Acquisition of multicellularity

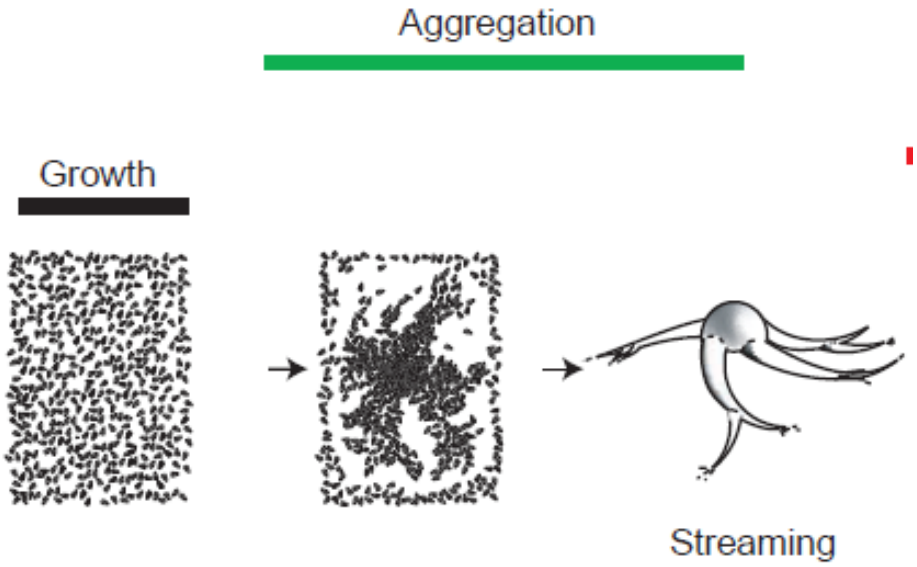
Signaling in Dictyostelium

Introduction

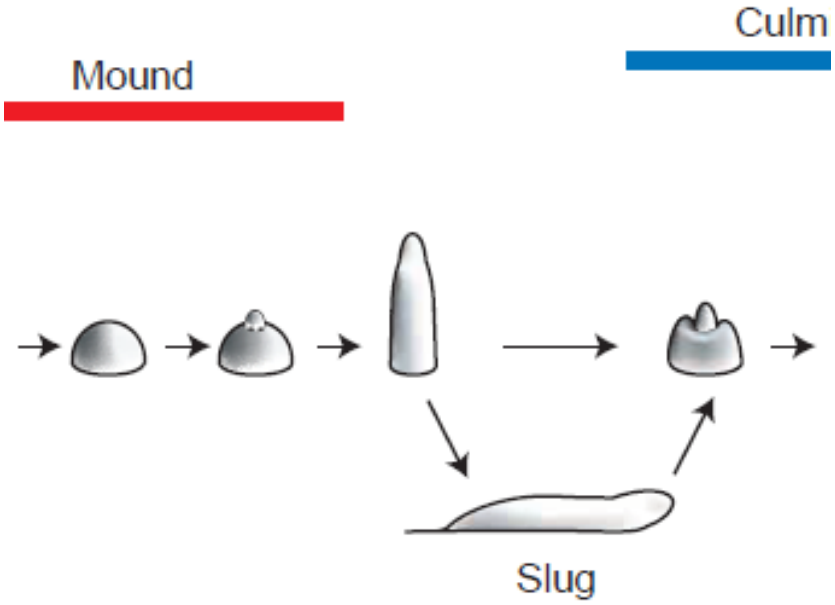
- Lineage› cellular organisms
  - › Eukaryota
  - › Amoebozoa
  - › Mycetozoa
  - › Dictyostelids
  - › Dictyosteliales
  - › Dictyosteliaceae
  - › Dictyostelium
- A type of multicellular organization derived from unicellular organisms is found in *Dictyostelium discoideum.*
- In its asexual cycle, solitary haploid amoebae called *myxamoebae.*
- They live on decaying logs, eating bacteria and reproducing by binary fission.

Introduction

- When they have exhausted their food supply, tens of thousands of these *myxamoebae* join together to form moving streams of cells that converge at a central point.
- They pile atop one another to produce a conical mound called a tight aggregate.
- A tip arises at the top of this mound, and the tight aggregate bends over to produce the migrating slug.

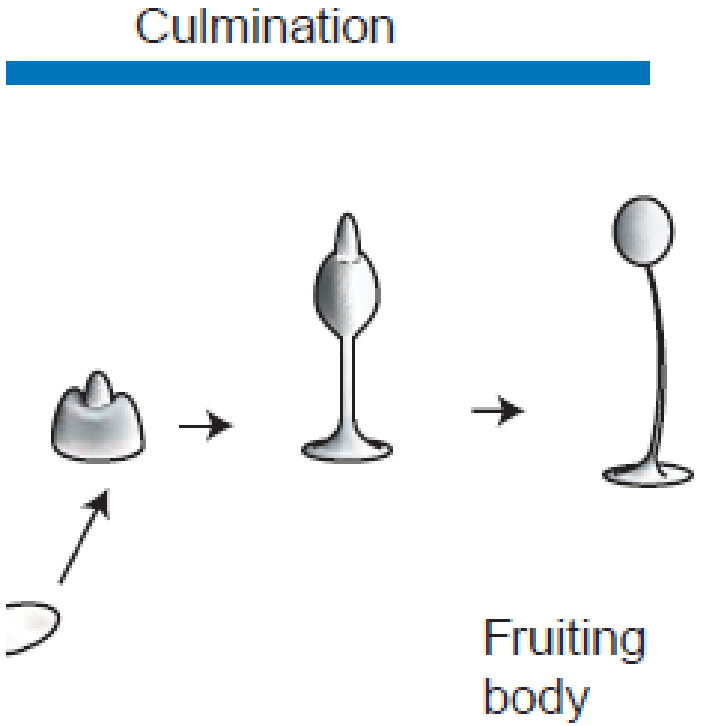


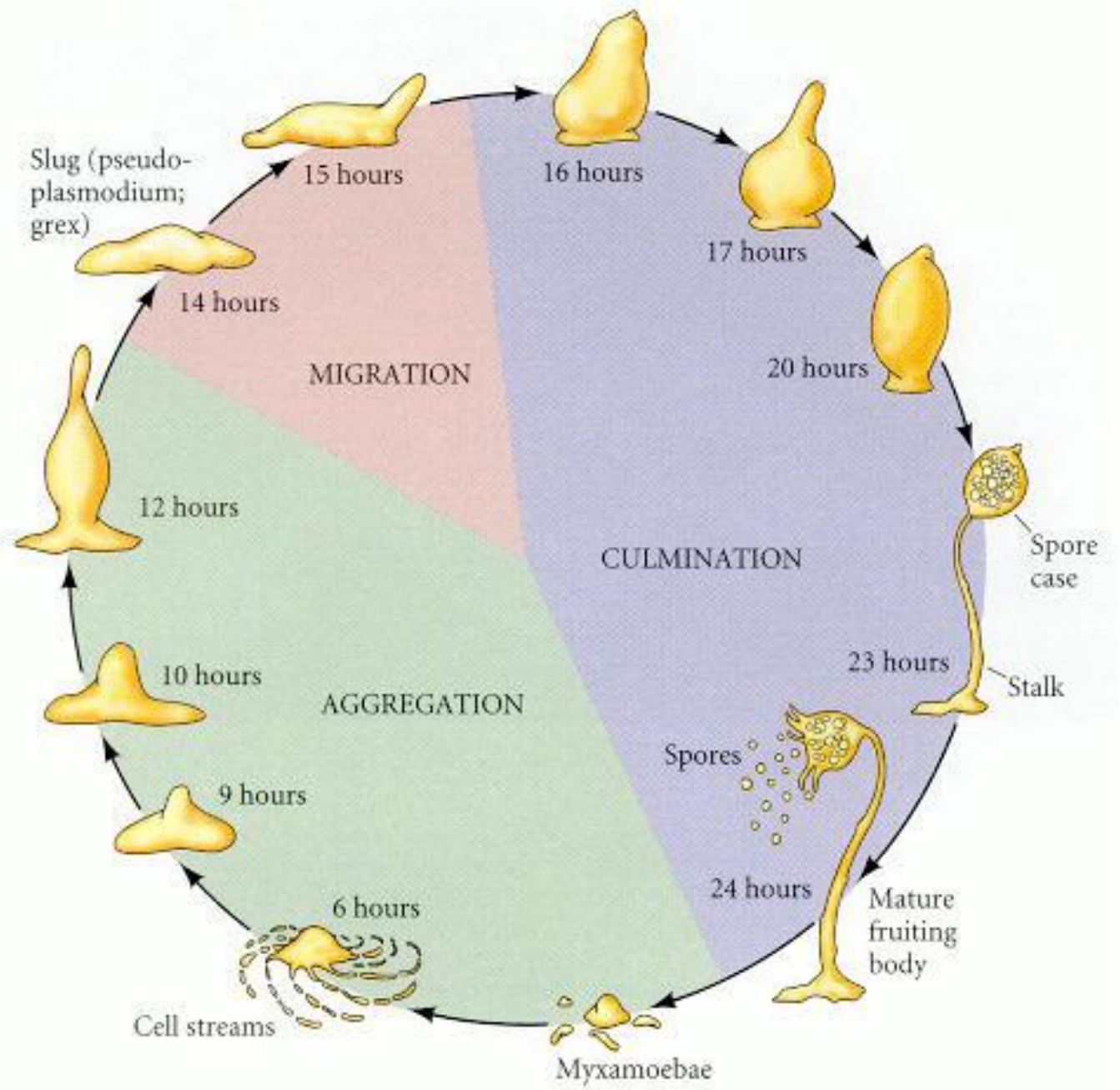
- The slug / pseudoplasmodium or grex) is usually 2- 4 mm long and is encased in a slimy sheath. The grex begins to migrate (if the environment is dark and moist) with its anterior tip slightly raised. When it reaches an illuminated area, migration ceases, and the grex differentiates into a fruiting body composed of spore cells and a stalk.



- The anterior cells, representing 15- 20% of the entire cellular population, form the tubed stalk. This process begins as some of the central anterior cells, the prestalk cells, begin secreting an extracellular coat and extending a tube through the grex. As the prestalk cells differentiate, they form vacuoles and enlarge, lifting up the mass of prespore cells that had made up the posterior four-fifths of the grex (Jermyn and Williams 1991).

- The stalk cells die, but the prespore cells, elevated above the stalk, become spore cells. These spore cells disperse, each one becoming a new myxamoeba.





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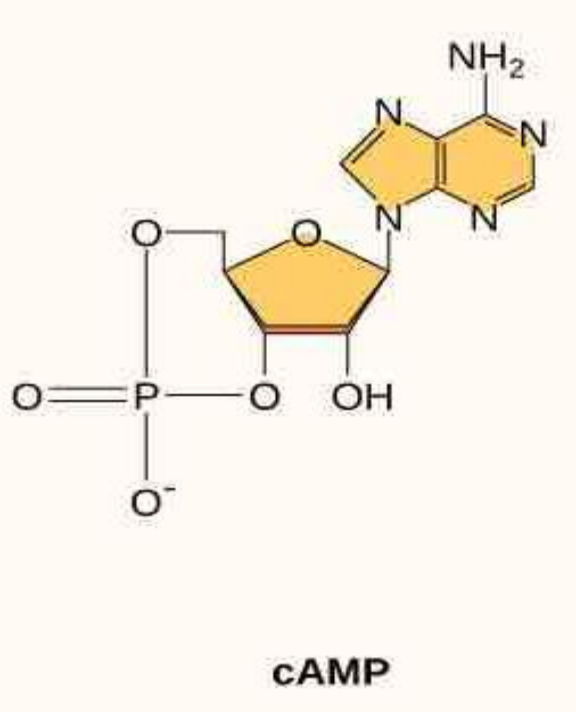
Aggregation

- Aggregation is initiated as each of the cells begins to synthesize cAMP (cyclic adenosine 3´,5´-monophosphate).
- Cells start secreting cAMP and the site of aggregation is determined by the density of myxamoebae.
- Neighboring cells respond to cAMP in two ways: they initiate a movement toward the cAMP pulse, and they release cAMP of their own.
- Cells now migrate and then move in a spiral to the a center.

Cell-cell adhesion

- *Dictyostelium* cells have adopted a strategy for multicellular development that differs from that of metazoa.
- *Dictyostelium* development, however, requires no growth, and multicellularity is achieved by aggregation of many unicellular amoebae.
- *Dictyostelium* cells must first aggregate to form a multicellular mass: the mound. The driving force behind this processes is chemotaxis towards a pulsatile source of extracellular cyclic AMP (cAMP).



cAMP

Cell-cell adhesion

- Amoebae move as individual cells towards the signal. Near the source, and cell density increases, cells coalesce into multicellular streams to form a mass of up to 10^5 cells.
- Cells enter the multicellular stage of development and begin to differentiate into pre-spore and pre-stalk cells, the precursors of spore and stalk cells, respectively

Cell-cell adhesion molecules

- Pioneering work in *Dictyostelium* identified several proteins that mediate cell-cell adhesion.
- Protein known to be expressed is DdCAD-1(cell adhesion molecule encoded by the *cadA gene*), also named contact sites B (csB) or gp24 (glycoprotein 24KD) it is present within the cytoplasm and is only slightly enriched at the plasma membrane. However, as aggregation proceeds, DdCAD-1 redistributes to the external surface of the plasma membrane.

DdCAD-1

- DdCAD-1-mediated cell adhesion is sensitive to both EDTA and EGTA, which suggests that Ca2+ is involved in this process.
- DdCAD-1 has been cloned, and it shares some homology with the extracellular domain of metazoan cadherins with two binding Ca^{++} sites (mammalian have 5 Ca^{++} binding domains).

Fruiting bodies

- Once this initial aggregation has occurred, it is stabilized by a second cell adhesion molecule.
- 80-kDa glycoprotein is also synthesized during the aggregation phase.
- The second cell adhesion system seems to be needed for retaining a large enough number of cells to form large fruiting bodies.

csA (aka gp80)

- This protein, csA has a mass of 54 kDa and is induced by the cAMP pulses that mediate chemotaxis.
- csA is a globular protein similar to the neural cell adhesion molecule N-CAM; molecular weight of 80 kDa.

Cell-cell adhesion mutants

- Blocking DdCAD-1 binding by antibodies and carnitine arrests *Dictyostelium* development.
- When cells that lack csA are mixed with wild-type cells in cell suspension, both cell types sort out to form strain specific aggregates.
- smlA mutants show reduced expression of DdCAD-1 and csA during streaming and early aggregation. This leads to reduced intercellular adhesion and causes *smlA* (Small aggregate formation protein) streams to break up and eventually form aggregates smaller than those of the wild-type.

Cell-cell adhesion mutants

- *Dictyostelium* possessing mutations in *countin*, limits the size of aggregates.
- Increased DdCAD-1 expression and cell-cell adhesion during early development leads to the formation of giant aggregates and fruiting bodies

Regulation

- The differentiation of individual myxamoebae into either stalk (somatic) or spore (reproductive) cells is a complex matter.
- Surgically removing the anterior part of the slug does not eliminate its ability to form a stalk.
- In fact, the cells that now at the anterior end (and which originally had been destined to produce spores) now form the stalk.
- Ability of cells to change their developmental fates according to their location within the whole organism and thereby compensate for missing parts is called ***regulation***.

Migration

- Third cell adhesion system is activated late in development, while the slug is migrating.
- This protein appears to be important in the movement of the prestalk cells to the apex of the mound

Differentiation

- Differentiation into stalk cell or spore cell reflects another major phenomenon.
- In *Dictyostelium* only two cell types are possible- stalk cell or a spore cell.
- The two major candidates are differentiation inducing factor (DIF) and cAMP.
- DIF appears to be necessary for stalk cell differentiation.

DIF

- Isolated myxamoebae or even to prespore (posterior) cells, form stalk cells when treated with DIF.
- Synthesis of this lipid is genetically regulated, mutant DIF^-, form only spore precursor cells but no stalk cells, when these are treated with DIF form stalk cells and new prestalk-specific mRNAs are seen in the cell cytoplasm.

cAMP

- During aggregation, starved cells typically move towards the aggregation center to form one multicellular aggregate.
- This coordinated migration is achieved by the self-organization of cAMP gradients and by chemotaxis to extracellular cAMP.
- cAMP is synthesized in response to external cAMP signals and secreted to induce neighboring cells to similarly produce cAMP.

cAMP

- During the aggregation cells form a loose mound, which is tightly packed due to secretion of extra cellular matrix leading to increase cell – cell contact.
- The tightly packed cells differentiate into pre stalk and pre spore cells.
- Conventional microscopic observations, show synchronous changes in cell shapes and act as an index of cAMP relay.
- Optical density waves have been detected in streams, mounds, and slugs, giving evidence of cAMP relay at these stages too.

cAMP

- Cell sorting to the tip of the mound also can be explained by cAMP relay.
- There is a difference in the response of chemotaxis toward cAMP between prespore and prestalk cells in mounds guided by cAMP relay in the sorting.
- It has been demonstrated cells of the slug produce cAMP upon extracellular cAMP stimulation and show chemotactic movement toward cAMP.
- Thus, cAMP relay is regarded as an essential mechanism for organized collective cell migration, such as cell sorting and multicellular movement, in *Dictyostelium* cells.

- Cells, which lack the ability of cAMP relay, normally cannot aggregate and form multicellular bodies.
- Mutant are rescued by constitutive activation of PKA, which is downstream of the cAMP signaling pathway, implying that *Dictyostelium* cells have developmental ability without cAMP oscillation.

References

- Collective cell migration of *Dictyostelium* without cAMP oscillations at multicellular stages . Hidenori Hashimura1,2, Yusuke V. Morimoto 2,3, Masato Yasui2 & Masahiro Ueda1,2,4. *COMMUNICATIONS BIOLOGY*

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