Signalling pathways which regulate eIF4E

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The eukaryotic cap-binding factor, eIF4E (reviewed in [1]), is known to be regulated by at least two different mechanisms: (i) eIF4E is a phosphoprotein and its phosphorylation state generally correlates positively with rates of protein synthesis; (ii) eIF4E is negatively regulated by the binding of small inhibitory proteins such as 4E-BPI (or PHAS-I) which inhibit the activity of eIF4E in vitro. 4E-BPI is also phosphorylated, e.g. in response to insulin and growth factors, causing it to dissociate from eIF4E and this is believed to be one mechanism by which such agents stimulate protein synthesis.

We are studying the signalling pathways which mediate changes in the phosphorylation state of both eIF4E and 4E-BPI under conditions where translation is stimulated, in response to insulin (1), or inhibited under conditions of cellular stress (2).

1. Studies into insulin-signalling have been performed using Chinese hamster ovary cells which overexpress the human insulin receptor (CHO.T cells). Contrary to earlier reports, we have found that, in these cells, classical isoforms of protein kinase C are not required for the insulin-stimulated phosphorylation of eIF4E. However, the compound PD98059 [2], which blocks the activation of MAP kinase, does prevent this effect of insulin on eIF4E, indicating the involvement of the MAP kinase pathway. In contrast, PD98059 does not prevent the insulin-stimulated phosphorylation of 4E-BPI or dissociation of 4E-BPI from eIF4E.

2. Conditions of cellular stress, such as heat shock, lead to a rapid inhibition of protein synthesis. One mechanism for this effect is via the phosphorylation and inactivation of the initiation factor eIF2. In addition, we find that exposure of Chinese hamster ovary (CHO.K1) cells to heat shock or sodium arsenite promotes dephosphorylation of 4E-BPI and its increased binding to eIF4E. As previously reported, following heat shock eIF4E is almost totally dephosphorylated. However, a surprising finding is that sodium arsenite causes a substantial increase in the phosphorylation of eIF4E. In this case, MAP kinase does not appear to be involved as arsenite does not stimulate MAP kinase in CHO cells. Instead, the stimulation of eIF4E phosphorylation by arsenite requires the activity of the stress-activated kinase p38RK, since it is blocked by the compound SB203580 (Smithkline Beecham) which inhibits p38RK [3]. However, eIF4E is not a direct substrate in vitro for p38RK. Thus eIF4E phosphorylation appears to be regulated by at least two different signalling pathways (involving MAP kinase and p38RK).