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ERK1/2, but not ERK5, is necessary and sufficient for phosphorylation and activation of c-Fos

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ABSTRACT

Growth factor-stimulated expression and activation of c-Fos is regulated by the ERK1/2 pathway. However, recent reports have also suggested a prominent role for the closely related ERK5 pathway in regulating the expression, transcriptional activation and nuclear localization of c-Fos. Here we have compared the role of ERK1/2 and ERK5 in regulating c-Fos using a combination of conditional protein kinases, selective biochemical inhibitors and ERK5 null fibroblasts. We demonstrate that activation of the ERK1/2 pathway, but not ERK5, is sufficient for c-Fos phosphorylation and transcriptional activation. Furthermore, growth factor-dependent expression of c-Fos is blocked by low doses of PD184352 that selectively inhibit the ERK1/2 pathway but proceeds normally in ERK5−/− 3T9 cells; in addition, nuclear localization of c-Fos is normal in ERK5−/− cells. ERK5−/− cells are, however, defective for c-Jun expression but this is reversed by re-expression of ERK5. In addition to ERK5, neither the JNK nor p38 pathways can substitute for ERK1/2 in the regulation of c-Fos transcriptional activity. These results demonstrate that c-Fos transcriptional activity is not regulated by the ERK5 pathway; rather, of all the MAPKs and SAPKs, c-Fos activation appears to be predominantly linked to the ERK1/2 pathway.

1. Introduction

The regulated expression of c-Fos serves as paradigm for growth factor-regulated gene expression [1], so an understanding of this system may inform other models of gene expression. The regulation of c-Fos is also important in the context of oncogenesis since: (i) the virally-transduced counterpart, v-Fos, is a transforming oncogene [2]; (ii) c-Fos is a major partner of c-Jun, which is required for cell proliferation and RAS-dependent transformation [3]; (iii) c-Fos, together with FosB, is required for optimal expression of Cyclin D1 [4] and (iv) c-Fos drives a programme of gene expression that controls tumour cell motility and invasiveness [5].

Mitogen-activated and stress-activated protein kinases (MAPKs or SAPKs) are phosphorylated and activated by specific MAPK kinases (MKKs, also called MAPK or ERK kinases or MEKs), which are in turn phosphorylated and activated by a MAPKK kinase (MKKK) in a three-tiered amplifying signal cascade [6]. Among the MAPKs there is a prominent role for the extracellular signal-regulated kinases 1/2 (ERK1/2) in regulating c-Fos expression in response to growth factors and oncoproteins [1]. Active ERK1/2 accumulates in the nucleus and phosphorylates and activates the Elk-1 and Sap1a transcription factors [1,7,8] to stimulate transcription of c-Fos. The newly expressed c-Fos protein forms obligate dimers with Jun proteins, including c-Jun, to form AP-1 transcription factor complexes [9]. The C-terminus of c-Fos is phosphorylated by ERK1/2 and the ERK1/2-dependent kinase RSK [10]; indeed, sustained ERK1/2 activation allows efficient phosphorylation of the C-terminus of newly synthesised c-Fos, thereby stabilizing it [11]. ERK1/2-catalysed phosphorylation of c-Fos also activates a C-terminal transcriptional transactivation domain (TAD) [12] and this domain is required for the transforming function of c-Fos [10].

ERK5 is activated by some growth factors and various forms of stress and is involved in cell differentiation and survival [13,14]. ERK5 is most closely related to ERK1/2, having the same T–E–Y activation motif, but is activated by a discrete MEK; MEK1 and MEK2 activate ERK1/2 but not ERK5, whilst MEK5 activates ERK5 but not ERK1/2 [15]. Few ERK5 substrates have been identified but it is known to phosphorylate and activate members of the myocyte enhancer factor-2 (MEF2) family of transcription factors [16], including MEF2D [17].

Many studies implicating ERK1/2 in c-Fos expression and activation have employed PD98059 and U0126 to inhibit MEK1/2 [18–20]. However, these drugs also inhibit the ERK5 pathway [21] and it has been suggested that ERK5 contributes to c-Fos transcription [21] and promotes nuclear retention of c-Fos [22]. It has also been suggested that ERK5 is even more effective than ERK1/2 at phosphorylating, stabilizing and activating c-Fos [23]. Here we have examined the role of ERK5 in the regulation of c-Fos using a combination of conditional protein kinases, selective biochemical inhibitors and ERK5-null fibroblasts. We find that of all the MAPK and SAPK pathways, c-Fos phosphorylation and transcriptional activation appear to be predominantly linked to the ERK1/2 pathway. Specifically,

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we find that ERK5 is neither necessary nor sufficient to phosphorylate and activate c-Fos nor is it required for c-Fos expression although it is required for optimal c-Jun expression.

2. Materials and methods

2.1. Materials

All cell culture reagents were purchased from Invitrogen. U0126 and EGF were from Calbiochem; PD184352 was obtained by custom synthesis. Mouse anti-HA was provided by the Babraham Institute Monoclonal Antibody Facility. Phospho-JNK (9251), phospho-p38 (9211), phospho-ERK5 (3371), phospho-ERK1/2 (9106) and total ERK1/2 antibodies were from Cell Signalling Technology; note that the phospho-JNK antibody also recognises P-ERK1/2 on western blots. c-Fos (sc-52), anti-GAL4 and total ERK5 antibodies were from Santa Cruz Biotechnology and mouse anti-FLAG was from Sigma. All other chemicals were purchased from Sigma unless otherwise stated in the text.

2.2. Cell culture

Culture of HR1 and HM3 cells has been described previously [24,25]. ERK5−/− 3T9 cells, provided by Dr Cathy Tournier, School of Biological Sciences, University of Manchester, were maintained under identical conditions. Stable re-expression of HA-ERK5 in ERK5−/− 3T9 cells was achieved by retroviral infection. pBabePuro HA-ERK5 (or

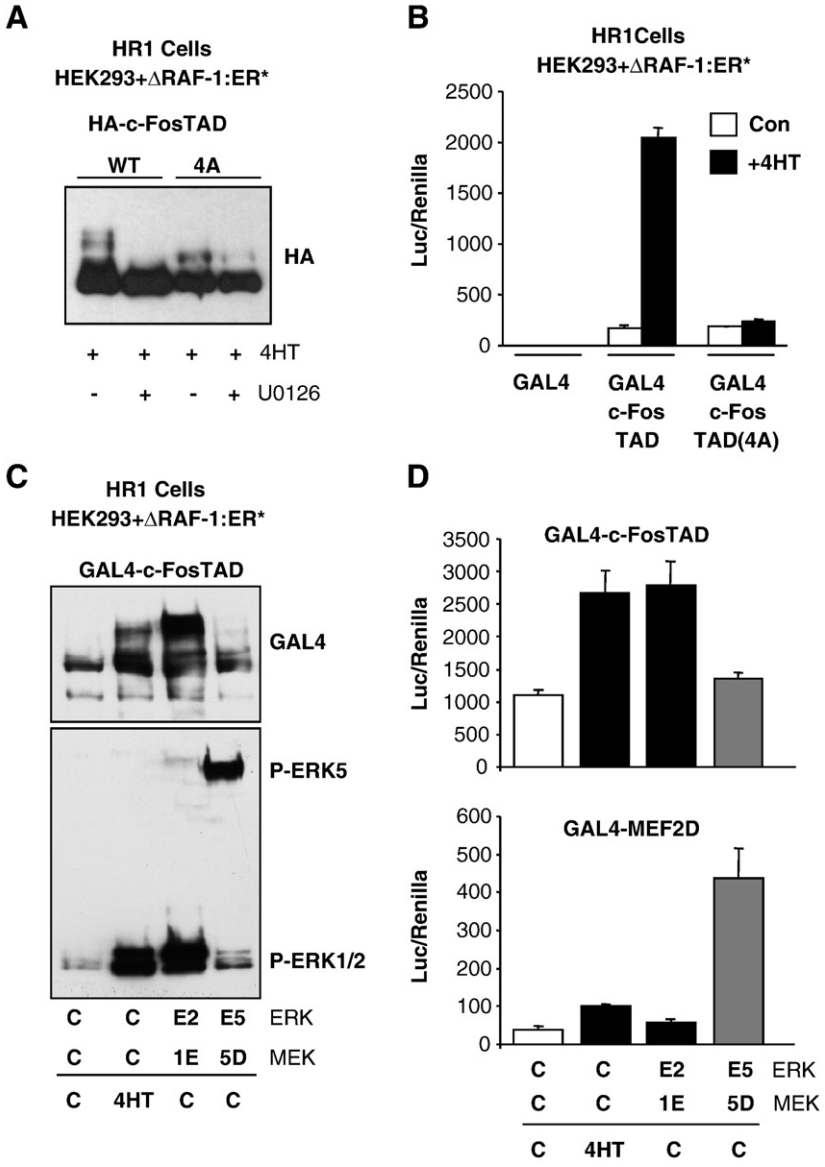

Fig. 1. Activation of ERK1/2, but not ERK5, is sufficient to activate the c-Fos transactivation domain. (A) HR1 cells were transfected with HA-c-FosTAD (WT or 4A) in serum-free media. After 18 h cells were pre-treated for 30 min with 20 μM U0126 (+) or a vehicle control (−) and then stimulated with 100 nM 4HT for 1 h. Cell lysates were immunoblotted for HA. (B) HR1 cells were transfected with GAL4 (control), GAL4-c-FosTAD WT or GAL4-c-FosTAD(4A) together with GAL4:LUC and a *Renilla* construct in serum-free conditions. After 3 h cells were stimulated by addition of vehicle control (−) or 100 nM 4HT (+) for 18 h. Firefly luciferase activity was normalised to *Renilla* and the results are the mean ± s.d. of triplicate samples from a single experiment representative of three. (C) HR1 cells were transfected with GAL4-c-Fos TAD together with MEK1E + ERK2 or MEK5D + ERK5 or empty vectors. Cells were treated with serum-free media containing either vehicle control (C) or 100 nM 4HT (4HT) for 18 h. Cell lysates were subjected to SDS-PAGE and immunoblotted with antibodies to GAL4 or P-ERK5 (which cross-reacts with p-ERK1/2). (D) HR1 cells were transfected with GAL4-c-FosTAD or GAL4-MEF2D together with GAL4:LUC, a *Renilla* construct (as described in the Methods) and either empty vectors pCAN-HA and pEGFP-C2 (C, control), HA-ERK2 and EGFP-MEK1E (E2 + 1E) or HA-ERK5 and EGFP-MEK5D (E5 + 5D). After 3 h serum-free media was added to the cells with either vehicle control or 4HT (100 nM) for 18 h and Firefly luciferase activity was normalised to *Renilla*. The results the mean ± s.d. of triplicate cell samples from a single experiment representative of three.