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Review

Extracellular matrix dynamics and functions in the social amoeba *Dictyostelium*: A critical review

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ABSTRACT

Background: The extracellular matrix (ECM) is a dynamic complex of glycoproteins, proteoglycans, carbohydrates, and collagen that serves as an interface between mammalian cells and their extracellular environment. Essential for normal cellular homeostasis, physiology, and events that occur during development, it is also a key functionary in a number of human diseases including cancer. The social amoeba *Dictyostelium discoideum* secretes an ECM during multicellular development that regulates multicellularity, cell motility, cell differentiation, and morphogenesis, and provides structural support and protective layers to the resulting differentiated cell types. Proteolytic processing within the *Dictyostelium* ECM leads to specific bioactive factors that regulate cell motility and differentiation.

Scope of review: Here we review the structure and functions of the *Dictyostelium* ECM and its role in regulating multicellular development. The questions and challenges that remain and how they can be answered are also discussed.

Major conclusions: The *Dictyostelium* ECM shares many of the features of mammalian and plant ECM, and thus presents an excellent system for studying the structure and function of the ECM.

General significance: As a genetically tractable model organism, *Dictyostelium* offers the potential to further elucidate ECM functions, and to possibly reveal previously unknown roles for the ECM.

1. Introduction

Comprised primarily of glycoproteins, proteoglycans, carbohydrates, and collagen, the extracellular matrix (ECM) of mammalian cells is more than just the sum of its parts [1]. It is the dynamic interface between the cell and its extracellular environment. As such, it plays a true intermediary role not only in protecting the cell that it surrounds, but also in directing cellular processes in response to environmental cues. The mammalian matrisome constitutes greater than 1% of the proteome, and the vast diversity of functions of the ECM and their mode of action are increasingly becoming clear [2]. As a mediator of cellular behaviour, development, and physiology, the mammalian ECM plays central roles in the regulation of cell motility, proliferation, shape, signalling, and survival, with new roles such as mechanochemical function coming to light [2]. The ECM is not just a physical sieve that molecules must make their way through. It is also a dynamic functionary that can trap, modify, or destroy those molecules. At times, the ECM can also malfunction by processing proteins in a way that is harmful [1,3].

Once considered to be primarily a structural element of metazoans, it should be recognized that even the cell walls of single bacteria, plant cells, and spores are forms of ECM. Organisms with less complex design and comparatively simpler lifestyles could be useful in analyzing the functions of the ECM. One such organism is the social amoebozoan *Dictyostelium discoideum*. *Dictyostelium* is a nucleated social microbe that feeds and grows as single cells. When prompted by starvation, it undergoes chemotactic aggregation to form a multicellular tissue called a pseudoplasmodium or slug. Differentiation of cells within the slug ultimately gives rise to a fruiting body consisting of a stalk supporting a droplet of spores [4]. During this transition to multicellularity, a true

Abbreviations: AcbA, acyl-CoA binding protein; AD, Alzheimer's disease; ALC, anterior-like cell; cAMP, cyclic adenosine monophosphate; CaBP, calcium-binding protein; Cad, calcium-dependent cell adhesion molecule; CaM, calmodulin; CaMBD, calmodulin-binding domain; CaMBP, calmodulin-binding protein; CV, contractile vacuole; DIF, differentiation-inducing factor; ECM, extracellular matrix; EGF, epidermal growth factor; EGFL, epidermal growth factor-like; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; GRASP, Golgi reassembly stacking protein; HD, Huntington's disease; PD, Parkinson's disease; Psa, puromycin-sensitive aminopeptidase; Psi, prespore-inducing factor; Pst, prestalk; SPARC, secreted protein acidic and rich in cysteine; SDF, spore differentiation factor; Sib, similar to integrin beta; Tenascin C, tenascin cytotactin; Tgr, transmembrane, IPT, IG, E-set, repeat; TipD, autophagy protein 16; TSP, thrombospondin; vWF, von Willebrand factor.

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dynamic ECM is produced that plays a central role in the transition of this tissue into a fruiting body. Many of the events that are mediated by the *Dictyostelium* ECM are the same as those seen in mammals—in fact, many more than were previously thought. Here we address them.

2. Extracellular matrices of social amoebozoans

As *Dictyostelium* cells initiate multicellular development, an event triggered by the diminishment of their microbial food supply, they begin to secrete an ECM called a slime sheath. ECM deposition becomes evident during cyclic AMP (cAMP)-mediated aggregation where cells at the center of the aggregate begin to secrete a complex ECM composed of cellulose, polysaccharide, protein, and glycoconjugates [5–8]. In other amoebozoan species such as *Polysphondylium pallidum*, the ECM surrounding both the central aggregate and incoming streams of cells is incredibly strong, so much so that both structures can be lifted off as a group from an agar surface [9]. Once aggregation is complete, the resulting multicellular pseudoplasmodium or slug continues to secrete ECM material from its tip, which is the site of cAMP synthesis and stalk cell formation [10,11]. The secreted material forms a sheath around the slug as the cells within begin the first stages of differentiation (Fig. 1). The ECM functions as a physical barrier, preventing the loss or entry of new cells into the multicellular aggregate, and may also function as a barrier to diffusion [12–13]. Terminal differentiation of prestalk and prespore cells into stalk cells and spores, respectively, occurs during culmination resulting in the formation of a fruiting body consisting of an unencased droplet of viable spores suspended atop a slender stalk. Cells within the stalk have thick walls and the stalk tube is surrounded by a cellulose-, protein-, and glycoconjugate-containing sheath, which represents another type of ECM in *Dictyostelium* [14–16].

Following culmination, each spore is surrounded by a rigid extracellular wall referred to as a spore coat, which is composed primarily of cellulose, protein, and polysaccharide [16,17]. The spore coat protects the spore from environmental conditions and allows it to remain viable for long periods of time [16]. During prespore cell differentiation, cells package all of the material required for spore coat formation, except cellulose, into prespore vesicles, which are then secreted outside the cell into the interspore matrix [16]. Both the spore coat and interspore matrix are additional forms of ECM in *Dictyostelium*. Cellulose is synthesized across the plasma membrane as differentiating spores rise up the stalk. The secreted components in the interspore matrix interact with the shrunken cell and form a coat around the newly formed spore. A layer of cellulose is sandwiched between a proteinaceous inner layer near the plasma membrane, and an outer protein-rich layer that acts as a permeability barrier towards exogenous macromolecules [16]. The function of the unincorporated material in the matrix is still unclear, however it is reasonable to suggest that this reservoir of ECM material helps to prevent dessication and maintain spore dormancy. In addition to spores and stalk cells, the amoebozoans form other types of ECM including the 2-layered walls of unicellular asexual microcysts, and the tripartite walls of sexual macrocysts [18,19]. These extracellular walls and coats provide structure and as yet unverified functions during cyst dormancy.

Clearly the ECM is a product of cell secretion, but just as evident, every protein that is secreted will not end up residing as an integral ECM component. Some secreted proteins will be reabsorbed by endocytotic mechanisms. Others will be lost to the extracellular environment, be broken down by proteases as the work their way through the ECM, or will use their proteolytic activity to modify the ECM. In *Dictyostelium*, proteomic analyses have revealed proteins that are secreted, present in the macropinocytic pathway, and retained within the sheath ECM [20–22]. In addition, the study of individual proteins has provided insight into proteins that are retained, modified, and excluded from the ECM. Here we review that information with a view towards gaining more insight into the *Dictyostelium* ECM, and how the proteins contained within regulate cell movement, differentiation, and morphogenesis in this model organism.

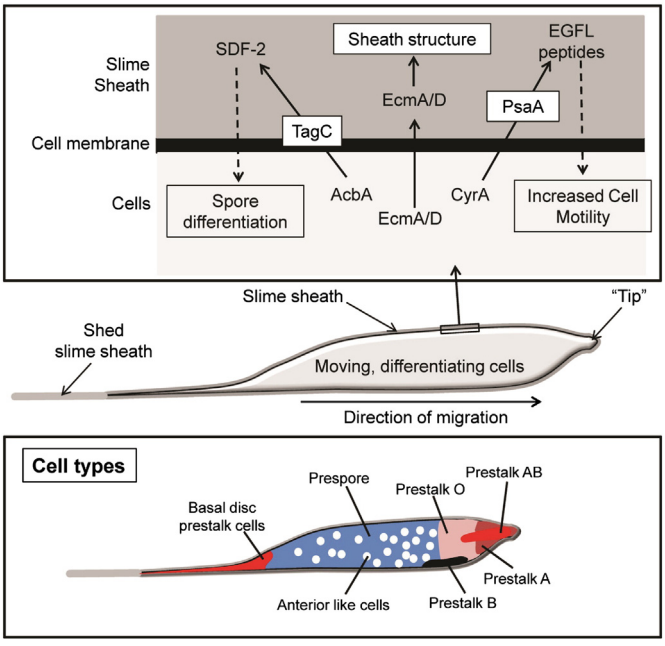

Fig. 1. The ECM surrounding the multicellular slug and the cell types contained within. The sheath ECM, which is synthesized from the tip of the multicellular slug, is shed from the back of the slug as it migrates along the substratum. (Top panel) a diversity of processes occur in the ECM during slug migration. Cells secrete EcmA and EcmD which provide structure to the ECM. Secreted proteins such as AcbA and CyrA are processed into bioactive fragments that in turn bind to the cell surface to modulate cellular processes (e.g., spore differentiation and cell motility, respectively). (Bottom panel) different cell types within the slug sort to specific locations.

3. The extracellular matrix of the *Dictyostelium* slug

As the *Dictyostelium* slug migrates along the substratum, the ECM is left behind as a collapsed tube, which has facilitated analyses of the specific components that make up the ECM [5,7,8,10,22,23] (Fig. 1). Based on the lack of detectable β-actin and α-tubulin, as well as several other cell-specific proteins in western blots, the sloughed off ECM contains few cells [6,22]. As further support, compared to cells within the slug, the ECM contains considerably more serine, glycine, alanine, and valine, and considerably less lysine, arginine, glutamic acid, proline, methionine, and phenylalanine [6]. Smith and Williams [6] showed that proteins comprise ~50% of the dry weight of the sheath ECM. Recent proteomic profiling of the sheath ECM revealed proteins involved in metabolic processes (~50%), transport (~9%), fruiting body development (~7%), biological adhesion (~4%), proteolysis (~3%), and cell motility (~2.5%) [22] (Table 1). In addition, of the over 300 proteins identified, ~48% are involved in some sort of binding, while ~30% are homologous to known enzymes [22] (Table 1). Together, these findings have provided valuable new insight into the primary functions associated with ECM proteins in *Dictyostelium*.

Several proteins have been well established as ECM components in *Dictyostelium* including the structural proteins EcmA and EcmD, as well as discoidins I and II that are involved in cell adhesion [24–27] (Fig. 1). As anticipated, all of these proteins were detected in a proteomic analysis of the sheath ECM [22]. EcmA has a signal sequence and is composed of 66 CTDC domains (Pfam accession number: PF00526) (www.dictybase.org) (Fig. 2). CTDC domains are ~25 amino acid cysteine-rich domains that are unique to *Dictyostelium* and are found in a large number of *Dictyostelium* ECM proteins [28]. Proteins that harbor these domains usually have multiple copies. EcmD contains a