Meeting Report – Workshop 'Actin-based mechanosensation and force generation in health and disease'

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Abstract

International experts in the fields of cellular motility, force generation and mechanosensation met in Baeza, a UNESCO World Heritage city, from the 10th to the 13th of November, 2019. The meeting, part of the "Current Trends in Biomedicine" series, took place at the “Sede Antonio Machado”, a beautiful XVII-century building turned into a Conference Center of the Universidad Internacional de Andalucía (UNIA), which sponsored the event. The meeting was organized by Alexis Gautreau, Pekka Lappalainen and Miguel Vicente-Manzanares, with the support of the European Molecular Biology Organization (EMBO) and the Spain-based company IMPETUX. Fifty scientists presented recent results during the talks, poster sessions, and thematic discussions. As Baeza itself served as a crossroads of medieval Christian, Moorish and Jewish cultures, the meeting brought together cell biologists, biochemists, biophysicists and engineers from around the world that provided an integrated vision of the role of the actin cytoskeleton, force generation and mechanosensation in diverse physiological processes and pathologies.

Actin pushes

The first session of the meeting, titled “Actin pushes” was focused on the role of actin in cell protrusion and motility as well as the functional significance of this phenomenon in a variety of biological models *in vitro* and *in vivo*. The talks addressed the importance of actin and/or actomyosin structures for cell-cell and cell-substrate interactions, individual or collective migration, and tissue specification in early embryogenesis or organ development. In addition, a number of talks concentrated on the molecular mechanisms underlying these events, and provided detailed explanations and *in vitro* reconstitutions of the molecular interactions. This combination of *in vitro* and *in vivo* approaches created a rich, complex and integrated overview of the field.

Analyzing the strength of cell-cell adhesions, Bill Brieher (University of Illinois, Urbana-Champaign, USA) showed that actin-based and Arp2/3 complex-dependent microspikes protrude from one epithelial cell to its neighbor, and contribute to repairing failing cell-cell junctions (Li et al., 2020). The importance of these microspikes was demonstrated by inactivating the key molecules involved (Arp2/3 complex, the Ena/VASP protein EVL, as well as Collapsing Response Mediator Protein 1, CRMP1). Depletion of any of these molecules led to the tearing of cadherin-cadherin bonds at cell junctions. Interestingly, this tearing, and the resulting ‘blistering’ of the junctions was dependent on myosin II.

Roberto Dominguez (University of Pennsylvania, Philadelphia, USA) addressed the mechanism of activation of Arp2/3 complex by nucleation promoting factors (NPFs) of the WASP family. In its inactivated state, the seven-subunit Arp2/3 complex is in a splayed conformation, whereas its activation by the conserved WCA domain of NPFs leads to a conformational change that pushes the two actin-repeated subunits (Arp2 and Arp3) into a filament-like, short-pitch conformation. Finely, dissecting the activation process by using recombinantly expressed human Arp2/3 complex, his team determined the cryo-EM structure of a human Arp2/3complex bound to two N-WASP WCAs, revealing for the first time in this meeting the specific interactions of NPFs with the Arp2/3 complex. One actin-bound NPF interacts with higher affinity with subunits Arp2 and ArpC1 of the complex, triggering the short-pitch conformational change and thus opening a second binding site for the binding of an actin-NPF complex at the

barbed-end of Arp3. Only then, the complex becomes fully activated **(Fig. 1)**. Together the two Arp subunits and the first two actin subunits form a filament-like tetrameric seed that can then grow into a branch once the complex binds on the side of a pre-existing (mother) filament. This stepwise activation process, requiring NPF-mediated delivery of actin subunits to both Arps for activation, is considerably different from previous models based on studies on the budding yeast complex.

Klemens Rottner (HZI, Braunschweig, Germany) studies the mechanisms that produce forces through protrusive actin polymerization. In his talk, he demonstrated how, in mouse melanoma cells, formins of FMLN subfamily (specifically FMLN2 and FMLN3) contribute to the protrusive forces generated by lamellipodia **(Fig.1)** (Kage et al., 2017). The team is also interested in the role of the WAVE complex in activating Arp2/3 at lamellipodia. Generation of stable knock-out cell lines, followed by specific rescue experiments elucidated the relative importance of each subunit of this pentameric complex, as well as of their family members. In fact, four out of five WAVE complex subunits are represented by two to three paralogous proteins, but the functional significance of this variety is far from clear. To this end, they compared the relative importance of the CYFIP1 subunit compared to NCKAP1. In addition, they have dissected the upstream lamellipodia-activating mechanisms, notably by establishing, for instance, that the recently discovered, second RAC1-binding domain located on CYFIP1 is critical for lamellipodia morphology and efficiency of protrusion, rather than WAVE complex activation (Schaks et al., 2018).

Feng-Ching Tsai (Institut Curie, Paris, France) addressed the initiation of filopodia in cells, concentrating on the key role of the I-BAR protein IRSp53. Using purified IRSp53, VASP and actin, the team had successfully reconstituted the process of filopodia initiation and growth on giant unilamellar vesicles. Formation of IRSp53 clusters recruits VASP and allows for local actin polymerization on the membranes. IRSp53 thus can be seen as a main driving force for protrusion initiation **(Fig.1)**, and the roles of other possible co-factors (ezrin, fascin) are currently investigated.

Pekka Lappalainen (University of Helsinki, Finland) talked about the mechanisms by which cyclase-associated protein (CAP) and twinfilins promote actin filament

depolymerization. By using structural approaches, his team revealed that CAP binds to the pointed ends of cofilin-decorated actin filaments. Moreover, they discovered that CAP promotes the rapid pointed-end disassembly of cofilin-actin filaments, and subsequently re-charges the resulting ADP-actin monomers with ATP **(Fig.1)**(Kotila et al., 2019). Their studies on twinfilin-1 and twinfilin-2, which accumulate at the lamellipodia edge, endosomes and cell-cell contacts, revealed that twinfilins are also important for F-actin disassembly in cells, but through a very different mechanism from the one mediated of CAP. Indeed, Pekka presented work demonstrating that twinfilins efficiently dissociate capping protein (CP) from actin filament barbed ends in cells and *in vitro*, and subsequently allow depolymerization of the 'un-capped' filaments from their barbed ends **(Fig.1)**. These latter studies also provide an explanation for why CP, which binds to barbed ends of actin filaments with very high affinity and a slow dissociation dynamics *in vitro*, displays dynamic interactions with actin filament arrays in cells.

Hugo Wioland (Institut Jacques Monod, Université de Paris, France) presented data on the role of ADF/cofilin in actin dynamics, showing that it generates a torque on twist-constrained filaments, which strongly promotes their severing. He was also able to directly observe that ADF/cofilin locally increases actin helicity (Wioland et al., 2019).

Alba Juanes-Garcia (IST, Klosterneuburg, Austria) presented a study focused on the function of a pericentrosomal actin structure (PAS) in dendritic cells. This structure is tightly regulated and has a key role in 3D migration, MT cytoskeletal network regulation and nuclear deformation and integrity.

Arnaud Echard (Institut Pasteur, Paris, France) addressed the role of actin oxidation in cell division. MICAL1 is activated by the GTPase Rab35 and oxidizes actin on Methionine 44, which promotes actin filament depolymerization and thus cytokinetic abscission (Fremont et al., 2017). He also showed that a cytosolic pool of the reductase MsrB2 delays abscission and counteracts MICAL1 function. MsrB2 appeared critical for preventing cytokinetic failure and tetraploidy when the NoCut checkpoint is activated by lagging chromatin. Importantly, MsrB2 only reduces G-actin monomers and MICAL1 only oxidizes F-actin.

Actin pulls

The session titled “Actin pulls” focused on actomyosin contraction and force generation and regulation in cells and tissues. The first speaker was Dylan Burnette (Vanderbilt University School of Medicine, Nashville, USA), who presented his views on contractile systems in muscle and non-muscle cells. Working in iPSC-derived human cardiomyocytes, which are close to embryonic stem cells in their physiology, the team had shown a role for muscle stress fibers in setting up the pattern of forming sarcomeres **(Fig.2a)**. This process does not require Arp2/3, but depends on formins and non-muscle myosins IIA and IIB, the two most widely-expressed NMII paralogs, which show no functional redundancy in this case (Fenix et al., 2018). The conclusion is that muscle stress fibers, which are similar in structure to actin arcs in non-muscle cells, are essential templates and precursors for sarcomeres in embryonic cardiomyocytes.

Miguel Vicente-Manzanares (Centro de Investigación del Cáncer, CSIC, Salamanca, Spain) showed that specific regulatory events in different domains and subunits of the NMII molecular motor control front-rear migratory polarity. Differential phosphorylation of several residues of the regulatory light chain (RLC) along the polarity axis of the cell defines a gradient of NMII activation that controls focal adhesion assembly and turnover, formation of actomyosin filaments and global processes such as cell migration and division. Most stable NMII filaments reside at the trailing edge and it is necessary for its formation **(Fig.2b)**. The different phosphorylations of the light chain control the ability of NMII to exert its roles in cellular physiology at several levels, including assembly of a NMII hexamer as well as the conformational change that enables NMII filament assembly and turnover.

François Fagotto (CRBM, Montpellier, France) demonstrated how changes in actomyosin contractility defined the morphogenetic properties of *Xenopus laevis* embryonic layers, inducing an ectoderm-to-mesoderm transition. Ectoderm cells appeared more rigid and less capable of movement than mesoderm cells, whereas other cellular features such as cadherin expression or cell polarization, were similar in both cell types. By treating the ectoderm explants in culture with Rho-associated protein kinase (Rock) inhibitor, the team obtained an immediate mesoderm-like switch, complete with adhesion, spreading,

and migration. Moreover, some Rho regulators, such as Rnd1 and Shirin, were identified as the key factors for the mesoderm-like phenotype, as their depletion resulted in cells returning to the round, static, and blebbing appearance typical for the ectoderm.

Jiong Chen (Nanjing University, China) talked about supracellular actomyosin structures and their roles in organized collective migration of border cells in *Drosophila melanogaster* ovaries. The relationship between leading and non-leading cells in the cluster, as well as protrusion formation, was found to be regulated by tension in myosin II supracellular structures, and their interactions with actin network. Depletion of myosin II by directed chromophore-assisted laser inactivation (CALI) in individual cells interfered with these control mechanisms, allowing the formation of large protrusions in distant, non-leading cells of the migrating cluster.

Adel Al Jord (CIRB, Collège de France, Paris, France) showed that oocyte growth requires the efficient transmission of mechanical forces from the cytoplasm to the nucleus during development. He demonstrated that cytoplasmic forces contribute to nuclear compartmentalization by controlling the dynamics of subnuclear membraneless organelles, highlighting the role of cytoskeletal-driven force in nucleus organization.

Ronen Zaidel-Bar (Tel Aviv University, Israel) described the role of the Rho GEF RHGF-1 in embryo transit through the *Caenorhabditis elegans* reproductive tract. Working *in vivo*, the team studied the passage of oocytes through the spermatheca of the worm, where the oocyte is fertilized, and then pushed into the uterus to pursue its development. The entire process must be fine-tuned, and actomyosin contractility is a key element. The exact timing of the contraction is regulated by an apically localized RhoGAP protein SPV-1, which prevents premature Rho activation and early contractility of the spermatheca (Cram, 2015), and by its counterpart, a Rho activator RHGF-1, which acts as a mechanotransducer. Upon the stretching of the spermatheca by the incoming oocyte, RHGF-1 translocates from the cytoplasm to the basal stress fibers, activating Rho and inducing the contraction, which will send the oocyte on its way.

These two sessions highlighted the intrinsic complexity of the regulation of actin polymerization and actomyosin-driven contractility in a variety of cellular systems and

phenomena. From the data presented, it became apparent that actin pushing (and polymerization-driven protrusion) is mainly controlled by molecular regulation and small- or moderately-sized protein complexes and has (relatively) local effects. On the other hand, myosin-driven contraction requires not only a similarly intricate molecular regulation, but also a tight control of larger-scale protein complexes that have dramatic effects on the form and function of cells and tissues.

Adhesion

This session was devoted to the role of adhesion as an orchestrator of actin organization and mechanosensation, with emphasis on the molecular regulation of adhesion by mechanical forces and the role of other cytoskeletal systems in the orchestration of force-generating cellular events.

Christophe Le Clainche (Institute for Integrative Biology of the Cell, Gif-sur-Yvette, France) aims to reconstitute the mechanical events that control adhesion formation and evolution over time, using purified components (Ciobanasu et al., 2015). His team showed how the mechanical stretching of talin by actomyosin controls the binding of RIAM and vinculin to trigger the maturation of nascent adhesions into mature focal adhesions **(Fig.2b)**. They also probed the role of the vinculin-talin complex in actin assembly, showing decisively that the force-dependent association of vinculin to talin forms a complex that controls actin polymerization. Finally, they propose a model suggesting a positive feedback loop between actomyosin-dependent talin-vinculin association and actin polymerization in focal adhesions.

Grégory Giannone (University of Bordeaux, Interdisciplinary Institute for neuroscience, France) addressed the acute mechanical response of individual proteins inside integrin-based adhesions using a novel cell stretching device compatible with super-resolution microscopy and single protein tracking. He showed that while integrins follow the elastic deformation of the substrate, inelastic responses are observed for actin filaments and talin, which connects integrins to the cytoskeleton. He also demonstrated that blocking myosin II inhibited the inelastic behavior of actin. These results show that

inelastic responses of actin depend on myosin II activity and trigger lagged and transient (≤5 s), local (≤250 nm) displacements associated with talin deformations. Finally, his data proved that stretch recruits zyxin, but not vinculin, to mature focal adhesions, while vinculin is recruited to early nascent adhesions upon stretching. In the proposed model, cells respond to external forces by amplifying transiently and locally cytoskeleton displacements triggering protein stretching and recruitment to mechano-sensitive structures.

Alexander Bershadsky (Mechanobiology Institute, National University of Singapore, and Weizmann Institute of Science, Israel) first presented their characterization of myosin IIA-filament assemblies in non-muscle cells (Hu et al., 2017). They further compared and contrasted the two types of integrin-mediated adhesions, focal adhesions and podosomes, and found that assembly of myosin IIA-filaments favors the former, but disrupts the latter **(Fig.2b)**. Next, they addressed the molecular mechanism underlying interactions between integrin-containing cell-matrix adhesions and microtubules, explaining a classic observation from Victor Small's group, who reported that microtubule-targeting promoted focal adhesion disassembly (Kaverina et al., 1999). The work of Alexander's group now showed that disconnecting microtubules from focal adhesions or podosomes by depletion of the adaptor KANK-protein reproduced the effect of microtubule disruption on these structures in a myosin IIA- and GEF-H1-dependent manner (Rafiq et al., 2019). Finally, he present recent work addressing the role of myosin II in filopodia, which showed that myosin IIA localizes to the base of these actin structures, increasing their lifetime and adhesion **(Fig.2b)**(Alieva et al., 2019).

Antonina Alexandrova (N.N. Blokhin Russian Cancer Research Center, Moscow, Russia) reported her observations regarding blebbing motility of cancer cells. They analyzed actin cytoskeleton reorganization during the life cycle of a bleb using correlative platinum replica electron microscopy. Their beautiful imaging demonstrated the spatial association of blebbing induced by Arp2/3 complex inhibition with pre-existing filopodia, which explained the preferential initiation of blebbing at areas with a weaker actin cortex at filopodial bases **(Fig.2b)**(Chikina et al., 2019).

Claire Hivroz (Institut Curie, Paris, France) demonstrated that antigenic presentation to T cells depends on the mechanical properties of the antigen-presenting cells. Her data elegantly demonstrated that increased stiffness or force application (by using micropipettes and polyacrylamide gels) promoted the expression of markers of T cell activation such as CD69 and proteins involved in mechanical resistance to deformation, e.g. lamins.

Sari Tojkander (University of Helsinki, Finland) presented data on the role of cytokeratin-5 (CK5) and cytokeratin-14 in cell mechanics. She showed that CK5 depletion inhibits expression of smooth muscle myosin and P-cadherin, leading to deficient myoepithelial cell maturation; this positions CK5 as a key regulator of myoepithelial morphogenesis.

This session highlighted the key role of mechanics in coordinating diverse cellular phenomena, and how different cytoskeletal systems contribute to mechanical homeostasis at a structural or regulatory level. It also served as an introduction for the following session, which was oriented to the development of methods to measure forces at a cellular and molecular scale, and the description of biophysical parameters that define cells and tissues at a mechanical level.

Physics of the cell

This session was devoted to illustrate recent breakthroughs at the interface between cellular biophysics, cell biology and signal transduction. The first presenter was Khalid Salaita (Emory University, Atlanta, USA), who summarized his recent work using diverse types of molecular probes to study forces in living cells (Ma and Salaita, 2019). His talk emphasized how molecular force probes can be used to reveal the concept of "mechanical proofreading", which states that cells can harness mechanical forces to enhanced the fidelity of information transfer. To explain this idea, his presentation described two examples where DNA-based force probes were used to study mechanical proofreading. The first focused on the mechanism by which a platelet distinguishes between soluble and immobile fibrinogen to trigger activation (Zhang et al., 2018). Using

DNA-based force probes with differing GC content, they could show that platelets apply forces through their integrin ligands and that these are associated with platelet activation. Interestingly, laterally mobile ligands inhibit platelet activation, demonstrating that lateral force is most physiologically relevant to activate platelet integrins; this concept had been proposed by Tim Springer ten years ago (Zhu et al., 2008), but was never proven experimentally until now. Further confirming the application of lateral forces by platelet integrins, Khalid presented molecular force microscopy which uses DNA force probes to map the orientation of integrin force (Brockman et al., 2018). The second demonstration of mechanical proofreading highlighted the role of forces in podosome formation. In this context, actin-pushing forces generated in podosomes depend on specific pN-scale pulling forces that are applied by integrin receptors in the ring complex (Glazier et al., 2019).

Next, Giorgio Scita (IFOM, the FIRC Institute of Molecular Oncology and University of Milan, Department of Oncology and Hemato-Oncology, Milan, Italy) reported his observations of solid-to-liquid phase transitions in cancer. He showed that Rab5 is a master regulator of the mesenchymal invasive program, maintaining the fluidity of cancer cell masses that otherwise become immobile (solid). Rab5-expressing cells display increased collective motility without affecting cadherin expression. However, their dynamics are much faster, suggesting a weaker tethering to actin. Also, these cells display dynamic microspikes that reminded the audience of the data presented by Bill Brieher the previous day. Analysis of Rab5 signaling in cancer spheroids revealed increased endosomal trafficking, Erk1/2 signaling and increased WAVE phosphorylation, which likely contribute to the microspikes they described (Malinverno et al., 2017). Finally, Rab5 promotes centrifugal cell gradients in 3D and promotes the separation of so-called ‘scout’ cancer cells (Palamidessi et al., 2019). Importantly, Rab5 expression is a predictor of poor outcome in some cancer cell types, and accordingly, it is elevated in invasive ductal carcinoma (IDC) towards the more invasive region.

Núria Gavara, (Impetux, Barcelona, Spain) presented the company's latest user-friendly optical tweezers for mechanobiology (called Sensocell); these can be used to trap objects of different sizes, including whole cells or intracellular structures. The main advantages of their system are that it does not require calibration to achieve

quantitative absolute force measurements, experiments can be carried out without using beads as trapped probes, and tens of traps can be created and manipulated using their software, which conjoins sample imaging and trapping.

This session illustrated the ability of careful biophysical measurements to extract novel information from cellular and molecular systems, increasing the diversity of techniques and approaches that enable the interaction of engineers, physicists, biochemists, cell biologists and clinicians, thus serving as the perfect link to the final session, devoted to actin and disease.

Actin and disease

The last session of the meeting addressed the role of actin deregulation in human disease, with emphasis on cancer. Laura Machesky (CRUK Beatson Institute and Institute of Cancer Sciences, University of Glasgow, UK) presented the EMBO-sponsored keynote of the meeting. She reminded the audience of the role of mechanics in the formation of acini by pancreatic ductal adenocarcinoma cells (PDAC) on very compliant polyacrylamide gels. Interestingly, PDAC tumors display high levels of YAP and fibronectin in more fibrotic regions. The key question of the talk, though, was focused on the origin of the energy that cancer cells use to disseminate. A proteomics screening identified the creatine phosphagen system as a mechanoresponsive, metabolic element. She showed that creatine kinase B (CKB) is a YAP target that controls the ADP:ATP ratio in PDAC gels. When inhibiting creatine shuttling, her team found that cells displayed slower actin dynamics, migration and wound healing. Along the same lines, collagen-rich (i.e. stiff) tumors are strongly positive for CKB, and its levels increased as tumors progressed, along with fibrosis. Finally, most of this energy is used in generating protrusions, as mitochondria are found polarized towards the leading edge, where they produce ATP and work with the phosphocreatine shuttle to maintain actin turnover.

Inés M. Antón (Centro Nacional de Biotecnología, Madrid, Spain) extended the YAP/TAZ subject, this time addressing its regulation by the WASP regulator WASP-interacting protein (WIP). After showing that high expression of WIP correlates with poor prognosis

in glioblastoma, she presented evidence that WIP downregulation lowered invasiveness and decreased proliferation. Interestingly, removal of WIP also decreased YAP/TAZ levels, but not *vice versa* (Gargini et al., 2016). WIP also correlates with the levels of mutant p53, and the oncogenic effects of p53 mutants require WIP expression downstream of Akt2, which phosphorylates WIP, controlling glioblastoma transformation (Escoll et al., 2017).

Fanny Jaulin (IGR, Villejuif, France) presented a new mode of collective migration in patients with metastatic colorectal carcinoma. These organized cell spheres are characterized by amoeboid-like, rapid migration without evident leader cells or protrusions, and have inversed polarity and tumor-initiating properties. Their migration is independent of adherence, and the cells do not appear to move or change position within the structures. This migration appears to depend on the multicellular, polarized actomyosin cortex localized at the back of the moving cellular clusters.

Anna Polesskaya (Institut Polytechnique de Paris, France) analyzed the roles of WAVE complex subunits and their paralogs in metastasis-free survival of breast cancer patients, demonstrating an anti-migratory role for CYFIP2, a highly homologous member of the CYFIP family. CYFIP2, in contrast to CYFIP1 and NCKAP1, inhibits protrusion formation, as well as the migration of breast cancer cells, normal breast epithelial cells, but also that of the prechordal plate cells of zebrafish embryos. Knock-out experiments in human cells suggested a role for CYFIP2 in destabilizing or decreasing expression of all the subunits of WAVE complex, whereas CYFIP1 and NCKAP1 play the opposite role.

To end the proceedings, Alexis Gautreau (Institut Polytechnique de Paris, France) demonstrated that actin polymerization controls cell cycle progression. In particular, Arp2/3 inhibition by CK-666 blocks cell cycle progression, stopping it at G1. This effect can be ascribed to a specific role of ARPC1B-containing Arp2/3 complexes. His group showed that ARPC1B-containing Arp2/3 complexes are critical for branched actin formation at the cell cortex, downstream of WAVE, whereas ARPC1A-containing Arp2/3 complexes are critical for branched-actin formation at the surface of endosomes (Molinie et al., 2019). Cortical branched actin appears to integrate soluble signals from

growth factors with mechanical signals from cell adhesions. This pathway accounts for the larger size of acini formed by breast cells when ARPC1B is overexpressed, or when the Arp2/3 inhibitory protein ARPIN is down-regulated. This critical ability of branched actin to signal cell cycle progression is necessary for untransformed cells, but unnecessary in most tumor cells that are generally mutated on cancer genes regulating the G1/S transition. A relevant exception are tumor cells with mutated RAC1, mostly melanoma, which still require downstream signaling by cortical branched actin.

This session emphasized that, despite the fact that actin regulation is considered an over-investigated field, the deregulation of actin-driven phenomena in diseases with a migratory component is still underdeveloped, and merits intense scrutiny as the search for novel therapeutic intervention points cast a wide net over the regulatory circuits that control cancer cells.

Concluding remarks

This workshop was concise, but yet presented an in-depth picture of this exciting field. Several trends emerged, for example the intimate relationship between force generation at a cellular level and the occurrence of mechanosensitive events at a molecular level. The need of precise biophysical measurements and their correlation with biochemical readouts was also highlighted, and some talks emphasized the emergence of novel approaches and techniques to do so. It also became clear that, more than ever, actin microfilaments are the dynamic backbone of cells; they not only produce mechanical forces, but also react to force produced outside the cell. Indeed, actin orchestrates and coordinates the responses of single cells within tissues in both physiological and pathological contexts linking form and function, and explaining how deregulation of function results in a disorganization of form, and vice versa.

Finally, it is important to highlight that this event was made possible by the UNIA-sponsored open call for their ongoing meeting series "Current Trends in Biomedicine". The application process is simple; it only requires an exciting topic and two or three

leaders in the field to spearhead the process and produce a proposal (for guidelines see https://www.unia.es/biomedicine, «Calls for proposals»).

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Legend to the figures

Fig. 1. Actin pushes

The graphic depicts the most relevant advances described in the meeting with regard to actin polymerization and depolymerization. For actin polymerization, the diagram indicates the role of cortical branched actin as a controller of cell cycle progression (discussed by Gautreau); the mechanism of activation of Arp2/3 by the WAVE complex and other NPFs (presented by Dominguez, Polesskaya, and Rottner), FMNL2/3(formin)-dependent protrusive force (Rottner) and IRSp53-VASP-dependent actin polymerization at filopodia (highlighted by Tsai). In the context of actin depolymerization, twinfilin promotes barbed-end (+) actin depolymerization (demonstrated by Lappalainen), whereas cofilin depolymerizes actin at the pointed end (-) by a mechanism that involves twisting and severing (highlighted by Wioland, and Lappalainen). Furthermore, CAP recharges actin monomers with ATP (indicated by purple arrow) to maintain treadmilling (discussed by Lappalainen).

Fig. 2. Actin pulls

A Scheme depicting non-muscle myosin II (NMII) filament-driven maturation into beta-myosin II (betaMII, cardiac isoform)-containing sarcomeres in iPSC-derived cardiomyocytes. NMII (in blue) forms mini-filaments close to protrusive areas as it interacts with actin (in red). As these areas are left behind and become more central, NMII mini-filaments incorporate betaMII (in gold) that later evolve into proto-sarcomeres containing only the latter isoform (discussed by Burnette). **B** Schematic representation of a polarized migrating cell, and the main functions of actomyosin filaments in this context. NMII is represented as dashed blue lines, actin as solid red lines. Actomyosin-driven pulling forces are represented by blue arrows at specific sites. Actomyosin filaments drive the maturation of focal adhesions (FAs, green ellipses), as discussed by Berdshasky and Vicente-Manzanares. NMII induces mechanotransduction events in proteins present in FAs (presented by LeClainche, and Giannone). NMII also promotes filopodia (Fp) stability by localizing to their base (emphasized by Berdshasky), triggers blebb formation preferentially where filopodia had emerged beforehand (shown by Alexandrova) and maintains front-rear cell polarity (discussed by

Vicente-Manzanares). Conversely, NMII inhibits podosome formation (yellow dots), as illustrated by Berdshasky. (Rq) indicates that NMII is required for the indicated process; (+), positive regulation; (-) negative regulation of the indicated processes. NMII*, highly stable non-muscle myosin II.