The absence of nuclear eIF2α

Background

Eukaryotic translation initiation factor 2 (eIF2) is necessary for binding of the methionyl-tRNA to the small ribosomal subunit and therefore is essential to the commencement of protein translation. It is of particular interest since it is a site of regulation of protein synthesis. Phosphorylation of its α subunit by a family of stress-dependent kinases leads to rapid inhibition of protein synthesis.

There has been evidence to suggest that eIF2 is not limited to the cytoplasm where one might expect a factor involved in protein synthesis to reside. Electron and light microscopy have both suggested the presence of immunoreactive eIF2α within the nucleus (1 and Heather Harding, unpublished observations) and recently evidence has been offered for the occurrence of intra-nuclear protein biosynthesis (2), which one would imagine would require nuclear eIF2α.

We set out to try to detect eIF2α within nuclear fractions and determine whether it is under the control of the eIF2α kinases.

Results

We have at our disposal cell lines expressing a fusion protein FV2E-PERK, PERK being an ER-resident eIF2α kinase triggered by ER stress. The fusion protein, by contrast, is cytosolic and is activated by the compound AP20187 at nanomolar concentrations. This provides a simple tool to investigate phosphorylation of eIF2α in the absence of actual ER stress.

When HT22 cells expressing FV2E-PERK were treated with AP20187 immunoreactive phopshorylated-eIF2α (P-eIF2α) could easily be detected within the cytosolic fraction. This was maximal at 30 minutes (figure 1). Drug treatment had no measurable effect upon total eIF2α levels as judged by Western blotting and had no effect on parental HT22 cells. Nuclear extracts, by contrast, failed to demonstrate either total eIF2α or P-eIF2α by Western blotting. Since it is conceivable that our procedure for the extraction of nuclear proteins (high-salt) failed to draw out eIF2α from the nucleus, the post-extraction nuclear pellet was solubilised by heating in SDS-loading buffer. This also failed to demonstrate either eIF2α or P-eIF2α (figure 1). Both the high-salt nuclear extract and the post-extraction nuclear pellet contained easily detectable CREB, attesting to the integrity of these nuclear fractions (figure 1)

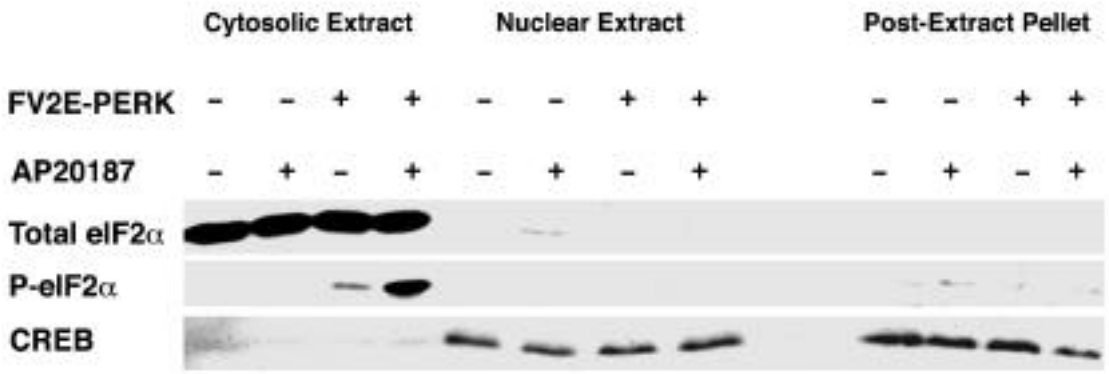

Figure 1. Western blot for total and phosphorylated eIF2α and CREB; 30 µg protein per lane. Parental or FV2E-PERK-transfected HT22 cells were treated for 30 minutes with 1 nM AP20187 or diluant. Cytsol and nuclear extracts were obtained. The post-extraction pellet was solubilised by heating to 95^{o}C in SDS loading buffer. A single blot was probed sequentilly with each antibody.

To ensure that tissue-specific differences were not to blame for our failure to detect nuclear eIF2α we carried out similar experiments with FV2E-PERK-expressing fibroblasts. The results were unchanged; no nuclear eIF2α was detected (figure 2).

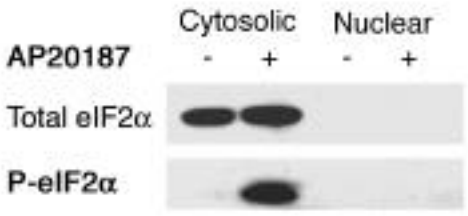

Figure 2. Western blot for total and phosphorylated eIF2α; 30 µg protein per lane. FV2E-PERK-transfected mouse embryonic fibroblasts were treated or 30 minutes with 1 nM AP20187 or diluant. Cytsol and nuclear extracts were obtained. A single blot was probed sequentilly with each antibody.

Discussion

We were unable to detect eIF2α within the nuclei of two cell lines. This would tend to argue against the presence of eIF2α within the nucleus and, by extension, against the phenomenon of nuclear protein translation. There are, however, two important caveats to these observations: (i) our techniques might fail to detect very low levels of protein, say <0.1% compared to cytosolic fractions, which might be all that is required for low levels of nuclear translation (ii) it is conceivable that preparation of nuclei from our cells led to the loss of nuclear eIF2α. The latter is unlikely in our opinion since the transcription factor CREB was not lost to the cytosolic fraction during preparation of the nuclei and, indeed, was still present within the nuclear debris following a lengthy wash in a high salt buffer.

References

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2. Iborra FJ, Jackson DA, Cook PR.
Coupled transcription and translation within nuclei of mammalian cells. Science. 2001 Aug 10;293(5532):1139-42.

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