A new ultracentrifugation technique for analysis and isolation of polysomes

Leslie Kelso-Winemiller, Julie Drawbridge and Matthew M. Winkler*

Center for Developmental Biology, Department of Zoology, University of Texas, Austin, TX 78712, USA
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Isolation of polysomes is usually accomplished by density gradient centrifugation. However, the centrifugation conditions required to effectively separate polysomal mRNA from nontranslated mRNA causes most of the polysomes to pellet. We have developed a method which compresses polysomes into a small region near the bottom of the gradient, without pelleting them. This is accomplished by underlaying conventional gradients with a "cushion" of 80% glycerol or 60% sucrose containing EDTA. When polysomes reach this cushion, the EDTA causes them to disaggregate, greatly reducing their rate of sedimentation.

Figure 1 illustrates the separation of nontranslated and polysomal mRNA using "cushion" gradients. The dashed line indicates the position of mRNA 53, an abundant sea urchin maternal mRNA, and the solid line represents the OD₂₅₄ tracing. The polysomes are concentrated into a single fraction at the bottom of the gradient well separated from the nontranslated RNA which sediments above the 80S monosome peak. We have found that these gradients are especially useful for isolation of polysomes from tissues which have small amounts of polysomes, for quantification of polysomal material for study of translational regulation, and for preparing polysomal mRNA templates for construction of cDNA libraries. Gradients are very tolerant to overloading. As much as 200 OD₂₆₀ units can be layered onto one SW 28 gradient.

Fig. 1 Fractionation and concentration of polysomes from sea urchin embryo post-mitochondrial supernatants.

Linear gradients were prepared by pouring 26-ml of a 15-50% (w/v) glycerol (or 15-40% sucrose) in Cold IV (0.25 M NaCl, 5 mM EGTA, 5 mM MgCl₂, 1 mM DTT, 10 mM Pipes, pH 6.8) into SW28 (Beckman) centrifuge tubes and then underlaying with a 7-ml 80% (w/v) glycerol-EDTA cushion (or 60% sucrose-EDTA) (10 mM Pipes, pH 6.8, 10 mM EDTA). A post-mitochondrial supernatant from 15-minute *Strongylocentrotus purpuratus* embryos, diluted to a final volume of 1 ml with Cold IV and adjusted to 0.1% Triton X-100, was layered onto the gradient and centrifuged for 5 hours at 28,000 rpm in a Beckman SW 28 rotor at 4° C.

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*To whom correspondence should be addressed