0014-4754/95/121189-08$1.50 + 0.20

The extracellular matrix of the *Dictyostelium discoideum* slug

M. R. Wilkins and K. L. Williams*

School of Biological Sciences, Macquarie University, Sydney, N.S.W. 2109 (Australia), Fax +61 2 850 8174

Abstract. In this review, we detail the current understanding of the extracellular matrix (ECM) of the migratory slug phase of the cellular slime mould, *Dictyostelium discoideum*. We describe some structural and non-structural molecules which comprise the ECM, and how these molecules reflect both plant and animal ECM systems. We also describe zones of the multicellular slug that are known to make ECM components, including the role of the prestalk cells and the slug epithelium-like layer. Finally, we review the contributions of studies on mutants to our understanding of the ECM of *D. discoideum*, and relate this to differentiation and development in more complex eukaryotic systems.

Key words. *Dictyostelium discoideum*; extracellular matrix; cellulose; cell-ECM interactions; slime; glycoproteins.

Introduction

A ubiquitous feature of multicellular organisms is the extracellular matrix (ECM). In animals the major components of this matrix include the fibrous element collagen, adhesive glycoproteins such as fibronectin and laminin, and a gel of proteoglycans and water[44]. In plants the ECM forms the cell wall, which is composed of a cellulosic framework, xyloglycans which interlock the cellulose, strengthening glycoproteins such as extensins, and a hydrated gel of pectic polysaccharides[11]. Extracellular matrices are also produced by some prokaryotes[30,40,41,48,71], but these are less well understood.

The cellular slime mould *Dictyostelium discoideum* is a simple eukaryote. In response to starvation, vegetative amoebae form aggregates of up to 100 000 cells, which in certain environmental conditions[4,46] form a migratory, multicellular organism known as a slug (fig. 1). The slug is partially differentiated simplistically into pre-stalk, and pre-spore cells[50], and contains an electron dense epithelium-like layer up to a few cells thick[22] (fig. 2). When migration ceases each slug culminates to form a ball of spores, held in the air by a stalk of vacuolated, cellulose-encased cells within a cellulosic stalk tube, and a basal disc also comprising cellulose-encased cells[51].

From late aggregation onwards, and shortly after the formation of the slug epithelium (S. Eliott, unpublished observation), the multicellular mass of *D. discoideum* is covered in a thin ECM of protein and cellulose[50,52,58]. During slug migration this ECM is continuously synthesized, forming the "slime sheath" through which the slug cells move[50,52]. The ECM is left behind as a collapsed tube or trail after the slug moves forward, providing a history of ECM synthesis and deposition (figs. 1 and 2). As such, it supplies clues into cell-ECM interactions that have occurred during slug movement[7,60,61]. For example, inspection of the ECM shows if a slug has been migrating in close apposition to the substratum, or if it has been migrating by raising itself completely above the substratum with each forward movement[60].

In this review, we present our view of the ECM of *D. discoideum*. We restrict discussion to the slug stage of the organism, as the ECMs of the spore mass and the stalk tube cells have been reviewed elsewhere[3,66,67], and while vegetative amoebae have been reported to secrete ECM material[5,49,52], nothing is known of this at the molecular level. After a brief historical perspective, we show that the *D. discoideum* ECM is unusual in having

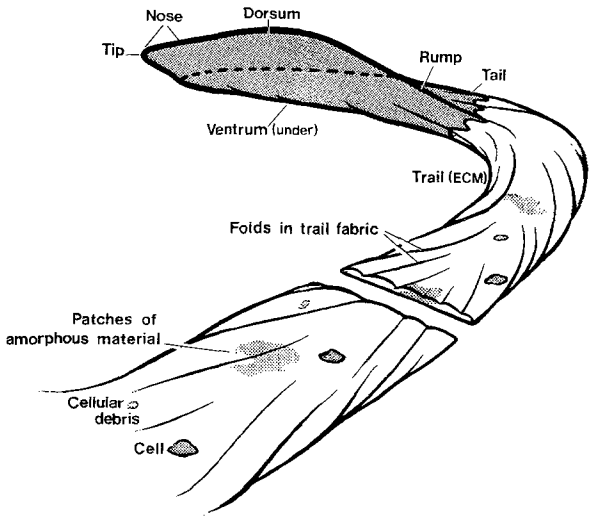

Figure 1. Cartoon of the *D. discoideum* slug and ECM morphology. The slug, which is about 1 mm long and 0.1 mm wide, is made of approximately 100 000 cells and shows a distinct tip, nose, dorsum and ventrum. The ECM is left behind the slug after migration, and is actually a collapsed tube.

* Corresponding author.

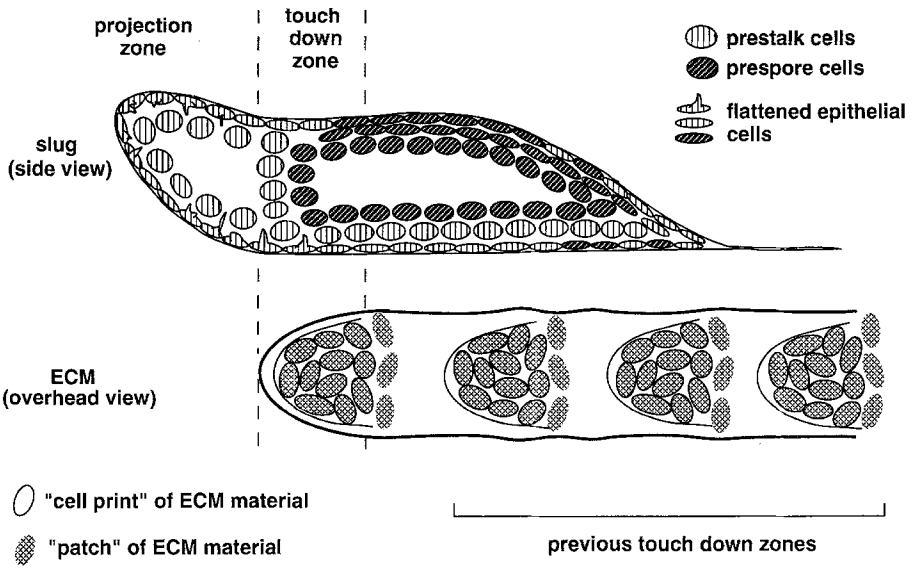

Figure 2. Detail of the *D. discoideum* slug and ECM. The slug shows an epithelium-like layer of flattened cells, a prestalk cell zone (slug tip and nose) and prespore cell zone (rear dorsal area). The ECM shows cell prints and patches in the touch down zones whereby cell prints are always found in association with patches, but patches can exist independently. The slug is approximately 1 mm in length, but cells and cell prints are not drawn to scale here.

compositional properties of both plant and animal systems, containing cellulose as well as proteins that have homology to animal extracellular proteins. We propose that the different classes of cells in the slug have well-defined roles in the synthesis of ECM molecules, and that this synthesis is strictly regulated. Finally we discuss studies on mutants which address the role that the ECM has in the *D. discoideum* slug, and relate this to the roles of ECMs in other systems.

A brief historical perspective of the *D. discoideum* ECM

Until recently, the widely-held view of the ECM of *D. discoideum* could best be summarised as "... a two-phase system with a fibrillar component, most likely cellulose, embedded in an amorphous, protein-containing matrix"[32]. Chemical studies on the ECM, whilst not always agreeing on the exact proportions of the components, were consistent with this view[19,20,54]. With the advent of the production of monoclonal antibodies to the *D. discoideum* ECM[27,63] and use of these in immunofluorescence microscopy experiments, it soon became apparent that the ECM was much more complex than previously thought[8,60,61]. Some proteins are found throughout the ECM and appear to form a structural framework[42,43,45], whilst other proteins are secreted only to specific zones of the ECM in a highly organised manner[8,60,79]. A further group of proteins appears to serve no structural role in the ECM, but may instead act as a lubricant for slug movement within the ECM[8]. Whilst this early view of cellulose was that it was present throughout the ECM, cellulose has recently been shown to be present only in particular zones[61,79].

These complexities of the ECM have impacted on our understanding of the slug of *D. discoideum*, how it may interact with the ECM, and how it functions as a multicellular organism.

What molecules comprise the ECM of the *D. discoideum* slug?

The ECM of *D. discoideum* is known to be a complex of cellulose, proteins, and polysaccharide[19,20,32,37,54]. The exact proportions of these constituents are unknown, as they can vary widely under different growth conditions[19], from strain to strain, and under different methods of ECM preparation[54]. The *D. discoideum* slug ECM is composed of structural (intrinsic) components and non-structural (extrinsic) components, in much the same manner as plant and animal ECMs[11,44]. The structural components form the meshwork of the ECM and can be further divided into generalised molecules that are found throughout the ECM, and molecules that are found only in specific zones. The non-structural components are proteins that are extracellular, yet are not fixed in the ECM. Molecules from each of these groups will be discussed in turn, and the potential functions of these molecules will be discussed in a later section.

Generalised structural molecules

Two generalised molecules that have been molecularly characterised in *D. discoideum* are the proteins EcmA and EcmB, encoded by the genes *ecmA* and *ecmB* (these were previously known as proteins ST430 and ST310, respectively encoded by the mRNAs pDd63 and pDd56[42,43]). These proteins are localised to both the slime sheath and the stalk tube with monoclonal or polyclonal antibodies[42,43]. EcmA is distributed throughout the slime sheath in a non-specific manner as an integral part of the ECM, and can be seen within prestalk cells as punctate labelling near the nucleus (M. Fuchs, unpublished observations). EcmB is largely confined to the stalk ECM rather than the ECM of the slug. The *ecmA* and *ecmB* genes are expressed only by prestalk cells of the slug[34], but show different temporal patterns of expression[33,72]. The *ecmA* gene is expressed maximally during slug formation and migration, whilst the *ecmB* gene is expressed maximally after slug migration, during the 'mexican hat' stage of culmination. The *ecmA* and *ecmB* genes have become the markers of choice for the prestalk cells of the slug.

The *ecmB* gene has been cloned and sequenced, and the *ecmA* gene has been cloned and partially sequenced[12,72], revealing that the primary structure of the EcmA and EcmB proteins is highly homologous[12]. Both proteins are largely composed of tandem arrays of a cysteine-rich, 24-amino acid repeat. EcmA contains approximately 70 copies of the repeat. EcmB contains 41 copies of the repeat, that can be further divided into type A and type B repeats which are mostly organised in an A A B pattern throughout the protein. Both EcmA and EcmB show putative secretion signal sequences, which is in accordance with their extracellular localization.

The proteins EcmA and EcmB therefore share features with other extracellular structural molecules, including collagens and fibronectins, showing extracellular localization and the presence of repeats[1]. Interestingly, the cysteine-rich repeat shared by EcmA and EcmB is now known to be homologous to domains of other extracellular proteins from *D. discoideum*, and animal-like organisms. The *D. discoideum* secreted cyclic nucleotide phosphodiesterase inhibitor contains internal repeats that show homology to the EcmA/B repeating unit[76], as does the available sequence of the ECM sheathin glycoproteins[78,79]. Similarly, the 170 kDa surface lectin of *Entamoeba histolytica* contains internal repeats that show homology with the repeating units of the *ecmA* and *ecmB* genes[59], as does the extracellular 12D3 protein from *Babesia bovis* (ref 75, and G. Harper, unpublished observations). These observations raise the possibility that all of the above proteins may share an ancestral repeat unit, or be derived from a single ancestral gene.

Specifically localised structural molecules

Molecules that have been found to be localised to specific regions of the *D. discoideum* ECM are cellulose and the sheathin glycoproteins. Cellulose has long been acknowledged to be part of the ECM, being first made in late aggregation[19,32,58], but more recently it has been shown to be found almost exclusively in specific areas of the ECM of the migrating slug[61,79]. The sheathins are a group of glycoproteins that colocalise with cellulose in the ECM[79].

Initially identified as proteins that are recognised by the monoclonal antibody MUD51[26], the sheathins can be detected by MUD51 in the ECM of the slug[61], but not within slug cells[2]. The sheathins are four oligomers of molecular mass 53, 55, 62 and 68 kDa, which are assembled from subunits of size 12, 31, 32 and 35 kDa[79]. Protein sequence analysis of the sheathins shows that the three main subunits (31, 32 and 35 kDa) are closely related, and that they show some homology to EcmA and EcmB. The sheathins have been defined as products of the genes *ecmC*, *ecmD* and *ecmE*. The sheathins are tightly but non-covalently associated with the ECM, requiring strong denaturing conditions for disruption. Treatment of the ECM with cellulase can also release the sheathins[27].

The sheathins and cellulose, visualised with the monoclonal antibody MUD51 and calcofluor respectively, are found in elliptical or U-shaped 'touch down zones', on the ventral side of the ECM[61,79] (figs. 2 and 3). Ellipses are formed by hurdling slugs that project for a considerable distance into the air with each forward movement, while U-shaped zones are formed by shuffling slugs[60] (fig. 3). The 'touch down zones' include 'cell prints', which refer to clear polygonal shapes that are approximately cell-sized and visualised by both MUD51 and calcofluor[61]. The 'touch down zones' also show 'patches', that are deposits of cellulose which are diffuse in nature, approximately the same size as 'cell prints' and often but not always found within the shapes defined by the 'cell prints' (fig. 2). These localization patterns are also observed with monoclonal antibody MUD50[8,60], which recognises a *modB*-dependent glycoconjugate common to the sheathins and other ECM glycoproteins (fig. 3)[2,13,26,78].

The creation of a 'touchdown zone' results from contact of the slug tip and newly synthesized ECM with the substratum[60]. As such, the deposition of the molecules comprising the 'touch-down zone' must occur over a very short time frame[9], probably less than one minute. This short time period available for secretion and print formation is impressive in the light of the fact that all cellulose must be extracellularly made de novo, and can not be an extension of existing cellulose fibers[35]. It is

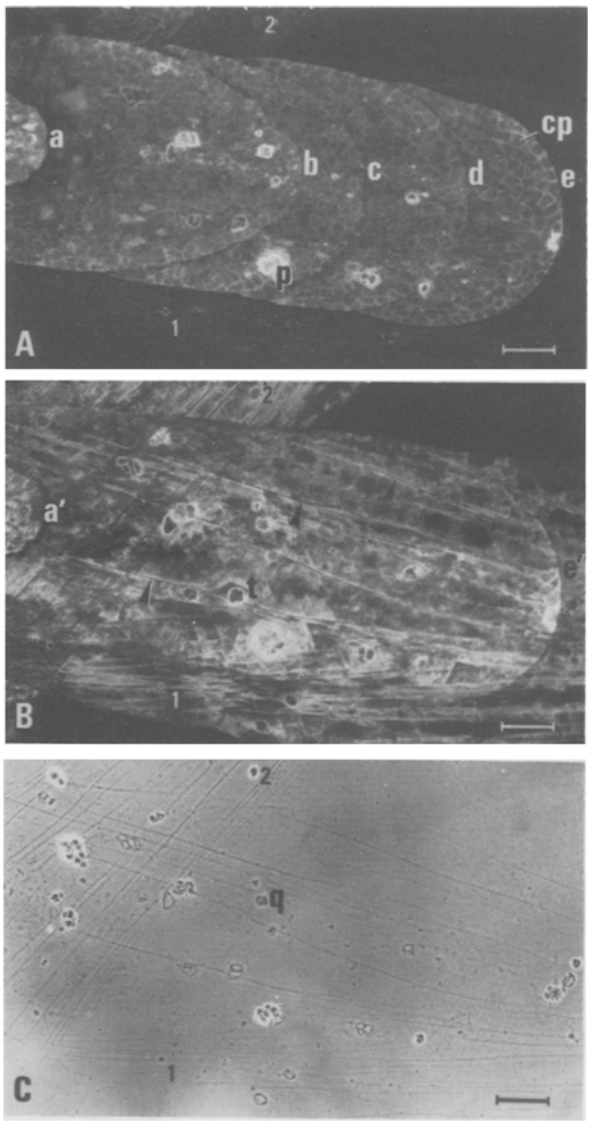

Figure 3. Different microscopic views of a single region of *D. discoideum* ECM. WS380B wild type ECMs from two slugs moving left to right and bottom to top respectively are shown overlapping here (#1 and #2). Bar = 50 μm. *A* Cell prints revealed with MUD50-FITC. Monoclonal antibody MUD50 reveals 'cell prints' (cp) in the ECM. The 'cell prints' occupy five clearly delineated 'touch down zones' (a–e). A prespore cell (p) can be seen inside the ECM. *B* MUD62-rhodamine reveals mobile proteins within the ECM. Monoclonal antibody MUD62 reveals proteins within the ECM, but no cell prints. The distribution is affected by longitudinal folds (black arrows) and parabolic folds (a', e') in the trail. Note that the borders of 'touch down zones' b,c,d in panel *A* have no counterpart in this panel or panel *C*. Stalk cells (t) are not labelled with MUD62, but displace the mobile MUD62 proteins, creating an area of no staining. *C*) Phase contrast view of the ECM. Folds in the trail are clearly visible, including a fold corresponding to e' (panel *B*). Cells appear with a bright halo, while debris and plaques (q) appear as dark areas without a halo.

likely that the ventral deposits of cellulose, sheathins and other MUD50 reactive proteins in part explains why the ventral ECM may be thicker than the dorsal ECM[21].

Non-structural ECM molecules

A group of glycoproteins that are recognised by the monoclonal antibody MUD62[8,27,61] are mobile within the ECM and hence are non-structural molecules. When examined by immunofluorescence microscopy, they are found inside the tube defined by the structural molecules of the ECM, accumulating along folds in the trail (fig. 3B). The identity of these proteins is currently unknown, but it is known that the MUD62 antibody and an equivalent antibody 83.5[68] recognise a *modC/D/E*-dependent O-linked carbohydrate epitope that is found on a number of spore coat proteins as well as a number of cysteine proteinases secreted by vegetative amoebae (refs 13, 68, and A. Champion, unpublished observations).

A second non-structural ECM substance that has recently been described is one that stains positively with Nessler's reagent[17]. This substance is manifested as clear outlines that conform to profiles of surface cells, as well as tiny globules over the extracellular surface of these cells. These outlines and globules are present over the entire surface of the slug, but are not evident on internal cells of the slug, the slime sheath in apposition with the slug, or the slime trail. Because of this, the Nessler-positive substance is unlikely to be related to any of the molecules described above, although it is possible that the substance is smeared into the slime trail as the slug migrates. As Nessler's reagent is used to detect ammonia, it is likely that the substance found is either polysaccharide, protein, or glycoprotein[17]. This may be related to the presence of a mucopolysaccharide in the sheath around the slug, which has been suggested elsewhere[23].

Where is the ECM produced in the slug?

There have been numerous theories formulated to explain the synthesis of the slug ECM. These theories have increased in complexity as our understanding of the nuances of the ECM and slug morphology has increased. Most simplistically, it has been proposed that all cells of the slug (being amoebae that are efficient secretors of proteins) may contribute to the ECM[5,49]. More recently, it was suggested that the ECM must be secreted by the outer layer of cells in the slug[37,65,70], although there has been conflicting evidence in defining whether all surface cells of the slug are involved in ECM synthesis. Radioisotope labelling experiments undertaken to show incorporation into the ECM by cells of the slug, and thus define the cells synthesizing the ECM, have been interpreted differently as to whether the incorporated label reflects synthesis or exchange of ECM components[37,65]. Therefore it is not clear what these experiments mean. Similarly, whilst one electron microscope study found that the ECM increased in thickness along the length of the slug[16], suggesting that the ECM

is continually synthesized by all cells of the slug surface, another study has shown that there is no apparent difference in the thickness of the sheath from anterior to posterior regions along the ventrum or dorsum of the slug (although the ventral ECM is slightly thicker than the dorsal ECM)[21].

We propose that the high degree of tissue differentiation found in the *D. discoideum* slug is reflected in a division of tasks with respect to the synthesis of specific components of the ECM. Prestalk cells from the anterior 20% of the slug[10], and prestalk-like cells (also known as anterior-like cells) are also found in some posterior regions of the slug[39,56,57,62]. The prestalk cells are a diverse group which can be divided into subclasses[73]. The importance of the prestalk cells is that they are almost certainly responsible for synthesis of the ECM components described above, including EcmA, EcmB, the sheathins and cellulose.

The expression patterns of the *ecmA* and *ecmB* genes have been well defined[34,73]. The expression of *ecmA* gene is restricted to the prestalk cells in the front 10% of the slug, as well as some prestalk-like cells found in the prespore zone of the slug. The *ecmB* gene, whilst also expressed in the front of the slug, seems to be predominantly seen later in culmination[73], and is less relevant to studies on the slug ECM. As EcmA is seen in the ECM surrounding the slug per se[21], the prestalk cells at the slug tip must be responsible for the majority of synthesis of the EcmA protein[42].

The genes encoding cellulose synthase and the sheathins are yet to be cloned in *D. discoideum*, so there is no information available to describe their mRNA expression patterns. But as cellulose and the sheathins are colocalised exclusively to the 'touch down zones' where the tip of the slug comes into apposition with the substratum[61,79], it is probable that prestalk cells are responsible for the synthesis of these molecules, and that they are made simultaneously. This is a logical prediction for cellulose, as the prestalk cells are responsible for stalk cell wall and stalk tube cellulose synthesis during fruiting body formation[24]. The monoclonal antibody MUD51, which identifies the sheathins, only recognises extracellular material, so the intracellular site of synthesis of sheathins is currently unknown[21].

Cellulose shows a complex synthesis pattern, as it can be made as 'cell prints' or as 'patches' (fig. 2)[61]. It is possible that these 'cell prints' and 'patches' correspond to the two modes of cellulose synthesis (DIF-dependent and DIF-independent) observed in isolated prestalk cells[3]. The 'cell prints' are similar to the intercellular cellulosic material that is initially synthesized in DIF-induced prestalk cultures, whilst the 'patches' are homologous to cell wall synthesis per se in the prestalk cell culture system. The contact-dependence of cellulose synthesis in the slug reflects a similar situation to the stalk tube, where only prestalk cells that are tightly appressed to the tube synthesize cellulose[24].

There is an epithelium like layer of cells that covers the entire slug[22]. These cells show a great abundance of rough endoplasmic reticulum, suggesting that they may produce a protein-rich secretion. We predict that the substances that are secreted by the epithelium-like cells will include those visualised by Nessler's reagent[17], as this is the only material described to date that is found over the entire surface of the slug. The epithelium-like cells will possibly also secrete the MUD62 reactive proteins. It is interesting to note that Nessler-positive substances are also found on the surface of culminating slugs[17], which may correlate with the presence of an electron-dense epithelium-like layer that has been recently described around the edge of fruiting bodies and culminating slugs (L. Blanton, unpublished observations).

The function of the ECM in *D. discoideum* – a genetic approach

The roles of three classes of ECM molecules have been studied either directly or indirectly using mutants. Two of these groups of molecules, the sheathins and cellulose, are localised within the ECM and are clearly involved in slug movement. The third molecule, EcmA, is a generalised structural protein which, while not involved in movement per se, has a number of effects on slug behaviour and differentiation.

Molecules involved in slug movement – sheathins

Genetic analysis of the role of sheathins has revealed the importance of the O-linked oligosaccharides attached to threonine residues in proline-rich domains[25,69]. A defect in the *modB* locus[38] leads to the failure to glycosylate these threonines[2,25,31]. The sheathins are present in the ECM of strains carrying the *modB* mutation[61,79] and cellulose is still synthesized[61], but the lack of the *modB* O-linked glycan leads to the slug effectively stalling during migration[7]. The shape of the slug is also somewhat altered, with a pronounced tip region that is quite distinct from the body of the slug. The key feature of a *modB* slug is its failure to gain traction on the subtratum. The tip projects in the air in a normal fashion, but the slug moves forward at approximately 10% of the wild-type speed[7], and the 'touch down zones' are distorted[61]. We propose that the failure to move forward in the *modB* mutant slug results from a failure to form the appropriate sheathin glycoprotein-cellulose complex, that in the wild-type slug is a rigid platform from which the cells of the slug project. In support of this, there is now evidence that *modB*-dependent O-sugars on the spore coat protein PsB bind or strongly interact with cellulose (ref 64, and S. Alexander, unpublished observations).

The above view is different from our earlier view that the 'touch down zone' and 'cell prints' are sites of cell traction which allow propulsion of the slug forward by a squeezing action of peripheral cells[74]. The finding that cells remain stationary for only a very short time at the 'touch down zone'[9] caused us to abandon this idea. We now view the 'touch down zone' in more structural terms. The elucidation of the formation of these structures as a result of cell-ECM-substratum contact remains one of the most interesting challenges for *D. discoideum* ECM researchers.

Molecules involved in slug movement – cellulose

From the above described studies, it is a clear prediction that a mutant lacking cellulose would not be able to form a motile slug. While there are no mutants described that are deficient in cellulose synthase activity, mutants have been isolated that show no or little UDP glucose pyrophosphorylase activity[15]. This enzyme produces UDP glucose, which is the precursor necessary for cellulose synthesis. The ECM of these mutants was shown not to have any urea/SDS insoluble material that contains cellulose, and whilst phenotypically normal slugs could be formed under the appropriate conditions, they could not migrate and soon lost their integrity. However it was noted that some aspects of development were able to proceed, as stage-specific enzymes were expressed and the yellow pigment found in spores was present in some cells. These data shows that cellulose plays a very important role in slug formation, maintenance of slug morphology, and migration.

ECM molecules with roles other than slug movement – EcmA

The development of techniques for homologous recombination[41] has meant that in principle any cloned gene can be deleted. This has been done for the *ecmA* gene, allowing a definition of the phenotype of strains lacking the EcmA protein[45]. EcmA is the only known generalised structural protein of the slug ECM, but is clearly not critical for slug movement as *ecmA* null mutants migrate in a normal fashion, showing normal aerial projection and speed. However, the ECM is weakened as cells sloughed off the slug can escape from the *ecmA* null mutant ECM; this does not occur in the wild type[45]. There are several phenotypes relating to developmental transitions and behaviour that are apparent in the *ecmA* mutant.

Two developmental transitions are altered in *ecmA* null slugs. Slugs normally develop by first making a tip region which commences migration, sometimes before the rear of the slug is properly formed[6]. The *ecmA* null mutants exhibit the phenotype of delayed posterior maturation, such that young slugs are elongated[45]. Another developmental transition delayed by lack of EcmA is the change from migration to fruiting modes of development[45,21].

The final *ecmA* mutant phenotype is intriguing. Slugs usually migrate in a bimodal fashion such that half the slugs approach a light source from the left, and the other half from the right[18]. In the case of the *ecmA* null mutant, slugs prefer to migrate towards light from the right only[21]. This handedness in movement is reminiscent of handedness in *rol-6* collagen mutants of *C. elegans*, where the nematode has a helical twist[36]. Slugs move in a spiral motion at the tip[53,55] and we interpret the *ecmA* null phototaxis phenotype to mean that whereas in wild type strains the slugs commence migrating with either left hand or right hand spirals, the null mutant almost exclusively chooses a right hand spiral.

The above examples of using mutants to probe aspects of the role of the ECM in slug movement provide new insights into what was once thought of as a conglomerate of cells, all moving individually[47]. It is now apparent that the 'simple' *D. discoideum* slug portrays many key features of all multicellular tissues.

How does the ECM of *D. discoideum* relate to other multicellular systems?

Much still remains to be discovered about the *D. discoideum* ECM. For example, as EcmA is not essential for ECM formation, the nature of the major structural component of the ECM remains an open question. Interestingly the modified amino acid hydroxyproline, exclusively associated with collagen domains in animal proteins, is present in small amounts in the *D. discoideum* ECM (M. Wilkins, unpublished observation), but a protein containing collagen domains has not been isolated to date.

Cell-ECM interactions appear to be involved in numerous multicellular and developmental events in *D. discoideum*. The mechanism for these interactions in *D. discoideum*, and how they relate to those in other multicellular systems, is currently unknown, but there are startling parallels to some animal systems. For example, cellular phenotype and ECM secretion has been shown to be dramatically influenced by the ECM in mammals. When differentiated epithelial cells are grown on collagen plates they develop into polarized epithelia and form a type IV collagen-containing basal lamina, but if suspended and grown in a collagen gel the cells become motile and fibroblast like, secreting a fibrillar type I collagen[28,29,80]. The ECM has also been shown to be involved in the regulation of symmetry in *Xenopus* development[77], whereby injection of Arg-Gly-Asp (RGD) peptides into the blastocoel ECM resulted in global randomisation of left/right symmetries. If the mechanisms of cell-ECM interactions are similar in *D. discoideum* and vertebrate systems, we would predict *D. discoideum* to have ECM receptors belonging to the

integrin family, interacting with RGD peptide binding sites on ECM proteins, and an internal coupling of the integrins to signal transduction pathways and the nucleus[1]. Alternatively, there may be other systems of cell-ECM interaction that are present.

The most interesting thing about the discoveries of the role of the *D. discoideum* ECM is that the phenotypes observed in mutants lacking particular molecules transcend single cell behaviour. *EcmA* mutants, *modB* mutants and strains lacking cellulose all impact on aspects of multicellularity. This provides exciting insights into the role of the ECM, which can complement traditional studies on plant or animal ECMs which are often based on in vitro systems of single cell-ECM interactions.

Acknowledgements. We thank Larry Blanton and Ira Feit for helpful discussions, and thank Phil Vardy for supplying figures 1 and 3 which appeared in his Ph.D thesis. MRW is the recipient of an Australian Postgraduate Research Award, and KLW acknowledges funding by Program Grant # A08700100 and Project Grant #A09330428 from the Australia Research Council for studies on the *D. discoideum* ECM.

1 Adams, J. C., and Watt, F. M., Regulation of development and differentiation by the extracellular matrix. Development *117* (1993) 1183–1198.
2 Alexander, S., Smith, E., Davis, L., Gooley, A. A., Por, S. B., Browne, L., and Williams, K. L., Characterization of an antigenically related family of cell-type specific proteins implicated in slug migration in *Dictyostelium discoideum*. Differentiation *38* (1988) 82–90.
3 Blanton, R. L., Prestalk cells in monolayer cultures exhibit two distinct modes of cellulose synthesis during stalk cell differentiation in *Dictyostelium*. Development *199* (1993) 703–710.
4 Bonner, J. T., Davidowski, T. A., Hsu, W.-L., Lapeyrolerie, D. A., and Suthers, H. L. B., The role of surface water and light on differentiation in the cellular slime molds. Differentiation *21* (1982) 123–126.
5 Bonner, J. T., Koontz, P. G., and Paton, D., Size in relation to the rate of migration in the slime mould. Mycologia *45* (1953) 235–240.
6 Breen, E. J., Eliott, S., Vardy, P. H., White, A., and Williams, K. L., Length regulation in the *Dictyostelium discoideum* slug is a late event. J. exp. Zool. *262* (1992) 299–306.
7 Breen, E. J., Vardy, P. H., and Williams, K. L., Movement of the multicellular slug stage of *Dictyostelium discoideum*: an analytical approach. Development *101* (1987) 313–321.
8 Breen, E. J., and Williams, K. L., Movement of the *Dictyostelium discoideum* slug: models, musings and images. Devl Genet. *9* (1988) 539–548.
9 Breen, E. J., and Williams, K. L., Optical flow analysis of the ventral cellular layer of the migrating *Dictyostelium discoideum* slug. Microbiology *140* (1994) 1241–1252.
10 Browne, L. H., and Williams, K. L., Gradients in the expression of cell surface glycoproteins in a simple tissue, the *Dictyostelium discoideum* slug. J. gen. Microbiol. *139* (1993) 847–853.
11 Carpita, N. C., and Gibeaut, D. M., Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J. *3* (1993) 1–30.
12 Ceccarelli, A., McRobbie, S. J., Jermyn, K. A., Duffy, K., Early, A., and Williams, J. G., Structural and functional characterisation of a *Dictyostelium* gene encoding a DIF inducible, prestalk-enriched mRNA sequence. Nucleic Acids Res. *15* (1987) 7463–7476.
13 Champion, A., Gooley, A. A., Callaghan, M., Carrin, M. I., Bernstein, R. L., Smith, E., and Williams, K. L., Immunodominant carbohydrate determinants in the multicellular stages of *Dictyostelium discoideum*. J. gen. Microbiol. *137* (1991) 2431–2438.
14 DeLozanne, A., and Spudich, J. A., Disruption of the *Dictyostelium* myosin heavy chain by homologous recombination. Science *236* (1987) 1086–1091.
15 Dimond, R. L., Farnsworth, P. A., and Loomis, W. F., Isolation and characterisation of mutations affecting UDPG pyrophosphorylase activity in *Dictyostelium discoideum*. Devl Biol. *50* (1976) 169–181.
16 Farnsworth, P. A., and Loomis, W. F., A gradient in the thickness of the surface sheath in pseudoplasmodia of *Dictyostelium discoideum*. Devl Biol. *46* (1975) 349–357.
17 Feit, I. N., Cell prints on the surface of the slug of *Dictyostelium discoideum*: a Nessler-positive matrix substance. Devl Biol. *164* (1994) 345–360.
18 Fisher, P. R., and Williams, K. L., Bidirectional phototaxis by *Dictyostelium discoideum* slugs. FEMS Microbiol. Lett. *12* (1981) 87–89.
19 Freeze, H., and Loomis, W. F., Isolation and characterisation of a component of the surface sheath of *Dictyostelium discoideum*. J. Biol. Chem. *252* (1977) 820–824.
20 Freeze, H., and Loomis, W. F., The role of the fibrillar component of the surface sheath in the morphogenesis of *Dictyostelium discoideum*. Devl Biol. *56* (1977) 184–194.
21 Fuchs, M., Cell interactions and the ECM of *Dictyostelium discoideum*. Ph.D. Thesis, Macquarie University, Sydney, Australia 1993.
22 Fuchs, M., Jones, M. K., and Williams, K. L., Characterisation of an epithelium-like layer of cells in the multicellular *Dictyostelium discoideum* slug. J. Cell Sci. *105* (1993) 243–253.
23 Garrod, D. R., Palmer, J. F., and Wolpert, L., Electrical properties of the slime mould grex. J. Embryol. expl Morph. *23* (1970) 311–322.
24 George, R. P., Hohl, H. R., and Raper, K. B., Ultrastructural development of stalk producing cells in *Dictyostelium discoideum*, a cellular slime mould. J. gen. Microbiol. *70* (1972) 477–489.
25 Gooley, A. A., Marschalek, R., and Williams, K. L., Size polymorphisms due to changes in the number of O-glycosylated tandem repeats in the *Dictyostelium discoideum* glycoprotein PsA. Genetics *130* (1992) 749–756.
26 Grant, W. N., Welker, D. L., and Williams, K. L., A polymorphic, prespore-specific surface glycoprotein present in the extracellular matrix of *Dictyostelium discoideum*. Molec. cell. Biol. *5* (1985) 2559–2566.
27 Grant, W. N., and Williams, K. L., Monoclonal antibody characterisation of the slime sheath: the extracellular matrix of *Dictyostelium discoideum*. EMBO J. *2* (1983) 935–940.
28 Greenburg, G., and Hay, E. D., Cytodifferentiation and tissue phenotype change during transformation of embryonic lens epithelium to mesenchyme-like cells in vitro. Devl Biol. *115* (1986) 363–379.
29 Greenburg, G., and Hay, E. D., Cytoskeleton and thyroglobulin expression change during transformation of thyroid epithelium to mesenchyme-like cells. Development *102* (1988) 605–622.
30 Harshey, R. M., Bees aren't the only ones – swarming in gram negative bacteria. Molec. Microbiol. *13* (1994) 389–394.
31 Haynes, P. A., Gooley, A. A., Ferguson, M. A., Redmond, J. W., and Williams, K. L., Post-translational modifications of the *Dictyostelium discoideum* glycoprotein PsA. Eur. J. Biochem. *216* (1993) 729–737.
32 Hohl, H., and Jehli, J., The presence of cellulose microfibrils in proteinaceous slime track of *Dictyostelium discoideum*. Arch. Mikrobiol. *92* (1973) 179–187.
33 Jermyn, K. A., Berks, M., Kay, R. R., and Williams, J. G., Two distinct classes of pre-stalk enriched mRNA sequences in *Dictyostelium discoideum*. Development *100* (1987) 745–755.
34 Jermyn, K. A., Duffy, K. T. I., and Williams, J. G., A new anatomy of the prestalk zone in *Dictyostelium*. Nature, Lond. *340* (1989) 144–146.
35 Jones, T. H. D., and Gupta, M., A protein inhibitor of cellulases in *Dictyostelium discoideum*. Biochem. biophys. Res. Commun. *102* (1981) 1310–1316.

36 Kramer, J. M., French, R. P., Park, E., and Johnson, J. J., The *Caenorhabditis elegans rol-6* gene, which interacts with the *sqt-1* collagen gene to determine organismal morphology, encodes a collagen. Molec cell. Biol. *10* (1990) 2081–2089.

37 Loomis, W. F., Role of the surface sheath in the control of morphogenesis in *D. discoideum*. Nature New Biol. *240* (1972) 6–9.

38 Loomis, W. F., Wheeler, S. A., Springer, W. R., and Barondes, S. H., Adhesion mutants of *Dictyostelium discoideum* lacking the saccharide determinant recognised by two adhesion-blocking monoclonal antibodies. Devl Biol. *109* (1985) 111–117.

39 MacWilliams, H. K., and David, C. N., Pattern formation in *Dictyostelium*, in: Microbial Development, Cold Spring Harbor Monograph 16, pp. 225–274. Eds. R. Losick and L. Shapiro. Cold Spring Harbor Laboratory Press, New York 1984.

40 Maresz-Babczyszyn J., and Sokalska, M., Immunogenic properties of slime produced by *Pseudomonas aeruginosa*. Arch. Immunol. Ther. Exp. *27* (1979) 585–589.

41 Martinez, J. P., Gil, M. L., Casanova, M., Rico, M., Sentandreu, R., and Ruiz-Herrera, J., Characterization of a proteinaceous extracellular coat synthesized by the 'slime' variant of *Neurospora crassa*, Archs Microbiol. *152* (1989) 25–32.

42 McRobbie, S. J., Jermyn, K. A., Duffy, K., Blight, K., and Williams, J. G., Two DIF-inducible, prestalk-specific mRNAs of *Dictyostelium* encode extracellular matrix proteins of the slug. Development *104* (1988) 275–284.

43 McRobbie, S. J., Tilly, R., Blight, K., Ceccarelli, A., and Williams, J. G., Identification and localization of proteins encoded by two DIF-inducible genes of *Dictyostelium*. Devl. Biol. *125* (1988) 59–63.

44 Morris, P. J., The developmental role of the ECM suggests a monophyletic origin of the kingdom animalia. Evolution *47* (1993) 152–165.

45 Morrison, A., Blanton, R. L., Grimson, M., Fuchs, M., Williams, K. L., and Williams, J., Disruption of the gene encoding the EcmA, extracellular matrix protein of *Dictyostelium*, alters slug morphology. Devl Biol. *163* (1994) 457–466.

46 Newell, P. C., Telser, A., and Sussman, M., Alternative developmental pathways determined by environmental conditions in the cellular slime mold *Dictyostelium discoideum*. J. Bacteriol. *100* (1969) 763–768.

47 Odell, G. M., and Bonner, J. T., How the *Dictyostelium discoideum* grex crawls. Phil. Trans. R. Soc. *B312* (1986) 487–525.

48 Omar, A. S., Weckesser, J., and Mayer, H., Different polysaccharides in the external layers (capsule and slime) of the cell envelope of *Rhodopseudomonas capsulata* Sp11. Archs Microbiol. *136* (1983) 291–296.

49 Paddock, R. B., The appearance of amoebach tracks in cultures of *Dcityostelium discoideum*. Science *188* (1953) 597–598.

50 Raper, K. B., Pseudoplasmodium formation and organisation in *Dictyostelium discoideum*. J. Elisha Mitchell Sci. Soc. *56* (1940) 241–282.

51 Raper, K. B., The Dictyostelids. Princeton University Press, New Jersey 1984.

52 Shaffer, B. M., Cell movement within aggregates of the slime mould *Dictyostelium discoideum*, revealed by surface markers. J. Embryol. exp. Morph. *13* (1965) 97–117.

53 Siegert, F., Weijer, C. J., Nomura, A., and Miike, H., A gradient method for the quantitative analysis of cell movement and tissue flow and its application to the analysis of multicellular *Dictyostelium* development. J. Cell Sci. *107* (1994) 97–104.

54 Smith, E., and Williams, K. L., Preparation of slime sheath from *Dictyostelium discoideum*. FEMS Microbiol. Lett. *6* (1979) 119–122.

55 Steinbock, O., Siegert, F., Mueller, S. C., and Weijer, C. J., Three-dimensional waves of excitation during *Dictyostelium* morphogenesis. Proc. natl Acad. Sci. USA *90* (1993) 7332–7335.

56 Sternfeld, J., and David, C. N., Cell sorting during pattern formation in *Dictyostelium*. Differentiation *20* (1981) 10–21.

57 Sternfeld, J., and David, C. N., Fate and regulation of anterior-like cells in *Dictyostelium* slugs. Devl Biol. *93* (1982) 111–118.

58 Sussman, M., The program of polysaccharide and disaccharide synthesis during development of *Dictyostelium discoideum*, in: Biochemistry of the Glycosidic Linkage, pp. 431–448. Eds. R. Piras and H. G. Pontis. Academic Press, New York 1972.

59 Tannich, E., Ebert, F., and Horstmann, R. D., Primary structure of the 170-kDa surface lectin of pathogenic *Entamoeba histolytica*. Proc. natl Acad. Sci. USA. *88* (1991) 1849–1853.

60 Vardy, P. H., Fisher, L. R., Smith, E., and Williams, K. L., Traction proteins in the extracellular matrix of *Dictyostelium discoideum* slugs. Nature, Lond. *320* (1986) 526–529.

61 Vardy, P. H., The extracellular matrix and locomotion in the *Dictyostelium discoideum* slug. Ph. D. Thesis, Macquarie University, Sydney, Australia 1992.

62 Voct, L., Krefft, M., Bruderlein, M., and Williams, K. L., Flow cytometer study of anterior-like cells in *Dictyostelium discoideum*. J. Cell Sci. *75* (1985) 423–435.

63 Wallace, J. S., Morrissey, J. H., and Newell, P. C., Monoclonal antibodies specific for stalk differentiation in *Dictyostelium discoideum*. Cell Differ. *14* (1984) 205–211.

64 Watson, N., Williams, K. L., and Alexander, S., A developmentally regulated glycoprotein complex from *Dictyostelium discoideum*. J. biol. Chem. *268* (1993) 22634–22641.

65 Watts, D. J., and Treffry, T. E., Incorporation of N-acetylglucosamine into the slime sheath of the cellular slime mould *Dictyostelium discoideum*, FEBS Lett. *52* (1975) 262–264.

66 West, C. M., and Erdos, G. W., Formation of the *Dictyostelium* spore coat. Devl Genet. *11* (1990) 492–506.

67 West, C. M., and Erdos, G. W., Incorporation of protein into spore coats is not cell autonomous in *Dictyostelium*. J. Cell Biol. *116* (1992) 1291–1300.

68 West, C. M., and Erdos, G. W., The expression of glycoproteins in the extracellular matrix of the cellular slime mould *Dictyostelium discoideum*. Cell Differ. *23* (1988) 1–16.

69 West, C. M., and Loomis, W. F., Absence of a carbohydrate modification does not affect the level of subcellular localisation of three membrane glycoproteins in *modB* mutants of *Dictyostelium discoideum*. J. biol. Chem. *260* (1985) 13803–13809.

70 Whitfield, F. E., The use of proteolytic and other enzymes in the separation of the slime mould grex. Expl Cell Res. *36* (1964) 62–72.

71 Wilkinson, B. J., Staphylococcal capsules and slime, in: Staphylococci and Staphylococcus Infections 2, pp. 481–523. Academic Press, London 1983.

72 Williams, J. G., Ceccarelli, A., McRobbie, S., Mahbubani, H., Kay, R. R., Early, A., Berks, M., and Jermyn, K. A., Direct induction of *Dictyostelium* prestalk gene expression by DIF provides evidence that DIF is a morphogen. Cell *49* (1987) 185–192.

73 Williams, J. G., and Jermyn, K. A., Cell sorting and positional differentiation during *Dictyostelium* morphogenesis, in: Cell-Cell Interactions in Early Development, pp. 261–272. Wiley-Liss, Inc. 1991.

74 Williams, K. L., Vardy, P. H., and Segel, C. A., Cell migrations during morphogenesis: some clues from the slug of *Dictyostelium discoideum*. BioEssays *5* (1986) 148–152.

75 Wright, I. G., Casu, R., Commins, M. A., Dalrymple, B. P., Gale, K. R., Goodger, B. V., Riddles, P. W., Waltisbuhl, D. J., and Abetz, I., The development of a recombinant *Babesia* vaccine. Vet. Path. *44* (1992) 3–13.

76 Wu, L., and Franke, J., A developmentally regulated and cAMP-repressible gene of *Dictyostelium discoideum*: cloning and expression of the gene encoding cyclic nucleotide phosphodiesterase inhibitor. Gene *91* (1990) 51–56.

77 Yost, H. J., Regulation of vertebrate left-right asymmetries by extracellular matrix. Nature, Lond. *357* (1992) 158–161.

78 Zhou-Chou, A. T., Characterisation of cell surface and ECM glycoproteins in *Dictyostelium discoideum*. Ph. D. Thesis, Macquarie University, Sydney, Australia.

79 Zhou-Chou, A. T., Wilkins, M. R., Vardy, P. H., Gooley, A. A., and Williams, K. L., Glycoprotein complexes interacting with cellulose in the 'cell print' zones of the *Dictyostelium discoideum* extracellular matrix. Devl Biol. *168* (1995) 332–341.

80 Zuk, A., Matlin, K. S., and Hay, E. D., Type I collagen gel induces Madin-Darby canine kidney cells to become fusiform in shape and lose apical-basal polarity. J. Cell Biol. *108* (1989) 903–920.