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Nonlinear dynamics of cell orientation

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The nonlinear dependence of cellular orientation on an external, time-varying stress field determines the distribution of orientations in the presence of noise and the characteristic time, τ_c, for the cell to reach its steady-state orientation. The short, local cytoskeletal relaxation time distinguishes between high-frequency (nearly perpendicular) and low-frequency (random or parallel) orientations. However, τ_c is determined by the much longer, orientational relaxation time. This behavior is related to experiments for which we predict the angle and characteristic time as a function of frequency.

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I. INTRODUCTION

The dynamics and mechanics of cells are important for wound healing, muscle growth, tissue assembly, and development. Cells respond to their mechanical environment in an active manner (e.g., by actively adjusting their contractility) [1,2] and by reorganizing their stress fibers, adhesions and traction forces to maintain a tactile set point in the adjacent matrix [3–6]. For static or quasistatic strain, some cells tend to orient along the direction of applied stress [7–10] while some cells maintain a random orientation [11]. However, for rapidly varying strains, cells tend to orient away from the stress direction [12,13]; in some cases, this can be identified with the zero-strain direction.

In this Rapid Communication, we predict the dynamics of cell orientation as a function of the frequency and amplitude of the externally applied stretch and random "noise" (modeled as an effective temperature). At low frequencies, the cell orientation can be random or nearly parallel to the stress, depending on whether the cell-matrix mechanical interaction is, respectively, smaller or larger than the noise. At high frequencies, the cell regulation forces [14,15] typically dominate the noise and the perpendicular (or zero-strain direction [15,16]) orientation is predicted. A simple nonlinear analysis is used in these calculations and this approach also predicts the characteristic time, τ_c, required for cells to obtain their steady-state orientation [11]. This time is strongly frequency dependent for stretch frequencies smaller than about 1 Hz; at larger frequencies, τ_c is frequency independent. We explain these observations using the dynamical theory that identifies the 1 Hz time scale with the time for local, molecular reorganization of the cytoskeletal components. The much larger value of τ_c itself—on the order of 10^3-10^4 s, is related to the much longer time scale for the cooperative reorientation of the cytoskeleton.

II. THEORETICAL MODEL

For simplicity, we consider stationary, mechanically active cells that have already established mature focal adhesions and are in mechanical equilibrium with the surrounding matrix. In a coarse-grained picture, we focus on the sum of the forces exerted by the cell via its focal adhesions, modeled as a pair of equal and oppositely directed acto-myosin contraction forces [14] that form a dipole that is the product of the distance, $\vec{l}$, between the oppositely directed forces, $\vec{f}$. The typical magnitude of the dipole strength for contractile cells, $P \sim |f||l| < 0$, is $\simeq 10^{-11}$ J [14]. This can be generalized to include interactions of many cells [17]. For simplicity, we focus on needlelike cells, e.g., muscle cells and fibroblasts; for these cells (aligned along x on the surface of a substrate), the dipole is: $P_{ij} = P\delta_{ix}\delta_{jx}$.

The local activity of the cell is an important part of our model that distinguishes cells from nonliving matter. Cells actively adjust their contractility by reorganizing the focal adhesion and stress fibers to maintain an optimal (or set-point) xx component of the stress, $\sigma^\star$, or strain $U^\star$ in the adjacent matrix [14–16]. We convert these quantities to energies by defining $P^\star \sim \sigma^\star a^3 \sim U^\star E a^3 > 0$ where a^3 is the cell volume and E is the Young's modulus; the expressions are also proportional to a function [15] of the Poisson ratio of the matrix, ν, whose detailed form is different for cells in three dimensions or on substrates. This is not important for the scaling relationships discussed here. We take $P^\star > 0$ and note that the cellular contraction dipole $P < 0$. In the presence of external stretch (converted to energy units by multiplying by a^3), with magnitude $P_a(t) > 0$ (that can be time dependent), acting at an angle θ relative to the cell axis, the homeostatic, set-point *total* local stress in the matrix is achieved when the cellular force dipole obeys

$$P = -P^\star + \alpha_0 P_a(t)(\phi - \phi_1), \tag{1}$$

where $\phi = \cos^2\theta$. This form is a generalization of the cases [15,16] in which (i) the cellular dipole is controlled by the matrix stress where $\phi_1 = 0$ (ii) the dipole is controlled by the matrix strain and $\phi_1 = \cos^2\theta_0 = \phi_0$, where θ_0 is the zero-strain direction given by $\cos^2\theta_0 = \nu/(1+\nu)$. In both cases, α_0 is a function of only ν; in general it can be either positive or negative corresponding to matrix stretch that causes either a decrease or an increase in the cytoskeletal forces, respectively. The experimental situation is not yet clear, with some reporting a decrease [4,18] while others measuring an increase, at least of the passive elastic response of the cytoskeleton [18,19]. The general response function treated here allows both the parallel and perpendicular components of the

external stress to modulate the cellular dipole [17,20].

Deviations from the set-point result in internal forces within the cell that reestablish the optimal force condition. These forces can be derived from derivatives of an effective, harmonic free energy [21] due to cell activity, F_a, that includes the active processes within the cell that establish cellular response to its *local* environment,

$$F_a = \frac{1}{2}\chi P^{\star 2}[-p + p_a(t)(\phi - \phi_1) - 1]^2, \tag{2}$$

where $\chi P^{\star 2}$ (with units of energy) is a measure of cell activity that establishes the set point. We define dimensionless quantities: $p = P/P^{\star}$, $p_a(t) = P_a(t)/(\alpha_0 P^{\star})$ which are the cell dipole and external stress relative to the set point, $P^{\star}$. We define the dimensionless energy: $f_a = F_a/(\chi P^{\star 2})$ whose minimum (zero force condition) determines both the dipole magnitude and orientation.

In addition to the cell activity, we also consider the effect of the mechanical matrix forces [15] that is proportional to the product of $P_a(t)$ and P. This energy, f_m, is written [15] in terms of the dimensionless quantities defined above,

$$f_m = cpp_a(t)(\phi - \phi_0), \tag{3}$$

where $c = (1+\nu)\alpha_0/(Ea^3\chi)$. Since cells are contractile ($p<0$) the mechanical energy, f_m, is minimized for dipoles parallel to the applied stretch ($p_a>0$). The total effective, dimensionless free energy, f is $f = f_m + f_a$.

In the absence of external stresses, it has been observed that cells align in a random manner [11–13]. At low frequencies, both random [11] and parallel [4,7,28] alignment are observed; this suggests competition between the elastic energy given by f and noise that can arise from either thermal motion of cellular proteins or from active forces such as myosin contractility. We consider a simple model of active noise similar to that of [22] in which the noise, $\zeta(t)$, decorrelates in time with a rate $1/\tau_n$ so that $\langle \zeta(0)\zeta(t)\rangle \sim T^* \exp[-t/\tau_n]/\tau_n$ (where T^* is a constant that in the limit of $\tau_n \to 0$, is the effective temperature). The system is described by an order parameter ψ, a free energy, $g(\psi)$, and an intrinsic relaxation time, τ_0. The noise [22] results in fluctuations of the temporal Fourier transform of the order parameter, $\psi(\omega)$, given by $\langle |\psi(\omega)|^2\rangle \sim T^*[(\omega^2+\omega_0^2)(1+\tau_n\omega^2)]^{-1}$. Here ω_0 is proportional to $g''(\psi)/\tau_0$ where the second derivative of g is evaluated at the mean-field value of the order parameter, where $g'(\psi)=0$. In the limit that the noise decays on short time scales (relative to the experimental time scale characterized by ω; see below), $\omega\tau_n \ll 1$, the fluctuations are the same as those of equilibrium systems with an effective temperature, T^*. For slowly varying noise, the effective temperature becomes frequency dependent due to the factor $(1+\tau_n\omega^2)^{-1}$. For simplicity, and as an attempt to motivate experiments that measure the character of the effective noise via the angular orientations, we consider the case where τ_n is small and use a Boltzmann-like distribution with a competition between the elastic energy given by f and T^*. Experiments [23] have found that a Boltzmann-like distribution can describe cell orientations on curved substrates. The effective temperature has been estimated [24] as $\sim 10^6$ room temperature since the randomization of the adhesions involves the disruption of $\sim 10^5$ bonds, each of which has an energy of ~ 10 times room temperature. The large number of bonds involved may account for the long times (5000 s) needed for cells to reach their steady-state orientation [11].

III. DYNAMICS

We now predict the orientational response of cells in the presence of time-varying stress: $p_a(t) = p_a(1-\cos\omega_a t)$. In general, the dynamics of the cytoskeleton are governed by complex, viscoelastic processes [2] involving actin polymerization and assembly via crosslinkers into contractile bundles due to the action of myosin motors. In addition, these contractile forces are responsible for the stabilization and growth [25,26] of focal adhesions that connect the cytoskeleton to the substrate or matrix. The representation of an entire cell as a force dipole, as described above, coarse grains over all these dynamics. In Ref. [15] the force dipole was treated as a rigid rotor (assuming that cell size and shape were fixed and ignoring fluctuations). This predicts dynamics with a single, intrinsic time scale.

However, experiments show that cells respond to stress on multiple time scales [2] due to the various processes mentioned above. It is thus more reasonable to model the dynamics by considering separately, the relaxation to steady state of the dipole magnitude (related to the concentration of actin bundles and myosin motors) and its orientation (related to the correlations between the assembly of neighboring bundles in response to stress). This suggests the following equations (noise effects are discussed later) for relaxation that are linear in the generalized forces [15] given by the derivatives of the free energy with respect to the appropriate degree of freedom. Due to the separation of time scales, discussed below, we ignore cross terms in which the force, $\partial f/\partial p(t)$ affects the dynamics of $\theta(t)$ and vice versa,

$$\frac{dp(t)}{dt} = -\frac{1}{\tau_p} f_p; \frac{d\theta(t)}{dt} = -\frac{1}{\tau_\theta} f_\theta; \frac{d\phi}{dt} = -\frac{1}{\tau_\theta} 4\phi(1-\phi) f_\phi, \tag{4}$$

where $f_x = \partial f/\partial x$. The last equation for $\phi = \cos^2\theta$ is obtained using the chain rule.

Based on experiments [2,18,19], it has been suggested that the liquification and repolymerization of the actin after stress is applied, occurs on a short time scale of the order of several seconds, while the correlated reorientation occurs on much longer time scales (of the order of many minutes) [4,7,11]. We thus assume that $\tau_p \ll \tau_\theta$: the time scale associated with changes in the magnitude of the dipole is much faster than that associated with the dynamics of its highly correlated reorientation. In this approximation, the dipole magnitude reaches a steady-state value in a short time; this value may be time dependent and oscillatory due to the cyclic nature of the applied time-dependent stress.

We therefore first solve for $p(t)$ treating $\phi(t)$, which is much more slowly varying, as a constant. The dynamical equation for $p(t)$ is linear. For times long compared with τ_p we can neglect a term proportional to $\exp(-2t/\tau_p)$ and find

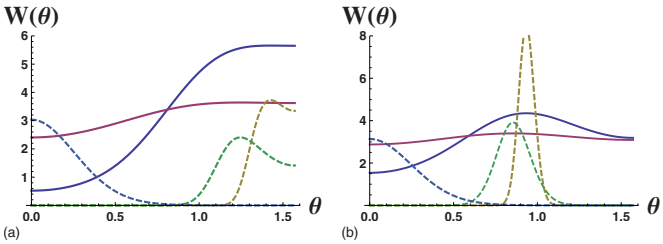

FIG. 1. (Color online) $W(\theta)$, the distribution of angles vs angle (in radians) of cells (in steady state) controlled by stress (left hand) and strain (right hand). The dashed curves are for $T_s=0.001$ and scaled frequencies $\omega=10,0.5,0.001$ (uppermost right, lower right and left, respectively). The solid curves are for $T_s=0.1$ with $\omega=10,0.5$ (upper right and lower right, respectively); for the solid curves, we show $5W(\theta)$ for clarity. The distributions are normalized to unity in the physical interval $0\leq\theta\leq\pi/2$. We take $\nu=1/2$, $c=0.001$, and $p=0.1$. For smaller values of ν, the peaks in the right-hand panel move to the right.

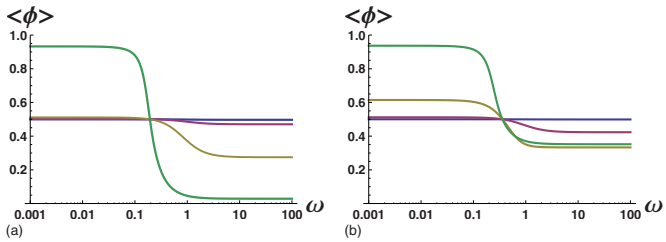

FIG. 2. (Color online) The average of $\langle\phi\rangle=\langle\cos^2\theta\rangle$ (in steady state) vs the scaled frequency $\omega=\omega_a\tau_p$ for cells controlled by stress (left-hand plot) and strain (right-hand plot). The scaled temperatures are $T_s=10,1,0.1,0.001$ as one looks at the graphs from top to bottom in the left-hand panel at high frequencies and in the right-hand panel from the bottom to the top at low frequencies. Here $\nu=1/2$, $c=0.001$, $p=0.1$.

$$p(\tau)=-[1+g(\phi)]-g(\phi)\left[\frac{\cos\omega\tau+\omega\sin\omega\tau}{(1+\omega^2)}\right], \quad (5)$$

where $g(\phi)=p_a(c(\phi-\phi_0)+(\phi-\phi_1))$; the dimensionless frequency $\omega=\omega_a\tau_p$ and time $\tau=t/\tau_p$ both scale with the short relaxation time τ_p. Thus, differently oriented stress fibers have different force magnitudes. We now use this expression for $p(t)$ in Eq. (4) for $\phi(t)$.

The nonlinear dependence of the dynamics on the external stress can be treated approximately by noting that we are interested in the dynamics for time scales of the order or larger than τ_θ. If the lowest frequencies of the cyclic stress satisfy $\omega_a\tau_p=\omega\gg\tau_p/\tau_\theta$, we can average the sinusoidal terms over one cycle since the response of $\phi(t)$ occurs on much larger time scales (even for relatively small values of $\omega\ll1$). The linear sinusoidal terms vanish but there is a nonlinear, frequency-dependent contribution from the quadratic terms.

The effective free energy, using Eq. (5), averaging over a cycle, and keeping terms linear in $c\ll1$ is

$$f^e=-cp_a\Delta\phi_0+\frac{p_a^2\Delta\phi_1}{4(1+\omega)^2}[2c(3+2\omega^2)\Delta\phi_0+\omega^2\Delta\phi_1], \quad (6)$$

where $\Delta\phi_i=\phi-\phi_i$. We use this expression for f in Eq. (4) for $\phi(t)$; the results compare well with numerical solutions of Eq. (4) when $\tau_p\ll\tau_\theta$. Note that the frequency dependence in f arises from the nonlinear dependence of the dynamics on the external stress, p_a.

The dimensional free energy, $F^e=f^e\chi P^{\star2}$, competes with the noise in the system that we model as an effective temperature, $T^*=T_s\chi P^{\star2}p_a^2$ where T_s is a scaled temperature. The probability of a given cellular orientation, $W(\theta)$, as a function of the dimensionless frequency, ω, is proportional to $\exp[-f^e/(T_sp_a^2)]$ and is shown for various frequencies and temperatures in Fig. 1. For $T_s=0.1$, at low frequencies, the angular distribution is relatively broad, while for high frequencies, the distribution has a maximum at an angle close to $\pi/2$ for the case of stress as a set point and at the zero-strain direction, θ_0, for strain as a set point [27]. At larger values of the effective temperature, the distribution flattens out even for relatively high frequencies. The nearly perpendicular orientation for high frequencies has been interpreted [14–16] in terms of the dynamic frustration of the cell: at high frequencies $\omega_a\tau_p\gg1$, the cell cannot respond to the external stress and would be dynamically frustrated if it were to remain parallel to the applied stress (which does lower the mechanical energy) or even random (in response to the noise). The cell thus reorganizes its cytoskeleton to the nearly perpendicular (or for the case of strain as the set point, the zero-strain) direction where there is no time-varying stress (or strain) and homeostasis can be achieved. The present calculations show how the orientation is a function of the cell activity, the mechanical energy and the noise.

The average value of $\phi=\cos^2\theta$ is calculated as a function of the frequency of the applied stress for several different effective temperatures in Fig. 2 for the cases of both stress and strain as set points. At very low effective temperatures, the average angle is nearly perpendicular (or in the zero-strain direction, θ_0, for cells whose set point is determined by matrix strain) at high frequencies, due to the dynamical frustration discussed above. More generally, the frequency-dependent orientation angle at low temperatures is given by the minimum of Eq. (6); for the case of stress as a set point we can write an approximate expression that is correct in both the high ($\omega\gg1$) and low-frequency limits: $1\gg\omega\gg\sqrt{c}$: $\langle\phi\rangle\approx(2c/p_a)(1+\omega^2)/\omega^2$. The full analytic expression is shown in the left-hand plot of Fig. 2 as a dashed line that compares very well with the numerical solution. At very low frequencies, the average angle is nearly parallel and for both the cases of stress and strain as set points: $\langle\phi\rangle\approx1-p_aT_s/(2c)$, consistent with experiments [4]. At higher effective temperatures, the orientation distribution is random and the average value of ϕ (in two dimensions) is 1/2 for all frequencies. At intermediate temperatures, in our model where both the cell activity forces and the noise dominate the mechanical forces (i.e., $c=1/(\chi Ea^3)\ll1$, $T^\star\gg P^{\star2}/(Ea^3)$ but $T^\star\ll\chi P^{\star2}$) we have the interesting case of nearly perpendicular orientation for high frequencies, but nearly random orientation for low frequencies (see Fig. 2). This regime seems to be applicable to recent experiments reported in [11]; however, some cell types studied [28] show a somewhat parallel and nonrandom orientation at low frequencies.

These differences may be attributed to different values of the scaled, effective temperatures for various cell types.

The dynamical theory is now used to calculate the relaxation of the system from a random orientation to the steady-state orientation. As in the experiments [11], we determine the characteristic time, τ_c, for this relaxation. In the absence of fluctuations, it can be calculated from Eq. (4) by solving for the steady-state value of $\phi=\phi_s$ where $\partial f^e/\partial\phi=0$, and writing the dynamical equation in terms of ϕ_s; since the free energy, Eq. (6) is quadratic in ϕ, the dynamical equation is exactly written as

$$d\phi(t)/dt = -4\phi(1-\phi)\tau_\theta^{-1} f^e_{\phi_s\phi_s}(\phi-\phi_s), \tag{7}$$

where the double subscript indicates a second derivative. This equation can be integrated analytically to find the time as a function of $\phi(t)$. The results, that depend on the frequency-dependent effective free energy, f^e, are in very good agreement with a numerical integration of the full equations of motion for $p(t)$ and $\phi(t)$ starting from the original (frequency-independent) free energy, f.

From the analytical integration, we define the characteristic time as the time at which the orientation is close to its steady-state value: $\phi(t)=\phi_s(1+\epsilon)$, where $|\epsilon|\ll 1$. For small ϵ, this is approximately

$$\tau_c = [\tau_\phi \log 1/|\epsilon|]/[4f^e_{\phi_s\phi_s}\phi_s(1-\phi_s)]. \tag{8}$$

For frequencies $\omega^2 \gg c \ll 1$ (note that ω can still be less than 1), $f^e_{\phi_s\phi_s} \approx (1/2)p_a^2\omega^2/(1+\omega^2)$ and $\phi_s=(2c(1+\omega^2)+p_a\omega^2\phi_1)/(p_a\omega^2)$; using this in Eq. (8) predicts the scaling of the characteristic time with frequency and magnitude of the external stress. At high frequencies τ_c approaches a constant value that scales with the long time scale τ_θ and is amplified further by a factor of $1/p_a^2$ for the case of strain and $1/(cp_a)$ for the case of stress as a set point, assuming $1>p_a\gg c$ At low frequencies, the time τ_c increases as $1/\omega^2$ with a divergence when $\phi_s=1$ which occurs when $2c/(\omega^2 p_a)\approx 1$. This transition does not occur at higher effective temperatures where the orientation is random at low ω (Fig. 2).

This behavior is in qualitative agreement with experiment [11]. Note that since typically $p_a<1$ and $cp_a\ll 1$, the characteristic time is even larger than the intrinsic relaxation time, τ_θ and this might explain the long times observed in experiments [11]. Thus, although the crossover between low and high-frequency behavior occurs at $\omega=\omega_a\tau_p\sim 1$ and is determined by the relatively short time scale associated with liquification and repolymerization of the cytoskeleton, here termed τ_p, the time it takes for the cell to reach its steady-state orientation is much longer and is related to $\tau_\theta\gg\tau_p$; in addition this time is amplified by a large factor related to $1/p_a$ and $1/c$, and becomes even larger as the frequency is decreased.

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