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Review

Cell–matrix adhesion complexes: Master control machinery of cell migration

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Abstract

Cell–matrix adhesion complexes (CMACs) are foci of cellular attachment to the extracellular matrix (ECM). This attachment, mediated by integrins and adaptor proteins, provides both physical and regulatory links between the ECM and the cellular microfilament system. Through continual regulation and rearrangement of both ECM adhesion and actin structures, CMACs constitute core machineries of cell migration. To fulfill this role, CMACs are exceptionally flexible and dynamic complexes, and their components undergo rapid and regulated turn-over to maintain delicately balanced streams of mechanical and chemical information. Besides the critical role of CMACs in cell migration, signaling through these complexes provides influence over virtually every major cellular function, including for example cell survival, cell differentiation and cell proliferation. This review depicts the roles of CMACs in cell migration and discusses how CMACs integrate with other sub-cellular systems involved in cell motility. Importantly, we also present a rationalized view of CMACs as information handling machines, and suggest strategies that may facilitate better understanding of the complex cell migration phenomenon as a whole, through quantitative and integrative (systems biology) approaches.

Keywords: Cell motility; Cell adhesion; Integrin; Signaling; Polarity; Focal contacts; Focal adhesions; Systems biology; Actomyosin

Contents

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1. Introduction

Cell migration is a central process in development, as well as in many physiological and disease states. For example, mortality in cancer is mainly caused by metastatic dissemination from the original tumor. Tumor cells gain invasive capacity at the conversion from a benign to a malignant state and therefore invasive capacity is a key hallmark of malignant tumors. To gain an invasive capacity, tumor cells need to acquire capacities both for cell migration through and proteolysis of the extracellular matrix (ECM). At the core of the migratory machinery are cell–matrix adhesion complexes (CMACs) (see Fig. 1). CMACs are composed principally of integrins, the cytoplasmic tails of which interact with a wide array of recruited factors that: regulate integrin clustering and ECM-binding; interface with signaling networks; and provide a physical linkage between integrins and the microfilament system. Ultimately, CMACs emerge as diverse protein networks that dynamically link the ECM to filamentous actin and thus directly facilitate cell migration through the continual regulation and rearrangement of both ECM adhesion and actin structures. Accordingly, CMACs are highly flexible and dynamic complexes, the components of which undergo rapid and regulated turn-over to maintain delicately balanced streams of mechanical and chemical information. CMACs are able to transmit these signals in different directions, including from the ECM via integrins into the cell (outside–in integrin signaling), as well as from inside the cell to regulate integrin extracellular domain-mediated attachment to the ECM (inside–out integrin signaling) [1,2]. Information flowing into CMACs may directly influence cell migration by, for example, modifying mechanical integrin–ECM interactions. Conversely, CMAC chemical signaling outputs can regulate a range of additional cellular machineries fundamentally required during cell migration. Thus, CMACs are dominant regulatory entities in the cell migration system, and in this review we will attempt to clarify how CMACs control and coordinate cell migration and invasion as a whole.

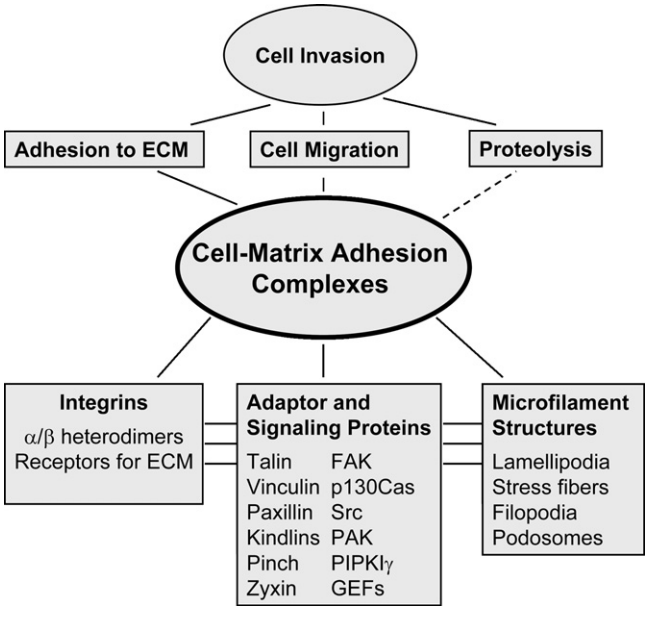

Fig. 1. Cell–matrix adhesion complexes as core machineries in cell migration and invasion. Cell–matrix adhesion complexes (CMACs) are at center stage during cell migration, when they attach cells to the ECM, physically link the ECM with actin microfilaments via integrins and adaptor proteins, and coordinate the processes of cell attachment and cytoskeletal rearrangement required for cell migration. CMAC are composed of transmembrane integrin receptors and an array of adaptor and signaling proteins (examples given in figure) which link to actin microfilaments. In addition to controlling cell adhesion and migration, CMACs are also functionally connected to proteolysis. Because cell adhesion, cell migration and proteolysis are the key components of cellular invasion, CMACs therefore represent core machineries of invasion.

1.1. Cell–matrix adhesion complexes

Cell–matrix adhesion complexes mechanically link the cell to the ECM [3,4]. CMACs form upon integrin ligation to the ECM and subsequent integrin clustering. This rapidly induces the recruitment of an array of CMAC signaling and adaptor proteins, forming large intracellular protein complexes bound both to clustered integrin cytoplasmic tails and to actin microfilaments. CMACs fulfill a variety of functions in the cell. Besides physically attaching cells to the ECM, CMACs also play important roles in the creation, mediation and sensing of tension [5]. In addition, CMACs are influential signaling hubs that detect, coordinate, transmit, adapt to and generate various signals regulating virtually all core cellular functions. Importantly, the concurrent association of CMACs with microfilaments and the ECM provides coordinated influence over both integrin-mediated cell–ECM attachment and microfilament remodeling, thus affording CMACs extensive control over cell migration.

CMACs differ significantly in features such as size, shape, location, componentry, dynamics and linkage to F-actin. Based on this, CMACs have been divided into a number of different categories [6,7]. Most of these CMAC categories represent different adhesion maturation states, including: focal points or nascent adhesions—small, often newly formed CMACs in the cellular periphery that link to an F-actin meshwork; focal complexes (FCs)—mid-size stationary contact sites linked to the cortical actin and/or the actin meshwork within lamellopodia; focal adhesions (FAs)—large adhesion sites that elongate along the axis of force application by actin stress fibers to which they connect; fibrillar adhesions—elongated adhesions that connect microfilament stress fibers with extracellular fibronectin fibers; and podosomes—invasive ring structures composed of adhesion machinery and filamentous actin. However, no unambiguous, commonly used collective term currently exists for the inclusive discussion of all these complexes. Somewhat confusingly, the terms focal adhesion and focal contact are sometimes used

for this purpose despite their more specific implications. We therefore propose and herein use the term cell–matrix adhesion complexes (CMACs (pronounced as "Seemacks")) as a collective term for all integrin-mediated cell–ECM adhesion complexes.

Despite being historically categorized into just a few discontinuous subclasses (as described above), CMACs necessarily exist along a dynamic, multi-dimensional continuum of evolution and devolution. CMACs respond inherently to stimuli, modifying their core properties through the alteration of their biochemical and physical content, topology and kinetics, i.e. by changing and modifying their protein and lipid composition, as well as the distributions and dynamics of these components. Different combinations of environmental cues may enforce selective adaptation of individual CMACs, as well as of the entire CMAC population, by differentially altering properties such as size, shape, intensity, localization, turn-over and composition. Ultimately, this creates unique CMAC patterns and functional features that reflect ever changing environmental conditions. Understanding this process in all its complexity demands a systematic and multi-parametric characterization of CMACs so as to better classify them and elucidate the processes involved in their remodeling. Such systematic characterization would also facilitate more detailed functional studies of different CMAC structures, including their respective roles in cell migration, as well as, for example, the relationship between CMACs observed in 2D and 3D environments—which appear to be very different [8]. To this end, a promising strategy was recently presented for detailed CMAC characterization using purpose developed high throughput/high content imaging and automated image analyses [9,10].

1.2. Cell–matrix adhesion complex components

1.2.1. Integrins

Members of the heterodimeric, transmembrane integrin family constitute the major cellular receptors for ECM proteins. In humans, variable combinations of 18 alpha and 8 beta subunits compose 24 distinct integrin alpha–beta heterodimers [11,12]. By binding to the ECM, integrins play a direct role in cell adhesion and also mediate cell migration by linking, via adaptors, the ECM to the actin microfilament system. Each integrin has a unique expression pattern, distinct specificities for different ECM protein ligands and a distinct capacity for bi-directional signal transduction [11,12]. Importantly, integrins generate signaling cascades from the ECM into the cell that control key cellular functions such as cell proliferation, differentiation, cell survival and cell migration [13]. Integrins are also themselves targets for signaling that regulates their ability to interact with the ECM, and this signaling is mediated through various CMAC components. These signals control both integrin affinity and integrin valency, the latter of which refers to the capacity to form integrin clusters [2,14].

1.2.2. Adaptor and signaling proteins

Given the large variety in size, shape, localization, etc. among CMACs, it is not surprising that CMAC composition is also highly variable. Thus, small nascent adhesions contain just a few components, while mature focal adhesions may contain more than a 100 different proteins [7,15]. Intracellular CMAC components function either as adaptors or as signaling molecules, or both. Adaptors may form physical links between, for example, integrins and actin, and/or may act as scaffolds for signaling molecules. While CMAC proteins generate, coordinate and transmit both mechanical and chemical signaling, the division between adaptor and signaling components is not so clear, since individual CMAC proteins have diverse functions as physical links, scaffolds, and signal transmitters. For example, talin serves as a physical link between integrins and F-actin, is a scaffold coordinating other CMAC components such as vinculin and PIPKIγ [16], and may be involved in transduction of tension signaling [17,18]. Likewise, while focal adhesion kinase (FAK) displays inherent kinase activity, the main functions of FAK appear to be connected to its capacity as a signaling scaffold [19]. Other important examples of CMAC components include paxillin, a highly regulated and dynamic scaffold protein [20]; kindlins, which may associate with integrins and form a link to actin via migfillin and filamin [21,22]; Pinch, that forms a ternary complex with ILK and parvin and functions to regulate gene transcription and cell–cell adhesion [23]; zyxin, which incorporates into CMACs in a tension-dependent manner [24]; p130Cas, which may serve as a transducer of both chemical and mechanical signaling [25,26]; c-Src, a key modifier of many CMAC scaffolds, such as paxillin and FAK, allowing them to transmit signaling further [27]; and various regulators of small GTPase signaling, including the alpha- and beta-PIX guanine nucleotide exchange factors (GEFs) [28].

1.2.3. Microfilament structures

The actin microfilament system structure and dynamics are to a large extent regulated by CMACs and their signaling, and the tension created jointly by the CMACs and microfilaments affects both structures. Different dynamic microfilament structures are physically linked to CMACs, and each is connected to distinct types of CMACs. These microfilament structures include lamellipodia, filopodia, stress fibers and podosomes, reviewed in [29,30].

2. Cell–matrix adhesion complexes as signaling hubs

2.1. An informational view of CMACs—seeing the forest through the trees

As discussed above, CMACs provide a physical connection between the cell and ECM through which mechanical and chemical signals are transmitted. These functionalities derive from extremely complicated and dynamic biological interactions, each with their own physicochemical properties and multifaceted regulation. The study of this biological apparatus is thus extraordinarily challenging.

As illustrated in Fig. 2, an alternative perspective identifies the CMAC essentially as an information handling machine or hub, and involves the identification of information fluxes or streams that traverse this system. These information streams

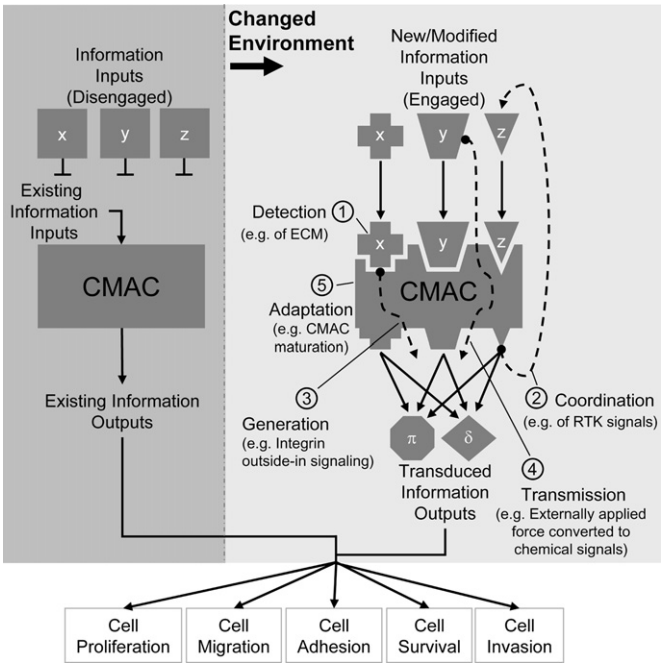

Fig. 2. An informational view of CMACs as signaling hubs. This schematic depicts the CMAC informational environment under an initial condition (left; dark grey background) and following environmental changes (right; light grey background). The polarity of the system depicted here relates only to the direction of *information flow* (solid arrows) relative to the CMAC, i.e. inputs and outputs. Informational inputs enter the CMAC from the top and outputs exit at the base. In the initial condition (left, dark grey background), existing information inputs interact with the CMAC to produce existing outputs, which regulate many higher order cellular features. This condition constitutes a theoretical *status quo* resulting in a specific CMAC state (represented as a rectangle) defined by precise morphological, biochemical and dynamic properties. Three example inputs (discussed in text) not yet engaged with the CMAC are also depicted. These represent*: ECM binding* (*x*); *tensile force transduction* (*y*); and *RTK signaling* (*z*). In the altered condition (right, light grey background), environmental variations modify these information inputs, causing them to engage with, and imprint changes upon, the CMAC, which integrates these collective inputs to produce new information outputs (π and δ). These outputs combine with existing outputs (also possibly modified) to alter the regulation of higher order features. Five numbers highlight important CMAC *information handling capabilities* (discussed in text): (1) *Detection* of new or modified information inputs; (2) *Coordination* of signaling partner inputs/outputs; (3) *Generation* of new information fluxes; (4) *Transmission* of incoming information fluxes; and (5) *Adaptation* of CMAC properties to integrate and respond to a changing informational environment (represented here by altered CMAC shape).

can be classified, relative to the CMAC, as either information inputs or outputs, and should ideally be ascribed experimentally derived, quantitative values. Such a perspective relieves a significant amount of complexity from the interpretation of the system as a whole and how it responds to environmental changes. Importantly, a quantitative aspect provides the potential to use mathematical language to interrogate and describe the system. This may facilitate the discarding of much supplementary information of limited significance to a systems level understanding. Accordingly, a key to this perspective is to identify functionally significant information fluxes as primary and their biological mediation or mediators as secondary in order to understand the system in its entirety. Overall, we believe that the overlaying of an informational perspective onto existing methodologies may be essential to unraveling and understanding the phenomenally complex mechanisms that mediate higher order cellular behaviors such as cell adhesion, migration, metastasis and beyond. This section is designed to address how such theories might be applied to the interpretation of the CMAC system in the context of a few existing data examples. An eloquent and enjoyable introduction to related concepts has been presented by Yuri Lazebnik [31].

2.2. *CMACs are highly dynamic signaling hubs that modulate multi-directional information flow*

CMACs are flexible machines, dynamically tuned to their environment. They are able to receive and integrate a diverse range of inputs and to respond by producing a variety of outputs. Thus, they act as both initiators and intermediaries within numerous signaling pathways and as a fulcrum for force application to drive the physical aspects of cell adhesion and migration. To achieve these varied capabilities, the biochemical and morphological features of CMACs must be modulated along a variety of axes for each component. For example, paxillin may be recruited more or less (concentration axis) to different sub-CMAC localizations (asymmetry axis) and may be phosphorylated more or less on different amino acids (phosphorylation axis) [32]. Paxillin phosphorylations (as on Ser273) can induce gross morphological changes to the CMAC structure (morphological axis) [33] and may promote faster or slower paxillin turn-over (kinetics axis) [34–36]. Each of these features depends upon and reflects the numerous informational inputs acting on the CMAC, while also contributing to the outputs of the complex in conjunction with all the other CMAC components present. In this way, CMACs constitute a hub for the integration and control of information flow.

The information inputs that interact with CMACs are manifold, but, for the purposes of simplicity, may be classified as either: mechanical—relating particularly to tension forces such as those applied by the actomyosin system to sliding adhesions during cell migration; or chemical—such as the signaling of activated Rac1 via c-Src and FAK to phosphorylate the paxillin-binding ArfGAP PKL (paxillin kinase linker), thereby altering CMAC structure and cell migration [37]. In truth, virtually all information exchanges that occur at CMACs probably contain both mechanical and chemical elements, and many CMAC components are implicated in both mechanical and chemical signaling pathways. Nonetheless, as regulatory elements within this information flow, CMAC function depends on a number of information handling and response capabilities, including the capacities to detect, coordinate, generate, transmit and adapt to different information fluxes. In the following, we discuss brief examples of each of these information handling functions. For more detailed, biologically oriented information, please see the specific reviews referenced in each section.

(1) *Detection* defines any process whereby a CMAC (or CMAC component) identifies or incorporates a new or modified information input. Detection can be exemplified by the initial binding of integrin to ECM. This event marks a major

modification of the informational environment, coinciding by definition with the earliest existence of a nascent CMAC. Alternatively, existing CMACs may detect incoming information via mechanical or chemical modification of CMAC components. For example, FAK may be phosphorylated by c-Src (reviewed by [38]), or dephosphorylated by Shp2 [35,39]. This marks the preliminary interaction of incoming and potentially antagonistic information streams with the CMAC, resulting in both information transmission through and also adaptation of the CMAC.

(2) *Coordination* describes the capacity of CMACs to regulate the informational output (i.e. signaling) of additional entities, with a prime example being the information transfer between, and regulatory interaction of, CMACs and receptor tyrosine kinases (RTKs). This relationship is experimentally revealed by the different cellular responses observed to equivalent growth factor environments in the presence or absence of ECM binding (reviewed by [1,40,41]). For example, the epidermal growth factor receptor (EGFR) is phosphorylated in a c-Src and p130Cas-dependent fashion in response to integrin αvβ3-ECM binding. When combined with low concentrations of EGF exposure, greatly increased EGFR phosphorylation is observed leading to downstream signaling for cell survival and proliferation. In the absence of CMAC–ECM interactions, the EGF responsiveness of EGFR is greatly reduced [42]. In another case, direct interaction between αvβ3 integrin and vascular endothelial growth factor receptor 2 (VEGFR2) also promotes RTK phosphorylation and significant signal enhancement [43]. Thus CMACs are able to coordinate the informational outputs of RTKs to enforce an additional layer of regulation onto these potent signaling machineries.

(3) *Generation* occurs following detection and results in the initiation of new information streams that originate within the CMAC. A clear example of this occurs during initial integrin–ECM interaction preceded by integrin activation. High affinity ECM ligand binding by integrin extracellular domains requires a structural shift to an open conformation, which is coincident with the separation of the integrin alpha and beta cytoplasmic domains (reviewed by [44,45]). Such separation promotes the association of intracellular binding partners involved in numerous signaling pathways as well as microfilament connectivity [12,13]. Integrin activation and ECM binding thereby initiate new information fluxes as well as initiating the formation of a nascent CMAC.

(4) *Transmission* also follows detection. Notably, information transmission through CMACs can occur in opposing directions: from within the cell to the extracellular domain – particularly to regulate integrin–ECM-binding affinity (inside–out signaling – reviewed by [2]); and from the extracellular domain into the cytosol (outside–in signaling—reviewed by [1]. During transmission, mechanical properties can also be converted to chemical information or vice versa.

One intriguing pathway that potentially mediates the transmission of both chemical and mechanical signals through CMACs includes the tyrosine kinases c-Src and FAK, and two c-Src substrates, paxillin and p130CAS. In response to information inputs such as growth factor signaling, c-Src is recruited to bind CMAC-localized, Y397 tyrosine phosphorylated FAK via the c-Src Src-homology 2 (SH2) domain (reviewed by [38]). This binding induces transphosphorylation of FAK and activates c-Src, which then phosphorylates numerous targets including paxillin and p130CAS. p130CAS phosphorylation drives downstream signaling to activate Rac1 via CrkII and the DOCK180/ELMO complex (reviewed by [25]). CrkII may also bind directly to phosphorylated paxillin, and by similar means activate Rac1 [46]. Furthermore, Rac1 can be activated by Rap1 [47], which itself lies downstream of p130CAS and its binding partner C3G. Active Rac1 encourages FC formation and is thought to antagonize RhoA signaling [48], thereby inhibiting FA maturation through downregulation of the RhoA–Rho kinase–LIM kinase–myosin II axis, which promotes actomyosin contractility. Intriguingly, this pathway may also participate in the transmission of mechanical force information based on stretch-induced conformational changes within p130CAS—resulting in the promotion of c-Src-mediated p130CAS phosphorylation [26]. This may represent a negative feedback loop in the context of FA-mediated force transduction, since p130CAS phosphorylation typically results in Rac1 and Rap1 stimulation (FC formation), resulting in RhoA inhibition (reduced actomyosin contractility, FA disassembly). Such a negative feedback does not correlate immediately with the typically observed responses of FAs to force application, which tend to include FA growth and RhoA upregulation (reviewed by [49]). Alternative findings demonstrating the dephosphorylation of p130CAS under mechanical shear stress [50] appear to fit more easily with our current understanding of CMAC adaptations and information outputs downstream of force application. However, it is salient to note here that Rac1 may also positively regulate RhoA at the rear of migrating neutrophils [51], while Rap1 inhibition of Rac1 [46] may provide another explanation for this apparent contradiction. Negative feedback of this type may, in fact, serve to maintain the finely balanced equilibrium of CMAC stability, dynamics and informational output. Such a consideration rests on the premise that information transmissions through CMACs do not exist in isolation. In fact, many potentially antagonistic signals may be integrated simultaneously through CMAC adaptation (discussed below) to generate distinct outputs dependent not only on the individual inputs received, but also on the combination, location, timing, and relative strength/frequency of inputs.

(5) *Adaptation* occurs inherently as CMACs respond to changing cues from their environment and is fundamental for CMAC information handling. The core role of adaptation is to control the connectivity of CMACs to the surrounding informational environment, by forming, breaking and modulating (via strength, density, frequency) links to incoming and outgoing information streams, be they physical or chemical. Overall, adaptive changes take the form of altered

CMAC composition, morphology and dynamics, and physically constitute the modified informational content and outputs of CMACs. Adaptation can be viewed at a number of resolutions: from the CMAC population level (e.g. shifts in the relative distribution of CMAC subpopulations such as FCs, FAs, fibrillar adhesions, etc.); or the individual CMAC level (e.g. evolution or devolution of single CMACs, altered morphology or dynamics); to the CMAC component level (e.g. post translational modification of individual components, altered dynamics, concentration, distribution or interactions). We hereafter highlight some interesting examples describing aspects of CMAC adaptation at different resolution levels, but we provide a separate analysis of CMAC dynamics as a means of highlighting the importance of this adaptable feature. We will later discuss how CMAC adaptation integrates with, and drives, cell migration.

An archetypal process of CMAC adaptation occurs when increasing actomyosin contractility is combined with sufficient ECM rigidity to oppose these forces. Under these conditions, actin-linked CMACs undergo general growth and maturation (*CMAC population level adaptation*). In FAs, CMAC growth occurs axially in a polarized manner towards the direction of force application (*individual CMAC level adaptation*—for review see [49]). Force-induced maturation of CMACs from FCs to FAs results from the heightened recruitment or retention of integrins and the incorporation of new CMAC components such as zyxin (*CMAC component level adaptation*) [52]. In contrast to this example, which results from macro level informational inputs (i.e. generalized mechanical force increases), it is also clear that more specific information inputs, such as CMAC component modifications, can cause adaptations that extend throughout CMAC populations at all resolution levels. For example, serine 273 phosphorylation of paxillin induces altered actin cytoskeleton dynamics and disrupts CMAC maturation, resulting in smaller, less stable CMAC populations, thus significantly altering whole cell dynamics [33]. Similarly, we have also found that the kinase activity of PAK4 functions to downregulate CMAC growth and condensation, resulting in smaller and less dense CMACs (Li et al., unpublished data). Thus, adaptation provides the mechanism by which diverse, variable and concurrent information inputs are integrated to create information outputs that reflect specific contextual changes in the CMAC environment.

2.3. Spatio-temporal dynamics of CMACs and CMAC machinery—a hidden layer of adaptive regulation

Variation in the spatio-temporal dynamics of CMACs and CMAC components represents a powerful, yet poorly understood mechanism for CMAC adaptation to an ever changing informational environment. While changes in CMAC composition clearly have important influences on CMAC function, relatively hidden changes in dynamics can have equally critical roles and implications. This is exemplified by the differential dynamics of αvβ3 integrin in FCs and FAs, where αvβ3 recruited to stationary, short lived, low density FCs has longer residence times (reduced turn-over) than αvβ3 recruited to much longer lived, high-density FAs [53]. While αvβ3 is obviously present in both types of complex, the low turn-over of αvβ3 in FCs is thought to contribute to the stationary nature of these CMACs, while rapid turn-over in FAs is likely to facilitate, amongst other things, the polarized assembly and disassembly mechanisms critical to FA sliding and force-responsiveness [54,55]. Thus, altered component dynamics provide powerful regulatory influences over CMAC function. This example illustrates a number of additional points, including the surprising observations that: changes in whole CMAC and CMAC component dynamics are not always parallel; increased component concentrations do not necessarily correlate to reduced turn-over; and, sites of increased force application can correspond to more dynamic complexes, which might intuitively be assumed less force resistant. To further reinforce these points, it is notable that our recent findings show that PAK4 overexpression induces more rapid αvβ5 integrin turn-over in adhesions while simultaneously driving them to a more FC-like morphology (in contrast to slower αvβ3 integrin turn-over in FCs described above) indicating clearly that CMAC morphology and component dynamics are relatively independent features at least under some conditions (Li et al., unpublished data). Importantly, the apparent uncoupling between whole cell dynamics, CMAC dynamics and CMAC component turn-over suggests strongly that research in this area should involve quantitative, comprehensive and integrated analyses at all of these resolution levels, as changes at one level cannot be reliably extrapolated to characterize the overall system.

Overall, whole CMAC dynamics, including assembly, maturation, disassembly and sliding rates, simultaneously facilitate and limit the overall mobility of cells. At the CMAC component level, flexible dynamics provide spatio-temporal control to the activities of CMAC components, be they signaling proteins, integrin–actin adaptors, or integrins themselves. Many informational inputs are known to impact on CMAC dynamics. As noted earlier, increased mechanical tension consistently correlates with reduced whole CMAC turn-over [49,56,57], accelerated integrin turn-over [53] and reduced zyxin turn-over [58], while having little impact on talin [59] or vinculin [58] kinetics. At greater biological resolution, mutagenic analyses of, for example, integrin activation and clustering, have revealed that specific activation of the integrin ectodomain results in greatly reduced integrin turn-over, while facilitation of integrin cytoplasmic domain interactions can stimulate enhanced integrin clustering without altering integrin turn-over [55]. This suggests that the integrin ectodomain conformation plays the major role in regulating the overall stability of integrins within CMACs (reviewed by [60]). In contrast to these fluorescence recovery after photobleaching (FRAP) oriented studies, two exciting new investigations focus on characterizing integrin–actin adaptor protein dynamics, to assess their roles in regulating the variable strength and information transmission capacity of the integrin–actin linkage [61,62]. These studies were performed using advanced new imaging methodologies, namely correlational fluorescent speckle microscopy [62], and spatio-temporal image correlation spectroscopy (STICS—[61]). By detecting the

degree of motion correlation between retrograde flowing actin and dynamic CMAC components, it was revealed that a "molecular clutch" mechanism exists to link CMACs to F-actin. This clutch regulates the efficiency of motion and probably force transmission from F-actin to CMACs, and defines an important new dynamic feature of CMACs. Due to space restrictions, these few examples demonstrate but a fraction of the depth and breadth of regulatory influence imparted by variable CMAC and CMAC component dynamics. Nonetheless, these insights argue strongly for the significance of this dimension of CMAC biology and further support the notion that dynamic, quantitative, and comprehensive research methodologies are critical to the fulsome understanding of cell migration.

3. CMACs as a core machinery of cell migration

3.1. Cell migration is an emergent process

Cell to matrix adhesion and cell migration are emergent processes that ultimately derive from a diverse network of molecular interactions between, for example, extracellular matrix components and integrins, integrins and adaptor proteins, adaptor proteins and actin, adaptor proteins and kinases, etc. These interaction networks increase in complexity in a hierarchical fashion to produce recognizable systems of intermediate complexity (such as those controlling actin polymerization, cytoskeletal tension, and CMAC organization and turn-over). Likewise, these intermediate systems interact to eventually produce adhesive and migratory cellular behaviors, which themselves are components of even more complex biological phenomena such as tumor metastasis. Thus, CMACs actively interact with and regulate other "sub-cellular systems" of cell migration, including the microfilament system; vesicular trafficking; cell polarity; plasma membrane composition and dynamics; microtubuli; and the ECM. Likewise, these sub-cellular systems actively influence CMAC properties and functions. Each sub-cellular system is composed of and regulated by an array of molecular components. Together, these sub-cellular systems shape the emergent system of cell migration (Fig. 3). However, current knowledge of cell migration is for the most part fragmented and relatively little is known about how different sub-cellular systems are integrated within cell migration as a whole.

In a migrating cell, protrusion at the front and retraction at the rear exist in a fragile equilibrium that has to be continually readjusted through feedback between external guidance cues and signals emanating from maturing or disassembling CMACs. In turn, the behavior of, and signals mediated by, CMACs are critical to maintaining the polarity of migrating cells. Here we describe cell migration as a cycle of spatio-temporal CMAC remodeling (Fig. 4), and discuss how CMACs integrate with other sub-cellular systems to mediate and control cell migration.

3.2. Initiation of polarity

Various external signals have been recognized to induce migratory polarity in cells, including chemokines and growth factors that are captured by transmembrane receptors on the

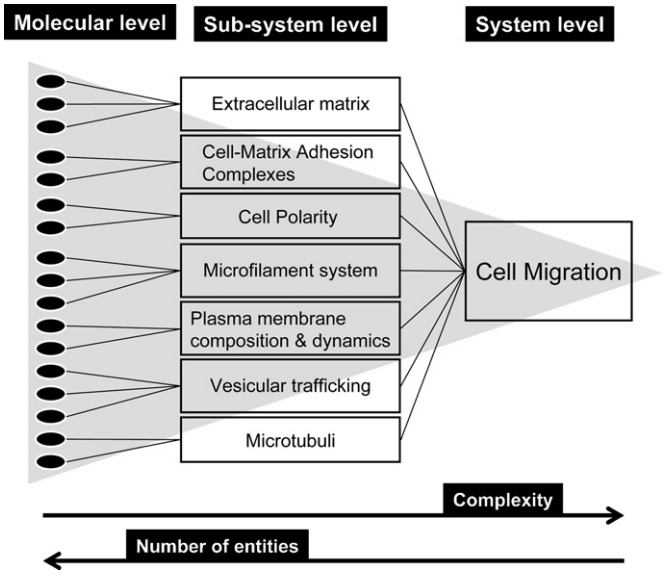

Fig. 3. Cell migration as an emergent process. Recognition of cell migration as an emergent process facilitates conceptualization of the informational flow and organizational hierarchy that produce complex cell migration behaviors. Cell migration derives from a vast diversity of molecular interactions that combine progressively to produce the intermediate complexity information outputs and behaviors of a number of semi-independent sub-cellular systems. These include: CMACs; the ECM; the cell polarity system; the microfilament system; microtubuli; vesicular trafficking; and the composition and behavior of the plasma membrane. Each of these sub-cellular systems interact with and affect one another, and the informational and mechanical outputs of these interactions coalesce to mediate cell migration, which itself is a component of even more complex biological phenomena such as tumor metastasis. This view of cell migration as emergent has important implications for the types of investigative approaches required to achieve a systems level understanding of the process. It is clear that such studies must quantitatively analyze and integrate not only molecular level events, but also the outputs and behaviors of the various sub-cellular systems involved. Ultimately, these comprehensive and quantitative findings must be integrated into the bigger picture of cell migration. Thus, given that CMACs act at the core of cell migration, quantitative analyses of CMAC features and dynamics, as well as of CMAC influences on other sub-cellular systems, will be prerequisite to a global and actionable understanding of cell migration.

cell surface. These signaling systems are designed to induce a series of second messengers as well as actin-dependent cellular protrusions that form the molecular scaffold required for the initiation and maturation of CMACs. However, in addition to soluble factors, polarity can also be induced more directly by CMACs in their role as sensors of exposed extracellular matrix. For example, during the wounding of a monolayer of astrocytes, the reorientation of the microtubule-organizing center and thus the development of cellular polarity is induced by integrin clustering-dependent activation of Cdc42 [63]. This in turn activates and recruits the components of the PAR polarity complex to the leading edge, resulting in APC interaction with the membrane-localized Dlg [64]. In parallel, Cdc42 activation of the F-actin linker protein IQGAP1 drives the formation of a ternary complex including APC and CLIP-170, both microtubule–tip binding proteins, which in turn allow capturing and reorientation of the microtubule-based vesicular transport system, and its direction to the leading edge of the migrating cell [65,66].

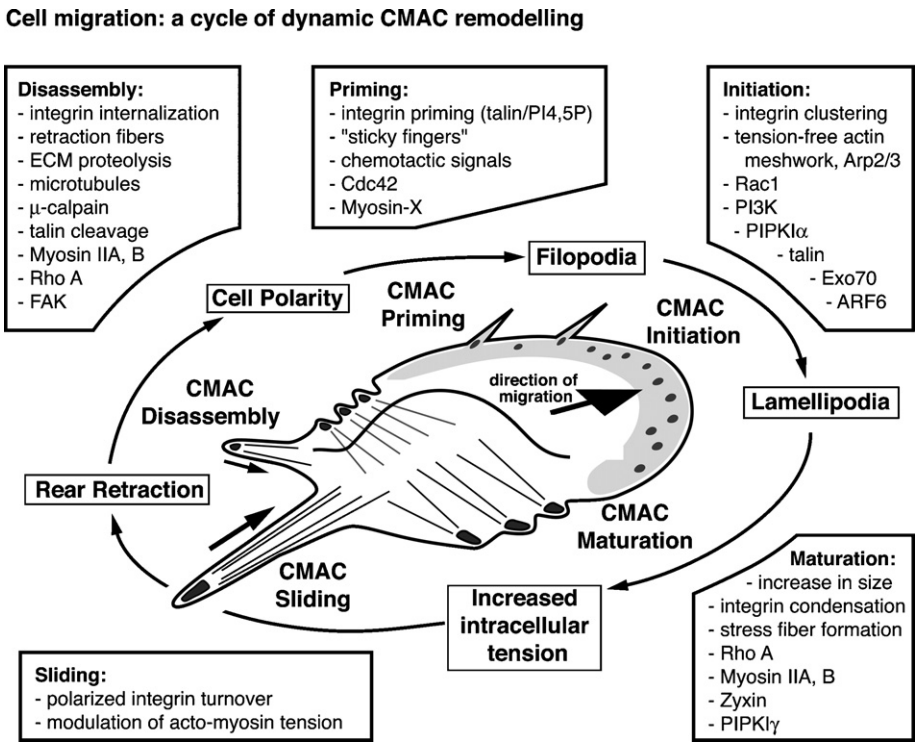

Fig. 4. Cell migration—a cycle of dynamic CMAC remodeling. This graphical representation of a migrating cell highlights protruding and retracting features, such as filopodia, lamellipodia and retraction fibers, respectively. Different types and states of CMACs are represented by dark dots or elongated shapes, either embedded within the lamellipodial actin meshwork (grey crescent) or attached to stress or retraction fibers (black lines). Each different CMAC state is described by an adjacent text box (refer to the text for more detailed descriptions). Please note that the different CMAC states are dynamically linked to morphological (filopodia, lamellipodia, rear retraction) or cellular features (increased intracellular tension, cell polarity).

In the case of chemotactic signaling through G-coupled receptors or receptor tyrosine kinases, the initial polarity cue also involves the direct modification of the actin cytoskeleton to create cellular protrusions. These can be initiated by the generation of PLC/cofillin-dependent foci of barbed ends (see below) or Cdc42 and Rac1-dependent PAK activation, which localizes the Rac-GEF βPIX to the leading edge or nascent adhesions via the adaptor protein GIT1 [33,67] (also see below).

3.3. Priming of CMACs

Before CMACs can form within protruding organelles such as filopodia or lamellipodia at the front of migrating cells, a series of conditions have to be fulfilled that we summarize under the term "priming". On the one hand, the polymerization of actin filaments into filopodia or lamellipodia requires Cdc42 and PI(4,5)P-dependent activation and recruitment of N-WASP or phospho-inositol lipid-assisted recruitment of the Rac1/WAVE complex, respectively [68]. On the other hand, the facilitated adhesion of these protrusions to ECM components may require surface exposed integrins with an extended (or activated) conformation, transforming these protrusions into "sticky fingers" [69,70]. The mechanisms of this integrin priming are not known, but active integrin transport by myosin-X [71] and/or a spatially controlled activation of the adaptor protein talin (see below) could potentially be involved. Alternatively to the extended conformation of individual integrins, the clustering of primed integrin receptors, as observed in membranes of monocytes, could further reduce the energy threshold required for the formation of CMACs [72]. Importantly, considering filopodia or lamellipodia as the sensing organelles of cells, integrin exhibiting an extended conformation would be ideal to interact with exposed ECM, and thereby trigger the subsequent formation of CMACs in response to ligand binding and outside–in activation of integrins.

3.4. Initiation of CMACs

In both filopodia and lamellipodia, the formation and behavior of CMACs are controlled by the convergence of several sub-cellular systems. For example, a lamellipodia that is initiated in response to growth factor/RTK stimulation requires the activation of the small GTPase Rac1 by Rac-GEFs (VAV, TIAM-1, SOS) that themselves respond to the local synthesis of PIP3 by the phosphotyrosine-dependent activation of PI3K [73]. Importantly, the same receptor system also activates PLC, which is responsible for two significant signaling pathways. On the one hand, cleavage of PIP2 generates IP_3, which serves to increase cytoplasmic Ca^{2+} levels, thereby stimulating the calmodulin-dependent linking of the Ral GTPase to the exocyst [74]. In turn, growth factor-dependent interaction of exocyst components with the actin branch promoting Arp2/3 complex are critically involved in the activation of Rac1 and the stimulation of cell protrusion [75]. Similarly, recruitment of inactive FAK to the ARP2/3 complex-localized within F-actin rich cellular protrusions, and subsequent release of activated FAK into nascent adhesions controls the spatio-temporal pattern of lamellipodia

extension [76]. This provides a mechanism for coordination of actin branching and polymerization and CMAC assembly at the front of migrating cells. On the other hand, the PLC induced drop of PIP2 at sites of receptor kinase activation leads to a transient activation of cofilin that severs pre-existing actin filaments, creating free barbed ends required as initiation sites for subsequent actin polymerization [77]. A cofilin activity gradient is further stabilized by the LIMK-dependent inactivation of cofilin throughout the cell body [78]. However, the transient drop of PIP2 at the plasma membrane is rapidly compensated for by the ARF6-dependent recruitment of PIPKIα [79], thus generating a membrane environment allowing the polymerization of the F-actin meshwork within lamellipodia. In turn, the F-actin-dependent recruitment of the LIM-domain containing protein Ajuba, which activates PIPKIα, serves to maintain high PIP2 levels in the advancing lamellipodia [16,80].

In order to allow the formation of CMACs, integrins have to be incorporated and clustered within this actin meshwork. In addition to the required presence of immobilized integrin ligands in the ECM, PIP2 synthesis provides a suitable microenvironment to stimulate the activation of the integrin adaptor protein talin, thereby promoting its interaction with ligand-bound integrins [81]. Due to the allosteric structure of integrins, simultaneous ECM ligand binding and PIP2-stimulated talin binding is required for lateral integrin clustering [55]. Interestingly, the formation of integrin clusters does not require the F-actin binding domain of talin. Thus, an alternative mechanism may be cholesterol-dependent lipid domain-mediated integrin clustering into nascent CMACs [72,82,83]. Recently, the clustering of PIP2 has been induced on giant unilamellar vesicles by PIP2 and N-WASP-dependent actin polymerization [84]. Combined, these details suggest the possible involvement of an actin and membrane-dependent self-organization system during the clustering of integrins into nascent CMACs.

As discussed previously, integrin turn-over within clusters formed in a Rac1-induced F-actin network is dramatically decreased compared to that observed in stress fiber-linked FAs [53]. The intrinsic stability of ligand-bound integrins within lamellipodia may thus explain the small size of FCs and their immobilized state with respect to the substrate. Once integrins are clustered via talin, additional structural and signaling components are recruited to turn these mechanical anchor points into signaling platforms, whose major output is the maintenance of Rac1-dependent lamellipodia formation. For example, paxillin-dependent recruitment of the GIT1/PIX/PAK complex to nascent adhesions results in a robust activation of lamellipodia [33]. As a consequence of the rapid progression and turn-over of the advancing actin polymerization front, the life-time of CMACs in this zone is very short. This may result from the particularly fast turn-over of the F-actin scaffold that maintains integrin clusters in advancing lamellipodia. This short life-time is in striking contrast to the longer life-time of CMACs in slow migrating cells [85]. This apparent difference can be explained by the alternative fates of the CMAC associated F-actin network, which either turns-over rapidly or matures in response to intracellular myosin II-dependent tensioning of stress fibers—a process resulting in high-density integrin-containing focal adhesions [49,53]. CMAC maturation, as a result of periodic stress fiber tensioning then serves to stabilize the slowly protruding lamella [86].

3.5. Maturation of CMACs

In the past, the maturation of CMACs has been described as the conversion of focal complexes into focal contacts/focal adhesions. Importantly, this maturation can be induced by applying external or internal tension to nascent adhesions [49,53,54,56,87,88]. Thus, an obvious role of CMAC maturation in response to internal or external tension is to probe, transmit and respond to the mechanical properties of the cellular environment. Correspondingly, increased mechanical resistance by the ECM leads to the augmentation of CMAC size and phosphotyrosine content [89,90]. This provides cells with immediate environmental feedback, including the generation of signals for reotaxis [91] and for the cell–substrate-dependent survival of cancer cells [92]. The molecular pathways that control the mechanical sensing at the level of CMACs are not well established, but they may involve enzymatic as well as stress-dependent conformational changes in adaptor proteins (described above). Moreover, in order for CMACs to further enhance this information relay function, the status of mechanical tension within microfilaments may also be communicated to the cell nucleus by adaptor proteins such as the LIM domain-containing protein zyxin or the ILK–PINCH–parvin complex [23,93].

3.6. Sliding of CMACs

Contrasting with the stability of mature CMAC structures, manifested as long life-times, analyses of integrin dynamics has, as discussed earlier, demonstrated a high integrin exchange rate in mature CMACs such as focal adhesions. This suggests that mature CMACs undergo continuous rebuilding to allow a rapid response to changes in the intracellular or extracellular microenvironment. It is unclear what causes the increased turn-over of integrins within mature CMACs. Nevertheless, we can propose that the overall life-time or remodeling rate of a particular CMAC is controlled by one or several rate-limiting steps influencing assembly and disassembly. Interestingly, integrin activation appears not to be rate-limiting for integrin exchange rates. Instead, integrin recruitment and PIP2-dependent association with talin, which is linked to the talin-dependent recruitment of PIPKIγ (for review see [16]), may set an upper limit for how fast new CMACs can be either built or modulated by integrin association to pre-existing CMACs.

One of the examples where remodeling of CMACs has an important impact on the direction and speed of migration, concerns the sliding of CMACs [94]. This process results from the polarized assembly and disassembly of CMAC components, suggesting that the functional CMAC unit is moving in response to, and in the direction of, stress fiber-induced tension, by simultaneously assembling new molecules at the front and disassembling at the rear. At the same time, individual components undergo a constant exchange within all parts of the sliding CMAC [54].

Not much is known about the molecular mechanisms that control the sliding of CMACs. It has been observed that the sliding speed of CMACs is proportional to intracellular tension [95]. Interestingly, in retracting portions of the cell, the tension between substrate and cytoskeleton is lower than in mature CMACs at the cell front. These data suggest a critical connection between the role of myosin II in controlling local contractility of the actin microfilaments and a cross-talk to sub-cellular systems that produce cellular polarity—such as microtubules [96]. Accordingly, inward sliding of CMACs is transiently inhibited by localized lamellipodia extension [53], which could either influence mechanical properties or stress reception within local CMACs, or alternatively, modify the local concentrations of CMAC components by altering their stability, signaling capacity or trafficking.

3.7. Disassembly of CMACs

Similar to the controlled retraction of sliding CMACs at the cell rear, CMAC disassembly has an important impact on the overall speed of cell migration. As mentioned above, CMAC disassembly may occur at different locations in the cell. At the cell front, older CMACs become obsolete when new fully functional adhesions have been formed. At the cell rear, CMAC disassembly is often initiated by the process of sliding. For this reason, it is likely that the inherent dynamic assembly and disassembly of CMAC components is critically linked to CMAC disassembly. Thus, processes that accelerate CMAC turn-over may contribute to their disassembly, such as RhoA and associated myosin II contractility [97]. However, CMAC turn-over and disassembly are also controlled by protein stability, either at the level of the extracellular matrix or by the Ca^{2+}-dependent intracellular protease calpain. For example, the stabilization of talin against calpain cleavage slows down CMAC turn-over and migration [98]. Another important pathway involves signaling via FAK and Src family kinases that are both important accelerators of CMAC dispersal and are counteracted by their respective phosphotyrosine phosphatases [99]. Interestingly, the initial observation of microtubule-targeting-dependent focal adhesion disassembly was recently connected to the phosphotyrosine-dependent interaction of dynamin and FAK, thus potentially linking the process of CMAC dispersal to vesicle formation and endocytic transport pathways [100]. Accordingly, α5β1 integrin has been observed to be internalized from the cell rear while recycling [101]. In fact, the targeted delivery of integrins, and the vesicular transport of CMAC components in general, may play a fundamental role in CMAC priming, formation and turn-over [102]. For example, the signaling of integrins αvβ3 and α5β1 to the small GTPases Rac and RhoA, respectively, can be specifically modulated by their differential vesicular trafficking, leading to dramatically altered cell adhesion and migration [103]. These events are regulated by both growth factor- and ECM-derived signals, and their elucidation provides an exciting new example of the sub-cellular system integration that underlies cell migration. In fact, this example illuminates linkages between all the sub-cellular systems discussed herein, with a functional regulatory cascade flowing from plasma membrane-derived ECM and RTK signals to regulate vesicular trafficking of integrins and microtubule-dependent cell polarity features, thereby altering Rho GTPase signaling and subsequently CMAC and microfilament dynamics to ultimately modify cell adhesion and migration.

4. Outlook

In this review, we present a relatively novel perspective for the conceptualization and analysis of cell migration as an emergent process derived from regulated information flow amongst sub-cellular systems and molecular components. Due to the incredible density of information that now exists within this field of research, and since many related studies analyze only one functional endpoint, e.g. cell migration, it is currently difficult to resolve the implications of *isolated* biological findings within the entire cell migration system. Thus, a focus on the significance of biological events as information fluxes may provide a means to rationalize the complexity of the system. When combined with the integration of information within and between molecular, sub-cellular system and system level resolutions, this approach may facilitate significant advances in our fundamental and mechanistic understanding. Therefore, we believe that research in this area would benefit from being, as much as possible: *quantitative*—to allow mathematical interrogation and accelerated information communication; *comprehensive*—to address the broad spectrum of components and sub-systems involved in cell migration; and *integrated*—to link adaptations detected throughout the entire system to specific information inputs and outputs. In practical terms, this means that we need to perform concurrent, quantitative analyses of localized molecular and sub-cellular system events, together with analyses of migratory characteristics. These approaches may perhaps provide the best hope of producing an actionable comprehension of cell migration and thereby facilitate the development of treatments for many related pathologies, including cancer metastasis.

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