A novel assay for DNA ligase

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Submitted May 21, 1991

Replication of the lagging strand in DNA biosynthesis is discontinuous and requires a ligase activity to join together the Okazaki fragments (1). A ligase enzyme may therefore be part of the multiprotein complex involved in DNA synthesis. Here we report a new assay for DNA ligase that is more direct and simple than the usual methods (2, 3). The new method was used to search for ligase that co-fractionates with the multiprotein DNA polymerase α -primase complex from HeLa cells (4).

DNA ligase was detected by its ability to join together two synthetic deoxyoligonucleotides in the presence of their complement. Polynucleotide kinase was used to 5'-phosphorylate just one of the oligonucleotides using a ³²P-label.

5'-TAGTCTCAAT pGGGCTGATAA
3'-ATCAGAGTTA-CCCGACTATT

As only one of the oligonucleotides was 5'-phosphorylated, only one product was possible from ligation, in this case a 20-mer from the two 10-mers. Blunt-end ligations were excluded by the lack of 5'-phosphates on the other oligonucleotides. Further overlapping-end ligations were also excluded by the sequences of the substrates. Formation of a 20-mer, indicating the presence of ligase, was detected by denaturing polyacrylamide gel electrophoresis that separated product from starting 10-mer. Some of the fractions assayed contained nuclease. In order to see evidence of ligase in these cases, the full length complementary strand was superior to a shorter chain that left the substrates with single-stranded ends.

The new assay was used to examine fractions from the reported preparation of DNA polymerase α_2 complex from HeLa cells (4) (Figure). Ligase activity in the starting nuclear extract (Lane 1) co-purified with the polymerase α_2 complex (Lane 11) while a nuclease activity also present in the nuclear extract and other fractions (Lanes 1, 2 and 10) was absent from the purified complex (Lane 11).

ACKNOWLEDGEMENTS

This work was supported by a grant from the G.Harold and Leila Y.Mathers Foundation, NIH grant CA15187 and by Cancer Center Support Grant P30 12708. We thank Dr Earl F.Baril for the facilities for J.K.V. to perform these studies.

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Figure. Assay for ligase activity during fractionation of HeLa nuclear extracts to give DNA polymerase α_2 . Each reaction contained 83 pmol of labelled oligonucleotide, 250 pmol of each unlabelled oligonucleotide and 4 μ L of test solution in a total volume of 20 μ L containing 50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 10 mM DTT and 250 μ M ATP. After incubation at 25°C for 5 hr, samples were electrophoresed on 15% polyacrylamide gel containing 8 M urea and autoradiographed. Mobilities of 10- and 20-mer oligonucleotides are indicated on the right. The fractionation of nuclear extract was described previously (4). Lane 1, starting nuclear extract. Lane 2, eluent from coupled DE-52, dsDNA and ssDNA columns. Lanes 3–9, various higher salt washes of the decoupled cellulose columns. Lanes 10–12, 0, 0.15 and 0.3 M salt washes of the DEAE–Biogel respectively. Lane 11 contains DNA polymerase α_2 .

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