REPLICATION OF HETEROLOGOUS DNA IN *XENOPUS LAEVIS* OOCYTES

E. Z. GOLDBERG, B. S. NARODITSKY, P. E. FELGENHAUER, M. M. GARAEV and T. I. TIKCHONENKO
D. I. Ivanovsky Institute of Virology, USSR Academy of Medical Sciences, Moscow, USSR

Received 3 November 1980; revised version received 5 January 1981

1. Introduction

Xenopus laevis oocytes have become a valuable tool for molecular biology. Nuclei from various eukaryotic cells and mRNA from different sources may direct synthesis of origin-specific proteins in oocytes [1,2]. Purified viral, phage or plasmid DNAs injected into the nucleus of *Xenopus* oocytes serve as templates for the transcription of specific RNA sequences [3–5]. Moreover, the synthesized RNA is processed and translated in oocytes [6,7]. The fact that SV40 RNA can be translated into the expected large and small T-antigens is good evidence of splicing activity in oocytes [8].

In [9–11], purified DNA was replicated in the cytoplasm of the *Xenopus laevis* egg. However, few attempts were made to investigate the replication of DNA injected into the germinal vesicle (GV) of *Xenopus* oocytes.

Although in [10] double-stranded DNA was not replicated when injected into the cytoplasm of the oocyte (V–VI stage), this result was explained in [11] by degradation of the DNA in the cytoplasm of the oocyte. Lack of synthesis of nuclear DNA, a characteristic of the oocytes at stage V–VI [12], together with the replication of injected genome, would make *Xenopus* oocytes a unique experimental system.

Here, we report that *Xenopus* oocytes may replicate injected heterologous pro- and eukaryotic DNAs from different sources.

We injected DNAs of human (Ad6) and simian (SA7) adenoviruses (Ad), SA7 and Ad6 DNA–terminal protein complexes (DNA–T_p), fragments of these DNAs after hydrolysis with the restriction endonuclease *Sal*I, the isolated *Bgl*II fragment of SA7 DNA, calf thymus DNA and plasmid pBR322 DNA.

2. Materials and methods

Adenovirus SA7 and Ad6 DNAs and DNA–terminal protein complexes were isolated from purified virions as in [13,14]. Digestions by restriction nucleases *Eco*RI, *Bam*HI, *Bgl*II, *Sal*I and *Hin*dIII were performed as in [15]. Fragments of Ad DNAs were isolated from agarose gel as in [15]. Plasmid pBR322 DNA was isolated as in [16]. Genetic transformation of *Escherichia coli* C 600 was done as in [17].

Stage V–VI oocytes [12] obtained from female frogs were prepared as in [3]. Oocytes were injected with 50–80 nl of sample into the nucleus, GV or cytoplasm of an oocyte. The DNA samples contained 1–100 μg DNA diluted into 'injection medium': 10 mM Hepes (pH 7.4), 88 mM NaCl, 0.5 mM EDTA and 10 Ci/ml d[α-^{32}P]NTP (2000–3000 Ci/mMol, Amersham). After incubation the oocytes were defolliculated following treatment with pronase (500 μg/ml for 3 min at 19°C) [4]. DNA-injected oocytes were stored frozen in dry ice. For DNA isolation oocytes were homogenized in 50 mM Tris–HCl (pH 7.5), 10 mM EDTA, 0.5% SDS, 500 μg proteinase K/ml (Merck).

3. Results and discussion

Table 1 shows the results of an experiment in which oocyte nuclei were injected with DNA and d[α-^{32}P]-NTP. One can observe that the injection of heterologous DNA directs the synthesis of DNA in such a system. Ad, Ad-TP and pBR322 DNAs cause a 3–6-fold stimulation of dNTP incorporation into the trichloroacetic acid-precipitable DNA fraction as compared with the control. The injection of labeled precursors into the GV or the injection of the mixture of d[α-^{32}P]NTP with DNA into the cytoplasm of the oocyte served as controls. In both control preparations

Table 1
Stimulation of DNA synthesis in *Xenopus laevis* oocytes injected with heterologous DNA

Injected DNA[a]	cpm/oocyte	Stimulation
None	1500	1
Calf thymus DNA	3600	2.4
DNA-TP Ad[b]	8600	5.73
Ad DNA	6700	4.5
Ad DNA cleaved by *Sal*I	1800	1.2
Isolated *Bgl*II fragment of SA7 DNA	1300	0.87
Plasmid pBR322 DNA	3800	2.55
SA7 DNA, injected into the cytoplasm	1600	1.06

[a] DNA was injected into the GV of the oocyte, except when injection into the cytoplasm is mentioned. The incubation period was 22 h. DNA in the injection medium was 100 μg/ml

[b] Values of ^{32}P incorporation for SA7 and Ad6 DNAs were practically the same, thus these DNAs are indicated as Ad DNA

we obtained rather low level of d[α-^{32}P]NTP incorporation (1500 and 1600 cpm/oocyte, respectively; table 1).

Two facts underline the specific character of α-^{32}P-incorporation:

(i) The fact that injection of SA7 DNA cleaved with *Sal*I or of the isolated *Bgl*II fragments of the same DNA did not cause any stimulation. This negative result is understandable, since the adenoviral DNA replication begins from the ends of the molecule [18].

(ii) The best results were obtained after injection of the DNA–TP complex [14,18]. Such complexes possess high infectivity, when tested in susceptible cell cultures. They also have a higher replicative potential than deproteinized DNA. These data practically exclude the possibility that the observed results are due to repair of the heterologous DNA in oocytes.

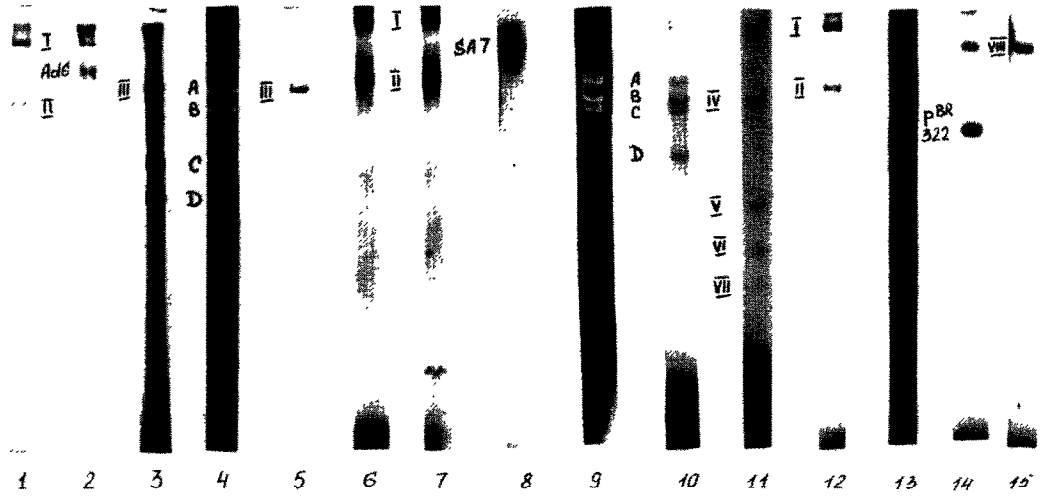

Fig.1. Electrophoretic analyses of restriction endonuclease digests of DNA isolated from *Xenopus laevis* oocytes in agarose gel. Bands marked by roman numerals correspond to the intact or digested oocyte mitochondrial DNA. Bands marked by letters correspond to the restriction fragments of Ad DNA. For the isolation of the DNA oocytes were homogenized in 50 mM Tris–HCl (pH 7.5), 10 mM EDTA, 0.5% SDS, 500 μg proteinase K/ml (Merck, FRG), incubated at 37°C for 2 h, and extracted twice with phenol–chloroform (1:1). Nucleic acids were recovered from the aqueous phase by precipitation with ethanol. RNA was eliminated by incubation with pancreatic RNase (Reanal) 50 μg/ml for 30 min at 37°C; the DNA was re-extracted with phenol–chloroform as above, and reprecipitated with ethanol. Samples were layered on 1% agarose slab gels for electrophoresis [15]. Gels were stained with ethidium bromide and photographed under UV light; if necessary they were dried and autoradiographed. Autoradiographs of: (1) DNA isolated from uninjected oocytes; (2) DNA isolated from oocytes injected with Ad6 DNA–TP; (3) *Bam*HI digests of DNA isolated from oocytes injected with Ad6 DNA–TP; (5) *Bam*HI digest of DNA from uninjected oocytes; (6) DNA isolated from oocytes injected with SA7 DNA hydrolysed by *Sal*I; (7) DNA isolated from oocytes injected with A *Bgl*II fragment SA7 DNA; (8) DNA isolated from oocytes injected with SA7 DNA–TP; (10) *Hin*dIII digest of DNA isolated from oocytes injected with SA7 DNA–TP; (11) *Hin*dIII digest of oocyte DNA; (12) DNA isolated from oocytes injected into the cytoplasm with SA7 DNA; (14) *Eco*RI digest of DNA isolated from oocytes injected with pBR322 DNA; (15) *Eco*RI digest of oocyte DNA. Photographs of: (4) *Bam*HI digest of Ad6 DNA; (9) *Hin*dIII digest of SA7 DNA; (13) *Eco*RI digest of plasmid pBR322 DNA.

The radioactive material isolated after 22 h incubation was analysed by agarose gel electrophoresis in order to determine the fidelity of incorporation of labeled precursors into the injected DNA. We analysed the intact DNA as well as the DNA cleaved by *Bam*HI, *Sal*I, *Hin*dIII, *Eco*RI. Fig.1 shows that injection of viral DNA–TP directs the synthesis of labeled DNA whose molecular weight and cleavage pattern is the same as that of DNA isolated from purified virions. However, in the track where viral DNA isolated from oocytes was analysed, one can see additional bands (roman numerals, fig.1), present also in control samples and corresponding to oocyte mitochondrial DNA (E. Z. G., unpublished).

Independent data confirming the possibility of heterologous DNA replication in *Xenopus* oocytes were obtained in experiments where the plasmid pBR322 DNA was injected into the GV. The DNA extracted after 22 h incubation was tested for its ability to produce genetic transformation of *E. coli* C 600. It is clear that if amplification of injected DNA takes place in oocytes, one can expect an increase of the transforming activity of the plasmid DNA. Table 2 shows that incubation of plasmid DNA in GV of *Xenopus* oocytes leads to an increase of the amount of pBR322 DNA capable of transforming bacterial cells. In oocytes injected with 0.5 ng plasmid DNA we observed that the amount of amplicyllin and tetracyclin-resistant clones increases 4–5-fold. The increase of transforming activity correlated well with stimulation of α-^{32}P incorporation into the trichloroacetic acid-precipitable DNA (table 1). It was important to investigate if the DNA preparation extracted from the oocytes contains any component that might influence the biological activity of plasmid DNA. Table 2 clearly indicates that DNA extracted from oocytes added to a known amount of pBR322 DNA does not increase the transforming activity of purified plasmid DNA. We would like to emphasize that the total amount of DNA in the samples extracted from oocytes and used for transformation was $\leqslant$0.1–0.2 μg/10^9 bacterial cells, i.e., there was a nearly linear increase in number of colonies with increasing amount of DNA [17].

We could not notice significant stimulation of transforming activity when 5 ng plasmid DNA were injected. The saturation obtained can be explained by the limited replicating capacity of the oocyte. In fact the amount of newly synthesized DNA according to our data is the same in both cases: 1 ng/22 h incubation. If one presumes that the rate of replication is a linear function of the incubation time then it is 50 pg/h; this corresponds to the rate of synthesis of nuclear DNA in *X. laevis* eggs [10].

Table 2
Biological activity of pBR322 DNA isolated from *Xenopus laevis* oocytes

Injected DNA (ng)[a]	Incubation time (h)	No. colonies/oocyte[d]	DNA isolated from 1 oocyte[b]	Stimulation
0.5 ng	0–1	9.9	0.265	1
	22	41.9	1.119	4.23
0.5 ng + 5 ng control DNA[c]	0–1	209		
	22	250		
0.5 ng cytoplasm	0–1	5.5	0.15	1
	22	7.0	0.18	1.25
5 ng	0–1	91.4	2.44	1
	22	128	3.41	1.4

[a] DNA was injected into the germinal vesicle (GV) of oocytes, except when injection into the cytoplasm is mentioned

[b] The amount of DNA was calculated from the transforming activity of the DNA with the use of a calibration curve. This curve was made in the presence of DNA from uninjected oocytes. The difference between the amount of injected and of isolated DNA can be explained by losses during injection and isolation

[c] As a control we used purified plasmid pBR322 DNA producing a known number of colonies, mixed with DNA from injected oocytes. 5 ng plasmid DNA in our conditions induce ~200 colonies in the presence of DNA from uninjected oocytes, while plasmid DNA alone induces 400 colonies

[d] The standard deviation in our experiments was 0.18

Replication of DNA takes place in the GV of oocyte only, because according to our data and [11], DNA is not replicated in *X. laevis* oocyte cytoplasm (fig.1, table 2).

These data indicate that heterologous pro- and eukaryotic DNAs injected into the GV of *X. laevis* oocytes are replicated. Taking into consideration the well known data about transcription and translation of injected DNA together with the fact that this DNA may replicate in oocytes one may conclude that *X. laevis* oocytes are a unique system for investigation of the peculiarities of replication and expression of various genomes.

As far as a mechanism of replication of prokaryotic DNA in a eukaryotic cell is concerned we would like to suggest the following:

Computer analysis (A. S. Borovik, unpublished) of the nucleotide sequences of the pBR322 DNA

allowed us to estimate in the zone of the nucleotide pairs 979–995, sequences homologous to the SV40 origin of replication [19]. In pBR322 DNA this may be recognized by the oocyte DNA polymerase system with subsequent replication of a prokaryotic DNA in a eukaryotic cell.

References

[1] De Robertis, E. M., Partington, G. A. and Gurdon, J. B. (1978) Phil. Trans. Roy. Soc. Lond. B. 283, 375–377.
[2] Lebleu, B., Hubert, E., Contout, I., De Wit, L., Braude, I. A. and De Clercq, E. (1978) Biochem. Biophys. Res. Commun. 82, 665–673.
[3] Mertz, I. E. and Gurdon, J. B. (1977) Proc. Natl. Acad. Sci USA 74, 4, 1502–1506.
[4] Colman, A. (1975) Eur. J. Biochem. 57, 85–96.
[5] Naroditsky, B. S., Derlaf, T. A., Goldberg, E. Z., Felgenhauer, P. E. and Chulitskaya, E. N. (1979) in: Viruses of cancer and leukosis, pp. 9–11, Moscow.
[6] De Robertis, E. M. and Olson, M. V. (1979) Nature 278, 137–143.
[7] Hubert, E., Huez, G. and Marbaix, G. (1978) Nature 275, 339–341.
[8] Rungger, D. and Turler, H. (1978) Proc. Natl. Acad. Sci. USA 75, 6073–6077.
[9] Laskey, R. A. and Gurdon, J. B. (1973) Eur. J. Biochem. 37, 467–471.
[10] Ford, C. C. and Woodland, H. R. (1975) Dev. Biol. 43, 189–199.
[11] Wyllie, A. H., Gurdon, J. B. and Price, J. (1977) Nature 268, 150–152.
[12] Dumont, J. N. (1972) J. Morphol. 136, 153–180.
[13] Bello, Z. I. and Ginsberg, H. S. (1969) J. Virology 3, 106–119.
[14] Rekosh, D. M. K., Russell, W. C., Bellett, A. J. D. and Robinson, A. J. (1977) Cell 11, 283–295.
[15] Zavizion, B. A., Goldberg, E. Z., Naroditsky, B. S., Zolotarskaya, E. E. and Tikhonenko, T. I. (1979) Vopr Virusol. 6, 595–599.
[16] Clewell, D. B. (1972) J. Bacteriol. 110, 667–676.
[17] Cohen, N., Chang, A. C. J. and Hsu, L. (1972) Proc. Natl. Acad. Sci. USA 69, 2110–2114.
[18] Winnacker, E. L. (1978) Cell 14, 761–773.
[19] Subramanian, K. N., Dhar, R. and Weissman, S. M. (1977) J. Biol. Chem. 252, 355–367.