Expression of Simian Virus 40 Early and Late Genes in Mouse Oocytes and Embryos

LORRAINE E. CHALIFOUR,[†] DANA O. WIRAK, PAUL M. WASSARMAN,[‡] AND MELVIN L. DEPAMPHILIS*

Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115

Received 24 February 1986/Accepted 9 June 1986

Simian virus 40 (SV40) large- and small-tumor antigens (T-Ag, t-Ag) are normally synthesized early after infection of either permissive (monkey) or nonpermissive (mouse) fibroblasts, whereas an equivalent amount of viral coat protein (V-Ag) is observed late after infection of permissive cells and only after viral DNA replication has occurred. To determine whether or not expression of these genes is regulated in the same manner during early mammalian development, SV40 DNA was injected into the nuclei of mouse oocytes and one- and two-cell embryos. In oocytes, about three times more V-Ag was produced than T-Ag, and both were synthesized concomitantly in the same cells. Viral mRNA and proteins synthesized in oocytes comigrated during gel electrophoresis with the same products synthesized in SV40-infected monkey cells. Viral gene expression required circular DNA molecules injected into the nuclei of transcriptionally and translationally active cells. Injected DNA was stable and underwent conformational changes consistent with chromatin assembly. Oocytes did not replicate either polyomavirus or SV40 DNA. Thus, the temporal order of viral gene expression is circumvented in mouse germ cells, allowing these proteins to be expressed concurrently and in equivalent amounts with no requirement for DNA replication. However, in preimplantation embryos, neither T-Ag nor V-Ag was detected by immunoprecipitation although T-Ag synthesis was demonstrated as a specific requirement for SV40 DNA replication. Thus, viral gene expression in mouse embryos as early as the one-cell stage was reduced at least 500-fold relative to that in oocytes. Similarities between SV40 gene expression in mouse oocytes and that in *Xenopus* oocytes suggest that germ cells in higher animals share common regulatory mechanisms.

Simian virus 40 (SV40) provides a well-characterized genome containing two sets of genes that are temporally expressed and stringently regulated during infection of mammalian cells. SV40 normally replicates in the nuclei of permissive lines of monkey and human cells, where host cell components transcribe and translate viral early genes soon after infection to produce large- and small-tumor antigens (T-Ag and t-Ag, respectively). T-Ag induces synthesis of cellular proteins required for DNA replication and chromatin assembly, induces cellular DNA replication, initiates replication of viral DNA, represses transcription from the early gene promoter, and participates in activation of the late gene promoter (N. P. Salzman, *The viruses: polyoma viruses*, in press). The products of the late (V-Ag) genes consist of three viral coat proteins (VP-1, VP-2, and VP-3). Greater than 95% of the SV40 mRNA present early during infection (prior to viral DNA replication) represents expression of early genes, whereas late in infection greater than 98% of SV40 mRNA comes from late genes (5, 24, 34). These differences reflect synthesis rather than stability of mRNA and result in a substantial increase in synthesis of viral coat proteins following the onset of viral DNA replication (28).

Activation of late gene transcription in permissive cells is a complex phenomenon that involves T-Ag, cellular proteins, and viral DNA replication (6, 20, 22, 34). SV40-infected or -transformed mouse fibroblasts (nonpermissive

cells) synthesize T-Ag, but viral DNA replication has not been detected (9) and V-Ag genes are expressed only at their basal level (11). Failure to express V-Ag genes may result from a low concentration of T-Ag, absence of a posttranslational modification of T-Ag, or a missing permissive cell factor. Synthesis of SV40 V-Ag can be observed in mouse fibroblasts, but it requires about 1,000-fold more viral DNA per nucleus than that required to observe T-Ag synthesis (15). The role of DNA replication in V-Ag gene expression may be twofold. First, it increases the number of gene copies, and second, it may activate the late gene promoter as it does the late-early gene promoter (8).

Whatever the roles of T-Ag and DNA replication in V-Ag gene expression in differentiated mammalian cells, they appear to have been bypassed in germ cells. *Xenopus laevis* oocytes injected with SV40 DNA synthesized V-Ag gene mRNA and protein at substantially higher levels than T-Ag and t-Ag gene products (31, 33, 41) despite the fact that oocytes replicate neither their own DNA nor the injected viral DNA (3, 18). Furthermore, V-Ag gene expression did not require T-Ag (10, 31, 33). Unfortunately, the relationship of these results to the studies carried out in mammalian cell lines is difficult to evaluate. Although some workers observed differences only in the frequency at which late mRNA start sites were used (10), others observed major changes in the locations of start sites (31) and some changes in the pattern of RNA splicing (33, 41). Moreover, differences between amphibian and mammalian transcriptional machinery as well as differences in the biology of their early development (35) may result in significant differences in the requirements for gene expression in germ cells, as it does for DNA replication in preblastomere embryos (42).

* Corresponding author.

[†] Present address: National Research Council Biotechnology Research Institute, Montreal, Quebec H3A 1A1, Canada.

[‡] Present address: Department of Cell Biology, Roche Institute of Molecular Biology, Nutley, NJ 07110.

To help resolve this question, we injected SV40 DNA into the germinal vesicles (i.e., nuclei) of mouse oocytes and into the nuclei of one- and two-cell mouse embryos. In oocytes, both early and late SV40 genes were expressed concomitantly, with more V-Ag than T-Ag synthesized. T-Ag, t-Ag, and V-Ag were synthesized at rates in excess of those observed in SV40-infected permissive monkey cells. When the same experiments were carried out in mouse embryos, neither T-Ag nor V-Ag could be detected by immunoprecipitation of radiolabeled proteins or blotting-hybridization of mRNA. However, active T-Ag was detected as a requirement for replication of plasmid DNA containing the SV40 origin of DNA replication (*ori*). These data are consistent with similar experiments carried out in amphibian oocytes, suggesting that mammalian and amphibian germ cells control gene expression by similar mechanisms. Moreover, the ability to express a variety of genes in abundance is lost in preimplantation embryos, including genes that are normally expressed in differentiated cells of the same species.

MATERIALS AND METHODS

Injection of DNA into mouse oocytes and embryos. Growing oocytes were dissected from ovarian follicles of 13- to 18-day-old CD-1 Swiss mice (39) and cultured in standard egg culture medium (SECM) (4) containing 100 μ g of dibutyryl cAMP (db-cAMP) per ml (36). Microinjection procedures and isolation of one- and two-cell embryos have been described previously (42).

Immunoprecipitation of SV40 proteins. Newly synthesized proteins were labeled by incubating oocytes or embryos in 20 to 100 μ l of SECM containing 1 mCi of [^{35} S]methionine (~1,100 Ci/mmol; New England Nuclear Corp.) per ml for the times indicated. Ova were then washed free of exogenous radiolabel with fresh SECM and transferred to 100 μ l of lysis buffer (20 mM Tris [pH 8.0], 137 mM NaCl, 1% Nonidet P-40, 25 μ g of phenylmethylsulfonyl fluoride per ml, 10 mM sodium metabisulfite [pH 7.0]). Samples were either frozen at -70°C or processed immediately.

Anti-SV40 T-Ag sera from tumor-bearing animals were obtained from the National Institutes of Health and shown to immunoprecipitate SV40 T-Ag and t-Ag. Rabbit anti-SV40 sera (Miles Laboratories) immunoprecipitated SV40 T-Ag, t-Ag, and capsid protein (VP-1). Monoclonal antibody PAB101 specifically precipitated T-Ag (17). Samples were immunoprecipitated from a total volume of 500 μ l of 50 mM Tris (pH 7.8)–150 mM NaCl–0.1% Nonidet P-40–2 mg of bovine serum albumin. Samples were preincubated twice with *Staphylococcus aureus* (Enzyme Centre Inc., Boston, Mass.). After the second preabsorption, 1 to 5 μ l of antibody was added and incubation proceeded for 2 h on ice with occasional gentle shaking. Immunoabsorption was achieved by adding an *S. aureus* suspension and further incubation for 1 h as described by Gottesman and Cabral (14). Pellets were solubilized by boiling (26). *S. aureus* was removed by centrifugation, and the supernatant proteins were fractionated by electrophoresis in 12% polyacrylamide containing sodium dodecyl sulfate (SDS) as described by Laemmli (26), except that glycerol was omitted. Protein bands were visualized by fluorography (7) with preflashed XAR-5 film (Kodak).

Authentic SV40 T-Ag, t-Ag, and V-Ag were generated by SV40 infection of CV-1 cells. Cells were washed free of virus either 24 or 48 h postinfection and incubated in methionine-free Dulbecco modified Eagle medium for 30 min at 37°C and then for 3 h in methionine-free Dulbecco modified Eagle

medium supplemented with 50 μ Ci of [^{35} S]methionine per ml (~1,100 Ci/mmol). Washed cells were incubated with lysis buffer for 30 min at 4°C and scraped from dishes, and the clarified supernatants were frozen at -70°C . Mock-infected CV-1 cells were processed in the same manner.

Identification of SV40 mRNA. RNA was extracted from CV-1 cells infected with SV40 18 to 19 h (early RNA) and 42 to 48 h (late RNA) after infection. Washed cells were centrifuged, and the cell pellet was lysed in 1 ml of sterile 10 mM Na_2HPO_4 (pH 7.3)–170 mM NaCl–34 mM KCl–1% SDS–20 mM vanadyl ribonucleotides (Promega Biotec). Lysates were incubated with proteinase K (200 μ g/ml; predigested), extracted with phenol-chloroform and chloroform, and then precipitated in ethanol at -70°C . Half of each sample was incubated in 0.5 N NaOH–0.5% SDS at 80°C for 1 h to hydrolyze RNA. These samples were then neutralized and precipitated in ethanol. RNA was isolated from mock-infected CV-1 cells in the same way. Oocytes 6 to 8 h postinjection and embryos were lysed in lysis buffer supplemented with 5 to 10 μ g of tRNA and then treated as described above.

RNA samples (from about 10^5 CV-1 cells or about 100 ova) were fractionated by electrophoresis in agarose gels containing formaldehyde (21). Each gel included a lane with *Escherichia coli* and CV-1 rRNAs as size standards. The gel was prepared for blotting as described by Huang et al. (21). GeneScreen Plus (New England Nuclear) membrane was prepared as recommended by the manufacturer. Capillary transfer was continued for 12 to 16 h. Each baked membrane was prehybridized in 50 ml of 50% formamide–1% SDS–1 M NaCl–10 mM NaPO_4 (pH 6.5)–100 μ g of heat-denatured salmon sperm DNA per ml for 4 to 6 h at 55°C . Heat-denatured RNA probe (1 to 5 ng/ml; 10^9 cpm/ μ g of RNA) and 10% dextran sulfate were added to the membrane in 0.2 volume of prehybridization buffer and then incubated at 55°C for 12 to 16 h. The membrane was washed with $2\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at room temperature, twice with $2\times$ SSC–0.1% SDS at 60°C , and then twice with $0.2\times$ SSC at 60°C . The membrane was exposed to preflashed Kodak XAR-5 film with an intensifying screen (7). SV40 RNA from 10,000 virus-infected CV-1 cells was easily detected in an overnight exposure.

RNA probes. SV40 DNA was cloned into the *Bam*HI site of pT7-1 in both orientations (gift from C. C. Richardson) (37). RNA probe complementary to both DNA strands of SV40 was transcribed from the pT7-1-SV40 clones in a reaction mixture containing 250 to 500 μ M [α - ^{32} P]UTP (3,000 Ci/mmol; New England Nuclear) and 1,000 U of T7 RNA polymerase (gift from C. C. Richardson) at 37°C for 45 min as described by Tabor and Richardson (37). DNA was then degraded by 1.5 μ g of DNase I (DNase-DPFF, Worthington Diagnostics). The reaction was stopped in 0.1% SDS, and free label was removed by gel filtration through sterile BioGel P10 (Bio-Rad Laboratories). About 0.4 to 0.6 μ g of RNA (100 to 400 bases long) was synthesized per microgram of DNA and had a specific radioactivity of 9.8×10^8 to 2×10^9 cpm/ μ g of RNA.

RESULTS

Expression of early and late SV40 genes in oocytes. Circular, superhelical SV40 DNA (form I DNA) was injected into the germinal vesicles (i.e., nuclei) of growing mouse oocytes to determine the abilities of mammalian germ cells to express both early and late viral genes. The survivors were incubated with [^{35} S]methionine for 16 h before immunoprecipitation of

FIG. 1. SV40 T-Ag, t-Ag, and V-Ag synthesis in mouse oocytes. SV40 DNA was injected into growing oocytes, and the survivors were metabolically labeled with [³⁵S]methionine (lanes c to q). The oocytes were then washed and lysed, and proteins were immunoprecipitated with anti-T or anti-V sera or both as indicated (Ab); the precipitated proteins were fractionated by electrophoresis in SDS-polyacrylamide gels, and the ³⁵S-labeled proteins were detected by fluorography. SV40-infected CV-1 cells were treated similarly at either 24 (lane b) or 48 (lane a) h postinfection. The number of injected oocytes used per lane (#/lane) is indicated for lanes c to i; 20 injected oocytes per lane were used in lanes j to q. Oocytes in lanes d, e, g, h, and i were injected with a solution

T-Ag and t-Ag with anti-T sera, V-Ag (VP1) with anti-capsid sera, or both. The precipitated proteins were then fractionated by electrophoresis in SDS-polyacrylamide gels. All three viral proteins were synthesized when form I DNA was injected directly into germinal vesicles (Fig. 1A, lane d). T-Ag, t-Ag, and VP-1 synthesized in oocytes had apparent molecular weights that were indistinguishable from the same proteins synthesized in SV40-infected CV-1 cells (Fig. 1A, lanes a and b; see Fig. 6; data not shown), although the ratio of T-Ag to t-Ag was generally higher in infected CV-1 cells than in oocytes. Proteins of this size were not synthesized in noninjected oocytes (lanes c and f). All three viral proteins could be detected in a single oocyte (Fig. 1A, lane i). Expression of viral genes was not particularly sensitive to the concentration of DNA injected. The amount of DNA injected per germinal vesicle was measured by dot blot analysis (42). When the concentration of injected DNA was varied 100-fold (2 μ l of 0.01 to 1 mg/ml), the amounts of viral ³⁵S-proteins synthesized varied only threefold (0.3:1:0.55; Fig. 1B, lanes j to m), and the ratio of T-Ag, t-Ag, and V-Ag observed at each DNA concentration remained the same. Therefore, DNA concentrations between 0.1 and 0.5 mg/ml were routinely used in these experiments. These data demonstrate that mouse oocytes can express both early and late SV40 genes.

Expression of SV40 genes in mouse oocytes required injection of a circular genome into a transcriptionally active germinal vesicle. Only about 5% as much viral protein synthesis was detected when linear form III DNA was injected into germinal vesicles (Fig. 1A, compare lanes d and e), and viral proteins were not detected when form I DNA was injected into the cytoplasm instead of germinal vesicles (T-Ag, t-Ag, Fig. 1A [compare lanes g and h]; V-Ag, data not shown). Furthermore, transcriptional activity in oocyte nuclei could be terminated by omitting db-cAMP from the culture medium. Under these conditions, 80% of the growing oocytes underwent meiotic maturation within 5 h, resulting in disintegration of germinal vesicles, inhibition of cellular transcription, and reduction of translation to 20% of normal levels (36, 38). SV40 form I DNA was injected into the germinal vesicles of growing oocytes isolated from 19-day-old animals. Half of the surviving oocytes were cultured in media containing db-cAMP, and half were cultured in the absence of db-cAMP. After 24 h, oocytes were separated into groups that retained their germinal vesicles and those that had undergone meiotic maturation. Regardless of the presence or absence of db-cAMP in the medium, those oocytes that retained their germinal vesicles synthesized viral proteins (Fig. 1B, lanes o and q), whereas those oocytes that underwent meiotic maturation did not (lane p).

Synthesis of SV40 mRNA. The number of SV40 mRNAs synthesized in oocytes and their relative sizes were essentially the same as those synthesized during a normal virus infection of permissive CV-1 cells. RNA was isolated 6 h

containing 0.5 mg of DNA per ml, resulting in about 0.5 pg of DNA per germinal vesicle. Otherwise, the concentration of DNA is indicated in milligrams per milliliter ([DNA]). Form I DNA (F1) was injected into nuclei (N) in all lanes except h, where it was injected into the cytoplasm (C), and in all samples except those in lane e, where oocytes were injected with form III DNA (F3) produced by cleavage of form I DNA at its single *Bam*HI site. Injected oocytes were routinely cultured in the presence of cAMP analog except in lanes n to p. Thus, all injected oocytes normally retained their nuclei (i.e., germinal vesicles [GV]) except for those pooled in lane p. The positions of T-Ag, t-Ag, and V-Ag (VP-1) are indicated.

FIG. 2. SV40 RNA synthesis in oocytes injected with SV40 DNA. RNA was isolated from 120 uninjected oocytes (lane U) and from 120 oocytes 6 h after injection of either form I SV40 DNA (lane SV40) or pJYM DNA (lane pJYM) into the germinal vesicles. The material in lane A initially contained an equivalent amount of RNA as in the SV40 lane but was digested with alkali. RNA was isolated from SV40-infected CV-1 cells 30 h postinfection. After fractionation by gel electrophoresis, SV40-specific RNA was identified by blotting-hybridization with both SV40 early and late [^{32} P]RNAs. The RNA from about 5,000, 10,000, and 20,000 SV40-infected CV-1 cells was applied to lanes 5, 10, and 20, respectively. Four ribosomal RNA standards were included in a parallel lane, and their positions are indicated as 28S, 26S, 18S, and 16S. Mock-infected and alkali-treated CV-1 RNA samples were identical to lanes U and A.

after injection of oocytes or 18 h after virus infection of CV-1 cells, fractionated by gel electrophoresis, and detected by blotting-hybridization with [^{32} P]RNA that was complementary to both early and late SV40 mRNAs. Three SV40-specific RNA species were observed in the injected oocytes that were not present in uninjected oocytes or uninfected CV-1 cells (Fig. 2). Their sizes (approximately 5,000, 2,500, and 2,000 nucleotides) and their sensitivities to alkali were essentially the same in both oocytes and CV-1 cells. The 2,500-nucleotide (19 to 20S) SV40 RNA was the size expected of early viral mRNA, and the 2,000-nucleotide (16S) SV40 RNA corresponded in size to late mRNA encoding VP-1 (2). Injection of pJYM, a plasmid DNA whose late gene coding region is interrupted by pML-1 DNA (29), produced only the 2,500-nucleotide RNA species (Fig. 2). Since T-Ag and t-Ag, but not V-Ag, were synthesized in oocytes injected with this plasmid (manuscript in preparation), the 2,500-nucleotide species most likely represented early SV40 mRNA. The 5,000-nucleotide band found in injected oocytes and infected CV-1 samples was presumably genome length RNA that resulted from failure to terminate transcription at the normal termination site (2).

Conformation, stability, and replication of injected DNA. SV40 form I DNA injected into mouse oocytes underwent conformational changes consistent with its conversion into viral chromosomes. At various times after injection, DNA was fractionated by gel electrophoresis and detected by blotting-hybridization. The relative abundances of form I (circular and superhelical), II (circular and relaxed), and III (linear) DNAs were determined by densitometry of the resulting autoradiograms. Prior to injection, the DNA was greater than 95% form I. However, immediately after injection most of it was converted into form II and then converted

slowly back into form I so that by 7 h postinjection about 65% of the DNA was form I, 20% was form II, and 15% was form III (Fig. 3). Similar changes in conformation have been observed with DNA injected into *Xenopus* oocytes and eggs and was shown to result from assembly of nucleosomes on the injected DNA (13, 32, 43). Injected DNA was stable for at least 48 h.

Neither SV40 nor polyomavirus DNA replication was detected in oocytes. pJYM or pB1a DNA (pML-1 containing an intact origin of replication and T-Ag gene from either SV40 or polyomavirus, respectively) was injected into germinal vesicles. At 24 (data not shown) and 48 h after injection, plasmid DNA was extracted and analyzed for replication by its change in sensitivity to cleavage by *DpnI* (42). The injected plasmid DNA, which was produced in *Dam*⁺ *E. coli*, contained 17 to 25 *DpnI* restriction sites that were sensitive to cleavage because they were fully methylated. A single round of DNA replication in mammalian cells, which lack the appropriate methylase, makes these sites resistant to *DpnI*. In as many as 96 oocytes, no *DpnI*-resistant DNA was observed with either pJYM or pB1a (Fig. 4, lanes a and b), whereas under the same conditions pB1a replication could be detected in a single embryo (42) and pJYM replication was detected in 10 embryos (42; Fig. 4, lanes c, d, f, and g). In addition, embryos from the one-cell to blastula stage readily incorporated [^{3} H]thymidine into their DNA, whereas oocytes did not (data not shown), confirming the inability of oocytes to replicate DNA.

Time course for early and late SV40 gene expression. Early and late SV40 genes were expressed concomitantly in mouse oocytes. At different times following injection of SV40 form I DNA into germinal vesicles, oocytes were pulse-labeled for 4 h with [^{35} S]methionine. The relative amounts of T-Ag, t-Ag, and V-Ag were then determined by densitometry of fluorograms taken of immunoprecipitated ^{35} S-labeled proteins fractionated by SDS gel electrophoresis. These data revealed that both early (Fig. 5A) and late (Fig. 5B) genes were expressed during the first 4 h and that their synthesis

FIG. 3. Conformation and stability of SV40 DNA injected into oocytes. About 0.5 pg of SV40 form I DNA was injected into the germinal vesicles of mouse oocytes, and the oocytes were incubated in vitro for the times indicated. The earliest time point was 5 min. Viral DNA was purified, fractionated by electrophoresis in 0.6% agarose gels, transferred to nitrocellulose filters, and hybridized with SV40 [^{32} P]DNA (4×10^8 cpm/ μ g) as described by Wirak et al. (42). DNA from 5 to 10 oocytes was pooled for each time point. The composition of DNA prior to injection is shown as PRE-INJ. F1 is covalently closed, superhelical, circular SV40 DNA. F2 is topologically relaxed, circular SV40 DNA. F3 is linear, double-stranded SV40 DNA one genome long.

FIG. 4. Activation of SV40 DNA replication as an assay for T-Ag production in mouse oocytes and embryos. Oocyte and two-cell embryo nuclei were injected with the plasmid DNA mixtures indicated (0.1 mg/ml for each plasmid). pML-1 is a derivative of plasmid pBR322 (29). pB1a and pJYM contain the entire genomes of polyomavirus and SV40, respectively, inserted into the *Bam*HI site of pML-1 (42). pSVoriH3 contains SV40 nucleotides 5171 to 1046 spanning the origin of replication inserted into the *Hind*III site of pML-1 (kindly provided by J. Hassell, McGill University, Toronto, Ontario, Canada). Surviving oocytes and ova were cultured in vitro for 48 h (lanes a to c and e to g) or 72 h (lanes d) (42). Plasmid DNA was purified from embryos and oocytes, and one half of each sample was digested with *Sall* to convert all DNA into linear monomers that were one genome in length (-). The remaining half (+) was digested with both *Sall* and *Dpn*I to destroy all DNA that had not replicated (42). DNA was fractionated by agarose gel electrophoresis and detected by blotting-hybridization with ^{32} P-labeled pML-1 DNA (42). Panels A and B contained DNA from 96 and 94 oocytes, respectively. Panels C, D, E, F, and G contained DNAs from 29, 20, 33, 34, and 30 embryos, respectively.

reached a maximum rate 7 to 8 h postinjection. The fact that all three antigens were synthesized in the same cell allowed their relative rates of synthesis to be calculated. T-Ag contains 22 methionine residues and VP-1 contains 7 (Salzman, in press). Therefore, the amount of ^{35} S label incorporated per T-Ag molecule was 3.14-fold greater than that incorporated per VP-1 molecule. Thus, the initial rate of V-Ag synthesis appeared to be about threefold greater than the rate of T-Ag synthesis (Fig. 5C). Incorporation of [^{35} S]methionine into T-Ag, t-Ag, and V-Ag began decreasing to a minimal rate reached around 20 h postinjection. Since total protein synthesis (acid-precipitable, ^{35} S-labeled material) also decreased 50% during this period, the decrease in SV40 protein synthesis resulted from a general decline in translation by oocytes cultured for longer than 10 h.

The relative stability of SV40 proteins synthesized in oocytes was determined over the same period of time to determine whether the data in Fig. 5 reflected primarily rates of protein synthesis or degradation. Injected oocytes were cultured with [^{35} S]methionine for 14 h, washed, and then cultured with unlabeled methionine for the times indicated in Fig. 6. Proteins were immunoprecipitated with anti-T and anti-V sera and fractionated by SDS gel electrophoresis (Fig. 6), and the resulting fluorographs were analyzed by densitometry. The half-life for T-Ag and t-Ag was about 20 h, and the half-life of V-Ag was about 40 h. Since these half-lives were 5 to 10 times longer than the 4-h period during which newly synthesized proteins were radiolabeled in the experiment described in Fig. 5, the rate of V-Ag synthesis was at least twice that of T-Ag synthesis (Fig. 5C).

The synthesis of T-Ag and V-Ag was also measured in virus-infected CV-1 cells (African green monkey cells permissive for SV40 replication) and 3T6 cells (nonpermissive mouse fibroblasts) to determine whether the methods used to detect these proteins in mammalian oocytes and embryos would distinguish the various temporal patterns of viral gene expression previously reported for differentiated mammalian cells. Synthesis of V-Ag in SV40-infected CV-1 cells was delayed at least 10 h relative to that of T-Ag, and the period of maximum V-Ag synthesis was at least 10 h later than the period of maximum T-Ag synthesis (Fig. 7). The subsequent increase in T-Ag synthesis marked a second round of virus infection in these cell cultures. SV40 T-Ag was also synthe-

FIG. 5. Concomitant expression of early and late SV40 genes in oocytes. Every hour for 20 h after injection, 20 to 30 oocytes were incubated for 4 h in [^{35} S]methionine and then lysed. T-Ag ($\blacktriangle$) and t-Ag ($\circ$) were immunoprecipitated with anti-T sera (panel A), and V-Ag was immunoprecipitated with anti-V sera (panel B). The precipitated proteins were fractionated and detected as described in the legend to Fig. 1, and the relative amount of each antigen was determined from densitometer tracings of fluorographs with a Joyce Lobel densitometer. Data from three experiments were averaged. The actual ratio of V-Ag to T-Ag was calculated by multiplying the values for V-Ag by 22/7, the ratio of T-Ag methionine to V-Ag methionine (Salzman, in press).

FIG. 6. Stability of SV40 T-Ag, t-Ag, and V-Ag in oocytes. About 0.5 pg of SV40 form I DNA was injected into the germinal vesicles of growing oocytes. The oocytes were cultured for 14 h with [^{35} S]methionine and then washed in SECM without radiolabel and incubated in SECM. At the times indicated in hours, groups of 20 oocytes were removed and lysed, proteins were immunoprecipitated with anti-T and anti-V sera and fractionated by electrophoresis in SDS-polyacrylamide gels, and ^{35} S-proteins were detected by fluorography. SV40-infected CV-1 cells (30 h postinfection) and 20 uninjected oocytes (U) were treated the same way. The positions of T-Ag, V-Ag (VP-1), and t-Ag are indicated.

sized in 3T6 cells but at a rate fivefold lower than that observed in CV-1 cells; SV40 V-Ag was not detected in 3T6 cells (Fig. 7). Similar results have been observed when SV40 DNA was injected into the nuclei of permissive and nonpermissive fibroblasts (15, 16). Therefore, regulation of SV40 gene expression in mouse oocytes was distinctly different than in differentiated mammalian cells.

SV40 gene expression in preimplantation mouse embryos. To determine whether SV40 genes were expressed in early mouse embryos as well as mouse oocytes, SV40 form I DNA was injected into male pronuclei of one-cell embryos or the nucleus of one of the two blastomeres in two-cell embryos. The injected embryos were cultured for up to 72 h, sufficient time for 80% of two-cell embryos to develop into blastocysts (42). Embryos were harvested at the one-cell, two-cell, four-cell, morula (16 to 32 cells), and blastocyst (64 to 128 cells) stages and then incubated in medium containing [^{35} S]methionine for an additional 24 h before immunoprecipitation with anti-T and anti-V sera. No viral antigens were detected in embryos at any of these developmental stages (Fig. 8). The large number of embryos used in each sample ($n = 100$) and the long exposure times emphasized nonspecific precipitation of cellular proteins. Based on the time allowed for fluorography and the number of embryos used per sample, preimplantation embryos synthesized less than 0.2% of the T-Ag observed in oocytes. DNA injected into one- or two-cell embryos was rapidly assembled into nucleosomes and was stable for at least 72 h (42). Nevertheless, SV40 mRNA was not detected (data not shown), suggesting that

embryos were much less proficient than oocytes at transcribing injected DNA.

Despite the absence of detectable viral antigens in mouse embryos, T-Ag activity was detected in embryos as a specific requirement for initiation of SV40 DNA replication. Circular DNA molecules containing either the SV40 or polyomavirus origins of replication (*ori*) have been shown by Wirak et al. (42) to replicate when injected into the nuclei of one- or two-cell mouse embryos that were then incubated in vitro, although SV40 replicated only about 3% as well as polyomavirus. Polyomavirus DNA replication required both the polyomavirus *ori* sequence and polyomavirus T-Ag (42). Similarly, the SV40 *ori* sequence was active in embryos only in the presence of SV40 T-Ag (Fig. 4). DNA replication was detected as plasmid molecules completely resistant to cleavage by *DpnI* (42). pJYM, a plasmid containing an intact SV40 *ori* region and T-Ag gene inserted into pML-1, replicated in mouse preimplantation embryos (i.e., lanes c, d, and g). The SV40 *ori* did not function in replication when the SV40 T-Ag gene was deleted; pSVoriH3, containing only the SV40 *ori* region inserted into pML-1, failed to replicate (lanes e). However, pSVoriH3 did replicate when SV40 T-Ag was provided in *trans* by coinjection with pJYM (lanes g). Polyomavirus T-Ag, provided by coinjecting a plasmid containing the complete polyomavirus genome (pB1a), did not activate the SV40 *ori* region (lanes f). However, polyomavirus T-Ag did activate the polyomavirus *ori* region for multiple rounds of replication in the same cells which did not support pSVoriH3 replication (lanes f). Thus, SV40 T-Ag and polyomavirus T-Ag were specific for their respective viral *ori* sequences in embryonic cells. Neither pML-1 nor pBR322, plasmids replicated in embryos in the presence (lanes c, d, f, and g) or absence (lanes e) of either viral T-Ag. Therefore, mouse preimplantation embryos, in contrast to

FIG. 7. Synthesis of SV40 T-Ag and V-Ag in permissive and nonpermissive fibroblasts. Subconfluent monolayers of monkey CV-1 and mouse 3T6 cells were infected with SV40 (5 to 10 PFU per cell) for 1 h. At 10, 25, 34, 48, and 60 h postinfection, the medium was changed to [^{35} S]methionine medium and incubation was continued for an additional 4 h at 37°C in a CO₂ incubator. The relative amounts of newly synthesized T-Ag (T) and V-Ag (V) were then determined as previously described (Fig. 5). The maximum amount of T-Ag or V-Ag synthesized in CV-1 cells was defined as 100%, and all other antigen values were calculated relative to this value. Data were plotted at the midpoint of the pulse-labeling period.

FIG. 8. Expression of SV40 genes in mouse preimplantation embryos. About 0.5 pg of SV40 form I DNA was injected into male pronuclei of mouse one-cell embryos or into one of the nuclei of mouse two-cell embryos (lanes 0.5), and then embryonic development was continued in vitro for up to 72 h (42). The same number of uninjected embryos were analyzed in parallel (lanes 0). Embryonic proteins were metabolically labeled with 1 mCi of [^{35} S]methionine per ml for the times postinjection indicated below and then sorted into groups by stage of development. T-Ag, t-Ag, and V-Ag were immunoprecipitated and fractionated by gel electrophoresis as described in the legend to Fig. 1. Fluorograms were overexposed for 2 months in an effort to detect trace amounts of viral proteins. Lanes: a, SV40-infected CV-1 cells radiolabeled at 30 h postinfection under the same conditions as embryos and immunoprecipitated with anti-V serum; b, same as a except immunoprecipitated with anti-T serum; c, 100 uninjected one-cell embryos labeled for 0 to 24 h that remained at the one-cell stage; d, same as c except that embryos were injected with SV40 DNA; e, 100 uninjected two-cell embryos labeled for 0 to 24 h that remained at the two-cell stage; f, same as e except that embryos were injected with SV40 DNA; g, 100 uninjected two-cell embryos that developed to the four-cell stage and then were labeled for 24 to 48 h; h, same as g except that embryos were injected with SV40 DNA; i, same as a and b together; j, 100 uninjected two-cell embryos that developed to the morula stage and then were labeled for 48 to 72 h; k, same as j except that embryos were injected with SV40 DNA; l, same as i but overexposed; m, 100 uninjected two-cell embryos that developed to blastocyst stage and then were labeled for 72 to 96 h; n, same as m except that embryos were injected with SV40 DNA; o, same as i but overexposed. The positions of T-Ag, V-Ag (VP-1), and t-Ag are indicated.

mouse oocytes, provide sufficient permissive cell factors to support a low level of SV40 T-Ag-dependent, *ori*-dependent DNA replication.

DISCUSSION

The data presented in this paper demonstrate that three viral genes (T-Ag, t-Ag, and V-Ag), whose expression is temporally regulated during infection of mammalian differentiated cells, are synthesized concomitantly when injected into the germinal vesicles (i.e., nuclei) of growing mouse oocytes and that equivalent amounts of these proteins are synthesized regardless of the fact that viral DNA replication does not occur. Both early and late proteins were detected within 4 h of injecting the DNA, and their rates of synthesis were comparable to one another throughout the 25-h period in which the injected oocytes were cultured in vitro. Based on size alone, T-Ag, t-Ag, V-Ag (VP-1), and SV40 mRNAs synthesized in oocytes were indistinguishable from those synthesized during lytic infection of permissive monkey cells. The rate of V-Ag synthesis was actually two to three times greater than that of T-Ag or t-Ag. This was surprising in view of the fact that neither SV40, polyomavirus, nor oocyte DNA replication was detectable under these conditions. Viral replication most likely did not occur because oocytes were in the diplotene stage of meiotic prophase and therefore did not replicate their own DNA. Furthermore, injection of SV40 DNA containing deletions in the origin of DNA replication synthesized as much T-Ag, V-Ag, and t-Ag as did wild-type SV40 DNA (manuscript in preparation). In contrast, SV40-infected mouse fibroblasts, which are

nonpermissive for SV40 DNA replication, synthesized only T-Ag and t-Ag. Infection of permissive CV-1 monkey cells resulted in expression of late viral genes, but the onset of V-Ag synthesis was delayed at least 10 h, the time required to initiate viral DNA replication (2). Therefore, mouse oocytes have circumvented the requirement of SV40 DNA replication for V-Ag gene expression at a level comparable to that of T-Ag genes. If activation of V-Ag gene expression by T-Ag requires a permissive cell factor (6, 22), then that factor may be present at sufficiently high levels in oocytes to compensate for the lack of DNA replication. The SV40 DNA sequences required for viral gene expression in mouse oocytes will be discussed in a separate publication.

Mouse oocytes synthesized about the same amounts of T-Ag and V-Ag as did 10^3 SV40-infected CV-1 cells and about the same amount of viral RNA as observed in 150 virus-infected CV-1 cells. Therefore, transcription and particularly translation of SV40 DNA in mouse oocytes was comparable to, if not significantly greater than, that in permissive monkey fibroblasts. This did not result simply from injecting large amounts of DNA into each cell. We routinely injected 0.1 to 0.5 pg of DNA per oocyte (17,000 to 85,000 SV40 genomes) or about the same amount of DNA per cell found late during infection of CV-1 cells ($10 \mu\text{g}/6 \times 10^6$ cells). Reducing the amount of DNA injected by 10-fold (0.01 pg or 1,700 genomes per cell) reduced protein synthesis only threefold, and the ratio of T-Ag to V-Ag was the same. Graessmann et al. (15) found that 2,000 to 4,000 copies of SV40 DNA had to be injected into each nonpermissive mouse fibroblast nucleus to detect late gene expression, whereas only one to two copies per cell were needed to

detect T-Ag. Correcting for the smaller volume of nuclei compared with germinal vesicles (about 200 $\times$), 1,700 copies of SV40 DNA per oocyte was equivalent to about 8 copies of DNA per mouse fibroblast. Moreover, when we injected the same amount of SV40 DNA into the nuclei of mouse embryos that was routinely injected into mouse oocytes (0.5 pg), neither T-Ag nor t-Ag synthesis was detected. Therefore, the efficient, concomitant expression of SV40 early and late genes in oocytes was not a result of the amount of DNA injected but of a difference in the control of gene expression in mouse oocytes compared with mouse embryos and differentiated cells.

SV40 (31, 33, 41) as well as other genes (reviewed in reference 12) have also been injected into *X. laevis* oocytes. The large size of amphibian oocytes makes them a convenient system in which to study the regulation of eucaryotic gene expression in germ cells, although interpretation of results is complicated when nonamphibian genes are used. Moreover, regulation of gene expression during early amphibian development may differ significantly from early mammalian development, as was in fact observed with DNA replication (42). However, the fact that the characteristics of SV40 gene expression in mouse oocytes is similar to that observed in frog oocytes suggests that *X. laevis* oocytes are representative of other germ cells.

Temporal regulation of SV40 gene expression was absent in both mouse and frog oocytes, and V-Ag genes were more efficiently expressed than T-Ag and t-Ag genes. V-Ag mRNA was 5- to 10-fold more abundant than T-Ag mRNA in frog oocytes, and V-Ag was 3-fold more abundant than T-Ag in mouse oocytes. SV40-specific RNA was present in both cases for at least 24 h postinjection (33), and gene expression was observed only when DNA was injected into germinal vesicles. The conformational changes observed after injecting SV40 form I DNA into mouse oocytes (Fig. 3) were also observed after injection of form I DNA into *Xenopus* oocytes, where they were shown to result from nucleosome assembly (13, 32, 43). Nucleosome assembly was completed more quickly in *Xenopus* oocytes (2 h) than in mouse oocytes (5 h), consistent with the higher concentrations of histones in *Xenopus* oocytes (27, 40). SV40-specific RNA was detected in mouse oocytes as early as 3 h postinjection (data not shown), suggesting that nucleosome assembly need not be completed prior to transcription. Gene expression from linear DNA was substantially reduced compared with circular DNA in either *Xenopus* (19, 32) or mouse (Fig. 1) oocytes. This may be attributed to the poor ability of linear DNA to form regularly spaced nucleosomes (30) as well as its lack of torsional strain (19). The small amount (5%) of protein synthesis from linear SV40 DNA likely occurred by reannealing of its staggered ends produced when it was cut by *Bam*HI (25).

Expression of SV40 genes in mouse preimplantation embryos was at least 500-fold lower than in mouse oocytes. Immunoprecipitation assays failed to detect either T-Ag or V-Ag at a level equivalent to 0.2% of that observed in oocytes (Fig. 8), and blotting-hybridization assays failed to detect mRNA. Nevertheless, both SV40 and polyomavirus T-Ag were synthesized at a low level in embryonic cells because plasmids containing the SV40 *ori* sequence (Fig. 4) or the polyomavirus *ori* sequence (42) replicated only when a functional SV40 or polyomavirus T-Ag gene was also present in the same nuclei. Similarly, the rate of transcription for *Xenopus* globin genes was about 100-fold lower in *Xenopus* eggs than in *Xenopus* oocytes (3).

As mouse embryonic cells undergo differentiation, their

ability to express SV40 and polyomavirus genes is greatly enhanced. Both SV40 T-Ag and polyomavirus T-Ag were detected by immunofluorescent staining in nuclei of trophoblast cells (differentiated cells in mouse blastocysts), but not in the inner cell mass (stem cells), after infection of mouse blastocysts with high multiplicities of either virus (1). Similarly, neither SV40 nor polyomavirus express their genes in mouse embryonal carcinoma cell lines unless these cells first undergo differentiation (23). Embryonal carcinoma cells are thought to represent pluripotent cells of early mouse embryos. Thus, both the temporal regulation and efficiency of expression of SV40 and polyomavirus genes vary significantly as mammalian development progresses from germ cells to fully differentiated cells, apparently reflecting changes in the ability of a cell to recognize specific transcriptional elements.

ACKNOWLEDGMENTS

We are indebted to Claude Lechene (Harvard Medical School) for providing the facilities to make micropipettes and to George Salzman and Jeffrey Greve for their advice and technical expertise.

This work was supported by a Public Health Service grant from the National Institutes of Health (HD 122275) and a grant from the American Heart Association (850-745). L.E.C. was supported by a fellowship from the Leukemia Society of America, Inc. D.O.W. was supported by a fellowship from the National Institutes of Health.

LITERATURE CITED

1. Abramczuk, J., A. Vorbroat, D. Solter, and H. Koprowski. 1978. Infection of pre-implantation embryos with SV40 and polyoma. *Proc. Natl. Acad. Sci. USA* 75:999-1003.
2. Acheson, N. H. 1981. Lytic cycle of SV40 and polyoma virus, p. 125-205. In J. R. Tooze (ed.), *DNA tumor viruses*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. Bendig, M. M., and J. G. Williams. 1984. Fidelity of transcription of *Xenopus laevis* globin genes injected into *Xenopus laevis* oocytes and unfertilized eggs. *Mol. Cell. Biol.* 4:2109-2119.
4. Biggers, J. D. 1971. New observations of the nutrition of the mammalian oocyte and the preimplantation embryo, p. 319-327. In R. J. Blandau (ed.), *The biology of the blastocyst*. University of Chicago Press, Chicago.
5. Birkenmeier, E. H., E. May, and N. P. Salzman. 1977. Characterization of simian virus 40 *tsA58* transcriptional intermediates at restrictive temperatures: relationship between DNA replication and transcription. *J. Virol.* 22:702-710.
6. Brady, J., and G. Khoury. 1985. *trans* activation of the simian virus 40 late transcription unit by T-antigen. *Mol. Cell. Biol.* 5:1391-1399.
7. Bonner, W. M., and R. A. Laskey. 1974. A film detection method for tritium-labelled protein and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* 46:83-88.
8. Buchman, A. R., M. Fromm, and P. Berg. 1984. Complex regulation of simian virus 40 early-region transcription from different overlapping promoters. *Mol. Cell. Biol.* 4:1900-1914.
9. Chia, W., and P. W. J. Rigby. 1981. Fate of viral DNA in nonpermissive cells infected with simian virus 40. *Proc. Natl. Acad. Sci. USA* 78:6638-6642.
10. Contreras, R., D. Gheysen, J. Knowland, A. van de Voorde, and W. Fiers. 1982. Evidence for the direct involvement of DNA replication origin in synthesis of late SV40 RNA. *Nature (London)* 300:500-505.
11. Ernoult-Lange, M., and E. May. 1983. Evidence of transcription from the late region of the integrated simian virus 40 genome in transformed cells: location of the 5'-ends of late transcripts in cells abortively infected and in cells transformed by simian virus 40. *J. Virol.* 46:756-767.
12. Etkin, L. D. 1982. Analysis of the mechanisms involved in gene regulation and cell differentiation by microinjection of purified genes and somatic cell nuclei into amphibian oocytes and eggs. *Differentiation* 21:149-159.

13. Gargiulo, G., F. Razvi, and A. Worcel. 1984. Assembly of transcriptionally active chromatin in *Xenopus* oocytes requires specific DNA binding factors. *Cell* 38:511–521.
14. Gottesman, M. M., and F. Cabral. 1981. Purification and characterization of a transformation-dependent protein secreted by cultured murine fibroblasts. *Biochemistry* 20:1659–1665.
15. Graessmann, A., M. Graessmann, and C. Mueller. 1976. Regulatory mechanism of simian virus 40 gene expression in permissive and nonpermissive cells. *J. Virol.* 17:854–858.
16. Graessmann, A., M. Graessmann, and C. Mueller. 1977. Regulatory function of simian virus 40 DNA replication for late viral gene expression. *Proc. Natl. Acad. Sci. USA* 74:4831–4834.
17. Gurney, E. G., R. O. Harrison, and J. Fenno. 1980. Monoclonal antibodies against simian virus 40 T antigens: evidence for distinct subclasses of large T antigen and for similarities among nonviral T antigens. *J. Virol.* 34:752–763.
18. Harland, R. M., and R. A. Laskey. 1980. Regulated replication of DNA microinjected into eggs of *Xenopus laevis*. *Cell* 21:761–771.
19. Harland, R. M., H. Weintraub, and S. L. McKnight. 1983. Transcription of DNA injected into *Xenopus* oocytes is influenced by template topology. *Nature (London)* 302:38–43.
20. Hartzell, S. W., B. J. Byrne, and K. N. Subramanian. 1984. The simian virus 40 minimal origin and the 72-bp repeat are required simultaneously for efficient induction of late gene expression with large tumor antigen. *Proc. Natl. Acad. Sci. USA* 81:6335–6339.
21. Huang, P. L., B. E. Roberts, D. McMahon-Pratt, J. R. David, and J. S. Miller. 1984. Structure and arrangement of the β -tubulin genes of *Leishmania tropica*. *Mol. Cell. Biol.* 4:1372–1383.
22. Keller, J. M., and J. C. Alwine. 1985. Analysis of an activatable promoter: sequences in the simian virus 40 late promoter required for T-antigen-mediated *trans* activation. *Mol. Cell. Biol.* 5:1859–1869.
23. Kelly, F., and H. Condamine. 1982. Tumor viruses and early mouse embryos. *Biochim. Biophys. Acta* 651:106–141.
24. Khoury, G., and E. May. 1977. Regulation of early and late simian virus 40 transcription: overproduction of early viral DNA in the absence of a functional T-antigen. *J. Virol.* 23:167–176.
25. Kopchick, J. J., and D. W. Stacey. 1984. Differences in intracellular DNA ligation after microinjection and transfection. *Mol. Cell. Biol.* 4:240–246.
26. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680–684.
27. Laskey, R. A., A. D. Mills, and N. R. Morris. 1977. Assembly of SV40 chromatin in a cell-free system from *Xenopus* eggs. *Cell* 10:237–243.
28. Leibowitz, P., and S. M. Weissman. 1979. Organization and transcription of the SV40 genome. *Curr. Top. Microbiol. Immunol.* 87:43–172.
29. Lusky, M., and M. Botchan. 1981. Inhibition of SV40 replication in simian cells by specific pBR322 DNA sequences. *Nature (London)* 293:79–81.
30. Mertz, J. E. 1982. Linear DNA does not form chromatin containing regularly spaced nucleosomes. *Mol. Cell. Biol.* 2:1608–1618.
31. Michaeli, T., and C. Prives. 1985. Regulation of simian virus 40 gene expression in *Xenopus laevis* oocytes. *Mol. Cell. Biol.* 5:2019–2028.
32. Miller, T. J., and J. E. Mertz. 1982. Template structural requirements for transcription *in vivo* by RNA polymerase II. *Mol. Cell. Biol.* 2:1595–1607.
33. Miller, T. J., D. L. Stephens, and J. E. Mertz. 1982. Kinetics of accumulation and processing of simian virus 40 RNA in *Xenopus laevis* oocytes injected with simian virus 40 DNA. *Mol. Cell. Biol.* 2:1581–1594.
34. Parker, B. A., and G. R. Stark. 1979. Regulation of simian virus 40 transcription: sensitive analysis of the RNA species present early in infections by virus or viral DNA. *J. Virol.* 31:360–369.
35. Slack, J. M. W. 1983. From egg to embryo. Cambridge University Press, Cambridge.
36. Stern, S., and P. M. Wassarman. 1974. Meiotic maturation of the mammalian oocytes *in vitro*: effect of dibutyryl cyclic AMP on protein synthesis. *J. Exp. Zool.* 189:275–281.
37. Tabor, S., and C. C. Richardson. 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. *Proc. Natl. Acad. Sci. USA* 82:1074–1078.
38. Wassarman, P. M. 1983. Oogenesis: synthetic events in the developing mammalian egg, p. 50–76. *In* J. F. Hartmann (ed.), Mechanism and control of animal fertilization. Academic Press, Inc., New York.
39. Wassarman, P. M., and W. J. Josefowicz. 1978. Oocyte development in the mouse: an ultra-structural comparison of oocytes isolated at various stages of growth and meiotic competence. *J. Morphol.* 156:209–235.
40. Wassarman, P. M., and S. C. Mrozak. 1981. Program of early development in the mammal: synthesis and intracellular migration of histone H4 during oogenesis in the mouse. *Dev. Biol.* 84:364–371.
41. Wickens, M. P., and J. B. Gurdon. 1983. Post-transcriptional processing of SV40 late transcripts in injected *Xenopus* oocytes. *J. Mol. Biol.* 163:1–26.
42. Wirak, D. O., L. E. Chalifour, P. M. Wassarman, W. J. Muller, J. A. Hassell, and M. L. DePamphilis. 1985. Sequence-dependent DNA replication in preimplantation mouse embryos. *Mol. Cell. Biol.* 5:2924–2935.
43. Wyllie, A. H., R. A. Laskey, J. Finch, and J. B. Gurdon. 1978. Selective DNA conservation and chromatin assembly after injection of SV40 DNA into *Xenopus* oocytes. *Dev. Biol.* 64:64–178.