Cell. Mol. Life Sci. (2012) 69:3989–3997
DOI 10.1007/s00018-012-1068-4

Cellular and Molecular Life Sciences

REVIEW

A matricellular protein and EGF-like repeat signalling in the social amoebozoan *Dictyostelium discoideum*

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Received: 16 May 2012 / Revised: 18 June 2012 / Accepted: 19 June 2012 / Published online: 11 July 2012

Abstract Matricellular proteins interact with the extracellular matrix (ECM) and modulate cellular processes by binding to cell surface receptors and initiating intracellular signal transduction. Their association with the ECM and the ability of some members of this protein family to regulate cell motility have opened up new avenues of research to investigate their functions in normal and diseased cells. In this review, we summarize the research on CyrA, an ECM calmodulin-binding protein in *Dictyostelium*. CyrA is proteolytically cleaved into smaller EGF-like (EGFL) repeat containing cleavage products during development. The first EGFL repeat of CyrA binds to the cell surface and activates a novel signalling pathway that modulates cell motility in this model organism. The similarity of CyrA to the most well-characterized matricellular proteins in mammals allows it to be designated as the first matricellular protein identified in *Dictyostelium*.

Keywords Matricellular · EGF-like repeat · Extracellular matrix · Signal transduction · Cell motility · *Dictyostelium* · CyrA

Abbreviations

AcbA	Acyl-CoA binding protein A
ADAMTS	A disintegrin and mettaloproteinase with thrombospondin motifs
carA	cAMP receptor A
carC	cAMP receptor C
CaM	Calmodulin
CaMBP	CaM-binding protein
CyrA	Cysteine-rich protein A
CyrA-C40	40 kDa CyrA cleavage product
CyrA-C45	45 kDa CyrA cleavage product
ECM	Extracellular matrix
EGFL	Epidermal growth factor-like
EGFL1	CyrA EGFL repeat 1
EGFR	EGF receptor
ER	Endoplasmic reticulum
MHC	Myosin II heavy chain
MMP2	Matrix metalloproteinase 2
PaxB	Paxillin B
PI3K	Phosphatidylinositol-3-kinase
PKA	Protein kinase A
PLA2	Phospholipase A2
PLC	Phospholipase C
SDF-2	Spore differentiation factor 2
SPARC	Secreted protein acidic and rich in cysteine
TalB	Talin B
Ten14	14th EGFL repeat of tenascin C
Tenascin C	Tenascin cytotactin
TSP	Thrombospondin
VinB	Vinculin B

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Matricellular proteins

Matricellular proteins belong to a group of extracellular matrix (ECM) proteins that function as adaptors and modulators of cell–matrix interactions [1, 2]. The most

well-studied examples of these proteins include tenascin cytotactin (tenascin C), thrombospondin (TSP) 1 and 2, and SPARC (secreted protein acidic and rich in cysteine) [3–5]. The evolutionary history of these proteins as well as the ECM was recently reviewed [2, 6]. A matricellular protein is defined by a number of characteristics. They associate with extracellular proteases and growth factors can function as both soluble and insoluble proteins are expressed at high levels during development, and unlike other components of the ECM do not appear to contribute directly to the organization of extracellular structures or their physical properties [1, 2, 7]. Matricellular proteins tend to be rapidly turned over and contain binding sites for some major structural elements in the ECM and specific cell surface receptors [3]. Another important attribute of matricellular proteins is their ability to modulate cellular processes by binding to cell surface receptors and initiating intracellular signal transduction [1, 3, 7–10].

The proteolytic cleavage of matricellular proteins to produce small signalling polypeptides and peptides has received much attention in recent in years and has been observed in many systems. Members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) superfamily of matricellular proteins contain a metalloprotease domain that can cleave specific substrates in the ECM or on the cell surface [3, 11]. As evident by recent studies on tenascin C and TSP-1 (discussed below), the literature on protein processing to release functional cleavage products is currently on the rise.

EGF-like repeats as motility-enhancing domains

Many of the matricellular proteins that have been characterized contain epidermal growth factor-like (EGFL) repeats, which are cysteine-rich motifs that share sequence similarity with EGF [12]. EGF is a 6-kDa, 53 amino acid, cysteine-rich polypeptide that binds to an EGF receptor (EGFR) and activates signalling pathways involved in mediating a diversity of cellular processes (e.g., cell motility, chemotaxis, proliferation, growth, survival, differentiation, morphogenesis) [13, 14]. The EGFL repeat is a widespread, highly variable domain that contains amino acids in specific positions relative to conserved cysteine residues [12, 15]. EGFL repeats can appear as single entities or be arranged as multiple tandem repeats [12]. They are found extensively in human proteins and have also been detected in lower eukaryotes, including the model organisms *Drosophila melanogaster* and *Dictyostelium discoideum* [16–21].

The most well-studied examples of EGFL repeat-containing ECM proteins include tenascin C, TSP-1, and laminin-5, which have all been shown to modulate cell movement via one or more of their EGFL repeats. Tenascin C is a member of an extensive family of ECM glycoproteins. In its monomeric form, this 190–300 kDa protein contains 14 EGFL repeats (Ten 1–14) [22]. It is expressed during tissue development and regeneration, is involved in cell proliferation and migration, and has been linked to tumor progression [22–25]. Some EGFL repeats of tenascin C (e.g., Ten14) have been shown to increase cell motility by binding to the EGFR and activating EGFR-dependent signalling [26, 27]. Although the sequence and structure of Ten14 is similar to EGF, this 31 amino acid polypeptide has been shown to be a low affinity ligand for the EGFR, since micromolar concentrations are required to increase the rate of cell motility [26, 28]. Activation at a micromolar concentration contrasts with the nanomolar concentrations of classical growth factors such as EGF that are required to elicit similar responses [26]. Unlike EGF, binding of Ten14 to the EGFR is transient and the Ten14–EGFR complex is not internalized, thus restricting the activated receptor to the cell surface [27, 28]. Internalization of receptor–ligand complexes functions as an attenuation mechanism for the signal. In the case of transient binding of Ten14 to the EGFR, the lack of internalization allows for a continuous activation of the receptor thus maintaining the signal for cell motility [27].

TSP-1, an angiogenesis inhibitor, is a 420-kDa ECM glycoprotein composed of three identical 145-kDa monomers that are held together via disulphide linkages [29, 30]. Each 145-kDa monomer possesses three EGFL repeats. Several cell types such as smooth muscle and endothelial cells secrete TSP-1 into the ECM, where it participates in processes related to tissue regeneration and cell differentiation such as re-epithelialization after injury [29]. Its activity has also been linked to cancer progression [30]. The EGFL repeats of TSP-1 have recently been shown to increase epithelial cell migration by activating intracellular signalling; however, the binding of the repeats to the cell surface has not yet been shown [29]. Although the TSP-1 EGFL repeats activate the EGFR by inducing autophosphorylation of the receptor, they do not directly bind to the receptor suggesting that not all EGFL repeats function by binding to the EGFR [29].

Laminin-5 is an ECM glycoprotein that possesses EGFL repeats in its ectodomain [31]. Although its function as major structural component of basement membranes prevents it from being classified as a matricellular protein, the EGFL repeats of laminin-5 have also been shown to modulate cell movement. Intact laminin-5 has been shown to support cell adhesion, but when cleaved by matrix metalloproteinase 2 (MMP2), the EGFL repeat-containing cleavage products promote cell migration by binding to the EGFR and activating downstream signalling pathways [31, 32]. Several

studies have reported the enhanced expression of laminin-5, especially of the cleaved EGFL repeat-containing products at sites of tumor cell penetration [33, 34]. Together, studies on tenascin C, TSP-1, and laminin-5 have indicated that a primary function of cysteine-rich, EGFL repeat-containing ECM proteins may be to modulate cell motility.

Dictyostelium as a model eukaryote for studying cell motility

Dictyostelium discoideum is a fascinating organism that is used as a model system for studying a number of cell and developmental processes [35]. During feeding, *Dictyostelium* amoebae find their food source by chemotactically responding to folic acid that is secreted by bacterial cells. Upon starvation, *Dictyostelium* cells begin to secrete cAMP which acts as a chemoattractant causing individual cells to aggregate into mounds. Aggregated cells then develop into a motile, multicellular structure known as a pseudoplasmodium or slug that can migrate on the substratum for an indefinite period of time in response to light and temperature. When conditions are suitable, the slug will culminate and develop into a fruiting body composed of a mass of spores that is supported by a stalk of dead stalk cells [36].

The mechanisms underlying cell motility and chemotaxis in *Dictyostelium*, as well as the molecular components and interactions involved are similar to those observed in mammalian cells making it a very useful system for studying this cellular process [37]. The movement of *Dictyostelium* amoebae has been extensively studied; however, all lines of evidence suggest that movement in this model eukaryote is a highly complex process likely involving a number of pathways that have yet to be identified [38]. It is thought that these unidentified pathways may also involve redundant components.

Phosphatidylinositol-3-kinase (PI3K) and phospholipase A2 (PLA2) are two proteins that have been shown to mediate cAMP chemotaxis in parallel compensatory pathways [39, 40]. PI3K controls F-actin polymerization, which has been linked to pseudopod extension [41, 42]; however, the activation mechanism and the targets of PLA2 are still unknown [39, 43]. In addition, studies have shown that phospholipase C (PLC) and Ca^{2+} are essential for PI3K- and PLA2-mediated signalling, respectively [40]. In mammalian cells, PLA2 induces Ca^{2+} signalling through the release of arachidonic acid; however, the ability of this fatty acid to mediate the PLA2-dependent chemotactic response in *Dictyostelium* has not yet been verified [39]. In addition to PI3K and PLA2 activity, Ca^{2+}- and calmodulin (CaM)-mediated signalling via interaction with specific CaM-binding proteins (CaMBPs) have both been shown to be required for efficient cell motility and chemotaxis in *Dictyostelium* [44, 45]. A recent study described the ability of aggregation-competent *Dictyostelium* amoebae to directionally respond towards a gradient of Ca^{2+} [46], suggesting the existence of other motility regulatory components and mechanisms in *Dictyostelium* that have yet to be identified.

Although much less well studied compared to cAMP chemotaxis, *Dictyostelium* can also chemotax towards folic acid, which is secreted by bacteria and allows *Dictyostelium* amoebae to find their food source during growth. The signal transduction regulating folic acid chemotaxis is different from the signalling that mediates chemotaxis towards cAMP. For instance, tyrosine kinase activity has been shown to be required for chemotaxis towards folic acid, but not cAMP, and unique CaM-dependent phosphoproteins have been shown to be linked solely to folic acid chemotaxis [45, 47]. These findings, therefore, provide further support for the existence of multiple motility-regulating pathways in this model eukaryote.

The *Dictyostelium* slime sheath

The *Dictyostelium* slug is covered by a thin ECM (i.e., slime sheath) that is continuously synthesized from the tip of the slug forming a sheath around the migrating slug cells [48]. As the slug migrates along the substratum, it leaves behind a trail of slime sheath that provides clues into the cell–ECM interactions that occurred during slug movement. Vegetative amoebae have also been shown to secrete ECM material; however, little is known at the molecular level about the ECM during the growth phase. The *Dictyostelium* slime sheath is similar in structure and composition to both animal and plant ECMs. It is composed of cellulose fibers and polysaccharide embedded within a protein-containing matrix that contains both structural and non-structural proteins. The most well-studied structural protein of the slime sheath EcmA is distributed throughout the slug ECM and has been shown to be an integral structural protein of the sheath. EcmA knockout cells are still able to form slugs that migrate normally; however, the structure of the ECM that surrounds the slug cells is weakened [49]. A group of glycoproteins called the sheathins (i.e., EcmC, EcmD, EcmE) co-localize with cellulose and have been shown to be involved in regulating slug migration [50]. Research on the non-structural components of the slime sheath, however, is limited. Although previous studies have identified a group of soluble, mobile glycoproteins within the slug ECM, the identity of these proteins remains unknown indicating that much still remains to be discovered about the *Dictyostelium* slug ECM [51, 52].

Identification of EGFL repeat-containing proteins in *Dictyostelium*

Bioinformatic analyses suggest that the *Dictyostelium* genome encodes a higher percentage of EGFL domains than any other sequenced eukaryote, including humans [18]. Despite their abundance, the function of EGFL repeats and of EGFL repeat-containing proteins in *Dictyostelium* has been relatively unstudied. The most well-studied structural proteins of the *Dictyostelium* ECM, EcmA, and EcmB possess extensive cysteine-rich regions composed of tandem arrays of a cysteine-rich 24 amino acid repeat that shares some sequence similarity with EGF [48]. SadA, the novel adhesion receptor in *Dictyostelium*, contains three conserved EGFL repeats in the predicted extracellular domain of the protein that are similar to regions in cell adhesion proteins such as integrins and tenascins [19]. SadA associates with the actin cytoskeleton, possibly by interacting with cortexillin I, a known actin bundling protein [53]. *sadA* knockout cells possess a disrupted actin cytoskeleton and cytokinesis defect. They also move with increased speed during vegetative conditions, suggesting that SadA-mediated cell–substrate adhesion acts as a brake during cell movement [19]. Therefore, as in mammals, these data suggest that a major function of EGFL repeats in *Dictyostelium* may be to modulate cell movement.

CyrA is a matricellular protein in *Dictyostelium*

CyrA is the first extracellular CaMBP identified and characterized in *Dictyostelium*. This 63-kDa cysteine-rich protein possesses four tandem EGFL repeats in its C-terminus and possesses attributes that allows it to be classified as a matricellular protein (Table 1) [21]. The expression and secretion of CyrA increases at a constant rate during the early stages of development, but peaks during mid-development which coincides with the time that cells are developing into a motile, multicellular slug [21, 54]. Consistent with the fact that CyrA possesses an N-terminal signal sequence and is secreted, the protein has been shown to localize to the endoplasmic reticulum (ER), particularly to the region of the ER that surrounds the nucleus (i.e., perinuclear region) [54]. CyrA-GFP was also shown to co-localize with calnexin an integral transmembrane protein of the ER [54, 55].

Like other matricellular proteins (e.g., tenascin C), CyrA is proteolytically cleaved during *Dictyostelium* development to release 45- and 40-kDa EGFL repeat-containing cleavage products (CyrA-C45 and CyrA-C40, respectively) (Table 1; Fig. 1) [21]. In *Dictyostelium*, this type of protein processing has previously been reported to occur in the acyl-CoA binding protein (AcbA), which is secreted and cleaved by the membrane-bound serine protease TagC to generate the bioactive peptide spore differentiation factor-2 (SDF-2) [56]. SDF-2 binds to a receptor on prespore cells in order to induce spore cell differentiation. During mid- to late *Dictyostelium* development, the multicellular mass of cells is covered by a thin ECM called a slime sheath, which is composed of protein, polysaccharide, and cellulose [48]. Full-length CyrA and its cleavage products localize to the slime sheath of the migrating slug (Fig. 1) [21, 54]. Interestingly, CaM has also been shown to be secreted and to localize to the slime sheath (Fig. 1) [21, 54, 57]. Full-length CyrA and its cleavage products bind CaM both intra- and extracellularly, and CaM antagonism has been shown to increase CyrA cleavage indicating that CaM regulates the release of EGFL repeats from CyrA and possibly other EGFL repeat-containing proteins during development (Fig. 1) [21, 54]. These findings are supported by studies that have shown that the activity and function of some EGFL repeats is dependent on Ca^{2+} binding, which is interesting considering that CaM is the primary sensor of Ca^{2+} within the cell [58]. Binding of Ca^{2+} to some EGFL repeats such as the EtMIC4 protein of

Table 1 Comparison of CyrA to the matricellular proteins tenascin C and thrombospondin-1

Characteristics	Tenascin C	Thrombospondin-1	CyrA
Secreted	Yes [1]	Yes [1]	Yes [21, 54]
Localizes to the ECM	Yes [1]	Yes [1]	Yes; slime sheath [21, 54]
Expressed at high levels during development	Yes [1]	Yes [1]	Yes [21, 54]
Contains EGFL repeats	Yes [28]	Yes [29]	Yes [21]
Releases cleavage products	Yes [23]	Unknown	Yes [21]
EGFL repeats modulate cell motility	Yes [27]	Yes [29]	Yes [20, 21, 60]
EGFL repeats bind to the cell surface	Yes [26, 28]	Unknown	Yes [54]
Receptor	Yes; EGFR [26, 28]	Unknown	Yes; uncharacterized [54]
Initiates intracellular signal transduction	Yes [27, 28]	Yes [29]	Yes [20, 21, 60]

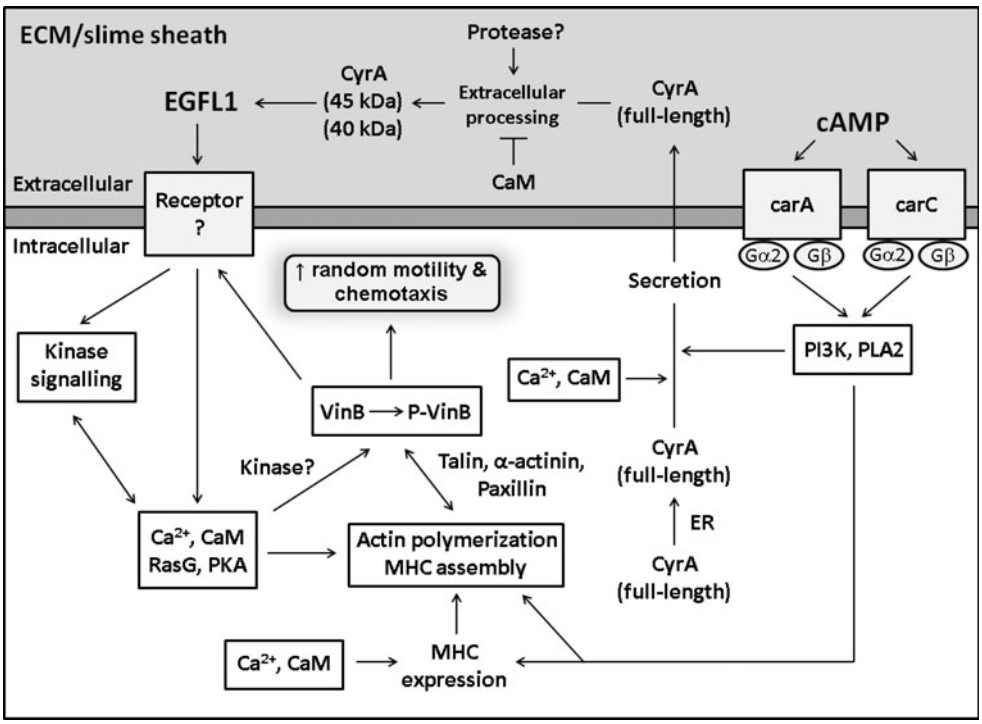

Fig. 1 Model for CyrA EGFL repeat signal transduction in *Dictyostelium discoideum*. Refer to the text for a thorough explanation of the signal transduction mediating the response of cells to DdEGFL1

Eimeria tenella has been shown to affect both the conformation of the repeat as well as its susceptibility to proteases [59]. Together, these data support an interaction between CyrA, CaM, and Ca^{2+} in *Dictyostelium*.

CyrA EGFL1 increases cell motility

A synthetic EGFL peptide (DdEGFL1), whose sequence is identical to the first 18 amino acids of the first EGFL (EGFL1) repeat of CyrA, functions extracellularly to increase both random cell motility and cAMP-mediated chemotaxis in a number of wild-type and parental strains of *Dictyostelium* (e.g., NC4, AX2, AX3, KAX3, DH1; Fig. 1) [20, 60]. The regulation of cell motility gives a matricellular function to CyrA (Table 1). DdEGFL1 shares sequence similarity with EGF, Ten14, and another C-terminal EGFL repeat in CyrA [20]. The DdEGFL1 sequence is highly conserved in *Dictyostelium* and shares sequence similarity with many regions of EcmA and EcmB, as well as many putative ECM proteins in *Dictyostelium*. A recent study showed that DdEGFL1 is not a chemoattractant for *Dictyostelium* cells, but instead functions in a supportive role to increase the rate of random cell movement and chemotaxis during development [61]. The response of cells to DdEGFL1 increases during starvation, supporting a function for DdEGFL1 during early developmental events (e.g., cAMP chemotaxis) [21]. CyrA over-expression has also been shown to increase the rate of cAMP-mediated chemotaxis, providing in vivo evidence linking CyrA function to *Dictyostelium* cell movement and confirming in vitro studies performed using DdEGFL1 [20, 21, 54, 60, 61]. In addition, since CyrA localizes to the *Dictyostelium* slime sheath during the later stages of multicellular development, this suggests that DdEGFL1 is involved in regulating the movement of cells within the slug during slug movement [21, 54]. DdEGFL1 also inhibits the cleavage of CyrA supporting the existence of a signalling pathway regulating the release of EGFL peptides (e.g., DdEGFL1) and/or CyrA cleavage products possessing functional EGFL domains (Fig. 1) [21].

Signalling pathways regulating the expression, secretion, and function of CyrA

Another criterion for establishing a protein as matricellular involves the ability of the protein and/or cleavage products to bind to the cell surface and initiate intracellular signal transduction (Table 1) [1]. DdEGFL1 increases the rate of *Dictyostelium* cell movement via a novel signalling pathway that does not require the heterotrimeric G-protein utilized in cAMP signalling or either of the two cAMP receptors that are active during early development cAMP receptor A or C (carA and carC, respectively; Fig. 1) [60]. The signalling pathway mediating cell movement in response to DdEGFL1 requires CaM activity and intracellular Ca^{2+} release, and DdEGFL1 stimulation increases the amount of polymeric actin and myosin II heavy chain (MHC) in the cytoskeleton (Fig. 1) [60]. In addition to

actin and MHC, DdEGFL1 signalling has also been shown to require the cytoskeletal proteins talin B (TalB) and paxillin B (PaxB), which are homologues of mammalian talin and paxillin, respectively (Fig. 1) [62].

DdEGFL1-enhanced cell movement requires the activity of both PI3K and PLA2, two signalling proteins that have been shown to mediate the chemotaxis of *Dictyostelium* amoebae in parallel compensatory pathways (Fig. 1) [20, 39, 40]. Although the activity of both proteins is required, PLA2 appears to be the more dominant regulator of DdEGFL1-enhanced random cell motility, since inhibition of PLA2 alone significantly suppresses the increased movement, whereas PI3K inhibition alone has no significant effect [20, 61]. Interestingly, the secretion of CyrA has also been shown to be dependent on intracellular Ca^{2+} release and the activity of PI3K, PLA2, and CaM, indicating that a common mechanism regulates the function of CyrA (Fig. 1) [54]. The dependence of DdEGFL1 function on PI3K and PLA2 signalling is likely due to the regulation of CyrA secretion by both of these proteins.

Both inter- and intracellular Ca^{2+} signalling as well as Ras signalling have been shown to be required for EGF-induced cell movement in normal and cancerous cells [63, 64]. In *Dictyostelium*, two members of the Ras protein family, RasC and RasG, have been shown to regulate chemotaxis towards cAMP [65]. Our research has shown that DdEGFL1-increased movement partially requires the activity of RasG, but not RasC (Fig. 1) [60].

Protein kinase A (PKA, cAMP-dependent protein kinase), a serine/threonine kinase whose activity is dependent on cellular levels of cAMP, and which has been shown to be involved in regulating cell movement and chemotaxis in *Dictyostelium*, is also required for DdEGFL1 function, showing that PKA kinase activity is required for DdEGFL1 signal transduction (Fig. 1) [62, 66]. PKA kinase activity was also shown to be required for the DdEGFL1-induced phosphorylation of a 90-kDa phosphotyrosine during cell starvation (Fig. 1) [62]. Finally, Huber and O'Day [62] reported the detection of two phosphotyrosine proteins in DdEGFL1 pull-down assays. Together, these results show that the motility-enhancing pathway activated by DdEGFL1 involves protein kinases, which fits with observations of EGFR-mediating signalling in higher eukaryotes (Fig. 1) [67, 68].

Prior to studies describing DdEGFL1 function and signalling, it was reported that *Dictyostelium* cells could undergo random migration in the absence of functional heterotrimeric G proteins [69, 70]. A previous study showed that PI3K and Ras were activated in Gβ null cells during random cell migration, suggesting the existence of a Gβ-independent signalling pathway regulating random cell motility [70]. Activation occurred in the absence of external stimuli (i.e., folic acid) and resulted in F-actin polymerization. Interestingly, like the DdEGFL1-mediated pathway, that study also implicated RasG function [60, 70]. Based on their data, Sasaki et al. [70] suggested the existence of two pathways involving activated PI3K and Ras. One was a chemoattractant/Gβ-dependent pathway that regulated chemotaxis and the other was a Gβ-independent positive feedback circuit that regulated random cell migration. During chemotaxis, the authors suggested that the Gβ-dependent pathway overrides or disrupts the positive feedback loop involving activated PI3K and Ras, thereby allowing the cell to directionally respond to the chemoattractant (i.e., folic acid) [70]. Although Sasaki et al. [70] showed that the Gβ-independent pathway functioned in the absence of chemoattractants, the authors did not address whether the pathway was activated by other secreted components (e.g., EGFL repeat-containing proteins). Subsequent research on DdEGFL1 function and signalling suggests that activation of this pathway may occur via the binding of secreted EGFL repeat-containing proteins to a surface receptor that initiates intracellular signal transduction.

CyrA EGFL1 has differential effects on chemotaxis to folic acid versus cAMP

As discussed above, the pathway regulating folic acid chemotaxis is different from the one that mediates chemotaxis towards cAMP. DdEGFL1 does not significantly increase folic acid chemotaxis, possibly due to cells moving at or near their maximal rate [45, 47, 61, 71]. However, DdEGFL1 is able to completely restore the normal chemotactic response to folic acid when one of PI3K, PLA2, or tyrosine kinase activity is inhibited and partially restore the response when CaM is inhibited [61]. This restoration of chemotactic ability was also observed in mutants of cAMP signalling. Untreated *carA*-, *carC*-, and *carA-/carC*-cells possess lower rates of random motility when compared to parental cells [60]. However, DdEGFL1 was able to significantly increase the movement of all three strains to amounts greater than that observed in parental cells [60]. Together, these results provide further support for the existence of a novel motility-enhancing pathway regulated by EGFL repeats in this model organism. When components of either the folic acid or cAMP-mediated pathways are inactivated (e.g., in a knockout mutant or by pharmacological means), the pathway regulated by EGFL repeats/peptides is allowed to function as the dominant motility regulating pathway.

Vinculin B is a downstream target of CyrA EGFL1 signalling

DdEGFL1 sustains the threonine phosphorylation of vinculin B (VinB) during cell starvation (Fig. 1) [20, 62].

VinB localizes to the cytoplasm and cytoskeleton of *Dictyostelium* amoebae and has been shown to interact with DdEGFL1, CyrA-C45, and established vinculin-binding cytoskeletal proteins (e.g., MHC, α-actinin, talin, and actin; Fig. 1) [54, 62]. In higher organisms, vinculin over-expression has been shown to reduce cell migration, whereas down-regulation of the protein increases cell motility [72, 73]. VinB over-expression suppresses DdEGFL1-increased cell movement fitting with observations in mammalian cells [62]. Although PI3K and PKA have both been shown to be important for *Dictyostelium* cell motility, VinB threonine phosphorylation was shown to be independent of PI3K and PKA kinase activity and PI3K/PLA2 signalling, therefore indicating that another kinase is responsible for phosphorylating VinB (Fig. 1) [62]. In addition, these results suggest that the phosphorylation of VinB is regulated upstream of both PI3K and PLA2.

Future directions

DdEGFL1 has been shown to localize extracellularly, and was not observed inside cells even after prolonged incubation (Fig. 1) [20]. A recent study showed that DdEGFL1-FITC could be detected on the surface of cells capped with concanavalin A, suggesting that a receptor for EGFL repeats/peptides exists in *Dictyostelium* [54]. The identification of an EGFR-like (EGFRL) protein in *Dictyostelium* would be an important, evolutionarily significant finding, since disturbances in EGFR signalling have been associated with the development of malignant tumors and many forms of cancer [67, 74]. Therefore, understanding the evolution of this signalling may provide insight for future biomedical research. The existence of a membrane-bound EGFL repeat/peptide receptor possessing kinase activity is strengthened by the fact that DdEGFL1-activated signalling does not require the cAMP receptor or the heterotrimeric G-protein utilized in cAMP-mediated signalling [60]. A putative EGFRL protein was previously identified by inputting the amino acid sequence of EGFR from 14 different organisms into the BLASTp server of the online *Dictyostelium* resource dictyBase (http://www.dictybase.org/tools/blast). This candidate receptor possessed a putative kinase domain that shared sequence similarity with the intracellular tyrosine kinase domain of mammalian EGFR, suggesting that it may be an evolutionary precursor to mammalian EGFR (DDB0229955, http://www.dictybase.org) [20]. However, preliminary studies into the localization and binding partners of this protein suggest it is not the EGFL repeat/peptide receptor in *Dictyostelium* [62]. Although the search for the EGFL repeat/peptide receptor in *Dictyostelium* remains elusive, its identification would yield further insight into the attributes and evolution of receptors involved in mediating matricellular function.

To fully understand ECM dynamics in *Dictyostelium*, future research should be aimed at identifying the extracellular proteases that cleave CyrA and potentially other proteins in the ECM of *Dictyostelium* to generate EGFL repeat-containing cleavage products or peptides. The presence of a large number of extracellular proteases was reported in a recent study describing the secreted proteome profile of developing *Dictyostelium* cells [57]. Of the 349 proteins identified, 29 were linked to proteolysis including a putative dipeptidyl aminopeptidase (DDB0001159), a putative vitellogenic-like carboxypeptidase (DDB0167727), cysteine protease 4 (DDB0214999), cysteine proteinase 5 (DDB0185092), cysteine proteinase 6 (DDB0001517), and cysteine proteinase 7 (DDB0215005). These findings suggest that future studies should be performed to determine the function of these proteins during *Dictyostelium* development.

Conclusion

CyrA is the first extracellular CaMBP identified in *Dictyostelium*. Since it fulfills the criteria required to establish a protein as matricellular, it is designated as the first matricellular protein to be identified in *Dictyostelium* (Table 1) [1–3]. The first EGFL repeat (EGFL1) of CyrA has been well characterized. Our current knowledge of DdEGFL1-enhanced cell movement has allowed us to develop a model for the signal transduction mediating the response of cells to DdEGFL1 (Fig. 1). The dependence of CyrA secretion and DdEGFL1-enhanced cell movement on PI3K, PLA2, CaM, and intracellular Ca^{2+} release shows that these intracellular signalling components mediate the function of CyrA and form the basis of a novel motility-regulating pathway in *Dictyostelium*. In order to fully characterize the function of CyrA, it will be necessary to assess the ability of the other EGFL repeats of the protein to increase the rate of cell motility. The presence of repeated DdEGFL1-like sequences in EcmA, EcmB, and many putative ECM proteins in *Dictyostelium* suggest that these highly conserved sequences possess an important function for ECM proteins in this model organism. The abundance of EGFL domains in *Dictyostelium* and the ability of some EGFL repeats/peptides to increase cell movement suggests that this simple eukaryote could be used as a model system for studying the evolution of matricellular proteins, their receptors, and EGFL repeat signalling. The findings described here set the stage for further study into ECM proteins in *Dictyostelium*, specifically those that modulate cell motility.

Acknowledgments This review was supported by a Discovery Grant (D.H.O'D.; A6807) and a Canada Graduate Scholarship (R.J.H.) from the Natural Sciences and Engineering Research Council of Canada.

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