

This Week's Citation Classic

Konijn T M, Barkley D S, Chang Y Y & Bonner J T. Cyclic AMP: a naturally occurring acrasin in the cellular slime molds. *Amer. Naturalist* **102**:225-33, 1968. [Dept. Biology, Princeton Univ., Princeton, NJ]

With the help of a new, quantitative assay, it was possible to show that supernatants from *Escherichia coli* contained an attractant for the amoebae of *Dictyostelium discoideum*. The attractant, which was identified as cyclic AMP, was also secreted by amoebae. Cyclic AMP, which is a second messenger in hormone reactions in mammals, serves as a first messenger in the cellular slime molds. [The *SCF*[®] indicates that this paper has been cited over 150 times since 1968.]

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"My interest in the social behaviour of *Dictyostelium* amoebae was aroused by Kenneth B. Raper of the University of Wisconsin, who introduced me in the late 1950s to the fascinating process of cell aggregation. At that time Bonner had firmly established that cell aggregation was mediated by a chemotactic agent which he named acrasin.¹ Shaffer isolated acrasin from pseudoplasmodia of *D. discoideum* and showed with his ingenious sandwich technique that the attractant was a small heat stable molecule that was inactivated by an enzyme.²

"Perhaps luckily, not aware of all the pitfalls that one may encounter during characterization of specific molecules, I developed a semiquantitative bioassay to purify crude amoebal extracts. Small drops (0.1 µl) of an amoebal suspension were applied next to another small population of amoebae on a water-repellent agar surface. The hydrophobic agar surface prevented cells from crossing the boundaries of the

drops, but allowed them to move to the site closest to the neighboring attracting drop. Such a positive response could be observed with aggregates of amoebae or with extracts of cells. Soon it became clear that the chemists' request for milligrams of pure attractant molecules never could be satisfied with the acrasin isolated from amoebae. Therefore, bacteria were used as an attracting source. *E. coli* was grown in large trays, washed, centrifuged, and purified by gel filtration, paper chromatography, and paper electrophoresis. While van der Meere and I isolated the attractant in Utrecht, Bonner and co-workers at Princeton University had started to purify the amoebal attractant from bacteria with the cellophane square assay. In 1967 we pooled our efforts which resulted in the identification of cyclic AMP as the attractant. Dreaming of the all attracting power of this nucleotide I asked at the infirmary if I could draw a pint of blood; alas, the chemotactic agent for aggregating amoebae did not seem to be attractive to leukocytes. The fact that the amoebae of the slime mold *Polysphondylium pallidum*, which secreted cyclic AMP, did not respond to it, showed its specificity. The results became outright discouraging when the supernatant of *D. discoideum* amoebae did not attract cells of their own species. Chang, however, explained the low yield of cyclic AMP by demonstrating the presence of the hydrolyzing enzyme phosphodiesterase.

"The frequent citations to this paper are mainly due to the coincidence that a molecule that just became a versatile regulatory agent initiated the multicellular stage of an organism which Trinkaus called 'far and away the best analysed example of directional cell movement and clearly a morphogenetic movement in the best sense.'³ *Dictyostelium* can be used as a model for studies on hormone action and differentiation and it is particularly satisfying to see that through the efforts of several laboratories progress has been made in the elucidation of the molecular mechanisms of cell locomotion in *Dictyostelium*."

1. **Bonner J T.** Evidence for the formation of cell aggregates by chemotaxis in the development of the slime mold *Dictyostelium discoideum*. *J. Exp. Zool.* **106**:1-26. 1947.
2. **Shaffer B M.** Acrasin, the chemotactic agent in cellular slime moulds. *J. Exp. Biol.* **33**:645-57, 1956.
3. **Trinkaus J P.** The mechanisms of metazoan cell movement. (Poste G & Nicolsen G L, eds.) *The cell service in animal emhrvogenesis and development*. New York: Elsevier North-Holland, 1976. p. 225-329.