



## **Experimental Techniques in Developmental Biology: On-Line Self-Study**

### **Goals of this exercise**

- To understand how the fields of developmental biology and human embryology developed as a result of old and recent technologies
- To understand how some of the concepts were developed those these techniques
- To learn about a number of major techniques that are employed in the field
- To learn how to read the results of these techniques (e.g., examine images and determine localization of gene or protein expression).
- Some of the terms will also become clearer as they are used during lecture

### **Descriptive Embryology**

- Understanding the normal and abnormal events that happen during embryological events through direct study of the embryos without experimental intervention
- Comes about through observation using light and phase contrast microscopy of living, fixed and stained tissues, electron microscopy for fine structural details.
- Confocal microscopy which uses laser technology is increasingly used because it allows for thin optical sectioning of living and stained tissues providing very high resolution and precise localizations. It's also possible to put all the optical sections together from such imaging to produce 3D images of tissues and organs.

### **Experimental Embryology**

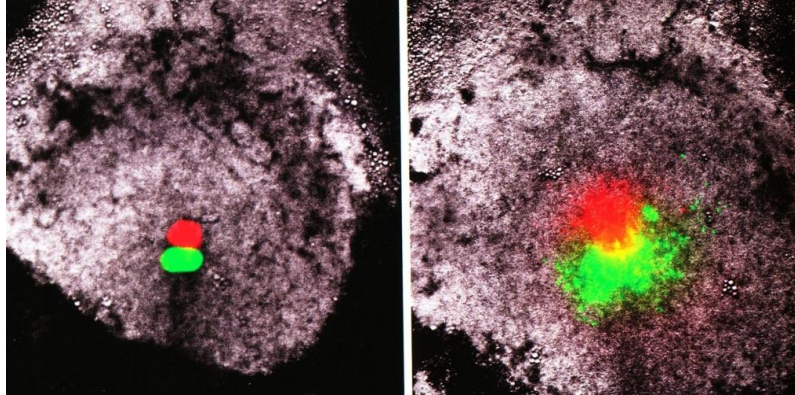
- Insight into normal and abnormal events that happen during embryological events through experimental intervention of embryos

#### Classical Experimental Embryology

- Early work involved microsurgical approaches (i.e., cutting and pasting of embryonic tissues and regions): parts of the embryo were removed or cut out and transplanted to other places in the same or different embryos
- Embryologists also stained cells and cell groups with vital dyes and followed their movements to gain insight into how cells and tissues moved within the embryo (e.g., during gastrulation)
- Green fluorescent protein (GFP) sequences can be inserted into the start or end of a sequence for a specific gene and then an expression vector containing the GFP-labelled gene can be inserted into the egg or embryo to follow its expression and localization. This is a primary tool used by cell and developmental biologists.
- Today genetic approaches involve inserting GFP-expression vectors allow more precise determinations of where cells move to and what they become. Several examples will be discussed in lectures.

## Experimental Techniques

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Fluorescent dyes used to map cells during gastrulation (from Larsen's Embryology, 4<sup>th</sup> ed, Elsevier)

- This led to the development of "fate maps": maps of the embryo showing what tissues were destined to develop into
- Further experiments clarified if the "fate" of a cell, cell group or tissue was determined (i.e., couldn't form anything else; it's removal led to the loss of specific structures; adding it to an embryo caused specific alterations) or regulative (i.e., the missing part didn't affect further development or an added part incorporated into the embryo resulting in normal development).
- Such experiments led to the concept of induction where one cell influences another to develop into a specific embryonic component (e.g., chordamesoderm induces neural tube; optic cup induces lens of the eye) as detailed in lecture

### Modern Experimental Embryology

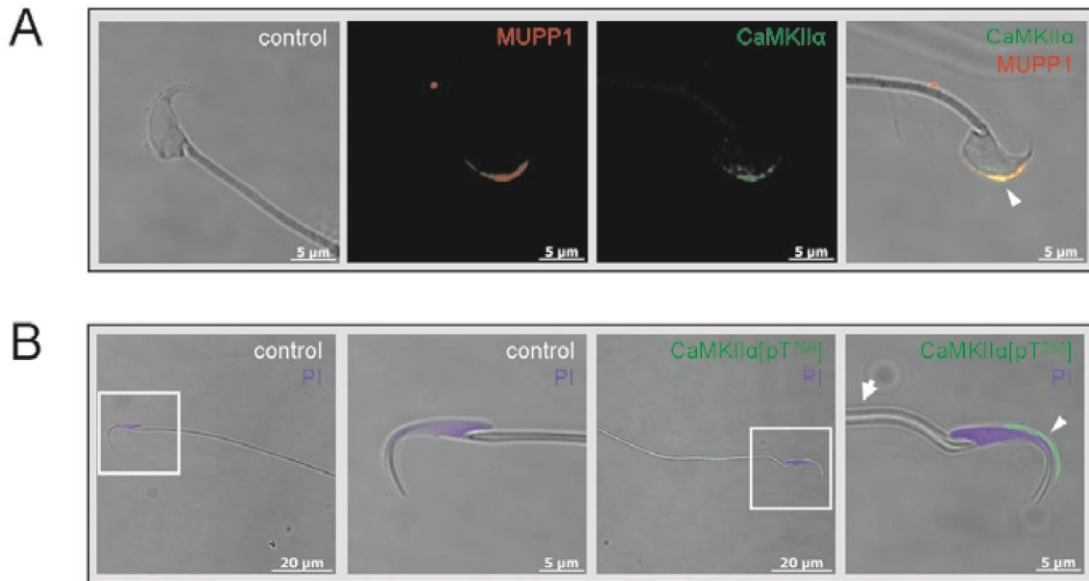
- A diversity of current techniques have been developed which have led to further understanding of the concepts developed through early experimental and descriptive embryology
- These approaches have also led to new concepts and ideas

### **Current Approaches To Studying Embryonic Development**

#### Immunohistochemistry

- Embryos (normal, mutant, exposed to teratogens, etc.) are fixed, sectioned (if necessary) and probed with a primary antibody against a specific protein after which the bound antibody is visualized by binding to a secondary antibody. The secondary antibody is bound to a marker (e.g., horse radish peroxidase) that can be detected by specific methods (e.g., a colour reaction).
- Co-localization with several antibodies can also be done if the antibodies were made in different animals.
- Such immunolocalization is often coupled with other immunofluorescence (e.g., Rhodamine-phalloidin for F-actin) or other kinds of staining (DAPI or Hoescht for nuclear DNA). Not shown in the following figure.

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Colocalization of CaMKII $\alpha$  and MUPP1 in rodent spermatozoa. MUPP1 is a tethering protein that links the acrosomal vesicle to the cell membrane. In brain MUPP1 also binds to calmodulin-dependent kinase II alpha (CaMKII $\alpha$ ). The research here was done to reveal the relationship between these proteins in mammalian spermatozoa. To determine the subcellular localization of MUPP1 and CaMKII $\alpha$  in spermatozoa, freshly isolated epididymal mouse sperm were simultaneously probed with a rabbit anti-MUPP1 and a mouse anti-CaMKII $\alpha$  antibody (A). Note the colocalization of MUPP1 (red) and CaMKII $\alpha$  (green) in some regions of the crescent-shaped acrosomal region. Samples incubated with only the secondary antibodies were unstained (control). A polyclonal anti-CaMKII $\alpha$ -Thr286-P antibody was used to visualize the subcellular localization of the identified CaMKII $\alpha$  in rat spermatozoa (B, CaMKII $\alpha$ -[pT286]). Immunostaining was restricted to the midpiece region of the sperm tail (arrow) and to the convex side of the sperm head, which represents the acrosomal region (arrowhead). Negative controls represent samples incubated with the secondary antibody only (control). The boxes indicate regions that are magnified in panels to the right. Fig. 2 from Ackermann et al, 2010.

## In Situ Hybridization

-Messenger RNA in embryos can be detected by binding to complementary RNA molecules (also called riboprobes). The riboprobe has typically been labelled with a marker such as digoxigenin (DIG) that can be detected by anti-DIG using immunohistochemistry. In the past radioactive riboprobes were common but are used less frequently as safer, more sensitive probes are being developed.

## **Manipulating Gene Expression**

### Gene Knockouts and Knock-ins

-Genes of interest can be deleted (gene knockouts) or changed to a mutant form (knocked in) through gene targeting after which researchers can: determine the effects on development in a diversity of ways

-The production of transgenic mice is discussed in a subsequent tutorial

### Knock-down experiments

-embryonic cells can be injected with morpholinos (stabilized mRNAs) or RNAi (interfering double-stranded RNA) which decrease gene expression rather than completely blocking it.

The genetically manipulated embryos are then studied using the diversity of techniques open to cell and developmental biologists.

We will cover examples of knockout mutants in lecture. Do a search for examples of knock-ins and knock-down mutants as they relate to development. You don't need to understand the molecular bases of these approaches just how they can be used to elucidate developmental issues. Later in the course we'll look at the

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use of these approaches in studying the genes involved in eye development (Analyzing Genes Involved in Eye Development: On-Line Exercise).

## References

Ackermann et al, 2010. CaMKII $\alpha$  interacts with multi-PDZ domain protein MUPP1 in spermatozoa and prevents spontaneous Acrosomal exocytosis. J. Cell Sci. 122: 4547-4557.

Smith et al, 2010. Which FGF ligands are involved in lens induction? Developmental Biology 337: 195-198.

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