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Abstract

Partially separated double-stranded RNA from purified Rous sarcoma virus, Schmidt.Ruppin strain, was observed by electron microscopy utilizing 8.M urea and protein monolayer technique. Furthermore, viruses in pair were frequently and viruses with two nuc1eoids were occasionally observed in ultrathin. sectioned specimens of chick cells transformed by RSV. From these results taking other reports in consideration, a possible mechanism of RNA replication in Rous sarcoma virus is proposed.

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A POSSIBLE MECHANISM OF RNA REPLICATION IN ROUS SARCOMA VIRUS

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RNA dependent RNA polymerase (EC 2. 7. 7. 6) has been detected in purified virions of vesicular stomatitis virus (1), influenza virus (2, 3) and Newcastle disease virus (4), which have single stranded RNA in their virions. The same enzyme was detected in *E. coli* infected with the RNA bacteriophage MS 2 (5) and Q_β (6). In these viruses, replication of the nucleic acid is mediated by virus-induced RNA dependent RNA polymerase.

Recently RNA dependent DNA polymerase has been proved *in vitro* in the virions of RNA type oncogenic viruses (7, 8), which have also single stranded RNA. It strongly supported the idea of participation of DNA (9) in transformation and replication of these viruses.

The activity of RNA dependent RNA polymerase from myeloblasts (10, 11) infected with avian myeloblastosis virus and from tumors induced by Friend virus (12) was reported, while the enzyme activity in chick embryo cells infected with Rous associated virus was not detected (13). The presence of the enzyme in RSV-infected cells was also suggested (14).

At the present time, very little is known concerning replication mechanisms of oncogenic viral RNA in permissive cells.

In our electron microscopic studies of RSV and its RNA, interesting figures were observed. On the basis of these findings, a possible mechanism of RSV-RNA replication is proposed.

MATERIALS AND METHODS

The source of RSV, Schmidt-Ruppin strain, and purification procedures were as described before (15). The purified viruses through sucrose density gradient were treated with 0.05% deoxycholic acid and 1% sodium dodesyl sulfate for 10 min at 37°C to release viral RNA from virions (16). The released viral RNA was precipitated with 2 vol. of cold ethanol. Discarding the supernatant after centrifugation, the precipitate was dissolved in a small amount of saline sodium citrate (pH 7.4). The RNA solution in saline sodium citrate was

diluted with 8 M urea (17), and protein monolayer technique (18) was applied for electron microscopic preparation.

Transferred cultures of chick cells transformed by RSV were fixed with glutaraldehyde and osmium tetroxide. The fixed samples were ultrathin sectioned, and were stained with uranyl acetate and lead citrate. The specimens were examined in an electron microscope, Hitachi HU-11C.

RESULTS AND DISCUSSION

Among linear structures of many RSV-RNA isolated from purified RSV through sucrose density gradient (15), partially separated double stranded RNA of 3.86 μ in length (Fig. 1) was observed. At the separated parts, both of the strands were the same in length. RSV with two nucleoids (Fig. 2) was rarely observed in extracellular space in an ultrathin-sectioned specimen of cultured chick cells transformed by RSV. The size of the RSVs with two nucleoids were 81 $\sim$ 100 mμ in diameter and the shape of the two nucleoids were elongated showing an ovoid shape. Furthermore, RSVs appeared in pair were frequently observed in extracellular spaces (Fig. 3).

It has been reported that the cell nuclei transformed by murine sarcoma-leukemia virus contain RNA complementary to viral RNA and some nuclear RNA are double stranded (19). In pair arrangement of virus particles in the specimens of Rous sarcoma cells (20) and avian leukosis virus-infected cells (21) were reported before. Virus particles with two nucleoids were also reported in the specimens of avian leukosis virus-infected cells (21) and passenger C-type viruses with two nucleoids in "mirror image" were sometimes observed in adenovirus-transformed hamster cells (22). In those reports, a possibility of virus replication by fission was suggested. The hypothesis on division of virus particle is interesting but lacks experimental evidence yet. The virus particles with two nucleoids might, however, be explained better as malformation of virus particles during the formation or maturation of virions. Partially separated double-stranded RNA from purified virus may be interpreted as RNA from malformed RSV which is formed in the process of double-stranded RNA formation by RNA dependent RNA polymerase and is

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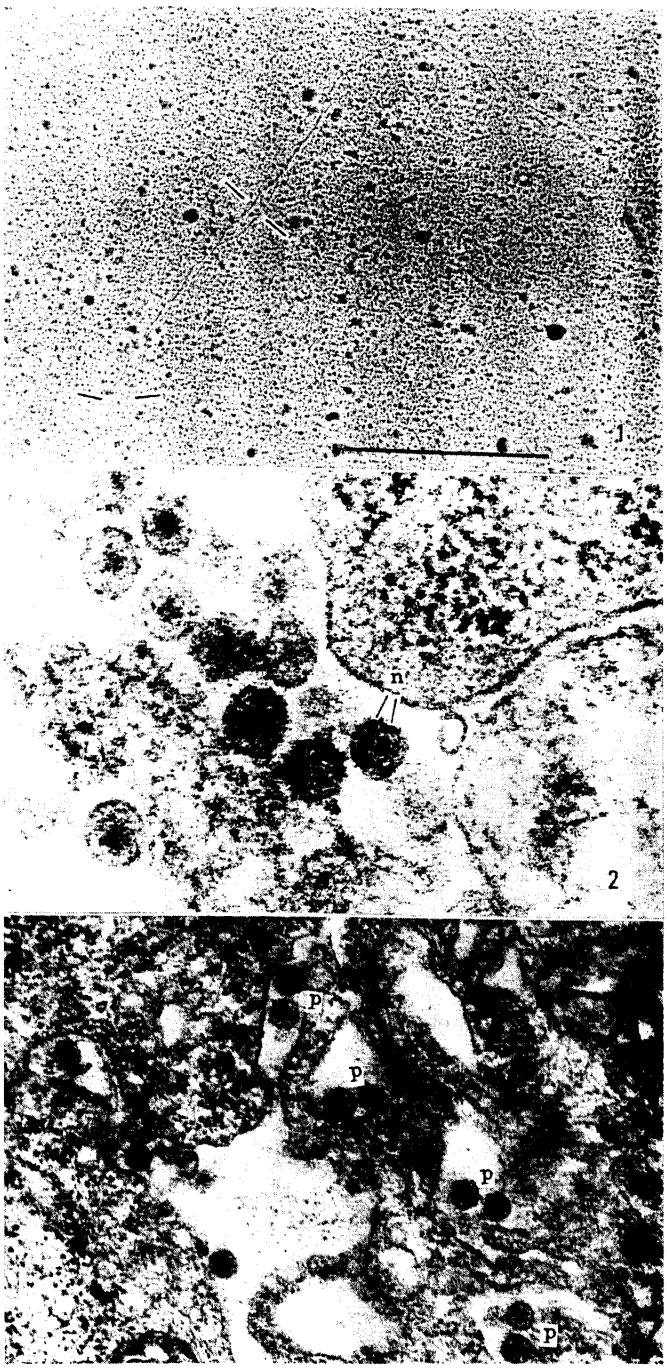
Fig. 1 Electron microscopic figure of double stranded RSV-RNA isolated from purified RSV (Schmidt-Ruppin strain). Arrows indicate the parts of a partially separated RNA strand. Marker presents 1 μ.

Fig. 2 RSV with two nucleoids appeared in an extracellular space in ultrathin-sectioned specimen of cultured chick cells transformed by RSV. n: nucleoids

Fig. 3 In pair appearance of RSVs in extracellular spaces. p: RSVs in pair


1
n
2
p
p
p
p

covered with coat protein before the complete separation of double-stranded RNA. It is not yet clear whether one of RSVs appearing in pair contains complementary minus strand RSV-RNA or not. From these observations, we propose a possible model of RSV-RNA replication as shown in Fig. 4. In order to clarify these hypothesis, experiments are now in progress.

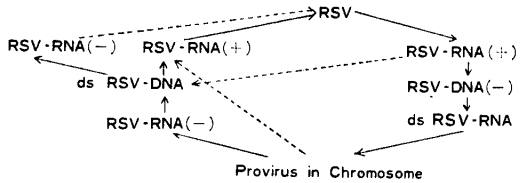


Fig. 4 A possible model of RSV-RNA cycle.
(+): plus strand of viral RNA, (−): complementary minus strand of viral RNA, ds: double stranded

SUMMARY

Partially separated double-stranded RNA from purified Rous sarcoma virus, Schmidt-Ruppin strain, was observed by electron microscopy utilizing 8-M urea and protein monolayer technique. Furthermore, viruses in pair were frequently and viruses with two nucleoids were occasionally observed in ultrathin-sectioned specimens of chick cells transformed by RSV.

From these results taking other reports in consideration, a possible mechanism of RNA replication in Rous sarcoma virus is proposed.

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