New and Notable

Chromosome Condensation: Amorphous or Structured

John E. Hearst
Department of Chemistry, University of California, Berkeley, California 94720 USA

Since eukaryotic chromosome condensation and segregation was first observed in the light microscope more than a century ago, the process has attracted the imagination of nearly all students with the opportunity to observe the dynamics of this process. As the understanding of the structural components of the metaphase chromosome has become clarified, molecular reductionism has been increasingly challenged by this process. A chromatid of a human chromosome contains a single DNA molecule with a length of several centimeters and a diameter of 20 angstroms. In the course of the cell cycle, this DNA molecule is confined to a volume with a largest linear dimension of approximately 5 microns, and upon replication, the two daughter molecules are resolved or segregated with the complete elimination of entanglements. It is not surprising that the mechanisms of condensation and decondensation, and the mechanisms of packing the DNA molecule into structural fibers would attract the attention of polymer theorists. The article by Sikorav and Jannink (1993) entitled "Kinetics of chromosome condensation in the presence of topoisomerases: a phantom chain model" discusses the condensation of chromosome as though it is caused by the change of solvent from good solvent to poor solvent. On the basis of existing theories that deal with polymer aggregation accompanied by solvent exclusion in polymer melts, the observed kinetics of chromosome condensation are far too rapid to fit the theoretical condensation rates predicted by this model.

The "phantom chain model" allows polymer chains to pass through each other, thus enhancing the predicted rates of the condensation process to values consistent with chromosome condensation rates. The topo II activity known to be associated with chromosomes is considered to be the rationalization of this "pass through" or "phantom" model. The application of classical polymer hydrodynamic theories has been historically important for our understanding of the properties of biological macromolecules, and for this reason alone, such analyses should be encouraged and accepted for publication. Only in this way will they be subjected to scientific scrutiny. There are, however, serious issues, ignored in this manuscript, that are an important part of the DNA and chromatin literature.

First, the concentration of nucleoprotein in metaphase chromosomes is difficult to estimate, but I believe it is less than that of a pure melt that would exclude all solvent. In fact, my estimate is that even at metaphase, the nucleoprotein occupies, at best, 6% of the volume of the chromosome, the rest being occupied by solvent.

Second, a theory based on reptation is very sensitive to the existence of polymer ends and to the mechanical properties of the polymer fibers. The interphase and metaphase chromosome fibers contain essentially no ends, making reptation in the absence of further assumptions of looping and pairing of fibers a very speculative model to deal with.

Third, there is a rather unusual oversimplification and classification of topological constraints in this paper into two categories: "plectonemic supercoiling of the DNA duplex" or an "ordinary polymeric constraint." Such an analysis ignores the possibility of higher order helicity, for example, plectonemic forms of the 11-nm- or 30-nm fibers, which I consider to be far more likely mechanisms for chromosome condensation than simple "precipitation."

Finally, the cleavage and rejoining by topo II in a DNA melt environment is a very hazardous mechanism to advocate for chromosome condensation, for at such high concentration, what provides the free energy to create a systematic direction for such an activity? Extensive entanglement and knotting of the DNA would be likely, and undoing such a process might very well be the undoing of life itself.

Although the article by Sikorav and Jannink is intellectually of interest and of high quality, I am of the opinion that a more systematic mechanism must be at the heart of chromosome condensation.

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The Complexities of Cardiac Cables: Virtual Electrode Effects

John P. Wikswo, Jr.
Living State Physics Group, Department of Physics and Astronomy, Vanderbilt University, Nashville, Tennessee 37235 USA

When the heart is stimulated by a cathodal (negative) current pulse applied by a point electrode, the resulting depolarization of a small region of tissue at the electrode triggers a propagating, depolarization wavefront that expands outward from the electrode. This wavefront can be detected by optical measurements of the transmembrane potential, microelectrode measurements of the intracellular potential, extracellular measurements of the voltage gradients associated with the extracellular current, or magnetic measurements of the combined intracellular and

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extracellular currents. From the perspective of biophysical electrodynamics, the depolarization wavefront can be thought of as a solitary wave propagating through an active, nonlinear, three-dimensional, anisotropic cable. The wavefront is considered solitary because once initiated, it is not periodic; yet it propagates with a uniform waveshape and velocity in a particular direction. The active, nonlinear membrane properties that support the depolarization are the result of the time- and voltage-dependent conductances of the membrane ion channels. A single cardiac cell acts as an electrical cable because it is long (100 μm) and narrow (15 μm) and has two separate conductors—the intracellular and extracellular spaces—separated by a dielectric—the membrane. In contrast to skeletal muscle, in which the muscle fibers are millimeters to centimeters long but have intracellular spaces that are not directly interconnected, the intracellular spaces of cardiac cells in bulk myocardium are tightly coupled by gap junctions. Hence, a block of skeletal muscle acts as an ensemble of independent, one-dimensional cables, whereas a block of ventricular myocardium acts as a single, three-dimensional cable or syncytium that can support propagation of a wavefront in any direction.

Now we come to the complexity worthy of note: the anisotropy of cardiac tissue. With regard to the heart, this term is used in several different contexts. Because the long and thin myocardial fibers are arranged in an ordered fashion, the mechanical properties of the tissue are anisotropic in that they depend upon whether the tissue is stretched along the local fiber axis or across it. Also because of the fiber architecture, the propagation velocity of the wavefront is directionally dependent, i.e., anisotropic, with the conduction velocity parallel to the fiber direction being twice that across the fibers. Similarly, the time constant of the foot of the action potential (which is a measure of the electrotonic cable properties of passive tissue before it reaches the threshold for the nonlinear behavior) and the rate of rise of the nonlinear action potential show differing directional dependencies. The measurement of an effective electrical conductivity of bulk myocardium, as might be accomplished with the standard technique of four co-linear electrodes, would show a conductivity that varied by a factor of four or so depending upon whether the linear electrode array was oriented parallel or transverse to the fibers, so that Ohm's law would have to be written as a tensor equation $\boldsymbol{J} = \tilde{\sigma}\boldsymbol{E} = \tilde{\sigma}\boldsymbol{\nabla V}$, where $\tilde{\sigma}$ is the anisotropic conductivity tensor. However, cardiac tissue is not a single conductor but a three-dimensional cable, so the effects of the fibrous architecture must be included into the description of both the intracellular and extracellular spaces, yielding two separate versions of Ohm's law and two anisotropic conductivity tensors $\boldsymbol{J}_i = \tilde{\sigma}_i\boldsymbol{\nabla V}_i$ and $\boldsymbol{J}_e = \tilde{\sigma}_e\boldsymbol{\nabla V}_e$. If we assume cylindrical symmetry along the local fiber axis, the conductivity tensors each have only a longitudinal and a transverse component, i.e., σ_{le}, σ_{ti}, σ_{le}, and σ_{te}, with values thought to be 200, 20, 800, and 200 μS/mm, respectively. These four numbers are the source of much of the mischief.

The mathematical models that incorporate these four conductivities generally require numerical solution (Sepulveda et al., 1989). A major simplification of the equations occurs with the assumption of equal anisotropy ratios: $\sigma_{le}/\sigma_{ti} = \sigma_{le}/\sigma_{te}$, for which there is only a single nonlinear equation to be solved over space and time rather than a coupled pair, but, as we will see, at significant cost in terms of physiological reality. Models that include the realistic conductivities predict several surprising phenomena, most of which are associated with strong stimuli or small, expanding wavefronts (reviewed by Wikswo, 1993). The first of these predictions to be confirmed experimentally relates to the virtual cathode effect: upon strong cathodal stimulation, a region of tissue adjacent to the electrode is immediately depolarized electrotonically, and the resulting dogbone-shaped virtual cathode is often twice as large ($\approx$3 mm) in the transverse direction than longitudinally. The relevant experiments are discussed by Knisley et al., 1994. This is in stark contrast with the predictions by models with equal anisotropy ratios of an elliptical virtual cathode that is twice as large longitudinally than transversely. It is difficult to describe in a few words why the differing anisotropies of the intracellular and extracellular conductivities are responsible for the oddly shaped virtual cathode; most simply, the extracellular currents cannot flow with the same spatial distribution as the intracellular ones. The differences in the two current distributions also produce a quatrefoil pattern in the net current, which in turn produces a measurable magnetic field. Models with equal anisotropy ratios would predict equal and opposite intracellular and extracellular currents and no magnetic field. The realistic, nonlinear model may also provide an explanation of the confusing directional differences of the time constant of the foot and the rate of rise of the action potential.

The realistic model also predicts that a **cathodal** stimulus would produce a 1- to 2-mm region of **hyperpolarization** along the fiber axis on either side of the transversely oriented dogbone. Knisley et al. (1994), in this issue of the *Biophysical Journal*, present the first definitive confirmation of this prediction, with data that clearly demonstrate the existence of a longitudinal hyperpolarization for cathodal stimulation of mammalian heart. Although fully consistent with the model, this finding would be particularly surprising to anyone accustomed to using a one-dimensional cable model to explain the behavior of a block of cardiac tissue.

Whence the excitement over these recent results? First, the observed cathodal hyperpolarization and anodal depolarization are a more direct confirmation of the predicted effects of differing tissue anisotropies than are back-extrapolations of extracellular potentials to obtain virtual cathode shape, or magnetic measurements of action currents. More importantly, they bring into question the concept of using a linear four-electrode array and a one-dimensional cable model to determine tissue conductivities in two- and three-dimensional tissue preparations, which

thereby suggests that the accepted numbers for the four conductivities may be inaccurate. The three independent confirmations of the fully anisotropic model would indicate that it may be unwise hereafter to use models with equal anisotropies or a grounded extracellular space, at least at spatial scales of several millimeters.

Experimental attention now needs to be turned to the predictions of quatrefoil spiral-wave reentry and anodal stimulation (Roth, 1994, in press), as well as to the accurate determination of the four anisotropic conductivities. It may be worthwhile to revisit the DC injury potentials, which are also described by the bidomain model (Tung, 1978). Another challenge will be to increase the spatial resolution of each of the experimental techniques used so far to assess for the effects of cellular discontinuities in bulk tissue (Fast and Kléber, 1993). Theorists need to extend their realistic nonlinear models toward the scale of the entire heart while also including the discontinuous structure at the cellular scale. The success of this model and the existence of these new phenomena suggest that we are on the path towards gaining a more complete understanding of the propagation of cardiac action potentials on the several-millimeter spatial scale, which in turn should help us understand the complex interaction between cardiac membrane channels, the cable-like properties of bulk cardiac tissue, and the macroscopic response of the heart to stimulation, defibrillation, and certain antiarrhythmic drugs.

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