EXPRESSION OF A DNA ANIMAL VIRUS GENOME IN A PLANT CELL

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1. Introduction

It has been shown that heterologous plant RNA [1] as well as heterologous animal RNA [2] can be translated in the unicellular and uninuclear green alga *Acetabularia*. The evidence came from experiments in which the nucleus was isolated from an *Acetabularia* cell, loaded with RNA by microinjection and reimplanted into the cytoplasm. In some experiments the loaded nucleus was fused with isolated *Acetabularia* cytoplasm instead of being implanted. Similar experiments in which whole TMV particles were injected directly into the cytoplasm have been reported [3].

These experiments raised the question whether a heterologous DNA could also be expressed in an *Acetabularia* cell. The DNA which was used in order to study this problem was an animal virus DNA, namely adenovirus type 2 (Ad2) DNA.

2. Materials and methods

Cells of *Acetabularia major* and *A. mediterranea* were grown [4] and nuclei from *A. major* were microinjected [1,2] as described. Preparation of 10 mm long anucleate posterior fragments was performed by cutting the cells with scissors. The isolated nuclei were injected with ~150 pl Ad2 DNA solution [5] containing 220 μg DNA/ml in 0.01 M Tris buffer (pH 7.3) and 0.001 M EDTA. Control injections were performed with 150 pl 0.01 M Tris buffer (pH 7.3) and 0.001 M EDTA. The contents of the cell fragments were squeezed out into 0.1 M Tris buffer (pH 7.4) containing 2.5% NaCl (Tris–NaCl) at different times (0,2,4,5,7,9,12,15,22 and 30 days) after injection and implantation of the loaded nuclei. The broken cytoplasm forms small spherical cytoplasts under these conditions [6]. The cytoplasts were washed once in Tris–NaCl and fixed in 4% isotonic formaldehyde containing 1.2×10^{-4} M digitonin for 30 min. The fixed cytoplasts were then washed 4 times in Tris–NaCl with 400 ml total vol. The cytoplasts were treated with diluted rabbit anti-Ad2 protein serum for 30 min in each washing step. After a 4 step washing as described above the cytoplasts were treated for 30 min with a solution of a 1% fluorescein-conjugated antirabbit gamma globulin from swine which had been diluted 1:75. After this treatment, the cytoplasts were washed 4 times with 400 ml Tris–NaCl and 4 times with 0.1 M Tris–HCl buffer (pH 7.4) embedded in polyviol and viewed under a Zeiss Axiomat microscope.

3. Results and discussion

Implantation of Ad2 DNA-loaded nuclei into anucleate *Acetabularia* fragments results in the appearance of Ad2 protein specific immunofluorescence in the cytoplasm (fig.1, table 1). This result is in agreement with the idea that the heterologous animal virus DNA introduced into the plant cell via the nucleus is recognized and handled like endogenous DNA and that the heterologous animal virus genome is transcribed and translated in the plant cell.

The controls which include sham-injected nuclei and zerotime experiments indicate that the immuno-

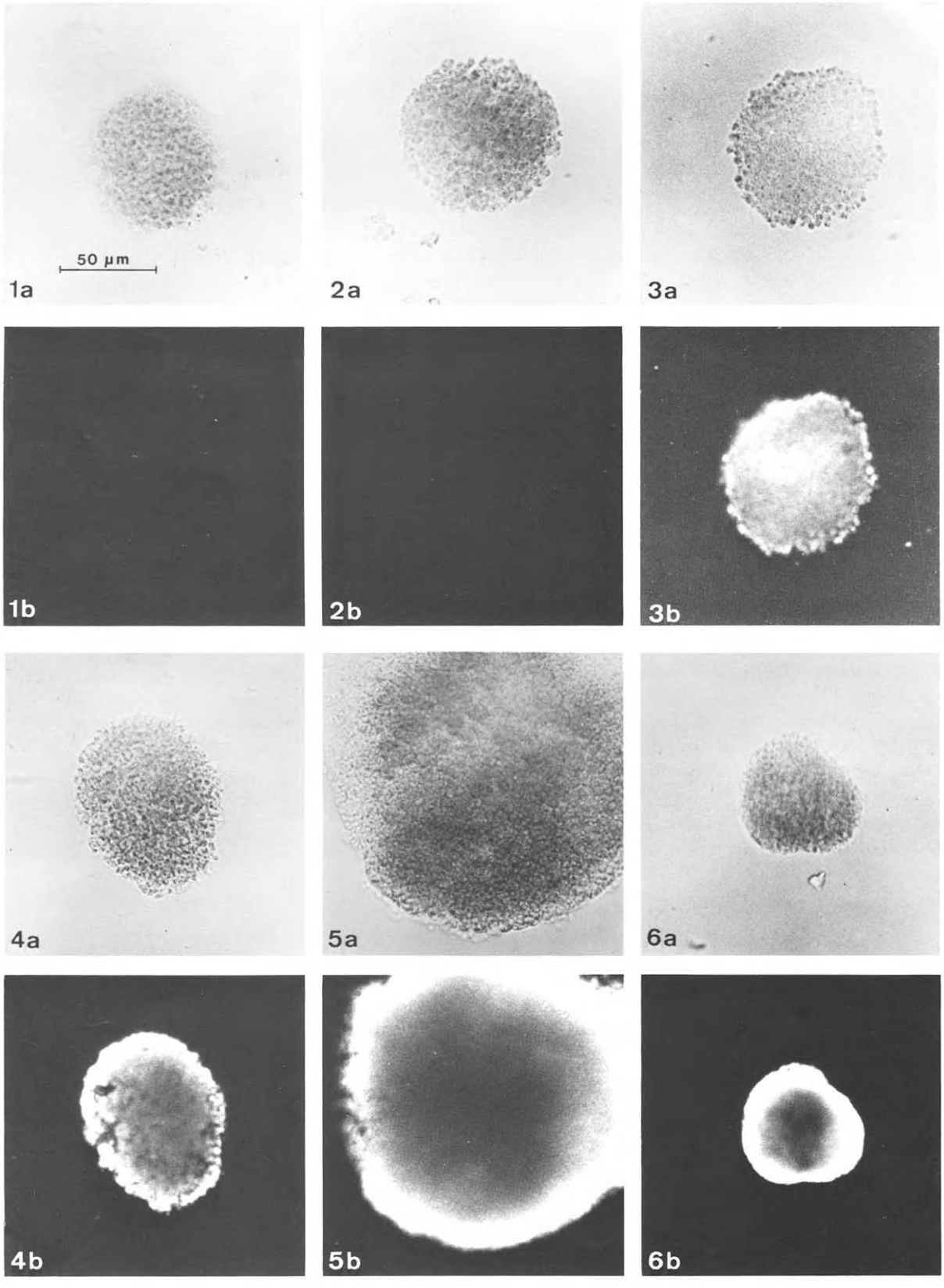
50 µm
1a
2a
3a
1b
2b
3b
4a
5a
6a
4b
5b
6b

Table 1
Appearance of Ad2 protein specific immunofluorescence in cytoplasts

Days after implantation	Replicate no.		Number of negative controls
	1	2	
0	0	0	2
2	0	0	2
4	0	0	2
5	+	0	2
7	+	+	2
9	+	+	2
12	+	0	2
15	0	+	2
22	+	+	2
30	+	+	2

The cytoplasts were prepared from *Acetabularia* cell fragments at different times after implantation with Ad2 DNA-injected nuclei

fluorescence reaction is specific although the possibility of incomplete or erroneous transcription or translation of the Ad2 genome cannot be ruled out by these methods. Quite an interesting point is the time course of appearance of the immunofluorescence. Immunofluorescence has not been detected earlier than day 5 but in the 30 day experiments no decline in intensity was observed at the end of the experimental period. This might mean that the injected DNA is transcribed for a long period and therefore is stable. Experiments with tobacco mosaic virus RNA [1] and with mengovirus RNA [2] have shown that the translation of these RNAs starts immediately after implantation of the loaded nucleus and is performed preferentially before the 12th day.

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Figs. 1–6. Ad2 protein specific immunofluorescence in cytoplasts. The cytoplasts were prepared from *A. mediterranea* cell fragments by cutting the stalks and letting cytoplasm droplets separate from the cell walls [6]. The 'a' series was exposed under white light. The 'b' series was exposed under fluorescing conditions.

Fig.1. Cytoplast prepared from an *A. mediterranea* basal cell fragment 30 days after an *A. major* nucleus had been isolated, loaded with control solution and implanted. The cytoplast was exposed under white light.

Figs. 2–6. Cytoplasts prepared from *A. mediterranea* basal cell fragments immediately (fig.2), 5 days (fig.3), 7 days (fig.4), 15 days (fig.5) and 30 days (fig.6) after implantation of an Ad2 DNA-loaded *A. major* nucleus.