Relaxation intervals alter the mobility of large DNA molecules in pulsed field gel electrophoresis

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We have used the programmable, autonomously-controlled electrode (PACE; ref 1) electrophoresis apparatus to study the mechanism which underlies the differential mobility of large DNA molecules in agarose pulsed field gels (PFG). Schwartz and Cantor (2) proposed that DNA molecules undergo conformational changes prior to migration in a new direction following a field reorientation. The time required for this conformational change is proportional to its length. This was suggested as the basis of PFG separation. We have examined the effects on the PFG mobility of yeast chromosomes of inserting zero field intervals of various lengths between two active fields (See figure). The mobilities of the 1,051 and 930 (unmarked) kilobase pair (Kb) bands dramatically increase with increasing zero field intervals. The mobility of the bands from 260 to 830 kb remain relatively unchanged. The size of the molecules whose mobility is affected by a zero field interval depends on the duration of the active fields. When 45 second (sec.) active fields are used the affected size is approximately 600 kb (data not shown). Increased DNA mobility with a zero field interval is consistent

with Schwartz and Cantor's model which postulated that some portion of the time of each new field is spent in molecular reorientation. Reorientation or relaxation which takes place during the zero field interval may reduce the time required for the molecules to begin moving in the new electric field. The relaxation of DNA in an agarose gel when an electric field is removed has been directly measured (3,4). The time scale of this process is about 30 seconds for DNA of 250 kb (4). We find that more than 90 sec. is needed for complete relaxation of the 1051 kb molecules, as the mobility increases further with a 180 sec. zero field interval.

Figure: Yeast chromosomes were separated in 1% agarose gels. The two active states of 6 V/cm and 90 sec. duration differed in orientation by 120°. The duration of the zero electric field inserted between each active state is indicated above the lanes. The total length of the active states in each case was 30 hours.

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References

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