Answers to Group 3 Questions

1. Histone deacetylase is a catalytic component of transcription silencing machinery.Its function is to remove acetylation marks from histones. Increases in histone acetylation result in increased gene expression, whereas decreases result in decreased gene expression.

2. Royal jelly contains an inhibitor of HDACs.

3.

a) The authors used a K-ras-transformed NIH 3T3 cell reporter system to measure the epigenetic regulatory activity. Cells were treated with two concentrations of royal jelly for up to three days. Western blots were conducted for loci associated with Fas, ras, and Actin.

b) A Western Blot analysis showed that 0.5% and 1.0% royal jelly suspensions are sufficient to restore FAS expression in NIH 3T3 and K-ras cells.

c) Royal jelly can reactivate expression of an epigenetically silenced Fas locus.

d) The conclusion supports the notion that royal jelly allows queen bees and worker bees to be phenotypically different from each other even though they are genetically identical.

4.

a) Royal jelly was fractionated using an ultra-filtration membrane with a 3 kDa cut-off. Cells were treated with either a >3 kDa or <3 kDa fraction. Western blots were conducted for loci associated with Fas, ras, and Actin.

b) The fraction of royal jelly that is smaller than 3 kDa in size contains epigenetic regulatory activity that reactivates the silenced Fas loci.

c) 10HDA is the main component of royal jelly that reactivates the Fas loci in NIH 3T3 K-ras cells.

d) The conclusion supports the notion that royal jelly allows queen bees and worker bees to be phenotypically different from each other even though they are genetically identical.

5.

a) Is 10HDA a HDAC inhibitor?

b) NIH 3T3 K-ras cells were treated with sodium butyrate, 10HDA, or a 5% royal jelly suspension for three days. Core histones from these cells were isolated analyzed with pan-acetyl-lysine antibodies to observe changes in acetylation levels through a Western blot analysis. Then, an in vitro assay was conducted using an HDAC colorimetric activity assay kit to test whether 10HDA and royal jelly inhibit HDAC activity.

c) Increased histone acetylations levels were observed in samples treated with sodium butyrate, 10HDA, and royal jelly. 10HDA and diluted royal jelly inhibit HDAC activity in vitro.

d) Most of the HDAC inhibitor activity in royal jelly is due to 10HDA.

e) The conclusion supports the notion that there is histone deacetylase inhibitor activity in royal jelly that allows queen bees and worker bees to be phenotypically different from each other even though they are genetically identical.

6. Based on Figure 1C, I do not think that royal jelly affects DNA methylation. When comparing the group that received 0.5% RJ for 72 hours to the group that received only 10HDA for the same amount of time, the data shows that the amount of Fas expression is approximately the same. Therefore, it appears that the reactivation of the silenced locus by royal jelly is entirely due to 10HDA. The paper also states that 10HDA functions synergistically with DNA demethylating agents. Thus, I infer that 10HDA would not be involved in DNA methylation; otherwise the synergistic function of 10HDA with DNA demethylating agents would be counter-productive.

7. HDAC inhibitor activity in royal jelly allows reactivation of genes that are necessary for caste switching in bees would be epigenetically silenced in the absence of royal jelly in the diet. Although all larvae are fed royal jelly for the first three days of development, worker bees switch to worker jelly on the fourth day while larvae that will become queen bees will continue to be fed royal jelly. As a result, there will be loci that will be silenced within worker bees that halt the developmental pathway necessary for the queen phenotype, whereas these loci will continue to be expressed in the larvae destined to become queens.