Early Ultrastructural Changes Induced in BHK Cell Nuclei by Frog Virus 3. A Possible Mechanism for the Impairment of Cellular DNA Synthesis

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SUMMARY

Evidence is presented that, in FV-3 infected BHK cell nuclei, the inhibition of RNA synthesis is paralleled by chromatin condensation. Inhibition of DNA synthesis could be due to the unavailability of the condensed chromatin to act as template.

Frog virus 3 (FV-3) is a deoxyribovirus which replicates in the cytoplasm of amphibian and mammalian cells at temperatures ranging from 11 to 31 °C. Cells infected by FV-3 exhibit an early inhibition of RNA, DNA, and protein synthesis, occurring at both permissive and non-permissive temperatures (for review, see Granoff, 1969).

We have shown that the inhibition of RNA synthesis is paralleled by, and is very likely dependent upon, the decrease of the activity of the host-cell DNA-dependent RNA polymerase II (Costanzo et al. 1970; Campadelli-Fiume et al. 1972).

The recent evidence that the inhibition of RNA synthesis brings about a condensation of the uncoiled parts of chromosomes (Beermann, 1971; Mancino et al. 1971; Marinozzi & Flume, 1971) prompted us to check the ultrastructural changes in nuclei of cells infected by FV-3 at both permissive and non-permissive temperatures.

Chromatin condensation occurs in FV-3 infected cell nuclei (Darlington, Granoff & Breeze, 1966; Bingen-Brendel et al. 1972) but has been observed only occasionally and much later than the beginning of the inhibition of RNA synthesis.

BHK 21/13 cells were grown at 37 °C, on mica coverslips in Leighton tubes, in Eagle's basal medium containing 10% foetal calf serum. Confluent monolayers were infected with FV-3 at an input multiplicity of 5 to 10 p.f.u./cell. Infected cell cultures were then incubated at 26 or 37 °C (see text). At various times after infection, duplicate samples were fixed with 1.7% glutaraldehyde in 0.1 M-phosphate buffer and thereafter treated according to the technique described by Laschi & Rizzoli (1968).

As soon as 30 min after infection, in the nuclei of infected cultured cells kept at the permissive temperature (26 °C), the small masses of chromatin, normally scattered throughout the nucleoplasm (Fig. 1), became larger, more electron-dense and compact (Fig. 2). This process continued as the nucleoplasm became clearer. At 2.5 h after infection, as condensation developed, the masses of condensed chromatin tended to collect mostly around the inner nuclear membrane, while the nuclear shape became irregular (Fig. 3). Virus inclusions, which are the first morphological sign of virus replication, started to appear in the cell cytoplasm at 2.5 h after infection in cultures kept at 26 °C.

The incubation of infected cell cultures at the non-permissive temperature (37 °C) induced nuclear lesions which appeared as early as in the infected cell cultures kept at 26 °C and developed in a very similar way (Fig. 4). At 37 °C no sign of virus replication was observed in the cell cytoplasm within 24 h, even as virus inclusions (Fig. 4).

In conclusion, nuclei of BHK cells infected by FV-3 showed nuclear lesions similar to those observed in various cells in which RNA synthesis had stopped. The major damage
Fig. 1. Nucleus of a normal BHK cell. Small masses of chromatin are scattered throughout the nucleoplasm.

Fig. 2. Nucleus of a BHK cell 30 min after infection with FV-3 at 26 °C. The small masses of chromatin are larger, more electron-dense and compact.

was represented by chromatin condensation, which appeared soon after infection and was independent of virus replication, since it was observed clearly before any morphological sign of virus replication and appeared also in the cells incubated at the non-permissive temperature.

This observation, together with the finding that RNA synthesis is blocked even at 37 °C (Gravell, 1969; Guir, Braunwald & Kirn, 1971), is consistent with the evidence that chromatin condenses as RNA synthesis is stopped (Beermann, 1971; Marinozzi & Fiume, 1971).
Fig. 3. Nucleus of a BHK cell at 2.5 h after infection with FV-3 at 26 °C. Condensed chromatin has collected mostly around the inner nuclear membrane. Nuclear shape is irregular.

Fig. 4. BHK cells at 24 h after infection with FV-3 at non-permissive temperature (37 °C). Chromatin has condensed at the borders of the nuclei. No virus inclusions have developed in the cytoplasm.

Electron microscopic radioautography of nuclei isolated from calf thymus lymphocytes, and in vitro studies on the template activity of chromatin fractionated into extended (eu-) chromatin and condensed (hetero-)chromatin, demonstrated that RNA and DNA synthesis were localized within the euchromatin, whereas the synthetic activities of heterochromatin were very low (Frenster, 1969). As the assumption that the chromatin which condenses in
the nuclei of cells infected by FV-3 is unable to serve as template, we propose the following sequence of events to explain the inhibition of nucleic acid synthesis in FV-3 infected cells:

the penetration of the virus into the cells brings about a decrease in the activity of the host cell DNA-dependent RNA polymerase II; the resulting inhibition of RNA synthesis induces the condensation of chromatin which, therefore, is no longer available as template for DNA synthesis. The observation that such inhibition begins later than that of RNA synthesis (Maes & Granoff, 1967) is consistent with this model.

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