Polarity patterns of stress fibers

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Stress fibers are contractile actomyosin bundles commonly observed in the cytoskeleton of metazoan cells. The spatial profile of the polarity of actin filaments inside contractile actomyosin bundles is either monotonic (graded) or periodic (alternating). In the framework of linear irreversible thermodynamics, we write the constitutive equations for a polar, active, elastic one-dimensional medium. An analysis of the resulting equations for the dynamics of polarity shows that the transition from graded to alternating polarity patterns is a nonequilibrium Lifshitz point. Active contractility is a necessary condition for the emergence of sarcomeric, alternating polarity patterns.

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Stress fibers are bundles of actin filaments made contractile by interaction with myosin minifilaments and elastic by crosslinking with α-actinin and other proteins. They are formed in the cytoskeleton of nonmuscle animal cells that need either to exert or to resist a mechanical force (see [1] for a recent review). Their biological function is exemplified in vivo by myofibroblasts that use the contractility of stress fibers to remodel the extracellular matrix during wound healing [2], or by vascular endothelial cells in blood vessels that align stress fibers parallel to the direction of flow to resist shear forces [3].

Actin filaments are polar structures, with a plus (or barbed) end and a minus (or pointed) end. The polarity of actin filaments inside a bundle is determined by decoration of actin with myosin subfragment-1 and electron microscopy imaging. Noncontractile (or passive) actin bundles often present uniform polarity, with barbed ends predominantly facing the same direction along the bundle, as seen in filopodia [4] or in the retraction fibers of postmitotic cells [5]. The polarity profile of stress fibers is alternating, with a majority of barbed ends successively pointing in opposite directions [6] [7] [7]. This periodic structure, with a wavelength of the order of 1 μm, is reminiscent of the sarcomeric organization of muscle cells. The actomyosin filament bundles of motile fibroblasts, however, exhibit a graded polarity profile [7] [8]. The proportion of barbed ends in the bundle facing one direction varies gradually and monotonically between 0 and 100% from one end to the other. The direction of the bundle sets the orientation of locomotion [9]. The formation of graded polarity bundles is an early event in the polarization of fibroblasts prior to migration. The spatial dependence of polarity in contractile actomyosin bundles contrasts with the uniform polarity observed in structures such as cilia, filopodia, and retraction fibers, in which motors do not play an organizing role.

In this Letter, we aim at identifying the physical mechanisms responsible for the existence of variable polarity patterns [10] in elastic [11] [12] actin bundles. We show that active contractility, the chemomechanical transduction from ATP hydrolysis to motion by myosin motors “pulling” on actin, can generate periodic, sarcomere-like polarity patterns provided that it exceeds a well defined threshold. We focus our attention on ventral stress fibers [1] that span the cell ventral surface and transmit force to the underlying substrate through end-point focal adhesions. We consider existing actomyosin bundles, with constant total material content, and ignore the intriguing questions raised by their assembly [13].

Recent theoretical descriptions of stress fibers focus on their mechanical properties, e.g., viscoelastic recoil after photoablation [11]. The question of polarity sorting was addressed in early models of contractile bundles of actin filaments [15] that consider a liquid state in the (dilute) limit of low concentration. In a recent extension, density patterns for viscoelastic actomyosin bundles are studied in the same limit [16]. The effect of coupling the density and polarity fields in the liquid state is investigated in detail in Ref. [17]. Conditions for the spontaneous oscillations of muscle sarcomeres are determined within the context of active elasticity in Ref. [18]. We propose to formulate the constitutive equations for an active, polar, elastic one-dimensional medium, following the prescriptions of linear irreversible thermodynamics [19] [20], valid in principle for arbitrarily large densities. This work is directly inspired by Ref. [21], where constitutive equations were proposed for an active polar viscoelastic liquid (see [22] for a review). Active polar liquid crystals share the same invariance properties [23] [24]. We treat here the case of a one-dimensional viscoelastic solid (Kelvin

![FIG. 1.](image-url) (Color online) Schematic representation of an actomyosin bundle with graded (A) and periodic (B) filament polarity profile.
model), with an emphasis on how polar degrees of freedom couple to elastic strain. Within this formalism, we restrict our attention to lowest-order terms in the free energy and in the constitutive equations.

In the spirit of Ref. [21], we model a stress fiber as a one-component material. The description is mesoscopic, valid over length scales large compared to the typical mesh size of the polymer network (\(\xi \approx 10\) nm). The geometry is one-dimensional: The stress fiber of (constant) length \(L\) occupies the interval \(x \in [-L/2, L/2]\). Our hydrodynamic description is built on three conserved fields: the mass, momentum and energy densities \(\rho(x,t), \vec{g}(x,t), \) and \(u(x,t)\), respectively; and one slow mode, the polarity \(\vec{p} = p(x,t) \vec{e}_x\), defined as the coarse-grained average of the polarity of actin filaments present at time \(t\) in the vicinity of \(x\). By convention, \(p = +1\) (respectively, \(p = -1\)) corresponds to a bundle where all (respectively, no) barbed ends point towards positive \(x\) (see Fig. 1).

The relevant boundary conditions for the polarity field are “antisymmetric”:

\[ p(-L/2) = -1, \quad p(L/2) = +1 \]  

since the barbed ends of actin filaments face the end-point focal adhesions [11,17].

The stress fiber is a viscoelastic solid [12], with velocity and strain fields \(v(x,t)\) and \(\epsilon(x,t)\). We focus on time scales for polarity ordering that are long compared to the typical turnover time of actin and other dynamic protein components: \(t \gg \tau_{TO} \approx 10^2\) s [13]. The density field \(\rho\) is a fast variable, slaved to the strain field: \(\delta \rho / \rho = -\epsilon\). The free energy density, expanded to quadratic order about \(p = e = 0\), reads [20]:

\[ f = \frac{a}{2} \rho^2 + \frac{K}{2} (\partial_x p)^2 + \frac{G}{2} e^2 + w e \partial_x p, \]  

where all terms are invariant under inversion of space and polarity. The positive coefficients \(a, K, \) and \(G\) respectively denote the susceptibility, the energy cost of inhomogeneities of the polarity field, and the elastic modulus. Symmetries of the problem allow for a coupling between strain and the polarity gradient, with a coefficient \(\beta\), as first introduced in Ref. [25]. For simplicity, we ignore the punctate spatial pattern of myosins in stress fibers [11,17]: \(\Delta \mu\) is a constant in the following.

We further assume that the time scale for polarity ordering is much longer than that for strain relaxation, of the order of 1 s as measured in laser ablation experiments [12]. In this long time limit the velocity field \(v\) and its gradient are negligible. The pressure field is uniform and equal to the pressure in the surrounding cytosol \(P = P_0\). Newton’s law, \(\partial_t \sigma = 0\), yields:

\[ G \partial_x e + (w + \beta \Delta \mu) \partial_x p = 0, \]  

which eliminates the strain field \(e\) in Eq. [4]. This yields the evolution equation for the polarity field:

\[ \partial_t p = -a \Gamma (p - l^2 \partial_x^2 p), \]  

where the length scale \(l^2 = (K/a) [1 - w^2/(KG)] \geq 0\) has been introduced. Using antisymmetric boundary conditions, we find a linearly stable, stationary, monotonic solution of Eq. [4]: \(p(x) = \sinh(x/l) / \sinh(L/2l)\) that describes the graded polarity profile of actin bundles in the absence of myosin-induced contractility.

In the active case \(\Delta \mu > 0\), Eq. [4] yields a damped Burgers equation:

\[ \partial_t p + \alpha \Delta \mu \rho \partial_x p = -a \Gamma p + D \partial_x^2 p, \]  

with a diffusion constant \(D = \Gamma K \left[ 1 - \frac{w^2}{K G} \left( 1 + \frac{\beta \Delta \mu}{w} \right) \right]\). For weak activity, i.e. when \(\beta \Delta \mu/w < KG/w^2 - 1\), \(D\) is positive, and monotonic stationary solutions qualitatively similar to the above analytical expression are obtained numerically (see Fig. 2). These linearly stable solutions describe the graded polarity patterns observed in contractile stress fibers.
For strong activity, i.e., when $\beta \Delta \mu / w > KG/w^2 - 1$, the diffusion coefficient changes sign to become negative. In the particular case $a = 0$, we obtain periodic stationary solutions: $p(x) = \sin(qx) / \sin(qL/2)$, with a wave number $q^2 = -a\Gamma/D$. Since $D < 0$, stationary solutions of Eq. (7) are linearly unstable. We are led to include a stabilizing, higher-order polarity gradient term $\nu/2(D_p^2 p)^2$ in the free energy density, where $\nu$ is a positive higher-order diffusion constant. This is reminiscent of a Lifshitz point \[21\] \[27\], where an equilibrium phase transition between a uniform and a modulated phase occurs as a diffusion coefficient changes sign. The field $h$ is supplemented with the term $-\nu D_p^2 p$ and Eq. (4) becomes a damped Kuramoto-Sivashinsky (KS) equation:

$$\partial_t p + \alpha \Delta \mu p \partial_x p = -a\Gamma p + D \partial_x^2 p - \nu \partial_x^4 p, \quad (8)$$

For large enough damping (here $a\Gamma$), this equation possesses stable stationary solutions periodic in space \[28\]. The transformation $\tilde{t} = a\Gamma t$, $\tilde{x} = a\Gamma x / |a| \Delta \mu$, makes Eq. (8) dimensionless, with only two parameters $\tilde{D} = a^2 D / (\alpha \Delta \mu)^2$ and $\tilde{\nu} = \nu / (a^2 / \alpha \Delta \mu)^4$. Alternating polarity patterns are obtained when the uniform solution $p(x) = 0$ is linearly unstable, i.e., for $\tilde{D} < \tilde{D}_c = -2\sqrt{\tilde{\nu}}$ (see Fig. 2). Their wavelength $\lambda$ is obtained where the growth rate of the linearized damped KS equation is maximal:

$$\lambda^2 = -8\pi^2 \Gamma \nu D / K = 8\pi^2 \nu \left[ w^2 KG / (1 + \beta \Delta \mu / w) - 1 \right]^{-1}. \quad (9)$$

We predict that the inverse square wavelength $\lambda^{-2}$ of alternating polarity patterns is a linear function of $\Delta \mu$, as may be checked experimentally by up- or down-regulating the contractility of fibers. The wavelength increases with $G$ and is independent of $a$, and also of $L$ when $L \gg \lambda$. We checked numerically that the periodicity of patterns is robust to sufficiently weak spatial modulations of $\Delta \mu$ mimicking the punctate profile of myosins, as well as to the addition of a noise term of small amplitude.

The case of “mixed” polarity ($p = 0$) is also accounted for \[10\]. When $D > 0$, the uniform solution $p(x) = \epsilon(x) = 0$ is stable but irrelevant because of incompatible boundary conditions. Mixed polarity may, however, be observed locally, far from the tips, when the penetration length $\delta \propto \sqrt{D}$ is small compared to the total length $L$ (see Fig. 2). When $D < 0$, mixed polarity is also obtained far from the tips in the range $D_c < D < 0$, with alternating polarity near the boundaries. Simulations suggest that the penetration length is of the order of $\delta \propto (D - D_c)^{-1/2}$. To the best of our knowledge, this type of pattern has not been observed in experiments. These results are summarized in a phase diagram (Fig. 3).

In the above derivation, we implicitly assumed that polarity is a nonconserved field. By disregarding the possibility of spontaneous polarity reversal of a filament, which we deem extremely unlikely in a one-dimensional cross-linked bundle, this implies that relevant time scales are longer than the characteristic time $\tau_C$ for nucleation/annihilation and insertion/removal of actin filaments. Equations (9) and (8) are valid only when $t > \tau_C$.

We now derive the constitutive equations for a conserved polarity field, valid for shorter time scales $t < \tau_C$.
with a damping coefficient $\gamma$. The conservation equation reads $\dot{\rho} = -\partial_x j^p$, and $j^p$, the polarity current, is the flux conjugate to the force $\partial_x h$. The constitutive equations read:

$$j^p = \Gamma \partial_x h + (-\zeta + \beta \partial_x p + \alpha p^2) \Delta \mu - \chi \partial_x v$$
$$\sigma = -P + \sigma^a + \eta \partial_x v + (-\zeta + \beta \partial_x p) \Delta \mu + \chi \partial_x h$$

where $\zeta$, $\beta$, and $\chi$ are additional cross-coupling coefficients of unknown sign and the nonlinear term $\alpha p^2 \Delta \mu$ is included in analogy with the nonconserved case. An Onsager relation sets the reactive nondiagonal coupling between stress and the gradient of the molecular field.

For a passive bundle ($\Delta \mu = 0$), we obtain the differential equation obeyed by stationary polarity profiles:

$$\partial_x^2 \left(p - \frac{\sigma}{\rho} \partial_x^2 p\right) = 0,$$

in the limit where $v = \partial_x v = 0$. Linear stability of the uniform, zero polarity steady-state imposes the condition $w \chi > 0$. For antisymmetric boundary conditions [Eq. (6)], we find a linearly stable stationary solution the linear profile $p(x) = 2x/L$.

For an active bundle ($\Delta \mu > 0$), we obtain a set of two coupled equations for $p$ and $h$:

$$\partial_t p + \alpha \Delta \mu p \partial_x p = -\Gamma \partial_x^2 h - \beta \Delta \mu \partial_x^2 p,$$
$$\left(1 - \frac{w \chi}{\Gamma} \partial_x^2 \right) h = -\alpha p + \frac{D}{\Gamma} \partial_x^2 p - \nu \partial_x^2 p,$$

where the higher-order term $\nu^2 / 2 \left(\partial_x^2 p\right)^2$ was included in the free energy density. An exhaustive study of the phase diagram of the system [10][11] is beyond the scope of this Letter. We naturally expect the graded polarity patterns obtained in the passive case to be preserved for weak enough activity. We shall restrict our analysis to the limit $w \chi >> \Gamma$, where the molecular field may be eliminated. In this limit, we obtain a damped KS equation:

$$\partial_t p + \alpha \Delta \mu p \partial_x p = -\epsilon_c p + D_c \partial_x^2 p - \nu_c \partial_x^4 p,$$

with a damping coefficient $\epsilon_c = GT a / w \chi$, a diffusion constant $D_c$ given by:

$$D_c = \frac{\Gamma w \chi}{\epsilon_c} \left(1 - \frac{w \chi^2}{\Gamma} \right) - \Delta \mu \left( \frac{P_b}{\chi} + \beta \right),$$

and a higher-order diffusion constant $\nu_c = GT v / w \chi$. In this regime, beyond the nonequilibrium Lifshitz point ($D_c < 0$), and for large enough damping $\epsilon_c$, we shall again find alternating polarity patterns. The transition may be driven either by the active stress (coefficient $\beta$) or by the active current (coefficient $\tilde{\beta}$).

A key ingredient of our model of an active, polar, one-dimensional elastomer is the coupling between the elastic strain and polarity gradient, allowed by symmetry in the free energy expansion [Eq. (9)]. We show that an alternating polarity profile emerges in an active medium with a uniform spatial distribution of myosins: Coupling with the myosin density field is expected to lead to myosin patterns with the same wavelength. While graded polarity patterns are allowed for both passive and active bundles, active contractility is a necessary condition for the self-organization of stress fibers into sarcomerelike, alternating polarity patterns. The alternating polarity pattern of an active actomyosin bundle may therefore become graded upon treatment with drugs that inhibit contractility. Whereas alternating profiles occur far from the boundaries beyond the instability threshold, graded profiles are made possible by nonzero values of the polarity field at the tips. These conclusions hold irrespective of the conserved or nonconserved nature of the polarity field.

Linear irreversible thermodynamics is a powerful framework within which coupling terms are deemed relevant according to their invariance properties. The numerical values of coupling constants are beyond the scope of the theory and may well depend here on the cell type. The only measurement we are aware of is that of the elastic modulus $G \approx 10^5$ $- 10^6$ Pa [11]. Giving a microscopic interpretation to hydrodynamic coefficients such as $w$, which couples elastic and polar degrees of freedom, will require models that relate behavior at hydrodynamic scales with the microscopic interaction of actin filaments with active and passive cross-linkers. One possible interpretation of $w$ is that a preference of cross-linkers to parallel or antiparallel pairs of actin filaments may lead to a different value of the stress at constant strain.

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Since the boundary conditions on $p$ are fixed, we may ignore the polar term $\partial_x p$ in the free energy density.