Discussion questions:

1. Describe the stages of C. elegans development. How many stages were you able to identify?

The worm begins as an embryo, which develops quickly. It only takes about 3 days for them to reach the adult life stage. The embryo develops for around 14 hours until it becomes an L1 larvae. From there it takes about 12 hours to become a L2 larvae, another 7 hours to become an L3 larvae, another 8 hours to become an L4 larvae. This last stage before becoming an adult lasts around 10 hours. The adult- the largest of the worms- has embryos inside. The worms can be male, female or hermaphrodite. In the wild-type strain however, there are mostly hermaphrodites because there are no females produced. As the worm moves through the life cycle, it gradually becomes bigger and bigger.

1. Why is it necessary for C. elegans to pass through several larval stages, and how is this type of development different from the development of humans?

It is necessary for C. elegans to pass through several larval stages because they need to molt between each stage. The molting allows them to continue growing. Humans do not have this because we do not need this process to continue to grow.

1. How does a hermaphrodite produce offspring without mating? Can you think of a way this could happen? Explain your hypothesis.

Hermaphrodites self fertilize- they contain both of the male and female components necessary to create an embryo. This means that in beneficial environments, they will maintain the same genetic makeup and continue to thrive. However, the males can fertilize the hermaphrodites. When the worms reach a particular life stage, their bodies will be mature and ready to reproduce, which will trigger a response in the worm to create eggs and to fertilize them.

1. What physical (morphological) differences did you observe in the mutant worms? What differences in behavior or movements did you notice? Did you classmates identify the same characteristics of the mutant C. elegans?

The N2 worms were the normal ones. The dumpy worms were comparatively shorter than the N2 ones and moved more slowly. The blister worms have a bump near their head, but I did not notice any significant differences in the way they moved. The RRF3 worms looked and acted the same as the N2 worms.

1. For each mutant phenotype you observed, what do you think would be the function of the protein produced by the wild type gene?

The function of the protein that is affected in dpy-11 is probably responsible for the growth of the worm. With the mutation, the worm is not able to develop correctly, which explains why it is shorter and moves more slowly.

The function of the protein that is affected in the bli-1 is alsp probably a protein that is responsible for part of the growth of the worm. In this case with the mutation, the cuticle is developed in a different way- caused by the incorrect function of the protein- that creates the bump on its head.

1. The mutant dpy-11 and bli-1 strains contain mutations that affect the cuticle, the other layer of the worm that is secreted by the epidermal (skin) cells. The gene dpy-11 codes for an enzyme that modifies other proteins, and bli-1 encodes a collagen. What is collagen and what do enzymes do? How can mutations in a collagen or an enzyme cause such different phenotypes that affect the cuticle?

Collagen is a structural protein for connective tissues. Enzymes are molecules that aid in reactions by catalyzing them or helping them occur. If there are mutations in collagen, the cuticle will form differently- which we can see in the worms, and if there are mutations in the enzyme, the reactions within the worm will not occur as efficiently or not at all. This explains why the dumpy worm moves more slowly than the N2 one.

Part 2

1. Have any eggs been laid? Have any eggs hatched? If so, are the worms at a larval or adult stage?

dpy-11:

1. Several eggs have been laid and hatched. Most of the C. elegans are in the L3, L4, or adult phase at this point.
2. The worms treated with RNAi are dumpy- smaller and moving more slowly. These differences are observed in the older worms since they are bigger and it is clearer what differences there are.
3. There are 15 total worms.
4. 11 of the worms appear to be dumpy and 4 normal.
5. We were not able to come in this day but when we did come in the next day, the number was much larger.
6. The first day, 73% of the worms were affected by the RNAi and were dumpy. By the next day we were able to look at the worms, almost all of the worms appeared to be affected by the RNAi.

Bli-1:

1. Several eggs have been laid and hatched. Most of the C. elegans are in the L3, L4, or adult phase at this point.
2. Looking at the worms, we noticed some blistered worms among those affected with the RNAi. We saw this trait in the older, bigger worms because it is easier to observe.
3. There are 17 worms.
4. 12 look to be blistered. 5 look to be normal.
5. We were not able to come in this day but when we did come in the next day, the number was much larger than we had thought they would be.
6. The first day we looked, around 70.6% of the worms seemed to be affected by the RNAi on the plate. By the second day we could observe them, almost all of the worms were affected by the RNAi.

Dpy-10:

1. Several eggs have been laid and hatched. Most of the C.elegans are in the L3, L4, or adult phase at this point.
2. Looking at the worms, only a few of the worms appear to be dumpy/moving more slowly. We saw this trait in the older, bigger worms because it is easier to observe.
3. There are 11 total worms.
4. 4 are dumpy and 7 look normal.
5. We were not able to come in this day but when we did come in the next day, the number of worms affected was larger than the first day.
6. The first time we observed the worms, around 36% of the worms were affected by the RNAi. The next time we were able to observe it appeared that around half of the worms were affected by the RNAi.
7. Given the phenotypes you observed, what can you deduce about the function of each gene that had its expression decreased?

Looking at the phenotypes of the resulting worms, we can come to the conclusion that the genes we worked with were responsible for some part of the cuticle. Both the dpy-10 and dpy-11 mutations affected the worm in the same way, making them somewhat smaller than the N2 worms. The bli-1 mutation causes the presence of a blister on their head. The RNAi that we fed the worms contained the mutations and which each of the successive generations produced in higher and higher quantity.

1. NGM-lite plates on which the RNAi feeding strains are grown include ampicillin. Why is ampicillin added to these plates? How are the RNAi feeding strains of bacteria different from the OP50 strain, which is grown on plain NGM-lite plates?

The ampicillin in the plates looks for the bacteria that contains the particular section of DNA that has the gene sequence that we use the RNAi to silence. This includes the gene that codes for ampicillin.