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Molecular motors make waves and sculpt patterns

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Networks of dynamic actin filaments and myosin motors, confined in cell-like droplets, drive diverse spatiotemporal patterning of contractile flows, waves, and spirals. This multiscale active sculpting is tuned by the system dynamics and size.

Cellular processes from motility to growth rely on networks of actin filaments, which grow, shrink, and are actively pulled on by myosin motors. Actin filament turnover — achieved by monomers continuously binding to one end of a filament and dissociating from the other — is equally critical to cellular processes, allowing filaments to recycle monomers and 'recreate' themselves on demand. However, how this molecular-level restructuring patterns global cellular dynamics remains poorly understood. Now, writing in *Nature Physics*, Ashwini Krishna and co-authors elucidate the interplay between network dynamics, architecture, activity and confinement, which is harnessed by cells to produce the spatiotemporal patterns that they need to function, adapt, and survive[1].

Actomyosin networks consist of a finite number of molecular building blocks: actin filaments, force-generating myosin motors, and accessory proteins that alter the connectivity and turnover of actin filaments. Actomyosin networks use these building blocks to adopt a wide range of architectures, mechanical properties and active dynamics, as required by cellular processes ranging from division to morphogenesis[2].

One of the hallmarks of actomyosin networks is their contractility, generated by molecular-level activities and coordinated over cell-spanning length scales. Studies on reconstituted actomyosin networks have demonstrated how molecular composition tunes network contraction, with dynamics ranging from global contraction, to local aster formation, to disordered flow and rupturing[3,4]. The primary molecular tuning knobs of this activity are crosslinking proteins that promote connectivity and myosin motors that generate forces between actin filaments. However, the activity in most of these studies simply moves the system from one steady-state to another, with no mechanism for reversibility, repetition or sustainable non-equilibrium activity, despite the necessity for these features in cells.

The key functionality that these simplified systems lack is the ability for actin filaments to turn over by continuously polymerizing, depolymerizing, and exchanging monomers. Incorporating turnover has been shown to enable sustainable steady-state contractile flows or periodic contractile waves, with contraction rates that scale with the turnover rate[5,6]. But how do molecular components that modulate turnover alter the emergent steady or pulsatile contractility and the transition between the two? Moreover, turnover and motor activity work in concert to restructure actomyosin networks, which,

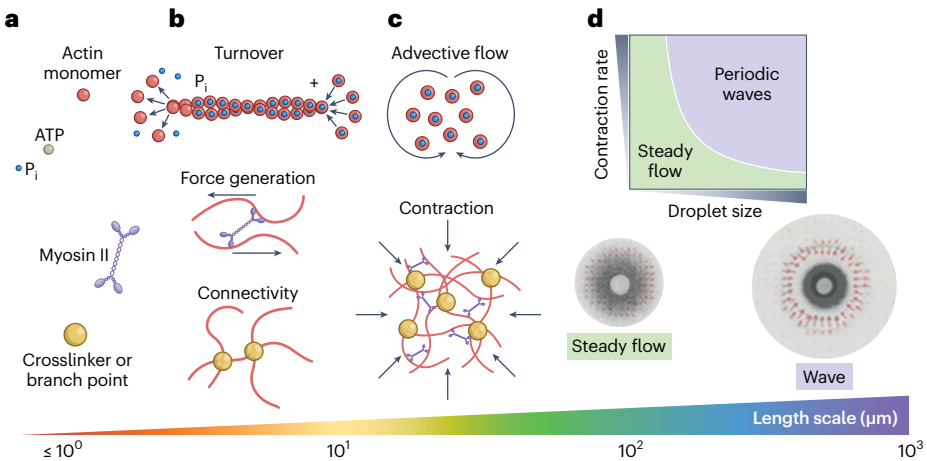

Fig. 1 | Transition from steady global flow to periodic contractile waves in actomyosin networks. a,**b**, Molecular components, including actin monomers (red), ATP, phosphate (P_i), myosin II motors (purple), and associated proteins that enhanced connectivity (yellow) (**a**), work together to generate molecular-level activities, including filament turnover, myosin-driven force-generation, and connectivity (**b**). **c**, These molecular-level activities give rise to mesoscale advective flow and network contraction. The dynamic patterning of contractile and advective regions under confinement allowed for global contractility that was sustained for hours. **d**, The size and shape of the confining droplets dictated the geometry and dynamics of sustained contractility, with steady flows in small droplets transitioning to periodic waves in larger droplets, by modulating the rate of contraction.

in turn, modulate their dynamics. However, this feedback loop, critical to allowing cells to morph, move, rigidify, and flow 'on demand', is poorly understood.

Another critical component that sculpts cellular actomyosin networks is their confinement by the cell. Confinement size has been shown to dictate the propensity for encapsulated actomyosin networks without turnover to contract to central clusters or form peripheral rings or asters[7]. Connectivity of confined actomyosin networks has also been shown to play a critical role in enabling cell-spanning contraction[3]. However, theories that predict the emergence of steady-state contractile flows do not consider confinement[8]. Therefore, how these key features —connectivity, turnover, and confinement — cooperate or compete to drive changes in contractile length scales, patterns and rates remains unclear.

To probe these open questions, Krishna and co-authors developed a reconstituted actomyosin system comprising cell extracts encapsulated in water-in-oil droplets with a distribution of sizes. The extracts included actin, crosslinkers, and myosins, exhibited rapid turnover rates, and allowed for selective addition of molecular components that alter filament turnover and network architecture. These robust system features enabled the authors to elucidate the coupled effects of turnover, architecture, and confinement on the emergence of sustained contractility and, importantly, the transition between steady flows and periodic waves (Fig. 1).

By imaging fluorescent-labeled actin in the extracts, researchers quantified the contraction rate, periodicity and geometry of contractile waves, as well as the emergence of structures, such as spirals, asymmetric contractile cores, and lateral waves. Dynamics transitioned from steady flow in smaller droplets to pulsatile waves in larger ones, with the transition size being inversely related to the contraction rate. Moreover, the wavefront patterns were sculpted by droplet geometry, with spherical and pancake-like droplets forming concentric spherical shells and rings, while straight wavefronts developed in elongated droplets.

By incorporating molecular components that altered the contraction rate, the authors revealed that the size-dependent transition from steady to pulsatile flow was surprisingly robust to changing network compositions. They also showed that the transition size universally scaled inversely with the contraction rate. Interestingly, the wavelength of periodic patterns was independent of contraction rate or droplet size, but was controlled by the actin assembly rate, which had surprisingly little impact on the contraction rate.

To predict the size-dependent transition to pulsatile flow, Krishna and co-authors incorporated confinement into models that balanced connectivity with motor-driven restructuring and turnover-mediated flow[8]. Their model recognized that networks contract faster at the edge of larger droplets than smaller ones because of the increased surface area that motors can populate, which causes network densification at the contractile front and dissolution in its wake. This density modulation is alleviated by turnover, which promotes advective flow and restructuring to reconnect and homogenize the network. Once a critical connectivity is reached, the network once again contracts, generating another wave. For smaller droplets, the contraction rate is slow enough that turnover can fill in disconnected regions or voids on the timescale of contraction, resulting in homogeneous steady flow.

An imposing question in cellular biophysics is how cells can harness disordered molecular components and processes to drive global coherent patterns and motions. More generally, understanding how individual uncorrelated components can self-organize to enable emergent system-spanning structure and dynamics — such as in bee swarms, bird flocks, and tissue cells — is at the heart of active-matter research.

Bridging across scales and programming global dynamics in cells is often thought to require biochemical patterning, chemo-electrical signalling, and the like[9]. However, the work of Krishna and co-authors showcases the emergence of diverse cell-spanning architectures and patterned dynamics sculpted from a finite number of molecular building blocks, requiring only modifications in mechanical properties. Moreover, this work brings to light the importance of cell size and shape on the dynamic patterning of the cytoskeleton, and how boundary conditions can be imprinted in the geometry, periodicity and speed of contractile wavefronts.

Future work may consider the interactions between microtubules and actomyosin networks, now recognized as essential to cellular processes such as division and motility. Interpenetrating microtubules have been shown to promote actomyosin connectivity in vitro, shifting dynamics from local disordered contraction and rupturing to slower global contraction[10]. These results[10] suggest that microtubules may play a critical role in the transition from steady contractile flow to pulsatile waves. Actomyosin adhesion to the cell membrane is also critical to cell motility, shape change and membrane rigidity, and controls localization and patterning of actomyosin networks[7]. However, how adhesion impacts the patterning of steady contractile flows remains an important unanswered question.

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Competing interests

The author declares no competing interests.