

Photosensory and thermosensory responses in *Dictyostelium* slugs are specifically impaired by absence of the F-actin cross-linking gelation factor (ABP-120)

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Chemotactic aggregation of starving amoebae of *Dictyostelium discoideum* leads to formation of a motile, multicellular organism — the slug — whose anterior tip controls its phototactic and thermotactic behaviour. To determine whether proteins that regulate the *in vitro* assembly of actin are involved in these responses, we tested phototaxis and thermotaxis in mutant slugs in which the gene encoding one of five actin-binding proteins had been disrupted. Of the proteins tested — severin, α -actinin, fimbrin, the 34 kDactin-bundling protein and the F-actin cross-linking gelation factor (ABP-120) — only ABP-120 proved essential for normal phototaxis and thermotaxis in the multicellular slugs. The related human protein ABP-280 is required for protein phosphorylation cascades initiated by lysophosphatidic acid and tumor necrosis factor α . The repeating segments constituting the rod domains of ABP-120 and ABP-280 may be crucial for the function of both proteins in specific signal transduction pathways by mediating interactions with regulatory proteins.

Results and discussion

With its well characterized actin cytoskeleton and molecular genetics, *Dictyostelium discoideum* has become a model organism for studying *in vivo* the biological roles of cytoskeletal proteins in non-muscle cell motility [1,2]. With few exceptions [3], disrupting genes that encode the actomyosin cytoskeleton has only subtle effects on single cell motility and chemotaxis [1,2]. More marked effects of cytoskeletal mutations have been observed at the stage where starving amoebae aggregate to form a motile, multicellular organism — the slug. Thus myosin II-deficient mutants [4,5] and double mutants lacking both α -actinin and ABP-120 [6,7] fail to develop normally in the multicellular stages of the *D. discoideum* life cycle. What presents as a subtle phenotype in the unicellular state can thus become a serious

defect in the multicellular stages. Multicellular slugs exhibit oriented motility responses to external stimuli — they migrate at average speeds from 0.2 to 2.0 mm h⁻¹ and are strongly phototactic (Figure 1) and thermotactic. These oriented movements involve the products of at least 20 genes and all of the common eukaryotic second messengers (cAMP, cGMP, Ca²⁺ and inositol polyphosphates) may participate in transducing the signals[8]. To evoke a motility response in the slug, signals presumably regulate the cytoskeletal system. In the experiments reported here, five major actin-binding proteins have been examined as potential targets for photosensory and thermosensory transduction pathways. Of these, severin, α -actinin, fimbrin and the 34 kD actin-bundling protein are regulated by Ca²⁺ *in vitro* [1,2] while ABP-120 [9,10] is not. ABP-120 is a homodimer and is related by sequence homology to the ABP-280/filamin family of mammalian actin cross-linking proteins [10–12].

Qualitative tests revealed that slug phototaxis was normal in each strain lacking one of the actin-binding proteins with the exception of the ABP-120-deficient mutant, which was severely defective (Figure 1). As with other reported phototaxis mutants [13,14], the slugs of this strain were disoriented and their phototaxis was bimodal, with two preferred directions ($\pm\alpha$) either side of the direction towards the light source. In qualitative tests of phototaxis [14], the initial inoculum density is not accurately controlled and some growth occurs at the inoculation site as a result of carry over of the bacterial food source. It was previously reported that phototaxis is impaired when *Dictyostelium* slugs are allowed to form and migrate at high cell densities[15]. We therefore performed quantitative phototaxis tests using defined cell densities of washed cells (Figure 2). Phototaxis of the wild-type strain AX2 was not significantly affected by cell densities up to about 6×10^6 cells cm⁻². Orientation in the ABP-120-deficient mutant (GHR) [16] was severely impaired at all cell densities (Figure

2). Similar results (not shown) were obtained in quantitative phototaxis experiments with HG1264 [17] and HG1270 [18], two strains bearing, in different genetic backgrounds, a chemically induced mutation in the ABP-120 gene that was also shown to affect phototaxis in qualitative tests (E. Wallraff, personal communication). Quantitative phototaxis experiments with the α -actinin- and severin-deficient mutants verified that their phototaxis was not impaired (results not shown). Thus only the ABP-120 protein was critical for normal phototaxis. Since none of the four Ca^{2+} -regulated actin-binding proteins tested were essential for phototaxis, we conclude that either the Ca^{2+} -regulated actin filament cross-linking proteins are functionally redundant or intracellular Ca^{2+} signals do not perform their postulated roles in slug orientation behaviour [8] by regulation of these particular proteins.

Darcy *et al.* [14] have shown that almost all phototaxis mutations also cause defects in thermotaxis. This is part of the evidence that the photosensory and thermosensory transduction pathways converge early so that most of the proteins involved are required for both phototaxis and thermotaxis. We therefore carried out qualitative thermotaxis assays with mutants lacking each of the five actin-binding proteins under study. Only the ABP-120-deficient mutant GHR exhibited abnormal thermotactic behaviour. Quantitative thermotaxis experiments were then carried out with the α -actinin- and severin-deficient mutants and with three strains lacking ABP-120. Thermotaxis was severely deranged in both GHR and in the ABP-120 deficient strains HG1264 [17] and HG1270 [18], both of which carry a chemically induced mutation in the ABP-120 gene (Figure 3). In contrast, AHR (α -actinin deficient) [16] and DS211C (severin deficient) behaved in a wild-type manner. Clearly ABP-120 is involved in both phototactic and thermotactic orientation in *Dictyostelium* slugs. Like phototaxis, the extent of slug migration and the final stages of morphogenesis are under tip

control [8]. Accordingly, many phototaxis mutants exhibit additional defects in morphogenesis and/or do not migrate as far as normal strains because of delayed development, slower migration or earlier cessation of migration prior to culmination [8,14]. In all phototaxis and thermotaxis experiments we found that the ABP-120 deficiencies also caused shorter migration paths.

The slug behavioural defects in ABP-120-deficient mutants are the first example in *Dictyostelium* of a dramatic impairment of sensory responses caused by the absence of a single, known cytoskeletal protein. This raises the question as to what distinguishing features this protein might possess compared to those eliminated in the other mutants studied. First, ABP-120 is not the most abundant or most active F-actin cross-linker in wild-type cells; this distinction belongs to α -actinin whose activity *in vitro* is at least five times greater [19,20]. Second, three of the proteins studied — gelation factor, α -actinin and fimbrin — have conserved actin-binding domains typical of the α -actinin/spectrin family of cytoskeletal proteins (Figure 4), yet only the gelation factor is essential for normal sensory responses in slugs. A tempting hypothesis is that the function of ABP-120 in slug behaviour is related to its unusual rod domain. An immunoglobulin-like fold has been recently identified in the six repeated segments that constitute this domain [10,11]. Sequence homologies suggest that a similar structure is present in each of the 24 repeats in the rod domain of the related human actin binding protein, ABP-280 (nonmuscle filamin, Figure 4) [11,12]. ABP-280 interacts with a number of other proteins [21–25] including SEK-1, a protein kinase that specifically phosphorylates the SAPK (stress-activated protein kinase) and p38 subfamilies of ERKs (extracellular signal-regulated kinases) [21]. In those cases where it has been tested, this interaction requires the rod domain [21–23]. It is possible that each repeated

immunoglobulin-like fold of ABP-120 (and of ABP-280) mediates specific binding interactions with particular proteins.

How might such interactions be regulated? Of the actin binding proteins studied here, ABP-120 is the only one whose *in vitro* function is unaffected by Ca^{2+} , suggesting that its activity in photosensory and thermosensory signalling pathways is regulated by some alternative means such as phosphorylation. Human ABP-280 is not only necessary for SAPK activity in specific protein phosphorylation cascades [21], but is itself phosphorylated at serine residues by multiple protein kinases in intact, stimulated cells [26]. Phosphorylation of ABP-280 regulates its actin cross-linking activity [27] and its translocation from the cell periphery to the cytosol [28]. By analogy with human ABP-280, *Dictyostelium* ABP-120 could interact specifically with other proteins in the photosensory and thermosensory transduction pathways including the receptors, could be necessary for specific protein phosphorylation cascades involved in transduction of signals from these receptors to the cytoskeleton, and could itself be regulated by phosphorylation.

Supplementary material

The Materials and methods for the experiments described here are published together with this paper on the internet.

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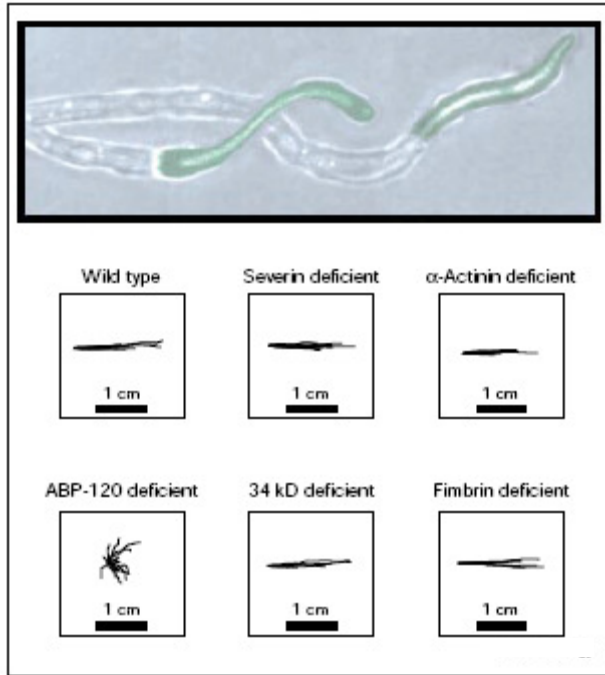


Figure 1 Phototaxis by wild-type

Dictyostelium discoideum slugs and mutants lacking actin-binding proteins.

The top panel shows a pseudocolour image of two wild-type slugs migrating on water agar towards a light source that was to the right of the figure. Each slug was about 1 mm long and was migrating at about 1 mm h⁻¹ through a secreted extracellular matrix that collapsed behind it to form a trail. The

lower panel shows digitized trails of slugs of the parental strain AX2 and of mutants lacking the indicated actin-binding proteins — α -actinin, AHR (*abpA* replacement) [16]; severin, DS211C (*sevA* disruption); ABP-120, GHR (*gela* disruption) [16]; 34 kD actin-bundling protein, 34 kD–(*abpB* replacement) [29]; fimbrin (plastin), HG1629 (*fimA* disruption). Plates with inocula of the indicated strains were exposed to a lateral light source for 24 h in qualitative phototaxis tests (see Supplementary material), blotted onto clear PVC discs, stained and digitized. Trails are plotted from a common origin such that the direction towards the light source was towards the right of the figure. Only the ABP-120-deficient mutant showed impaired phototaxis, which was both disoriented and significantly ($P < 0.05$) bimodal (i.e. two preferred directions, $\pm \alpha$, either side of the direction towards the light source). Statistical analysis of the directions of migration by ABP-120-deficient slugs yielded a maximum likelihood estimate of $\alpha = 49^\circ$ with an accuracy of orientation (κ_2) of 3.46.

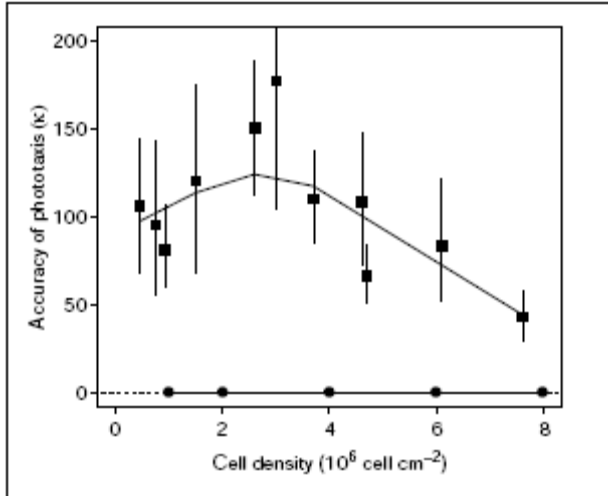


Figure 2 Phototaxis by wild-type and ABP-120-deficient slugs formed at defined cell densities. Measurements of phototaxis by slugs formed at the indicated cell densities are shown for the parental wild-type strain AX2(■) and the ABP-120-deficient mutant GHR (●).

Washed amoebae were exposed to a lateral light source for 48 h at 21°C. Trails were digitized and analysed using directional statistics [14,15] (see Supplementary material). In two experiments, pooling results for AX2 at cell densities less than $6 \times 10^6 \text{ cells cm}^{-2}$ yielded accuracies of phototaxis (κ) of 109 ± 13 and 119 ± 25 . For GHR, pooled data over all cell densities yielded an accuracy of phototaxis (κ) of 1.1 ± 0.25 and, because of the extreme disorientation, the bimodal model for phototaxis did not fit the data significantly better than the unimodal model. Vertical bars and indicated errors represent 90% confidence limits.

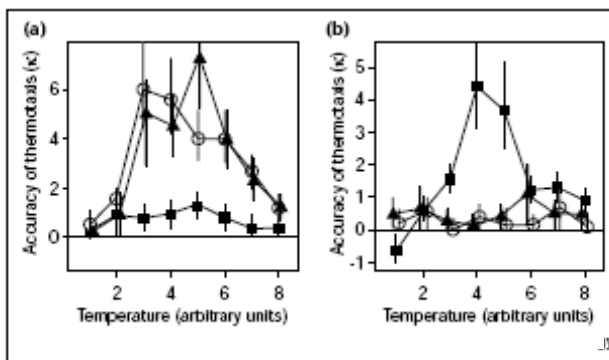
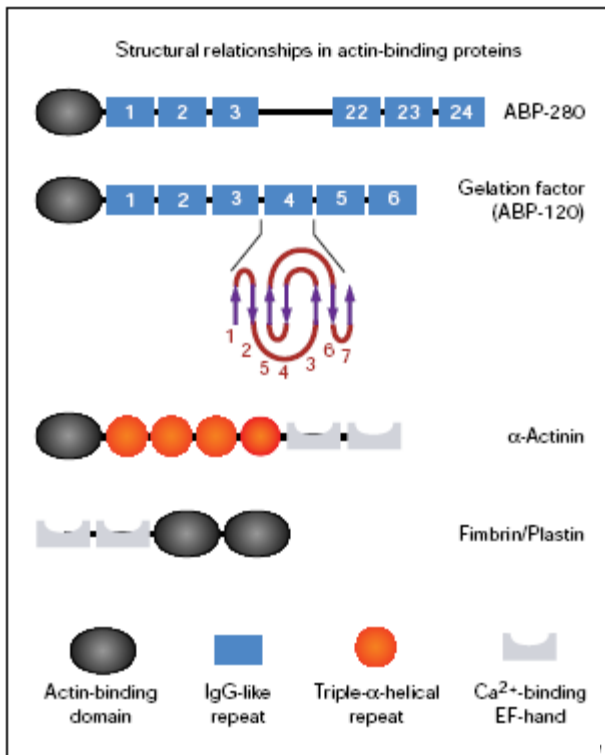


Figure 3 Quantitative measurement of thermotaxis by slugs of wild-type and mutant strains lacking specific actin-binding proteins. (a) Thermotaxis by mutants in which the genes encoding the actin binding proteins

α -actinin (strain AHR,○, $3.0 \times 10^6 \text{ cells cm}^{-2}$), severin (strain DS211C,▲, $3.0 \times 10^6 \text{ cells cm}^{-2}$) or ABP-120 (strain GHR,■, $3.0 \times 10^6 \text{ cells cm}^{-2}$) had been disrupted or replaced by homologous recombination.(b) Thermotaxis by AX2 (■, $2.3 \times 10^6 \text{ cells cm}^{-2}$) and the

mutants HG1264 [17] (○, 3.3×10^6 cells cm^{-2}) and HG1270 [18] (▲, 3.6×10^6 cells cm^{-2}), which carry the *gela1000* mutant allele generated by chemical mutagenesis of the ABP-120 gene. Washed amoebae were inoculated onto water agar plates and incubated for 72 h in darkness on a heat bar that provided a temperature gradient of $0.2^\circ\text{C cm}^{-1}$ at the agar surface. Temperatures at the centres of the plates are indicated in arbitrary units that corresponded in separate calibration experiments to 14°C (T = 1) through 28°C (T = 8). Trails were analysed using directional statistics [15,30] (see Supplementary material). In the experiment shown, AX2 thermotaxis at T = 1 was weakly negative (that is, towards the cold) so the previously used convention [30] is followed of assigning negative values to κ for the accuracy of significantly negative thermotaxis. In other experiments (not shown) the AX2 slugs at T = 1 showed no significant thermotaxis (that is, κ not significantly different from 0) as the transition to negative thermotaxis for this strain occurs close to this temperature. In such cases there is, by definition, no significantly preferred direction, and estimates of κ are



assigned positive values (with a lower confidence limit of 0) [30].

Figure 4 Structural relationships among proteins carrying a common actin binding region typical of the α -actinin/spectrin family. The diagram shows the modules constituting the human actin cross-linking protein ABP-280 and three *Dictyostelium* actin-binding proteins used in this study. The structure of the

fourth repeat in *Dictyostelium* ABP-120 has been solved by nuclear magnetic resonance and shown to consist of an immunoglobulin-like fold [11], which is represented schematically.