QL1.A53

Location:

DateReq: 4/25/2003 Yes

Date Rec: 4/28/2003 No

Borrower: JHW Conditional

ILL: 5998377 Maxcost: 25.00IFM LenderString: *JHE,JHE

Title: American zoologist.

Author:

Edition:

Imprint: [McLean, VA] Society for Integrative and Comparative Biology [

Article: Theibert, A., Fontana, D., Wong, T-Y. and Devreotes, P.: Cell-cell interactions in the development of Dictyostelium

Vol: 26 **No.:** **Pages:** 549-551 **Date:** 1985

Borrowing Notes: QL 1 .A53

Fax:

ILL: 5998377 :Borrower: JHW :ReqDate: 20030425 :NeedBefore: 20030525
:Status: IN PROCESS 20030425 :RecDate: :RenewalReq:
:OCLC: 1480990 :Source: OCLCILL :DueDate: :NewDueDate:
:Lender: *JHE,JHE
:CALLNO: :TITLE: American zoologist. :IMPRINT: [McLean, VA] Society for Integrative and Comparative Biology [etc.] :ARTICLE: Theibert, A., Fontana, D., Wong, T-Y. and Devreotes, P.: Cell-cell interactions in the development of Dictyostelium :VOL: 26 :NO: :DATE: 1985 :PAGES: 549-551 :VERIFIED: <TN:199044>OCLC ISSN: 0003-1569 [Format: Serial]
:PATRON: Antol, Pamela :SHIP TO: ILL/JHU/Welch Medical Library/1900 E.Monument St./Balto. MD 21205 :BILL TO: Same :SHIP VIA: Library Rate :MAXCOST: 25.00IFM :COPYRT COMPLIANCE: CCL :BORROWING NOTES: QL 1 .A53 :LENDING CHARGES: :SHIPPED: :SHIP INSURANCE: :LENDING RESTRICTIONS: :LENDING NOTES: :RETURN TO: :RETURN VIA:

ShipVia: **Library Rate**

Return To:

Interlibrary Services Department
Milton S. Eisenhower Library
Johns Hopkins University
Baltimore, MD 21218 USA

Ship To:

ILL

JHU

Welch Medical Library

1900 E.Monument St.

Balto. MD 21205

NeedBy: 5/25/2003

ILL: 5998377 **Borrower:** JHW

Req Date: 4/25/2003 **OCLC #:** 1480990

Patron: Antol, Pamela

Author:

Title: American zoologist.

Article: Theibert, A., Fontana, D., Wong, T-Y. and Devreotes, P.: Cell-cell interactions in the development of Dictyostelium

Vol.: 26 **No.:**

Date: 1985 **Pages:** 549-551

Verified: <TN:199044>OCLC ISSN: 0003-1569 [For

Maxcost: 25.00IFM **Due Date:**

Lending Notes:

Bor Notes: QL 1 .A53

AMER. ZOOL., 26:549–551 (1986)

Cell–Cell Interactions in the Development of *Dictyostelium*[1]

ANNE THEIBERT, DONNA FONTANA, TIT-YEE WONG, AND PETER DEVREOTES

Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland

The life-cycle of *Dictyostelium discoideum* consists of two distinct phases, a growth phase in which cells feed on bacteria, and a developmental phase which results in the formation of sorocarps (encapsulated spore cells held aloft by vacuolized stalk cells). In the developmental phase, which is initiated by removal of nutrients, the uniform cell monolayer divides into many territories of approximately 100,000 cells each, which aggregate to form multicellular structures. Aggregation is mediated by chemical signaling. Waves of adenosine 3′,5′ cyclic monophosphate (cAMP), initiated at aggregation centers, propagate through the cell-monolayer and provide transient cAMP gradients for the chemotactically responsive cells. Several parameters of the cAMP waves have been determined by both dark-field, time-lapse photography and an isotope dilution technique. The waves are initiated at 7–10 min intervals and propagate outwardly at a velocity of 300 μm/min. The cAMP concentration at the wave peak is 1 μM and the width of the wave at half-height is 700 μm. When the leading edge of the wave passes a cell, it responds by moving toward the center for about a 2-min interval. After the wave passes, the cells move randomly for 5 min until the next wave passes and triggers another center-directed movement step. Since cells at the distal edge of the territory must move as far as 1 cm, many waves and steps are required for aggregation. Early aggregation is complete when the cells have reached the "tight-aggregate" stage which usually takes about 10 hr. It has been postulated that cAMP either in the form of a stable gradient or waves may be involved later in development in both morphogenesis and differentiation of the aggregate into the sorocarp.

The cAMP waves are generated by a cell-to-cell signal relay system in which cells produce and secrete cAMP in response to cAMP. High-affinity receptors on the cell surface bind cAMP; perception of the signal results in activation of a membrane associated adenylate cyclase. The production of intracellular cAMP is followed by its secretion. The presence of both intracellular and extracellular phosphodiesterase can lead to degradation of the cAMP signal and response. In addition, activation of the cyclase is transient, even in the continued presence of exogenous cAMP, due to a process known as adaptation. Adaptation is reversible if the exogenous cAMP is removed.

Much research has focused on the activation and adaptation processes, however, unlike the analogous systems in higher eukaryotic cells, *in vitro* activation of *Dictyostelium* adenylate cyclase has not been observed. This has forced investigators to turn to other methods, which include the use of inhibitors of *in vivo* activation and identification and characterization of the known components in order to understand this response. In addition, many other responses to cAMP have been identified. They include a rise in intracellular cGMP, a decrease in extracellular pH, an increase in cell associated Ca^{2+}, changes in the methylation state of some proteins and phospholipids, a decrease in optical density of a cell suspension, changes in the phosphorylation state of some proteins, and an increase in the number of vesicles near the cell surface as seen by electron microscopy. Activation of adenylate cyclase and activation of the chemotactic machinery might

[1] From the Symposium on *The Cell Surface in Development and Cancer* presented at the Annual Meeting of the American Society of Zoologists, 27–30 December 1983, at Philadelphia, Pennsylvania.

involve any number of these responses in addition to those molecular events which have yet to be elucidated.

Studies using several inhibitors of *in vivo* activation of adenylate cyclase have yielded some interesting results which may relate to the mechanisms of activation and adaptation. First, the plant lectin, Concanavalin A (Con A) blocks the activation of adenylate cyclase in intact cells but does not alter cAMP binding to its surface receptor, inhibit basal cyclase activity, or reduce the ATP pool. Other surface binding agents, poly and monoclonal antibodies, and a chemical cross-linking reagent also inhibited the signaling response. However, the monovalent derivative of Con A, succinylated Con A, and the unlinked, monovalent derivative of the chemical cross-linking reagent were relatively ineffective as inhibitors, suggesting that cross-linking was the cause of inhibition. It was postulated that perhaps a membrane rearrangement such as endocytosis or exocytosis or a lateral or transverse movement of molecules within the plasma membrane is necessary for activation of adenylate cyclase.

A second inhibitor of adenylate cyclase activation, caffeine, has proven useful in understanding the molecular basis of adaptation. Caffeine rapidly and reversibly blocks activation of adenylate cyclase and was used to test whether adaptation depended on activation of adenylate cyclase. Caffeine blocked the initial response to cAMP and if adaptation depended on activation, adaptation should have been blocked as well. Hence the cells should have remained sensitive and responded when the caffeine was removed from the cAMP stimulus. However, the cells did not respond, indicating that adaptation had proceeded even in the absence of cyclase activation. Since adaptation is independent of activation, it is not caused by the rise in intracellular cAMP. Other studies have shown that adaptation is also independent of protein synthesis, and is not due to a loss of surface cAMP binding sites or a reduction in the substrate (ATP) pool. A conclusion consistent with these observations is that adaptation could be the result of a reversible covalent modification of a component in the signaling response. It is interesting to note here that adaptation is apparently a universal feature of the cell surface cAMP mediated responses, perhaps indicating that it may occur at a common, early step in the pathway of signal transduction.

A common control point of the responses mentioned earlier is binding of cAMP to the cell surface. A fundamental question is whether cellular responses are mediated by a single set of cAMP receptors or if each response is linked to a separate receptor. In an attempt to answer this question, the specificities of the receptors which mediate chemotaxis, cGMP accumulation, the cAMP signaling response and cAMP binding were determined and compared. This comparison reveals that the three responses and binding are mediated by receptors of nearly identical specificity. Clear molecular identification of the receptor(s) should resolve this question. Previous attempts at identification, plagued with problems which include the rapid dissociation rate, low affinity of photo-affinity labels, and presence of phosphodiesterase, have produced equivocal results.

Recently, however, some of these technical problems were overcome using a binding stabilization technique. The result was specific photolabeling of a single major cAMP binding protein that migrates as a doublet between 40 and 43,000 daltons in SDS-PAGE. The photolabeling of one major membrane species is consistent with the conclusion drawn from the specificity comparison. However, since a few minor proteins (5%) were also specifically photolabeled, it could not be ruled out that there exist other cAMP receptors on the surface which could be responsible for eliciting the responses.

The photolabeling studies yielded another exciting result. The electrophoretic mobility of the photolabeled receptor was dependent on whether the cells had been exposed to cAMP prior to photolabeling. The higher mobility form (MW = 40,000) was observed in the absence of cAMP treatment and a lower mobility form (MW = 43,000) was observed if cells were pretreated with cAMP. Furthermore, the

pattern of modulation (kinetics and dose dependence) of this cAMP-induced receptor modification correlates with the signaling adaptation process. The receptor is phosphorylated both *in vivo* and *in vitro* and phosphorylation increases upon cAMP stimulation. An exciting speculation is that the mechanism of adaptation is modification at the receptor level. It is possible for such a mechanism to mediate adaptation of all the cAMP mediated responses.

Molecular characterization of another component of the signaling response, the adenylate cyclase, has been hindered by the extreme instability of this enzyme. Recently, however, methods have been developed to purify the enzyme about 300 fold and greatly increase its stability. All the activity is membrane bound, and remains associated with an insoluble residue after extraction with the detergent CHAPS. This residue probably contains lipid and a large amount of cytoskeletal proteins. Unfortunately, solubilization of these detergent resistant membranes with retention of cyclase activity has been difficult. Therefore, alternative methods may have to be employed to identify this enzyme, including photoaffinity labeling of and preparation of monoclonal antibodies against these partially purified membranes. Regarding another component of the signaling system, a possible coupling protein between receptors and cyclase, little is known since the *in vitro* activation has not been successful. However, the occurrence of an apparent N_s protein in *D.d.* in both membranes and cytosols has been observed. This protein specifically binds N_s-GTP, is ADP-ribosylated by cholera toxin and NAD, and focuses on a 2-D gel in a position similar to the MW = 42,000 subunit of the N_s regulatory protein which links the hormone and neurotransmitter receptors to adenylate cyclase in vertebrates. A "coupling" function of this protein has yet to be shown in *Dictyostelium.*

This article has summarized some of the studies of the cAMP-mediated cell–cell communication which directs the transition from single cells to a multicellular form. The life cycle of this simple eukaryotic organism consists of both single and multicellular phases and the developing cells exhibit phenomena which include pattern formation, morphogenesis, differentiation, and specific cell death. The ease of culture, ability to generate mutants, and development on a monolayer make it an amenable model that may apply to the three-dimensional development in higher systems.