Virally Coded Noncapsid Protein Associated with Bovine Parvovirus Infection

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A phosphorylated protein (NP-1) with an Mr of 28,000 has been detected in nuclei of bovine parvovirus (BPV)-infected cells in association with chromatin. No protein in this size range was detected after infection of appropriate cells with several autonomous rodent parvoviruses although the BPV-specific protein is similar in size to noncapsid proteins associated with rabbit parvovirus or adeno-associated virus infection. Structural homology between BPV-1 and a BPV capsid protein could be detected by electrophoretic analysis of the products of proteolysis with chymotrypsin. This protein can be detected after in vitro translation of RNA from BPV-infected cells and BPV-specific RNA. Homology between the in vivo- and in vitro-synthesized species was shown by the similarity of the chymotryptic products.

Materials and Methods

Cell culture, virus infection, and radiolabeling. Cells used in this investigation included secondary bovine fetal lung or spleen cells, buffalo lung cells (ATCC CCL 40), bovine turbinate cells (ATCC CRL 1390), NB (SV40-transformed newborn human kidney) cells, and normal rat kidney cells. Cells were cultured and infected with 10 PFU per cell of autonomous parvoviruses as described by Parris and Bates (14). KB cells in suspension culture were infected with adenovirus and AAV as described by Buller and Rose (4). For 32P labeling, the medium was changed to phosphate-free minimal essential medium containing 10% dialyzed fetal calf serum and 10 μCi of 32P per ml at 6 h postinfection. For [35S]methionine labeling, the medium was changed to minimal essential medium containing 0.1% of normal amount of methionine and 10% dialyzed fetal calf serum at 6 h postinfection, and [35S]methionine (5 μCi/ml, 1,200 Ci/mmol) was added 10 h postinfection. Cells were harvested 24 to 26 h postinfection. Preparations of NP-1. Mock- and virus-infected cells were washed twice in a modified phosphate-buffered saline (20), and, for electrophoresis, the pellets were resuspended directly in gel application buffer (8) and sonicated to disrupt DNA.

Studies of the subcellular distribution of NP-1 followed a modification of the fractionation protocol of Tremblay et al. (22). Cells were fractionated into nuclei and cytoplasm as described by Pritchard et al. (16). The nuclei were resuspended in 50 mM Tris-chloride (pH 7.5)-5 mM MgCl2-25 mM KCl-3 mM DTT and lysed by addition of an equal volume of 0.2% Sarkosyl. The suspension was layered over 4 ml of 15% sucrose over 40% sucrose in the same buffer and centrifuged in an SW27 rotor at 25,000 rpm for 60 min at 4°C. The nuclear membrane (the band forming at the interface), the chromatin (pellet), and the nucleoplasm (material remaining at the top of the gradient) were electrophoresed on 7 to 15% linear gradient sodium dodecyl sulfate (SDS)-polyacrylamide gels, and the distribution of NP-1 was determined from densitometric tracings of autoradiograms. Electrophoresis and autoradiography. SDS-polyacrylamide gel electrophoresis was carried out by the method of Laemmli (8). Gels containing 32P were exposed to Kodak XAR-5 X-ray film either directly or with a Cronex Lightning-Plus intensifier screen at ~ -80°C. Gels containing [35S]me-
fying

1.3
to

rabbit
preimmune
by

Stained
proteins,
NP-1
bovine
Corp.,
Nuclear
Laboratories,
(13).
316 LEDERMAN
munoprecipitation
of
BPV
BPV-infected
10%
rabbit
anticapsid
cells.

the
gel

specific
in

32P-labeled
BPV-specific
was

used
Staphylococcus
V8
protease
(9).

However,
NP-1
had

a
similar
electrophoretic
mobility
to

a
protein

originally
detected
by
Buller
and
Rose
(4, 5) which
appeared
in
KB
cells
after
coinfection
with
human
adenovirus
and
AAV
type
2
(data
not
shown).

Proteolytic
analysis
of
the
relation
of
NP-1
to
BPV
capsid
proteins.
To
detect
possible
structural
homology
between
NP-1
and
BPV
capsid
proteins,
the
partial
proteolysis
products
of
NP-1
and
both
the
80,000-
(VP1)
and
62,000-dalton
(VP3)
capsid
proteins
were
compared.
Previous
studies
on
amino acid
homology
among
the
BPV
capsid
proteins
had
used
Staphylococcus
 aureus
V8
protease
(9).
However,
only
two
species
very
close
in
molecular
weight
were
detected
as
proteolysis
products
of
NP-1
with
this
enzyme.
The
action
of
chymotrypsin
on
NP-1
gave
numerous
bands,
two
of
which
were
identical
in
migration
to
bands
obtained
after
treatment
of
VP1
with
this
enzyme
(2).
No
homologous
bands
between
NP-1
and
VP3
were
detected
with
this
enzyme
(data
not
shown).

Determination
of
the
genome
coding
for
NP-1.
The
appearance
of
NP-1
after
viral
infection
and
its
amino
acid
homology
to
a
capsid
protein
suggested
that
this
protein
was
coded
by
the
viral
genome.
We
have
previously
detected
three
polypeptides
in
the
Mr.
range
of
25,000
to
30,000
as
immunoprecipitation
products
of
in
tro
translation
of
cytoplasmic
RNA
from
BPV-infected
cells
with
either
anticapsid
IgG
or
IgG
from
BPV-infected
calves
and
of
BPV-specific
RNA

gels
and
compared
with
a
parallel
lysate
of
uninfected
cells,
a
protein
(NP-1)
of
apparent
Mr.
of
28,000
was
detected
in
infected
cell
material
by
staining
(Fig.
1A).
This
protein
was
in
high
concentration
in
infected
cells
about
250
µg/10
cells
as
estimated
by
the
intensity
of
Coomassie
brilliant
blue
R
staining
and
was
as
prominent
as
the
major
capsid
protein,
VP3.
Although
no
phosphorylation
of
BPV
capsid
proteins
was
detected
after
electrophoresis
on
gels
of
different
acrylamide
percentages
(data
not
shown),
NP-1
was
found
to
be
phosphorylated,
as
shown
by
the
comigration
of
32P,
incorporated
in
vivo
between
6
and
24
h
postinfection
(Fig.
1B).
NP-1
was
detected
after
BPV
infection
of
bovine
fetal
spleen
cells,
buffalo
lung
cells,
and
bovine
turbinate
cells.
To
determine
the
cellular
localization
of
this
protein,
infected
cells
were
fractionated
as
described
above.
At
least
95%
of
the
32P
label
associated
with
NP-1
was
found
in
a
nuclear
pellet,
and
greater
than
85%
of
the
nuclear
NP-1
was
associated
with
chromatin
(Table
1).

No
increase
in
mass
of
a
protein
in
this
molecular
weight
range
was
detected
in
nuclear
lysates
of
appropriate
host
cells
infected
with
the
other
autonomous
parvoviruses
Lukievi-H-1, or Kilham
rat
virus
(data
not
shown).
No
in
vivo
incorporated
32P
was
detected
in
the
28,000-dalton
trane
nuclear
lysates
of
Kilham
rat
virus-infected
normal
rat
kidney
cells
as
compared
with
uninfected
cells.
However,
NP-1
had

a
similar
electrophoretic
mobility
to
a
protein
originally
detected
by
Buller
and
Rose
(4, 5) which
appeared
in
KB
cells
after
coinfection
with
human
adenovirus
and
AAV
type
2
(data
not
shown).

Partial
proteolytic
cleavage
of
NP-1.
Partial
proteolysis
of
in
vitro
[35S]methionine
labeled
NP-1,
in
vivo
[35S]methionine-labeled
NP-1
and
BPV
capsid
protein,
or
35S-labeled
NP-1
was
carry
out
by
the
method
of
Cleveland
et
al.
(7).
Stained
proteins,
cut
from
a
preparative
gel
of
a
lysate
of
BPV-infected
cells,
were
stored
frozen
overnight
in
buffer
containing
1.3
U
of
aprotinin
per
ml
Sigma.
In
vitro
translational
reaction
(50 µl)
containing
RNA
from
infected
cells
were
immunoprecipitated
as
described
above.
Eluates
of
the
immune
precipitates
were
electrophoresed,
and
areas
of
the
gel
corresponding
to
marker
NP-1
were
cut
out
and
processed
as
described
above.

RESULTS

Detection
and
distribution
of
NP-1.
When
lysates
of
bovine
parvovirus-infected
bovine
fetal
lung
cells
prepared
24
h
postinfection
were
electrophoresed
on
SDS-polyacrylamide

FIG. 1. Detection of NP-1 in BPV-infected bovine fetal lung cells. (A) Coomassie
brilliant
blue
R-stained
10% SDS-polyacrylamide
gel
of
3P-labeled
lysates
do
mock-infected
(lane
1)
or
BPV-
infected
(lane
2)
cells.
The
positions
of
the
molecular
weight
markers
phosphorylase
b
(92,500
daltons),
human
transferrin
(75,000
daltons),
bovine
serum
albumin
(68,000
daltons),
aovalbumin
(43,000
daltons),
carbonic
anhydrase
(30,000
daltons),
and
soybean
trypsin
inhibitor
(21,500
daltons)
are
indicated.
(B)
Autoradiogram
of
the
gel
shown
in
A.
The
position
of
NP-1
is
indicated.
VP1,
VP2,
and
VP3
mark
the
positions
of
the
capsid
proteins.

TABLE 1. Intracellular distribution of 3P-labeled NP-1 in BPV-infected bovine fetal spleen cells

<table>
<thead>
<tr>
<th>Subcellular fraction</th>
<th>% Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasm .............</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Nuclei .................</td>
<td>&gt;95</td>
</tr>
<tr>
<td>Nucleoplasm ............</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Nuclear envelope .......</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Chromatin ..............</td>
<td>&gt;90</td>
</tr>
</tbody>
</table>

* Percentages
were
obtained
from
densiometric
scans
of
tautograms
of
electrophorograms
of
cell
fractions
prepared
as
described
in
the
text.
with anticapsid IgG (9). To determine whether one of these low-molecular-weight species might be NP-1, RNA from BPV-infected cells was translated in vitro in a messenger-dependent rabbit reticulocyte lysate. the reaction immunoprecipitated first with preimmune IgG and then with anticapsid IgG. An eluate of the immune precipitate was electrophoresed (Fig. 3, lane 2) in parallel with a [35S]methionine-labeled lysate of BPV-infected cells (Fig. 3, lane 1). One of the three low-molecular-weight in vitro translation products had the same electrophoretic mobility as NP-1.

The position of NP-1 is indicated by the arrows.

To determine the genome coding for NP-1, a portion of the same RNA preparation was hybridized to BPV virion DNA which had been covalently attached to epoxycellulose (13). Of the input RNA, 0.5% bound to this material. The nonhybridizing RNA (presumably bovine cell RNA) and the BPV-specific RNA were also translated and immunoprecipitated as described above for total cytoplasmic RNA. The α-peptide corresponding to NP-1 was detected as a translation product of BPV-specific RNA (Fig. 4, lane 3) but not as a product of RNA which did not hybridize with BPV DNA (Fig. 4, lane 4). No polypeptides were observed in a translation reaction from which RNA was omitted (Fig. 3, lane 3).

Proteolytic analysis of the relation of in vivo-labeled NP-1 to in vitro translation product. To confirm the identity of the in vitro product with NP-1 synthesized in infected cells, the chymotryptic peptides of these species were compared (Fig. 4). There are several bands in common (arrows) between the [35S]methionine-labeled protein isolated from a gel of a lysate of infected cells (Fig. 4, lane 3) and the [35S]methionine-labeled in vitro translation product (Fig. 4, lane 2). There are also bands (crossed arrows) with the same mobility as proteolysis products of in vivo 32P-labeled NP-1 (Fig. 4, lane 1) and [35S]methionine-labeled in vitro translation product (Fig. 4, lane 2).

DISCUSSION

A phosphorylated protein (NP-1) with an apparent Mr, of 28,000 has been detected in nuclei of BPV-infected cells. Within the nucleus, this protein is associated with chromatin. No protein in this size range increased substantially in mass after infection of appropriate cells with the autonomous rodent parvoviruses we have tested or with LuIII. Astell et al. (1) report that Cotmore and Tattersall have detected a 25,000-dalton noncapsid protein after infection with minute virus of mice. Noncapsid proteins of this size are associated with infection with the autonomous rabbit parvovirus (11) and with the defective AAV (4, 5). We have
not been able to demonstrate reproducibly a BPV noncapsid protein in the 70,000- to 85,000-dalton range, although such virus-specific proteins have been found for H-1 (17, 18). Aleutian mink disease (3), and minute virus of mice (1). However, both BPV (9) and rabbit parvovirus (11) contain capsid proteins in this size range.

The appearance in infected cells of a protein absent from uninfected cells suggests that it is virally coded. Alternatively, the synthesis may result from viral activation of a cellular gene. For NP-1, the homology with a capsid protein, its translation from BPV-specific RNA and lack of synthesis by bovine cell RNA, the similarity between the partial proteolysis products of in vivo- and in vitro-synthesized NP-1, as shown here, and the immunoprecipitation of in vivo- and in vitro-synthesized protein with antiscapsid IgG and IgG from a field-infected calf (9) strongly suggest that NP-1 is a viral gene product.

From the data presented, it cannot be determined whether NP-1 is a cleavage product of the 80,000-dalton capsid protein which is post-transcriptionally modified by phosphorylation or whether NP-1 is coded by a unique mRNA. Translation of cytoplasmic RNA from BPV-infected cells fractionated by size on a sucrose gradient (M. Lederman, unpublished data) suggests that NP-1 might be coded by a BPV-specific RNA with a size of about 1.1 kilobases. S1 nuclease analysis of RNA from BPV-infected cells reveals a spliced molecule of 1.05 kilobases. Transcription mapping of this RNA with restriction endonuclease fragments of the BPV genome (P. R. Burd, J. T. Patton, R. C. Bates, and E. R. Stout, manuscript in preparation) shows that it is transcribed from the left half (3' end) of the genome and overlaps the region of the genome coding for the putative mRNAs for the capsid proteins by about 250 bases. The 80 amino acids in common would be sufficient to account for the observed amino acid homology and antigenic cross-reactivity.

The determination of the RNA coding for NP-1 is being investigated by hybrid-arrested translation and translation of mRNAs selected by hybridization to restriction fragments covering the left and right halves of the BPV genome. If NP-1 is coded by the 1.05-kilobase RNA, this organization of the BPV genome, i.e., noncapsid proteins being coded from the left half of the genome and the capsid proteins being coded from the right half of the genome, would be in agreement with the arrangement of other parvovirus genomes (15, 18; B. J. Carter, C. A. Laughlin, and C. J. Marcus-Sekura, in K. Berns, ed., The Paroviruses, in press), although the number and sizes of transcripts and capsid and noncapsid proteins differ between individual viruses.

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LITERATURE CITED


