Ultrastructural Changes in Cells Induced by Temperature-sensitive Mutants of Fowl Plague Virus at Permissive and Non-permissive Temperature

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SUMMARY
Ultrastructural changes developing in chick embryo fibroblast cultures infected with a wild-type strain of fowl plague virus (FPV) or one of six FPV temperature-sensitive (ts) mutants belonging to different complementation groups were studied. Cells infected with wild-type FPV and incubated at optimal (36 °C) or non-permissive temperature (42 °C) displayed changes similar to those described for orthomyxoviruses. The same patterns of changes were observed at 36 °C in cells infected with ts mutants belonging to five of the complementation groups. Mutant ts 303, possessing mutation-altered haemagglutinin, induced at 36 °C the formation of virions carrying a considerably reduced number of spikes on their surfaces. At 42 °C, cells infected with ts mutant 131, with a defective primary transcription stage, showed no morphological changes and no formation of electron-dense inclusions. Cells infected with ts mutants with defective secondary transcription or replication displayed nuclear inclusions but no formation of filamentous cytoplasmic structures or virions. Mutant ts 5 with defective late morphogenesis induced formation of considerably enhanced numbers of nuclear inclusions.

INTRODUCTION
Previously we described some biochemical characteristics of a number of temperature-sensitive (ts) mutants of fowl plague virus (FPV; Ghendon et al. 1973, 1975; Markushin & Ghendon, 1973; Ghennkina & Ghendon, 1979). These ts mutants belonged to six complementation groups and exhibited distinct features of cRNA, vRNA and protein synthesis, ribonucleoprotein formation and virion assemblage at non-permissive temperature. The present study is concerned with ultrastructural changes in cells induced by these FPV ts mutants at permissive and non-permissive temperatures. The data obtained disclose certain differences in the cellular changes induced by these ts mutants that correspond well with their biochemical and genetic characteristics.

METHODS
Viruses. Fowl plague virus, strain Weybridge and six ts mutants of this virus belonging to different complementation groups (Markushin & Ghendon, 1973; Ghennkina & Ghendon, 1979) were used. The viruses were propagated in 11 day-old chick embryos.

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Cells. Forty-eight h primary trypsin-treated cultures of chick embryo fibroblasts (CEF) were used throughout.

Infection of cells. Cultures of CEF were infected at 10 to 30 p.f.u./cell. After adsorption for 1 h at 20 °C the cells were rinsed three times with cold medium 199, the same medium warmed to 36 or 42 °C added and the cells incubated at 36 or 42 °C in a water bath. Tests were performed twice at a simultaneous infection of one lot of cells by all the ts mutants studied.

Electron microscopy. After 8, 16 or 24 h incubation, cells were scraped off the glass with a rubber policeman and sedimented by spinning at 400 g for 7 min. Specimens for electron microscopy were prepared as described previously (Anisimova et al. 1977). A Philips EM 300 electron microscope was used for observation and photography.

RESULTS

Virus-induced structural alterations of cells infected with wild-type FPV

A characteristic feature of CEF infected with wild-type FPV and incubated at 36 °C was the development of nuclear electron-dense inclusions of variable shape and size (Fig. 1a). The inclusions were of fibrillar nature with the fibril diam. 4.5 to 5.0 nm. Within 8 h of infection these inclusions were found both in the nucleolus area and all over the nucleus. At later stages (16 and 24 h) inclusions were found all over the nucleus up to the nuclear membrane and in most instances the nucleolus was reduced. Morphologically similar inclusions in cells infected with orthomyxoviruses have been repeatedly observed (Compans & Dimmock, 1969; Ciampor, 1972; Anisimova et al. 1973, 1977; Reinacher & Weiss, 1975).

Changes observed in infected-cell cytoplasm were of two types. First, there were the dense inclusions which differed from the nuclear ones in being usually surrounded by ribosomes (Fig. 1a, thick arrows). Morphologically similar structures have repeatedly been found in the cytoplasm of cells infected by orthomyxoviruses and have been characterized as accumulations of non-structural virus protein, NS (Morrongiello & Dales, 1977; Shaw & Compans, 1978).

The second type of structure detected in the cytoplasm includes filamentous formations 8 to 9 nm in diam. which were chiefly seen at sites of virus-particle budding, that is around vacuoles and at the cell membrane (Fig. 1b). Similar structures of about the same size have been described in cells infected by influenza viruses (Apostolov et al. 1970; Ciampor, 1972; Anisimova et al. 1973) and as a rule are considered to be virus ribonucleoprotein.

Virus-particle maturation took place at the cell membrane. The main mass of virions was of relatively homogeneous shape and size and morphologically appeared as complete virus with a dense core (Fig. 1b).

Wild-type FPV-infected cells incubated at 42 °C showed specific changes analogous to those at 36 °C. The population of virions forming at 42 °C did not, in terms of morphology, differ from that produced at 36 °C (Fig. 1c).

Virus-induced structural alterations of cells infected with FPV ts mutants

Chick embryo fibroblasts infected with ts 131 mutant (complementation group D) and cultivated at 36 °C displayed identical virus-specific changes in the cells and identical morphology of newly formed virions to CEF infected with wild-type FPV (Fig. 2a). At the non-permissive temperature (42 °C), the great majority of cells infected by this mutant remained without any visible morphological change. Dense inclusions were not seen in the nucleus and cytoplasm, nucleolus morphology stayed unchanged, the cytoplasm
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Fig. 1. Fragments of CEF cultures infected with wild-type FPV and incubated at (a) 36 °C for 16 h, (b) 36 °C for 24 h or (c) 42 °C for 24 h. (a) Dense inclusions in the nuclei and cytoplasm (thin arrows) and cytoplasmic inclusions surrounded by ribosomes (thick arrows). (b) Filamentous structures 8 to 9 nm in diam. are situated in the vicinity of the cell membrane (arrows) and mature virions with a dense core are visible. (c) Newly synthesized virions at 42 °C, morphologically identical to virions formed at 36 °C.

exhibited no filamentous structures and no virus maturation was observed at the cell membrane (Fig. 2b).

When infected with ts 29 mutant (complementation group C) CEF also presented, at 36 °C, a picture of intracellular changes and nascent virion morphology similar to that observed in CEF infected with wild-type FPV. At 42 °C, about 80% of cells also displayed
Fig. 2. Fragments of CEF cultures infected with ts 131 mutant and incubated at (a) 36 °C or (b) 42 °C for 24 h. (a) Budding of virus particles on the surface of cells incubated at 36 °C. (b) Intact nuclei and cytoplasm of infected cells incubated at 42 °C.
dense inclusions in the nucleus and cytoplasm. The total sum and frequency of presence of these inclusions were about the same as in cells incubated at 36 °C. Filamentous structures and virions at the cell membrane were nevertheless not found in the vast majority of cells.

Cells infected with ts 43 and ts 166 mutants (complementation groups A and B, respec-
tively) showed closely similar changes. At 36 °C, the cells displayed morphological identity both as regards specific intracellular changes and newly-formed virions with cells infected with wild-type FPV. At 42 °C, nuclear and cytoplasmic inclusions were a little more numerous than at 36 °C. Filamentous structures in the cytoplasm and newly synthesized virions upon the cell surface were not observed.

Infection of CEF with ts 5 mutant (complementation group E) and incubation at 36 °C resulted in the same morphological picture (Fig. 3a) as that obtained with wild-type FPV at this temperature: dense inclusions in the nucleus and cytoplasm and the presence of filamentous structures in the cytoplasm. The morphology of newly formed virions was also the same. At 42 °C, the nuclei and cytoplasm of ts 5-infected cells also contained dense inclusions, similar to those observed at 36 °C, but they were much more numerous, especially in the nuclei, when compared with cells infected with wild-type FPV and incubated at 42 °C (Fig. 3b, arrow). They were also larger in size. The cytoplasmic location of these dense inclusions was frequently in the vicinity of, or adjacent to, the membrane. The majority of these inclusions were either not surrounded by, or were associated with only small numbers of ribosomes. Filamentous structures in the cytoplasm were difficult to identify and newly synthesized virus particles were not detected in the majority of cells.

The photomicrograph of CEF infected with ts 303 mutant (complementation group F) and incubated at 36 °C also showed great similarity with the specific changes seen in cells infected with wild-type FPV. However, the population of newly synthesized virions exhibited a peculiarity of the envelope. Most of the ts 303 virions carried considerably fewer spikes, which were distributed thinly or irregularly and were occasionally missing altogether (Fig. 4). These data were confirmed in the experiments with virions by negative staining (S. G. Markushin et al. unpublished data). On incubation at 42 °C the nuclei and cytoplasm contained large numbers of dense inclusions. Filamentous structures in the cytoplasm were not detected, nor were newly synthesized virions.
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DISCUSSION

As is evident from the data presented above, CEF cultures infected with FPV ts mutants of different complementation groups displayed, at non-permissive temperature, somewhat variable patterns of ultrastructural changes. These changes correlate well with previously established defects in the reproduction of these mutants (Ghendon et al. 1973, 1975). For instance, cells infected with ts 131 mutant, in which the primary transcription stage is defective and virus-specific protein synthesis considerably reduced (Y. Z. Ghendon et al. unpublished data), displayed no changes, not even formation of nuclear inclusions. At the same time, cells infected with ts mutants of other complementation groups, in particular ts 29, in which secondary transcription is impaired but protein synthesis takes place (Ghendon et al. 1975), showed formation of nuclear inclusions. These data strongly suggest that the appearance of nuclear inclusions in cells infected with influenza virus is a virus-specific process, that their formation presupposes the synthesis of virus proteins and that those proteins which are synthesized on the cRNA templates formed in primary transcription suffice for this.

The investigations showed that virions were not produced at 42 °C in cells infected with ts mutants. However, virions morphologically indistinguishable from wild-type FPV did form at 36 °C in experiments with mutants of five different complementation groups. The only exception was mutant ts 303, whose virions, formed at 36 °C, carried less spikes on their envelopes. These data show good correlation with the results of a detailed study of the characteristics of virions isolated from cells infected with ts 303 mutant, which possessed mutation-modified haemagglutinin (S. G. Markushin et al. unpublished data).

Cells infected with ts 43, ts 166 or ts 5 mutants displayed enhanced numbers of nuclear inclusions at the non-permissive temperature; this was particularly striking with ts 5 mutant. Whether this accumulation of nuclear inclusions was due to impairment of their nucleus-to-cytoplasm migration or to greater production is difficult to decide. Nevertheless, it seems that the more probable cause as regards ts 43 and 166 mutants was an increase in the production of nuclear inclusions since the cytoplasm of cells infected by these mutants also contained an increased number of dense inclusions. It cannot be excluded that cells infected with the latter two ts mutants, in which vRNA synthesis is defective (Ghendon et al. 1975), have an impaired regulation of protein synthesis at the non-permissive temperature, which leads to an enhanced production of the protein(s) forming nuclear inclusions. It is however more probable that in the case of ts 5 mutant a relative impairment of nuclear inclusion migration into the cytoplasm was involved, since the number of inclusions in the nuclei increased 3 to 5 times when compared to wild-type virus whereas the nucleus-to-cytoplasm migration of inclusions did not increase more than 1:2 to 1:4 times (Y. Z. Ghendon et al. unpublished data).

REFERENCES


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