Development 107, 945-956 (1989)
Printed in Great Britain © The Company of Biologists Limited 1989

Transactivation of the adenovirus EIIa promoter in the absence of adenovirus E1A protein is restricted to mouse oocytes and preimplantation embryos

THOMAS P. DOOLEY[1,*], MIRIAM MIRANDA[2], NICHOLAS C. JONES[1] and MELVIN L. DePAMPHILIS[2]

[1]*Gene Regulation Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England*
[2]*Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110, USA*

* Author for correspondence; current address: The Upjohn Company, Unit 7235-25-12, Kalamazoo, Michigan 49001, USA

Abstract

Undifferentiated mouse embryonal carcinoma (EC) cells are capable of transactivating the adenovirus EIIa promoter in the absence of its normal transactivator, E1A protein, suggesting that EC cells contain an E1A-like activity. In an effort to identify where this activity appears during normal mouse development, mouse oocytes and preimplantation embryos were injected with plasmids containing the EIIa promoter coupled to various reporter genes. These expression vectors were fully active in human 293 cells where E1A is present, but were inactive in differentiated fibroblast cell lines unless cotransfected with the *E1A* gene. In mouse oocytes and preimplantation embryos, EIIa promoter activity in the absence of adenovirus E1A protein was equivalent to or greater than activity of the HSV thymidine kinase promoter coupled to a strong enhancer. Coinjection of the *E1A* gene failed to stimulate EIIa activity further, perhaps because c-myc protein, which has been reported to transactivate this promoter, was already present at high levels in mouse oocytes. Activation of the EIIa promoter in the absence of E1A was unique to mouse oocytes and preimplantation embryos because gene expression from an EIIa promoter introduced into transgenic mice was observed only in the adult ovary, and particularly in the oocytes. In addition, post-implantation transgenic embryos failed to express the E1A-activatable reporter gene, thereby indicating that expression from the EIIa promoter is restricted to the relatively undifferentiated stages of oogenesis and preimplantation development. These data suggest that cellular promoters of the class that can be transactivated by E1A may serve uniquely to initiate transcription of genes that are needed for preimplantation development.

Key words: embryonal carcinoma cells (EC cells), E1A, EIIa, mouse oocytes and embryos, transcriptional activation, transgenic mice, microinjection, c-myc, adenovirus, chloramphenicol acetyltransferase, β-galactosidase, luciferase.

Introduction

At present little is known about the transcriptional regulatory mechanisms that govern the processes of differentiation and early embryogenesis in mammals, although genetic and molecular approaches are beginning to reveal insights into some of these mechanisms in certain invertebrates, such as *Drosophila* and *Caenorhabditis elegans*. One approach to studying mammalian embryogenesis and differentiation involves the use of embryonal carcinoma (EC) cell lines. An intriguing property of undifferentiated EC cells, such as F9 and PCC4, is that they are capable of activating transcription from the human adenovirus EIIa promoter, in the absence of its natural transcriptional activator, the *E1A* gene product (Imperiale *et al.* 1984). The observation that the EIIa promoter is significantly more active in undifferentiated *versus* differentiated cells has resulted in the suggestion that certain EC cell lines express an 'E1A-like' transcriptional regulatory property, which is lost upon differentiation of EC cells (Imperiale *et al.* 1984). Furthermore, EC cells fail to efficiently express genes linked to a number of viral enhancer elements, (derived from SV40, polyoma, MLV, etc.) which are known to be repressed by E1A in differentiated cell lines, consistent with the presence of an E1A-like activity (Schneider *et al.* 1987; Gorman *et al.* 1985; Sleigh and Lockett, 1985; Borrelli *et al.* 1984; Jones,

1986; Cremisi and Babinet, 1986; Barklis *et al.* 1986; Taketo and Tanaka, 1987). Whether the two E1A-like activities, transactivation and enhancer repression, are encoded for by the same endogenous gene product remains to be seen, for the identities of both cellular putative transcriptional regulators are as yet unknown. At present, no cellular gene has been reported that encodes a cDNA homologous to *E1A*. Therefore, these putative cellular E1A-like activities are likely to be cellular proteins whose properties are functionally analogous to E1A, rather than a cellular homologue of this viral oncogene. Furthermore, although the mechanism by which the EIIa promoter is transactivated is not yet fully understood, it may represent a class of transcriptional promoters that is uniquely regulated during differentiation.

The purpose of the experiments described in this paper was to determine whether or not the E1A-like environment exhibited by undifferentiated EC cells is representative of normal mouse embryonic cells. If so, is the activity restricted to one or more specific stages in mouse development? We have addressed these questions by introducing various EIIa–reporter gene constructions into cultured cell lines, mouse oocytes and preimplantation embryos, and transgenic mice to monitor expression from the EIIa promoter. Our results demonstrate that an E1A-activatible promoter is expressed, in the absence of E1A, specifically during early embryonic development and in the adult mouse ovary.

Materials and methods

Construction of EIIa-reporter genes

pEIIa–*CAT* and pEII–65/75 *ls-CAT* (this linker-scanning mutant promoter is referred to as pEII65–*CAT*) were provided by B. Thimmapaya (Murthy *et al.* 1985). These constructs contain the Ad5 EIIa early promoter fused to the bacterial chloramphenicol acetyltransferase gene (*CAT*). The wild-type EIIa promoter was fused to the *Escherichia coli* β-galactosidase *lacZ* gene to form pEII–*lacZ*. This was accomplished by insertion of a 3 kb *Bgl*II-linked *lacZ* fragment from pZip *ras* βgal (Thompson *et al.* 1989) into *Bgl*II-cut pEII–*CAT*. pEII–*luciferase* (pEII–*luc*) was constructed by inserting the 350 bp EIIa promoter (*Xho*–*Bgl*II fragment from pEII–*CAT*) into a pML-1 vector containing the firefly *luciferase* gene encoded by a 2572 bp *Hin*dIII–*Bam*HI fragment from pSV232AL-AD5 along with SV40 splicing and polyadenylation signals (de Wet *et al.* 1987). The Ad5 E1A-expression plasmid, pCE, has been previously reported (Haley *et al.* 1984).

Microinjection of DNA into mouse oocytes and embryos

Isolation, culture and microinjection of mouse oocytes and embryos were carried out as previously described (DePamphilis *et al.* 1988; Martinez-Salas *et al.* 1989). Oocytes were obtained from 16 to 18 day old CD1 mice and cultured in minimal essential medium in the presence of 100 $\mu g\,ml^{-1}$ dbcAMP so that about 85 % retained their germinal vesicle. 1-cell embryos were isolated 17 to 18 h after hCG was injected into CD1 or B6SJL females 7 to 8 weeks old. Embryos were cultured in Whitten and Bigger's medium supplemented with 40 μM-EDTA and 2 $\mu g\,ml^{-1}$ of aphidicolin. 2-cell embryos were isolated 40 to 42 h post-hCG and cultured in Whitten and Bigger's medium plus EDTA.

Groups of 100 to 150 embryos at a time were injected with 2 pl of DNA sample into one of their nuclei using the same needle and an automated injection system (Eppendorf microinjector 5242). The amount of DNA injected was determined as previously described (Martinez-Salas *et al.* 1988). Surviving oocytes were cultured for 24 h, and surviving 1-cell and 2-cell embryos for 42 h before measuring luciferase or β-galactosidase activity in individual oocytes and embryos.

Transfection of promoter – luciferase constructs into cultured cell lines

Mouse NIH/3T3 and human 293 cells grown in Dulbecco's modified Eagle's Medium (DMEM) and 10 % fetal calf serum on 60 mm dishes to 40–60 % confluency were transfected with plasmid DNA using the calcium phosphate technique (Wigler *et al.* 1978). At 6 to 18 h after addition of DNA, the cells were incubated in 20 % glycerol in DMEM for 2 min at 37 °C. This solution was removed and fresh medium was added. At 48 h after addition of DNA, the cells were rinsed twice with cold 20 mM-Tris pH 7.5, 137 mM-NaCl, 5 mM-KCl, 1 mM-Na_2HPO_4 buffer, then scraped into 1 ml of cold 100 mM-potassium phosphate (pH 7.8), 1 mM-dithiothreitol followed by a 2 min centrifugation at 4000 *g* at 4 °C. The pellet was resuspended in 100 μl of the same buffer and immediately analyzed for luciferase activity or frozen and stored at −70 °C.

Staining cells for β-galactosidase activity

Oocytes and embryos were harvested at 24 to 72 h post-injection and rinsed through six 100 μl drops of PBS+0.4 % PVP-40 under oil. This was followed by a 20 min fixation in cold 2 % formaldehyde, 0.2 % glutaraldehyde, 0.4 % PVP-40 in 15 mM-$NaPO_4$ (pH 7.4), 150 mM-NaCl (phosphate-buffered saline; PBS). They were rinsed as before, then transferred to 0.5 ml of reaction mix containing 5 mM-potassium ferrocyanide, 5 mM-potassium ferricyanide, 25 mM-$MgCl_2$, 2 $mg\,ml^{-1}$ X-gal (Bethesda Research Labs) in PBS. The fixed embryos were incubated in the dark for 16 to 24 h at 37 °C in a humidified chamber to prevent evaporation (Sanes *et al.* 1986).

Luciferase assay

Individual embryos were harvested in 50 μl of reaction mix (25 mM-glycylglycine (pH 7.8), 15 mM-$MgSO_4$, 5 mM-ATP (pH 7), 100 $\mu g\,ml^{-1}$ BSA, 1 mM-dithiothreitol), subjected to three cycles of freeze-thawing (−70 °C to 37 °C) and then assayed in the presence of 1 mM-luciferin. Light emission from extracts of microinjected embryos or transfected cells were integrated for the initial 10 s of emission at 25 °C in a luminometer (Monolight 2001, Analytical Luminiscence). Each group of assays was standardized using aliquots of purified luciferase (Sigma) stored at −70 °C, and light unit data were corrected for small variations from one experiment to the next (1506±46 light units/pg luciferase). This assay was linear from 10 fg to 200 pg of luciferase per assay.

c-myc immuno-blotting analysis

Differentiated cells (2.4×10^6 HL60 and 4.4×10^6 NIH/3T3) were washed twice in cold PBS by centrifuging for 5 min at 300 *g* (4 °C) and discarding the supernatants. Laemmli sample buffer was added to the pellet to give a final concentration of 2×10^4 cells μl^{-1}. Since the cell suspension was too viscous to pipette, it was sonicated for 60 s while bathed in cool water and then incubated for 5 min at 100 °C. Oocytes and embryos were rinsed twice in PBS containing 0.4 % PVP-40. Embryos were collected in this buffer (100 embryos/5 μl) and trans-

ferred to a 1.5 ml centrifuge tube containing an equal volume of 2X Laemmli sample buffer. These were then incubated for 5 min at 100°C. Lysates were fractionated by electrophoresis in a 10% polyacrylamide gel containing SDS. Polypeptides were electrophoretically transferred onto a nitrocellulose membrane in a buffer containing 12.5 mM-Tris (pH 8.3), 96 mM-glycine, 20% methanol, and 0.01% SDS. The membrane was incubated for 90 min at 37°C in PBS containing 5% non-fat milk, followed by 1 h at room temperature in 10 $\mu g\,ml^{-1}$ of affinity-purified rabbit-anti-c-myc (Miyamoto *et al.* 1985) diluted in PBS containing 5% non-fat milk and 0.05% Tween 20. This was followed by six 5 min washes in PBS and 0.05% Tween 20, and then a 1 h incubation in 0.2 $\mu Ci\,ml^{-1}$ ^{125}I-Protein-A diluted as above. The membrane was then washed eight times for 5 min in PBS and 0.05% Tween 20, air-dried and subjected to autoradiography using Kodak X-Omat AR film and a Dupont Cronex Lightning Plus intensifying screen.

*Transfection of pEII–*CAT *into differentiated cell lines and CAT assays*

Approximately 30–50% confluent 9 cm plates of HeLa, NIH/3T3, BHK hprt−, Rat 1, and Ltk- cell lines, grown in DMEM+10% fetal calf serum, were transfected by the calcium phosphate technique of Graham and Van der Eb (1973). These cells received precipitates that contained 3 μg pEII-*CAT* or pEII65-*CAT* with or without 5 μg of the Ad5 E1A-expressing plasmid, pCE. About 16–24 h later the transfected cells were shocked with 20% glycerol in PBS for approximately 2–3 min, followed by a PBS rinse, and were then refed with DMEM+10% FCS. Two days later these samples were harvested by scraping in 0.25 M-Tris, pH 8.0 and either freeze thawing or mildly sonicating. Soluble extracts were obtained by centrifugation and CAT assays were performed using 100 μl of extract, 2.5 μl of 33.3 $mg\,ml^{-1}$ acetyl CoA (Sigma), and 1.5 μl of [^{14}C]chloramphenicol (Amersham; 53 $mCi\,mmol^{-1}$, 0.2 $\mu Ci\,\mu l^{-1}$) for 3 h at 37°C. Samples were extracted in ethyl acetate, and thin layer chromatograms (TLC) were resolved in 95% chloroform:5% methanol. The TLCs were exposed to Kodak XAR film overnight.

*Generation of E1165–*CAT *transgenic mice*

The no. 42-5 transgenic mouse line was produced by microinjection of C57/CBA fertilized egg pronuclei (obtained at day 0.5 post-coitus) with the 1.95 kb *Xho*I–*Xba*I pEIII65–*CAT* fragment at a concentration of 2 $\mu g\,ml^{-1}$. Six offspring were obtained from surrogate mothers that received microinjected 1-cell embryos. DNA samples were prepared from 1 cm tail biopsies of these offspring, by mincing in 0.7 ml 50 mM-Tris pH 8.0, 100 mM-EDTA, 0.5% SDS, 35 μl 10 $mg\,ml^{-1}$ proteinase K and incubating at 55°C overnight. These samples were phenol/chloroform-extracted, ethanol/sodium acetate-precipitated, and resuspended in 100 μl Tris–EDTA buffer at 65°C. *Bgl*II restriction digests of 16 μl samples were electrophoresed on 1% agarose gels in Tris/borate/EDTA gels. Then southern blots were performed using nitrocellulose and a random-primed CAT-specific probe, which was ^{32}P-labelled. The southern blots (and subsequent DNA slot blots) confirmed that the offspring of the transgenic mouse no. 42-5 (female) contained the transgene at low copy number (data not shown). This transgenic line was then propagated in a heterozygous state, by mating with non-transgenic C57/CBA mice.

CAT assays on transgenic tissues

Adult and embryonic tissues from transgenic mice were examined for expression by CAT assays. Freshly dissected tissues isolated from numerous transgenic offspring were sonicated in cold 0.25 M-Tris pH 8.0, heated at 65°C for 5 min (to inactivate potential deacetylases), centrifuged to collect soluble extracts, and protein concentrations were determined using the Bradford protein assay (Biorad). Then 200 μg of each sample was subjected to the CAT assay using 100 μl total of extract+Tris buffer, 2.5 μl 33.3 $mg\,ml^{-1}$ acetyl CoA, 1.5 μl [^{14}C]chloramphenicol (Amersham; 54 $mCi\,mmol^{-1}$, 0.2 $\mu Ci\,\mu l^{-1}$), and to incubation at 37°C for 90 min. Purified CAT (Boehringer) was used as a positive control in these assays. Ethyl acetate-extracted samples were run on TLC plates in 95% chloroform: 5% methanol and visualized by autoradiography. For the embryo assays, pooled embryo extracts (200 μg and 1000 μg) contained 12, 10, and 6 embryos from 5.5, 7.5, and 9.5 days, respectively.

Immunohistochemical localization of CAT in ovaries

The ovary from a transgenic adult mouse was formalin-fixed, paraffin-embedded, sectioned, and dewaxed in xylene/ethanol. The mouse monoclonal antibody, CAT1, raised against purified chloramphenicol acetyltransferase (gift from J. Gannon and D. Lane) was used as the primary antibody at dilutions ranging from 1:1 to 1:5000 in PBS containing 5% FCS. A secondary goat anti-mouse antibody that was conjugated to colloidal gold was applied and subsequently silver-enhanced (Jannsen). Following a light toluidine blue staining, coverslips were mounted, and the sections were photographed with an Olympus BH-2 compound light microscope system. Two primary antibody controls (PBS alone and mouse anti-actin) were included for comparison. The PBS control yielded no staining, whereas abundant staining was obtained throughout the ovary section with anti-actin antibody.

Results

Expression of EIIa-promoter in mouse oocytes and preimplantation embryos

The ability of mouse oocytes and preimplantation embryos to express the Ad5 EIIa promoter was revealed by injecting plasmids that express the *lacZ* gene when fused to the EIIa promoter. Supercoiled plasmid DNA was injected into the oocyte germinal vesicle, the male pronucleus of 1-cell embryos, and one of the zygotic nuclei in 2-cell embryos. The amount of DNA injected per nucleus and the time of incubation in culture after injection were those previously shown to give the optimum levels of gene expression using the HSV thymidine kinase (tk) promoter (Martinez-Salas *et al.* 1989). DNA injected into the nucleus was stable for at least 3 days, while DNA injected into the cytoplasm was rapidly degraded (Wirak *et al.* 1985; Martinez-Salas *et al.* 1988). Maturation of oocytes was prevented by culturing them in dbcAMP (Chalifour *et al.* 1986, 1987), and 1-cell embryos were prevented from entering S phase by incubation with aphidicolin (Martinez-Salas *et al.* 1989). These embryos retained their two pronuclei and did not cleave into 2-cell embryos. Injected 2-cell embryos continued development to the morula and blastocyst stages. Previous studies using these conditions revealed that promoters such as the tk-promoter respond to the same controls that regulate endogenous gene expression during pre-

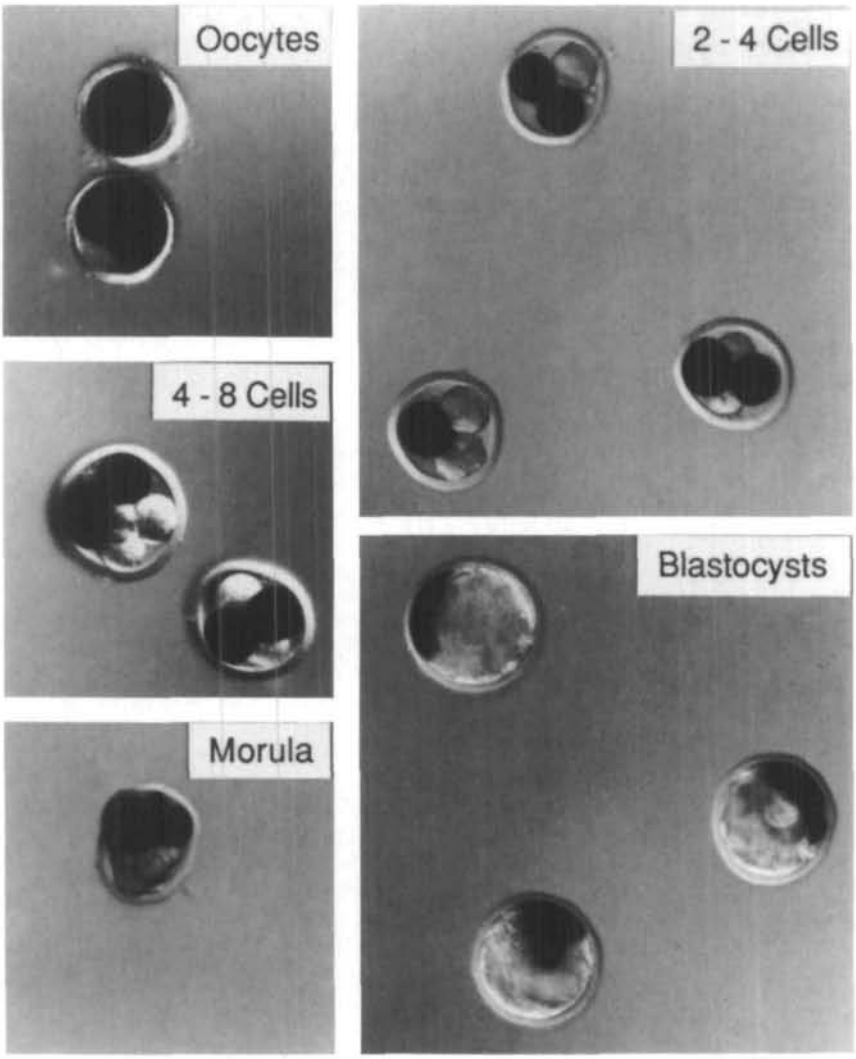

Fig. 1. Expression of pEII–*lacZ* in microinjected oocytes and early embryos. β-galactosidase-positive cells exhibit dark X-gal staining (refer to Materials and methods for staining protocol).

implantation development, and that the level of gene expression and the need for enhancers to activate promoters or origins of DNA replication depends upon the type of nucleus in which the DNA exists (Martinez-Salas *et al.* 1988, 1989).

Oocytes and embryos were stained for β-galactosidase activity at various times post-injection. The results revealed that the EIIa promoter was very active at all stages of preimplantation development from the oocyte to the blastocyst (Fig. 1). No β-galactosidase activity was detected under these conditions when the same plasmid without a promoter was injected. Furthermore, β-galactosidase activity was restricted to those cells receiving the injected gene and their progeny; in 2 to 8 cell embryos where individual blastomeres were readily identified, only half were stained. In all of the blastocysts derived from injected 2-cell embryos, staining was restricted to one portion of the embryo consistent with pEII-*lacZ* expression in the inner cell mass but not in the trophectoderm. Attempts to produce blastocysts from either 2-cell embryos injected in both nuclei or from injected 1-cell embryos that were allowed to develop in the absence of aphidicolin were unsuccessful. Therefore, we could not conclusively demonstrate that this restricted pattern of pEII-*lacZ* expression was cell type-specific.

The levels of EIIa-promoter activity in oocytes and preimplantation embryos were quantified by injecting a plasmid in which the EIIa promoter was fused to the firefly luciferase gene. This assay allowed quantitative measurements of luciferase activity in individual oocytes and embryos (Martinez-Salas *et al.* 1989). Analogous data from Martinez-Salas *et al.* (1989) using the HSV tk-promoter was included for comparison. The HSV tk-promoter represents a promoter that does not respond to E1A, but does respond to enhancers and has been well characterized in a variety of cell lines as well as mouse and *Xenopus* oocytes and embryos (Weeks and Jones, 1985; Martinez-Salas, 1988; McKnight and Kingsbury, 1982).

At least 90% of the oocytes and embryos injected with pEII–*luc* produced levels of luciferase substantially greater than the level observed with the same expression vector without a promoter (Fig. 2A and B). In general, EIIa promoter activity paralleled that of the tk-promoter; it was lowest in oocytes and highest in 1-cell embryos arrested in S phase. In oocytes and developing 2-cell embryos, the EIIa-promoter was

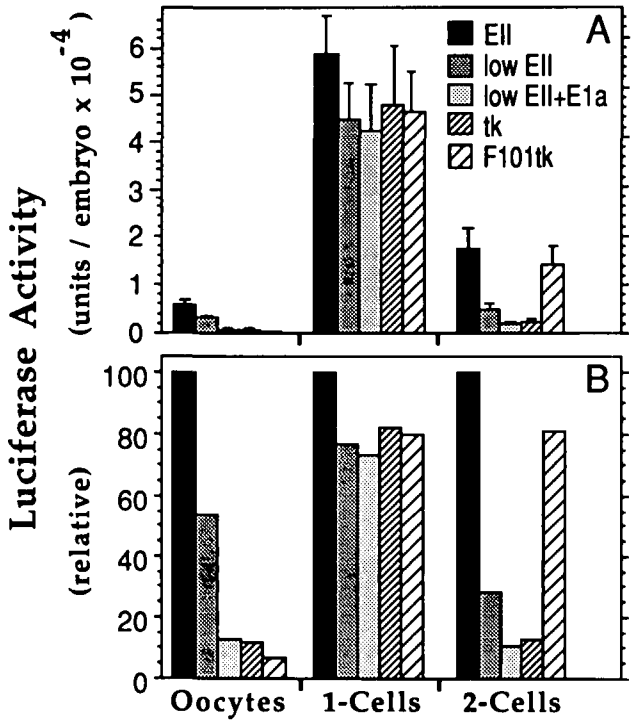

Fig. 2. Activity of adenovirus *EIIa–luciferase* fusion gene in mouse oocytes and preimplantation embryos. Plasmid DNA was injected into the germinal vesicle (GV) of mouse oocytes, the male pronucleus (MP) of 1-cell embryos, and one of the zygotic nuclei (ZN) of 2-cell embryos. Surviving ova were cultured as described in Materials and methods, lysed and then luciferase activity measured in individual ova. (A) 'EII' represents injection of 1 pg pEIIa–*luc*/GV, 0.6 pg/MP and 0.6 pg/ZN. 'Low EII' represents injection of 0.5 pg pEIIa–*luc*/GV, 0.2 pg/MP, and 0.3 pg/ZN. 'Low EII+E1a' represents the same experiment as 'low EII' but with an equal amount of pCE. The standard error of the mean (error bars) for the groups of oocytes and embryos varied from 15 % to 25 % of the mean. 'tk' and 'F101tk' represent the same experiment as 'EII', but using ptk–*luc* and pF101tk–*luc*. The promoterless control plasmid, pluc, yielded activities from 0.03 to 0.05×10^{-4} luciferase units per oocyte or embryo. These data were reproduced from Martinez-Salas *et al.* (1989). (B) the 'EII' level of luciferase activity in each group of oocytes and embryos was defined as 100 % and the other data in that group were expressed relative to this value.

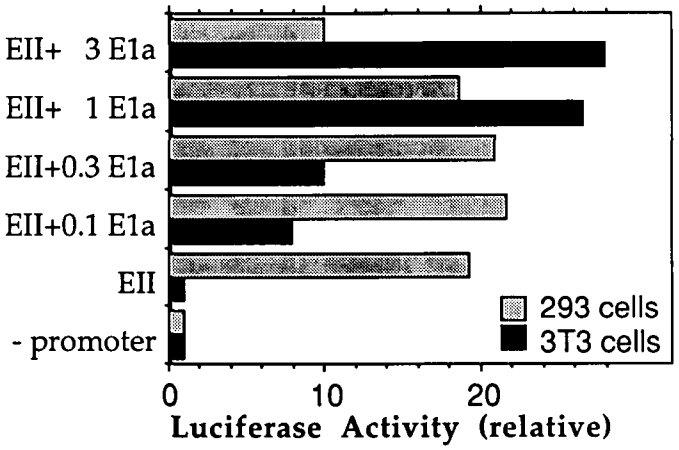

Fig. 3. Activity of the EIIa–*luciferase* gene in mouse NIH/3T3 cells and human 293 cells. 3T3 and HeLa cells were transfected with pEIIa–*luc*±pE1a (pCE) at the molar ratios indicated (Materials and methods). Levels of luciferase activity are presented relative to the control plasmid, pluc, lacking the EIIa promoter within the same cell type. Note that direct comparisons of actual levels of expression cannot be made from this relative data, because the actual expression levels of pEIIa–*luc* are significantly higher in 293 cells than 3T3 cells.

8-fold more active than the tk-promoter. In 2-cell embryos, the polyomavirus (PyV) F101 enhancer stimulated tk-promoter activity to a level comparable to that of the EIIa-promoter alone. However, in oocytes, this enhancer further reduced tk-activity 2-fold. The PyV enhancer mutation F101, which was selected for its ability to support virus growth in mouse embryonal carcinoma F9 cells, was particularly effective in stimulating tk-promoter activity in developing 2-cell embryos (Martinez-Salas *et al.* 1989). Therefore, this mutation is perhaps recognized by a novel enhancer-activating protein expressed from 2-cell embryos through to the inner cell mass, which compensates for the presumed repressive effects of the 'E1A-like' environment of undifferentiated cells. And in 1-cell embryos, EIIa promoter activity was equivalent to tk-promoter activity with or without an enhancer (Fig. 2). Previous studies have shown that replication origins and promoters that normally require an enhancer for activity in mouse differentiated cells function efficiently without an enhancer when present in the pronuclei of 1-cell embryos arrested in S phase (Martinez-Salas *et al.* 1988, 1989). Thus, in all three developmental stages (oocytes, 1-cell embryos and 2-cell embryos) the EIIa promoter activity was equal to or greater than the activity of the reference tk promoter either with or without an enhancer element.

Coinjection of lower amounts of pEII–*luc* with an equal amount of pCE, a plasmid encoding the Ad5 E1A gene, did not stimulate EIIa-promoter activity, but, in fact, reduced luciferase expression about 3-fold in oocytes and developing 2-cell embryos (Fig. 2B). Since cotransfection of pEII–*luc* with pCE stimulated luciferase expression in mouse 3T3 cells up to 28-fold (Fig. 3), the EIIa-promoter was already fully active in mouse oocytes and early embryos and could not be superstimulated by E1A. Furthermore, the response of mouse oocytes and early embryos to over expression of E1A protein was similar to 293 cells, a transformed human cell line that expresses Ad5 E1A from an integrated viral genome (Fig. 3). All three cell types express the EIIa promoter at full activity and all three cell types reduced the level of expression in the presence of additional E1A, for some yet unknown reason.

pEII–65 CAT *transgenic mice*

Having established that EIIa promoter activity was high in the undifferentiated stages of oogenesis and early embryogenesis, we then created a transgenic mouse line to further characterize the pattern of EIIa expression.

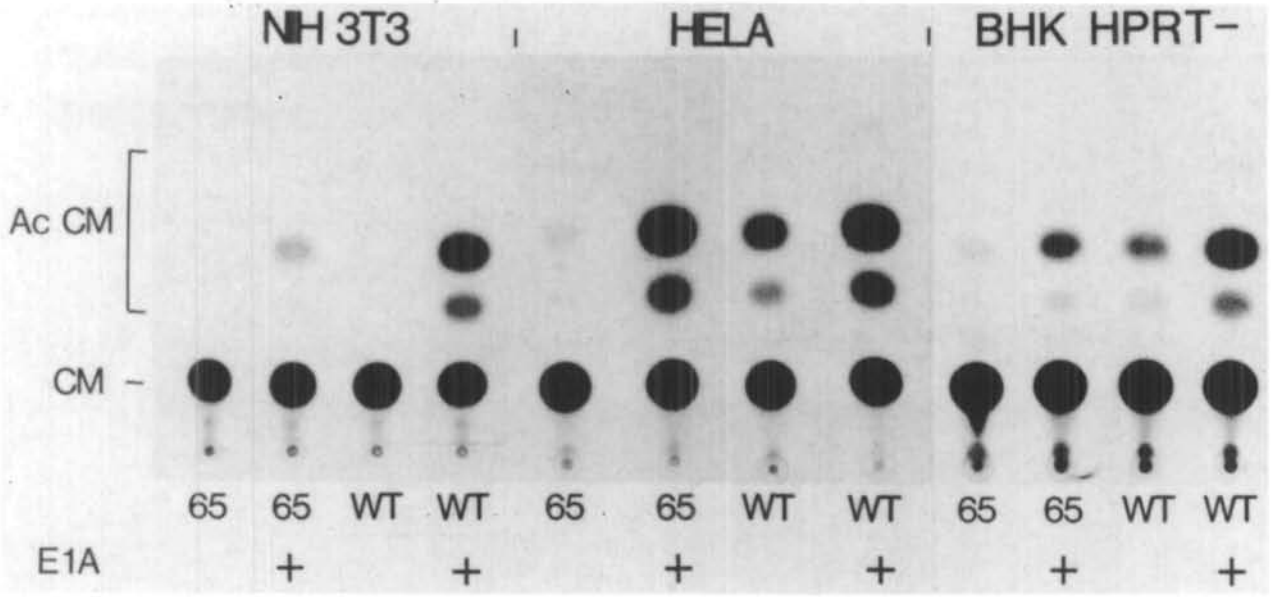

Fig. 4. CAT assays on transfected differentiated cell lines. Calcium phosphate transient transfections of pEIIa–*CAT* (WT) or pEII65–*CAT* (65) either with (+) or without the Ad5 E1A-expressing plasmid, pCE, were analyzed by CAT assays. NIH 3T3, HeLa, and BHK hprt− represent three of the differentiated mammalian cell lines which were transfected. The positions of the unmodified and acetylated forms, of chloramphenicol on the TLC are indicated by 'CM' and 'Ac CM', respectively.

In order to demonstrate the extent to which the EIIa-reporter gene used in the following transgenic studies could be transactivated by E1A, a variety of differentiated cell lines were transfected with pEII–*CAT* either in the presence or absence of an E1A-expressing plasmid. Transient expression of the pEII–*CAT* gene in transfected cell lines, derived from four different species, was assessed by CAT assays (NIH/3T3, Hela, and BHK hprt−, in Fig. 4; Ltk- and Rat 1, data not shown). This assay monitors the enzymatic conversion of [^{14}C]chloramphenicol to its acetylated forms. Both the wild type and -65/-75 linker-scanning mutant EIIa promoters (WT and 65, respectively) were transcribed in all cell lines at low basal rates in the absence of an E1A-expression plasmid (Fig. 4). However, the level of EIIa expression was elevated dramatically in the presence of E1A, consistent with previously reported results. The linker-scanning mutant form of this promoter was also E1A-inducible, albeit both the basal and induced levels of transcription in several cell types (NIH 3T3, Hela, and BHK hprt−) were less than those obtained with the wild-type promoter. pEII–*CAT* gene expression was also observed in stably-transformed PCC4 undifferentiated EC cells, but was drastically reduced upon differentiation (data not shown).

To determine the tissue specificity of an EIIa promoter activity, a transgenic mouse line was established by microinjecting a pEII65–*CAT* restriction fragment into fertilized mouse eggs. This promoter was chosen because it exhibited a lower basal level of transcription in the absence of E1A in some cell types, yet was still transactivated by E1A (Murthy *et al.* 1985; see pEII65–*CAT* data above). Thus the EII65 promoter should be a sensitive tool for identifying the primary sites of high-level transactivation. By microinjection, we obtained a transgenic line of mice (derived from the no. 42-5 female) that contained this reporter gene integrated at low copy number (data not shown). The pEII65–*CAT* transgenic line transmitted this allelle as a single autosomal 'dominant' trait lacking any noticeable teratogenic or dysplastic abnormalities.

Soluble extracts from various transgenic adult somatic tissues were subjected to CAT assays (see Fig. 5A). The transgenic line did not express CAT in any of the adult somatic tissues that were analyzed. The tissues chosen for examination contained a large variety of endo-, ecto-, and mesodermally-derived cell types and yet none of these somatic tissues was positive in the CAT assays. These results suggest that this promoter was transcribed at non-detectable levels under the conditions chosen, in non-germ line tissues. Of more significant interest was the observation that all (8/8) females were CAT-positive in this assay in ovarian tissue (Fig. 5B). The amount of CAT activity in the ovaries was estimated to be at least 20× higher than in the 'non-expressing' somatic tissues of the same mice. This activity was not due to a natural endogenous CAT-like activity in non-transgenics, since CAT activity was present only in the transgenic females. The absence of this activity in the testis of male transgenics (see Fig. 5B) demonstrates that this ability to form active transcription complexes was not germ line-specific, but rather ovary-specific. Transactivation of this EII-reporter gene in adult ovaries indicates that at least one type of cell within the ovary is capable of making active transcription complexes, presumably *via* the *cis*-acting transcription factor binding sites within this E1A-activitable promoter. Alternatively, the site of inte-

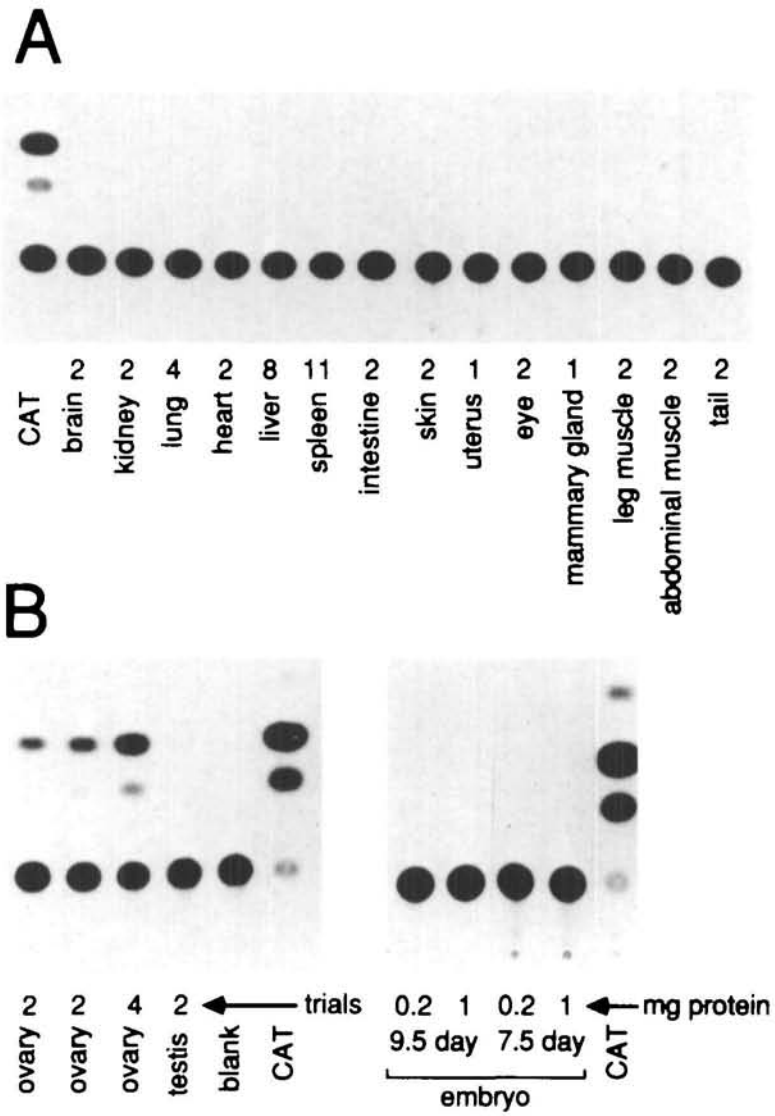

Fig. 5. CAT assays on transgenic mice tissues. CAT assays were performed on 200 μg of soluble protein from individual EII65–*CAT* transgenic mice tissues. (A) Lack of activity in somatic tissues: lane 1, 0.05 units CAT (Boehringer); lane 2, brain; lane 3, kidney; lane 4, lung; lane 5, heart; lane 6, liver; lane 7, spleen; lane 8, intestine; lane 9, skin; lane 10, uterus; lane 11, eye; lane 12, mammary gland; lane 13, leg muscle; lane 14, abdominal muscle; lane 15, tail. (B) Germ-line and embryonic tissues: lanes 1–3, ovaries from three different adults; lane 4, testis; lane 5, no protein-negative control; lane 6, 0.15 units CAT; lanes 7 and 8, pooled 9.5-day embryo extracts at 200 μg and 1 mg total protein, respectively; lanes 9 and 10, pooled 7.5-day embryo extracts at 200 μg and 1 mg total protein, respectively; lane 11, 0.2 units CAT. The numbers adjacent to each lane refers to the number of times that result was obtained from different transgenic offspring.

gration of this transgene might cause it to be aberrantly hormonally-activated and thus expressed only in the female ovaries. This alternative appears unlikely, because the phenotype of these transgenic mice was obtained by screening rather than direct phenotypic selection. Pedigree analysis of this transgenic line revealed that the pattern of pEII65–*CAT* expression was identical, regardless of the sex of the transgenic parent mouse. Thus, the ovaries from offspring of both transgenic males or females were CAT-positive, demonstrating that transmission of the transgene *via* the 'CAT-negative' testis of the male parent does not irreversibly inactivate this transgene. These results indicate that only the adult ovary contains the necessary 'activated' transcription factors to mediate the transactivation of an E1A-dependent promoter.

The fact that the EIIa promoter appeared to be fully active when injected into mouse oocytes suggested that the expression of CAT in the transgenic ovaries was due primarily to transactivation of the transgene in developing oocytes. To test this hypothesis, histological sections of transgenic ovaries were treated with an anti-CAT antibody preparation and were visualized by silver enhancement of immunogold-bound complexes (Fig. 6A). The specific staining of oocytes was due to the primary anti-CAT antibody, as controls lacking a primary antibody exhibited no staining (Fig. 6B), and an anti-actin primary antibody demonstrated strong immunostaining throughout the ovary sections (data not shown). Significantly lower levels of diffuse non-nuclear, cytoplasmic staining was also seen in some corpus lutea (data not shown). The presence of immuno-reactive cytoplasmic CAT enzyme in the oocytes of adult female ovaries confirmed our hypothesis that the pEII65–*CAT* transgene was expressed primarily in the oocytes.

To determine whether or not CAT activity was present in the post-implantation embryos as well as oocytes, embryos at days 5.5, 7.5, and 9.5 post-coitus from female heterozygous transgenics were examined. Presuming that only 50 % of the embryos contained the transgene, a large number of embryos were pooled to prepare mixed extracts for CAT assays. These extracts exhibited no CAT activity even when 5-fold higher than

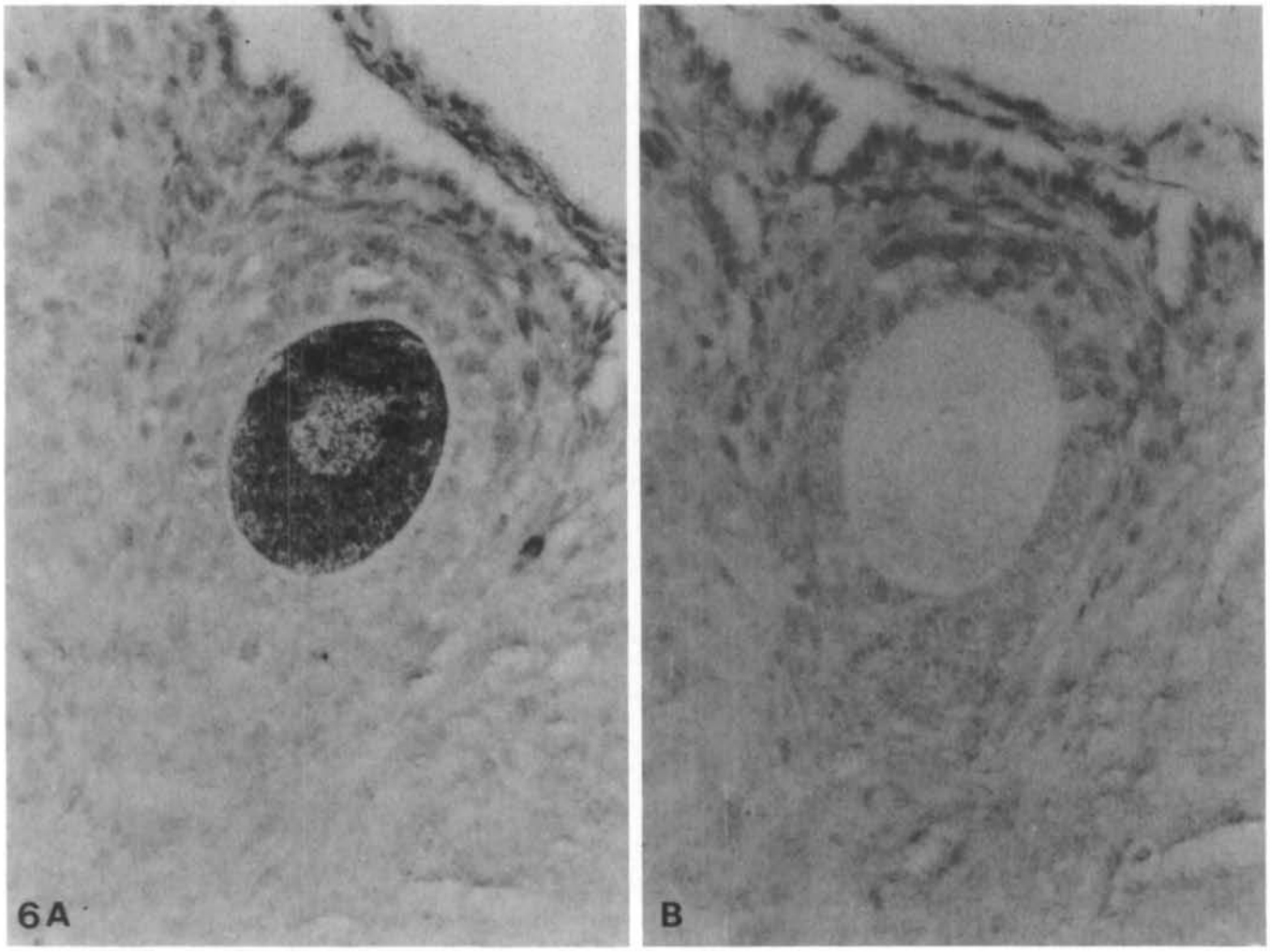

Fig. 6. Immunohistochemical detection of CAT in transgenic ovary. Photomicrographs are presented of sections (488×magnification) from the ovary of a transgenic female that were treated with (A) mouse anti-CAT antibody, CAT 1, or (B) no primary antibody. Samples were visualized by binding with gold conjugated goat anti-mouse IgG, and silver enhancement. Note the localization of the anti-CAT antibody within the cytoplasm of the developing oocytes (A).

normal amounts of extract were used (Fig. 5B; day 5.5, data not shown). This result is consistent with the findings of Suemori *et al.* (1988). By infecting *E1A*-deficient adenovirus into embryos and scoring the transactivation of EIIa, they also found evidence of an E1A-like activity that was restricted to preimplantation embryos, i.e. no activity was found after day 4.5.

Expression of c-myc during oogenesis and embryogenesis

Onclercq *et al.* (1988) have reported that c-myc protein, like E1A protein, is capable of transactivating the adenovirus EIV and EIIa promoters in HeLa and mouse EC cells. If c-myc were present at sufficiently high levels in mouse oocytes and embryos, it might play a role in the transactivation of E1A-inducible promoters. Therefore, the steady-state levels of c-myc in mouse oocytes, embryos and differentiated cell lines were determined by Western blot analysis (Fig. 7). Mouse oocytes contained 150 to 1000 pg of c-myc, depending on their age. Since mouse oocytes consist of 24 ng of protein, this was equivalent to 0.6 % to 4 % of total oocyte protein. The levels of c-myc in mouse preimplantation embryos were reduced by 3 to 20-fold relative to mouse oocytes. In comparison, cell lines such as HL60 that have amplified the *c-myc* gene contained only about 1.1 pg of c-myc per cell, and differentiated cells with a single copy of the *c-myc* gene such as mouse 3T3 fibroblasts contained only 0.03 pg/cell of c-myc. Since the level of c-myc mRNA in 3T3 cells is equal to or greater than the level of c-myc RNA found in a wide variety of tissues from newborn and adult mice (Zimmerman *et al.* 1986), the levels of c-myc protein in mouse embryos and oocytes are from 3000 to 30 000-fold higher per cell (or approximately 75-fold higher in concentration) than most mouse differentiated cells. Similar high levels of c-myc expression in *Xenopus* oocytes have been reported (Taylor *et al.* 1986; Godeau *et al.* 1986; King *et al.* 1986; Hourdry *et al.* 1988). The correlation between high c-myc levels, expression of the EIIa promoter, and the report that c-myc can substitute for E1A to transactivate EIIa

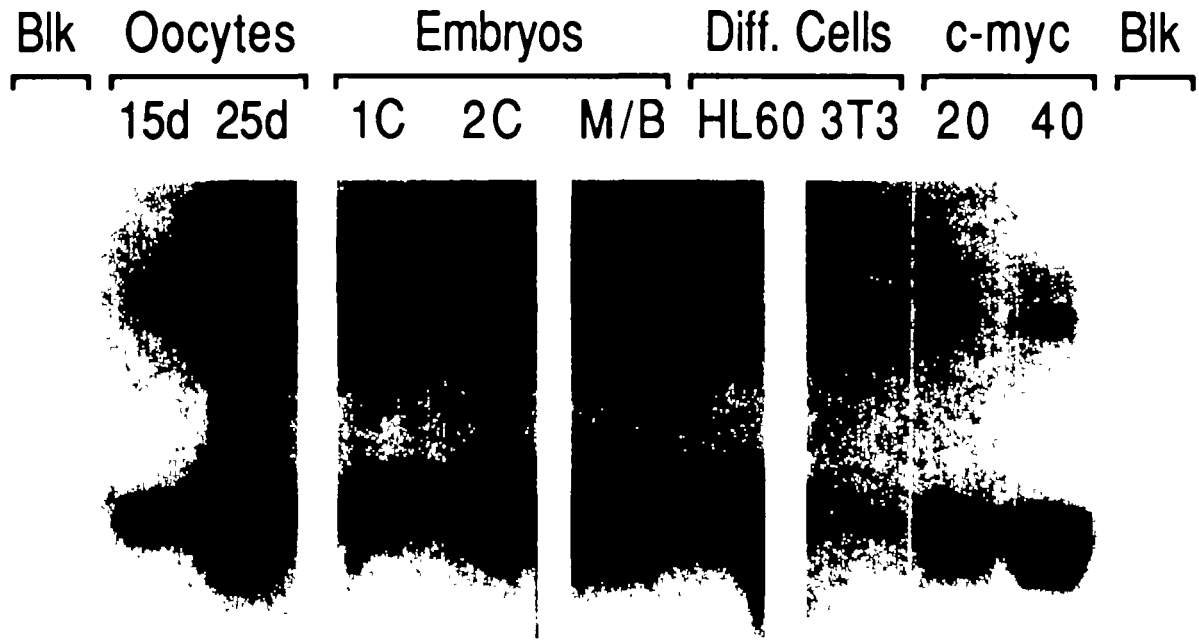

Fig. 7. Detection of c-myc protein in extracts from mouse oocytes, embryos and differentiated cells. Oocytes (100 per lane) were isolated from CD-1 females at 15 and 25 days after birth. Embryos were prepared from CD-1 females 6–8 weeks old: 1-cell embryos (1C, 20 per lane), 2-cell embryos (2C, 60 per lane), and morula and blastocysts (M/B, 60 per lane). Extracts were also prepared from mouse NIH/3T3 cells (3T3, $\sim 5 \times 10^4$ per lane) and human HL60 cells containing amplified copies of the *c-myc* gene (HL60, $\sim 1 \times 10^4$ per lane). Proteins were fractionated by gel electrophoresis, transferred to a nitrocellulose membrane, incubated with affinity-purified rabbit anti-c-myc antibody, and reacted with ^{125}I-protein-A. Blank reactions without cell extract (Blk) and standard aliquots of 20 ng and 40 ng of purified c-myc protein (Watt *et al.* 1985) were included as controls. The amounts of c-myc was calculated from these and similar data at 150 pg/15-day old oocyte (15d), $\geq$1000 pg/25-day old oocyte (25d), 300 pg/one-cell embryo (1c), 100 pg/two-cell embryo (2c), 50 pg/early morula, 300 pg/late morula and blastocyst (M/B). HL60 cells contained about 1.1 pg/cell and NIH/3T3 cells contained about 0.03 pg/cell. (Diff., differentiated).

(Onclereq *et al.* 1988), suggests that c-myc might account for the efficient use of this promoter during mammalian oogenesis and embryogenesis.

Discussion

The data presented above clearly demonstrate that the adenovirus EIIa early promoter is transcriptionally activated in mouse oocytes and preimplantation embryos. These findings were confirmed by different approaches: microinjection of EIIa promoter–reporter genes into ova and cleavage-stage embryos and construction of transgenic mice containing an integrated EII promoter–reporter gene (summarized in Table 1.) Microinjection of pEII–*lacZ* or pEII–*luciferase* revealed high levels of gene expression in all of the early cleavage stages of preimplantation embryos. Our results are in agreement with the findings of Suemori *et al.* (1988), who also demonstrated the presence of EIIa transactivation in 2.5- to 4.5-day-old mouse embryos following infection by an E1A-defective adenovirus. We have extended these studies to include mouse oocytes, fertilized ova, and early cleavage-stage embryos. The presence of the oocyte-specific activity was confirmed in the ovaries of adult female transgenics. The ability to transactivate EIIa was lost during the generation of post-implantation embryonic and adult

Table 1. *Summary of various cell types which are capable of EIIa transactivation*

Cell type	Transactivation	References
Differentiated non-EC	–	1; this work
Differentiated non-EC+E1A	+	1; this work
Undifferentiated EC	+	2
Differentiated EC	–	2
Murine oocytes	+	This work
Murine 1-cell embryos	+	This work
Murine 2-cell embryos	+	This work
Murine 4–8 cell embryos	+	This work
Murine morula embryos	+	This work
Murine blastocyst embryos	+	This work
Murine day 2.5–4.5 embryos	+	3
Murine day 5.5 embryos	–	3; this work
Murine day 7.5 embryos	–	3; this work
Murine day 9.5 embryos	–	This work
Murine adult somatic tissues	–	This work
Murine adult testis	–	This work
Murine adult ovary	+	This work

(1) Numerous reports have demonstrated the transactivation of EIIa by E1A in various differentiated cell lines (Jones & Shenk, 1979; Murthy *et al.* 1985; Strair *et al.* 1988; for review on transactivation see Jones *et al.* 1988). (2) Transactivation of EIIa in EC cells by an E1A-like activity (Imperiale *et al.* 1984). (3) Suemori *et al.* 1988.

somatic tissues. This lack of expression in somatic tissues of transgenic mice further strengthens our hypothesis that this activity is a marker of the undifferentiated state during murine development, i.e. the presence of this marker is restricted to the relatively 'undifferentiated' state of murine oogenesis and early embryogenesis.

Our data also correlate well with the reported behavior of the EIIa promoter in undifferentiated EC cells. Undifferentiated embryonal carcinoma cell lines express EIIa in the absence of E1A, suggesting that they may contain an endogenous factor which substitutes for E1A (Imperiale *et al.* 1984). The identity of the E1A-like activity in EC cells is currently unknown. Our microinjection and transgenic data lead us to believe that 'normal' undifferentiated oocytes and preimplantation embryos of mice also contain an 'E1A-like' environment resembling that found in undifferentiated EC cells. Thus, EC cells are not unique in their ability to transactivate the EIIa promoter, because mouse oocytes to blastocyst also exhibit this property. Blastocysts are composed of two cell types; the totipotent inner cell mass that gives rise to the embryo, and the differentiated trophectoderm that gives rise to the placenta. Mouse EC cell lines share many characteristics with primitive endoderm that arises from the inner cell mass during the late stage of blastocyst development (Hogan *et al.* 1983). Therefore, it is not surprising that 'E1a-like' activity appears to be restricted to undifferentiated cells such as the inner cell mass, since it is only found in EC cell lines prior to their differentiation. This conclusion is further supported by the fact that EIIa activity was barely detectable in expanded or hatched blastocysts (4.5-day embryos; Suemori *et al.* 1988) and undetectable in extracts of 5.5-, 7.5- and 9.5-day embryos (Fig. 5B).

What might the role of this transactivation potential be in undifferentiated cells during oogenesis and embryogenesis? It seems quite likely that this activity may be responsible for the transcriptional regulation of various cellular genes. E1A has been shown to be capable of transcriptionally regulating various endogenous genes, such as *heat shock*, *globin*, *MHCI*, and others (Imperial *et al.* 1984; Kingston *et al.* 1985). The strikingly high degree of similarity of the human brain creatine kinase promoter to the EIIa promoter suggests that both E1A and the E1A-like activity could transactivate this promoter and possibly others which share similar transcription factor binding sites (Daouk *et al.* 1988). Thus, the endogenous E1A-like activity we are monitoring during oogenesis and embryogenesis might be responsible for transcriptional regulation of numerous cellular genes. This activity may even possibly act as a developmental switch mechanism for determining the expression of a larger set of cellular genes, i.e. the loss of this activity during early development might signal the switching 'off' of genes which are E1A-activatible and the switching 'on' by depression of genes which are normally repressed by this presumed transregulator (assuming this E1A-like activity contains both the transactivator and the repression functions of E1A). A report by Montano and Lane (1987) addressed whether over-expression of exogenous E1A in undifferentiated EC cells would block their ability to undergo differentiation. They found that exogenous E1A is *not* capable of inhibiting EC cell differentiation and, surprisingly, E1A actually induced differentiation in their hands. This intriguing result suggests that the endogenous cellular E1A-like activity does not 'hold' EC cells in their undifferentiated state, whereas one might have predicted that release from the undifferentiated state is accomplished by the loss of the cellular E1A-like activity. Their perplexing results do not address whether the E1A-like activity is dispensable in the undifferentiated state. Thus, the ability to transactivate EIIa in cells lacking E1A appears to be a marker for the undifferentiated state during early development. EIIa represents an example of a promoter that is differentially regulated as mammalian cells undergo differentiation. As such, this information might result in the identification of cellular genes which are regulated in a similar manner, and which may play a role in the processes of differentiation.

How might EIIa transactivation occur in undifferentiated cells?

Transactivation of the Ad5 EIIa early promoter by E1A has been extensively studied *in vitro* (Siva Raman *et al.* 1986; Kovesdi *et al.* 1986; Murthy *et al.* 1985; and references therein). Thus far, E1A has not been shown to bind directly at the DNA level to the EIIa promoter. Mutational and biochemical analysis of this promoter has delineated the positions of a number of transcription factor binding sites, including the sites for the E2F and ATF transcription factors (revised in Jones *et al.* 1988). Two E2F binding sites are located within the region from -32 to -71 of the transcriptional start site, adjacent to a single ATF site (Reichel *et al.* 1987; Siva Raman *et al.* 1986). These transcription factors are thought to play similar roles in transactivating the EIIa promoter in both E1A-containing differentiated cells and undifferentiated EC cells. Adenovirus infection of HeLa or differentiated EC cells results in increased binding of E2F to the EIIa promoter (Reichel *et al.* 1987; Kovesdi *et al.* 1986; Lathangue and Rigby, 1987). Furthermore, the binding of E2F to this promoter occurs in undifferentiated EC cells, whereas this factor declines upon differentiation. ATF levels, on the other hand, are not affected by E1A. The precise mechanism by which E1A acts either directly or indirectly on these and other factors is not yet known, although Lillie and Green (1989) have proposed that E1A might directly interact with some DNA-bound transcription factor(s) resulting in complex formation and thus transactivation. Alternatively, E1A might stimulate modification of these factor(s) resulting in an increase in the initiation of transcription.

What might the identity of the 'E1A-like' activity be in oocytes, embryos and undifferentiated EC cells? The mechanism by which EIIa transactivation occurs in undifferentiated cells is not currently known and it might be accomplished in the absence of E1A by one of

several different plausible mechanisms. Some evidence provided by Onclercq *et al.* (1988) suggests that overexpressed c-myc protein can transactivate both the EIIa and EIV promoters. Our observation that c-myc levels are very high in oocytes and embryos (in terms of absolute amounts and relative concentrations) would be consistent with this interpretation, provided that the c-myc protein is available at sufficient levels in an active state within the nucleus. However, this correlation between elevated c-myc levels and EIIa expression should not be interpreted as a verification that c-myc is actually an E1A-like analog in oocytes and embryos, although this remains a valid possibility.

In addition to c-myc, numerous other non-E1A viral gene products have been demonstrated to transactivate the adenovirus early gene products. The list includes pseudorabies IE, HSV ICP4, EBV early transactivator, human cytomegalovirus, SV40 small T, HTLV I transactivator and HPV E7 (Feldman *et al.* 1982; Tevethia and Spector, 1984; Tremblay *et al.* 1985; Wong and Levine, 1986; Workman *et al.* 1988; Loeken *et al.* 1988; Phelps *et al.* 1988). Possibly, a putative cellular homologue to one of these genes exists, and its product is responsible for the promiscuous transactivation of the EIIa promoter during oogenesis and embryogenesis. Alternatively, one might also speculate that undifferentiated cells synthesize or activate a transcription factor which is capable of interacting with the promoter to initiate transcription (i.e. E2F or ATF, etc.), in an E1A-independent manner. Currently, studies to address the roles of transcription factors are technically difficult to perform on such limited quantities of cells derived from oocytes or early stage embryos. And, finally, undifferentiated cells might regulate the EIIa promoter by yet another mechanism independent of an E1A analog. Through this possible mechanism the EIIa promoter is merely expressed at a constitutive uninduced level in undifferentiated cells. And this promoter is repressed in differentiated cells, although currently no evidence exists in favor of this 'constitutive *vs* repressed' model, and if it were true one would expect exogenous E1A to enhance expression of EIIa in oocytes and preimplantation embryos, and yet the opposite result occurred in our hands. These perplexing alternative interpretations of the possible mechanism of apparent EIIa transactivation have not been fully addressed to date. Therefore, at this stage there is no direct evidence showing that the increased expression of EIIa in undifferentiated cells is being accomplished by a cellular E1A analog, such as c-myc. However, if an E1A-like transactivation effector does exist, it may be possible to identify it by a genetic approach. To this end, we have created E1A-dependent cell lines, which become drug-resistant when expressing E1A. These lines could be used as potential screening 'traps' for the isolation of novel cDNA clones which encode the putative E1A-like activity from undifferentiated cells (unpublished results, Dooley and Jones).

We wish to acknowledge Vyviene Attenburrow for assistance in generating transgenic mice, Sadhan Majumder for the construction of pEIIa–*luc* and pluc, Rita Tilley and George Elia for their help with the immunohistochemistry and Peg Kornacker for preparation of the manuscript. Plasmid constructs, cell lines, and antibodies were graciously provided by B. Thimmapaya, J. Morganstern, P. Goodfellow, M. Fried and D. Lane. T.P.D. was supported by a fellowship from the Helen Hay Whitney Foundation.

References

Barklis, E., Mulligan, R. C. and Jaenisch, R. (1986). Chromosomal position or virus mutation permits retrovirus expression in embryonal carcinoma cells. *Cell* **47**, 391–399.

Borelli, E., Hen, R. and Chambon, P. (1984). Adenovirus-2 E1A products repress enhancer-induced stimulation of transcription. *Nature, Lond.* **312**, 608–612.

Chalifour, L. E., Wirak, D. O., Hansen, U., Wassarman, P. M. and DePamphilis, M. L. (1987). *Cis*- and *trans*-acting sequences required for expression of simian virus 40 genes in mouse oocytes. *Genes Dev.* **1**, 1096–1106.

Chalifour, L. E., Wirak, D. O., Wassarman, P. M. and DePamphilis, M. L. (1986). Expression of simian virus 40 early and late genes in mouse oocytes and embryos. *J. Virol.* **59**, 619.

Cremisi, C. and Babinet, C. (1986). Negative regulation of early polyomavirus expression in mouse embryonal carcinoma cells. *J. Virol.* **59**, 761–763.

Daouk, G. H., Kaddurah-Daouk, R., Putne, S., Kingston, R. and Schimmel, P. (1988). Isolation of a functional human gene for brain creatine kinase. *J. biol. Chem.* **263**, 2442–2446.

DePamphilis, M. L., Herman, S. A., Martinez-Salas, E., Chalifour, L. E., Wirak, D. O., Cupo, D. Y. and Miranda, M. (1988). Microinjecting DNA into mouse ova to study DNA replication and gene expres ion and to produce transgenic animals. *BioTechniques* **6**, 662–680.

De Wet, J. R., Wood, D. V., DeLuca, M., Helinski, D. R. and Subramani, S. (1987). Firefly luciferase gene: structure and expression in mammalian cells. *Molec. cell Biol.* **7**, 725–737.

Feldman, L. T., Imperiale, M. J. and Nevins, J. R. (1982). Activation of early adenovirus transcription by the herpesvirus immediate early gene: Evidence for a common cellular control factor. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4952–4956.

Godeau, F., Persson, H., Gray, H. E. and Pardee, A. B. (1986). c-myc expression is dissociated from cDNA synthesis and cell division in *Xenopus* oocyte and early embryonic development. *EMBO J.* **5**, 3571–3577.

Gorman, C. M., Rigby, P. W. J. and Lane, D. P. (1985). Negative regulation of viral enhancers in undifferentiated embryonic stem cells. *Cell* **42**, 519–526.

Graham, F. L. and van der Eb, A. J. (1973). A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**, 456–467.

Haley, K. P., Overhauser, J., Babiss, L. E., Ginsberg, H. S. and Jones, N. C. (1984). Transformation properties of type-5 adenovirus mutants that differentially express the E1A gene products. *Proc. natn. Acad. Sci. U.S.A.* **81**, 5734–5738.

Hogan, B. L. M., Barlow, D. P. and Tilley, R. (1983). F9 Teratocarcinoma cells as a model for the differentiation of parietal and visceral endoderm in the mouse embryo. *Cancer Surv.* **2**, 115–140.

Hourdry, J., Brulfert, A., Gusse, M., Schoevalert, D., Taylor, M. V. and Mechali, M. (1988). Localization of c-myc expression during oogenesis and embryonic development in *Xenopus laevis*. *Development* **104**, 631–641.

Imperiale, M. J., Hung-Teh, K., Feldman, L. T., Nevins, J. R. and Strickland, S. (1984). Common control of the heat shock gene and early adenovirus genes: Evidence for a cellular E1A-like activity. *Molec. cell. Biol.* **4**, 867–874.

Jones, N. (1986). Negative regulation of enhancers. *Nature, Lond.* **321**, 202–203.

Jones, N. C., Rigby, P. W. J. and Ziff, E. B. (1988). *Trans*-acting protein factors and the regulation of eucaryotic transcription: lessons from studies on DNA tumor viruses. *Genes & Development* **2**, 267–281.

JONES, N. AND SHENK, T. (1979). An adenovirus type 5 early gene function regulates expression of other early viral genes. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3665–3669.

KING, M. W., ROBERTS, J. M. AND EISENMAN, R. N. (1986). Expression of the *c-myc* protooncogene during development of *Xenopus laevis*. *Molec. cell. Biol.* **6**, 4499–4508.

KINGSTON, R. E., BALDWIN, A. S. AND SHARP, P. A. (1985). Transcription control by oncogenes. *Cell* **41**, 3–5.

KOVESDI, I., REICHEL, R. AND NEVINS, J. R. (1986). Identification of a cellular transcription factor involved in E1A *trans*-activation. *Cell* **45**, 219–228.

LATHANGUE, N. B. AND RIGBY, P. (1987). An adenovirus E1A-like transcription factor is regulated during the differentiation of murine embryonal carcinoma stem cells. *Cell* **49**, 507–513.

LILLIE, J. W. AND GREEN, M. R. (1989). Transcription activation by the adenovirus E1a protein. *Nature, Lond.* **338**, 39–44.

LOEKEN, M., BIKEL, I., LIVINGSTON, D. M. AND BRADY, J. (1988). *Trans*-activation of RNA Polymerase II and II promoters by SV40 small t antigen. *Cell* **55**, 1171–1177.

MARTINEZ-SALAS, E., CUPO, D. Y. AND DEPAMPHILIS, M. L. (1988). The need for enhancers is acquired upon formation of a diploid nucleus during early mouse development. *Genes & Development* **2**, 1115–1126.

MARTINEZ-SALAS, E., LINNEY, E., HASSELL, J. AND DEPAMPHILIS, M. (1989). The need for enhancers in gene expression first appears during mouse development with formation of the zyotic nucleus. *Genes and Development*, (in press).

MCKNIGHT, S. L. AND KINGSBURY, R. (1982). Transcriptional control signals of a eucaryotic protein-coding gene. *Science* **217**, 316–324.

MIYAMOTO, C., SMITH, G. E., FARRELL-TOWT, J., CHIZZONITE, R., SUMMERS, M. D. AND JU, G. (1985). Production of human c-myc protein in insect cells infected with a baculovirus expression vector. *Molec. cell. Biol.* **5**, 2860–2865.

MONTANO, X. AND LANE, D. P. (1987). The adenovirus E1A gene induces differentiation of F9 teratocarcinoma cells. *Molec. cell. Biol.* **7**, 1782–1790.

MURTHY, S. C. S., BHAT, G. P. AND THIMMAPPAYA, B. (1985). Adenovirus EIIa early promoter: Transcriptional control elements and induction by the viral pre-early E1A gene, which appears to be sequence independent. *Proc. natn. Acad. Sci. U.S.A.* **82**, 2230–2234.

ONCLERCQ, R., GILARDI, P., LAVENU, A. AND CREMISI, C. (1988). C-myc products trans-activate the adenovirus E4 promoter in EC stem cells by using the same target sequence as E1A products. *J. Virol.* **62**, 4533–4537.

PHELPS, W. C., YEE, C. L., MÜNGER, K. AND HOWLEY, P. M. (1988). The human papillomavirus type 16 E7 gene encodes transactivation and transformation functions similar to those of adenovirus E1A. *Cell* **53**, 539–547.

REICHEL, R., KOVESDI, I. AND NEVINS, J. R. (1987). Developmental control of a promoter-specific factor that is also regulated by the E1A gene product. *Cell* **48**, 501–506.

SANES, J. R., RUBENSTEIN, J. L. R. AND NICHOLAS, J. F. (1986). Use of a recombinant retrovirus to study post-implantation cell lineage in mouse embryos. *EMBO J.* **5**, 3133–3142.

SCHNEIDER, J. F., FISHER, F., GODING, C. R. AND JONES, N. C. (1987). Mutational analysis of the adenovirus E1A gene: the role of transcriptional regulation in transformation. *EMBO J.* **6**, 2053–2060.

SIVA RAMAN, L., SUBRAMANIAN, S. AND THIMMAPPAYA, B. (1986). Identification of a factor in Hela cells specific for an upstream transcriptional control sequence of an E1a-inducible adenovirus promoter and its relative abundance in infected and uninfected cells. *Proc. natn. Acad. Sci. U.S.A.* **83**, 5914–5918.

SLEIGH, M. J. AND LOCKETT, T. J. (1985). SV40 enhancer activation during retinoic acid-induced differentiation of F9 embryonal carcinoma cells. *EMBO J.* **4**, 3831–3837.

STRAIR, R. K., MILLER, J. S. AND ROBERTS, B. E. (1988). Use of recombinant retroviruses to study the regulation of integrated adenovirus early promoters. *J. Virol.* **62**, 2143–2149.

SUEMORI, H., HASHIMOTO, S. AND NAKATSUJI, N. (1988). Presence of the adenovirus E1A-like activity in preimplantation stage mouse embryos. *Molec. cell. Biol.* **8**, 3553–3555.

TAKETO, M. AND TANAKA, M. (1987). A cellular enhancer of retrovirus gene expression in embryonal carcinoma cells. *Proc. natn. Acad. Sci. U.S.A.* **84**, 3748–3752.

TAYLOR, M. V., GUSSE, M., EVAN, G. I., DATHAN, N. AND MECHALI, M. (1986). *Xenopus myc* protooncogene during development: expression as a stable maternal mRNA uncoupled from cell division. *EMBO J.* **5**, 3563–3570.

TEVETHIA, M. J. AND SPECTOR, D. J. (1984). Complementation of an adenovirus 5 immediate early mutant by human cytomegalovirus. *Virology* **137**, 428–431.

THOMPSON, T. C., SOUTHGATE, J., KITCHENER, G. AND LAND, H. (1989). Multistage carcinogenesis induced by *ras* and *myc* oncogenes in a reconstituted organ. *Cell* **56**, 917–930.

TREMBLAY, M. L., YEE, S., PERSSON, R. H., BACCHETTI, S., SMILEY, J. R. AND BRANTON, P. E. (1985). Activation and inhibition of expression of the 72,000-Da early protein of adenovirus type 5 in mouse cells constitutively expressing an immediate early protein of herpes simplex virus type 1. *Virology* **144**, 35–45.

WATT, R. A., SHATZMAN, A. R. AND ROSENBERG, M. (1985). Expression and characterization of the human c-myc DNA-binding protein. *Molec. cell. Biol.* **5**, 448–456.

WEEKS, D. L. AND JONES, N. C. (1985). Adenovirus E3 early promoter: sequences required for activation by E1A. *Nucl. Acids Res.* **13**, 5389.

WIGLER, M., PELLICER, A., SILVERSTEIN, S. AND AXEL, R. (1978). Biochemical transfer of single-copy eucaryotic genes using total cellular DNA as donor. *Cell* **14**, 725–731.

WIRAK, D. O., CHALIFOUR, L. E., WASSARMAN, P. M., MULLER, W. J., HASSELL, J. A. AND DEPAMPHILIS, M. L. (1985). Sequence dependent DNA replication in preimplantation mouse embryos. *Molec. cell. Biol.* **5**, 2924–2935.

WONG, K. AND LEVINE, A. J. (1986). Identification and mapping of Epstein-Barr virus early antigens and demonstration of a viral gene activator that functions in *trans*. *J. Virology* **60**, 149–156.

WORKMAN, J. L., ABMAYR, S. M., CROMLISH, W. A. AND ROEDER, R. G. (1988). Transcriptional regulation by the immediate early protein of pseudorabies virus during *in vitro* nucleosome assembly. *Cell* **55**, 211–219.

ZIMMERMAN, K. A., YANCOPOULOS, G. D., COLLUM, R. G., SMITH, R. K., KOHL, N. E., DENIS, K. A., NAU, M. M., WITTE, O. N., TORAN-ALLERAND, D., GEE, C. E., MINNA, J. D. AND ALT, F. W. (1986). Differential expression of *myc* family genes during murine development. *Nature, Lond.* **319**, 780–783.

(*Accepted 6 October 1989*)