synthesis that can be inhibited to increase cellular ATP levels for use in other processes. One key factor in translation elongation is eEF2 (eukaryotic elongation factor 2), an enzyme that binds to ribosomes and facilitates ribosome function. A key regulation of eEF2 function is inhibitory phosphorylation at threonine 56 (T56) by the kinase eEF2K.

In turn, eEF2K is phosphorylated by other kinases to modulate its activity. For example, mitogen stimulation activates kinase cascades to phosphorylate eEF2K at sites that repress its kinase activity, stimulating eEF2 function, protein synthesis, and cell growth. During nutrient deprivation, eEF2K is subject to activating phosphorylation to inhibit eEF2 activity and protein synthesis. A new manuscript by Hizli *et al*, with author contributions from the Divisions of Human Biology and Clinical Research, explains a novel pathway for eEF2 regulation.

Previously, the authors used a mass spectrometry-based proteomics approach to identify Cdk2/cyclin A targets and identified eEF2 as a potential Cdk2 substrate. Cdk2 is a cyclin-dependent kinase that phosphorylates a plethora of proteins to drive cell cycle events. Cdks are proline-directed serine/threonine (S/T) kinases, allowing Hizli *et al.* to hone in on the six S/T eEF2 residues neighbored by a proline. Cdk2/cyclin A could not efficiently phosphorylate the eEF2 (S595A) mutant protein *in vitro*, making S595 the main Cdk2 site in eEF2. Phosphorylation of eEF2 S595 *in vivo* was confirmed by incubating cells with 32P, isolating eEF2, and performing phosphopeptide mapping. The authors then confirmed that Cdk2 was responsible for S595 phosphorylation *in vivo* by showing increased S595 phosphorylation upon Cdk2 overexpression.

Cdk activity oscillates throughout the cell cycle, and Hizli and colleagues show that eEF2 is hyperphosphorylated during mitosis, both at T56 (eEF2K-dependent) and S595 (Cdk2-dependent). The concurrence of T56 and S595 eEF2 phosphorylation during the cell cycle and the results from the phosphopeptide mapping suggested that the two post-translational modifications were linked. Strikingly, the eEF2 (S595A) phospho-mutant exhibited decreased T56 phosphorylation *in vivo* and *in vitro*. This indicated that Cdk2 phosphorylation of eEF2 somehow primes eEF2 for

subsequent phosphorylation by eEF2K. The authors further confirmed this paradigm by showing that eEF2K phosphorylation of eEF2 was substantially enhanced by previous phosphorylation of eEF2 by Cdk2. S595 phosphorylation likely promotes an eEF2K binding site on eEF2 to potentiate T56 phosphorylation.

The identification of Cdk2 regulation of eEF2 and ultimately protein synthesis is a significant advancement toward understanding the regulation of protein translation. Since Cdks associate with multiple cyclins that affect diverse cellular processes, future work will be aimed at discovering the relevant cyclin/Cdk2 complexes that impinge on eEF2 activity during specific cell cycle stages. However, Hizli *et al.* have found a critical link between the cell cycle machinery and protein translation control.

Hizli AA, Chi Y, Swanger J, Carter JH, Liao Y, Welcker M, Ryazanov AG, Clurman BE. 2012. Phosphorylation of Eukaryotic Elongation Factor 2 (eEF2) by Cyclin A-Cyclin-Dependent Kinase 2 Regulates Its Inhibition by eEF2 Kinase. *Mol Cell Biol*. 33(3):596-604.

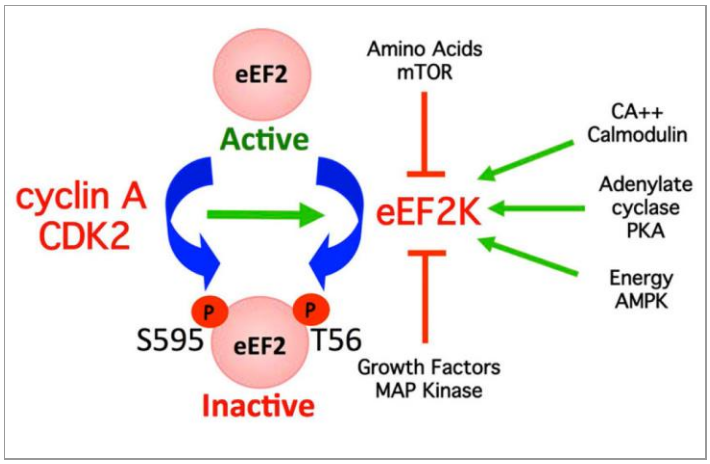

Figure obtained from manuscript

Figure: eEF2 inactivation by multiple phosphorylations. The eukaryotic protein translation elongation factor eEF2 is subject to a well-studied T56 phosphorylation and a newly identified CDK2-dependent S595 phosphorylation. eEF2K kinase is influenced by a variety of signals and phosphorylates T56 to inhibit eEF2 activity and decrease protein synthesis when ATP levels are low. Cdk2 is a cyclin-dependent kinase that the authors show has a surprising role in global protein synthesis regulation by promoting T56 phosphorylation of eEF2 by eEF2K.