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Isolation of ribosomes from Neurospora and their analysis using a vertical rotor.

Differential ultracentrifugation has enabled researchers to isolate and purify ribosomes. Conventional isopycnic centrifugation techniques to resolve ribosomal subunits have used swinging bucket rotors requiring long, time-consuming spins. This paper reports a new technique for ribosomal subunit separation in a sucrose density gradient by using a vertical rotor. We also report the effect of various storage condiditons on the stability of Neurospora crassa ribosomes.

The Dupont Sorvall TV850 vertical rotor contains eight fixed, vertically-positioned tube apertures. During controlled, slow acceleration, tube contents are reoriented 90°, producing a very narrow sample zone and increasing the slope of the gradient. Reorientation also significantly decreases run time due to shortening of the poth length through which the sample must travel.

The experiments reported here utilized 15 - 25% continuous linear sucrose (Beckman, ribonuclease-free) gradients mode in 50 mM Tris-HCl, pH 7.8 buffer containing 500mM KCl, 5mM $MgCl_2$ and 1 mM dithiothreitol (DTT). The gradients were refrigerated for 60 minutes at 10° C to stabilize them prior to centrifugation. The sample, ribosomes isolated from wild type Neurospora crassa and stored at -70° C (S.C. Schlitt and P.J. Russell 1974 J. Bacteriol, 120: 666-671), was thawed and immediately layered upon the gradient. Gradients were centrifuged in the TV850 rotor for 95 minutes, 47,000 rpm at 4° C. After centrifugation, gradients were displaced upwards through a flow cell to monitor nucleic acid absorbancy at 260 nm. This method of separation results in significantly greater resolution of the 60S and 37S ribosomol subunits (Fig. IA) when compared with that achieved with the usual separation technique in which gradients are centrifuged in a Beckman SW27. 1 swinging bucket rotor for 21 hours, 24,000 rpm at 4° C (Fig. 1B). Another advantage of this technique is that it overcomes the frustrating problem of partial degradation of the 37S subunit during centrifugotion of gradients in the SW27.1 rotor. Quantification of this 37S degradation, using 60S:37S peak amplitude ratios (PAR) indicates that TV850 gradients result in a mean PAR of 1.5:1 ond a substantially incrased resolution of the 60S and 37S subunits when compared to SW27. 1 gradients which result in a mean PAR of 3:1 for the two subunits.

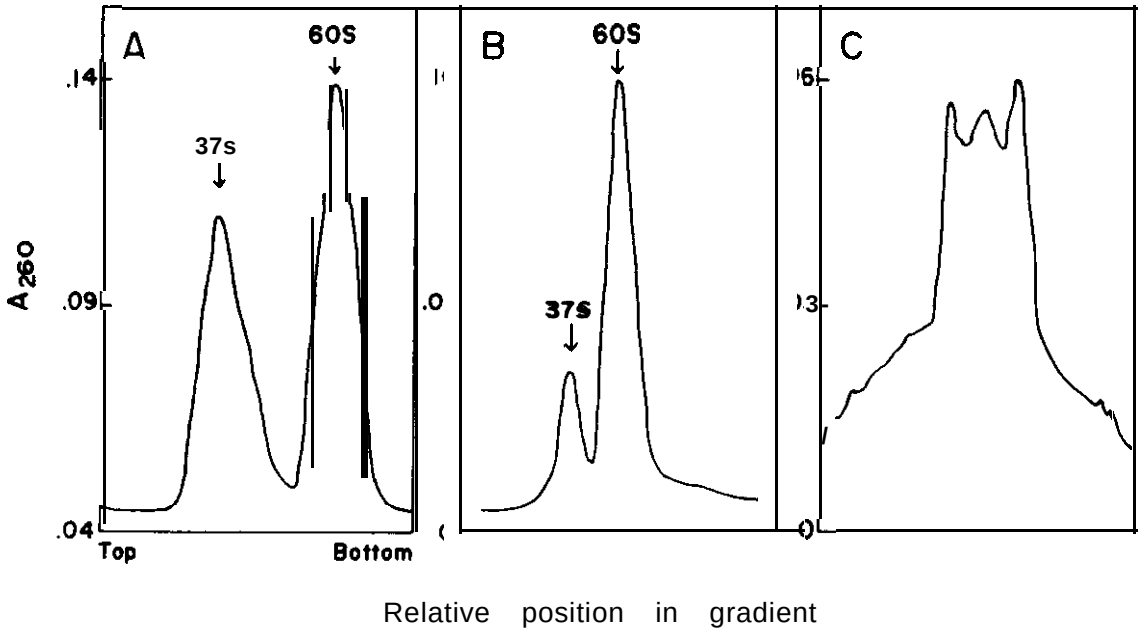

Figure 1. -- Zone sedimentation profiles of wild type ribosomal subunits produced by various means: A. Centrifugation in TV850 vertical rotor, 95 min, 47,000 rpm; B. Centrifugation in SW27. 1 swinging bucket rotor, 21 hr, 24,000 rpm; C. Maintenance of ribosome sample at 0° C for 3 hr, then centrifugotion in TV850 rotor as in A.

Having established an optimal method for ribosomal subunit separation and analysis, we examined the parameters for the preparation and storage of ribosomes prior to gradient analysis. We hove observed a correlation between the duration of handling ribosomes prior to layering on sucrose gradients and successful subunit separation. This led us to investigate the effects of temperature on subunit stability. After thawing, ribosomes were stored in on ice bath for 3 hours prior to layering on top of a 15 - 25% continuous gradient and centrifuging in the TV850 rotor as already described. A highly aberrant profile (Fig. 1 C) was obtained in comparison with the control (Fig. 1A). Repeated freeze-thawing of the sample also hod detrimental effects on both 37S stability and subunit separation. In summary, ribosomol subunits ore quickly and efficiently separated in a vertical rotor when layered on 15-25% sucrose gradients immediately after thawing. (Supported by Grant GM-22488 from N.I.H. and NSF Grant PCM76-21478.)

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