« History and choice of marine model species: Example of the ascidian \textit{Phallusia mammillata} »

Janet Chenevert
LBDV McDougall team « Cell cycle in eggs and embryos »

\textit{Phallusia and Paracentrotus development video: Christian Sardet}

\textit{Possible topics for discussion :}
What is a "model" organism?
Loss of diversity in models and scientific ideas.
Advantages of autonomous vs regulative development.
Relationship between developmental biology and cell biology.

“The orientation of intracellular movements by which different substances are segregated and localized and the mitotic spindles turned into particular positions so that cleavage takes a characteristic form for each species is one of the most important processes in development.”
\textit{E. Conklin 1929}
What is a “model organism”? Many definitions

Such as: “Any non-human species that is used to understand biological processes”

“Models are necessary lenses through which we focus on research problems” Ronald Jenner

The “Krogh principle” August Krogh 1929. For a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied.

Claude Bernard 1865. "Dans l'investigation scientifique, le choix heureux d'un animal suffit souvent pour résoudre les questions générales les plus élevées."

Wilhelm Roux 1894, a founding father of Developmental Biology
“The ultimate goal is to explain developmental events in terms of the laws of physics and chemistry. Because this new branch of biology (“developmental mechanics”) values all methods that give rise to causal knowledge, and utilizes all biological disciplines for its purposes, its area of investigation encompasses all living organisms from the lowest protestant to the highest animals and plants.”
What is a “model organism”? Many adjectives!

A few species are heavily studied by many labs (ie mouse, fly, worm, yeast)

Many species are studied by only a few labs (ie marine invertebrates)

Why we at marine stations are frequently preoccupied with this « Model » issue:
- requests for funding, training grants, aquarium renovations...
- keep alive the expertise (workshops, summer schools)
Timely:
- genomic coming of age (sequencing, molecular techniques)
- current interest in biodiversity/climate change/ocean ecosystems
- recent initiatives EMBRC, European Marine Biological Resource Center
  EFOR Etudes fonctionnelles organismes modeles

catalog of potential species useful for extending research in new directions
History of developmental biology

Choice of model

Philosophy?

History of *Phallusia*

History of developmental biology
Ascidian embryos are good models for: embryonic development, evolution of the vertebrate body plan, fundamental processes in cell biology.

Features of ascidians = seasquirts:
- Chordates, sister group to vertebrates,
- Hermaphroditic, External fertilization
- Rapid synchronous development to tadpole
- Small cell number, invariant lineage
- Genome is compact, unduplicated

Scanning electron micrographs of *Phallusia mammillata*
My favorite (emerging non-canonical genetically-intractable unusual alternative yet traditional and classical) model species: the European ascidian *Phallusia mammillata*

Abundant gametes year-round, hardy adults, Genome sequenced 2012, cDNA clones arrayed

Well suited for imaging, Transparent eggs which translate exogenous mRNAs such as those encoding fluorescent GFP proteins.

Unlike *Ciona intestinalis*, Ascidian species of reference, genome sequenced 2002
MÉMOIRE
SUR LES ASCIDIES
ET SUR LEUR ANATOMIE.
PAR M. G. CUVIER.

Je suis obligé, comme à mon ordinaire, de commencer mes recherches par un exposé historique des variations bizarres que la nomenclature de ces animaux a éprouvées.

Les Ascidies sont du petit nombre des mollusques dont le nom ancien ne laisse point de doute. Aristote les appelle thethyum; il les avait parfaitement bien observées, et la description générale qu'il en donne (Hist. An., lib. IV, cap. VI; et de Part. An., lib. VI, c. V) est aussi exacte que celles de nos auteurs modernes.

Rondelet paroit les avoir bien reconnus, quoique sa figure et sa description (de Ins. et Zooth., 127) ne puissent faire déterminer positivement l'espèce dont il a parlé. On distingue un peu mieux deux autres animaux dont il traite ensuite (p. 128 et 129) sous le nom de mentula marina, et qui sont également deux espèces d'ascidies. En effet, les pêcheurs de la Méditerranée, gens peu réservés dans leur langage, donnent encore aujourd'hui aux ascidies, dans leurs divers jargons, des noms qui équivalent à celui-là.

Je viens à présent aux ascidies où le sac branchial, après être descen du jusqu'au fond de la tunique propre, se recouvre et remonte jusque vers le milieu du corps pour prendre plus d'estension.

Nous en avons une belle et grande espèce de la Méditerranée que je crois proprement celle qui a servi de type au pudendum marinum alterum de Rondelet, et qui en conséquence seraient la véritable ascidia mentula de Linnaeus, mais non pas celle que Müller et Gmelin ont confondue ensuite avec elle.

L'espèce dont je parle a de 4 à 6 pouces de longueur sur 2 ou 3 de largeur; sa teinte est d'un iamnâtre clair; sa surface est toute marmelonnée ou comme bosselée, par grosses inégalités arrondies. Sa substance est cartilagineuse et épaisse, en quelques endroits, de plus de 6 lignes. Pour ne point donner lieu à de nouvelles confusions de synonymie, je lui assigne le nom d'ascidia bosselée, ascidia mammillata.

Le sac extérieur produit en dedans une arête saillante qui se continue et se termine par une petite pourrassure du corps proprement dit, pour maintenir l'une et l'autre en situation, et c'est vers le bas de cette arête qu'il reçoit les vaisseaux dont les ramifications pénètrent toute sa substance et y produisent un très-beau effet en se montrant au travers de sa demi-transparence.
Highlights of history of experimental work with *Phallusia mammillata*

**Cuvier (French) 1815**

**Kowalevsky (Russian) 1866, 1871** discovers that tunicates are chordates, using *Phallusia* and *Ciona* in Trieste Italy

**Driesch (German) 1895 -1905 Naples**

**Conklin (American) 1911** “The organization of the egg and the development of single blastomeres of *Phallusia mammillata* » JEZ Naples

**Italian school** 1931--1962: SZN Anton Dohrn Naples (Palermo, Milano) Reverberi, Ortolani, La Spina, Monroy

1956 Reverberi observes movement of mitochondria during development using vital dyes “it would be very useful to analyze by cinematography”

**French school** 1974, 1979, 1980

**Villefranche tradition:** Christian Sardet etc 1984-2013

Genome sequenced, collection of cDNA clones...

The transparency of *Phallusia* is the cause of a controversy over whether development is mosaic or regulative, whether the egg has any « differentiation »
Edwin Conklin (1905a, b, c) working in WoodsHole using *Styela, Ciona, Molgula* established the cell lineage up to gastrula stage. He observed localized domains partitioned into distinct lineages and proposed that « organ forming substances » (maternal determinants) direct fates of cells which inherit them.

This discovery was possible because the eggs of *Styela partita* happen to contain naturally pigmented regions which can be followed into various blastomeres of the dividing embryo and larva.

How did Conklin accidentally happen onto *Styela*?

“The very first lot of the living eggs of *Styela* which I examined showed a most remarkable phenomenon and one which modified the whole course and purpose of my work; for there on many of the unsegmented eggs, which were of a slate-gray color, was a brilliant orange-yellow spot, which in other eggs appeared in the form of a crescent or band.”

Yellow Myoplasm = posterior mitochondria-rich domain segregates into muscle cells of tadpole tail

Similarly, special pigmentation in *Parecentrotus* allowed Boveri (1901) to discover relationship between egg polarity, cleavage pattern and embryonic axes.
19th century Debate about Development

“Preformation”

Mosaic=determinant=invariant=
autonomous, independent.
Cell fate involves intrinsic factors ie
inherited determinants. (“maternal”)
Eggs are “differentiated” ie
inhomogeneous, compartmentalized.

versus

“Epigenetic”

Regulative=Indeterminate =
nonautonomous, dependent, conditional.
Cell fate depends on extrinsic factors ie cell
interactions. ("zygotic")
Eggs are “undifferentiated” ie
homogeneous, isotropic.

...changing terminology for same concepts...

Societal debate: these ideas about the fate of a cell in an embryo
were used to defend opposing schools of thought in
Religion: Creation (preformation)
Politics: Conservative, hereditary rights vs Liberal, all are equal
Philosophy, the properties of the whole can or cannot be predicted
solely from the properties of the component parts
Literature: Brenner dubs the European vs American plan
Psychology: Genetics vs environment; Nature vs nurture
“Preformation”
*Mosaic / autonomous* versus “Epigenetic”
*Regulative*

Roux 1885 “When distinguishing independent from dependent differentiation, what matters solely is to establish whether the specific cause of a developmental change is located within the part undergoing the visible change or outside of it”

Isolated blastomeres develop as they would in the intact embryo, giving only the cell types expected from the cell lineage

Isolated blastomeres compensate and produce more than the structures expected from the cell lineage

**Frog** Wilhelm Roux 1888

**Sea Urchin** Hans Driesch 1895

What about ASCIDIAN?

Chabry 1887 « Pour l’ascidie et les animaux dont les blastomeres sont différenciés de bonne heure, chaque blastomere contient en puissance certaines parties dont sa mort entraîne la perte irrémédiable et que les différentes parties de l’animal sont préformées dans les différentes parties de l’œuf... L’œuf d’ascidie se comporte comme si il contenait en puissance un seul adulte déterminé et que chaque partie de l’œuf contint une partie de cette adulte. »
Ascidian embryo separated into left and right blastomeres gives larvae which are:

PARTIAL (half larvae)
- Chabry 1887 (*Ascidiella aspersa*)
- Conklin 1905c (*Styela partita*, *Ciona intestinalis*, *Molgula manhattensis*)

COMPLETE
- Driesch 1895 (*Phallusia mammillata*)
- Crampton 1897 (*Molgula manhattensis*)
- Driesch 1905 (*Phallusia mammillata*)

Conklin 1906  Roux Archives “Does half an ascidian egg give rise to a whole larva?”

“In view of the fact that Driesch has stated that the eggs of *Phallusia mammillata* show none of the ooplasmic differentiations which I had observed in the eggs of other ascidians, and since he has maintained that 1 out of 2 or 4 blastomeres of *Phallusia* may give rise to an entire gastrula and larva, contrary to the observations of Chabry and of myself on other ascidians, it has seemed to me desirable to reinvestigate the egg of *Phallusia* in order to determine, if possible, whether it differs from other ascidian eggs in these respects.
1910: Conklin travels to Naples to resolve the debate. “The organization of the egg and the development of single blastomeres of *Phallusia mammillata*” J Exp Zool 1911

“The egg of *Phallusia mammillata*, although it appears perfectly homogeneous and as clear as glass in the living condition, shows when fixed and stained, the same fundamental differentiations and distribution of these ooplasmic substances as have been found in the egg of other ascidians.

½ larvae always lack the organs of the missing half.

“... the distinction between the various ooplasmic substances, such as ectoplasm, endoplasm, mesoplasm, etc., are not so easily seen in *Phallusia* as in *Styela* and it is doubtful whether they would have attracted my attention if I had not been acquainted already with ascidian eggs in which these distinctions are strikingly evident.”

“... in short *Phallusia* is not so favorable a form for the study of egg organization”...!
In interpreting ascidian experiments, Driesch was undoubtedly influenced by his urchin results. This same thing later happened to Conklin with respect to myoplasm and Amphioxus.

Driesch later abandoned developmental biology and became a philosophy professor. He felt that it was impossible to conceive of a biological machine that can be fragmented in space and the fragments still form a complete machine. He despaired of finding a causal explanation for regulative development and resorted to a philosophy of vitalism (“entelechy”) in which a supernatural life force/controlling consciousness directs embryonic development.
Today *Phallusia* is the ascidian species of choice for the study of spatial organization of the egg and orientation of cell divisions in the embryo.

From Sardet and Zalokar 1984

« Phases of cytoplasmic and cortical reorganizations of the ascidian zygote between fertilization and first division » Roegiers, Djediat, Dumollard, Rouviere, Sardet 1999

Dumollard and McDougall in press
Comparative Cell Biology (= Evo-Devo?)

1. **Cell polarity** « Cytoplasmic domains in eggs »
   Sardet, McDougall, Houliston; Trends in Cell Biology 1994
   « Comparative axis establishment »
   Medecine/sciences; Sardet et al

2. **Asymmetric Cell Divisions** generate daughter cells with distinct size, content, or fate

   **Meiosis** (polar bodies)
   Ascidian
   Germ line
   yeast    worms    flies    urchin    mammals

   Xenopus
   Phallusia
   C elegans
Comparative Cell Biology (Evo-Devo?)

3. Why is the invariant cleavage pattern of ascidian embryos so conserved among distantly related species?
Team « Cell cycle in eggs and embryos »
Alex McDougall
Remi Dumollard
Gerard Pruliere
Janet Chenevert
Lydia Besnardeau
Celine Hebras
Vlad Costache

Friends of Phallusia:
Christian Sardet
Fabrice Roegiers
Alexandre Paix
Solenn Patalano