PNAS Plus Significance Statements

Filament rigidity and connectivity tune the deformation modes of active biopolymer networks

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Living cells spontaneously change their shape in physiological processes like cell migration and division. Forces generated by molecular motors on biopolymers must underlie these dynamics, but how molecular-scale forces give rise to cellular-scale shape changes is unknown. We use experimental measurements on reconstituted actomyosin networks and computer simulations to show that polymer stiffness and connectivity regulate motor-generated stresses and, in turn, longer-length-scale shape deformations. Importantly, we find that filament rigidity controls whether stresses transmitted are uniaxial or biaxial and that, for rigid filaments, the connectivity can control a transition between extensile and contractile deformations. These results have implications for how conserved molecular mechanisms give rise to diverse morphogenic events in cells. (See pp. E10037–E10045.)

Highly scalable multichannel mesh electronics for stable chronic brain electrophysiology

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Implantable electrical probes have led to fundamental neuroscience advances and treatment of neurological diseases, yet are unable to stably track the long-term evolution of large numbers of individual neurons critical to brain functions. Here, we demonstrate a scalable scheme for highly multiplexed mesh electronics probes that overcomes this long-standing challenge. We illustrate this scheme through fabrication of 32 to 128 channel probes with macroporous neural network-like structure and flexibility comparable to the brain. Following implantation into rodent brains, we demonstrate chronic 128-channel recordings with single-neuron-level stability from multiple brain regions over 4 mo. These scalable mesh electronics probes represent an ideal platform for mapping, tracking, and modulating the single-neuron-level circuit changes associated with learning, aging, and neurodegenerative diseases. (See pp. E10046–E10055.)

Immature HIV-1 lattice assembly dynamics are regulated by scaffolding from nucleic acid and the plasma membrane

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In order for HIV to proliferate, viral proteins and genomic dimers are assembled at host cell membranes and released as immature virions. Disrupting this key intermediate step in viral replication is a potential target for treatment. However, a detailed molecular view of this process remains lacking. Here, we elucidate a network of constitutive interactions that regulate viral assembly dynamics through a combined computational and experimental approach. Specifically, our analysis reveals the active roles of nucleic acid and the membrane as scaffolds that promote the multimerization of Gag polyprotein, which proceeds through multistep and self-correcting nucleation. Our findings also illustrate the functional importance of the N-terminal, C-terminal, and spacer peptide 1 protein domains. (See pp. E10056–E10065.)

Aggregation control in natural brush-printed conjugated polymer films and implications for enhancing charge transport

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Shear-printing of electroactive polymers using natural brushes is a promising film deposition technique for printed electronics capable of microstructure control and electrical properties enhancement over large areas. Nevertheless, the interplay between film printing parameters, microstructure development, and charge transport is not well-understood. We report that natural brush-printing greatly enhances charge transport by as much as 5.7× through control of polymer nanofibril aggregate growth and backbone alignment, attributable to the oriented squamae of the natural hair. However, while brush shear-induced aggregation enhances charge transport, we show that backbone alignment alone does not guarantee charge transport anisotropy. These results provide additional understanding of shear-induced enhanced charge transport and set processing guidelines for high-performance printed organic circuitry. (See pp. E10066–E10073.)

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Cell-to-cell variation sets a tissue-rheology–dependent bound on collective gradient sensing

Brian A. Camley and Wouter-Jan Rappel

Cells cooperate to sense the direction of a chemical gradient by communicating with each other, which may be important when clumps of cancer cells metastasize or embryos develop. However, because each cell is distinct, we find these clumps are biased toward cells that are "loud"—sending inappropriately large signals. Cell clusters can reduce this bias by rearranging themselves so loud cells change their locations. This means the mechanical dynamics of the cluster matter—fluid, squishy clumps of cells are better at sensing than solid ones. If a single cell's motion gets noisier, it will make the cluster more fluid—so adding noise can actually make a cluster of cells a better sensor. (See pp. E10074–E10082.)

New free-exchange model of EmrE transport

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EmrE facilitates *Escherichia coli* multidrug resistance by coupling drug efflux to proton import. This antiport mechanism has been thought to occur via a pure-exchange model, which achieves coupled antiport by restricting when the single binding pocket can alternate access between opposite sides of the membrane. We test this model using NMR titrations and transport assays and find it cannot account for EmrE antiport activity. We propose a new free-exchange model of antiport with fewer restrictions that better accounts for the highly promiscuous nature of EmrE drug efflux. This model expands our understanding of proton-coupled transport and has implications for both transporter design and drug development. (See pp. E10083–E10091.)

GPCR-controlled membrane recruitment of negative regulator C2GAP1 locally inhibits Ras signaling for adaptation and long-range chemotaxis

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Eukaryotic cells migrate through a gradient with a huge concentration range of chemoattractant stimuli by employing "adaptation," in which cells no longer respond to the present stimuli, but remain sensitive to stronger stimuli. Many models agree on the "temporal adaptation": a rapid "excitation" that triggers cellular responses and a temporally delayed "inhibition" that terminates the responses to reach adaptation. The inhibitory mechanism largely remains elusive, although many molecules of the excitatory signaling pathway have been identified. In the present study, we showed that GPCR-activated Ras negative regulator C2GAP1 locally inhibits Ras signaling for adaptation and long-range chemotaxis. (See pp. E10092–E10101.)

Proof of region-specific multipotent progenitors in human breast epithelia

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We have devised a culture system with conditions that allow primary breast myoepithelial cells (MEPs) to be passaged in a manner that sustains either nonmyodifferentiated or myodifferentiated cell populations without permitting contaminating luminal cells to grow. We show that progenitor activity and potency of MEPs to generate luminal cells in culture and in vivo rely on maintenance of myodifferentiation. Specific isolation and propagation of topographically distinct MEPs reveal the existence of multipotent progenitors in terminal duct lobular units. These findings have important implications for our understanding of the emergence of candidate luminal precursor cells to human breast cancer. (See pp. E10102–E10111.)

Structure and function of yeast Atg20, a sorting nexin that facilitates autophagy induction

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Autophagy is a cellular process that results in the capture of cytosolic material in double-membrane vesicles, which subsequently fuse with lysosomes to degrade the captured contents. Autophagy is essential to maintain cellular homeostasis, respond to cellular stress, and prevent the accumulation of material that could damage the cell. The initiation of autophagy is carried out by the Atg1 complex. Whereas recent work has provided functional and mechanistic insight into many components of the Atg1 complex, one member of this complex—Atg20—has remained relatively uncharacterized. Here we report a detailed investigation into the structure and function of Atg20, including the identification of an amphipathic helix in Atg20 that is required for efficient autophagy and membrane tubulation. (See pp. E10112–E10121.)

Sea anemone model has a single Toll-like receptor that can function in pathogen detection, NF-κB signal transduction, and development

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Toll-like receptors (TLR) are involved in pathogen recognition and defense in organisms from fruit flies to humans. Recent genomic evidence suggests that TLRs and their downstream signaling components are present in more basal phyla. We characterize a TLR in a sea anemone model and demonstrate its ability to activate NF-κB signaling when exposed to a bacterial pathogen and a known human TLR activator. Moreover, this TLR has an early developmental role in anemones. We also identify a primitive sea anemone organ that expresses components of the TLR–to–NF-κB pathway. These results demonstrate that TLRs have ancient roles in NF-κB signal transduction, pathogen detection, and development, thus providing molecular insights into how simple marine invertebrates may respond to pathogens. (See pp. E10122–E10131.)

Periodic production of retinoic acid by meiotic and somatic cells coordinates four transitions in mouse spermatogenesis

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Male mouse sex cells mature into sperm through a 35-d process punctuated by four transitions, two occurring before meiosis (spermatogonial differentiation and meiotic initiation) and two after meiosis (spermatid elongation and sperm release). The four transitions occur in proximity spatially and temporally, with an 8.6-d periodicity. We describe how this coordination is achieved. The premeiotic transitions were known to be regulated by retinoic acid (RA). We show that RA also regulates the two postmeiotic transitions. RA levels change periodically, and meiotic cells contribute to its production. The two postmeiotic transitions require RA from meiotic cells while the premeiotic transitions require RA from somatic cells. These elements underpin the

spatiotemporal coordination of spermatogenesis to ensure constant sperm production throughout adult life. (See pp. E10132–E10141.)

Climate extremes and predicted warming threaten Mediterranean Holocene firs forests refugia

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Climate extremes are major drivers of long-term forest growth trends, but we still lack appropriate knowledge to anticipate their effects. Here, we apply a conceptual framework to assess the vulnerability of Circum-Mediterranean *Abies* refugia in response to climate warming, droughts, and heat waves. Using a tree-ring network and a process-based model, we assess the future vulnerability of Mediterranean *Abies* forests. Models anticipate abrupt growth reductions for the late 21st century when climatic conditions will be analogous to the most severe dry/heat spells causing forest die-off in the past decades. However, growth would increase in moist refugia. Circum-Mediterranean fir forests currently subjected to warm and dry conditions will be the most vulnerable according to the climate model predictions for the late 21st century. (See pp. E10142–E10150.)

SIRPα$^+$ dendritic cells regulate homeostasis of fibroblastic reticular cells via TNF receptor ligands in the adult spleen

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Homeostasis of Pdpn$^+$ fibroblastic reticular cells (FRCs) is thought to be regulated by hematopoietic cells. However, the cellular and molecular mechanisms of such homeostasis have been poorly understood, especially under the steady-state condition in adults. We show that dendritic cells, particularly CD4$^+$ conventional dendritic cells (cDCs), are crucial for the homeostasis of FRCs in the adult spleen under the steady-state condition. The production of TNF receptor ligands by CD4$^+$ cDCs regulates such homeostasis of FRCs, with SIRPα and CD47 likely being indispensable for the production of TNFR ligands by CD4$^+$ cDCs. The CD47–SIRPα interaction on CD4$^+$ cDCs may thus control the homeostasis of FRCs, which in turn maintain homeostasis of T cells in the white pulp of the spleen. (See pp. E10151–E10160.)

Blocking immunosuppression by human Tregs in vivo with antibodies targeting integrin αVβ8

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Immunosuppression by regulatory T cells (Tregs) is essential for the maintenance of self-tolerance, but it is detrimental in cancer because Tregs inhibit antitumor immunity. Development of therapeutic tools to block Tregs in patients with cancer requires a precise understanding of how human Tregs suppress immune responses. We recently identified an important mechanism implicating release of the active form of TGF-β1, a potently immunosuppressive cytokine, from GARP/latent TGF-β1 complexes on the surface of human Tregs. Here we unravel the molecular process leading to this release. We identify integrin αVβ8 as indispensable for TGF-β1 activation from GARP/latent TGF-β1 complexes. We show that anti-β8 monoclonals block immunosuppression by human Tregs in vivo and could thus serve in cancer immunotherapy. (See pp. E10161–E10168.)

Peptidyl arginine deiminase immunization induces anticitrullinated protein antibodies in mice with particular MHC types

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The presence and development of autoantibodies to citrullinated proteins (ACPAs) are highly associated with rheumatoid arthritis (RA). The mechanisms leading to the production of ACPAs are unknown. Here, we propose a model to explain the emergence of anticitrullinated protein autoantibodies in RA. Indeed, we could trigger the development of anticitrullinated fibrinogen autoantibodies in normal mice by immunization with peptidyl arginine deiminase (PAD), most likely through a hapten/carrier mechanism in which the carrier is the PAD enzyme that performs citrullination and the hapten is any protein being citrullinated (hence bound) by PAD. Our results allow us to understand the birth of anticitrullin autoimmunity. (See pp. E10169–E10177.)

HIF and HOIL-1L–mediated PKCζ degradation stabilizes plasma membrane Na,K-ATPase to protect against hypoxia-induced lung injury

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Exposure to hypoxia requires adaptive mechanisms for survival. During acute hypoxia, Na,K-ATPase endocytosis in alveolar epithelial cells occurs via protein kinase C zeta (PKCζ) phosphorylation of α_1-Na,K-ATPase independently of the hypoxia-inducible factor (HIF). However, exaggerated Na,K-ATPase down-regulation leads to cell death. Here we report that during prolonged hypoxia plasma membrane Na,K-ATPase levels were maintained at ∼50% of normoxic values due to HIF-mediated up-regulation of HOIL-1L, which targets PKCζ for degradation. Silencing HOIL-1L in the lung epithelium prevented PKCζ degradation, causing Na,K-ATPase downregulation. Accordingly, HIF regulation of HOIL-1L targets the phosphorylated PKCζ for degradation and serves as an hypoxia-adaptive mechanism to stabilize the Na,K-ATPase, avoiding significant lung injury. (See pp. E10178–E10186.)

DGR mutagenic transposition occurs via hypermutagenic reverse transcription primed by nicked template RNA

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Diversity-generating retroelements (DGRs) are in vivo sequence diversification machines that are widely distributed in bacteria, archaea, and their viruses. DGRs use a reverse transcriptase (RT)-mediated mechanism to diversify protein-encoding genes to facilitate adaptation of their hosts to changing environments. Here, we demonstrate that the *Bordetella* phage DGR-encoded RT uses the 3′-OH of a nicked template RNA to initiate reverse transcription, during which random nucleotides are incorporated when adenine residues in the template are copied into complementary DNA (cDNA). We further show that this mutated, covalently linked RNA-cDNA molecule is required for DGR-mediated sequence diversification, revealing a mechanism of accelerated evolution with broad practical applications. (See pp. E10187–E10195.)

Phosphatidylserine save-me signals drive functional recovery of severed axons in *Caenorhabditis elegans*

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Nervous system injury can cause lifelong disability, because repair rarely leads to reconnection with the target tissue. In the nematode *Caenorhabditis elegans* and in several other species, regeneration can proceed through a mechanism of axonal fusion, whereby regrowing axons reconnect and fuse with their own separated fragments, rapidly and efficiently restoring the original axonal tract. We have found that the process of axonal fusion restores full function to damaged neurons. In addition, we show that injury-induced changes to the axonal membrane that result in exposure of lipid "save-me" signals mediate the level of axonal fusion. Thus, our results establish axonal fusion as a complete regenerative mechanism that can be modulated by changing the level of save-me signals exposed after injury. (See pp. E10196–E10205.)

let-7 miRNA controls CED-7 homotypic adhesion and EFF-1–mediated axonal self-fusion to restore touch sensation following injury

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In many organisms axonal fragments can rejoin by self-fusion after neuronal injury. It is hypothesized that cell fusion would be an efficient way to repair functional loss after injury. In this study, we tested this hypothesis using the *Caenorhabditis elegans* sensory neurons that are responsible for gentle touch sensation. We found that fusion between the proximal and distal fragments of an injured posterior touch neuron (the posterior lateral microtubule) promotes functional recovery in an age-dependent manner. We also discovered that *let-7* miRNA inhibits functional restoration via EFF-1–mediated axonal self-fusion by reducing *ced-7* expression. Our work established that the axon fusion process has functional significance in the maintenance of neuronal integrity throughout the life span of an organism. (See pp. E10206–E10215.)

Definition of the hypothalamic GnRH pulse generator in mice

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Neural networks located in the hypothalamus are responsible for generating ultradian patterns of hormone secretion that control a wide variety of functions. How these neural networks generate pulsatile hormone secretion remains unknown. We report here that a population of hypothalamic kisspeptin neurons represents the gonadotropin-releasing hormone (GnRH) pulse generator. These cells have the remarkable ability to generate synchronized GnRH secretion every 9 min to drive pulsatile gonadotropin hormone secretion in the blood. These observations indicate the arcuate kisspeptin neurons as the origin of reproductive hormone pulsatility in mice and offer the prospect of better understanding and manipulating fertility in the clinic. (See pp. E10216–E10223.)

DSCAM-mediated control of dendritic and axonal arbor outgrowth enforces tiling and inhibits synaptic plasticity

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Adult neurons are not able to make new connections as easily as developing neurons can; however, future therapies aimed at regeneration and repair of neural circuits in the adult nervous system depend critically on the formation of such connections. Here, we studied a recently discovered cell population that has the unusual ability to make new connections into adulthood, but under normal conditions does not grow new axons or dendrites, so that no new cells are contacted. We manipulated this cell population to induce axon and dendrite outgrowth using transgenic methods and determined that this results in stable, functional connections with new cells. (See pp. E10224–E10233.)

M-current inhibition rapidly induces a unique CK2-dependent plasticity of the axon initial segment

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The axon initial segment (AIS), the region where neurons generate spikes, was recently shown to be a highly dynamic structure, exhibiting plasticity over wide timescales. Here we triggered a unique form of AIS plasticity in hippocampal pyramidal neurons by selectively targeting the AIS M-type K^+ channels. We uncovered the mechanisms whereby sustained cholinergic activation or direct M-channel block rapidly trigger a unique form of AIS plasticity. Minutes to hours of sustained M-current depression resulted in a compensatory reduction in intrinsic excitability associated with distal shift of the axonal spike trigger zone and distal relocation of both Na^+ and M-channels. These fast homeostatic changes, necessary to stabilize network excitability, were dependent on the crucial AIS protein, protein kinase CK2. (See pp. E10234–E10243.)

An RNA structure-mediated, posttranscriptional model of human α-1-antitrypsin expression

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Protein and mRNA expression are in most cases poorly correlated, which suggests that the posttranscriptional regulatory program of a cell is an important component of gene expression. This regulatory network is still poorly understood, including how RNA structure quantitatively contributes to translational control. We present here a series of structural and functional experiments that together allow us to derive a quantitative, structure-dependent model of translation that accurately predicts translation efficiency in reporter assays and primary human tissue for a complex and medically important protein, α-1-antitrypsin. Our model demonstrates the importance of accurate, experimentally derived RNA structural models partnered with Kozak sequence information to explain protein expression and suggests a strategy by which α-1-antitrypsin expression may be increased in diseased individuals. (See pp. E10244–E10253.)