Formation of proviruses in acute infection in vitro and in vivo

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Summary. Formation of DNA proviruses of RNA viruses, Sendai and Newcastle disease, was studied. DNA provirus of NDV was shown to be formed in mouse L-cells and Sendai DNA provirus in Ehrlich ascites carcinoma cells within 2–3 days post infection.

It has been shown in the study of chronic viral infections caused by RNA viruses that their genome is transcribed into DNA and integrated with DNA of chronically infected cells[1–4]. Therefore it was worthwhile to reveal how rapidly the formation of DNA proviruses occurs after an RNA virus was introduced into tissue cultures. This paper reports the results of such experiments in vitro and in vivo.

Materials and methods. The first series of experiments was conducted with mouse fibroblasts (the L-cell line) infected with the non-cytopathic Queensland strain of Newcastle disease virus (NDV) at m.o.i. of 10 PFU/cell. The infected cultures were incubated for various periods, then DNA was extracted from the cells and treated for molecular hybridization. The second series of experiments was carried out in mice with Ehrlich ascites carcinoma. The tumour was implanted into the peritoneal cavity of 18–20 g mice, and the ascites tumour developed in 1 week. Sendai virus was then inoculated i.p., 10^9 EID50 per mouse, the animals were sacrificed at various intervals, the tumour cells were removed, DNA was extracted from them and prepared for molecular hybridization.

DNA was extracted and prepared for molecular hybridization in the following way. Cell suspensions in TNE buffer (Tris HCl 0.01 M, pH 8.0; NaCl 0.1 M, EDTA 10^{-3} M) were treated with 0.2% SDS for destruction of membranes, then incubated at 37 °C for 3–4 h with pronase (0.5 mg/ml, predigested) for destruction of proteins. The nucleic acids were extracted twice with phenol, precipitated with ethanol and kept overnight at –20 °C. The next day DNA was fragmented in a MSE ultrasonic disintegrator, at the maximal amplitude, by 4 15-sec-cycles with the 30-sec-intervals, RNA admixtures were removed by alkaline hydrolysis (0.5 M NaOH, 37 °C, 16 h) and, after neutralization with HCl, the DNA was precipitated with ethanol. DNA preparation thus obteined had the $A_{260}:A_{280}$ ratio 1.9, they sedimented in the area of 4–8 S and banded at the density of 1.42–1.46 g/ml in caesium sulfate density gradients.

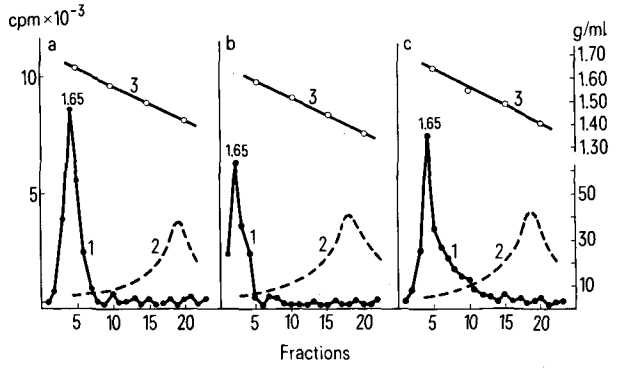

Fig. 1. Density banding of ^{3}H-labeled NDV-RNA annealed with itself (A), DNA from non-infected (B) and NDV-infected L-cells, 40 h post infection, after centrifugation in caesium sulfate density gradients at 35,000 rev/min for 70 h in a Ti 50 rotor of a Spinco L5-50 centrifuge.

1 V. M. Zhdanov, N. N. Bogomolova, V. I. Gavrilov and O. G. Andzhaparidze, Experientia *30*, 499 (1974).
2 V. M. Zhdanov and M. I. Parfanovich, Arch. ges. Virusforsch. *45* (3), 225 (1974).
3 V. M. Zhdanov, N. N. Bogomolova, V. I. Gavrilov, O. G. Andzhaparidze, P. G. Deryabin and A. V. Astakhova, Arch. ges. Virusforsch. *45* (3), 215 (1974).
4 H. W. Simpson and M. Jinuma, Proc. Nat. Acad. Sci. USA, *72* (8), 3230 (1975).

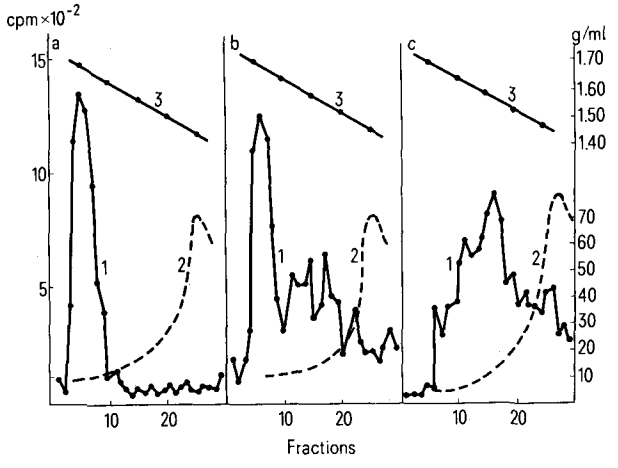

Fig. 2. Density banding of ^{3}H-labeled Sendai virus RNA annealed with DNA from non-infected Ehrlich ascites tumour cells (A) and from the virus-infected cells, 48 (B) and 72 (C) h post infection. Conditions of centrifugation are the same as in figure 1.

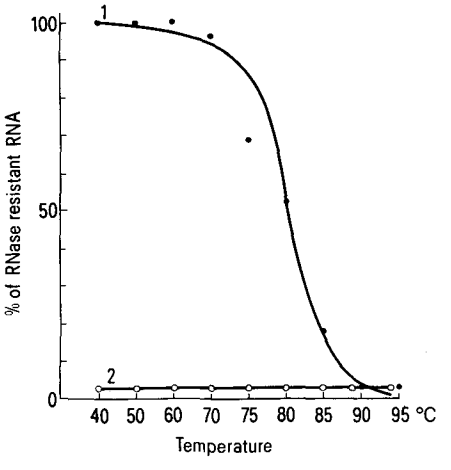

Fig. 3. Melting curves of the labeled NDV-RNA (about 2000 cpm per aliquot) annealed with DNA (about 4 mg/ml) from NDV-infected (1) and non-infected (2) L-cells.

The methods of preparation of tritium-labeled viral RNA are described elsewhere[2,3]. For elimination of possible interaction of admixtures of cell RNA with DNA preparations both viruses, NDV and Sendai virus, were grown in chick embryos, a cell system highly heterogenous for mouse fibroblasts, Preparations of Sendai virus RNA used in this work were labeled with ^{3}H uridine; they had a specific activity of $0.6–1.0 \times 10^6$ cpm/μg and after equilibrium centrifugation in caesium sulfate density gradients formed homogenous peaks at the density of 1.64–1.68 g/ml. ^{3}H-uridine-labeled ($3–4 \times 10^6$ cpm/μg) NDV-specific '18S' RNA complementary to virion RNA was obtained from NDA-infected chick embryo fibroblasts[5]. Molecular hybridization of the viral RNA with cellular DNA was performed in a buffer that contained in 0.5 ml: formamide 50%, NaCl 0.5 M, DNA 2–3 mg and RNA 8000–12,000 cpm. The mixture was incubated at 37 °C for 3 days, and thereafter was centrifuged in performed caesium sulfate density gradients 1.32–1.72 g/ml in a Ti50 rotor of a Spinco L2-50 centrifuge at 35,000 rpm and 20 °C for 70 h. After fractionation of the gradients, radioactivity of acid-precipitable material of each fraction was determined in a liquid scincillation counter Packard-Tricarb.

Results and discussion. Figure 1 presents the results of experiments in vitro It is seen from the figure that NDV-RNA bands at the density of 1.65 g/ml, and the position of the band does not change after annealing of RNA with DNA from non-infected L-cells. After incubation with DNA from virus-infected L-cells, part of radioactivity shifts to areas of lower densities that are characteristic of RNA:DNA hybrids. Figure 2 presents the results of experiments in vivo. It is seen that, after annealing with DNA from non-infected ascites tumour cells, Sendai virus RNA forms a peak with the density of 1.67 g/ml. After incubation with DNA from tumours of virus-infected animals, the essential part of the radiocative label shifts to the area of RNA:DNA hybrides, the amount of the latter increasing with an increase of durability of infection from 48 to 96 h. Figure 3 present a melting curve of NDV-RNA annealed with DNA from non-infected and virus infected cells. It is seen that Tm of the latter is about 78 °C that is characteristic of true RNA:DNA hybrides. Therefore the data obtained may be interpreted as the formation of DNA-proviruses in in vitro and in vivo systems, i.e. DNA transcripts homologous to RNA of the corresponding viruses. The question whether the proviruses are free or covalently linked to the cell DNA needs further investigation. Possible mechanisms of formation of proviruses, including interaction of infectious and oncogenic viruses, is discussed elsewhere in application to chronically-infected tissue cultures[6]. Here we have obtained the first evidence that formation of proviruses may take place in an acute virus infection caused by infectious RNA viruses.

5 N. V. Kaverin and N. L. Varich, Arch. ges. Virusforsch. *38* (2), 183 (1972).
6 V. M. Zhdanov, Intervirology *6*, 129 (1976).

Mise en évidence par dichroïsme circulaire d'une association intermoléculaire pour les complexes NADP(H)-Cu^{2+}

Evidence for intermolecular association of NADP(H)-Cu^{2+} complexes by circular dichroism study

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Summary. The presence of a conservative excitonic term in the ultraviolet circular dichroism spectra of NADP(H)-Cu^{2+} complexes in equimolar solution ($5 \cdot 10^{-4}$ M/l) shows the existence of an intermolecular association by formation of a stable Cu^{2+}-bridged stacked adduct between the purine moieties of 2 neighbouring dinucleotides.

Les pyridines nucléotides sont des coenzymes intervenant dans de nombreuses réactions enzymatiques d'hydrogénation-déshydrogénation qui mettent en jeu des cations métalliques. L'étude de leurs complexes peut donc aider à mieux comprendre les mécanismes de ces réactions. La RMN ^{1}H et ^{13}C des complexes des métaux de transition a permis de préciser les sites de fixation de l'ion Co^{2+} sur les dinucléotides[1,2]. Un large excès de ligand est nécessaire pour effectuer ces mesures et dans ces conditions expérimentales aucune différence n'a pu être mise en évidence entre NAD(H) et NADP(H). Par contre, le dichroïsme circulaire (DC) permet l'utilisation de concentrations équimolaires relativement faibles. Ainsi, le groupe phosphate en 2′ s'est révélé être un nouveau site de fixation pour Co^{2+}[3]. En utilisant l'ion cuivrique, notre étude conduit au même résultat et, de plus, met en évidence un phénomène d'autoassociation entre deux molécules voisines, exclusivement pour le dérivé phosphorylé en 2′.

Toutes les mesures sont effectuées à 25 °C en utilisant des concentrations ligand-ion équimolaires. Les solutions sont préparées immédiatement avant chaque expérience. Les spectres sont obtenus sur un dichrographe «J.Y. Mark III». L'intensité du DC est donné en $d° \cdot cm^2 \cdot dmole^{-1}$. Les dinucléotides ont tendance à former des dimères pour des concentrations élevées voisines de 0,1 M[4]. Pour des solutions millimolaires, ce phénomène n'existe plus et seule une forte interaction intramoléculaire intervient entre la base purique et le résidu nicotinamide ou dihydronicotinamide. Cet effet, dû à un fort couplage des moments de transition des deux chromophores voisins, est parfaitement décelable en DC car il donne naissance à des termes excitoniques[5].

Lorsque l'on ajoute des ions Cu^{2+} à une solution ($5 \cdot 10^{-4}$ M/l) de dinucléotides oxydés aucun nouvel effet n'est observé pour NAD aux environs de pH 7 dans la région aromatique (figure 1). Au contraire, dans des conditions

1 J. Torreilles et A. Crastes de Paulet, Biochimie *55*, 1077 (1973).
2 J. Torreilles, A. Crastes de Paulet et N. Platzer, à paraître.
3 J. Bolard, C. Zentz et G. Chottard, à paraître.
4 R. H. Sarma et R. J. Mynott, J. Am. Chem. Soc. *95*, 7470 (1973).
5 D. W. Miles and D. W. Urry, J. biol. Chem. *243*, 4181 (1968).