A simple method for isolation of extrachromosomal circular DNA in unicellular eukaryotes

Gautam Chaudhuri and K.-P. Chang*

Department of Microbiology and Immunology, University of Health Sciences/Chicago Medical School, 3333
Green Bay Road, North Chicago, IL 60064, USA
Submitted January 25, 1988

The presence of extrachromosomal covalently closed circular (ECCC) DNA elements has been described in unicellular eukaryotes like *Leishmania* made resistant to methotrexate (1) and tunicamycin (2). Here we describe a simple method for isolation of ECCC-DNA from tunicamycin-resistant *Leishmania* based on an apparently yet unexploited unique characteristic of orthogonal pulse field agarose gel electrophoresis (OPFAGE). Our method is developed entirely depending on the difference in the migration behavior of linear and circular DNA in OPFAGE using inhomogeneous electric field when a sample with mixed populations of these molecules is loaded in a well nearer to the cathod array (Fig.1a). Low melting agarose (FMC Co.) blocks containing total intact DNA molecules of tunicamycin-resistant *L. mexicana amazonensis* promastigotes (10^7 cells) were made as described (2). The blocks were loaded in slots closer to the cathod arrays of the gel unit. Electrophoresis was run in a low melting agarose gel (0.5%) at 13°C in 0.5 X TBE at constant voltage of 300 volt with a pulse interval of 40 sec for 20 h. The migration of the linear chromosomal DNA markedly skewed towards the cathod arrays, while that of the ECCC-DNA followed a relatively strait path (Fig. 1b). The agarose containing the ECCC-DNA was cut and stored in 1X TBE at 4°C. The block containing ECCC-DNA was melted at 65°C, diluted with water and subjected to conventional phenol extraction procedure for purification of the DNA from agarose. The DNA in the agarose was also used directly without any difficulty for all molecular biological experiments, including oligolabelling, digestion with restriction enzymes and ligation for cloning. The ECCC-DNA isolated by the technique described was free from contamination by any chromosomal DNA fragments. When used as probe in Southern blots containing linear chromosomal DNAs and ECCC-DNA, the isolated DNA only hybridized to the latter (not shown). The method can clearly be used not only for identification but also for rapid isolation of ECCC-DNAs from other eukaryotic cells.

Fig. 1: (a) Differential migration of linear chromosome sized DNAs and ECCC-DNA as shown in EB-stained OPFAGE gels. (b) Two agarose blocks were loaded into the same slot during the isolation of ECCC-DNA. Arrowheads indicate the ECCC-DNAs.

Acknowledgements Supported by NIH-NIAID grant AI-20486 to KPC.

*To whom correspondence should be addressed

References

1. Garvey, E. P. and Santi, D.V. (1986) Science 233, 539-540.
2. Detke, S., Chaudhuri, G., Kink, J. A. and Chang, K. -P. (1988) J. Biol. Chem. (in Press).