Biochimica et Biophysica Acta 965 (1988) 60–67
Elsevier

BBA 22901

A sensitive enzyme-linked immunosorbence assay for the c-*fos* and v-*fos* oncoproteins

John P. Moore, Trevor D. Littlewood, David C. Hancock and Gerard I. Evan

Ludwig Institute for Cancer Research, MRC Centre, Cambridge (U.K.)

(Received 10 November 1987)

Key words: c-*fos* protein; ELISA; v-*fos* protein

The c-*fos* nuclear oncoprotein is rapidly induced when the growth of normal cells is initiated by mitogens, and it is also synthesised in several cell systems in response to stimuli that do not cause cell proliferation. When expressed inappropriately, c-*fos*, and its retroviral counterpart v-*fos*, can transform susceptible cells in vivo and in vitro. We have developed a simple and sensitive ELISA for the c-*fos* and v-*fos* proteins. *Fos* proteins are captured from cell lysates by an antibody specific for an amino-terminal peptide substantially conserved between v-*fos* and c-*fos*; the captured proteins are recognised by a second antibody against a different peptide sequence also conserved in the two proteins. The second antibody has been conjugated to alkaline phosphatase to provide an enzyme label; bound alkaline phosphatase is measured with a sensitive cycling enzyme system that generates a coloured end-product. We show that the *fos* ELISA is immunologically specific and use it to monitor increased c-*fos* expression in serum-stimulated HeLa cells and human fibroblasts, and in mitogen-stimulated murine thymocytes.

Introduction

The c-*fos* gene is the cellular counterpart of the v-*fos* transforming gene of the FBJ and FBR murine osteosarcoma viruses [1,2]. These retroviruses induce bone tumours in mice [3,4] and transform cultured fibroblasts [5,6], which demonstrates the oncogenic potential of the v-*fos* gene. In adult tissue c-*fos* is normally expressed at high levels only in a subset of haematopoietic cells [7,8], but the gene is rapidly and transiently induced on addition of growth factors to several types of quiescent cells (e.g., fibroblasts) in culture [6,9–11]. In these systems c-*fos* expression appears to be essential for subsequent cell proliferation as this is blocked when c-*fos* mRNA translation is inhibited by antisense RNA [12,13]. Furthermore, deregulated expression of the c-*fos* gene can transform fibroblasts in vitro [14,15] and cause abnormal bone growth in vivo [16]. However, notwithstanding its role in proliferation, the c-*fos* gene is also expressed in cells and tissues in response to stimuli that do not cause cell growth (e.g., in cultured nerve cells and in brain [17–19]).

Both v- and c-*fos* genes encode nuclear oncoproteins of apparent M_r 55 000 ($p55^{c\text{-}fos}$ and $p55^{v\text{-}fos}$) that differ substantially only at the C-terminus [14,20,21]. Both *fos* proteins are extensively modified post-transcriptionally by phosphorylation [14,22,23], but their biochemical functions are at present uncertain. There is, however, some evidence that the c-*fos* protein is a component of the machinery that regulates the expression of genes mediating longer-term cellular responses [24].

The most common method for analysis of *fos*

Correspondence: J.P. Moore, Department of Veterinary Pathology, Glasgow University, Bearsden Road, Bearsden, Glasgow, G61 1QH, U.K.

gene expression has been to measure *fos* mRNA levels (e.g., by Northern blotting). This is slow and laborious, and gives no direct information on *fos* protein levels. The *fos* proteins themselves have been monitored by immunoprecipitation, immunoblotting and immunocytochemical analyses using polyclonal or monoclonal antibodies specific for v- and/or c-*fos* [10,11,14,18,20,22,25–27]. Each of these procedures is complex and time-consuming, and (except for immunocytochemistry) their sensitivity is poor. We have recently described rapid, simple and sensitive enzyme-linked immunosorbence assays (ELISA) for the c-*myc* and N-*myc* oncoproteins [28,29]. Here we report the development of similar assays for the human and murine c-*fos* proteins and for the v-*fos* protein, and demonstrate their use in monitoring *fos* protein expression in HeLa cells, thymocytes and fibroblasts.

Materials and Methods

Antibodies and peptides

The rabbit polyclonal antibody afos5 was raised against the peptide sequence MFSGFNADYEASSSRC which corresponds to amino acids 2–17 of the human and murine c-*fos* protein. The v-*fos* sequence is identical with the exception of a Ser to Phe change at position 15. The afos1 antibody is also a rabbit polyclonal and recognises the sequence NDPEPKPSLEPVKS, amino acids 245–258 of the murine c-*fos* protein. The corresponding v-*fos* sequence is identical, and the only change in the human c-*fos* sequence is Leu to Val at position 253 (all sequences taken from Refs. 21 and 30). The preparation and some properties of these antibodies have been described [18]: both antibodies recognise human and murine c-*fos* proteins and also v-*fos* protein when used in immunoprecipitation, immunoblotting and immunocytochemical analyses (G.I. Evan, unpublished data). The phosphorylation state of neither of the above peptide sequences is altered by serum or phorbol esters [22].

The afos5 antibody was purified from immune serum by affinity chromatography. Briefly, a small column was prepared from afos5 peptide coupled to agarose beads, and washed extensively with 144 mM NaCl, 25 mM Tris-HCl, pH 8.0 (TBS) containing 0.1% Nonidet P40 (NP-40). Antibodies were precipitated from serum using 45% ammonium sulphate and redissolved in TBS containing 0.1% NP-40 before application to the column. After extensive washing with the same buffer, then TBS, then 0.9% NaCl, the direction of flow through the column was reversed and afos5 antibody was eluted with 100 mM sodium citrate, pH 2.5. The eluate was neutralised with Tris base and antibody was precipitated in 50% ammonium sulphate. The pellet was dissolved at 4 mg/ml in TBS containing 0.1% sodium azide and stored in small aliquots at $-20\,^{\circ}C$.

The afos1 antibody was partially purified from serum by octanoic acid treatment followed by ammonium sulphate precipitation [31]. This fraction was conjugated to alkaline phosphatase [32] and the antibody-alkaline phosphatase conjugate (afos1-AP) was purified by fast protein liquid chromatography.

Cell culture

HeLa cervical carcinoma cells, MRC-5 human lung fibroblasts and RS2 cells (rat 208F fibroblasts transformed with FBJ.MSV [33]) were grown as adherent monolayers in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum. When confluent, HeLa and MRC-5 cells were washed twice in serum-free DMEM, then incubated for 18–24 h in DMEM containing 10 mM Hepes and 0.5% fetal calf serum before serum stimulation. Murine thymocytes were prepared in RPMI 1640 medium containing 10 mM Hepes as described previously [34,35].

Preparation of cell lysates for ELISA

Lysates were prepared essentially as described previously [29]. Cells from suspension cultures were adjusted to 0.1% sodium azide and pelleted by centrifugation ($1000 \times g$, 5 min, $4\,^{\circ}C$). Cells growing as monolayers were washed with ice-cold Hank's balanced salts solution containing 0.1% sodium azide (HBSS), removed with a scraper into ice-cold HBSS and collected by centrifugation as above. Cell pellets were carefully resuspended at $(5–10) \cdot 10^7$ cells/ml (thymocytes at $3 \cdot 10^8$ cells/ml) in lysis buffer (TBS containing 1% SDS, 50 mM dithiothreitol, 1% aprotinin and 0.5 mM phenylmethylsulphonyl fluoride) and boiled for 5

min. After addition of 100 mM iodoacetamide, the lysates were passed through a 26 gauge needle to shear DNA, incubated on ice for 30 min, then diluted with 9 vol. of TBS/NP-40 (TBS containing 1% NP-40, 1% aprotinin and 0.5 mM phenylmethylsulphonyl fluoride) and mixed by passage through the same needle and syringe used above. Lysates were stored at −70 °C and immediately before use were clarified by centrifugation (1 min at 14000 × *g*).

Conditions for ELISA

The afos5 antibody was adsorbed onto polystyrene flat-bottomed microtitre wells (Immulon 2, Dynatech Ltd.) by incubation (4 μg/ml in 100 mM sodium bicarbonate, 1 mM EGTA, pH 9.6) for 16 h at room temperature in a humid environment. Unbound antibody was removed, the wells were washed twice with TBS, blocked for 30 min with TBS containing 2% non-fat dried milk (Marvel, Cadbury Ltd.), and washed twice with TBS before addition of TBS/NP-40 (100 μl/well). Where appropriate this contained afos5 peptide. Cell lysates and/or mock lysate buffer (TBS/NP-40 containing 10% lysis buffer and 10 mM iodoacetamide) to a total of 100 μl were added to each well and incubated for 3 h at room temperature to allow maximal capture of *fos* proteins. Unbound protein was removed by washing twice with TBS. Bound *fos* proteins were recognised by incubation for 1 h at room temperature with 100 μl/well of afos1-AP (1 μg/ml in TMT, which is TBS containing 4% non-fat milk powder and 0.5% Tween 20). Non-specifically bound afos1-AP was removed by washing the wells six times with 200 μl/well of AMPAK wash buffer (Novo BioLabs, formerly IQ (Bio) Ltd., Cambridge, U.K.). Bound alkaline phosphatase was detected with the sensitive AMPAK system (Novo BioLabs). The design of the AMPAK system has been described elsewhere [36–38], as has its use in ELISAs for *myc* oncoproteins [28,29]. In the present assay the wells were incubated for 1 h at room temperature with 100 μl of 'substrate solution', followed by 5 min with 100 μl of 'amplifier solution'. The reactions were stopped with 50 μl of 0.5 M HCl and the absorbance (A_{492}) was determined using a conventional automated plate-reader (Titertek).

Results

The design of an ELISA for the c-*fos* and v-*fos* oncoprotein was similar to that used for the c-*myc* and N-*myc* ELISAs described previously [28,29]. Briefly, the afos5 antibody adsorbed to wells of a microtitre plate is used to capture c- and v-*fos* proteins from cell lysates. Bound *fos* proteins are recognised at a separate epitope by the afos1 antibody conjugated to alkaline phosphatase. Specifically bound alkaline phosphatase is detected using the AMPAK system [36–38] which generates a coloured end-product whose intensity is determined spectrophotometrically.

Preliminary experiments established that the conditions used previously to prepare cell lysates and assay them for *myc* proteins [29] were suitable for an ELISA for *fos* proteins. Lysates prepared from HeLa cells incubated for 18 h in 0.5% serum then stimulated for 1 h with 15% serum were used to characterise and optimise the c-*fos* ELISA. HeLa cells treated in this way contain large amounts of c-*fos* mRNA [39] and protein (as assayed by immunoblotting with afos1 and afos5 antibodies [18]).

Titration of the afos5 capture antibody showed that the optimal concentration for ELISA was approximately 4 μg/ml (Fig. 1a). Higher concentrations were less effective, possibly due to aggregation of excess antibody on the plate surface with consequent reduction of available binding sites for *fos* proteins. A small background signal (A_{492} of 0.10–0.15 in the absence of any cell lysate) was found at high afos5 concentrations, probably caused by non-specific binding of afos1-AP to afos5. The background was, however, minimal (less than 0.10 A_{492}) at the afos5 concentration used routinely (4 μg/ml), where the signal-to-noise ratio was 15–20 (Fig. 1a).

In a similar experiment (not shown), the afos1-AP detection antibody was titrated at a fixed afos5 concentration (4 μg/ml) in the presence or absence of a lysate of serum-stimualted HeLa cells. The maximal signal-to-noise ratio was found at an afos1-AP concentration of 1 μg/ml, and this concentration was used routinely. Higher concentrations of afos1-AP gave progressively higher background signals in the absence of a cell lysate.

To demonstrate that the coloured end-products

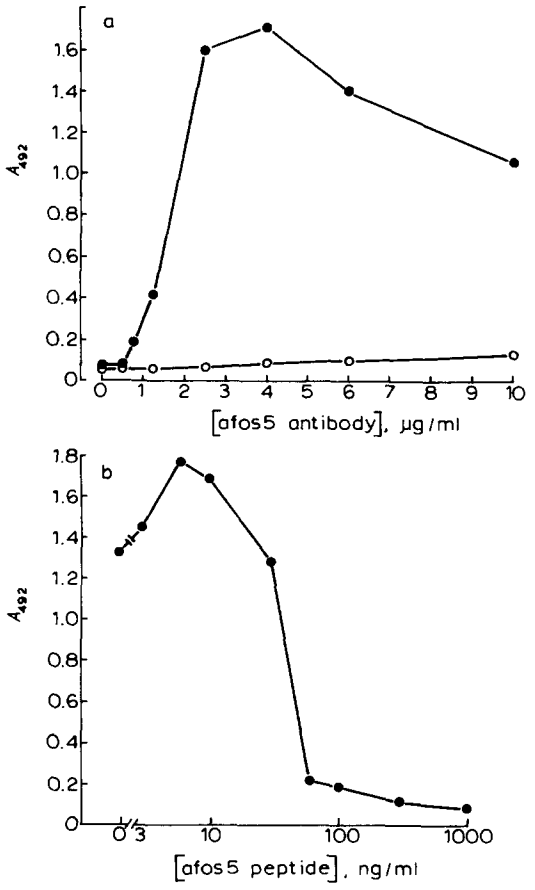

Fig. 1. Capture of c-*fos* protein by afos5 antibody and its inhibition by afos5 peptide. (a) The afos5 antibody was adsorbed onto microtitre wells at the input concentrations indicated. The wells were treated as described in Materials and Methods, then 100 μl aliquots of mock lysate buffer (○) or lysate containing $1 \cdot 10^6$ serum-stimulated HeLa cells (●) were added. (b) The afos5 antibody was adsorbed onto microtitre wells at 4 μg/ml. The afos5 peptide was added to the wells at the concentrations indicated (●) for 30 min prior to the addition of a lysate of serum-stimulated HeLa cells. In both experiments bound c-*fos* protein was detected with afos1-AP (1 μg/ml) and the AMPAK system.

of the *fos* ELISAs were caused by immunologically specific reactions, various components of the assays were omitted, the capture and detection antibodies were varied, and the peptides against which the antibodies were raised were included in the assays. The signal (A_{492}) derived from serum-stimulated HeLa cells was completely dependent upon the presence of afos5 capture antibody (Fig. 1a). Omission of this antibody or replacement by an equivalent concentration of irrelevant antibody (pan-*myc* antibody [29] or rabbit immunoblobulins; Sigma) prevented any significant signal (more than 0.10 A_{492}) when afos1-AP was used to detect captured *fos* proteins. Similarly, there was no signal when afos1-AP was omitted. Pre-incubation of the afo5 antibody with the peptide against which it was raised blocked binding (and hence detection) of c-*fos* protein from serum-stimulated HeLa cells (Fig. 1b) and also of v-*fos* protein from RS-2 cells (not shown). Furthermore, pre-incubation of the afos1-AP detection antibody for 30 min with its immunogenic peptide (10 μg/ml) completely inhibited detection of c-*fos* and v-*fos* proteins. We conclude that the ELISAs for *fos* proteins are immunologically specific and that they can be used to monitor *fos* expression in cells.

To assess the sensitivity of the *fos* ELISA we varied the number of cell equivalents in lysates added to wells containing adsorbed afos5, then detected bound *fos* proteins with afos1-AP and AMPAK (Fig. 2). The $p55^{c\text{-}fos}$ content of as few as $3 \cdot 10^4$ serum-stimulated HeLa cells could be detected by ELISA, and the signal (A_{492}) increased in an approximately linear manner with the number of cell equivalents assayed. In contrast, no

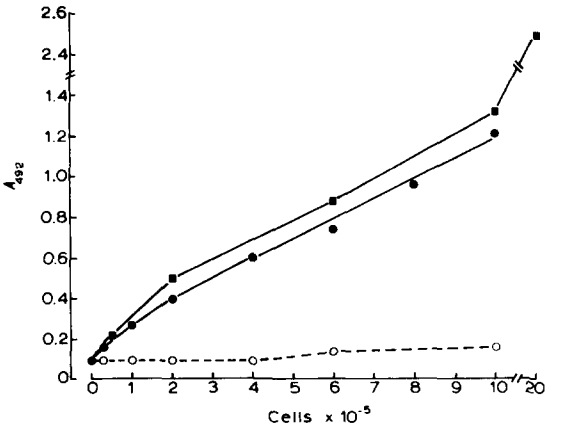

Fig. 2. Detection of c-*fos* and v-*fos* proteins by ELISA. Conditions for the ELISA were as described in Materials and Methods. Lysates were prepared at $(1–2) \cdot 10^7$ cell equivalents/ml, and the number of cell equivalents indicated were assayed for *fos* protein. The lysates contained: HeLa cells incubated for 18 h in 0.5% serum (○); HeLa cells incubated for 18 h in 0.5% serum, then stimulated for 1 h with 15% serum (●); RS-2 transformed rat fibroblasts harvested in log phase (■).

signal could be detected from $4 \cdot 10^5$ serum-deprived HeLa cells, and only a small signal (less than 0.20 A_{492}) from $1 \cdot 10^6$ serum-deprived HeLa cells. The signals derived from $1 \cdot 10^6$ serum-deprived and $3 \cdot 10^4$ serum-stimulated HeLa cells were similar, which implies that the induction of c-*fos* protein by serum is at least 30-fold and probably much greater. Log-phase HeLa cells grown continuously in the presence of 10% serum contained no more c-*fos* protein than serum-deprived cells, which agrees with previous immunoblotting data [18]. In contrast, log-phase RS-2 fibroblasts (which have been transformed by the FBJ murine sarcoma virus [33]) contained abundant *fos* protein (Fig. 2). The *fos* protein detected by ELISA in RS-2 cells is assumed to be $p55^{v\text{-}fos}$ rather than $p55^{c\text{-}fos}$. On the further assumption that the affinities of afos5 and afos1-AP are similar for both the v-*fos* and c-*fos* proteins, we estimate that there is approximately the same amount of v-*fos* protein in RS-2 cells as in serum-stimulated HeLa cells and slightly less than the amount of $p55^{c\text{-}fos}$ in serum-stimulated MRC-5 fibroblasts (see below).

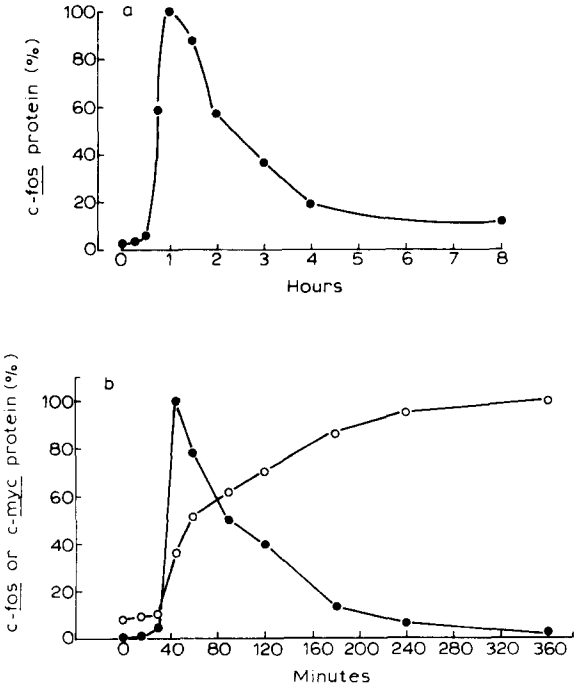

Fig. 3. Induction of c-*fos* protein by serum in HeLa cells and fibroblasts. (a) HeLa cells were grown to confluence ($6 \cdot 10^6$ cells) in 75 cm^2 culture flasks in medium containing 10% serum, then incubated for 18 h in medium containing 0.5% serum. At time zero the cells were stimulated with 15% serum and lysates were prepared at the times indicated (●) and assayed ($5 \cdot 10^5$ cells per 100 μl) for c-*fos*. (b) The experiment was of similar design to that in (a) except that confluent, quiescent MRC-5 fibroblasts were stimulated with 10% serum. The same lysates ($5 \cdot 10^5$ cells per 100 μl) were assayed by ELISA for c-*fos* (●) and c-*myc* (○). Absolute values for the c-*myc* protein content of fibroblasts were obtained using bacterially expressed c-*myc* protein [29,41], but for clarity data are expressed relative to the highest c-*myc* protein level recorded (defined as 100%). Similar data were obtained in three experiments of the same design on each type of cell.

The rate of induction of c-*fos* protein in serum-stimulated HeLa cells was measured by ELISA (Fig. 3a). In this and subsequent experiments the A_{492} signals at each time-point were converted to percentage c-*fos* by reference to a calibration curve (similar to that shown in Fig. 2) prepared from the lysate containing the maximum amount of c-*fos* protein (defined as 100%). The A_{492} signals from all lysates were completely blocked by pre-incubation of the afos5 capture antibody with afos5 peptide (1 μg/ml). A small increase in $p55^{c\text{-}fos}$ was detectable within 30 min of serum addition to HeLa cells, and the amount of c-*fos* protein increased dramatically between 30 and 60 min after serum stimulation. There was a subsequent slow decline in $p55^{c\text{-}fos}$ levels, but a significant amount (approximately 10% of the peak response) of c-*fos* protein was still detectable as late as 8 h after serum stimulation. In contrast to the induction of the c-*fos* protein in Hela cells, there was no increase in the expression of the c-*myc* protein on serum-stimulation. The amount of $p62^{c\text{-}myc}$ in log-phase adherent HeLa cells was estimated as 100 000 molecules per cell by quantitative ELISA [29], and any change on serum-deprivation and re-addition was less than 2-fold (not shown).

Proliferating human MRC-5 lung fibroblasts are rendered quiescent by serum deprivation and accumulate in G_0. In most experiments, no $p55^{c\text{-}fos}$ was detectable by ELISA in $5 \cdot 10^5$ log-phase or quiescent fibroblasts, but the c-*fos* protein was always rapidly induced upon re-addition of serum to the quiescent cells (Fig. 3b). Whether c-*fos* protein is present at very low levels in log-phase or quiescent cells is presently under investigation. The $p55^{c\text{-}fos}$ response to serum was maximal after

45 min, and we estimate that the peak level of c-*fos* protein in fibroblasts was 2–3-fold greater than that in HeLa cells. The amount of $p55^{c\text{-}fos}$ in fibroblasts slowly declined over the following 4–6 h, and no c-*fos* protein could be detected later than 6 h after serum stimulation (not shown). Unlike HeLa cells, fibroblasts also respond to serum deprivation by a reduction in c-*myc* protein levels, and synthesis of $p62^{c\text{-}myc}$ is re-initiated on serum addition to the quiescent cells [29,40]. We monitored the increase in c-*myc* protein in response to serum using the same fibroblast lysates assayed for c-*fos* (Fig. 3b). An increase in $p62^{c\text{-}myc}$ from the level in quiescent cells (450 molecules/cell) was detectable after 45 min and the amount of c-*myc* protein increased for 6 h to a level of 5400 molecules/cell (defined as 100%). There was a subsequent slow decline in $p62^{c\text{-}myc}$ expression (not shown). Kinetically similar increases in the expression of c-*fos* and c-*myc* proteins were also found on addition of epidermal growth factor plus insulin to quiescent murine Swiss 3T3 fibroblasts (S.R. Pennington and J.P. Moore, unpublished data).

The c-*fos* gene is also inducible in freshly isolated murine thymocytes in response to a variety of defined mitogens, including the phorbol ester 12-*o*-tetradecanoyl phorbol 13-acetate (TPA) and the calcium ionophore A23187 [35]. The combination of TPA (10 nM) with A23187 (30 nM) is mitogenic for these cells and cause a very large increase in c-*fos* and c-*myc* mRNA. We therefore used the ELISAs to follow the synthesis of the c-*fos* and c-*myc* proteins in murine thymocytes (Fig. 4). An increase in $p55^{c\text{-}fos}$ was found within 10 min of addition of TPA plus A23187, and the amount of c-*fos* protein rose for 90–120 min before declining slowly. (Similar kinetics were also observed in response to A23187 alone.) The peak level of c-*fos* protein per cell in murine thymocytes stimulated by TPA plus A23187 was estimated to be approximately 10% and 25% of the peak levels in serum-stimulated MRC-5 fibroblasts and HeLa cells, respectively. There was also an increase in c-*myc* protein above the quiescent level of approximately 50 molecules/cell after 45 min, and synthesis continued for at least 3 h (Fig. 4), when the amount of $p62^{c\text{-}myc}$ was estimated as 600 molecules/cell (i.e., approximately 10% of

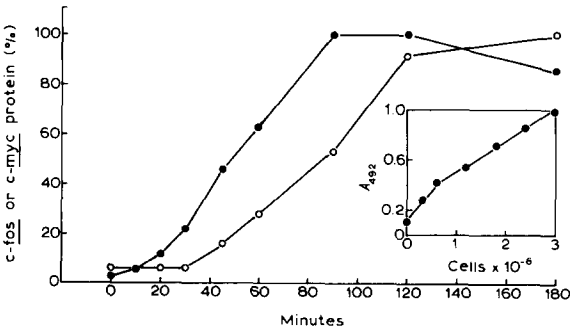

Fig. 4. Induction of c-*fos* and c-*myc* proteins in murine thymocytes. Thymocytes were incubated at $6 \cdot 10^6$ cells/ml for 2 h after isolation before stimulation of 5 ml aliquots with TPA (10 nM) and A23187 (30 nM) at time zero. Lysates were prepared at the times indicated and assayed ($3 \cdot 10^6$ cells per 100 μl) for c-*fos* (●) and c-*myc* (○) by ELISA. The data are expressed relative to the highest values recorded (defined as 100%): for c-*fos* the calibration curve prepared from the 90 min time point was used (inset); for c-*myc*, the assay was calibrated with bacterially expressed c-*myc* protein. Similar data were obtained in one other experiment.

that in serum-stimulated fibroblasts).

The kinetics of the c-*fos* protein response to TPA plus A23187 in thymocytes were somewhat slower than the responses to serum in HeLa cells and fibroblasts. This may be due, at least in part, to cellular heterogeneity in the thymocyte population. It should be noted, however, that although c-*fos* mRNA levels are maximal 30 min after addition of TPA plus A23187 [35], the decline from the peak is slow. Thus, 5–10% of the maximum amount of c-*fos* transcripts still remain in thymocytes even 4–8 h after addition of TPA plus A23187 (J.P. Moore, unpublished data).

Other mitogens also stimulated increases in $p55^{c\text{-}fos}$ in murine thymocytes; expressed relative to the maximum response evoked by TPA plus A23187 (defined as 100%), the responses to concanavalin A (10 μg/ml), TPA (10 nM) and A23187 (30 nM) after 90 min were typically 4–8%, 6–10%, and 30–50%. The relative increases in c-*fos* protein were consistent with the relative increases in c-*fos* mRNA in response to the same range of thymocyte mitogens [35].

Discussion

We have described a simple ELISA for c-*fos* and v-*fos* proteins and have used it to monitor

c-*fos* expression in several types of cell. The assay is simple, quick and sensitive. Thus, lysates of cultured cells are prepared easily and rapidly, and can be stored for several weeks at −70 °C prior to assay if desired. The ELISA itself takes a few hours to carry out, and hundreds of samples can be processed simultaneously. The reproducibility of the assay is good: assay of a single sample in triplicate produces an A_{492} measurement with a standard deviation that is typically less than 10% of the mean value. Assay of duplicate lysates is slightly more variable, as the principal sources of error precede the ELISA stage of the procedure. These errors include variation in the number of cells in replicate monolayer cultures, variable recovery of cells from monolayer by scraping, and losses of material during lysate preparation. Nonetheless, assay of duplicate lysates of stimulated fibroblasts or HeLa cells results in mean A_{492} values that differ by a maximum of 0.15, which does not usually cause large variations in relative c-*fos* values (see Fig. 2).

The *fos* ELISA complements existing assays for c-*myc* and N-*myc* proteins: use of the appropriate combination of antibodies allows simultaneous assay of, for example, c-*fos* and c-*myc* proteins in the same lysate. The present lack of purified c-*fos* proteins means that, unlike the ELISA for c-*myc*, the c-*fos* ELISA is not calibrated. Nevertheless, values for relative c-*fos* expression may easily be obtained by preparation of standard curves such as those shown in Fig. 2, and relative values are the usual aim of an experiment. Once a supply of purified c-*fos* protein becomes available, quantitation of $p55^{c\text{-}fos}$ expression in cells should be relatively straighforward by ELISA. Finally, modifications to the *fos* assay currently under development (principally preparation of a more highly purified afos1-AP detection antibody) could increase the sensitivity of the present assay by up to 10-fold, perhaps allowing determination of $p55^{c\text{-}fos}$ levels in quiescent and/or log-phase cells.

Acknowledgements

We wish to thank David Bates, Jim Fuller and Alison Jenner of Novo BioLabs for preparation of afos1-AP and for supplies of AMPAK reagents, and Paul Sheppard of Cambridge Research Biochemicals Ltd. for the gift of peptides. Steve Pennington is thanked for assistance with the experiment on Swiss 3T3 fibroblasts, and we are grateful to Carole Spencer for assistance with cell culture. This work was supported, in part, by a grant from the Medical Research Council to J.P.M.

References

1 Finkel, M.P., Biskis, B.O. and Jenkins, P.B. (1966) Science 151, 698–701.
2 Finkel, M.P., Reilly, C.A., Biskis, B.O. and Greco, I.L. (1973) Proceedings of the 24th Symposium of the Colston Research Society, Vol. 24, pp. 353–366, Butterworths, London.
3 Lee, C.K., Chan, E.W., Reilly, C.A., Pahnke, V.A., Bockus, G. and Finkel, M.P. (1979) Proc. Soc. Exp. Biol. Med. 162, 214–230.
4 Ward, J.M. and Young, D.M. (1979) Cancer Res. 36, 3985–3992.
5 Jenuwein, T., Muller, D., Curran, T. and Muller, R. (1985) Cell 41, 629–637.
6 Muller, R. (1986) Biochim. Biophys. Acta 823, 207–225.
7 Gonda, T.J. and Metcalf, D. (1984) Nature (Lond.) 310, 249–251.
8 Muller, R., Muller, D. and Guilbert, L. (1984) EMBO J. 3, 1887–1890.
9 Greenberg, M.E. and Ziff, E.D. (1984) Nature (Lond.) 311, 433–438.
10 Kruijer, W., Cooper, J.A., Hunter, T. and Verma, I.M. (1984) Nature (Lond.) 312, 711–716.
11 Muller, R., Bravo, R., Burckhardt, J. and Curran, T. (1984) Nature (Lond.) 312, 716–720.
12 Holt, J.T., Gopal, T.V., Moulton, A.D. and Nienhuis, A.W. (1986) Proc. Natl. Acad. Sci. USA 83, 4794–4798.
13 Nishikura, K. and Murray, J.M. (1987) Mol. Cell. Biol. 7, 639–649.
14 Curran, T., Miller, A.D., Zokan, L. and Verma, I.M. (1984) Cell 36, 259–268.
15 Miller, A.D., Curran, T. and Verma, I.M. (1984) Cell 36, 51–60.
16 Ruther, U., Garber, C., Komitowski, D., Muller, R. and Wagner, E.F. (1987) Nature (Lond.) 325, 412–416.
17 Curran, T. and Morgan, J.I. (1985) Science 229, 1265–1268.
18 Hunt, S.P., Pini, A. and Evan, G.I. (1987) Nature (Lond.) 328, 632–634.
19 Morgan, J.I., Cohen, D.R., Hempstead, J.L. and Curran, T. (1987) Science 237, 192–197.
20 Curran, T. and Teich, N.M. (1982) J. Virol. 42, 114–122.
21 Van Beveren, C., Van Straaten, F., Curran, T., Muller, R. and Verma, I.M. (1983) Cell 32, 1241–1255.
22 Barber, J.R. and Verma, I.M. (1987) Mol. Cell. Biol. 7, 2201–2211.
23 Sambucetti, L.C., Schaber, M., Kramer, R., Crowl, R. and Curran, T. (1986) Gene 43, 69–77.

24 Distel, R.J., Ro, H.-S., Rosen, B.S., Groves, D.L. and Spiegelman, B.M. (1987) Cell 49, 835–844.
25 Curran, T., Van Beveren, C., Ling, N. and Verma, I.M. (1985) Mol. Cell. Biol. 5, 167–172.
26 Giardina, S.L., Evans, S.W., Gandino, L., Robey, F.A., Bonvini, E., Longo, D.L. and Varesio, L. (1987) Anal. Biochem. 161, 109–116.
27 Ruther, U., Wagner, E.F. and Muller, R. (1985) EMBO J. 4, 1775–1781.
28 Moore, J.P. and Evan, G.I. (1987) Nature (Lond.) 327, 733–734.
29 Moore, J.P., Hancock, D.C., Littlewood, T.D. and Evan, G.I. (1987) Oncogene Res. 2, 65–80.
30 Van Straaten, F., Muller, R., Curran, T., Van Beveren, C. and Verma, I.M. (1983) Proc. Natl. Acad. Sci. USA 80, 3183–3187.
31 Evan, G.I., Lewis, G.K., Ramsay, G. and Bishop, J.M. (1985) Mol. Cell. Biol. 5, 3610–3616.
32 Ishikawa, E., Imagawa, M., Hashida, S., Yoshitake, S., Hamaguchi, Y. and Ueno, T. (1983) J. Immunoass. 4, 209–217.
33 Curran, T. and Teich, N.M. (1982) Virology 116, 221–235.
34 Moore, J.P., Smith, G.A., Hesketh, T.R. and Metcalfe, J.C. (1983) Biochem. J. 216, 207–213.
35 Moore, J.P., Todd, J.A., Hesketh, T.R. and Metcalfe, J.C. (1986) J. Biol. Chem. 261, 8158–8162.
36 Johannsson, A., Ellis, D.H., Bates, D.L., Plumb, A.M. and Stanley, C.J. (1986) J. Immunol. Methods 87, 7–11.
37 Self, C.H. (1985) J. Immunol. Methods 76, 389–393.
38 Stanley, C.J., Johannsson, A. and Self, C.H. (1985) J. Immunol. Methods 83, 89–95.
39 Treisman, R. (1985) Cell 42, 889–902.
40 Kelly, K., Cochran, B.H., Stiles, C.D. and Leder, P. (1983) Cell 35, 603–610.
41 Watt, R., Shatzman, A.R. and Rosenberg, M. (1985) Mol. Cell. Biol. 5, 448–456.