Department of Biological Sciences
Animal and Plant Physiology 2nd Year
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Chemotaxis Practical

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Questions to Section I and answers

1) Briefly describe what happens with the chemoattractant concentration over time, as it diffuses into the agar (less than 50 words)

The chemoattractant diffuses freely inside the agar (as seen with the dye), building a gradient that is steep at the start of the experiment and becomes shallower with time, later it all flattens down.

2) Do you observe a difference between wild-type and Gβ minus cells, both for vegetative and starved cells ? If yes, what do you think the starvation does to the cells ? Is the length of the starvation period critical, and why ? (less than 200 words)

The Gβ minus cells do not respond to any chemoattractant, being veg or starved. The symetrical radial movement of Gβ cells outside the initial drop is barely visible. The cells do not aggregate in any of the cells drops. The wt cells move outward in all conditions (only slightly visible), but a crescent of migration is clearly visible for veg cells to folate but not to cAMP, and for starved cells to both folate (better than veg cells, due to higher speed of intrinsic motility) and cAMP. There is a dose-dependence in the migration, for folate it is farthest at the highest folate conc., but for cAMP, it is highest for either 10 or 50 µM. At 250 µM, a slight inhibition of direct migration to the well is observed, due to saturation of the receptors. Therefore, the cells move "sideways" toward the well, creating a deformed drop or crescent (banana or ellipse). The cells aggregate at low cAMP conc, but at 250 µM the external cAMP inhibits aggregation. The starvation period used was close to optimal, shorter times would have resulted in a delay in setting off or a reduction of the numbers of cells migrating, because they need time to express the cAMP receptor. The starved wt move to folate because they have not yet lost the receptor.

3) Estimate the maximal speed of cell motility (in µm/min) towards folate and cAMP.

The cells maximally moved about 3-4 mm in 4 hours. This is the same as 10 to 15 µm/min

4) How would the cells behave if you were to fill a well with 5 mM of cAMP ? (less than 50 words)

A situation comparable but more dramatic than with 250µM, namely the cells would be unable to sense the gradient because their receptors are completely saturated. In addition, they would be unable to aggregate.

5) Describe what you think will happen to the cells in each of the "drops", for the four cell types (vegetative and starved wild-type and Gβ null cells) on both plates if you further incubate them overnight in the same conditions. What would happen if you left the plates in the fridge ? (less than 200 words)

Nothing would happen to the Gβ null cells … except that they would be almost dead of hunger. All the wt cells, both veg and starved will have aggregated and even have completed their development cycle. EXCEPT where the cAMP was too high, 250μM should completely inhibit differentiation (50 μM might be interfering but not completely).

6) Based upon what you learned at the lecture on chemotaxis, suggest two genes, ablation of which should disrupt chemotaxis to folate only, and chemotactic motility to any chemoattractant, respectively. Briefly explain your logical arguments. (less than 200 words)

The receptor to folate (a GPCR (G protein coupled receptor) protein, which is a seven transmembrane or serpentine receptor). Gα4 is a Gα subunit of trimeric Gs which is specifically coupled to the folate receptor. Both will disrupt (almost) exclusively the chemotaxis to folate. Mutations in the cAMP receptor (CAR1) or Gα2 will be specific to the cAMP chemotaxis and will not affect chemotaxis to folate. Any other protein downstream, PI3Kinase, PTEN, Ras, CRAC (or other PH domain containing proteins), PKB, PAK1 (all seen in the lecture) will likely affect both. Mutations in any effector, like in the actin dynamics or myosin contraction machineries, will affect both pathways.