A model for cell type localization in the migrating slug of *Dictyostelium discoideum* based on differential chemotactic sensitivity to cAMP and differential sensitivity to suppression of chemotaxis by ammonia

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The three basic cell types in the migrating slug of *Dictyostelium discoideum* show differential chemotactic response to cyclic AMP (cAMP) and differential sensitivity to suppression of the chemotaxis by ammonia. The values of these parameters indicate a progressive maturation of chemotactic properties during the transdifferentiation of slug cell types. We present a model that explains the localization of the three cell types within the slug based on these chemotactic differences and on the maturation of their chemotactic properties.

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1. Introduction

There are many examples of directed cell movement by chemotaxis during the development of *Dictyostelium discoideum*, beginning with the aggregation process that initiates the multicellular stage of the life cycle, and ending with the elaborate coordinated cell movements that create the fruiting body stalk and basal disc, the sorus, and the upper and lower cups that support the sorus (Bonner 1947; Siegert and Weijer 1992; Sternfeld and David 1982). These directed chemotactic movements contribute to cell sorting and to localization of the cell types.

We focus on the localization of the three major cell types within the migrating slug (figure 1) and address the roles played by differential chemotactic sensitivity to cAMP, and differential suppression of sensitivity of the chemotaxis by ammonia.

We previously reported that prestalk (pst) cells have higher chemotactic sensitivity to cAMP than anterior-like cells (ALC) (Feit *et al* 2001). We now present refined data for these cell types as well as for prespore (psp) cells. Psp cells have even lower chemotactic sensitivity to cAMP than do ALC.

The slug tip, which acts as a centre for initiation of waves of cAMP chemotaxis (Siegert and Weijer 1989, 1992), also produces an inhibitor of the forward chemotactic movement of ALC (Sternfeld and David 1981). We presented evidence that this tip-emitted suppressor of ALC chemotactic forward movement in the slug is ammonia (Feit *et al* 1990).

We previously reported that pst cells have higher resistance to ammonia suppression of cAMP chemotaxis than do ALC (Feit *et al* 2001). We now present refined data on ammonia suppression for these 2 cell types as well as for psp cells. Psp cells have even lower resistance to chemotaxis suppression by ammonia than ALC.

Within the migrating slug, psp cells are localized in the posterior part of the slug. ALC localize within the psp mass, and are dispersed uniformly longitudinally, but concentrated toward the substratum (Sternfeld and David 1982). Pst cells are localized at the anterior end, with pst cell subtypes forming a specific pattern within the prestalk region: pst A cells at the front of the prestalk region, pstO cells at the rear, and pstB (pstAB) cells forming a core at the centre of the prestalk region (figure 1).

Localization of the three major cell types within the migrating slug stage is a dynamic process (Sternfeld 1992;

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Abbreviations used: ALC, anterior-like cells; cAMP, cyclic AMP; pst, prestalk; psp, prespore

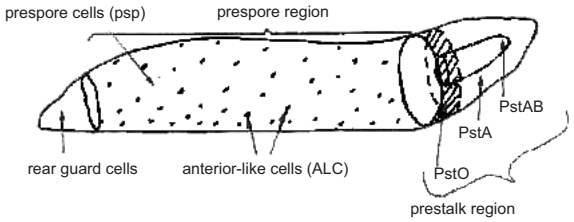

Figure 1. Location of the three major cell types in the slug, with the pst cells categorized by subtype within the prestalk region. The rear-guard region includes ALC and pstB cells.

Abe *et al* 1994). As the slug migrates, pstB (or pstAB) cells in the wedge-shaped area within the prestalk zone at the anterior end of the slug migrate or are pushed back through the mass and are incorporated into the basal disc or lost into the slime trail behind the slug. The replacement of these lost pstB (pstAB) cells involves a series of cell type transdifferentiations and relocalizations (Sternfeld 1992). Although the psp cells are localized in the slug posterior, a fraction of the psp cells transform into ALC. ALC move forward through the psp cell mass toward the anterior (pst region) of the slug. These ALC differentiate into pstO cells, localized in the posterior part of the pst zone. Within the pst zone, pstO cells differentiate into pstA cells, and pstA cells differentiate into pstB (pstAB), replacing the lost pstB (pstAB) cells. As these transdifferentiations occur within the slug, the cells relocalize.

We present a model that relates our data on differential chemotactic properties of the three cell types, and on maturation of these properties, to the localization of these cell types within the migrating slug during transdifferentiation.

2. Materials and methods

2.1 *Growth and harvesting of amoebae and formation of migrating slug*

Stocks of *Dictyostelium discoideum* (strain NC-4) were maintained on nutrient agar (Bonner l967) with *Escherichia coli* B/r as the food source. For experiments, the amoebae were grown in liquid culture at 22°C for 72 h using the method of Sussmann (1961) with substitution of *E. coli* B/r for *Enterobacter aerogenes.* Flasks were shaken at 1000 rpm in an EnvironShaker 3597 at 22°C. Amoebae were harvested by centrifugation on a Sorvall SP/X angle centrifuge for 2 min at 310 *g* (1500 rpm) and washed once with17 mM Na/K phosphate buffer (pH 6.6). The amoebae were suspended in 2 ml of the buffer, to which was added 0.1 ml of neutral red dye (0.2 mg /ml in17 mM Na/K phosphate buffer, pH 6.6, Sigma, St Louis, MO, USA). The suspension was immediately diluted to 12 ml by addition of the same buffer (procedure modified from Sternfeld and David 1982). The amoebae were washed twice more with the buffer before resuspension in the buffer as a thick paste at a final concentration of about $1x10^8$ cells/ml. The paste of amoebae was deposited in a line across the centre of the surface of 2% Difco Bacto-Agar in petri dishes (15 x 100 mm diameter, plastic). The dishes were covered with aluminium foil and incubated at 17°C; slugs began emerging from the lines of amoebae within 24 h.

2.2 *Drop assays for chemotaxis of individual psp, ALC and pst cells toward cAMP sources*

All procedures described below were performed under sterile conditions.

The red-stained anterior tips (the pst region) and the red-stained rear-guard cell regions at the extreme posterior ends of the neutral red-stained slugs were removed from each slug by using the tip of a B-D 24 gauge inoculating needle. The isolated prespore regions served as a source of ALC and psp cells; the isolated pst regions served as a source of pst cells. For each test of chemotaxis, 4–6 excised psp or pst masses were placed, using an eyelash attached to a Pasteur pipette, in a 10 μl drop of 17 mM Na/K buffers (pH 6.6) on the surface of 20 ml of agar made in the same buffer, in a 100 x 15 mm petri dish. Such drops are referred to as "response drops" (figure 2). We attempted to make the total dissociated cell mass in each response drop approximately equal. The excised cell masses were crushed and mixed together using a hair loop and the heat-rounded tip of a thin glass rod. A 10 μl "source" drop of 2 x 10^{-6} M cAMP (adenosine-3′,5′-cyclic monophosphate-sodium salt, Sigma, St Louis, MO, USA) in buffer was placed, using a plastic template, at a distance of 3 mm from the "response drop" (figure 2). The petri dishes were wrapped in aluminium foil and incubated at 22°C for 48 h, after which the plates were examined for movement of psp, ALC or pst cell amoebae outside the response drops (figures 2–4).

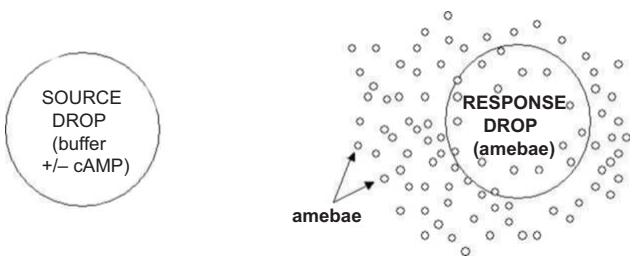

Figure 2. Arrangement of "source" (buffer ±1x10^{-6} M cAMP) and "response" (amoeba) drops 3 mm apart on the agar surface in a 100 x 15 mm petri dish. Amoebae are represented as small circles moving toward and away from the source drops.

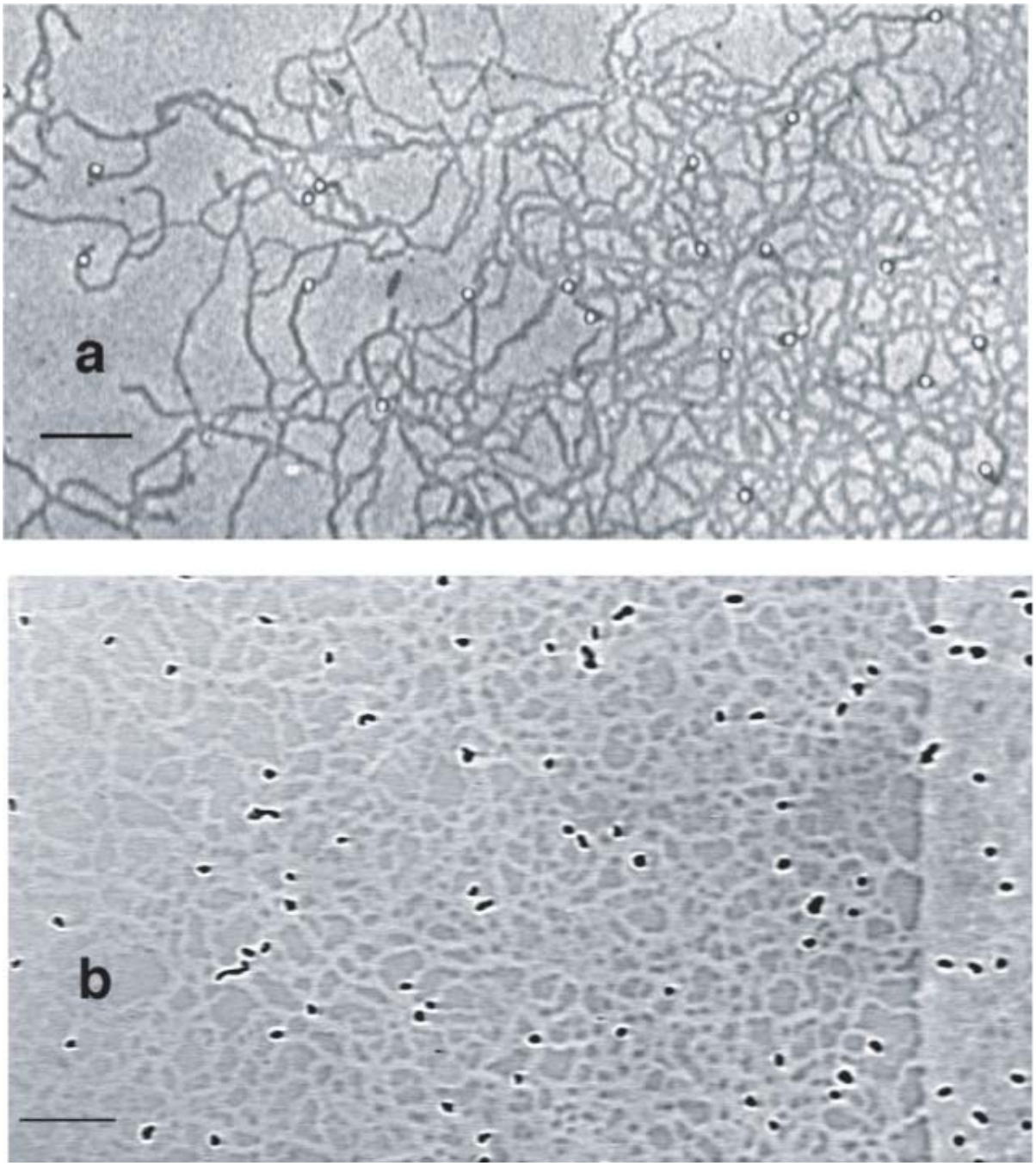

Figure 3. Micrograph of pst cells moving out of the response drop, which is positioned 3 mm from a source drop containing $2x10^{-6}$ M cAMP. **(a)** Cells are beginning to migrate out of the response drop. Amoebae appear as bright circles, since they were placed out of focus to facilitate fluorescence analysis. **(b)** A later stage in emergence of a cell "cloud". In both figures, the edge of the response drop is at the right, amoebae are moving toward the cAMP source drop, and amoeba trails are visible on the agar surface. Scale bar=50 μm.

The isolated prestalk masses contain only pst cells; the prespore masses contain both ALC and prespore cells. Since the ALC in the pst masses had fluorescent neutral red-stained autophagic vacuoles and the psp cells did not, we were able to determine which of the cells emerging from the prespore masses were ALC and which were psp. To facilitate the analysis we developed a protocol that allowed us to superimpose an image of optically enhanced and marked fluorescent ALC onto an image of non-fluorescent psp cells. For each plate, the distance migrated by the 5

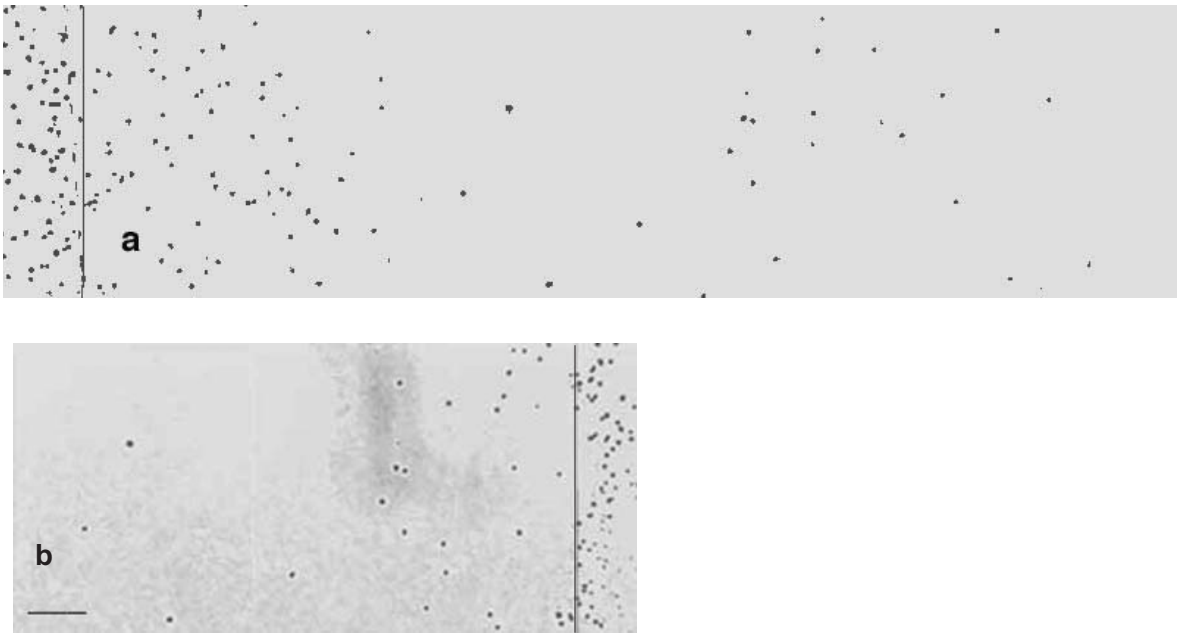

Figure 4. Lower magnification micrographs of pst cells moving out of a single response drop, which is positioned 3 mm from a source drop containing $2x10^{-6}$ M cAMP. **(a)** Cells migrating from the side of the response drop that faces toward the cAMP source drop. **(b)** Cells migrating from the side of the response drop that faces away from the cAMP source drop. In both figures, the edge of the response drop is marked with a line. Scale bar=100 μm.

farthest amoebae of each cell type moving towards, and the 5 farthest amoebae of each cell type moving away from the cAMP source drop was recorded using digital video microscopy. To avoid including possible atypical movement of the outermost cells, we used only the distance moved by the innermost cell of each set of five cells in the analysis of the data.

The differences between the distances amoebae moved toward and away from the source drop (figure 2) were analysed for significance by the use of a paired *t*-test (Data Desk). Amoebae were considered to show a positive chemotactic response to cAMP if there was a significant difference in toward versus away movement of amoebae (at a significance level of 0.05), and no significant difference in controls in which source drops lacked cAMP.

2.3 Drop assay tests for ammonia inhibition of psp, ALC and pst cell chemotaxis to cAMP

The tests for ammonia inhibition of chemotaxis were performed as described above, except that the agar substratum contained specified concentrations of NH_4Cl in Na/K buffer (pH 6.6) and the results of the tests were compared with those of the control tests in which the agar contained equivalent concentrations of NaCl. In these inhibition tests, both experimental and control source drops contained $2x10^{-6}$ M cAMP.

A given concentration of NH_4Cl was considered to suppress the chemotactic response to cAMP if there was no significant difference in movement of amoebae toward or away from the source drop (at a significance level of 0.05) and if there was a significant difference in the movement toward and away in the corresponding NaCl control plates.

3. Results

3.1 *Chemotactic sensitivity of the three cell types (psp, ALC and pst cells) to cAMP*

All three cell types show a chemotactic response to cAMP. In terms of the minimal concentration of cAMP in the source drop that elicits a response, ALC are more sensitive to cAMP than psp cells, and pst cells are much more sensitive than ALC. (Data for cAMP sensitivity are presented in tables 1–3 and summarized in table 4.)

Another way of expressing the relative chemotactic sensitivities of the three cell types to cAMP is that concentrations of cAMP in the source drop that elicit a response in one cell type fail to do so in another. Thus, a cAMP concentration that elicits a chemotactic response in ALC ($2x10^{-7}$ M) does not elicit a response in the less sensitive psp cells. cAMP concentrations that elicit a chemotactic response in pst cells ($1x10^{-9}$ M –$1x10^{-7}$ M) fail to elicit a response in the less sensitive ALC.

Table 1. Test for chemotactic response of psp cells to a cAMP gradient.

Treatment (concentration in source drop)	Distance of amoeba movement (mm)		*n*	*P*
	Toward cAMP (±SE)	Away from cAMP (±SE)		
$2x10^{-6}$ M cAMP	1.95 (±0.19)	1.29 (±0.16)	14	0.0086*
$1x10^{-6}$ M cAMP	2.73 (±0.17)	2.16 (±0.10)	19	0.0011*
$2x10^{-7}$ M cAMP	1.58 (±0.34)	1.04 (±0.17)	10	0.0694
$1x10^{-7}$ M cAMP	2.06 (±0.22)	1.78 (±0.16)	11	0.1725
Buffer, no cAMP (control)	1.83 (±0.18)	1.53 (±0.21)	16	0.0710

All plates contain cAMP in buffer or buffer alone (controls) in the source drop. The *P* value indicates the probability that the distance moved towards or away from the cAMP drop is due to chance. Ho: μ (toward–away)=0. Ha: μ (toward–away)>0. An * indicates a significant difference in movement toward versus away from the source drop at the 0.05 confidence level.
P values <0.05, with *P*>0.05 in the control, are interpreted as a chemotactic response to cAMP.

Table 2. Test for chemotactic response of ALC to a cAMP gradient.

Treatment (concentration in source drop)	Distance of amoeba movement (mm)		*n*	*P*
	Toward cAMP (±SE)	Away from cAMP (±SE)		
$2x10^{-6}$ M cAMP	2.19 (±0.22)	1.63 (±0.19)	14	0.0070*
$1x10^{-6}$ M cAMP	2.68 (±0.18)	2.20 (±0.11)	19	0.0023*
$2x10^{-7}$ M cAMP	1.45 (±0.33)	0.906 (±0.19)	10	0.0125*
$1x10^{-7}$ M cAMP	1.87 (±0.24)	1.78 (±0.20)	11	0.5172
Buffer, no cAMP (control)	1.98 (±0.19)	1.74 (±0.18)	16	0.2093

All plates contain cAMP in buffer or buffer alone (controls) in the source drop. The *P* value indicates the probability that the distance moved towards or away from the cAMP drop is due to chance. Ho: μ (toward–away)=0. Ha: μ (toward–away)>0. An * indicates a significant difference in movement toward versus away from the source drop at the 0.05 confidence level.
P values <0.05, with *P*>0.05 in the control, are interpreted as a chemotactic response to cAMP.

3.2 *Sensitivity of the three cell types to suppression of chemotaxis to cAMP by ammonia*

With regard to suppression of the chemotactic response to cAMP, the response of all three cell types is suppressed by ammonia. Based on the minimal concentration of ammonia in the agar substratum needed to suppress the chemotactic response to cAMP, ALC are less sensitive to ammonia than are psp cells, and pst cells are the least sensitive to ammonia of the three cell types. (Data for ammonia sensitivity are presented in tables 5–7 and summarized in table 8.)

Expressing the relative sensitivities in terms of ammonia concentrations that suppress cAMP chemotaxis of one cell type but fail to suppress a less sensitive cell type:

A concentration of NH_4Cl in the agar substratum that suppresses the chemotactic response in psp cells ($4x10^{-4}$ M)

Table 3. Test for chemotactic response of pst cells to a cAMP gradient.

Treatment (concentration in source drop)	Distance of amoeba movement (mm)		*n*	*P*
	Toward cAMP (±SE)	Away from cAMP (±SE)		
$2x10^{-6}$ M cAMP	1.79 (±0.27)	0.92 (±0.25)	11	0.003*
$2x10^{-7}$ M cAMP	2.69 (±0.26	1.69 (±0.19)	11	0.002*
$2x10^{-8}$ M cAMP	3.601 (±0.10)	0.991 (±0.11)	9	0.011*
$1x10^{-9}$ M cAMP	2.59 (±0.23)	2.08 (±0.01)	9	0.010*
$2x10^{-10}$ M cAMP	1.60 (±0.0.39)	1.99 (±0.88)	8	0.881
$1x10^{-10}$ M cAMP	2.45 (±0.39)	1.93 (±0.15)	8	0.154
Buffer, no cAMP (control)	2.16 (±0.34)	2.31 (±0.61)	4	0.640

All plates contain cAMP in buffer or buffer alone (controls) in the source drop. The *P* value indicates the probability that the distance moved towards or away from the cAMP drop is due to chance. Ho: μ (toward–away)=0. Ha: μ (toward–away)>0. An * indicates a significant difference in movement toward versus away from the source drop at the 0.05 confidence level.
P values <0.05, with *P*>0.05 in the control, are interpreted as a chemotactic response to cAMP.

Table 4. Chemotactic sensitivity of the three cell types (psp, ALC, and pst cells) to cAMP.

Cell type	The lowest concentration of cAMP in the source drop that elicits a chemotactic response from the response drop
Psp cells	Between $2x10^{-7}$ M and $1x10^{-6}$ M
ALC	Between $1x10^{-7}$ M and $2x10^{-7}$ M
Pst cells	Between $1x10^{-10}$ M and $2x10^{-9}$ M

does not suppress the response in ALC. A concentration of NH_4Cl that suppresses the chemotactic response in ALC cells ($5x10^{-4}$ M) does not suppress the response in pst cells.

We previously demonstrated (Feit *et al* 2001) that the suppression of cAMP chemotaxis by ammonia in ALC and pst cells by NH_4Cl was due to the molecular species (NH_3) rather than the protonated species $(NH_4)^+$.

4. Discussion

Several possible concerns about our design should be considered:

The first concern centres on the possibility that the cells may change their properties during the course of the assay. We distinguish between the three cell types based on their location in the slug anterior (for pst cells) or on a combination of location in the slug posterior plus presence or absence of neutral red-stained vacuoles (for ALC versus psp cells). The quantitative differences in response to cAMP and ammonia between these three cell types, so defined, are distinct and reproducible. Implicit in our analysis is the assumption that the properties of the cells do not change during the assay. However, if transdifferentiation or dedifferentiation occurs during the assay, the values that we have measured may differ from the values of these cell types at the time that the isolated slug regions were dissociated.

During the regeneration of isolated slug prestalk regions, after a 6-h period, redifferentiation of pst cells into psp cells is accompanied by a loss of neutral red-stained vacuoles (Bonner 1952; Sternfeld and David 1981). Our assumption of lack of change of cell properties during the assays is supported, but certainly not proved, by two observations. First, the pst cells in our assays retained their neutral red-stained vacuoles throughout the entire period of observation. Second, for the two cell types obtained from the prespore zone (ALC and psp cells), the fraction of neutral-red stained cells both within and outside the response drop did not seem to vary.

Another concern about our design might be the influence of the interior of the response drop on the outward movement of cells. In our experiments with ALC, pst and psp cells, we

Table 5. Test for NH_4Cl suppression of chemotaxis of psp cells to cyclic AMP (cAMP).

	Distance of amoeba movement (mm)			
Treatment	Toward cAMP (±SE)	Away from cAMP (±SE)	*n*	*P*
$5.0x10^{-4}$ M NH_4Cl	1.59 (±0.18)	1.09 (±0.14)	21	0.9992
$5.0x10^{-4}$ M NaCl (control)	1.56 (±0.21)	0.66 (±0.14)	11	0.0003*
$4.0x10^{-4}$ M NH_4Cl	0.31 (±0.14)	0.33 (±0.05)	6	0.4420
$4.0x10^{-4}$ M NaCl (control)	0.71 (±0.16)	0.11 (±0.07)	4	0.0347*
$2.5x10^{-4}$ M NH_4Cl l	1.19 (±0.13)	0.21 (±0.06)	19	≤0.0001*
2.5 $x10^{-4}$ M NaCl (control)	1.85 (±0.38)	0.16 (±0.05)	9	0.0011*
1.0 $x10^{-4}$ M NH_4Cl	0.95 (±0.12)	0.61 (±0.13)	24	0.0071*
1.0 $x10^{-4}$ M NaCl (control)	1.88 (±0.28)	0.60 (±0.12)	16	≤0.0001*

All plates contain 2 x 10^{-6} M cAMP in the source drop, and NH_4Cl or NaCl (control) in the agar substratum.
The *P* value indicates the probability that the distance moved towards or away from the cAMP drop is due to chance. Ho: μ (toward–away)=0. Ha: μ (towards–away)>0. An * indicates a significant difference in movement toward versus away from the cAMP source drop at the 0.05 confidence level. *P* values > 0.05, with $P < 0.05$ in the NaCl control, are interpreted as suppression of the cAMP chemotactic response.

rarely observed formation of mounds or later stages in the response drops. This is in striking contrast to our newer experiments in which we are attempting to use our assay to measure the response parameters of post-vegetative cells, as would be expected from many previous observations.

It should also be pointed out that the pst cells migrate out as a single cell type, while the ALC and psp cells migrate together and may possibly affect each other as they migrate.

The high chemotactic sensitivity of pst cells to cAMP that we found is in agreement with the results of studies using a variety of assays (Sternfeld and David 1981; Matsukuma and Durston 1979; Mee *et al* 1986; Early *et al* 1995; Traynor *et al* 1992).

Cells in all parts of the migrating slug respond to waves of cAMP originating in the slug tip (Siegert and Weijer 1992). At the same time, the cells are bathed in ammonia originating from the deamination of amino acids and nucleotides which occurs primarily in the prestalk region of the slug (Gregg *et al* 1954; Wilson and Rutherford 1978). The localization of these cells in the slug can be related to our data on the sensitivity of these cells to cAMP and ammonia. The relation between cell type sensitivity and localization is of special importance with relation to the temporal progression of transdifferentiation of these cell types as the slug migrates (Sternfeld 1992).

On the basis of our data on relative chemotactic sensitivities and ammonia sensitivities of the three cell types, we propose the following model.

3.2a *Model for localization of cell types in the migrating slug:* Psp cells have the lowest sensitivity to cAMP and their chemotaxis to cAMP is most easily suppressed by ammonia. Thus, they are localized throughout the slug posterior.

As some of the psp cells transdifferentiate into ALC, their sensitivity to cAMP increases and they are less suppressed by ammonia. Thus, they can move forward toward the prestalk zone.

As ALC transdifferentiate into pst cells, their sensitivity to cAMP increases sharply and they are much less suppressed by ammonia. ALC might become pst cells as they move toward the prestalk zone or only when they enter that zone. Either way, the high sensitivity of pst cells to cAMP and their low sensitivity to ammonia enable them to localize within the prestalk zone.

Table 6. Test for NH_4Cl suppression of chemotaxis of ALC to cAMP.

	Distance of amoeba movement (mm)			
Treatment	Toward cAMP (±SE)	Away from cAMP (±SE)	*n*	*P*
$5.0x10^{-4}$ M NH_4Cl	2.05 (±0.12)	2.51 (±0.17)	21	0.9979
$5.0x10^{-4}$ M NaCl (control)	2.71 (±0.16)	1.87 (±0.15)	12	≤0.0001*
$4.0x10^{-4}$ M NH_4Cl	0.52 (±0.10)	0.25 (±0.08)	8	0.0130*
$4.0x10^{-4}$ M NaCl (control)	0.31 (±0.0)	0.07 (±0.05)	5	0.0053*
$2.5x10^{-4}$ M NH_4Cl	2.69 (±0.14)	1.90 (±0.06)	19	≤0.0001*
2.5 $x10^{-4}$M NaCl (control)	3.26 (±0.35)	1.47 (±0.15)	9	≤0.0001*
1.0 $x10^{-4}$M NH_4Cl	1.8281 (±0.12)	1.5189 (±0.10)	24	0.0014*
1.0 $x10^{-4}$M NaCl (control)	2.93 (±0.28)	1.52 (±0.15)	16	≤0.0001*

All plates contain 2 x 10^{-6} M cAMP in the source drop, and NH_4Cl or NaCl (control) in the agar substratum.
The *P* value indicates the probability that the distance moved towards or away from the cAMP drop is due to chance. Ho: μ (toward–away)=0. Ha: μ (towards–away)>0. An * indicates a significant difference in movement toward versus away from the cAMP source drop at the 0.05 confidence level. *P* values > 0.05, with *P* < 0.05 in the NaCl control, are interpreted as suppression of the cAMP chemotactic response.

Table 7. Test for NH_4Cl suppression of chemotaxis of pst cells to cAMP.

	Distance of amoeba movement (mm)			
	Toward (±SE)	Away (±SE)	*n*	*P*
1.0 $x10^{-3}$M NH_4Cl	2.09 (±0.14)	2.36 (±0.17)	19	0.8838
1.0 $x10^{-3}$M NaCl (control)	2.17 (±0.26)	1.43 (±0.16)4)	10	0.0019*
5.0 $x10^{-4}$M NH_4Cl	3.00 (±0.22)	1.48 (±0.11)	10	≤0.0001*
5.0 $x10^{4}$M NaCl(control)	2.75 (±0.15)	1.50 (±0.11)	20	≤0.0001*

All plates contain 2 x 10^{-6} M cAMP in the source drop, and NH_4Cl or NaCl (control) in the agar substratum.
The *P* value indicates the probability that the distance moved towards or away from the cAMP drop is due to chance. Ho: μ (toward–away=0). Ha: μ (towards–away)>0. An * indicates a significant difference in movement toward versus away from the cAMP source drop at the 0.05 confidence level. *P* values >0.05, with *P*<0.05 in the NaCl control, are interpreted as suppression of the cAMP chemotactic response.

Our model concentrates on the role of chemotaxis in cell type localization. It is likely that differences in cell adhesion may also play a role (Tasaka and Takeuchi 1979).

The cAMP and ammonia parameters that we have determined suggests a progressive maturation of the chemotactic apparatus within these cells, based on the flow

Table 8. Sensitivity of the three cell types (psp, ALC, and pst) to suppression of chemotaxis to cAMP by ammonia.

Cell type	The lowest concentration of ammonium chloride in the agar substratum that suppresses a cyclic AMP chemotactic response in the response drop
Psp cells	Between $2.5x10^{-4}$ M and $4x10^{-4}$ M
ALC	Between $4x10^{-4}$ M and $5x10^{-4}$ M
Pst cells	Between $5x10^{-4}$ M and $1x10^{-3}$ M.

of cells and the transdifferentiation of cell types within the migrating slug (Sternfeld 1992).This maturation is reflected in the following progression:

Chemotactic sensitivity is weakest and ammonia sensitivity is highest in the psp cells. Chemotactic sensitivity is more highly developed and ammonia sensitivity is lower in the ALC. Chemotactic sensitivity is most highly developed and sensitivity to ammonia is lowest after ALC are transformed into pst cells.

The initial transdifferentiation of ALC into pst cells is to the pstO cell subtype (Jermyn *et al* 1989). PstO cells in the slug tip subsequently differentiate into pstA and then pstB (pstAB) cells (Jermyn *et al* 1989). We plan to investigate the sensitivity parameters of these subtypes to see whether they correlate with their localizations within the tip.

The chemotactic response movements of ALC within submerged oxygenated prespore masses and the effect of ammonia on that response (Feit *et al* 2001) were consistent with the movement of these cells when the same conditions were imposed on ALC in our plate assay. This indicates that the results we obtained based on the movement of cells on agar are relevant to the response of cells moving within the migrating slug.

One interesting aspect of the model is the coexistence of two control systems. Redundancy in biological control is not uncommon. It is possible that decreasing sensitivity to ammonia simply amplifies or fine-tunes the response to cAMP. On the other hand, given the high rate of deamination in the prestalk zone, and the relatively higher concentration of ammonia there (Wilson and Rutherford 1978), it is possible that it was selection for decreasing sensitivity to ammonia during maturation of the chemotactic apparatus that made localization of maturing ALC and pst cells possible in the multicellular stage of *Dictyostelium*.

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