frames, and a high level of homology in the amino acid sequences between corresponding proteins.

Only the replicase ORF (approx 223 kDa) is translated from the full-length genomic RNA. In vitro translation data from a range of carlaviruses suggested that this large ORF was proteolytically processed, with approx 30–40 kDa being removed. Recent elegant experiments by Lawrence et al. ***(8)***, using in vitro transcribed and translated replicase ORF and full-length infectious clones of BBScV, have clearly demonstrated that this is indeed the case.

The 3' terminal ORFs appear to be translated from two subgenomic mRNAs, which can be found in infected tissue, and, for some viruses, can be detected within purified virus preparations ***(9,10)***. The 3' ORFs encode proteins of 25, 12, and 7 kDa, which have been designated the triple gene block, the 34 kDa coat protein, and an ORF of approx 11 kDa, present at the 3' terminus, which is unique to carlaviruses ***(1)***. The sizes of reported RNAs and typical ORFs for carlaviruses are summarized in **Fig. 1**, with the position of the 34-kDa coat protein (CP) indicated.

2. Materials

2.1. Virus Isolation

1. 0.5*M* borate buffer, pH 7.8, with 5 m*M* EDTA.
2. Diethyl ether.
3. Polyethylene glycol (PEG), average mol wt 8000.
4. Thioglycolic acid.
5. Triton X-100.
6. Cesium chloride.
7. Sterile dH_2O.
8. High-capacity (500 mL) centrifuge, capable of 10,000*g*.
9. Ultracentrifuge capable of 110,000*g*.
10. Blender.
11. Stir plate and magnetic stirrers.

2.2. RNA Extraction

RNA dissociation buffer, TE buffer, and 3*M* sodium acetate should be autoclaved to avoid nuclease contamination.

1. RNA dissociation buffer: 40 m*M* Tris-HCl, 2 m*M* EDTA, pH 9.0, 2% sodium dodecyl sulfate (SDS) (w/v), 0.5mg/mL bentonite (*see* **Note 6**).
2. TE buffer: 10 m*M* Tris-HCl, 1 m*M* EDTA, pH 7.5.
3. Phenol saturated in TE, pH 7.5 (*see* **Note 1**).
4. Chloroform.
5. 3*M* Sodium acetate, pH 5.2, with acetic acid (autoclaved).
6. Ethanol (kept at –20°C).