Detection in non-erythroid cells of a factor with the binding characteristics of the erythroid cell transcription factor EF1

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The erythroid transcription factor erythroid factor-1 (EF1) plays a critical role in the transcription of erythroid-specific genes. Here we report the presence of a factor with the mobility and sequence-specific DNA-binding characteristics of EF1 at low abundance in a wide variety of non-erythroid cell types. This is the first report of an EF1-like activity in non-erythroid cells and indicates that this factor may play a role in the regulation of genes expressed in such cells.

INTRODUCTION

The transcriptional regulation of many cellular genes is mediated by the binding of cell-type-specific factors, present in only one or a few tissues, to regulatory elements within the promoters or enhancers of such genes (for review, see Maniatis *et al.*, 1987). In particular, the promoters and enhancers of many genes expressed specifically in chicken or mammalian erythroid cells, such as the globin genes and the porphobilinogen deaminase gene, contain a sequence with the consensus A/TGATAA/G (Evans *et al.*, 1988; Wall *et al.*, 1988; Plumb *et al.*, 1989; Mignotte *et al.*, 1989). This sequence acts as the binding site for a transcription factor which is present at high levels in cells of the erythroid lineage and has been variously referred to as EF1 (Perkins *et al.*, 1989), EryF1 (Evans *et al.*, 1988), NF-E1 (Wall *et al.*, 1988) and GF-1 (Tsai *et al.*, 1989). Here we refer to this factor as EF1.

A variety of evidence suggests that EF1 plays a critical role in the activation of erythroid-specific gene expression. Thus deletion or mutation of the EF1 binding sites within erythroid-specific genes reduces or abolishes their expression in erythroid cells (Kollias *et al.*, 1987; Behringer *et al.*, 1987; Reitman & Felsenfeld, 1988). Moreover, partially affinity-purified chicken EF1 protein has recently been shown to stimulate the transcription of a chicken β hatching globin gene in a site-specific manner upon co-injection into *Xenopus* oocytes (G. A. Partington, W. D. Perkins, R. H. Nicolas & G. H. Goodwin, unpublished work).

In agreement with its critical role in erythroid gene expression, EF1 has been detected at high levels in cells of all stages of the erythroid lineage, and has not previously been observed in non-erythroid cells (Evans *et al.*, 1988; Galson & Houseman, 1988; Wall *et al.*, 1988; Tsai *et al.*, 1988; Plumb *et al.*, 1989). We report here, however, that a factor with identical binding specificity and gel-retardation mobility to EF1 is present in a wide range of human and murine non-erythroid cells, although at levels considerably lower than those observed for EF1 in erythroid cells. In addition to its role in the control of erythroid gene expression, EF1 may also therefore play a role in the regulation of gene expression in non-erythroid cells.

EXPERIMENTAL

Gel retardation assays

Protein and DNA were mixed in a binding buffer that consisted

of 4% (w/v) Ficoll, 20 mM-Hepes (pH 7.9), 50 mM-NaCl or -KCl and 1 mM-MgCl₂ in a final volume of 20 μ l. The incubations were carried out on ice for 45 min. Typically, 5–10 fmol of ³²P-end-labelled probe DNA (> 10⁶ c.p.m./ μ g) was used in each incubation. For a 0.3 M-NaCl nuclear extract, approx. 1.5–2.0 μ g of protein was added to each incubation. For these concentrations of protein, 5 μ g of non-specific competitor, poly(dI-dC) DNA was added to each incubation. Competitor DNAs were added as detailed in Figure legends. The protein was always the final component to be added to the gel retardation mixture.

After incubation, each gel retardation was loaded directly on to a 4% (w/v) non-denaturing polyacrylamide gel containing 0.25 $\times$ TBE (0.089 M-Tris/borate, 0.089 M-boric acid, 0.002 M-EDTA), pre-run for 45 min. The gel was run at 4 $^{\circ}$ C for 90–180 min depending on the size of the probe DNA, at a constant 150 V and 5 mA. After electrophoresis the gel was dried and then autoradiographed.

Nuclear protein extractions

All solutions contained the proteinase inhibitors phenylmethanesulphonyl fluoride (0.5 mM) benzamidin (0.5 mM), aprotinin (1 μ g/ml), pepstatin A (1 μ g/ml), leupeptin (2 μ g/ml) and bestatin (5 μ g/ml), and the phosphatase inhibitors levamisole (2 mM) and β -glycerophosphate (10 mM). Sodium butyrate (10 mM) was included throughout. All procedures were carried out on ice or at 4 $^{\circ}$ C and were essentially those described in Perkins *et al.* (1989).

The number of cells was counted in a Coulter counter. A typical preparation used approx. 10⁷ cells. The cells were spun down at 1000 g and then washed in phosphate-buffered saline (PBS; 137 mM-NaCl/2.7 mM-KCl/8 mM-sodium phosphate, pH 7.2). The cell pellet was resuspended in 1 ml of 10 mM-Tris/HCl (pH 7.5)/5 mM-MgCl₂/0.05% (v/v) Triton X-100 and lysed with 20 strokes in a 1 ml Dounce homogenizer. Jurkat T-cells were first incubated on ice for 10 min in the above buffer with no Triton X-100 before being spun down and resuspended in 10 mM-Tris/HCl (pH 7.5)/5 mM-MgCl₂/0.25 M-sucrose (kept in buffers for all the Jurkat T-cell extraction procedure) and lysed as above in the absence of Triton. The nuclei were pelleted by spinning in an Eppendorf centrifuge at 10000 g for 15 min at 4 $^{\circ}$ C. The nuclei pellet volume was estimated and then the pellet was resuspended in an equivalent volume of 10 mM-Tris/HCl (pH 7.5)/5 mM-MgCl₂, followed by the addition of 1 nuclei pellet vol. of 1 M-NaCl/10 mM-Tris/HCl (pH 7.5)/4 mM-MgCl₂, so

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that the final concentration of NaCl was 0.33 M. The lysing nuclei were left on ice for 30 min before being centrifuged at 10000 *g* for 15 min in an Eppendorf centrifuge at 4 °C. The supernatant was removed and could then be used immediately. If it was to be stored, 80 % (v/v) glycerol was added, such that the final glycerol concentration was 20 % (v/v) and then the sample was frozen at -70 °C.

HeLa and K562 cell nuclear extracts were prepared in the presence of all proteinase and phosphatase inhibitors by the method of Dignam *et al.* (1983). Human T-cell nuclear extract, prepared as for Jurkat T-cells, was provided by Dr. M. Crompton (Institute of Cancer Research) and Dr. D. Cantrell (Imperial Cancer Research Fund).

RESULTS AND DISCUSSION

In order to detect EF1 binding activity, we carried out a gel retardation experiment using an oligonucleotide containing a high-affinity EF1 binding site from the mouse α -globin promoter and nuclear extracts from a variety of cell types prepared as previously described (Perkins *et al.*, 1989). As expected, an EF1 binding activity (labelled 'a' in Fig. 1) was readily detectable using small amounts of extracts prepared from the mouse erythroleukaemia cell line MEL (Fig. 1, track 1) or the human erythroleukaemia cell line K562 (Fig. 1, track 2).

More surprisingly, retarded bands indicative of proteins binding to the EF1 site were also detected using large amounts of extracts prepared from HeLa cells (Fig. 1, track 3), resting human T-cells prepared as described by Cantrell & Smith (1984) (Fig. 1, track 4), murine F9 embryonal carcinoma cells (Fig. 1, track 5) and the human T-cell line Jurkat (Fig. 2c). A variety of bands representing proteins binding to the probe were observed in these non-erythroid extracts, including a band of mobility identical to that of the major band seen in erythroid cells (labelled a), two bands of higher mobility (labelled b and c) and

Fig. 1. DNA mobility shift assay using a radiolabelled oligonucleotide containing an EF1 binding site from the mouse α_1 -globin promoter

Track 1 shows the pattern of retarded bands obtained with 0.4 μ g of protein of MEL cell nuclear extract; track 2 with 1 μ g of protein of K562 extract; track 3 with 40 μ g of protein of HeLa cell extract; track 4 with 20 μ g of protein of purified human T-cell extract; and track 5 with 20 μ g of protein of murine F9 embryonal carcinoma cell extract. Arrows labelled a, b, c, x and y indicate the various bands produced by binding of nuclear proteins to the labelled oligonucleotide.

Fig. 2. DNA mobility shift assay using the same oligonucleotide and extracts as in Fig. 1

Samples used were: (a) 1 μ g of protein of K562 extract; (b) 40 μ g of protein of HeLa extract; (c) 20 μ g of protein of extract from the human T-cell line Jurkat; (d) 20 μ g of protein of purified human T-cell extract; (e) 20 μ g of protein of F9 cell extract. The assays were performed in the absence of competing oligonucleotide (track 1), or in the presence of a 100-fold excess of oligonucleotides containing the identical sequence to the probe (track 2), the sequence of the EF1 binding sites B (track 3) and C (track 4) from the chicken β hatching globin gene promoter (Perkins *et al.*, 1989), a mutated site C which does not bind EF1 (track 5) or an octamer-binding motif (track 6). In track 7, competition was carried out using an equivalent mass of pBR322 plasmid DNA. The arrows indicate the bands labelled as in Fig. 1.

bands of lower mobility which appeared to be specific to non-erythroid cells (labelled x and y).

To investigate the relationship of these binding factors to the EF1 expressed in erythroid cells, we carried out a competition analysis of factor binding using the same cell extracts and also a

nuclear extract prepared from the human T-cell line Jurkat (Fig. 2). Interestingly, in the competition experiments, band a, which is identical in size to erythroid EF1, showed binding characteristics identical to those of erythroid EF1 in each cell type. Thus its binding was competed for by a 100-fold excess of identical unlabelled probe oligonucleotide (track 2) or by oligonucleotides containing two different EF1 binding sites from the chicken β hatching globin gene promoter (tracks 3 and 4), but not by an oligonucleotide containing the octamer motif which binds the unrelated transcription factors Oct-1 and Oct-2 (track 6) or by an equivalent molar mass of plasmid pBR322 DNA (track 7). Moreover, no competition for binding of the band a protein was observed with an oligonucleotide containing a mutated version of the EF1 binding site in the β hatching globin promoter which fails to bind erythroid EF1 (Fig. 2, track 5). Hence this protein, in addition to being the same size as erythroid EF1, binds specifically to the EF1 binding site in a manner similar to EF1.

In competition experiments using all the cellular extracts (see Fig. 2), bands b and c were competed with much less effectively than band a by the competitor DNAs, though to some extent they show the same specificity of competition and may be related to EF1. In K562 cells, a band labelled a' shows an identical pattern of competition with EF1 and may therefore represent a degradation product or alternative form of EF1. Bands x and y observed in non-erythroid cells did show a sequence-specific pattern of binding, but with different characteristics than EF1. Thus these bands were competed for by the probe EF1 site (track 2) and the mutated EF1 site (track 5), but not by the non-specific competitors (tracks 6 and 7) or by the EF1 sites from the chicken β hatching globin gene (tracks 3 and 4). They are relatively more abundant in T-cells than in Jurkat cells or F9 cells, and are barely detectable in HeLa cells. The nature of these proteins and their relationship to EF1 is unclear, although the factor we observe at a very low level in HeLa cells (Fig. 1, track 3) may be related to the factors observed in these cells by Brady *et al.* (1989) and Mignotte *et al.* (1989) which bind to an EF1 site but are of different mobility to erythroid EF1.

In conclusion, it is clear that non-erythroid cells contain a protein with the size and binding characteristics of erythroid cell EF1. The levels of this protein in non-erythroid cells are 10–100-fold lower than in erythroid cells, and much larger amounts of non-erythroid extracts must therefore be used in order to detect this factor. Our ability to detect this low level of non-erythroid EF1 whereas others have not is likely to be due to the use of a high-affinity binding site in the gel retardation assay, coupled with the preparation of nuclear extracts in the presence of a variety of proteinase and phosphatase inhibitors.

It is likely that the EF1-like factor we describe here is widely distributed in non-erythroid cells. We have detected it in cells as disparate as HeLa cells, T-cells and embryonal carcinoma cells, and have also observed an EF1-like activity in *Xenopus* oocytes (G. A. Partington, N. D. Perkins, R. H. Nicolas & G. H. Goodwin, unpublished work). Similarly, a factor binding to an EF1 site in the rat elastase gene promoter has been observed by others in pancreatic cells (Kruse *et al.*, 1988). Moreover, our detection of the EF1-like factor in resting T-cells obtained from human blood indicates that it is not confined to immortalized cell lines but is present also in normal human non-erythroid cells.

It is clear, therefore, that an EF1-like factor or family of factors is expressed in many non-erythroid cell types, although at a lower level than EF1 in erythroid cells, and may therefore play a role in the regulation of genes expressed in non-erythroid cells.

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