Volume 16 Number 21 1988 Nucleic Acids Research

Purification of nascent DNA chains by immunoprecipitation with anti-BrdU antibodies

Lyubomir Vassilev*+ and George Russev

Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
Submitted July 25, 1988

Commercially available monoclonal antibodies against 5-bromodeoxyuridine (BrdU) have been widely applied recently for cytological detection of replicating DNA (Gratzner,H. (1982) Science 218, 474-475). Here we show that they can also be used for preparative purification of newly replicated DNA strands from the vastly larger fraction of genomic DNA. Anti-BrdU antibodies (Becton-Dickinson) specifically bind single stranded DNA synthesized in the presence of BrdU. DNA-antibody complexes formed can be easily separated from bulk DNA by immunoprecipitation with secondary anti-mouse IgG antibodies. In order to see whether nascent DNA strands can be purified from total DNA we mixed DNA continuously labelled with ^{14}C-thymidine and DNA pulse labelled (5 minutes) with ^{3}H-deoxycitidine in the presence of 50 uM BrdU, both isolated from Ehrlich ascites tumour (EAT) cells. An aliquot containing 250 ug of DNA was heat denatured, cooled on ice and immediately mixed with anti-BrdU antibody (several fold molar excess over DNA to be precipitated) in 0.14M NaCl, 10 mM Phosphate buffer, pH 7.2, 0.05% Triton X100. After 1 hour incubation at room temperature under mild shaking, an excess of a second antibody (anti-mouse IgG rabbit IgG fraction, Sigma) was added and incubation continued for another 1 hour. The precipitate formed was collected by 5 minute centrifugation in an Eppendorf microcentrifuge, dissolved in 0.5% SDS, 1M NaCl, 10mM EDTA, 50mM Tris-HCl, pH 8, and treated with Proteinase K (0.5mg/ml) at 37°C for 2 hours. DNA was deproteinized by phenol-chloroform (1:1) and chloroform extractions and ethanol precipitated. Aliquots were taken before and after immunoprecipitation and differentially counted. About 85% of ^{3}H-DNA was found in the precipitate and the ^{3}H/^{14}C ratio, representing newly synthesized portion of DNA, showed more than 200-fold enrichment of nascent DNA chains in the pellet. The specific radioactivity of the purified DNA fraction was 1.56×10^{5} ^{3}H-cpm/ug (more than 200-fold increase from initial 7.5×10^{3} ^{3}H-cpm/ug) and approached the specific radioactivity of DNA uniformly labelled under the same conditions in exponentially growing EAT cells (1.61×10^{5} ^{3}H-cpm/ug). Taking into account that a 5 minute pulse labels approximately 0.5% of the total DNA in unsynchronized EAT cells this result demonstrates the ability of the immunoprecipitation procedure to efficiently purify newly synthesized DNA strands from bulk genomic DNA. The method is fast and simple. It gives better separation than equilibrium density gradient centrifugation techniques and can be used in different studies in which purified nascent DNA chains are required.

*To whom correspondence should be addressed

+Present address: Box 1194, Brookdale Center for Molecular Biology, Mount Sinai School of Medicine, 1 Gustave Levy Place, New York, NY 10029, USA