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Abstract

An explosive growth in knowledge, in the last two decades, has conferred a new dimension to the process of endocytosis. Endocytic circuitries have come into focus as a pervasive system that controls virtual all aspects of cell biology. A few years ago, we proposed the term “endocytic matrix” to define a cellular network of signalling wiring that is at the core of the cellular blueprint. A primary role of the endocytic matrix is the delivery of space- and time-resolved signals to the cell in an interpretable format, and, as such, it has profound consequences on polarized cellular and supra-cellular functions, first and foremost, cell motility. Here, we describe a set of recent results that expand this notion and illuminate how endocytic matrix dynamically controls the plasticity of migratory strategies. We further highlight the impact of inter-organelle contact sites on motility and the role of organelle positioning in this process. Finally, we illustrate how global perturbation of the endocytic circuitry influences cellular and supra-cellular mechanics, ultimately controlling a solid-to-liquid-like transition in the mode of motility with potential consequences on cancer dissemination.

Keywords	Endocytosis and recycling, cell migration, membrane trafficking, organelle positioning

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Milan, January 18th 2018

Dear Jasmine, Vania and Andrew,
We are submitting as per your invitation our review entitled "**The "endocytic matrix reloaded" and its impact on the plasticity of migratory strategies"** by Sara Sigismund and myself for the upcoming issue on "Cell Dynamics" in Current Opinion in Cell Biology edited by Vania Braga and Andrew Ewald.

As you will see, we focused on a set of recent evidence revealing novel aspects of the impact of the endocytic network on cell migratory strategies. We covered primarily work published in the past two/three years that illustrates how endocytic networks control dynamically migratory processes, the role of inter-organelle contact sites and organelle positioning on migration, the influence of key endocytic factors on collective motility strategies and solid-to-liquid tissue transition.

The review includes 3 figures.

We take this opportunity to thank Vania and Andrew for inviting us to contribute to this issue and hope our manuscript is suitable for the present issues of Current Opinion of Cell Biology.

Yours Sincerely,
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The "endocytic matrix reloaded" and its impact on the plasticity of migratory strategies

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Abstract

An explosive growth in knowledge, in the last two decades, has conferred a new dimension to the process of endocytosis. Endocytic circuitries have come into focus as a pervasive system that controls virtual all aspects of cell biology. A few years ago, we proposed the term "endocytic matrix" to define a cellular network of signalling wiring that is at the core of the cellular blueprint. A primary role of the endocytic matrix is the delivery of space- and time-resolved signals to the cell in an interpretable format, and, as such, it has profound consequences on polarized cellular and supra-cellular functions, first and foremost, cell motility. Here, we describe a set of recent results that expand this notion and illuminate how endocytic matrix dynamically controls the plasticity of migratory strategies. We further highlight the impact of inter-organelle contact sites on motility and the role of organelle positioning in this process. Finally, we illustrate how global perturbation of the endocytic circuitry influences cellular and supra-cellular mechanics, ultimately controlling a solid-to-liquid-like transition in the mode of motility with potential consequences on cancer dissemination.

Introduction

The last two decades of research on membrane trafficking have dramatically expanded our view of the relevance and functional consequences of this process, and of its broad interconnections within the cellular masterplan. Virtually all cellular and, more recently, supra-cellular, biological processes have been shown not only to be influenced, but also intimately controlled, by the pervasive nature of membrane trafficking networks. To reflect this, we previously coined the term "endocytic matrix" to define a hidden system of integration within the cellular blueprint. Stated differently, we proposed that "the trafficking network and signalling are actually two sides of the same coin and should be conceptualized as a single cellular process that is central to the eukaryotic cellular master plan" [1]. Several lines of evidence accumulated since then invariably reinforce this notion [2,3] and corroborate the idea that endo/exocytic circuitries are master organizers of signalling pathways, with one of their main roles being the resolution of signals in space and time. They also highlight how the flexible and dynamically adjustable nature of trafficking routes and fluxes critically contribute to the adaptability and plasticity of cells and cellular processes. Cellular plasticity has emerged as a general property essential for endowing cells with a degree of flexibility that is required by tissues and organs to adapt to diverse and dynamically changing micro-environmental conditions in a number of physiological and pathological processes.

A cellular process that has intrinsically plastic properties is cell motility. Different migratory strategies can be adopted by individual, or groups of collectively moving, cells [4]. These strategies are flexible, interconvertible, morphologically diverse, driven by biochemically distinct pathways, and governed by specific mechanical and physical cues. The plasticity and versatility of normal and tumour cell migration is, thus, the result of cells being able to switch between these different migratory modes.

Given the impact of the endocytic matrix, in the temporal and spatial control of signaling output, more and more evidence is accumulating of its widespread consequences on all polarized cellular function, and specifically on cell motility. Here, we will describe a set of recent examples that expand this notion and illuminate on the type of control exerted by various routes of internalization and endosomal signalling on individual and multicellular migration. We will further focus on novel emerging levels of physical integration in trafficking epitomized by inter-organelle contact sites, and discuss examples of the relevance of organelle positioning in the control of locomotion. Finally, we will illustrate how perturbation of endocytic molecules and circuitry may impact on cellular and supra-cellular mechanics, ultimately controlling a solid-to-liquid-like transition in the mode of motion with potential profound consequences on cancer dissemination.

The conventional endocytic routes and cell migration

Internalization of plasma membrane (PM) and PM-cargos, the first step of endocytosis, is executed through different entry routes that use clathrin-dependent or -independent mechanisms to initiate membrane invagination and vesicle scission, or through actin-based protrusions to engulf solutes and macromolecules.

The pathway that has been traditionally linked to cell motility is macropinocytosis [5]. This endocytic route is associated with actin-dependent ruffling of the PM [5], and is upregulated by oncogene activation, such as KRAS and v-SRC [6,7]. Not surprisingly, macropinocytosis has been implicated in cancer cell migration, extracellular matrix degradation and metastasis [5]. However, it is debated whether macropinocytosis exerts a positive or negative regulation on cell motility, and these effects might depend on cell types. For example, macropinocytosis is very active in macrophages and dendritic cells (DCs), and important in the chemotactic response of highly motile cells, such as neutrophils [8]. Notably, DCs, as they mature and become activated, switch from their macropinocytic, antigen-sampling, but sessile state to a highly migratory, chemotactic phase that promotes their travelling toward lymph vessels and nodes to mount an efficient adaptive immune response [9]. This switch in states depends on the differential usage of actin machineries, whereby a CDC42–Arp2/3 axis generates a branched actin network that limits migration speed, while enhancing membrane protrusions for macropinocytic engulfment [10], and a formin (mDia1)-dependent linear array of F-actin drives persistent and directed chemotaxis [10]. Thus, at least in some specialized contexts, macropinocytosis is a trade-off for efficient directed migration. In non-professional, migratory cells, however, macropinocytosis, or at least some form of this process has invariably been associated with enhanced crawling locomotion. For example, integrins have been shown to traffic rapidly via circular dorsal ruffles (a specialized set of ARP2/3-dependent protrusions that are sites of macropinocytic internalization) and macropinocytosis during migration of stimulated fibroblasts [11]. Additionally, we recently showed that circular dorsal ruffles display dynamic features (rapid and oscillatory wave-like behaviour) typical of an excitable system that can be biased by chemical cues, and are therefore acting as steering devices driving efficient chemotactic migration [12].

In addition to macropinocytosis, both clathrin-mediated endocytosis (CME, [13]) and non-clathrin endocytosis (NCE, [14]) have been implicated in membrane flow and spatial restriction of molecules needed for cell migration. Clustering of receptors in clathrin-coated pits (CCPs) is required to optimize receptor activation, and amplify and spatially constrain signalling [15,16]. Recently, a form of deregulated ‘adaptive CME’ has been proposed to be activated in non-small cell lung cancer (NSCLC) cells and to be responsible for enhanced migration and metastasis [17]. Adaptive CME is characterized by an increased rate of CCP initiation and internalization [17], due to the aberrant

activation of the neuronal dynamin, dynamin1 (Dyn1), and/or the overexpression of the clathrin-light chain b (CLCb)[17]. Deregulated adaptive CME affects EGFR trafficking, by increasing receptor recycling, and signalling, by augmenting AKT activity, ultimately promoting cancer cell survival, migration and invasion [17]. Consistently, elevated Dyn1 and CLCb expression in NSCLC and breast cancer correlates with poor prognosis and metastasis [18]. Thus, the ability of cells to migrate and invade is fine-tuned by the rate of endocytosis and trafficking via CME.

Differently from CME, NCE includes several endocytic mechanisms that have only recently begun to be dissected and that vary in terms of molecular players, lipid composition, internalizing cargo and cell type [19-23]. If and how these different NCE mechanisms are related is still a matter of intense investigation. This notwithstanding, some NCE mechanisms are spatially confined at the PM and have been linked to cell migration. For example, the fast endophilin-mediated endocytosis (FEME), involved in the internalization of G-protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs), was shown to be restricted at the leading edge of migrating cells [22]. Similarly, NCE of interleukin-2 receptors (IL-2R) is initiated at the base of PM migratory protrusions and depends on actin polymerization [23]. However, a direct link between these NCE mechanisms and cell migration remains uncertain. More solid evidence in this direction was provided for endocytosis via clathrin-independent carriers (CLICs, [19]) or GPI-enriched early endosomal compartments (GEEC, [24]). The CLIC/GEEC pathway was shown to be the major constitutive endocytic route involved in PM turnover at the leading edge of migrating fibroblasts [19]. Additionally, a decrease in surface tension due to acute reduction in cell volume was recently shown to be buffered by activation of CLIC/GEEC internalization [25]. Thus, CLIC/GEEC endocytosis might act as a tension-driven, mechano-sensory process that regulates PM dynamics during cell adhesion, spreading and migration [25,26]. The latter function might also be relevant to the plasticity of cancer migration and invasion. Indeed, the removal of the CLIC regulator and RHOGAP, GRAF1, proposed to act as a tumor suppressor in various cancers [27,28], causes extensive cellular blebbing and increases invasiveness [25]. In summary, asymmetric distribution of distinct endocytic mechanisms is an emerging cellular property with potential direct consequences on polarized cellular functions, including cell adhesion and migration.

Dynamic endocytic and exocytic cycles (EEC) spatially restrict signals for directed migration: Integrin turnover and beyond.

The impact of EEC on the establishment of a structural and dynamic polarity in migratory cells has long been hypothesized [29], and several striking reports have supported the validity of this contention. For example, a critical determinant of cells moving on 2D substrates or under confinement is their ability to couple propulsive forces, generated by actin tread-milling and actomyosin contraction, with

substrate adhesion forces. The latter forces can be weak and dynamic, such as those generated by non-specific substrate friction of cells moving in a non-adherent, blebbing-like motion [30]. Alternatively, mesenchymal moving cells exert strong propelling and contractile forces that elicit, and are coupled to, large focal adhesions (FA). In FA, heterodimeric integrin receptors connect the extracellular matrix with the F-actin cytoskeleton and transduce actin-based mechanical forces to the matrix through mechanosensitive FA proteins, thus acting as "molecular clutch" [31]. In the last 20 years, integrin endocytosis and recycling have emerged as major players in controlling integrin action [32,33]. Here, we illustrate recent discoveries that expand this notion. Integrins can be internalized via multiple endocytic routes [33]. Invariably, this event leads to the removal of the receptor from the cell surface, but it is also coupled with the polarized delivery of integrins back to the PM via recycling routes. Within this context, EECs impact directly on integrin turnover and FA dynamics, and are, thus, essential for regulating directional migration.

Upon ligand engagement, integrin receptors undergo structural changes and become activated, and as such are thought to be internalized, although internalization of unligated integrin has also been shown to be important in regulating their function [34]. Recently, however, endocytosed integrin was reported to remain in an active, but un-ligated state in endosomes [35]. The focal adhesion kinase, FAK, and the non-receptor tyrosine kinase, SRC, co-localize with α5β1 integrin on RAB11+ endosomes and critically contribute to maintaining the activation status of this integrin. This endosomal signalling complex also appears to be essential for ensuring the polarized delivery of pre-activated α5β1 integrin for rapid FA assembly, ultimately facilitating directional migration. These findings are remarkable as they reveal a novel function of endosomal-restricted signals in maintaining a conformationally active "memory state" of integrin that facilitates FA assembly and directional motility.

Another, previously unexplored intracellular itinerary, which impacts on polarized recycling of un-ligated β1, but not β3 integrin, is the retrograde transport route. Two main intracellular integrin trafficking pathways have been extensively characterized: the RAB4-dependent short-loop, used by the αVβ3 heterodimer [36] and the inactive conformation of β1 integrin [37]; and the RAB11-dependent long-loop, which also involves RAB21 and RAB25/RAB-coupling protein pathways and is mainly used by the activated α5β1 heterodimer [32,33,38]. Recently, however, un-ligated β1 was shown to be internalized from the PM and directed to the trans-Golgi for secretion in a polarized fashion through a retrograde route. This retrograde trafficking pathway, as opposed to the canonical recycling endosomal flux, appears to be the prevalent mode through which inactive adhesion receptors are polarized, particularly in cells moving in a persistent directional mode on fibronectin (FN)-coated stripes or on fibres [39]. These findings can be rationalized within a common framework, whereby

persistent directed motion requires rapid turnover of a pool of active or ready-to-be activated integrins [39]. This can be achieved through RAB11 recycling routes that ensure ligated or structurally pre-activated α5β1 is restricted to the leading edge, whereas retrograde routes provide the needed efflux of un-ligated adhesion receptors to initiate cell-matrix interactions at the migrating front (Fig. 1).

EEC roles extend beyond that of controlling integrin dynamics, function and activity in migration. Indeed, similar cycles of internalization/recycling also ensure spatial restriction of signals of key actin remodelling GTPases, including RAC1 [40-42] and CDC42 [43]. Both these molecular switches exploit EEC to acquire a polarized distribution and to promote a mesenchymal locomotory migratory mode in various cells, and invasion in carcinoma cell lines.

Similarly, EEC impacts on the activity and distribution of yet another class of adhesion molecules, the cadherins, that mediate homotypic cell-cell interactions, and thereby influence the collective migratory mode of epithelial, endothelial and neuronal N-cadherin-expressing cells. One important concept emerging from recent studies is that cell-cell adherens junctions (AJ) in cell collectives, sense mechanical cues by acquiring polarized cadherin-based adhesions [44]. The obvious implication of this notion is that AJ must adopt an asymmetric configuration between cells that is characterized by the different turnover and asymmetric recruitment of junctional components at either side of the cell-cell contact. This is clearly observed when comparing the distribution of AJ components in leader cells that extend polarized actin-based protrusions at their front, while engaging with their followers at the back by means of tight cell-cell contacts. Importantly, establishing and maintaining AJ polarity during cell migration is achieved by trafficking membrane cadherins through mechanisms that depend on interactions with the actin cytoskeleton. The most striking example of the impact of trafficking is on N-cadherin-based astrocyte migration. Astrocytes are glial cells that migrate collectively maintaining cohesive interactions through N-cadherin. Following wounding, leader cells extend actin-based protrusions, concomitantly triggering a rearward retrograde flow of actin. N-cadherins were shown to travel along this flow, from front to back, where N-cadherin is endocytosed to allow its anterograde trafficking to the front so that it can form new junctions [45]. This finding uncovers an unexpected coupling between spatially restricted EEC and actin retrograde flow that initiates junctional tread-milling, which is, in turn, essential for maintaining an asymmetric distribution of AJ during cohesive migration. Asymmetry in cell-cell junction topology has also been elegantly documented in migrating endothelial cells. These cells utilize VE-cadherin as a cohesive glue. Collectively migrating human umbilical vein endothelial cells (HUVEC), display serrated cell-cell junctions that arise from mechanical tension generated by actomyosin contractility [46]. These serrate junctions are polarized relative to the direction of movement, such that they point away from the rear of leader cells and are engulfed into the front of follower cells, like interdigitating fingers. Functionally, the formation of

engulfed VE-cadherin fingers precedes cell turning and has been proposed to serve as guidance cues for collective migration [46]. Topologically, these structures generate also asymmetric and opposite membrane curvatures in leaders *vs.* followers, suggesting that they might be sites for differential recruitment of curvature-selective molecules that could bias signalling and also endocytic trafficking.

Contact sites and organelle positioning in the control of polarized signalling and cell migration: the lysosomal case

The endocytic pathways are highly compartmentalized within the cell, with the different stages/types of endosomes and lysosomes localizing at specific cellular sites [47]. Their asymmetric positioning, which results from movements of organelles along microtubules and actin filaments, is critical for their function. More recently, contact sites with the endoplasmic reticulum (ER) have also been shown to contribute to the movement and positioning of organelles [47]. The ER controls the fusion and fission of endo-lysosomal organelles [48], and regulates the progressive concentration of late endosomes and lysosomes towards the perinuclear area via a ubiquitin-dependent tethering mechanism. Given their role in determining organelle asymmetry, the latter mechanisms are predicted to impinge on cell polarity and polarized cell functions, including cell motility. A case in point is provided by the link between lysosome positioning and migratory behaviour. In non-polarized cells, lysosomes are prevalently concentrated around the microtubule-organizing centre (MTOC), with scattered peripheral lysosomes localizing in proximity to the PM and cell protrusions [49]. In polarized cells, instead, lysosomes display a more diffuse peripheral distribution. More importantly, lysosomes that cluster at cell protrusions appear to regulate cell motility through the delivery of adhesion and signalling molecules to the PM and/or hydrolases to the extracellular space, possibly to degrade the extracellular matrix [47]. Direct evidence for a role of cortical lysosomes in cell migration, however, came with the discovery of the molecular machinery involved in their positioning. A multi-subunit complex, called BORC (BLOC-1–related complex), was shown to assemble on the lysosomal membrane, where it recruits the GTPase, ARL8, leading, in turn, to kinesin-and microtubule-dependent centrifugal movements of lysosomes [50,51]. Silencing BORC causes a collapse in lysosomal distribution to the peri-centriolar area and impairs cell spreading and migration [50,51]. These findings are important as they point to a positive signalling role for cortical lysosomes, as opposed to the canonical degradative function traditionally attributed to perinuclear lysosomes.

Migrating DCs further exemplify the critical role of lysosome positioning in cell motility. Upon bacterial sensing, DC undergo a switch in their motility behaviour from a sessile to a highly migratory state (for more details see previous Section). This switch has been recently shown to involve lysosome

positioning at the rear of DCs. At this site, local calcium release through the lysosomal Ca^{2+} channel, mucolipin-1, activates the actin-based motor protein, myosin-II, only at the rear of the cell, thereby locally increasing actomyosin contractility [52]. Lysosomal Ca^{2+} release also induces the nuclear translocation of the transcription factor, TFEB, and the TFEB-dependent transcriptional activation of mucolipin-1, triggering a positive feedback loop that allows persistent DC chemotaxis [52] (Fig. 2).

In conclusion, emerging evidence points to non-degradative signalling functions of lysosomes that not only control mTOR signalling and cell metabolism [53], but also cell migration, particularly when these organelles are asymmetrically positioned within the cell.

Endocytic control of collective motion: unjamming what is jammed.

Collective cell migration, a widely recognized mode of migration during embryogenesis, wound repair and cancer [54], refers to the process of cells migrating as a cohesive group, with each individual cell coordinating its own movement to that of its neighbours. A complex network of biochemical pathways governs cellular and multicellular dynamics and motility. In addition, most aspects of multicellular migration are ruled by the physical interactions that cells establish with each other and with their environment [55]. For example, physical forces, exerted locally by individual cells on their substrate or propagated long-range in multicellular cohorts through cell-cell adhesion, are principal determinants of multicellular dynamics [56]. Another obvious corollary to this notion is that processes and factors that impinge on the turnover, dynamics and amount of cell-cell adhesion molecules, are predicted to impact on supra-cellular force transmission and on the ability of multicellular entities to move in a coordinated fashion. One striking example of this tenet is observed during the collective motion of the neural crest (NC) in *Xenopus laevis*. Initiation of NC migration during embryonic development requires activation of a partial epithelial-to-mesenchymal (EMT) program, which involves a qualitative and quantitative change in cell adhesion [57]. In this system, cells become fully migratory before they complete cell-cell dissociation, enabling the role of adhesion strength in migratory behaviour to be assessed. A signalling axis impacting on junctional adhesion strength is triggered by lysophosphatidic acid (LPA), which controls a myriad of cellular activities by binding to six different cognate LPA receptors [58]. In the NC, the interaction of LPA with LPA receptor 2 specifically affects NC collective motion by modulating the extent of cell-cell cohesion through internalization of N-cadherin [59]. Remarkably, however, rather than promoting the generation of single, fully mesenchymal cells, this reduction of membrane N-cadherin only triggered a partial mesenchymal phenotype. Under this condition, NC collectives undergo a transition from a solid-like state, where cell exchange with neighbours is not permitted, to a fluid-like state, where reduced N-cadherin adhesion allows local cell rearrangement [59]. This change in state endows the NC with a degree of plasticity and fluidity that

facilitates its migration in narrow confined spaces, while ensuring cell cooperation during collective motion.

A variety of multicellular entities have been shown to acquire structural and dynamic physical properties that are surprisingly similar to those of amorphous viscoelastic materials [60,61]. During collective motility, epithelial cells can flow like a fluid, but as density rises due to proliferation, the motion of each cell is constrained by crowding by its neighbours, forcing them to move in groups [62,63]. At a critical density, motility ceases and collectives jam or rigidify undergoing a liquid (Unjammed)-to-solid (Jammed) Transition, herein referred to as UJT [60,61]. This transition has been proposed to ensure proper development of elasticity and of barrier properties in epithelial tissues, but also to act as a formidable suppressive mechanism for the aberrant growth of oncogenic clones.

Recently, global perturbation of endocytic processes and EEC by interfering with the master regulator of early endosome biogenesis, RAB5, was shown to impact on various biomechanical properties of cell collectives. More specifically, elevation of RAB5A was sufficient to re-awaken the motility of otherwise kinetically silent and fully jammed epithelial monolayers. RAB5A expression promoted millimetre-scale, coherent and ballistic locomotion of multicellular streams that flow like flocking fluids. Molecularly impairing endocytosis and micropinocytosis, or increasing fluid efflux, abrogated RAB5A-induced collective motility. At the same time, increased EEC, resulting from RAB5A elevation, directly influenced E-cadherin turnover rate at junctions [64]. The increased junctional dynamics enables cell neighbour exchange, while ensuring coordinated locomotion. RAB5A expression also caused the extension of oriented and persistent protrusions, in keeping with its ability to activate and spatially restrict RAC1 and RAC1-dependent actin-based protrusions [42]. The latter findings are consistent with earlier results relating to *Drosophila* border cell migration that showed how RAB5 is critical for polarizing RAC1 activity, while RAB11, via Moesin, ensures proper restriction of RAC1 activity to the leading cell through regulation of cell-cell communication [65].

In essence, endocytic-re-awakening of locomotion can be understood in terms of a combination of large-scale directed migration and local unjamming [66], leading to the acquisition of a flocking (or flowing) fluid mode of collective migration (Fig. 3). Numerical simulation of the process provided a quantitative framework supporting the notion that small variations in fundamental cellular properties, such as cell self-propulsion, junctional tension and packing densities, are sufficient to tip the status of collective entities from solid to liquid or to a flowing liquid, dynamic state. Importantly, tumour cells can exploit this “mechanical flexibility” to execute key steps in the metastatic process and unjam without the need to change genetic makeup and cell identity; thus, requiring significantly less drastic events than EMT (or the inverse MET) to disseminate. Within this context, the Jamming-to-Unjamming Transition (herein referred to as JUT) might represent a complementary gateway to EMT-

driven cell migration and dissemination that enables tissues to escape the caging imposed by the crowded cellular landscape of mature epithelia.

Figure Legends

Figure 1. Integrin turnover in polarized cell migration.

Integrins can be found in an inactive/close conformation or in an active/open one upon engagement with the extracellular matrix at focal adhesion (FA) sites. Both forms of integrins can be endocytosed and this event has been shown to regulate their function. Active integrins are internalized and recycled via a Rab11-positive recycling routes (red line), which also involves RAB21 and RAB25/RAB-coupling protein pathways (not shown) [32,33,38]. They have been shown to be maintained in an active, although un-ligated, state in endosome, so that they are primed for rapid assembly at FA sites [35]. Src and FAK colocalize with integrins in Rab11-postive endosomes and contribute to maintain them in an active state [35]. Inactive integrins are also internalized and can be recycled to the PM through a Rab4-positive recycling route [36,37] (not shown) or can transported from the early endosome (EE) to the trans-Golgi network (TGN) via a retromer-dependent mechanism, to be secreted through a retrograde route in a polarized fashion to the lamellipodium during directed cell migration (light blue line) [39]. Inactive integrins can also be localized to circular dorsal ruffles and they can be endocytosed from these sites. Thus, the integration between canonical recycling pathways of active integrins and the retrograde transport route of inactive integrins allows for the rapid turnover of a pool of active or ready-to-be activated integrins to initiate cell-matrix interaction at the migrating front, required for persistent cell migration.

Figure 2. Lysosome positioning in cell migration: the exemplar case of activated dendritic cells.

Activated DC display a polarized localization of lysosomes, which are concentrated at the rear of the cell. At this site, the release of Ca^{2+} from the mucolipin-1 channel (MCOLN1) generates a polarized Ca^{2+} signalling that exerts a dual function: i) on the one hand, it activates myosin II and stimulates acto-myosin contractility in a spatially –confined manner at the cell rear [52]; ii) on the other hand, it activates on the lysosomal membrane the calmodulin (CaM)/calcineurin (Calc) complex that dephosphorylates the transcription factor TFEB. Dephosphorylated TFEB can then translocate to the nucleus to promote the transcription of lysosomal genes, including MCOLN1, thereby generating a positive feedback loop that sustains polarized Ca^{2+} signalling [52].

Figure 3. Endocytic-dependent induction of a solid (jammed)-to-liquid (unjammed) transition (JUT) in the collective motion of epithelial entities.

Global perturbation of endosomal function through elevation of RAB5A, a master regulator of early endosome biogenesis, alters endosomal number and macropinocytic internalization detected by the uptake of large molecular weight fluorescently-labelled Dextran in various epithelial cells [66]. These alterations, in turn, affect E-cadherin turnover, junctional tension and topology, and further increase volume and density fluctuations that typically mark a liquid-to-solid-like transition. Finally, RAB5A elevation promotes the formation of RAC1-dependent, polarized, protrusions extending beneath neighbouring cells (also called cryptic lamellipodia) that promote cell self-propulsion. The impact of monolayer kinematics on the combination of the above cellular and mechanical alterations can be understood through mathematical modelling. The simulation is based on self-propelled Voronoi model with two main ingredients. The first is a target shape of each individual cell ($p_{0,}$ the ratio between perimeter and the square root of the area) that is the result of the competition between intracellular adhesion and cortical tension [66]. The second is the inverse of the reorientation time that each individual cell takes to align to the local direction of motion, τ^{-1}. These ingredients give raise to a rich phase diagram, qualitatively depicted on the bottom right, that explain endocytic-re-awakening of locomotion in otherwise jammed and solid epithelia, in terms of a combination of large-scale directed migration in the presence of local cell-re-arrangement, leading to a flocking (or flowing) liquid mode of migration. This transition in the mode of locomotion enables RAB5A-expressing epithelial monolayers to flow efficiently through micro fabricated narrows slits that mimic the confined channels encountered during interstitial migration.

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Annotated references

*10. Vargas P, Maiuri P, Bretou M, Saez PJ, Pierobon P, Maurin M, Chabaud M, Lankar D, Obino D, Terriac E, et al.: Innate control of actin nucleation determines two distinct migration behaviours in dendritic cells. Nat Cell Biol 2016, 18:43-53.
Dendritic cells to mount an immunogenic adaptive response undergo from an immature, antigen-sampling and macropinocytic, but sessile state to a highly migratory phase. This work elegantly shows that immature cells used an ARP2/3 branched actin machinery to promote macropinocytosis, while concomitantly limiting their directional migration. Following maturation, ARP2/3 machinery and macropinocytosis is turned off, and formin-mediated liner filaments promote their directed, chemotactic migration to the lymph nodes.

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Here, the concept of 'adaptive' CME, previously hypothesized by the same group, is rationalized and molecularly defined. This deregulated form of endocytosis is shown to be minimal in normal tissue, but drastically enhanced in breast and NSCLC cancer cells due to the aberrant activation of the neuronal dynamin, Dyn1, and/or the overexpression of the Clathrin Light Chain b, CLCb. As a consequence of the accelerated/deregulated EGFR endocytosis observed under these conditions, EGFR recycling and signalling are elevated, leading to increased cancer cell survival, migration and invasion.

**35. Nader GP, Ezratty EJ, Gundersen GG: FAK, talin and PIPKIgamma regulate endocytosed integrin activation to polarize focal adhesion assembly. Nat Cell Biol 2016, 18:491-503.
A RAB11 endocytic recycling pathway is shown to be critical for the polarized delivery of pre-activated, internalized β1 integrin. Following focal adhesion disassembly, β1 integrin is internalized and directed to RAB11+positive endosome where is kept un-ligated, but active through a FAK- and talin dependent pathway. The maintenance of integrin in an active conformation during recycling allows it to rapidly and locally reform FAs on return to the cell surface in a SRC and FAK-dependent fashion, promoting directional migration.

**39. Shafaq-Zadah M, Gomes-Santos CS, Bardin S, Maiuri P, Maurin M, Iranzo J, Gautreau A, Lamaze C, Caswell P, Goud B, et al.: Persistent cell migration and adhesion rely on retrograde transport of beta(1) integrin. Nat Cell Biol 2016, 18:54-64.

A novel and unexpected role of retrograde transport from the Golgi to the plasma membrane of non-ligand-bound conformation of β1 integrin is shown to be primarily responsible for the polarized delivery of this adhesion molecule. This trafficking routes is essential to ensure the flux of β1 integrin to the leading edge during the persistent migration of various cells in mammalian and during gonadal development in nematodes

*46. Hayer A, Shao L, Chung M, Joubert LM, Yang HW, Tsai FC, Bisaria A, Betzig E, Meyer T: Engulfed cadherin fingers are polarized junctional structures between collectively migrating endothelial cells. Nat Cell Biol 2016, 18:1311-1323.
Using collectively migrating endothelial cells, the authors show that leader cells extend finger like, VE-cadherin-rich, protrusions into the adjacent follower cells. The latter cells engulf these fingers by extending an ARP2/3 driven lamellipodia, which possess low contractility. Topologically, this arrangement results in a front-to-back polarization as an emergent property of the collective entity, that serves as guidance cues for the directed migration of endothelial sheets.

**52. Bretou M, Saez PJ, Sanseau D, Maurin M, Lankar D, Chabaud M, Spampanato C, Malbec O, Barbier L, Muallem S, et al.: Lysosome signaling controls the migration of dendritic cells. Sci Immunol 2017, 2.
This work using mature and highly migratory dendritic cells (DC) unveils an unexpected role of lysosomes signalling in controlling cell migration. Specifically, lysosomal calcium released through the calcium channel transient receptor potential cation channel, mucolipin subfamily, member 1 (TRPML1) is shown to activate myosin II motor activity at the rear of DC, promoting fast and directional migration. Lysosomal calcium also induces the activation of the transcription factor TFEB, which, ensures, the expression of TRPML1. Such lysosomal-based, feed-back positive loop is proposed to be critical in linking stimulus sensing with DC maturation and migration.

**66. Malinverno C, Corallino S, Giavazzi F, Bergert M, Li Q, Leoni M, Disanza A, Frittoli E, Oldani A, Martini E, et al.: Endocytic reawakening of motility in jammed epithelia. Nat Mater 2017.
In this work, the master regulator of endocytosis is shown to be sufficient promote the fluidization of jammed and otherwise kinetically arrested epithelial monolayers. RAB5A, through its endocytic and macropinocytic functions, overcomes epithelial jamming by promoting junctional line tension, increasing the alignment efficiency in the motion of each individual cells with respect to their neighbours. This combine effects lead to emergence of collective motility streams that flow like flocking currents, and facilitate the migration and dissemination of collective entities into confined spaces.

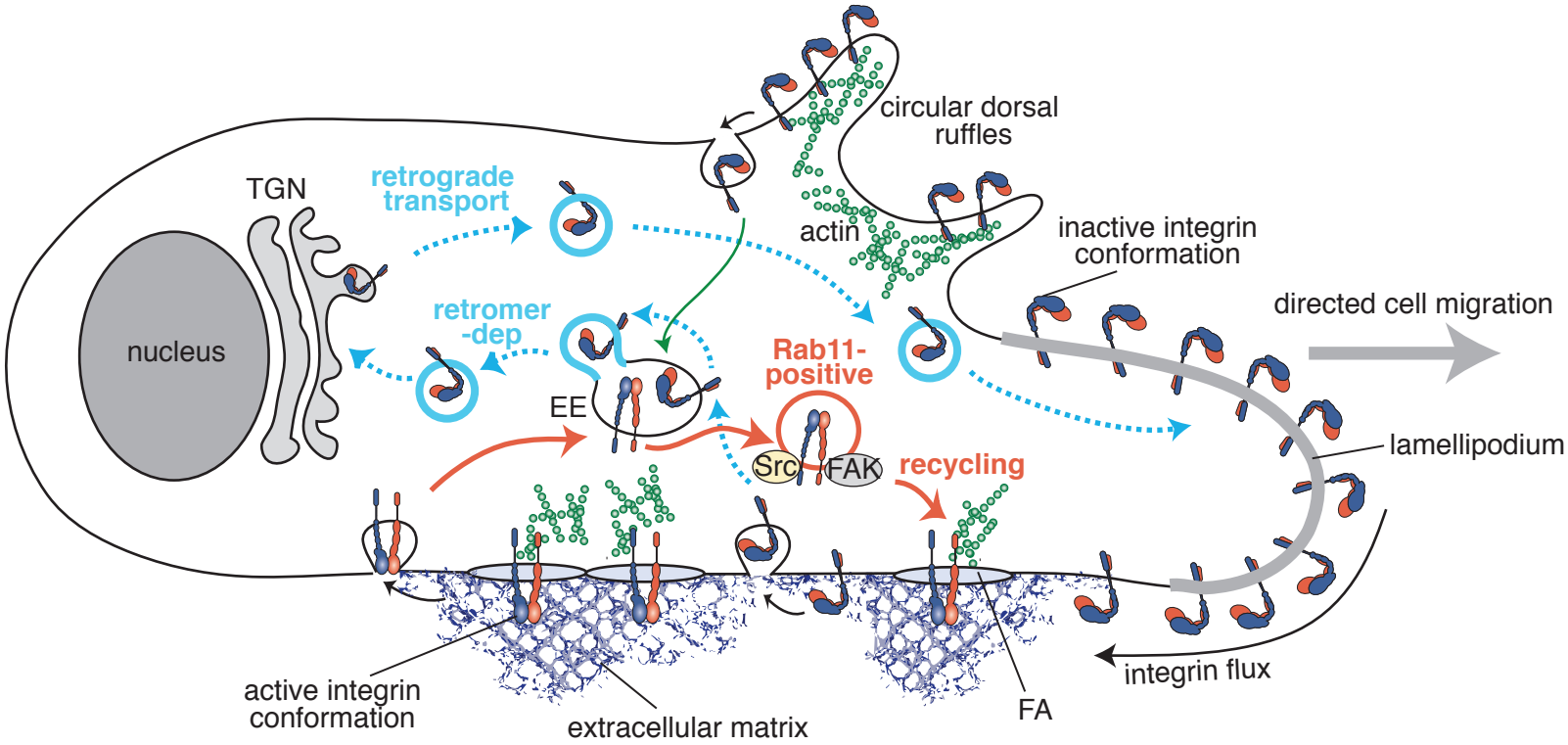

circular dorsal ruffles
TGN
retrograde transport
actin
inactive integrin conformation
directed cell migration
nucleus
retromer -dep
Rab11-positive
EE
Src
FAK
recycling
lamellipodium
integrin flux
active integrin conformation
extracellular matrix
FA

Figure 1

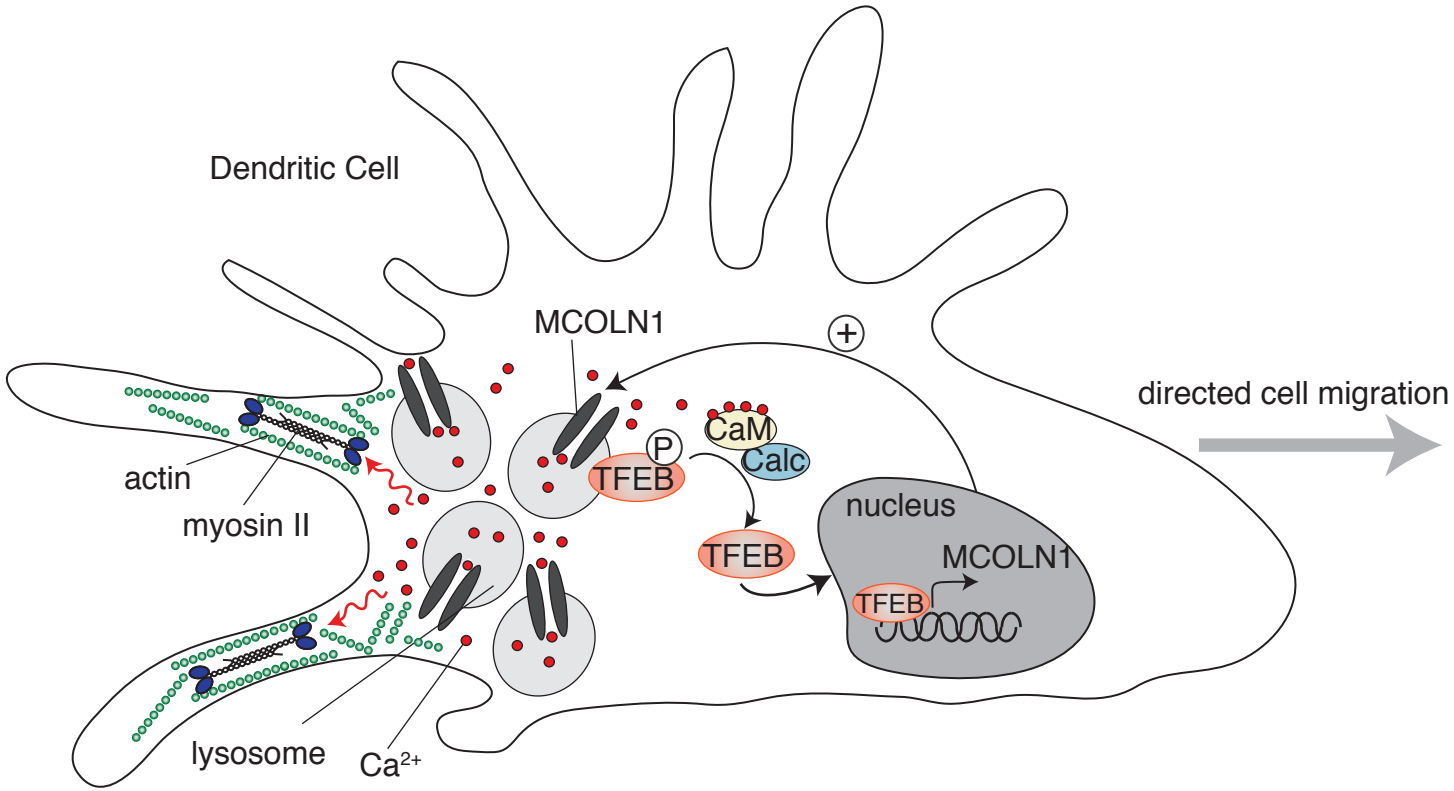
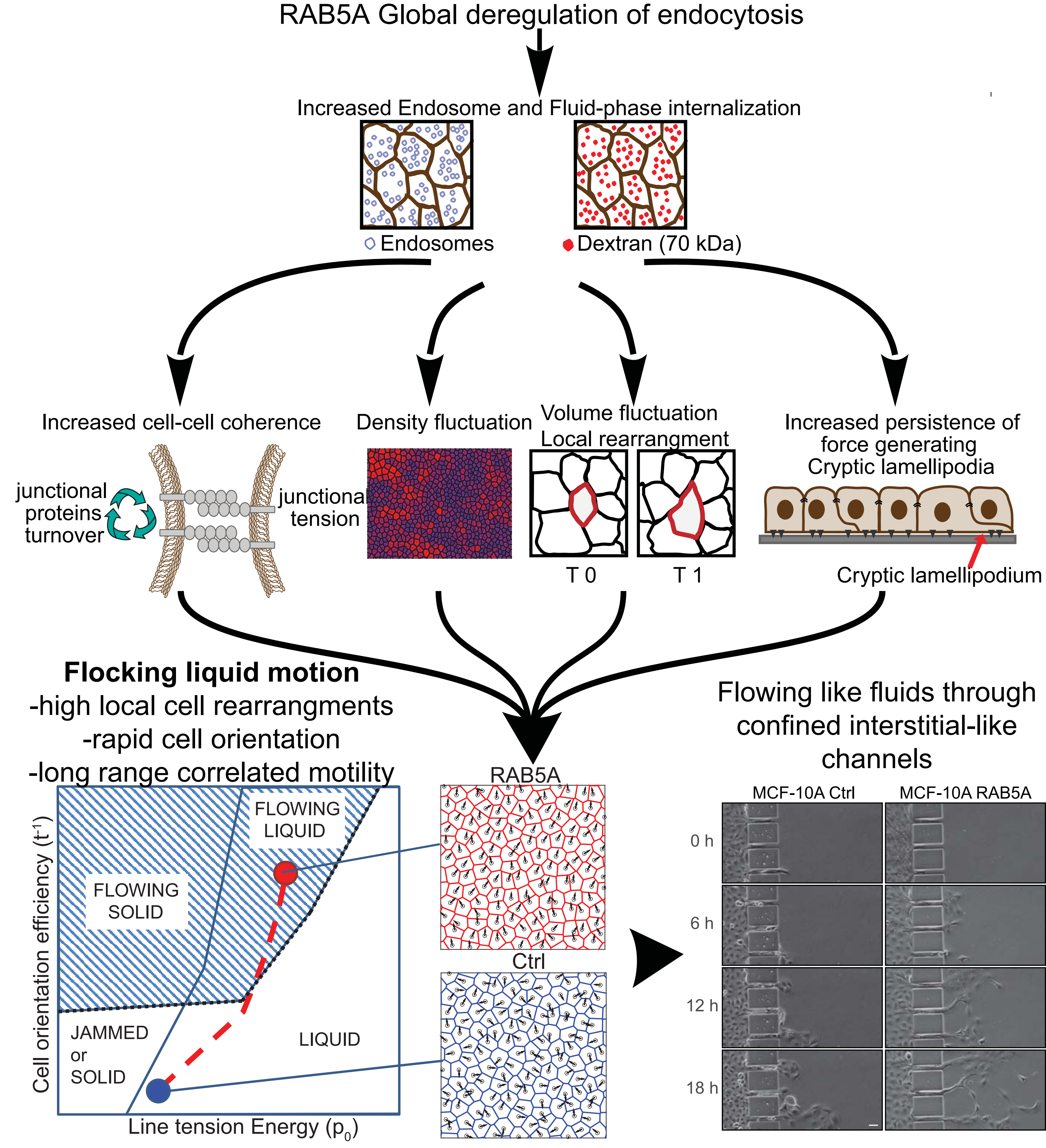

Dendritic Cell
MCOLN1
+
directed cell migration
actin
myosin II
P
TFEB
CaM
Calc
nucleus
TFEB
MCOLN1
TFEB
lysosome
Ca2+

Figure 2



RAB5A Global deregulation of endocytosis
Increased Endosome and Fluid-phase internalization
Endosomes
Dextran (70 kDa)
Increased cell-cell coherence
junctional proteins turnover
junctional tension
Density fluctuation
Volume fluctuation
Local rearrangment
T 0
T 1
Increased persistence of force generating Cryptic lamellipodia
Cryptic lamellipodium
Flocking liquid motion
-high local cell rearrangments
-rapid cell orientation
-long range correlated motility
FLOWING LIQUID
FLOWING SOLID
JAMMED or SOLID
LIQUID
Cell orientation efficiency (t^{-1})
Line tension Energy (p_0)
RAB5A
Ctrl
Flowing like fluids through confined interstitial-like channels
MCF-10A Ctrl
MCF-10A RAB5A
0 h
6 h
12 h
18 h