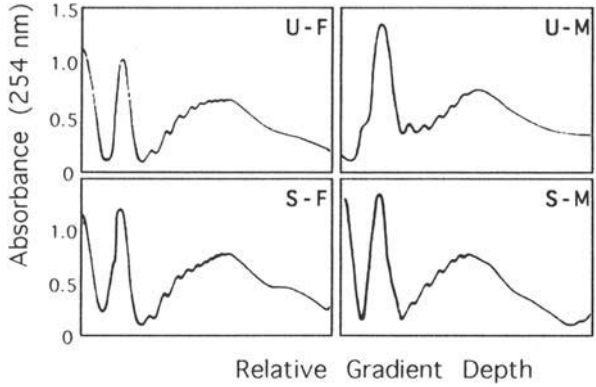

Fig. 3. Sucrose density gradient analysis of free and membrane-bound polyribosomes isolated from uninfected and SYNV-infected tobacco. The panels designated U-F, U-M, S-F, and S-M refer to free and membrane-bound polysomes isolated from leaves of uninfected and SYNV-infected plants, respectively. Although the profiles vary slightly, there is no major correlation between the number of monomers associated with the different classes of polysomes. However, we have shown ***(16,17)*** that about 2–5% of the messenger RNA in SYNV-infected plants is viral-specific. The free polysomes contain sequences hybridizing to nearly 100% of the viral RNA, but the RNA derived from membrane-bound polysomes hybridizes to only 40% of the RNA. These results suggest that a specific subset of the viral mRNAs are membrane-associated. Adapted from **ref. *17***.

0.66, 1.34, 1.34, and 0.66 mL layers of sucrose, as described above. The sample size should also be 0.2 mL with 1–2 A_{260} units. After centrifugation at 335,000*g* for 30 min at 4°C, the gradients should be fractionated in a density gradient fractionator attached to a UV monitor at 254 nm.

3.4. Notes on Polysome Procedure

1. The recovery of polyribosomes is critically dependent on maintaining the correct ratios of EGTA to Mg^{2+} in the solutions. Because of metals found in vacuoles, polyribosomes precipitate when insufficient amounts of EGTA are present. However, when the ratio of Mg^{2+} to EGTA is too low, the polyribosome subunits dissociate (*see* **ref. *9*** for a discussion of these variables).
2. Maintaining the temperature close to 4°C throughout the various steps is important for polysome stability. Minor amounts of RNase will quickly degrade polysomes, and, although the relatively high pH and ionic strength of the buffers mediate against RNase activity, transient increases in the temperature can affect the extent of polymerization observed in the polysome profiles.
3. For cereals ***(18)***, reduce the extraction buffer to 2 mL for each gram of leaf tissue. The relative proportion of vascular tissue is much higher in cereals than in dicots and this tissue cannot be easily removed. This results in lower recovery of cytosol from the cereal tissue.