INDUCTION OF CHROMOSOMAL ALIGNMENT BY HIGH FREQUENCY ELECTRIC FIELDS

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1. Introduction

Since the alignment of certain cells and subcellular entities has been attained by externally applied electric or magnetic fields [1–7] it seemed probable that chromosomes likewise will align in a specific manner depending on frequency and strength of applied electric fields. In this investigation, it is shown that chromosomal alignment may be induced by high frequency electric fields and that induction of chromosomal alignment by externally applied electric fields may be used for the estimation of dipole moments of normal and abnormal chromosomes.

2. Materials and methods

Chromosomal suspensions from human peripheral blood lymphocytes and established cultures of Chinese hamster cells (CHO), containing 10^6 cells/ml, were prepared using standard techniques:

1. Lymphocytes were cultured for 60–70 h in McCoy's SA medium (Gibco) supplimented with 15% (v/v) fetal calf serum and 2.5 μg/ml phytohaemagglutanin. The cultures were then incubated for 3 h in presence of 0.1 μg colchicine/ml. Following 30 min hypotonic treatment with 1.0% (w/v) sodium citrate the suspensions were centrifuged and pellets resuspended in 0.1 M sucrose containing 2.5% (w/v) citric acid and 0.1% (w/v) Tween 80.
2. Chinese hamster cells chromosomes were prepared as in [8]. Chromosome suspensions were used in 0.02 M Tris–HCl buffer containing 0.001 M each of $CaCl_2$, $MgCl_2$ and $ZnCl_2$ as well as 0.1% (w/v) Tween 80.

2.1. *Viewing cells and exposure conditions*

Fabrication of viewing cells consisting of two cover-slips separated from each other by a parafilm frame has been described [7]. Briefly, chromosome suspensions were pippeted between parallel, 0.064 mm diam. platinum wires fixed firmly in place 0.6 mm apart. The viewing cell was then sealed by heating parafilm. An electric field was generated by an alternating potential difference produced by a microdot Power Oscillator model M445.

In these experiments Chinese hamster and human chromosomes were exposed to field strengths of 50–600 and 50–800 V/cm, respectively, while the frequencies ranged from 2–50 MHz. The magnitude of the field between two wires was established from the peak-to-peak potential difference divided by the separation of the wires. Thus, for example, a potential difference of 24 V corresponds to an electric field of 400 V/cm.

2.2. *Visual observations and microphotography*

Visual observations were performed using phase-contrast optics, which however proved unsuitable for high contrast photography of chromosomes in motion. Therefore, a Nikon S series microscope equipped with Automatic Microflex (Model AFM) strobe light and camera was used for microphotography as follows: with the field turned off, the viewing cell was scanned visually to locate a cluster of chromosomes. The cluster was then photographed, the field turned on and its strength and/or frequency varied. When, in response to the field, the chromosomes began to move, they were photographed again and the field variables recorded.

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3. Results and discussions

Dispersed chromosomes did not respond to applied electric fields in the absence of an emulsifying agent, Tween-80. Two factors may be involved in account for this negative response. Clumping as well as adhesion of chromosomes to glass surfaces could have impeded chromosomal motion in an electric field. This explanation was, however, ruled out since mechanical agitiation of suspension failed to impart any detectable change of chromosomal mobility. Addition of Tween-80 to suspensions did in fact induce chromosomal motion by an external electric field. Orientation and motion of chromosomes was probably due to chromosomal dipole induction since formation of counterions on chromosomal surfaces evidently created requisite conditions for chromosomal motion in an electric field namely the tangential mobility of counterions leading to polarization of the system, i.e., dipole induction [9–11].

Induction of orientation thresholds of dispersed chromosomes by externally applied fields as a function of field strength and frequency are depicted in fig.1. At low frequencies, hamster chromosomes required much stronger field than lymphocytes chromosomes. Within 10–40 MHz range, the situation was reversed while in the ranges exceeding 40 MHz, the chromosomal response was essentially identical. The observed chromosomes orientation phenomenon appeared to be analogous to similar observations made on intact *Escherichia coli* [5] and to the orientation of isolated retinal rods and muscle fibers by applied magnetic field [4,12]. The fact that human and hamster chromosomes had markedly different orientation thresholds could not be attributed to size differences but suggests intrinsic dissimilarity of chromosome surface ionization potentials, i.e., the magnitude of induced dipoles. Whether or not this counter-distinction is phylogenetic or is attributable to state of chromosomes in established as opposed to primary cultures remains to be determined.

Increasing field strength of a set frequency to approximately twice the orientation threshold values resulted in chain formation of dispersed chromosomes (fig.2). In contrast to chromosome orientation, the threshold strength for chain formation was identical for both sets of chromosomes. Formation of chromosome chains was analogous to chain formation by a variety of biologic and non-biologic entities [1,13], and was induced by external electric fields

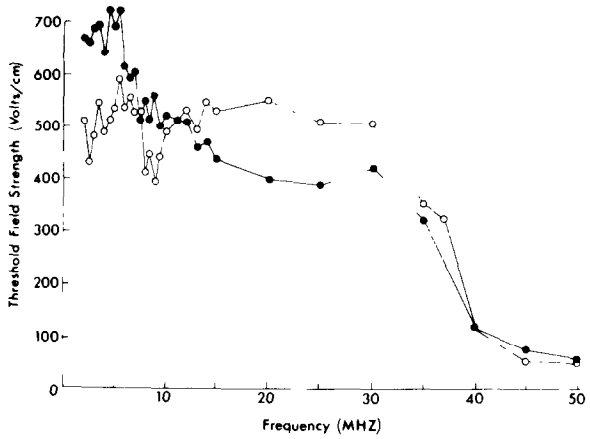
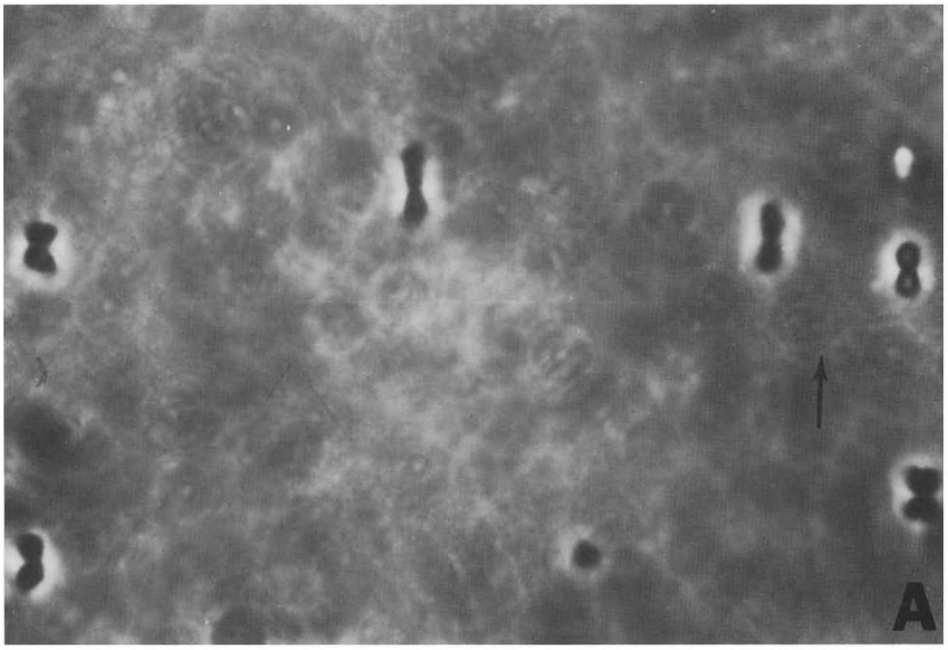


Fig.1. Frequency dependence of the orientation threshold of CHO Chinese hamster chromosomes (•) and human lymphocyte chromosomes (○). Each point represents an av. ⩾3 determinations.

changing charge configuration of chromosomes so that each chromosome may be regarded to behave as concentric dipole. Since a system of adjacent dipoles may be expected to induce less than perfect fields, their potential energy would be minimized by chain formation [6,15–17]. Chain formations were ruptured by increasing field to ~800 V/cm and chromosomes moved rapidly towards the wire poles. This translational motion of chromosomes was essentially dielectrophoresis [18] and could be attributed to variations in electric field between two parallel wires, since a particle in an inhomogeneous field would move in the direction of field gradient [19]. A possibility that translational electric forces could be used for chromosomes separation by size was tested by setting the wires at a slight angle to one another. In theory, this configuration should produce inhomogeneous fields in a direction along the wires with a translational force being exerted on chromosomes in the same direction. Only a slow drift of chromosomes was observed under stated conditions suggesting that simple rearrangement of electrodes was insufficient to yield usefully large field gradients.

Induced alignment of chromosomes by high frequency electric fields and the differences of orientation thresholds between hamster and human chromosomes was interpreted to signify the differences of respective dipole moments. Since chromosomal dipoles are the product of surface ions stabilized by an emulsifier it is suggested that selective degradation of chromosomal substrates by enzymes, such as



A

B

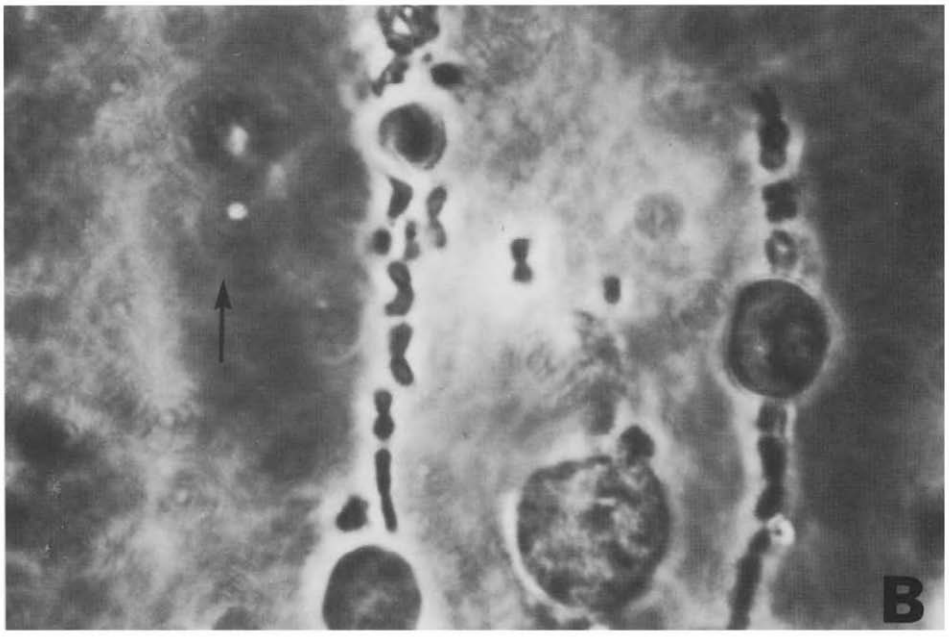


Fig.2. Orientation and chain formation of Chinese hamster chromosome suspensions: (A) Orientation of chromosomes along the field direction indicated by an arrow; (B) chain formation of chromosomes also along the field direction. ×1200.

DNase, prior to electric field exposure may prove to be useful in assessing relative contribution of chromosomal components to surface ionization. Practical aspects of this possibility remain to be determined.

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