DNAs of simian virus 40 and polyoma direct the synthesis of viral tumor antigens and capsid proteins in *Xenopus* oocytes

(microinjection/coupled transcription and translation/immunoprecipitation/mRNA splicing)

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ABSTRACT   Purified simian virus 40 and polyoma DNAs injected into nuclei of *Xenopus* oocytes were transcribed and subsequently translated into virus-specific tumor antigens and capsid proteins. Simian virus 40 large and small tumor antigens synthesized in the oocytes were indistinguishable, by gel electrophoresis and [³⁵S]methionine-labeled tryptic peptide mapping, from the corresponding polypeptides synthesized in CV-1 African green monkey cells. The synthesis of large simian virus 40 tumor antigen implies the correct splicing of its mRNA, which is complementary to nonadjacent nucleotide sequences in the early region of the viral genome. Polyoma DNA directed synthesis of two polyoma tumor antigen polypeptides, 57,000 M_r and small tumor antigen, and of the main capsid protein.

Nuclei from various eukaryotic cells injected into frog oocyte nuclei (germinal vesicles) continue to direct synthesis of origin-specific proteins (1). In addition, genes that were not expressed in the donor cell may be activated after injection (2). Purified viral, phage, or plasmid DNAs injected into the nucleus of *Xenopus* oocytes serve as templates for the transcription of specific RNA sequences (3). The synthesis of 5S RNA (4) and tRNA (5) is greatly stimulated upon injection of the corresponding templates. It has been claimed that, after injection of simian virus 40 (SV40) DNA I (6), much of the virus-specific RNA synthesized in the *Xenopus* oocyte has the same size and is transcribed from the same region as the late viral mRNAs present in lytically infected monkey cells (7, 8), and proteins that migrate on two-dimensional gels in the same manner as the SV40 capsid proteins VP 1 and VP 3 were detected (8).

In this article we report that *Xenopus* oocytes transcribe and translate most or all of the genetic information contained in the SV40 genome. In particular, we observed the synthesis of large (T) and small (t) SV40 tumor antigens having the same characteristics as those synthesized in monkey cells undergoing lytic infection. Injection of polyoma DNA I (6) resulted in the synthesis of three polyoma-specific polypeptides comigrating, during gel electrophoresis, with two minor polypeptides related to polyoma tumor antigen and with the major capsid protein, respectively.

MATERIALS AND METHODS

SV40 DNA I (covalently closed circular) was isolated from SV40-infected CV-1 African green monkey cells (Flow Laboratories, Irvine, Scotland) 40–48 hr after infection by selective extraction, deproteinized with phenol, and purified by CsCl/ethidium bromide density gradient equilibrium centrifugation (9). Polyoma DNA I was isolated from polyoma-infected mouse kidney cell cultures 30–35 hr after infection by the same procedure. For injection, the viral DNA was dissolved in 88 mM NaCl/1 mM KCl/15 mM Tris·HCl, pH 7.6 at concentrations between 300 and 500 µg/ml.

The procedure used for injecting viral DNA into the germinal vesicle of *Xenopus* oocytes was a modification of already established microinjection techniques for DNA (4, 10) and mRNA (11, 12). Pieces of ovaries were excised from *Xenopus laevis* females anesthetized with tricaine (MS 222; Sandoz, Basel). Stage 5 and 6 oocytes (13) were defolliculated by collagenase treatment (14), extensively washed with modified Barth's solution (15), and maintained in this buffer at 20°C during all further manipulations and incubations. The oocytes were placed onto a 700-µm mesh grid (Züricher Beuteltuch Fabrik, Rüschlikon) fixed to the bottom of a 6-cm petri dish and centrifuged (10) in a MSE 2-liter swing-out rotor at 400 × g for 10 min. During centrifugation the germinal vesicles rise to the surface of the oocytes and their position is indicated by a clear area in the dark animal pole. The oocytes were gently removed from the grid, transferred to a depression slide, and positioned for injection by means of watchmaker's forceps. The glass capillaries used for injections had a tip diameter of about 15 µm and were calibrated to deliver about 15 nl, containing 5–7 ng of viral DNA. A micromanipulator (Singer Instruments Co., Somerset, England) and a micrometer syringe (Agla Wellcome Research Laboratories, Beckenham, England) allowed rapid injection. The injected oocytes (about 50–100 per series) were incubated for about 24 hr in Barth's solution containing 50 units per ml each of streptomycin, penicillin, and kanamycin. Thereafter 1 ml of Barth's solution containing 300 µCi of [³⁵S]methionine (500–1000 Ci/mmol, the Radiochemical Centre, Amersham, England) was added and incubation was continued for another 24 hr (1 Ci = 3.7 × 10¹⁰ becquerels). The surviving oocytes (60–90%) were washed first with Barth's solution, then with distilled water, and stored at −80°C after removal of the water.

For extraction of viral proteins, the oocytes were disrupted in a small Dounce homogenizer in 2 ml of a buffer containing detergent (0.1 M Tris·HCl, pH 9/0.1 M NaCl/5 mM KCl/1 mM CaCl₂/0.5 mM MgCl₂/0.7 mM Na₂HPO₄/0.5% Nonidet P-40). The homogenate was sonicated in a MSE ultrasonic disintegrator (Mk 1) for 1 min with an amplitude of 10 µm and kept on ice for 20 min. After centrifugation at 15,000 × g for 30 min at 4°C the supernatant was divided into aliquots and 10 µl of antiserum was added to each. The immune complexes were isolated by means of staphylococcal protein A-Sepharose (16) (Pharmacia, Uppsala, Sweden) and the proteins were analyzed by electrophoresis on a 12.5% sodium dodecyl sulfate (NaDodSO₄)/polyacrylamide gel (17).

For tryptic peptide analysis, the 88,000 M_r and 18,000 M_r polypeptides shown in Fig. 1, slot 1, were eluted from a preparative 12.5% NaDodSO₄/polyacrylamide gel with 0.25 M ammonium carbonate containing 0.1% NaDodSO₄ and 50 µg

Abbreviations: SV40, simian virus 40; t antigen, small tumor antigen; T antigen, large tumor antigen; NaDodSO₄, sodium dodecyl sulfate.

of bovine serum albumin per ml as carrier. Large (88,000 M_r) and small (18,000 M_r) tumor antigens synthesized in SV40-infected CV-1 cells were isolated by the method of Schwyzer (16) from 10 cultures that had each been labeled with 100 μCi of [^{35}S]methionine from 27 to 31 hr after infection and were eluted after preparative gel electrophoresis as described above. The proteins were precipitated with trichloroacetic acid, oxidized with performic acid, and digested with trypsin (18, 19). The digests were dissolved in 10 μl of water and spotted on 20 $\times$ 20 cm Polygram Cell 300 precoated plastic sheets (Macherey–Nagel, Düren, Germany). The solvent for the first dimension was pyridine (175 ml)/isoamylalcohol (175 ml)/water (150 ml) and for the second dimension n-butanol (150 ml)/pyridine (120 ml)/acetic acid (30 ml)/water (120 ml) (20). For fluorography the sheets were run in acetone containing 20% 2,5-diphenyloxazole (PPO). Preexposed (hypersensitizing light flash) (21) Kodak RP Royal X-Omat films were exposed to the chromatograms for 2–3 weeks at −70°C.

RESULTS

Identification of SV40-specific polypeptides synthesized in the oocyte

Virus-specific polypeptides were isolated from the oocyte lysate by using specific antisera, characterized by NaDodSO$_4$/polyacrylamide gel electrophoresis (17), and compared to known virus-specific proteins extracted by the same methods from infected cells. In the experiment shown in Fig. 1, SV40-specific proteins were isolated from oocytes injected with SV40 DNA

I with two different antisera. Anti-SV40 T serum from tumor-bearing hamsters (22) strongly complexed two polypeptides with apparent M_r values of 88,000 and 18,000 (Fig. 1, slot 1). A commercial SV40 hyperimmune rabbit serum (Microbiological Associates, Bethesda, MD) also complexed the 88,000 and 18,000 M_r polypeptides, but in addition precipitated a polypeptide with an apparent M_r of about 46,000 (Fig. 1, slot 2). Fig. 1, slot 3, shows the pattern of all radioactive proteins present in the oocyte extract. Radioactive bands that might correspond to 18,000, 46,000, and 88,000 M_r SV40-specific polypeptides can be detected in this autoradiogram. As determined by trichloroacetic acid precipitation, the immune complexes represented in these experiments 0.25–1% of total radioactivity incorporated in the oocytes.

In order to test the specificity of the antisera used, a number of control experiments were done, using preimmune serum as well as anti-polyoma serum to precipitate extracts of SV40 DNA-injected oocytes. The results are shown in Fig. 2. The oocyte lysate (slot 1) was divided into aliquots that were treated with normal hamster serum (slot 2), hamster serum directed against polyoma T antigens (23) (slot 3), anti-SV40 T serum (22) (slot 4), and rabbit antiserum directed against purified SV40 capsids (slot 5) (a gift from E. May, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France). Slot 6 shows the pattern of [^{35}S]methionine-labeled proteins from purified SV40. Although in this experiment the isolated immune complexes were contaminated with oocyte proteins, Fig. 2 shows that the 46,000 M_r polypeptide was specifically bound by antiserum against SV40 capsid (slot 5) and that it comigrated with the main capsid protein (VP 1) of SV40 (slot 6). The 88,000 and 18,000 M_r polypeptides were specifically bound by anti-SV40 T serum (slot 4) but not by preimmune serum (slot 2), anti-
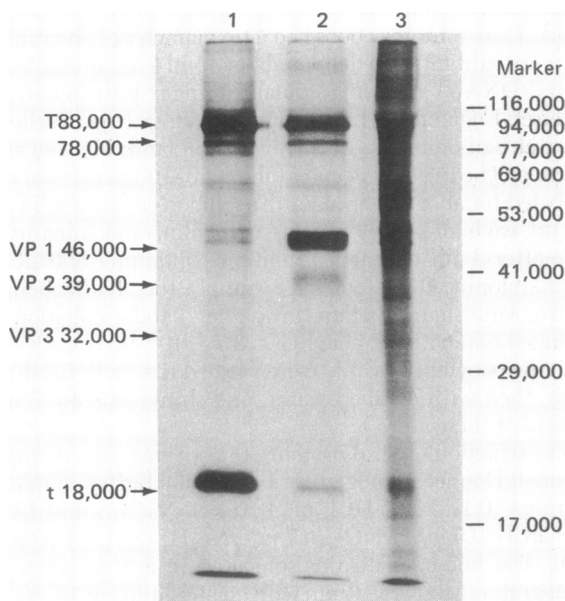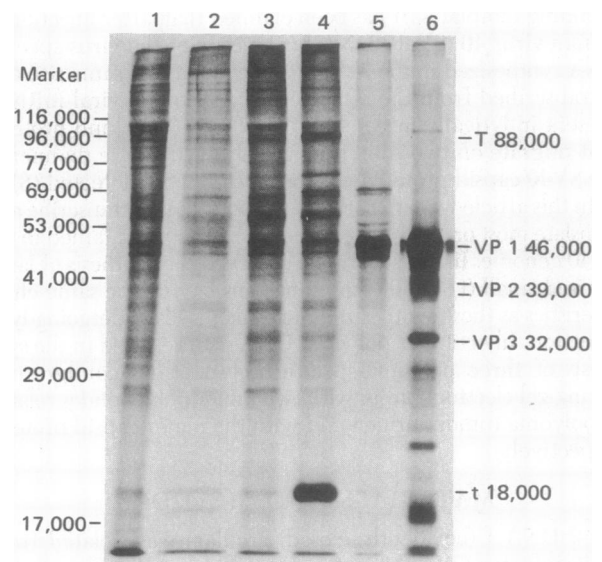

FIG. 1.   Polypeptides synthesized in oocytes injected with SV40 DNA I. The proteins of 50 oocytes, injected with SV40 DNA and incubated from 26 to 52 hr after injection with [^{35}S]methionine at 300 μCi/ml were extracted with 2 ml of extraction buffer. The extract was divided into two aliquots to which 10 μl of either anti-SV40 T serum (22) (slot 1) or serum directed against SV40 virus (Microbiological Associates) (slot 2) was added. The immune complexes were isolated by means of staphylococcal protein A-Sepharose (16) and the proteins were analyzed by electrophoresis on a 12.5% NaDodSO$_4$/polyacrylamide gel (17). Slot 3: 2 μl of the oocyte extract in 10 μl of loading buffer was directly put on the gel. The arrows to the left indicate the expected position of SV40 proteins (T, t, VP) and the figures give their nominal M_r. The figures to the right indicate the position of marker proteins: 116,000 M_r β-galactosidase, 96,000 M_r phosphorylase a, 77,000 M_r transferrin, 69,000 M_r bovine serum albumin, 53,000 M_r glutamate dehydrogenase, 41,000 M_r alcohol dehydrogenase, 29,000 M_r carbonic anhydrase, 17,000 M_r myoglobin.

FIG. 2.   Characterization of SV40-specific polypeptides synthesized in oocytes. From 47 oocytes, injected with SV40 DNA I and labeled with [^{35}S]methionine from 20 to 48 hr after injection, proteins were extracted with 2 ml of extraction buffer. A small aliquot of the extract was diluted in loading buffer for gel electrophoresis (slot 1). To 0.5-ml aliquots of the extract, 5 μl of serum was added. Slot 2: normal hamster serum; slot 3: anti-polyoma T serum (hamster) (23); slot 4: anti-SV40 T serum (hamster) (22); slot 5: anti-SV40 capsid serum (rabbit). The isolated immune complexes were subjected to NaDodSO$_4$/polyacrylamide gel electrophoresis (12.5% gel) together with a sample of [^{35}S]methionine-labeled SV40 (slot 6) purified from a lysate of SV40-infected CV-1 cells. The positions of the marker proteins (see legend to Fig. 1) are indicated to the left; to the right, the apparent M_r values of SV40-specific proteins synthesized in the oocytes or present in SV40 virus.

polyoma T serum (slot 3), or anti-SV40 capsid serum (slot 5). In this experiment a faint band representing most likely a contaminating oocyte protein that comigrated with the strong 18,000 M_r SV40-specific polypeptide can be seen in slots 2, 3, and 5. Furthermore, neither the 88,000 nor the 18,000 M_r polypeptide bands were observed when extracts of oocytes that had been injected with polyoma DNA I were treated with the same anti-SV40 T serum (see Fig. 5, slot 2). The specific reaction with antisera and the electrophoretic mobilities observed suggest that the strong bands of SV40-specific polypeptides synthesized in the injected oocytes correspond to large SV40 T antigen (88,000 M_r) (16, 22, 24), small t antigen (18,000 M_r (25, 26), and the main capsid protein VP 1 (46,000 M_r) (24, 27).

The electrophoretic mobilities of the SV40 T antigens and of the capsid proteins synthesized in the oocytes were compared to those of the corresponding proteins synthesized in monkey CV-1 cells undergoing lytic infection with SV40 and run on the same gel. The anti-T and anti-capsid sera used were the same as in Fig. 2, slots 4 and 5, respectively. As is shown in Fig. 3, the putative large T and small t antigens isolated from oocytes (slot 3) comigrated with large T and small t antigens isolated from SV40-injected CV-1 cells (slot 4). The main capsid protein (VP 1) was resolved into two or three bands, which differed in intensity in VP 1 isolated from cells or from oocytes (slot 2); this might reflect differences in the extent of modification of the polypeptides. In this experiment contamination of the immune complexes with oocyte proteins was very low and no contami-

nating 18,000 M_r oocyte polypeptide could be observed in immune complexes isolated with anti-capsid serum (slot 2). In all extracts of oocytes injected with SV40 DNA and treated with commercial anti-SV40 serum (Fig. 1) or anti-SV40 capsid serum (Figs. 2 and 3), a faint discrete band with an electrophoretic mobility corresponding to VP 3 (32,000 M_r) (24, 28) and a rather diffuse zone instead of a discrete band of VP 2 (39,000 M_r) (24, 28) were observed (see Fig. 1, slot 2, and Fig. 3, slot 2).

For reasons that will be discussed below, the formation of large SV40 T antigen in injected oocytes is of major interest. Therefore, the putative large and small SV40 tumor antigens were further identified by two-dimensional chromatographic analysis of the [35S]methionine-labeled tryptic peptides. The patterns obtained from the 88,000 and 18,000 M_r bands isolated in the experiment shown in Fig. 1, slot 1, are represented in Fig. 4 a and c, respectively, and are compared to those obtained from large and small SV40 tumor antigens synthesized in infected CV-1 cells (Fig. 4 b and d, respectively). Chromatography of the tumor antigens from the oocytes and from infected cells was done in different tanks and at different times, which might account for the minor differences in migration of some spots. Nevertheless, the overall picture clearly shows that the fingerprints represented in Fig. 4 a and c are very similar to those given in Fig. 4 b and d, respectively. In both cases the small t antigens (4 c and d) share spots with the large T antigens (4 a and b), and the same spots that are present in large T antigens (4 a and b) and indicated by arrows are absent in small t antigens (4 c and d). Analysis of the 78,000 M_r band (Fig. 1, slot 1) by the same method revealed that this band also is related to large SV40 T antigen (not shown).

These results demonstrate that injection of SV40 DNA I into Xenopus oocytes evokes the synthesis of SV40 large T and small t antigens that are indistinguishable by the analytical methods used (immunoprecipitation, gel electrophoresis, and fingerprinting) from the T and t antigens synthesized in SV40-infected monkey cells.
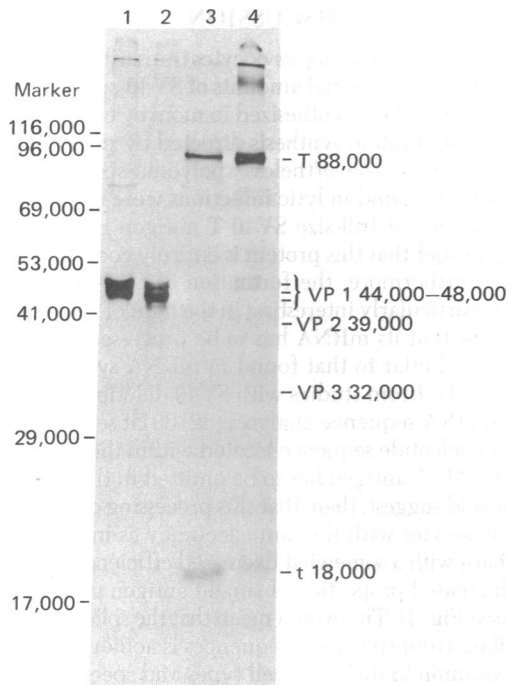

FIG. 3. Comparison of SV40 proteins synthesized in oocytes injected with SV40 DNA I and in SV40-infected CV-1 cells. Fifty oocytes, injected with SV40 DNA I and labeled with [35S]methionine from 36 to 60 hr after injection, were extracted as described for Fig. 1. One culture (9-cm diameter) of SV40-infected CV-1 cells was labeled with 50 μCi of [35S]methionine from 41 to 45 hr after infection and extracted as described earlier (16). The isolated immune complexes were analyzed by electrophoresis on a 12.5% NaDodSO₄/polyacrylamide gel. Slot 1: proteins isolated from CV-1 cells with serum directed against purified SV40 capsids. Slot 2: proteins isolated from oocytes with anti-SV40 capsid serum. Slot 3: proteins isolated from oocytes with anti-SV40 T serum (22). Slot 4: proteins isolated from CV-1 cells with anti-SV40 T serum (22).
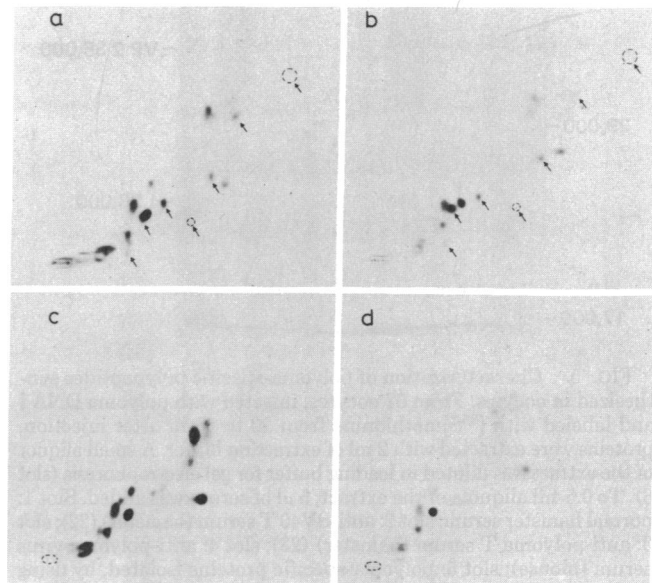

FIG. 4. Tryptic peptide analysis of large and small SV40 tumor antigens synthesized in oocytes and in CV-1 cells. (a) The 88,000 M_r T antigen synthesized in oocytes; (b) 88,000 M_r T antigen synthesized in CV-1 cells; (c) 18,000 M_r t antigen synthesized in oocytes; (d) 18,000 M_r t antigen synthesized in CV-1 cells. The arrows indicate spots present only in 88,000 M_r T antigen.

Polyoma-specific polypeptides synthesized in the oocytes

Using the same procedures, we also looked for the synthesis of polyoma-specific polypeptides in oocytes that had been injected with polyoma DNA I. These experiments also serve as a mock DNA injection control for the results obtained with SV40 DNA. Fig. 5 shows the autoradiographs of proteins isolated by means of various sera. Aliquots of the oocyte extract (slot 6) were precipitated with preimmune hamster serum (slot 1), anti-SV40 T serum (slot 2), anti-polyoma T serum (23) (slot 3), and a commercial mouse antiserum against polyoma virus (slot 4; Microbiological Associates, Bethesda, MD). Slot 5 was loaded with polyoma-specific proteins that were isolated from poly-oma-infected mouse kidney cells by using anti-polyoma T serum, which also binds the viral capsid proteins (23). In this extract the large polyoma T antigen (98,000 M_r), small polyoma t antigen (23,000 M_r), and VP 1 of polyoma virus (47,000 M_r) were clearly visible. Two additional, minor bands related to polyoma T antigen and migrating as if their M_r values were about 61,000 and 57,000 were rather weak in this experiment, but they can usually be detected in extracts of mouse kidney cells (23). Similar results on the characterization of polyoma tumor antigens in lytic infection differing slightly in the estimated M_r have been published by other groups (29, 30). The commercial polyoma virus antiserum (slot 4) bound a strong band comigrating with VP 1 present in infected mouse kidney cells (slot 5) and a slightly faster moving band. The same two bands were also bound by the anti-polyoma T serum (slot 3), which in addition bound a rather strong band at about 57,000 M_r and a band at 23,000 M_r; the latter two comigrated with 57,000 M_r and small polyoma t antigen from infected cells (slot 5). None of these bands were present in oocyte extracts treated with preimmune serum or anti-SV40 T serum, nor were they observed in an extract of SV40-injected oocytes treated with the same anti-polyoma T serum (see Fig. 2, slot 3). The specific reaction with the antiserum and the electrophoretic mobilities observed suggest that the 57,000 and 23,000 M_r polypeptides correspond to 57,000 M_r and small polyoma t antigen and that the 47,000 M_r band corresponds to VP 1 of polyoma virus. While these three bands were observed in all experiments, the band moving slightly faster than VP 1 was observed only in this experiment; it might represent unmodified or incompletely modified VP 1. So far none of the polyoma-specific polypeptides synthesized in the oocytes has been identified further. In the experiments done so far we were unable to detect the synthesis of a new protein corresponding to large polyoma T antigen. Its detection is, however, rendered difficult by a strong, Coomassie blue-staining, unlabeled oocyte protein migrating at 100,000 M_r that interferes with the resolution of the radioactive bands in this region (see Fig. 5, slots 1, 2, and 3). Although the total incorporation of [35S]methionine into proteins was similar for oocytes injected with SV40 DNA and polyoma DNA, synthesis of polyoma-specific polypeptides seems to be somewhat less efficient than that of SV40-specific polypeptides.
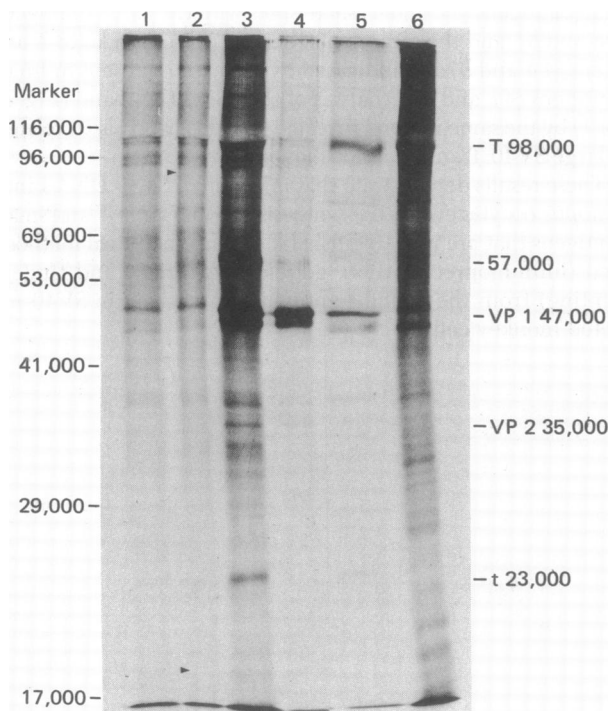

FIG. 5.   Characterization of polyoma-specific polypeptides synthesized in oocytes. From 67 oocytes, injected with polyoma DNA I and labeled with [35S]methionine from 20 to 48 hr after injection, proteins were extracted with 2 ml of extraction buffer. A small aliquot of the extract was diluted in loading buffer for gel electrophoresis (slot 6). To 0.5-ml aliquots of the extract, 5 μl of serum was added. Slot 1: normal hamster serum; slot 2: anti-SV40 T serum (hamster) (22); slot 3: anti-polyoma T serum (hamster) (23); slot 4: anti-polyoma virus serum (mouse); slot 5: polyoma-specific proteins isolated, by using anti-polyoma T serum (23), from mouse kidney cells infected with polyoma virus at 27°C and labeled with [35S]methionine from 40 to 48 hr after infection. On the left are indicated the positions of marker proteins, and on the right, the apparent M_r values of polyoma-specific proteins synthesized in polyoma-infected mouse kidney cells. The arrowheads (slot 2) indicate the positions of SV40 tumor antigens, which are not synthesized after injection of polyoma DNA.

DISCUSSION

Our results show that *Xenopus* oocytes transcribe and translate SV40 DNA into substantial amounts of SV40-specific proteins closely similar to those synthesized in monkey cells undergoing lytic infection. Protein synthesis directed by polyoma DNA I was less efficient; nevertheless, polyoma-specific proteins similar to those found in lytic infections were formed.

The synthesis of full-size SV40 T antigen in the oocyte is additional proof that this protein is entirely coded by the viral genome. Furthermore, the formation of 88,000 M_r SV40 T antigen is particularly interesting in the light of recent findings that suggest that its mRNA has to be processed by a splicing mechanism similar to that found in mRNA synthesis of adenovirus 2 (31). From studies with SV40 deletion mutants (26) and from DNA sequence analyses (32, 33) it seems likely that a certain nucleotide sequence located within the coding region for 88,000 M_r T antigen has to be omitted in the mRNA. Our results would suggest, then, that this processing of SV40 mRNA occurs in oocytes with the same accuracy as in monkey cells, but perhaps with a somewhat decreased efficiency, as indicated by the increased proportion of small t antigen produced in the oocyte (see Fig. 3). This would mean that the splicing of mRNAs transcribed from split gene sequences is achieved by a mechanism common to different cell types and species.

Our results do not allow us to draw conclusions on the relative amounts of correct or abnormal virus-specific transcripts synthesized in the oocytes. In experiments using purified mRNAs (11, 12, 34, 35), synthesis of similar proportions of specific proteins could be induced by injection of 1–10 ng of mRNA per oocyte, strongly depending on the type of mRNA used. Because we injected 5–7 ng of DNA per oocyte (i.e., about 10^9 molecules), one or a few correct transcripts per injected genome might give the observed amount of protein synthesis. There were, however, no clear indications of abnormal transcription, because the majority of the SV40-specific proteins detected by the immunoassay were of the correct size. In another coupled

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transcription and translation system, derived from *Escherichia coli* and using SV40 DNA I as template, several polypeptides ranging from 12,000 to 59,000 M_r were immunoprecipitated by the same anti-SV40 T serum (36).

We do not know yet whether SV40 or polyoma DNA is able to replicate in the oocyte. During lytic infections with these viruses, viral capsids are synthesized in large amounts only after the onset of viral DNA replication (6, 37, 38), whereas T antigens are synthesized early in infection and are required for the induction of host and viral DNA replication (39, 40, 41). Further experiments are needed to determine whether in the oocytes the control of late viral gene expression (i.e., synthesis of viral capsids) is similar to or different from that operating in mammalian cells undergoing lytic infection.

Coupled transcription and translation of viral DNAs in *Xenopus* oocytes might provide a tool for studies on the control of gene expression and on processing of mRNAs. In addition, this system might be useful for characterizing the structure and function of proteins synthesized from DNA of defective viruses, in particular of nonviable deletion mutants.

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