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Optimal Conditions for In Vitro Reconstitution of Small Nuclear Ribonucleoprotein Particles

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Aim. Optimization of conditions required for the in vitro reconstitution of small nuclear ribonucleoprotein particles (snRNPs) of the U-family using different parameters.
Method. Individual snRNPs were reconstituted by adding each small nuclear RNA of the U-family to the snRNP protein pool obtained from S 100 HeLa cytoplasmic extract. The mixture was then immunoprecipitated with anti-trimethylguanosine (aTMG) monoclonal antibody, followed by polyacrylamide gel electrophoresis and autoradiography.
Results. U1 required 0.5 nuclear extract equivalents of U1 snRNA, 6 mM MgCl2, and an incubation at 30 °C for 30 min; U2 required 0.5 nuclear extract equivalents of U2 snRNA under the above conditions, excepting Mg++ ions. U4 was reconstituted in a 20-minute incubation at a temperature of 30 °C, followed by a 10-minute one at 37 °C without Mg++ ions. U5 required 2 nuclear extract equivalents of U5 snRNA, 6 mM MgCl2, and an incubation at 30 °C lasting 30 min. U4/U6 needed 0.5 nuclear extract equivalents of each snRNA, and the same incubation duration and temperature as U5. U4/U5/U6 was reconstituted employing 0.5 nuclear extract equivalents of each snRNA, and a 30-minute incubation at 30 °C with 6 mM MgCl2. None of the snRNPs required ATP for reconstitution.
Conclusion. The optimal conditions reported in this paper should prove useful in elucidating the function of snRNP-specific polypeptides and in the potential reconstitution of the snRNPs active in splicing.

Key words: ribonucleoproteins, small nuclear; RNA splicing; RNA, small nuclear