

## Movement of the multicellular slug stage of *Dictyostelium discoideum*: an analytical approach

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### Summary

Time-lapse video recordings of migrating multicellular slugs of *Dictyostelium discoideum* were subjected to image analysis. A transient 'collar-like' structure was identified at the anterior end of the slug. This collar remains stationary in the wild-type strain WS380B; it is observed shortly after the advancing tip contacts the substratum. Stationary collars formed approximately every 12 min; they were matched with patterns revealed on the underside of slime trails with FITC-coupled monoclonal antibody MUD50. It is proposed that stationary collars are involved with the

forward movement of the slug. The mutant strain HU2421 lacks the MUD50-epitope and forms collars which do not remain stationary but move backwards along the slug to collect at a 'waist' region. The slipping-collars observed in the mutant correlated with very slow migration rates. We propose that HU2421 moves slowly because it lacks traction.

Key words: *Dictyostelium discoideum*, movement, monoclonal antibodies, time-lapse filming, extracellular matrix, cell–substrate adhesion, motive force.

### Introduction

The cellular slime mould *Dictyostelium discoideum* is used as a model system to study various aspects of eukaryote development such as cell–cell contact (Bozzaro, 1985), timing of development (Soll, 1983), developmental gene regulation (Gomer *et al.* 1985) and pattern formation (Schaap, 1986). Recently several groups have focused on how the multicellular slug moves (Clark & Steck, 1979; Inouye & Takeuchi, 1979; Odell & Bonner, 1986; Williams, Vardy & Segel, 1986). Early studies established that the front of the slug is the sensitive region for turns (Raper, 1940; Francis, 1962; Poff & Loomis, 1973; Fisher, Dohrmann & Williams, 1984) and that this region provides a greater motive force than other slug regions (Inouye & Takeuchi, 1980). Two groups have started to examine how the collective actions of 100 000 amoebae within the slug become coordinated to move the slug (Odell & Bonner, 1986; Williams *et al.* 1986).

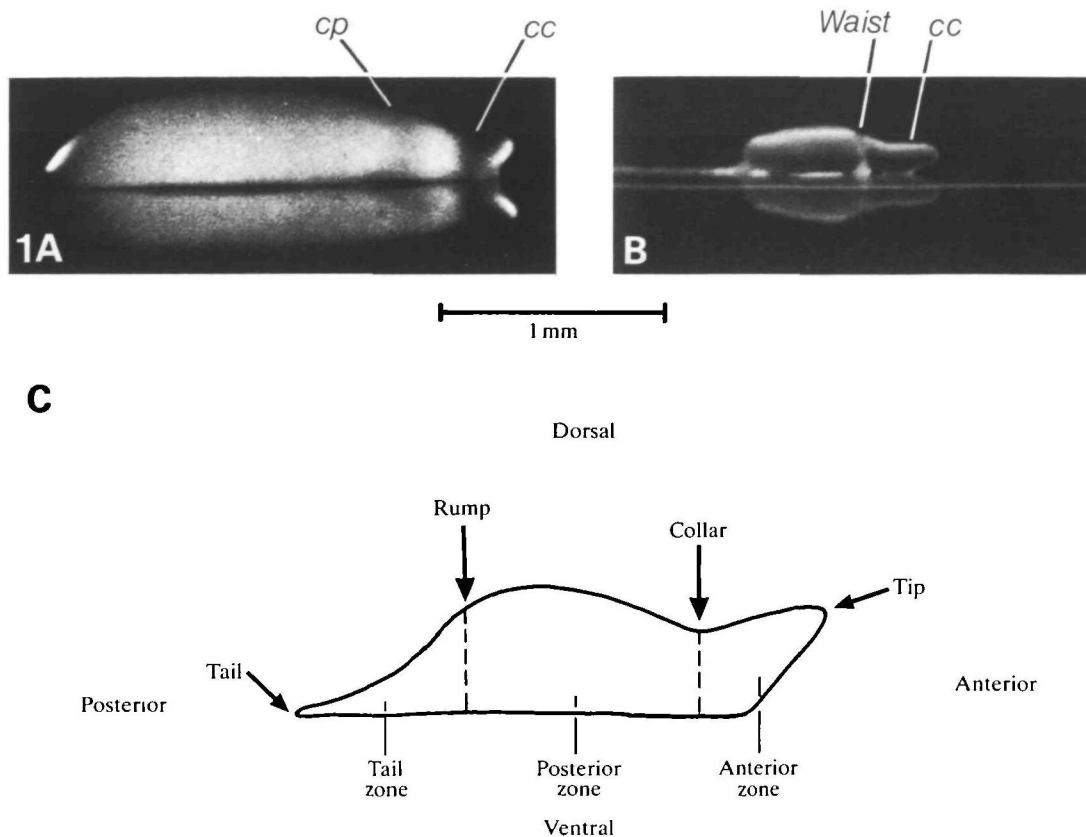
The amoebae comprising a *D. discoideum* slug migrate through a continuously produced extracellular matrix, which is left behind as a trail (Raper, 1940). This sheath encloses the entire slug and is stationary with respect to the substratum as the slug

amoebae advance (Bonner, 1967), except at the anterior where the sheath is thin and pliable (Raper, 1941; Francis, 1962; Loomis, 1972; Farnsworth & Loomis, 1975). The advancing slug leaves distinctive patterns in its trailing sheath. We have argued elsewhere (Vardy, Fisher, Smith & Williams, 1986) that these patterns, revealed by treatment with a monoclonal antibody MUD50, provide clues to how the slug migrates. In particular, we have proposed that there is a stationary component to slug movement (Williams *et al.* 1986). In this report, we film migrating slugs from the side rather than from above and thus observe the interaction between the slug and the substratum. Image analysis of the time-lapse video records is used here to demonstrate that there is a stationary part of the slug which may be involved in its adhesion to the substratum.

### Materials and methods

#### Strains

Two strains of *D. discoideum* were used in these experiments: a wild-type strain WS380B (Erdos, Raper & Vogen, 1973) and a mutant HU2421 which carries the *modB501* mutation (West & Loomis, 1985) in the genetic background of strain WS380B (Gooley & Williams, unpublished data).



**Fig. 1.** Video records of slug images. Single frames from a video cassette were captured on a Mitsubishi video copy processor P6OU(T). (A) Mature wild-type WS380B slug showing two collars: *cc*, current collar and *cp*, previous collar. (B) Mature mutant HU2421 slug showing prominent 'waist' and less-prominent current collar (*cc*); see text. (C) Schematic representation of slug, defining anterior, posterior and tail zones.

Slugs were prepared by scraping amoebae and bacteria (*Klebsiella aerogenes*) from a SM nutrient agar plate (Sussman, 1966) with a toothpick and placing them to one side in a Petri dish containing water agar (1.5% w/v Bacto agar and 250  $\mu\text{g ml}^{-1}$  dihydrostreptomycin sulphate). Each water agar plate was enclosed within a black PVC container with a 3 mm hole in the side opposite the amoebae. PVC containers were incubated at  $21 \pm 1^\circ\text{C}$  in an illuminated room. Slugs formed after approximately 24 h and migrated across the plate towards the light.

#### Conditions for time-lapse recording

Under subdued light a block of agar bearing a single slug, which had migrated for 2–7 cm towards the light, was cut from a plate of migrating slugs. The block was then mounted on the stage of a Zeiss (Oberkochen) dark-field/light-field stereomicroscope illuminator. A Schott KL 1500 lamp fitted with a Kodak long pass filter (red; greater than 600 nm) illuminated the slug from below. The stage was enclosed in an opaque plastic container with a front viewing port (40×20 mm) and (at 90° to this on the same level) a side light port 3 mm in diameter. A fibre-optic white-light source (3 mm diameter) was connected to this port to orientate the slug. The entire inner surface of the opaque plastic container was lined with moistened black filter paper (Schleicher & Schüll, no. 508) and a small moist

U-shaped sponge surrounded the agar block on three sides. This prevented desiccation of the slug.

#### Time-lapse video recording

A Zeiss OPM1 microscope with a Zeiss f 150 mm lens (×2.5) was focused on to the side of the slug through the viewing port. A Sanyo VC 3300S video camera captured the image, which was illuminated from below with red light. The movements of migrating slugs were recorded on a National time-lapse cassette recorder NV-8051, fitted with a Panasonic/National time/date generator NV-F85, set to a compression ratio of 1/40 real time.

#### Modelling *D. discoideum* slug shape

To obtain data from the video records, each slug was abstracted into a two-dimensional representation consisting of three zones: anterior, posterior and tail (Fig. 1C). These zones were bounded by the following marker points.

- (1) Tip – the anterior extremity of the slug.
- (2) Collar – a girdle surrounding an anterior region of the slug, which is most readily visualized as a saddle on the anterodorsal surface.
- (3) Rump – the posterior extremity of the dorsum of the slug.

(4) Tail – the posterior extremity of the slug *per se* (on the substratum).

The whole slug outline was digitized and stored in the computer as outlined below. In addition, the x,y coordinates of the four slug features described above were collected in data files.

#### *Data extraction and management*

Slug images were cabled from the video recorder *via* a Crestwood Video Arrowhead Indicator VS-100 to a Javelin video monitor. Typically, a slug image occupied 10–30 % of the monitor screen width, depending on the size of the slug. Slug images were calibrated by placing a scale in the field of focus.

The Crestwood Video Arrowhead Indicator was interfaced to a Summagraphics Intelligent Digitizer Series 2000 which in turn was linked to a VAX mainframe computer. The coordinates of slug position and slug shape were digitized directly from the Javelin video monitor *via* the video arrow indicator and directed to the computer for analysis and storage on disk. This process was repeated for video frames representing 30 s intervals (real time) so that a sequential time series was built up in the computer for each slug.

A data base program (which can be obtained on request from E. Breen) was designed specifically to store and retrieve slug coordinates sent to the VAX computer from the digitizer. The data base was written in the C programming language (Kernighan & Ritchie, 1978) and used the SCOPE technique of manipulating data and indexed files (Claybrook, 1983). The analysis of slug images was abstracted into C data structures (Pugh, 1986) which were then stored on disk and internally referenced as frames of information. All frames were double-linked together into a double-linked list (Sobelman & Kregelberg, 1985). Usually, a double-linked list represented 50–70 min of movement for each slug. All double-linked lists were controlled and accessed through separate header structures (Sobelman & Kregelberg, 1985).

#### *Monoclonal antibody treatment of slime trails*

After each video recording session, the agar block on which newly deposited trail had been laid down was overlaid with a microscope slide. The trail adhered to the slide and hence its underside was exposed when the slide was lifted from the block. Treatment with monoclonal antibody MUD50 tissue culture supernatant and FITC-coupled second antibody was as described previously (Vardy *et al.* 1986).

## Results

#### *Time-lapse recordings of moving slugs*

The easiest way to make time-lapse recordings of migrating slugs is to mount a camera on a microscope and to record from above. This approach was not satisfactory here because we wanted to study the interaction between the underneath of the slug and the substratum, which is obscured when recording from above. By using a microscope and camera

mounted on its side at the level of the slug, we were able to record slug–substratum interaction as the slug migrated across the field of view towards a side light source. This produced side-on images which clearly displayed the attitude of the slug to the substratum, particularly at the tip region (Fig. 1A,B).

Initial experiments were unsuccessful as slugs became desiccated and/or ceased moving and culminated to form fruiting bodies. The combination of humidity control and illumination from beneath with red light and from the side with white light caused slugs to migrate in a relatively straight line for periods up to 4 h. The behaviour of 60 slugs (49 WS380B and 11 HU2421) aged 1 to 3 days was recorded in this study. Of these, eight slugs were analysed in detail (six WS380B and two HU2421); data for three slugs are presented here. Although data for a small group of slugs are presented, we are confident that we are reporting general phenomena as our own and other films of *D. discoideum* slug movement (e.g. BBC Horizon program (1984) 'Professor Bonner and the Cellular Slime Moulds') exhibit the novel features to be described here.

#### *Slug shape and movement*

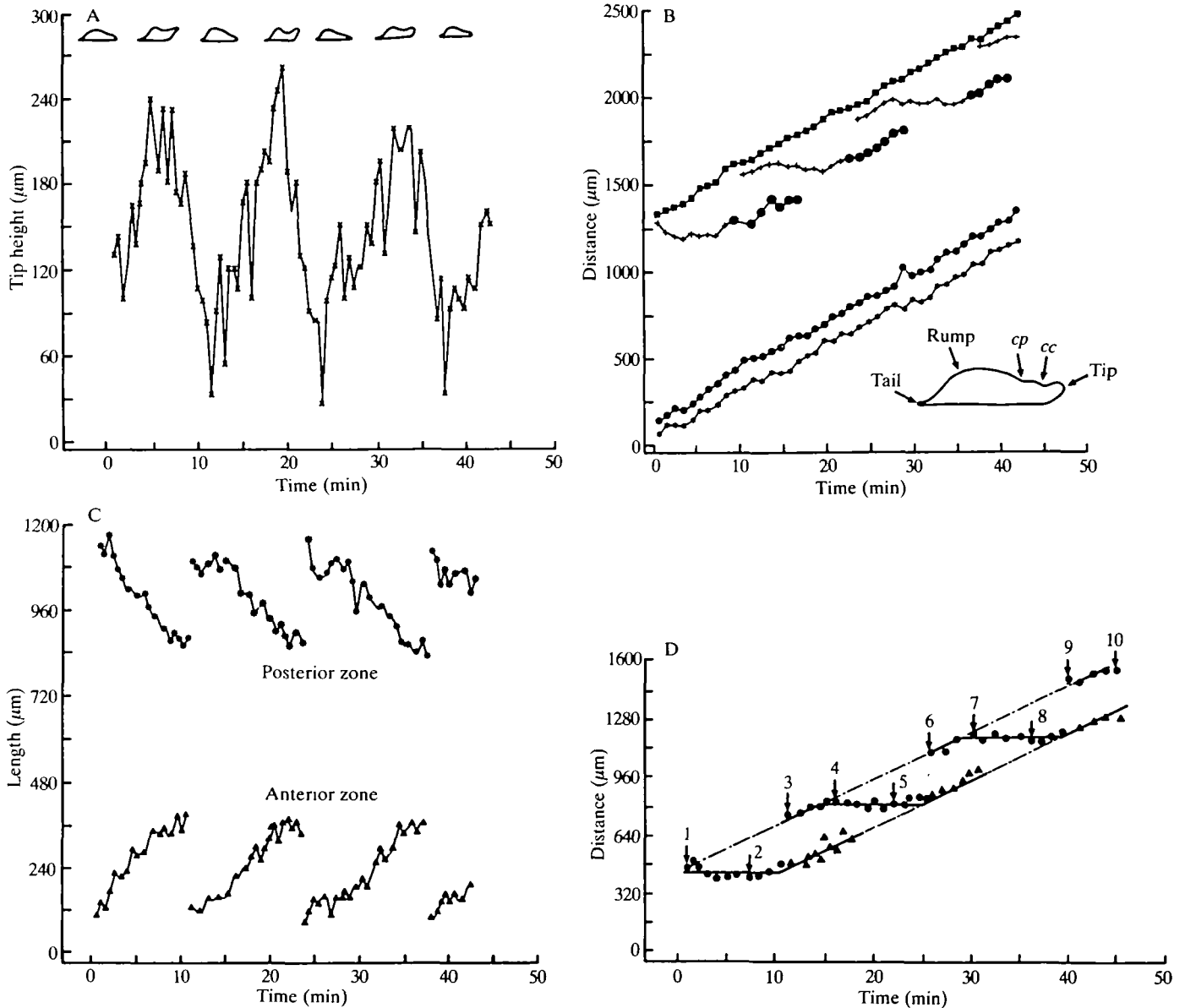
The tip, rump and tail (Fig. 1) are obvious features in both mature wild-type WS380B (Fig. 1A) and mutant HU2421 (Fig. 1B) slugs. To analyse slug movement, the features indicated in Fig. 1C were accumulated in the VAX computer for each slug at 30 s intervals of real time (Materials and methods). Manipulation of files allowed the construction of figures showing changes which occurred with time. For example, it was immediately apparent that slugs advance by periodically projecting their tips up and down (Fig. 2A); in strain WS380B a cycle of raising, projecting and lowering the tip is repeated approximately every 12 min. The slug illustrated raised its tip to a height approximately 20 % of its length (Fig. 2A). Mature wild-type slugs move at approximately constant speeds (e.g.  $30 \mu\text{m min}^{-1}$ , Fig. 2B), with tip, rump and tail moving in a coordinated fashion so that slug length remains relatively constant (Fig. 2B).

#### *The collar, a stationary feature of the migrating wild-type slug*

In addition to tip, rump and tail, another feature which we call the 'collar' was observed in migrating slugs. This loop-like structure was apparent as an indentation extending around the slug perpendicular to its long axis slightly behind the tip (Fig. 1A; cc). In some slugs, two or even three collars were detectable. No doubt further morphological features of slugs could be described, but only two are considered in this report: we use the phrase 'current collar' (cc) to

identify the most anterior structure and the phrase 'previous collar' (*cp*) to identify that collar posterior to the *cc* (Fig. 1A). In the mutant strain, previous collars accumulate in another structure called the 'waist' which will be discussed shortly (Fig. 1B).

Fig. 2B shows that unlike the tip, rump and tail, which move at relatively constant speeds, the collar of wild-type WS380B is markedly different. The figure shows a 'slip-stick-slip' profile in which the collar moves at the same speed as the tip initially, becomes



**Fig. 2.** Movement profiles for a mature WS380B slug approx. 1.3 mm long, moving at  $28 \mu\text{m min}^{-1}$ . (A) Height of tip recorded at 30 s intervals during migration. The illustrations depict the attitude of the slug at different times. Zero on the ordinate represents the level of the substratum. (B) Displacement over time of different slug parts (see Fig. 1C). At one minute intervals distance was measured from an arbitrary reference point. Distance to tip ■, rump ●, tail \* and collar + ⊕. The uncircled collar points refer to current collars and those circled refer to previous collars (see Fig. 1). Note that each new collar (*cc*) appears before the previous collar (*cp*) disappears. The illustration summarizes the regions of the slug measured. (C) Relative changes in length of posterior ★ and anterior ▲ zones at 30 s intervals.

The boundary between anterior and posterior zones is defined as a vertical line drawn through the current collar saddle point (Fig. 1C). The zone length is the distance from this line along the x axis to the tip (anterior) or tail (posterior) point. Note the inverse relationship between zone lengths. (D) Idealized collar movement profile. The collar profiles from B are replotted with additional time point measurements and with lines of slope  $30 \mu\text{m min}^{-1}$  (slug speed) joined by horizontal lines (indicating stationary collar). Note that current collars (●) form before the previous collars (▲) disappear. Points 1–10 correspond to slug images reproduced in Fig. 3D.

stationary with respect to the substratum (and therefore moves backwards along the slug with respect to its tip, Fig. 2C) for approx. 12 min and finally recommences movement at the same speed as the tip, until it disappears. This movement profile is idealized in Fig. 2D. Some variation is seen in this pattern in different films. In particular, sometimes there is no initial slip phase: the collar becomes stationary with respect to the substratum as soon as it is observed. However, during normal movement of WS380B slugs, there is always a stationary component followed by movement of the collar before it disappears. Fig. 2C shows the changes in length of anterior and posterior slug zones which reflect the movement of slug cells through the stationary collar. The relative lengths of the zones indicate that collars are anterior phenomena; anterior zones always remain shorter than the posterior zones (Fig. 2C).

When the collar becomes stationary, it increases in circumference as the cells from the rear of the slug move through it. The collar also becomes less prominent as an indentation until it becomes difficult to detect (Fig. 1A). Before the collar disappears however, a new collar (Fig. 1A, *cc*) appears anterior to the previous collar (*cp*). For WS380B slugs, previous collars can be observed for several minutes after the formation of new collars (Fig. 2B).

Fig. 2 shows different aspects of a single slug movement profile. From a comparison of Fig. 2A and B it is clear that the collar is formed immediately after the tip is lowered on to the substratum and that the collar becomes stationary as the tip is projected upwards and forwards.

#### *Movement and trail patterns*

A distinctive feature of the interaction between the slug and its substratum is revealed by treating the slime trail of *D. discoideum* with FITC-labelled monoclonal antibody MUD50; 'footprint-like' patterns are revealed in the trail (Vardy *et al.* 1986; Fig. 3A,B,C). We became interested in a possible relationship between the spacing of these MUD50 'footprints' and the appearance of collars. The movement profiles for the slug shown in Fig. 2 were matched with 'footprints' revealed in its trail by MUD50 treatment (Fig. 3C).

Fig. 3D shows the shape and position of the slug at various stages during its migration. Slug images 1–10 were selected because they correspond with significant points on the collar movement profiles in Fig. 2D. Two types of pattern are observed in the MUD50-treated trail: single 'footprints' (Fig. 3A) and multiple 'footprints' (Fig. 3B). Multiple 'footprints' are believed to be caused by the anterior zone (Fig. 1C) of the slug 'rolling' forward for several minutes before the tip is elevated. Note that the

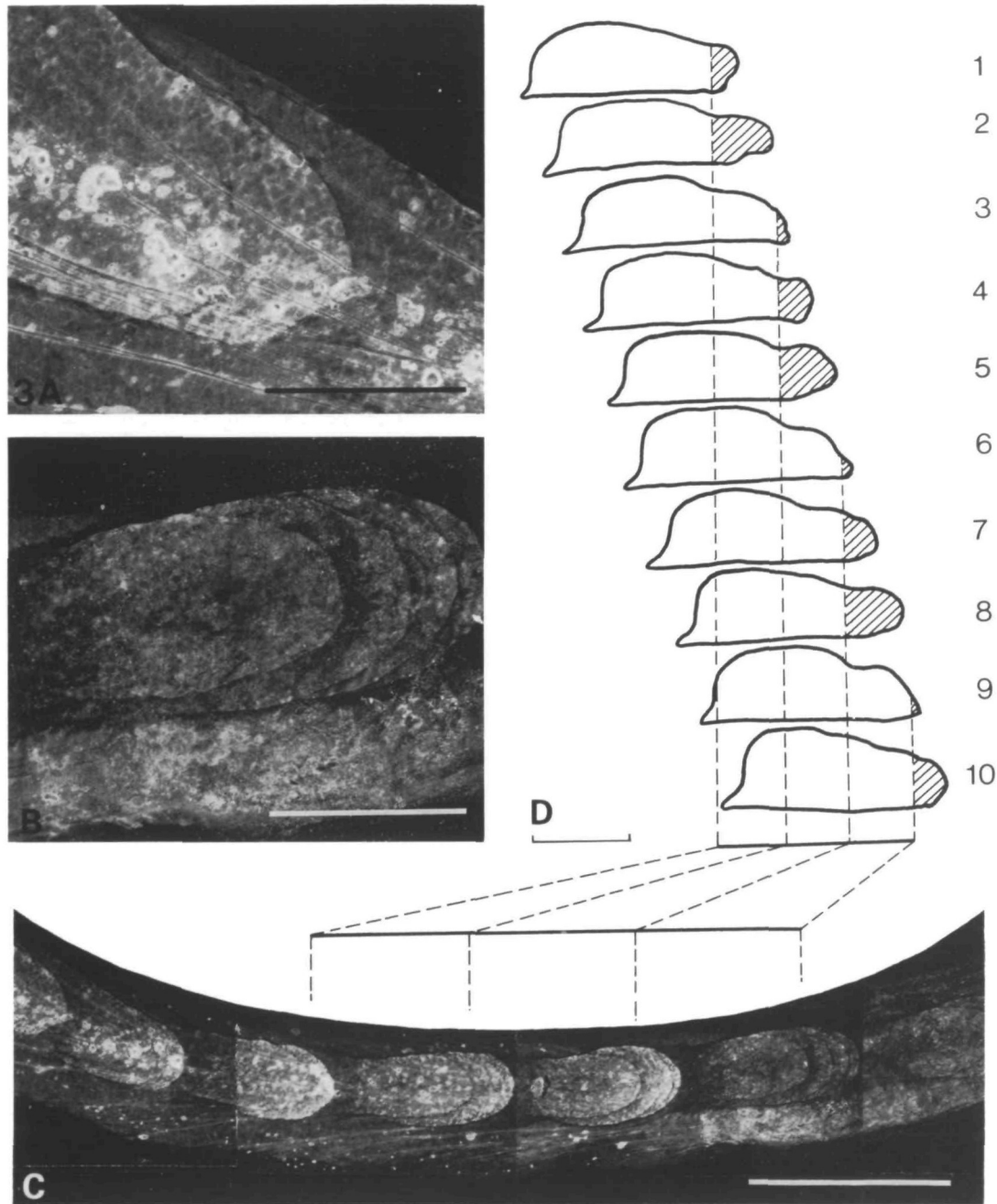
multiple 'footprints' in Fig. 3C correspond with the initial 'slip' phases of the collar profiles (Fig. 2D). Note also that the position of MUD50 patterns within the slime trail (Fig. 3C) matches with the appearance of the collar depression (Fig. 3D).

#### *Analysis of strain HU2421 which carries a modB mutation*

Recently, it has been shown that strains carrying the glycosylation defective mutation *modB* (West & Loomis, 1985) lack the epitope recognized by monoclonal antibody MUD50 (Alexander *et al.* 1987). Since the MUD50 epitope is implicated in slug movement, it was decided to study the movement of a strain carrying the *modB501* mutation. However, this mutation was isolated in strain AX3, which itself migrates poorly (Smith & Williams, 1980). We moved the *modB501* from strain DL118 into the genetic background of strain WS380B (A. Gooley & K. L. Williams, unpublished data) which has excellent migration characteristics. Several strains were constructed, all of which exhibit similar migration to strain HU2421, which is described in detail here.

Slugs of strain HU2421 are smaller than the parent strain WS380B (compare Fig. 1A,B) and they migrate for relatively short distances. The slugs often divide in two because the anterior forms two tips. Phototaxis of HU2421 is markedly bimodal (see Fisher & Williams (1981) for a discussion of bimodal phototaxis). Normal fruiting bodies are formed. Two features of HU2421 are particularly notable in relation to this study: mature HU2421 slugs migrate slowly (approx.  $2 \mu\text{m min}^{-1}$ ) and have a characteristic shape. A boundary is clearly evident between the anterior and posterior regions (Fig. 1B). We use the word 'waist' to describe this demarcation between the anterior projection and the rounded body.

Although HU2421 migrates slowly, the movement of tip, rump and tail are coordinated as they are in WS380B (Fig. 2B). Collars are formed every 12–15 min in HU2421 (Fig. 4A) which is comparable to that of WS380B (Fig. 2B). Collars are less obvious in HU2421 than in WS380B (compare Fig. 1A,B). However, movement profiles for the collars are quite different: in WS380B, the collars are either stationary with respect to the substratum or move *forward* at the speed of the slug; in HU2421, collars move *backwards* with respect to the substratum. This backward displacement proceeds at approx.  $16 \mu\text{m min}^{-1}$ , until the collar reaches the 'waist'. The position of the 'waist' remains constant with respect to the tip and tail (Fig. 4A); therefore it moves forward at the speed of the slug. Collars are not observed to move back beyond this structure.



**Fig. 3.** Movement and the slime trail as revealed by MUD50 treatment. (A) Enlargement from early part of slime trail (C). A single line defines the anterior lateral border of the pattern. Within the pattern, 'cell prints' are clearly evident (Vardy *et al.* 1986). Bar, 150  $\mu\text{m}$ . (B) Enlargement from latter part of slime trail (C). The pattern is composed of a short irregular series of crescentic lines. The width of the pattern corresponds to the width of the area of contact between the anterior of the slug and substratum. The patterns were laid down from left to right. Bar represents 150  $\mu\text{m}$ . (C) Slime trail laid down by the mature WS380B slug as it produced the movement profiles shown in Fig. 2. The slug migrated from left to right. The width of the trail corresponds to the maximum width of the slug. Bar, 500  $\mu\text{m}$ . (D) Slug images reconstructed from data records at various times during migration (see Fig. 2D). Vertical lines indicate collar positions; the hatched area is the anterior zone. Note that the collar position matches patterns in the slime trail. Bar, 500  $\mu\text{m}$ .

*Relationship between collar behaviour and slug speed in WS380B*

Adenosine has been reported to affect the distribution of cells within slugs (Schaap & Wang, 1986).

Therefore, we studied the movement of WS380B slugs on water agar containing adenosine. These findings are not reported here, but Fig. 4B shows serendipitous results in which a dramatic change of

speed was captured on video of a WS380B slug moving on water agar containing 5 mM-adenosine. At the start of the video recording, the slug migrated slowly ( $12 \mu\text{m min}^{-1}$ ), but after 25 min the slug commenced moving at normal speed ( $30 \mu\text{m min}^{-1}$ ). This change of speed was coordinated in that the tip and rump changed at the same time. However, the change

in speed of the tail lagged by approx. 7 min (arrows Fig. 4B).

The most striking feature of the change in speed involved the collar region. When the slug was moving slowly, the collars moved backwards along the slug at a speed faster than the slug moved forward (i.e. they moved backwards with respect to the substratum).

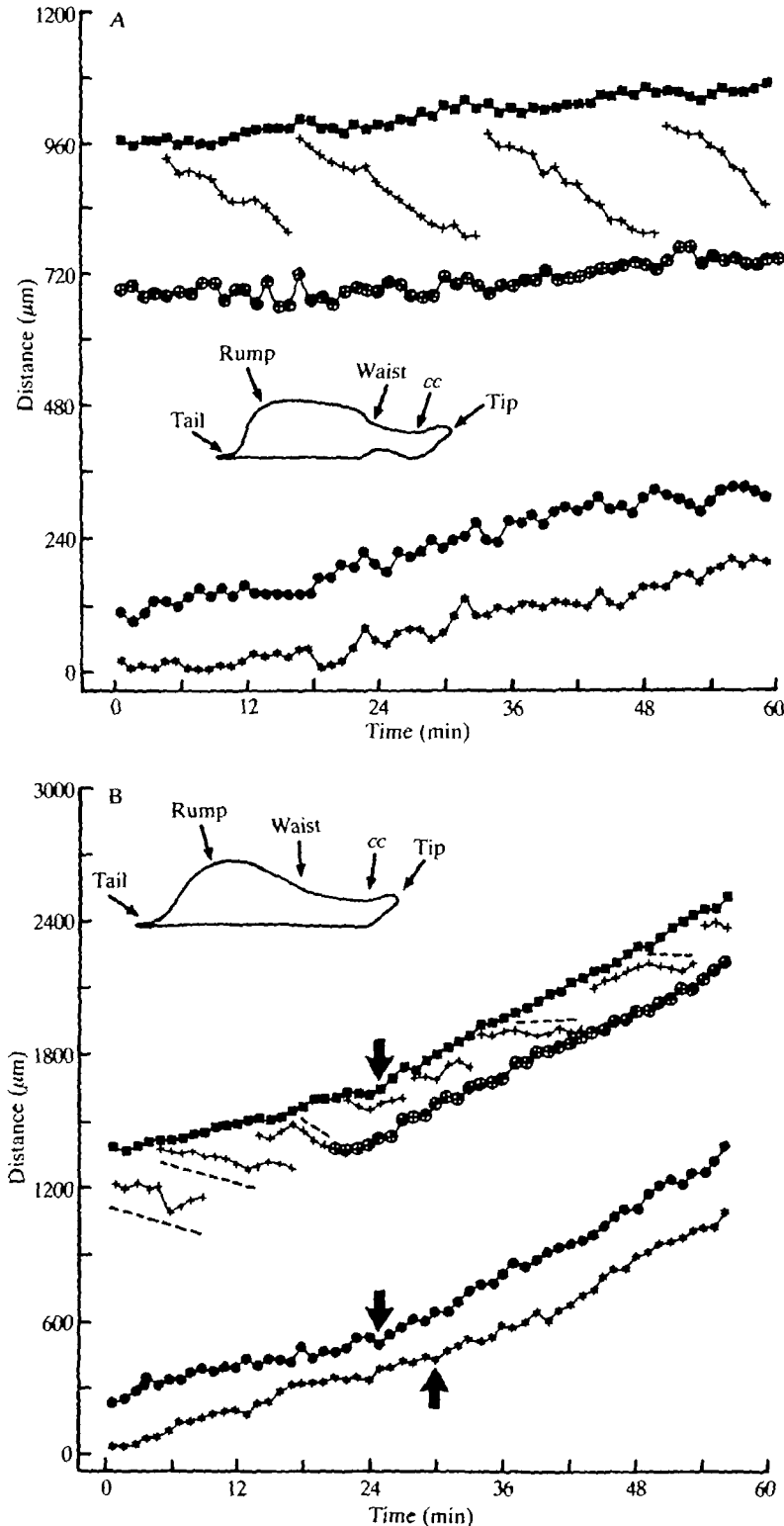


Fig. 4. Movement profiles for the mutant HU2421 migrating on water agar and the wild-type WS380B migrating on adenosine water agar. (A) Movement profile for HU2421; slug velocity  $2 \mu\text{m min}^{-1}$ , slug length 1 mm. The 'waist' is a prominent feature of HU2421 (see illustration and Fig. 1B); the 'waist' advances at approx. the same speed as the slug. Collars (cc) move backwards with respect to the substratum at approx.  $16 \mu\text{m min}^{-1}$  and can be observed to merge with the 'waist'. It was not possible to determine when the collar merged with the 'waist', thus the lower ends of the collar profiles do not join with the 'waist'. Distance of tip ■, collar +, waist ⊕, rump ● and tail \*, from an arbitrary starting point. (B) Movement profile of WS380B slug formed on water agar and then transferred to a 5 mM-adenosine water agar plate and allowed to migrate for 18 h (Breen & Williams, in preparation). Large arrows indicate points where the speed of tip, rump and tail increase from  $12 \mu\text{m min}^{-1}$  to  $30 \mu\text{m min}^{-1}$ . Note the change in collar behaviour (cc) which accompanies this change in speed and the appearance of a 'waist' (see text). The speed of the tail appears to increase some time after the simultaneous change in speed of the rump and tail. Distance to tip ■, collar +, waist ⊕, rump ● and tail \*, from an arbitrary starting point. Dashed lines indicate backwardly moving and stationary collars.

After a confused period when the speed was changing, the collars ceased to move backwards with respect to the substratum and became stationary as is normally seen with WS380B (compare Figs 4B, 2B). An unusual feature of WS380B migrating on 5 mM-adenosine was the existence of a 'waist' similar to that seen in mutant HU2421 (Fig. 4B).

## Discussion

Simplistically, it might be assumed that the small multicellular *D. discoideum* slug moved by the combined action of its 100 000 individual mobile amoebae. However, it is clear that amoebae above the layer in contact with the substratum have severe problems gaining traction. Different models of slug movement have been published recently. In the differential cell flow model (Odell & Bonner, 1986), it is proposed that slugs gain no traction on the sheath during migration, and that slug movement is brought about by interactions between the amoebae. In the squeeze-pull model (Williams *et al.* 1986), it is proposed that amoebae gain traction on the substratum and that some cells in the slug are specialized for locomotion. It is proposed that slug migration is brought about by peripheral cells contracting circumferentially to squeeze forward the anterior and longitudinally to draw up the posterior.

The squeeze-pull model predicts the existence of discrete zones of adhesion where the bottom layer of amoebae become stationary and attach to the substratum (*via* the slime sheath) so that longitudinal contraction will cause the rear to advance (Williams *et al.* 1986). Footprints in the trail (Vardy *et al.* 1986) are consistent with this prediction. Here we have provided more substantial evidence for the squeeze-pull model with the discovery of the collar, a stationary aspect of slug migration, which coincides with the position of the cellular footprints in the trail. Moreover, we have demonstrated that the *modB501* mutation leads to reduced rates of slug migration and is associated with a collar that moves backwards. In the squeeze-pull model (Williams *et al.* 1986), the rear of the slug is pulled forward towards anchored anterior cells (by longitudinal contraction). If the anchored anterior cells did not adhere, they would be pulled rearwards. We observe that in the *modB* mutant the collar in fact moves rearwards as might be predicted from the model.

While the collar is a distinctive morphological feature (Fig. 1A,B), its structure remains unexplained. The sheath is too thin to form such a distinct feature and thus the collar must be the result of a change in the slug amoebae. There is a strong possibility that it represents a circumferential band of contracting amoebae. If this is true, then the collar

could be involved with forward projection (squeeze) as well as the longitudinal contraction (pull) of slug motion (Williams *et al.* 1986).

A cellular contractile band provides a plausible explanation for the structure of the collar, but it does not address the dynamic properties of the collar. Collars move forward at the speed of the slug, are stationary or even move rearwards with respect to the substratum. We are testing two dynamic models: first, the collar may form by tetanic contraction of a band of cells, which remain contracted throughout its existence. Therefore, the cells comprising the collar do not change with time. Alternatively, the collar may reflect waves of contraction imposed on the slug in a fashion similar to the observation of Durston & Vork (1979) during *D. discoideum* culmination. In this model, the cells comprising the collar change with time, depending on the progression of the wave.

Clearly slug movement is periodic. Saltatory movement has been observed at the tip of the slug (Inouye & Takeuchi, 1979; Odell & Bonner, 1986; Williams *et al.* 1986). Collars are also formed periodically every approx. 12 min. There is increasing evidence for cyclic AMP pulses being involved in *D. discoideum* slug movement (Schaap, 1986); the periodicity observed here is comparable to that of cAMP pulses formed when amoebae begin to aggregate. Therefore, it is possible that collar formation is related to cAMP waves in the slug. Any relationship between the position of the collar and the prestalk-prespore boundary is yet to be established.

The work reported here is based on image analysis of time-lapse video recordings of moving slugs. Even as applied to the simple model of slug shape used here, image analysis is a powerful technique capable of providing new information on slug movement. In combination with other techniques, it will be possible to characterize the molecules involved with the movement of this simple multicellular organism. The reduced rate of migration of *modB* mutants is apparently due to failure of traction. Monoclonal antibody MUD50 which reveals the 'footprint' pattern in slime trails, recognizes a sugar epitope on a class of sheath glycoproteins. Strains carrying a *modB* mutation do not express the MUD50 epitope (Alexander *et al.* 1987). Experiments are in progress to identify the nature of the glycoconjugate recognized by MUD50 as this may prove to be the glue which sticks amoebae to the substratum.

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