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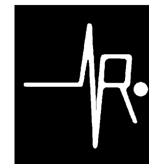

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Original Contribution

Signal transduction pathways leading to increased eIF4E phosphorylation caused by oxidative stress

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Abstract

Phosphorylation of eIF4E is associated with increased activity of the translational machinery. Oxidative stress of resident vascular cells and macrophages potently enhances eIF4E phosphorylation. Oxidative stress activates numerous intracellular signaling pathways, including MAP-family kinase pathways and pathways leading to S6 kinase activation. The activation of MAP-family kinase pathways leads to the activation of Mnk and hence eIF4E phosphorylation, whereas the S6 kinase pathway is not involved, based on insensitivity to its inhibitors rapamycin and wortmannin. Ca-dependent pathways have been implicated in eIF4E phosphorylation, but the oxidative stress response pathway targeting eIF4E does not appear to require their participation. The results suggest that the potent activation of ERK and p38 protein kinases is sufficient to account for the enhanced eIF4E phosphorylation. Either is independently sufficient to effect the change, as neither PD098059 (Erk pathway inhibitor) nor SB202190 (p38 pathway inhibitor) alone can block the response, but when combined the response is almost completely abrogated. Mnk activation by oxidative stress leading to enhanced eIF4E phosphorylation may play a role in promoting stress-induced hyperproliferative diseases, such as smooth muscle cell proliferation and hypertrophy in cardiovascular disease, as the synthesis of several key regulators of cell growth has been shown to be held in check by moderation of eIF4E activity.
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Keywords: Signal transduction; Initiation factors; Phosphorylation; Oxidative stress; Free radicals

Introduction

Cell growth is coupled to the rate of protein synthesis. Treatment of certain cancer cells with translational inhibitors restores cell growth to a controlled state [1]. Genetic ablation of key positive regulators of translation (TOR, S6K) causes a reduced body size in *Drosophila* [2,3], as does repression of translation due to overexpression of protein inhibitors such as eIF4E-BP [4]. A detailed understanding of the mechanisms and molecular components underlying inappropriate cell growth provides an avenue to therapeutic strategies in a wide variety of hyperproliferative diseases, such as cancer, atherosclerosis, and retinopathies [5].

Most situations that enhance global mRNA translation rates increase the activities of key initiation factor proteins

Abbreviations: BSO, buthionine sulfoximine; DMEM, Dulbecco's modified essential medium; EBSS, Earle's Balanced Salts Solution; EGF, epidermal growth factor; eIF, eukaryotic initiation factor; eIF4E-BP, eIF4E-binding protein; FBS, fetal bovine serum; GCS, glutathione cysteine synthase; HCR, heme-controlled repressor (eIF2α kinase); IP, immunoprecipitate; LDL (LDL−), low-density lipoprotein particle, total fraction (electronegative fraction [oxidized fraction]) isolated by HPLC; MAKKK, MAP kinase kinase kinase; MBP, myelin basic protein; PDK1, phosphoinositide-dependent protein kinase 1; PERK, PKR-like endoplasmic reticulum eIF2α kinase; PI3K, phosphatidylinositol 3-kinase; PI3KK, PI3K-related kinase; PKR, double-stranded RNA activated eIF2α kinase; PMA, phorbol 12-myristate 13-acetate; PP2, *src* nonreceptor tyrosine kinase inhibitor; RTK, receptor tyrosine kinase; S6K, ribosomal protein S6 kinase; TOR, target of rapamycin; TSC, tuberous sclerosis complex; 5UTR, 5′ untranslated region of mRNA; DPI, diphenlyeneiodonium chloride.

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(eIFs), which act as the molecular rheostats governing protein synthesis [6]. The principal eIFs identified as molecular rheostats are eIF2 and eIF4E [7,8]. Another protein that has been a focus of translational affects on growth control is ribosomal protein S6 (rpS6) [9], which, like the initiation factors, appears to function in initiation events involving the recruitment of mRNA to the polysome [9]. Phosphorylation plays a role in the regulation of each of these proteins. The regulatory subunit of eIF2, eIF2α, is phosphorylated to inhibit eIF2 activity when protein synthesis rates are low [6,7], and dephosphorylated during activation of translation. eIF4E, the mRNA cap-binding protein, is constitutively phosphorylated. Dephosphorylation occurs in many situations in which translation rate is inhibited (e.g., heat shock [10], viral infection [11]) and hyperphosphorylation is observed concurrent with enhanced translation caused by numerous agents including serum, growth factors, and hormones [8]. Evidence that the functional abundance of eIF4E is low has suggested it is the rate-limiting factor controlling translation rate [8,10]. Furthermore, specific ablation of eIF4E's single phosphorylation site (S→A mutation) significantly impairs embryonic development in *Drosophila* [12]. rpS6 is phosphorylated on multiple sites following numerous mitogenic signals. Evidence suggests this may enhance its mRNA binding activity [13], specifically toward mRNAs containing TOP sequences in their 5′ termini [14].

Two main pathways have been characterized that regulate eIF phosphorylation; these are diagrammed in Fig. 1. One emanates from PI3 kinase (PI3K), and prominently involves Akt and TOR. This pathway influences both rpS6 phosphorylation and eIF4E activity [15–18]. Activation of PI3K elevates inositol polyphosphates, recruiting Akt to the cytoplasmic membrane and activating it [19], which in turn phosphorylates, and inactivates, the tuberous sclerosis complex (TSC) inhibitor of TOR [20]. TOR, a PI3KK, phosphorylates S6K and eIF4E-BP via its raptor scaffold link [21]. eIF4E-BP is a specific inhibitor of eIF4E, which competes with eIF4G for eIF4E binding in its dephosphorylated state, but is inactive when phosphorylated [8]. Hence, PI3K and TOR activation stimulates the translational activator rpS6 via S6K, and blocks the translational repressor eIF4E-BP. This pathway, and specifically the TOR kinase, is inhibited by the macrolide immunosuppressant rapamycin, which provides a useful tool to analyze its significance in metabolic events. PI3K inhibitors also repress the pathway, but at a more global step. In lower eukaryotes, rapamycin profoundly and rapidly represses protein synthesis [22], though the precise effectors downstream of TOR have not been conclusively identified. In higher eukaryotes, there is a progressive inhibition of protein synthesis over many hours, which plateaus at about 50% inhibition [23]. However, the translation of certain key mRNAs, such as c-*myc*, is rapidly virtually abolished [24], suggesting that rapamycin's immediate effects are on specific mRNA translation, which may propagate to global translation inhibition with time. The rapamycin-sensitive pathway has been suggested to constitute an intracellular signal transduction pathway dedicated to translational control [25].

The other pathway that regulates eIF phosphorylation involves MAP family kinases, specifically ERK and p38. Treatments that activate *ras* target this pathway, as do treatments that activate the pathway downstream of *ras*, such as PMA [8]. Both ERK and p38 can phosphorylate and activate the integrating kinase Mnk, which in turn phosphorylates eIF4E [26]. Thus, eIF4E phosphorylation is enhanced by mitogenic agents that target the ERK branch of MAP family kinases, as well as by stress-producing agents that target the p38 branch. Activated eIF4E increases *ras* activity, creating a perpetuating loop [27]. Pharmacological agents that block ERK or p38 activity can influence eIF4E phosphorylation [28,29], but rapamycin typically has little or no effect. Conversely, ERK/p38 kinases antagonists do not inhibit rpS6 or eIF4E-BP phosphorylation [30].

Oxidative stress initially was viewed as a toxic event, an unwanted but unavoidable consequence of life in an oxygen-rich environment. Recent studies have necessitated a profound revision of this assessment. Low level, controlled generation of oxidative stress is now recognized as being a required component for the propagation of many critical cellular signals [31]. It manifests these roles by influencing the activities of key signal transduction pathways. For example, mitogenic activation by growth factors such as EGF is largely suppressed by antioxidants [32], suggesting the oxidant production is a requisite co-stimulatory signal. Ras activation transduced to Rac activation causes activation of membrane-localized NADPH oxidase activity, resulting in increased free radical generation [33]. These free radicals, in turn, inhibit tyrosine phosphatases [34] at a sensitive cysteine [35], allowing the maintenance of tyrosine phosphorylation generated by the growth factor-induced RTK activation [32]. Consistent with this view, exogenous inhibition of tyrosine phosphatases by agents such as sodium orthovanadate initiates a mitogenic signal based on tyrosine phosphorylation [36], which can be mimicked by treatment of cells with oxidants such as hydrogen peroxide [37,38].

We have recently shown that oxidative stress activates eIF4E [39]. During normal mitogenic stimulatory events this may serve to optimize the growth response through global and specific translational control, but when oxidative stress becomes chronic, as in atherosclerosis, for example, inappropriate prolonged activation of eIF4E may contribute to the hyperproliferative response. Furthermore, we have suggested that eIF4E hyperphosphorylation specifically enhances the translation of growth-regulatory mRNAs which are known to contain highly structured 5′ -untranslated regions (5UTRs) and have been shown to exhibit

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