

Using genetics to establish gene function and relationships between genes during *C. elegans* development**Reading: these notes**

Gilbert 8e: pp. 158-160, pp. 243-250

Gilbert 9e: pp 192-199; pp 99-100

Learning goals:

Compare the phenotypic consequences of maternal-effect and non-maternal-effect mutations

Interpret crosses between animals with mutations in maternal-effect genes.

Explain what studying maternal-effect mutants informed us about control of blastomere fates in the embryo.

Describe and interpret the experiments that demonstrated how the cells of the vulva take on their different fates.

Just as we discussed genes important for early cell fate determination events in vertebrate embryos, we will now investigate the experimental approaches taken to identify developmentally relevant genes in invertebrates as well. In fact, much of the work in invertebrates led to searches for similar genes in vertebrates that we have already discussed. We have also already discussed the importance of maternally contributed products to embryonic development, apparently in all organisms except mammals. From a broad perspective, removing the function of any gene involved in early developmental events lead to a similar phenotype: death of the embryo. An embryonic lethal mutation, then, is simply one that causes embryonic defects and death of the embryo before hatching. To actually analyze more carefully the contributions of these genes to cell fate determination, scientists figured out how to look for observable phenotypes. How can mutations in the genes that are required for cell fate determination (not just general sustainability of the embryo) be distinguished from those in the thousands of other genes required for embryonic development?

Maternal-effect embryonic lethal mutations identify genes that control very early events

**note: this topic is not really described in your book. Use the notes and class slides to achieve understanding!*

As you already know, very little transcription occurs in most embryos until the onset of gastrulation, when many genes become active at the mid-blastula transition. Therefore, most events during the cleavage stage, including determination of blastomeres, must depend on the products of genes that were transcribed in the mother during oogenesis and deposited into the egg. In contrast, gastrulation and subsequent organogenesis and morphogenesis require expression of the embryonic genome. This distinction becomes apparent in genetic analysis of embryogenesis. When an embryonic lethal mutation occurs in a gene encoding a maternally supplied component, it will show maternal effects, characterized by the following:

- It is the genotype of the mother, not the genotype of the embryo, that determines whether the embryo is defective.
- If the mutation is recessive, one wild type copy of the gene in the oocyte will be sufficient for survival of the embryos, no matter what their genotype. Thus, a mother heterozygous for such a mutation, $m/+$ (carrying one mutant and one wild-type copy of the gene) will produce only healthy embryos by self-fertilization, even though 1/4 of them will be homozygous for the mutation (m/m). If the gene is not required during later development, these m/m embryos will develop into adult hermaphrodites, which will produce *only* defective embryos, even if mated to a wild-type male. Such a mutation, in which only the mother's genotype determines the embryonic phenotype, is called a strict maternal-effect mutation.
- Less common are partial maternal-effect mutations. These behave as above, except that when an m/m embryo is mated to a wild-type male, all of the embryos, which are genotype $m/+$, will survive. This behavior means that the gene product is required somewhat later after fertilization, so that it can be supplied *either* by the maternal *or* by the embryonic genome. Such mutations are also called male-rescuable maternal-effect mutations.

[From last time: remember how fates in *C. elegans* are determined? ***Embryo manipulations demonstrate both cell-autonomous and inductive determination mechanisms (review)***

Cell isolation and laser ablation experiments showed that the gut, germ-line, and body-wall muscle lineages are programmed autonomously by the mid-4-cell stage. Other such experiments indicated that inductive events are also required. If one of the cells of the 4-cell stage embryo (EMS) is ablated, the ABa descendants fail to form anterior pharynx. If P₂ is ablated, the ABp descendants behave like ABa descendants. And finally, experiments with isolated blastomeres showed that gut development from the E lineage requires brief contact between P₂ and EMS (the parent of E) during the first few minutes of the 4-cell stage. These details (cell names and what the signals are) are not important; however, the way in which genetics can be used to help dissect these signaling events, is!]

Obviously, these components that are used at the 4-cell stage and earlier are maternally contributed! Thus, the mutants that were studied to help understand these early interactions were all “maternal-effect” mutants—the offspring only show the mutant phenotype if their mother contributes no mRNA or protein from that gene to the embryo. And, even when the mother was mated to a wild type male, the embryos will still show the mutant phenotype because the embryonic genome has not yet been activated. There are a few important examples described in your book: specification of the EMS cell by a Wnt homolog (*mom-2*), and the specification of the ABp cell by a Delta homolog (*apx-1*).

Non-maternal embryonic lethal mutations identify genes controlling later embryonic events

These mutations occur in genes that are required during or after gastrulation and must be transcribed from the embryonic genome. For these mutations, the genotype of the hermaphrodite parent is unimportant, and the genotype of the embryo determines its survival. For such a mutation, a heterozygous (*m/+*) mother will produce only 3/4 the normal number of viable embryos, because the 1/4 that are of genotype *m/m* will die.

By genetically testing an embryonic lethal mutation for maternal effects, one can determine whether the gene it defines is expressed only during oogenesis, only in the embryo, or both. This is an important first step in defining and characterizing the genes that control embryonic development.

Later events in C. elegans development: signaling involved in differentiation of the gonad and the egg-laying organ, the vulva

As mentioned earlier, the complete cell lineage of *C. elegans* is known, from egg to adult. In later stages of development, there are many characteristic sublineages, small patterns of cell divisions from particular founder cells that are repeated in several places in the animal. Sublineages have developmental significance, because the lineally homologous cells in a sublineage generally adopt functionally analogous fates; e.g. the anterior daughter of the anterior daughter of the founder cell will generally be similar in each of the repeated lineages. The sublineage that gives rise to neurons in the ventral nerve cord is repeated 12 times along the length of the animal, producing sets of similar neurons at each position. Although *C. elegans* is not a segmented animal, its development is *metameric*, that is, based on repeating patterns of lineage.

Inductive events in later *C. elegans* development often result in modification of particular sublineages so that lineally homologous cells adopt no similar fates, but instead different fates, in response to external signals. The best understood example is formation of the hermaphrodite vulva, in a process involving modification of somatic gonad and ventral hypodermal sublineages. We will go through them as examples of how signaling pathways can be elucidated by a combination of observation, experimental perturbations, genetic analysis, and finally molecular characterization of genes and their products.

Somatic gonad development:

The somatic gonad is derived from two sublineages (Z1 and Z4) derived from the embryonic MS blastomere. Both Z1 and Z4 give rise to various components of the somatic gonad, including two cells that sit adjacent to each other, ventral uterine (VU) cell and the central anchor cell (AC) of the gonad in hermaphrodites. Interestingly, these two cells constitute an *equivalence group*, because they have the same

developmental potential (molecularly, they are essentially equivalent). Both of them are capable of differentiating into the AC, but only one of them does. If either one of the two cells in the AC/VU pair is ablated before the choice is made, then the remaining cell always becomes the AC, suggesting that the two cells normally interact with each other to determine their fates. Sound familiar? The AC/VU fates are controlled by Notch-Delta signaling (G, 6.30). The *lin-12* gene encodes the relevant *C. elegans* Notch homolog. One of the two possible AC precursor cells (randomly) begins to produce more of the Delta ligand. The other cell then binds this ligand with its Notch (LIN-12) receptor, and responds by down-regulating its production of Delta and up-regulating its production of Notch. The cell that produces more Delta and less Notch becomes the AC. As you have heard already, this type of interaction is called lateral inhibition, and we will encounter it repeatedly as a mechanism of cell fate determination in many organisms and processes, including vulval development.

Formation of the hermaphrodite vulva:

The cells along the ventral midline of the L1 all generate similar post-embryonic sublineages. These cells are called the P cells (NOT related to the germline P cells in the embryo), and each P cell gives rise to daughters that divide and then differentiate in a stereotyped fashion: anterior daughters of the P cells give rise to ventral cord neurons, while posterior daughters become hypodermal cells or part of the vulva: the posterior daughters are all lineally related, and form an equivalence group as described above. In the hermaphrodite, the vulva is formed from the P cells called P5p, P6p, P7p. There are two kinds of vulval fates: primary (P6p, makes the central structure of the vulva), and secondary (P5p and P7p, surrounding vulval structure). P3p, P4p, and P8p remain hypodermal (sometimes called the tertiary fate). Although normally only three of these cells, P5p, P6p, and P7p are vulval precursor cells (VPC's), laser ablation of individual Pnp cells shows that five of them, P3p to P8p, constitute the *vulval equivalence group*: any of them can become VPC's if the normal VPC's are ablated, whereas Pnp cells outside this group cannot. (In the male, a different set of more posterior Pnp cells (P9p, P10p, P11p) generate specialized structures for mating, again by modification of the basic Pnp lineage). How do the cells that make up the vulva get specified? Initial evidence that there were cell interactions between the somatic gonad (specifically the anchor cells) and the vulval precursor cells came from the following results:

- 1) If the anchor cell is ablated, no vulva forms.
- 2) In mutants where the gonad is displaced toward the anterior or posterior relative to the VPCs, the vulva is also displaced accordingly.

Genetic analysis of vulval development:

Genetic analysis of these events provides an impressive example of the power of the genetic approach. To figure out how this all works, saturation screens were done to isolate egg-laying-defective mutants, in other words, worms that had non-functional vulvae. This phenotype is not lethal because embryos can hatch inside the mother and survive to reproduce themselves (the "bag of worms" phenotype). Analysis of a large number of such mutations identified about 70 genes (most named *egl* or *lin* genes), affecting many aspects of cell lineage, cell determination, differentiation and function, which have been characterized genetically and molecularly.

The Vul and Muv phenotypes: Mutations affecting vulval development generally result in one of two phenotypes: Vulvaless (Vul), in which the normal VPC's (P5-7p) do not form a vulva, or Multivulva (Muv), in which all cells of the equivalence group (P3-8p) behave as VPC's so that multiple (nonfunctional) structures are formed. We'll go on in the next class period to see how the genes that, when mutant produce these phenotypes, were discovered to work together in a signaling pathway to determine vulval fate. We'll talk more about how the genes were found to interact with each other in the next class period, but for reference sake, here are the genes involved (interestingly, many components of the famous Ras signaling pathway, conserved in virtually all organisms, were discovered by studying the formation of the vulva in *C. elegans*):

lin-3: encodes the AC signaling ligand, a growth factor of the TGF- α family related to EGF.

let-23: encodes a membrane protein homologous to the EGF receptor (a PTK receptor); it is the receptor for LIN-3.

let-60: encodes a homolog of the cellular oncogene Ras, which is mutated to cause loss of growth control in many mammalian cancers. In worms, it is activated by when *let-23* is bound.

lin-1: encodes a transcription factor which is inhibited by LIN-45 (another member of the pathway) and negatively regulates the initial choice between vulval and non-vulval fates of Pnp cells (see pathway diagram in PowerPoint slides). *lin-1* is active in cells that take on the non-vulval fate, and inactive in cells that take on the vulval fate.

A corollary pathway already mentioned above is involved in determining the subtleties of which of the VPCs adopts the primary fate (at the center of the developing vulva) and which the secondary fates on each side: the juxtacrine signaling pathway Notch/Delta. The cell (normally P6p) that encounters the highest concentration of *lin-3* ligand and adopts the primary fate sends out a lateral inhibitory signal that is received by *lin-12*-encoded (Notch-like) receptors on the two neighboring cells (P5p and P7p), which therefore adopt secondary fates.

Review questions

- 1) Describe what is meant by a “maternal-effect mutation,” and explain the difference between "strict" and "partial" ("male-rescuable") maternal-effect mutations. Explain how you would define these two types of mutations genetically.
- 2) Explain how you would set up a genetic cross to determine whether a gene product was contributed maternally, or transcribed in the embryo, and interpret the results of such a cross.
- 3) Describe how the maternally contributed genes that control cell fate in the early embryo were discovered, and how we know what they do.
- 4) How does the somatic gonad form in *C. elegans*, and what signaling molecules are involved?
- 5) What impact does the gonad have on the development of the vulva?
- 6) What determines which 5 of the 12 ventral precursor (Pnp) cells give rise to the modified sublineages that form the vulva? What is the evidence for this?
- 7) What is the experimental basis for calling the cells P3p - P8p members of an equivalence group? Are their subsequent fates determined by ancestry or cell interactions?
- 8) What familiar signaling pathway determines the AC vs. VU fate and helps to determine P5p and P7p fate? How does this process work?