4. To avoid polycarbonate tube breakage and subsequent run failure at these high gravational forces, always wash these alkali-sensitive tubes in very mild detergent approved for this purpose. Reuse the tubes only three times, and, before each centrifuge run, pipet ~100 µL of H_2O into the rotor receptacles before inserting the centrifuge tubes. This provides a cushion that helps create a uniform force around the base of the tubes during centrifugation. The cushion disperses the gravitational stress uniformly around the tube and reduces tube cracking and sample loss.
5. The yield of polyribosomes is 0.75 mg/g fresh weight of young tobacco leaves, assuming an extinction coefficient of ~15 A_{260} U/mg for ribosomes containing ~65% RNA.
6. Variations of the extraction procedure have been used for recovery of polyribosomes from many different tissues and species of plants, from fungi and from insects. The procedure thus has broad applicability that extends beyond its use in plants.

4. Preparation of Replicase Extracts from Nuclei of SYNV-infected Tobacco

4.1. Introduction

An indispensable tool in studies of the replication of rhabdoviruses and other negative-strand viruses has been the use of in vitro polymerases for the analysis of the factors that contribute to transcription of the viral messenger RNAs and to the replication of the genomic RNAs. These studies have been especially important in our understanding of the biochemistry and regulation of the replication processes of vesicular stomatitis virus ***(19)***. However, purified SYNV virions lack an active polymerase, and this has hampered research. Wagner et al. ***(10)*** have recently circumvented this problem by devising a procedure for isolating polymerase activity from the nuclei of infected tobacco leaves. We hope this procedure will be of general utility for studies of other nuclear associated rhabdoviruses.

4.2. Materials

1. Nuclei extraction buffer: Mix 40% v/v glycerol, 600 m*M* ribonuclease-free sucrose, 5 m*M* $MgCl_2$. Adjust the mixture to 480 mL with ddH_2O. Then, add 1 mL of diethylpyrocarbonate (DEPC), mixed with 1 mL of ethanol, to destroy RNase activity. **Caution:** DEPC is toxic, so carry out this step in a hood (*see* **Subheading 4.4., Note 1**). Let the DEPC-treated H_2O sit overnight at 37°C, and autoclave to destroy the DEPC. Store at 4°C. Shortly before use, add Tris-HCl, pH 8.0, to 25 m*M*, spermine to 2 m*M*, and β-mercaptoethanol to 10 m*M*. Immediately (<5 min) before use, add phenylmethane sulfonyl fluoride (PMSF) to 1 m*M* and add H_2O to 500 mL. The PMSF stock is stored at room temperature as a 200-m*M* solution in isopropanol.
2. Mannitol buffer: For a 500-mL solution, add 250 m*M* mannitol (22.9 g), 5 m*M* $MgCl_2$ (0.51 g) and H_2O to 480 mL. Add 0.5 mL DEPC, leave at 37°C overnight,