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CHEMOTAXIS : NOT A PREREQUISITE FOR PLASMODIA COALESCENCE IN PHYSARUM POLYCEPHALUM

Wung-Wai Tso* and Man-Yin Wong

*Department of Biochemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong and Radioisotope Unit, University of Hong Kong, Hong Kong

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SUMMARY

Unlike pseudoplasmodium formation in Dictyostelium discoideum which involves the aggregation of amaebae chemotactically responding to pulses of cyclic AMP released from pacemaker cells, the plasmodia and presumably the microplasmodia of Physarum polycephalum coalesce without the presence of a chemotactic stimulus.

INTRODUCTION

Myxomycetes, the acellular slime molds, are thought to be related to Acrasiales, the cellular slime molds (1). Both Myxomycetes and Acrasiales exhibit many similar behavioural features. Their vegetative motile phases perform tactically oriented movement in response to various external stimuli including those from heat (2,3), light (4,5) and chemicals (6-8, 1, 9-12).

When logarithmically grown amaebae of Dictyostelium discodeum, an Acrasiale, are washed free of nutrient medium and dispersed on filters humidified with a non-nutrient buffered salt solution, they aggregate to form a pseudoplasmodium which contains 10^3-10^5 cells (13). The formation of the pseudoplasmodium is indeed a well programmed biochemical event designed for social communication. Some autonomously cyclic AMP releasing pacemaker cells in the amaebae population generate the aggregation signals by sending out a pulse of cyclic AMP at intervals between 3 to 10 minutes (14,15). These pulsatile signalling molecules diffuse out to reach the peripherous cells which trigger an inward chemotactic movement. It is proposed that the signal reception

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mechanism involves a presumed association between cyclic AMP receptor and the cell bound phosphodiesterase molecules (12). The signal is relayed outward in waves of expanding rings or spirals (15,16) since the cells near the source go through a refractory period immediately after receiving a cyclic AMP signal, presumably from the center. The pulsatile movements last for about 100 seconds and cover approximately 20 μm towards these centers. As a result of this cleverly programmed inward cyclic AMP mediated chemotactic movement, the aggregated pseudoplasmodium, which is entirely surrounded by a slime sheath (17) moves in a co-ordinated fashion over the substratrum.

Similar to the aggregation of amaebae to form pseudophasmodium in Dictylstelium discodeum, the microplasmodium of Physarum polycephalum, a widely studied Myxomycete, coalesce into a large multinucleated plasmodium (18). This large plasmodium grows, sporulates or spherulates depending on the nature of the environment. Like the aggregation in D. discodeum, the coalescence in P. polycephalum involves the assembly of numerous separated microplasmodia. Not only microplasmodia coalesce, but the existing smaller plasmodia also coalesce when allowed to get into contact (2,19). The size of a coalesced plasmodium or a growing plasmodium can reach 14 cm diameter (20). Cyclic AMP has been reported to be one of the known attractants in P. polyceaphalum (21). In this report, we describe our observation that a chemotactic event is not required in the coalescence of plasmodia in P. polycephalum.

MATERIALS AND METHODS

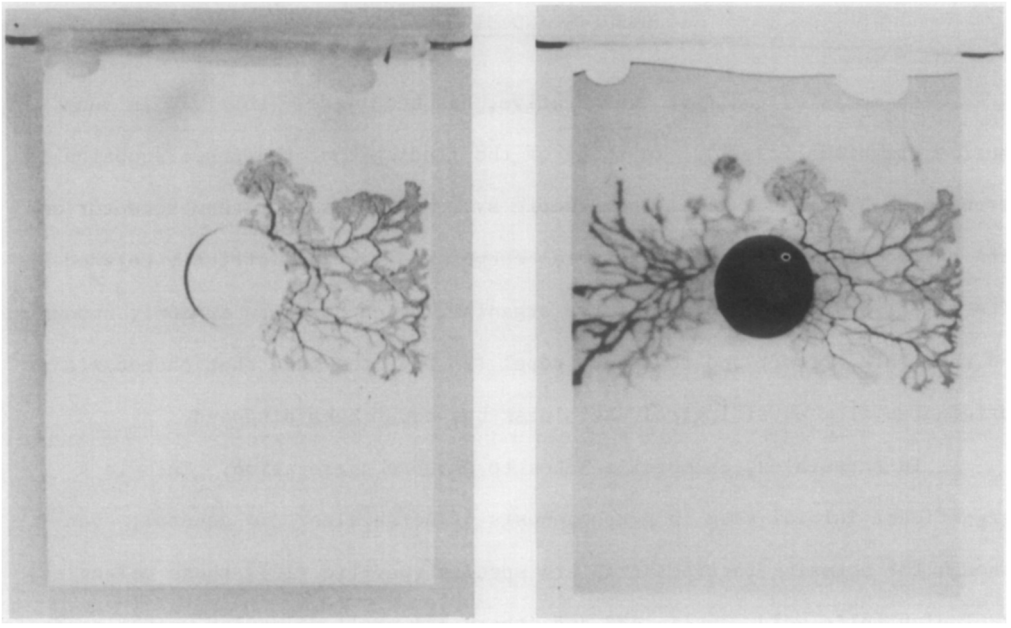
The microplasmodia of P. polycephalum M_3C were grown to logarithmic phase in liquid culture with adequate agitation similar to that described in Daniel and Balkwin (2,22). The harvested microplasmodia were washed three times in salt solution and dispersed onto sterile 13-mm Millipore circular (pore size: 0.45 μm) laid on the surface of water agar. After approximately 15 minutes, the excess water was removed by absorption into the supporting agar. Tow of these filter supported microplasmodia were lifted and attached onto a rectangular pre-moistened Millipore filter with one back to back to the other on both sides of the rectangular filter support. The whole support was then allowed to rest on a wire (see legend to the Figure) suspended in a humidity, light and temperature controlled environment such as a screw-cap bottle in a dark room. The microplasmodia on each circular filter coalesced in approximately one hour and the two coalesced plasmodia then migrated as two individuals. The migration pattern of these two plasmodia were observed at half hour intervals.

RESULTS AND DISCUSSION

Chemotaxis, positive and negative, has been widely observed in many motile organisms (23-28). In spite of the finding that the chemoreception is transduced by a specialized chemotactic system (such as the chemoreceptor and its auxillary components in bacterial chemotaxis) and not strictly related to its effect on the physiology of the organism, which has been commonly recognized as nutrients attract and toxicants repel (23,24), the idea that chemotaxis originates from physiological usefulness has never been disposed.

In Acrasiales, chemotaxis leads to amaebae aggregation. This is a significant initial step in morphogenesis of Aerasiales. In general, even though the acrasins (attractants) are species specific (12), these molecules, including folic acid, cyclic AMP and others are small molecular weight products which are readily diffusable, a good quality for communication signallers. On the rectangular filter support described in this study, the microplasmodia of P. polycephalum dispersed on each circular Millipore filter coalesced in approximately one hour and began independent migration afterwards by sending out pseudopodia. If chemotaxis is operating, we would expect that both plasmodia migrate in such a pattern that they superimpose on each other. Our observation throughout a time period longer than 10 hours was recorded (the 7 hour migration pattern was shown in the Figure). In NO instance did the patterns superimpose on each other. This suggests that small molecules which are diffusable through the three layers (2 circular filters and one rectangular filter support, if there is any) do not function as that documented in Acrasiales in inducing microplasmodia or plasmodia coalescence.

The plasmodia used in this study comes from the same culture, thus eliminating the possibility that noncoalescence is due to different physiological states of both individuals (29). In Acrasiales, only the amaebae state is sensitive to its acrasin; the formed pseudoplasmodia is not (1,30). The observation in the entire period (including the coalescence from microplasmodia)



Microplasmodia on each circular filter coalesced to give two separate small plasmodia migrating on both sides of the Millipore support. The migrating pattern of one plasmodium seven hours after set-up was shown on the left hand panel and that of both plasmodia were revealed by the opposite panel. Left hand panel: illumination from top; right hand panel: illumination from bottom.

showing definitely no instance whereby the back to back plasmodia migrate superimposably, does not support a state-dependent chemotaxis event. There is, however, an extracellular phosphodiesterase reported (31); but this enzyme should not explain the nonsuperimposed migration pattern of the two separate plasmodia as shown by the following: if one of the plasmodia is allowed to migrate around the support to the back of the filter, it did coalesce with the one on the back. This was observed in all experiments that were attempted. If the extracellular phosphodiesterase is indeed responsible for inhibiting chemotaxis, presumably due only to cyclic AMP attractant, we should have observed the same enzyme inhibiting coalescence in the latter case even though both individuals were in contact (30). It is apparent that chemotaxis is not required for plasmodial coalescence.

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REFERENCES

1. Konijn, T.M., and Koevenig, J.L. (1971) Mycologia 63, 901-906.
2. Tso, W.-W., and Mansour, T.E. (1975) J. Behav. Biol. 14, 499-504.
3. Poff, K.L., and Skokut, M. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 2007-2010.
4. Hato, M., Ueda, T., Kurihara, K., and Kobatake, Y. (1976) Cell Struct. Funct. 1, 269-278.
5. Bonner, J.T., Clarke, W.W., Jr., Neeley, C.L., Jr., and Slifkin, M.K. (1950) J. Cell. Comp. Physiol. 36, 149-158.
6. Coman, D.R., (1940) Arch. Pathol. 29, 220-228.
7. Carlile, M.J., (1970) Gen. Microbiol. 63, 221-226.
8. Ueda, T., Muratsugu, M., Kurihara, K., and Kobatake, Y. (1976) Expt. Cell Res. 100, 337-344.
9. Konijn, T.M., van de Meene, J.G.C., Bonner, J.T., and Barkley, D.S. (1967) Proc. Natl. Acad. Sci. U.S.A. 58, 1152-1154.
10. Pan, P., Hall, E.M., and Bonner, J.T. (1972) Nature New Biol. 237, 181-182.
11. Keating, M.T., and Bonner, J.T. (1977) J. Bacteriol. 130, 144-147.
12. Bonner, J.T. (1977) Mycologia 69, 443-459.
13. Raper, K.B. (1940) J. Elisha Mitchell Sci. Soc. 56, 241-282.
14. Shaffer, B. (1962) Advan. Morphog. 2, 109-182.
15. Gerisch, G. (1968) Curr. Top. Develop. Biol. 3, 157-197.
16. Cohen, M.H., and Robertson, A. (1971) J. Theoret. Biol. 31, 119-130.
17. Loomis, W.F. Jr. (1972) Nature New Biol. 240, 6-9.
18. Rusch, H.P. (1969) Federation Proc. U.S.A. 28, 1761-1770.
19. Guttes, E., and Guttes, S. (1967) Experientia 23, 713-715.
20. Mohberg, J., and Rusch, H.P. (1969) J. Bacteriol. 97, 1411-1418.
21. Ueda, T., Terayama, K., Kurihara, K., and Kobatake, Y. (1975) J. Gen. Physiol. 65, 223-234.
22. Daniel, J.W., and Baldwin, W.W. (1964) in Methods in Cell Physiology, Vol. 1 (Prescott, D.M. ed.) pp. 9-41, Academic Press, New York.
23. Adler, J. (1969) Science 166, 1588-1597.
24. Tso, W.-W., and Adler, J. (1974). J. Bacteriol. 118, 560-576.
25. Zigmond, S.H., and Hirsch, J.G. (1973) J. Exp. Med. 137, 387-410.
26. Snyderman, R., Shin, S.H., Philips, J.K., Gewurz, H., and Mergenhagen, S.E. (1969) J. Immun. 103, 413-422.
27. Hickman, C.J., and Ho, H.H. (1966) Ann. Rev. Phytopathol. 4, 195-200.
28. Miller, R.L. (1966) J. Exp. Zool. 162, 22-44.
29. Carlile, M.J. (1973) Ber. Deutsch. Bot. Ges. Bd. 86, S. 123-139.
30. Garrod, D.R. (1974) J. Embryol. Exp. Morph. 32, 57-68.
31. Chang, Y.Y. (1968) Science 160, 57-59.