Revisiting genetic interactions

Introduction

In genetic studies, the question often is: What is the relationship between genotype and phenotype? While in forward genetics, one tries to find the genes responsible for a certain phenotype, reverse genetics observes the changes in the phenotype depending on changes in the genotype. For both approaches, it is important to understand how a genotype can produce a phenotype on a molecular level. During the following discussion, keep in mind that the term 'genotype' is used in two different ways: When looking at a single gene, it describes the allele combination of an individual with respect to this gene; however, the term also describes the genetic makeup of a cell, i.e., its entire set of genes (its genome). The phenotype is often the result of interactions between more than one gene or allele that can lead to a phenotype (see also modifiers in the previous lesson).

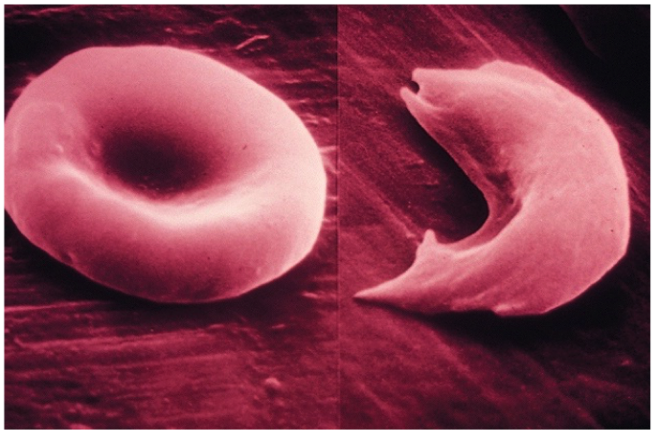
Allelic interactions

The first form of gene interactions you will have encountered during your studies is probably the interplay of alleles to produce a phenotype. A common example used to teach about dominant and recessive traits is sickle-cell anemia, where a single point mutation seemingly produces a recessive phenotype. Let's re-examine this example and look more closely whether it is that simple: A point mutation in *HbA*, the gene encoding the oxygen-carrying protein complex hemoglobin that is found in red blood cells, results in an abnormal shape and decreased functionality of the complex. This mutation, called *HbS* , has two harmful consequences: sickle-shaped red blood cells and anemia. However, people carrying the mutation are resistant to the disease malaria, since the pathogenic parasite normally spends part of its life-cycle inside red blood cells, but is unable to do so in individuals carrying the *HbS* allele. Figure 3-1 shows how the allele combination is linked to the three traits, anemia, sickle-shaped cells, and resistance to malaria. Surprisingly, we see that depending on which trait we look at, a point mutation can result in either a recessive, a dominant, or a co-dominant phenotype (both normal and sickle-shaped cells exist).

This example shows that the allele combination at a single locus can be responsible for at least three very different phenotypes. This is, in part, because similar to the ambiguity of the term 'genotype', we use the term 'phenotype' to describe observation on different levels: An altered protein complex, such as hemoglobin, is a phenotype on the molecular level, whereas misshapen red blood cells are a phenotype on the cellular level and a disease such as sickle-cell anemia is a phenotype on the organismal level. Hence, the terms recessive or dominant only apply to a given phenotype.

Another example of co-dominance, the phenomenon when the contributions of both alleles are distinguishably visible in the phenotype, is the ABO blood group system. Chemical modifications to a glycoprotein (called the H antigen) on the surface of blood cells are controlled by three alleles (I^A, I^B, and i) at the ABO locus, where the first two are co-dominant to each other. The modifications produced by the glycosyltransferases that are the gene products of alleles I^A and I^B are both dominant over the phenotype produced by the i allele, where no such modifications are made. While individuals with $I^A i$, $I^A I^A$, $I^B i$, or $I^B I^B$ show only one type of modification to the glycoproteins on blood cells, in individuals with genotype $I^A I^B$, both modifications are made, resulting in blood type AB.

A



B

Genotype	Shape of red blood cells	Anemia	Malaria resistance
Hb^A/Hb^A	round	No	No
Hb^A/Hb^S	few are sickle-shaped	No	Yes
Hb^S/Hb^S	sickle-shaped	Yes	Yes

Figure 3-1 Genotype-phenotype relationship in sickle-cell anemia. (A) Red blood cells have a round shape (*left*) in healthy individuals, but are sickle-shaped (*right*) in sickle-cell anemia patients. **(B)** Table linking allele combinations to three phenotypes related to a point mutation in the gene encoding hemoglobin. Hb^A and Hb^S are the wild-type and mutant allele, respectively.

Penetrance and expressivity

By analyzing family pedigrees, it is possible to predict the likelihood of a person inheriting a trait from their parents. One rule in the Mendelian concept of inheritance is that a dominant trait cannot "skip" a generation.

Let's look at figure 3-2, assuming that the pedigree shows the inheritance of a dominant trait. Obviously, the genotype of the person II-1 and his expected phenotype do not match. Therefore, when we say that a trait 'skips' a generation, we have to distinguish between i) the trait not being visible and ii) the allele not being passed on. But how is it possible that a person carrying an allele that encodes a dominant phenotype does not show the trait? This phenomenon is called incomplete penetrance.

The penetrance of an allele is the fraction of individuals carrying the allele that also expresses the associated trait. For example, if an autosomal dominant trait such as breast cancer linked to the gene *BRCA1* has a penetrance of 80%, 80% of females carrying the responsible allele will develop breast cancer and 20% will not.

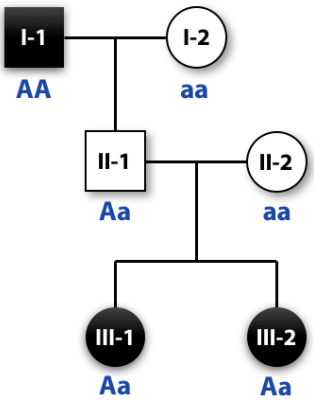


Figure 3-2 Pedigree showing an unusual pattern of inheritance of an autosomal-dominant trait. Males are indicated by squares, females by circles. Individuals showing the trait are colored black. Below each individual, the genotype is indicated, where A and a represent the alleles encoding the dominant and recessive traits, respectively.

What is the molecular basis of this phenomenon? One well-understood example of incomplete penetrance is the autosomal-recessive disease phenylketonuria, in which the amino acid phenylalanine cannot be hydrolyzed and therefore accumulates in the blood. Carrying two mutated alleles leads to intellectual disability in the context of a normal diet, whereas a life-long phenylalanine-restricted diet allows a relatively healthy life. Here, the determining factor, whether the phenotype linked to the genotype can be observed, is the phenylalanine-content of the diet, i.e., an environmental factor.

Unfortunately, only few other examples are as simple as the case of phenylketonuria, and the exact mechanism underlying the reduced penetrance are often unknown. What is known, however, is that it is a widespread phenomenon and that there are numerous different mechanisms by which incomplete penetrance can occur: It can depend on the specific mutations involved (i.e. how close they set a pathway activity to a threshold level) or whether an individual is homozygous or heterozygous (allele dosage, haploinsufficiency); it may result from differential allelic expression, modulating influence of additional genetic variants (modifiers) or it may be age- or sex-dependent.

A related phenomenon is the phenomenon of variable expressivity of a trait, which describes the variation of phenotypic expression across individuals that have a particular genotype. Expressivity differs from penetrance, because it does not characterize phenotypic variation qualitatively and in a binary manner (e.g., does or does not show the phenotype), but in a quantitative continuous manner. Note that the percentage indicating the penetrance of a trait describes a fraction of individuals of a population (e.g., 65% of people with a given genotype express the phenotype), while a percentage for the expressivity of a trait refers to the "intensity" of this trait in one individual.

In order to illustrate the two phenomena, consider the example of the egg color of the Hawai'ian Oo'Aa bird (see figure 3-3). In heterozygotes (Bb), different patterns of phenotypic variation resulting from varying penetrance and expressivity are possible.

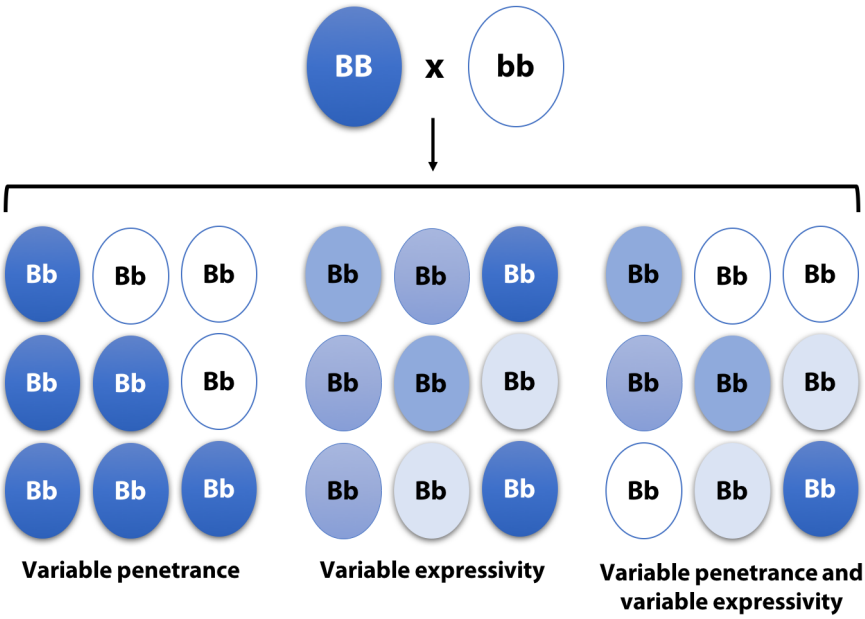


Figure 3-3 Different patterns of egg color in heterozygous Hawai'ian Oo'Aa birds. The egg color is controlled by a single locus, *BLU*, where the *B* allele encodes blue color, which is the dominant phenotype, and the *b* allele encodes the recessive white phenotype. The offspring of the cross shown here are all heterozygous (Bb). However, with varying penetrance and/or expressivity, three pattern of variation are possible.

Epistasis

Phenotypes are often not the result of the expression of a single gene, but of a combination of gene products that interact. In the lecture part on yeast genetics, you have already encountered gene interactions that are classified as epistatic interactions. Epistasis of two genes describes the phenomenon when the phenotype of the double mutant is the same as one of the single mutants, or, in more general terms, the effect of one gene is influenced by one or more other genes. Since epistasis analysis is such a widely used concept in genetics, we will introduce three more examples of epistasis, each in a different organism.

Interactions between mutations in cell-cycle regulators

The cell cycle is a highly regulated system to ensure the controlled transitions between different cell-cycle phases. The key regulators of the cell cycle are cyclins, a group of proteins that are differentially expressed during different phases, and cyclin-dependent kinases (CDKs), specific kinases that only show activity when in complex with their cyclin partner. In mammals, the cell-cycle transition from G1- to S-phase is regulated by the cyclin D-Cdk4 and -6 complex (see figure 3-4). This complex can be inhibited by CDK inhibitor proteins such as members of the INK family, resulting in cell-cycle arrest in G0 stage. If uninhibited, however, cyclin D-Cdk4/6 inhibits the retinoblastoma tumor suppressor protein (pRB) through phosphorylation. pRB is an important regulator of genes promoting G1/S-phase transition by repressing transcription factors of the E2F family. When pRB is inactivated, E2F members facilitate the transcription of proteins involved in DNA replication.

Let's consider different mutants in this regulatory pathway: Down-regulating mutations in proteins of the INK family (*ink*-mutants) or pRB (*pRB*-mutants) result in constitutive entry into S phase. Conversely, down-regulation of cyclin D, CDK4/6, or E2F by mutation prevents entry into S phase. The epistatic relationship between the genes of this regulatory pathway can be determined by generating double mutants and comparing the phenotypes to the phenotypes of the single mutants.

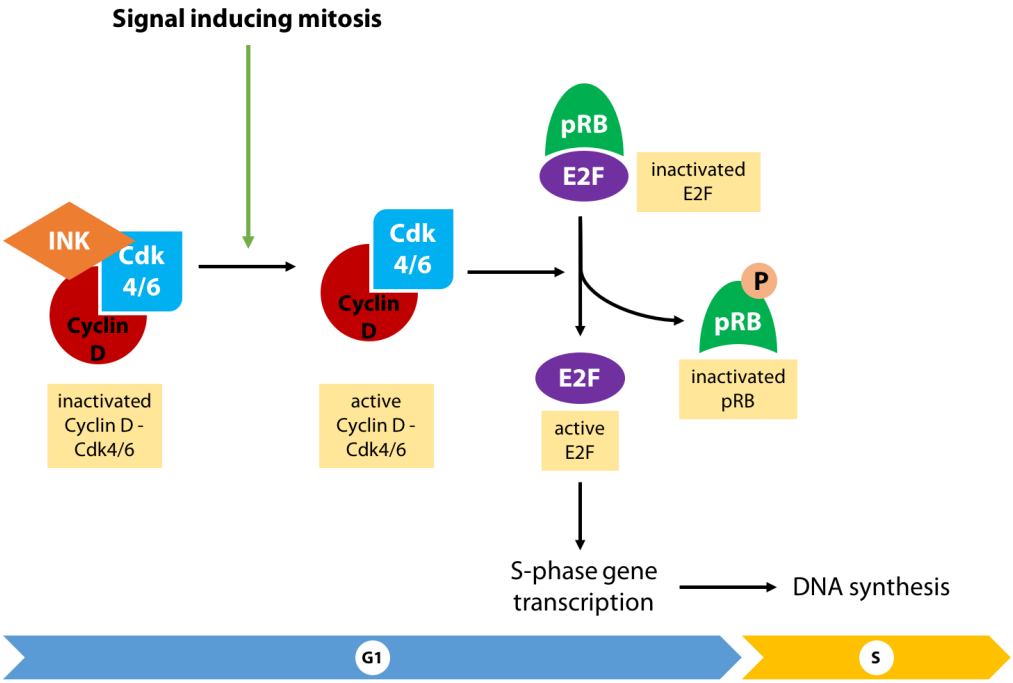
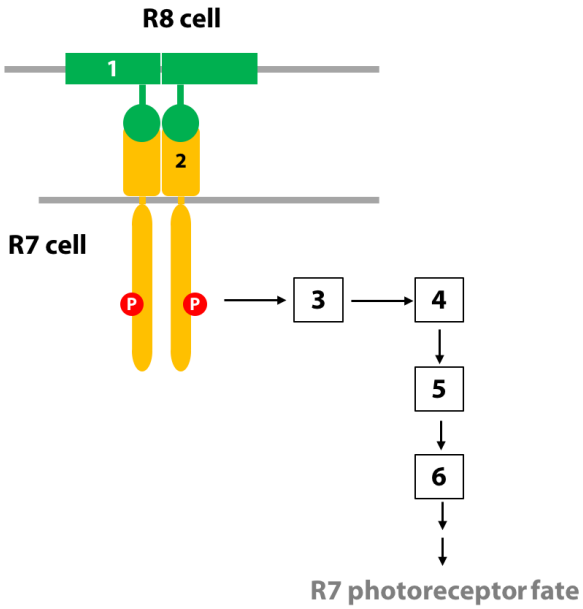


Figure 3-4 Control of cell-cycle entry. If a cell receives a growth signal, inhibition of the cyclin D-Cdk4/6 complex by a member of the INK family is stopped. Cyclin D-Cdk4/6 in turn inactivates pRB through phosphorylation. pRB releases E2F, which in its active form, acts as transcription factor for genes promoting G1/S-phase transition.

Interactions between mutations in the *Drosophila* sevenless pathway

In lesson 2, we have already discussed the sevenless pathway in *Drosophila* that regulates differentiation of precursor cells into fully functional R7 photoreceptor cells in the eye. Sevenless (encoded by *sev*) is a receptor tyrosine kinase that, when receiving a signal from its ligand Boss, dimerizes, self-activates through phosphorylation, and transduces the signal for cell differentiation into the nucleus. Important key players in this signaling pathway are SOS (Son of Sevenless), GTP exchange factor, and Ras1, a GTPase, and its target Raf, a kinase which initiates a phosphorylation cascade that transduces and amplifies the signal, resulting in activation of pathway targets. These downstream effectors have been identified in the dominant modifier screens using a sevenless sensitized background (constitutively active sevenless, Sev^{act}) as you have seen in lesson 2. These screens did not only identify the proteins involved in the pathway, but also aided to determine the order they function in the pathway and whether their interaction has an inhibiting or activating effect. The phenotypic effect of the Sev^{act} mutation can be reversed by loss-of-function mutations in *sos*, *ras*, *raf*, and the gene encoding MAP kinase (a kinase involved in the phosphorylation cascade), but not by a mutation in *boss*. A different gain-of-function mutation, this time in *ras*, was suppressed by mutations in *raf* and the gene encoding MAP kinase, but not by mutations in *sos*, *sev*, or *boss*. Mutations in the gene for MAP kinase suppressed the rough-eye phenotype induced by a constitutively active form of *raf*.

In the online lesson the following question concerning the epistasis of components of the sevenless pathway was asked: *Can you, analyzing the information above, infer the epistatic relationship between the key players of the sevenless pathway and draw a regulatory pathway using the template below?* You can use the scheme on the next page to indicate the components in the correct order.



Control of mitosis vs. meiosis in the *C. elegans* germ line

Another example for epistatic interactions is cell-line proliferation in *C. elegans*. In wild-type *C. elegans*, the germ line proliferates starting from two primordial germ cells (called Z2 and Z3), which are set aside in the early embryo into the gonadal primordium and only start proliferation after the worm has hatched. Continued rounds of mitosis take place in the two the somatic gonadal 'stem cell niches', which are each made up by one distal tip cell (DTC) that are located at the tip of the two gonad arms (see figure 3-6). The DTC is responsible for maintaining germ stem cells throughout the entire life of an animal. Once germ cells have left this niche, they start to differentiate and enter meiosis. That means that there are mitotically dividing cells at one end of the gonad, where the DTC is located, and cells that are meiotically dividing as they move away from the DTC.

But what makes germ cells that divide mitotically switch to meiosis? It has been shown that the Notch signaling pathway controls this transition: The DTC expresses the protein LAG-2 (a Delta homolog), which acts as a ligand for the Notch receptor GLP-1 (see figure 3-7). Upon activation, the intracellular domain of GLP-1 is cleaved off and enters the nucleus, where it activates the transcription of genes that promote the mitotic and prevent the meiotic cell cycle. If cells move away from the DTC, they lose their contact to the DTC and LAG-2 can no longer activate GLP-1 in these cells, thus, these cells enter meiosis. Interestingly, *glp-1* mRNA is present in all germ line cells, but GLP-1 protein is restricted to the mitotic region. This is accomplished, in part, by GLD-1, a translational repressor of *glp-1* mRNA and other targets relevant for staying in the mitotic cell cycle. GLD-1 binds directly to the *glp-1* 3'-untranslated region (UTR), thereby preventing GLP-1 translation in the meiotic region of the germ line. But why does GLD-1 not inhibit GLP-1 translation in mitotic cells, preventing the receptor from being transported to the plasma membrane? This is accomplished by the action of the FBF proteins. These proteins are expressed in the mitotic region and their expression is activated by GLP-1 signaling. Also the FBF proteins are sequence-specific RNA-binding proteins that bind to the 3'-UTR of target mRNAs and prevent their translation. Two of their targets are the *gld-1* and *gld-2* mRNAs, which encode proteins required for meiotic differentiation and act in a redundant fashion. This explains why GLD-1 and GLD-2 are not active in the mitotic region. When GLP-1 signaling is turned off in cells moving away from the DTC, FBF abundance is reduced and GLD-1 and GLD-2 proteins can be translated where they promote the entry of germ cells into meiosis.

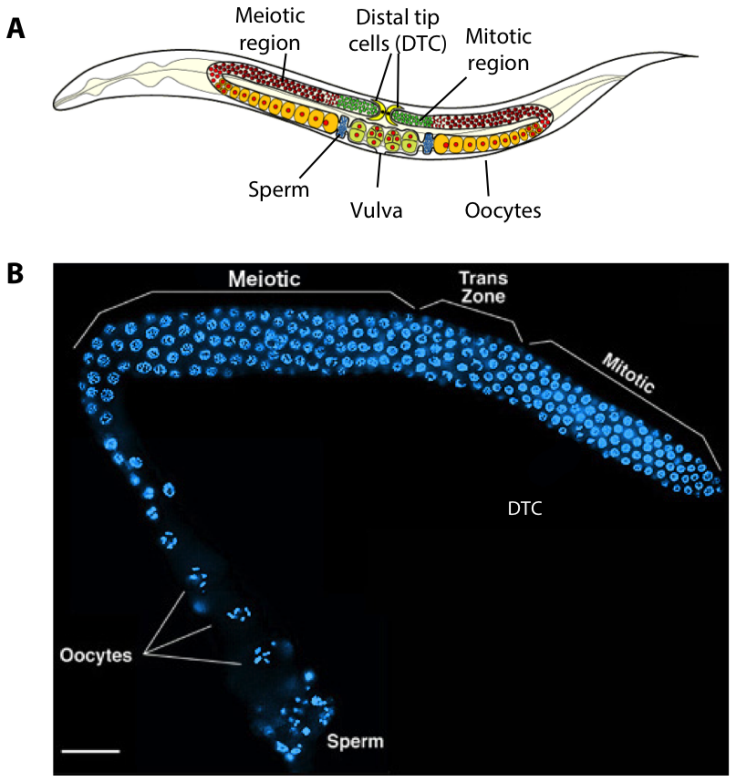


Figure 3-6 Germ line proliferation in the gonads of *C. elegans*. Hermaphroditic worms have two gonads, where the germ cells differentiate from germ stem cells that are maintained by one distal tip cell (DTC) at the distal end of each gonad. The cells first divide mitotically, but while moving away from the DTC, enter meiosis and differentiate into oocytes or sperm. **(A)** Schematic overview of the reproductive system. **(B)** Fluorescently labelled nuclei in one gonad.

In summary, in *C. elegans*, there exists a complex genetic network controlling the switch of germ cells from mitosis into meiosis. But how were researchers able to untangle these complex genetic interactions?

Now, let's again look at mutations in the genes that encode the key players of this regulatory system. Loss-of-function mutations in *glp-1*, *lag-2* or *fbf-1/2* result in immediate entry of germ cells into meiosis without prior mitotic expansion of the stem cell pool. Double loss-of-function mutations in *gld-1* and *gld-2* prevent germ cells from entering meiosis; instead, the germ line stem cells continue to divide mitotically, causing a germ cell tumor. Again, the question is: What is the order of these proteins in the regulatory pathway and what is the default state of a germ line stem cell in the absence of any input? As seen before, this question can be answered by generating double and triple mutants and performing epistatic analysis. The outcome of the experiment was that germ cells in *glp-1; gld-1; gld-2* triple mutants do not enter into meiosis and form a mitotic germ cell tumor. Thus, the default state of a germ line stem cell in the absence of any input is to divide mitotically.

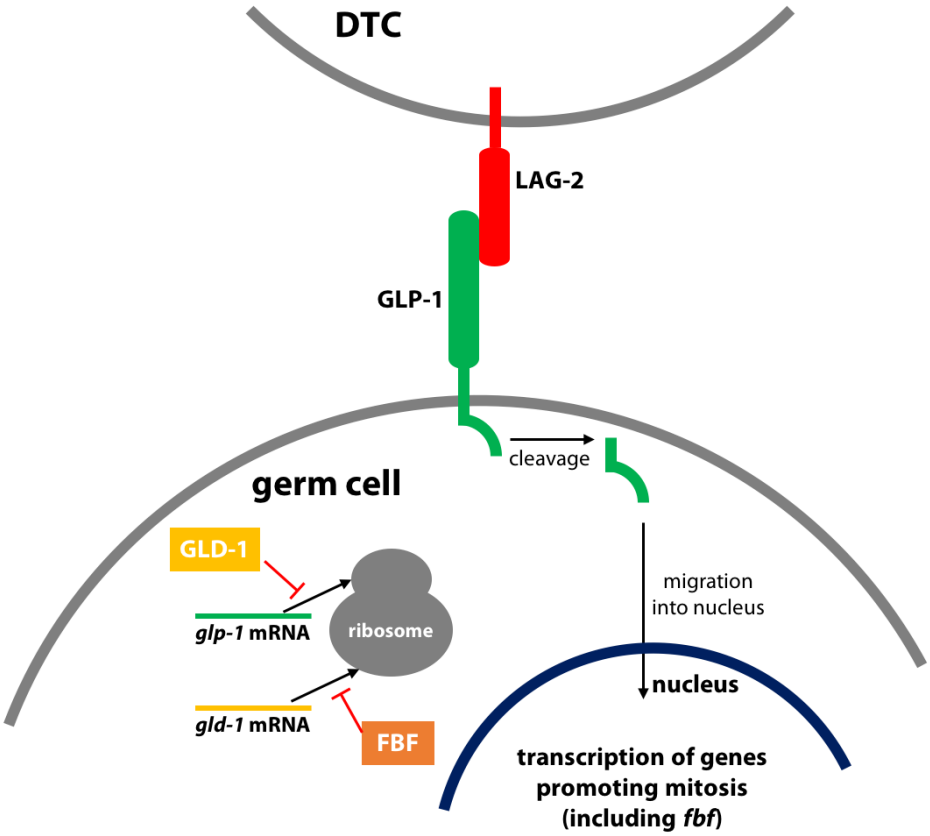


Figure 3-7 Signaling pathway controlling entry into meiosis of germ cells in *C. elegans*.

Summary

In this lesson, we have discussed different mechanisms how a genotype and a phenotype are related. The example of sickle-cell anemia shows that several phenotypes can result from a single point mutation and that their dominance can vary. We have further introduced the two concepts of penetrance and expressivity, in which one genotype can produce a range of phenotypes under the influence of other genetic or external factors. Finally, we have discussed three examples of epistatic interactions showing that often there are more than gene involved in producing a phenotype and that epistasis analysis helps elucidate the order of components of a pathway.