

Objective 1.5 – Genetic studies of BMSB

Objective 2.2.8 – RNAi (and Entomopathogens)



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Collaborating Institutions

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Objective 1.5 – Genetic studies of BMSB

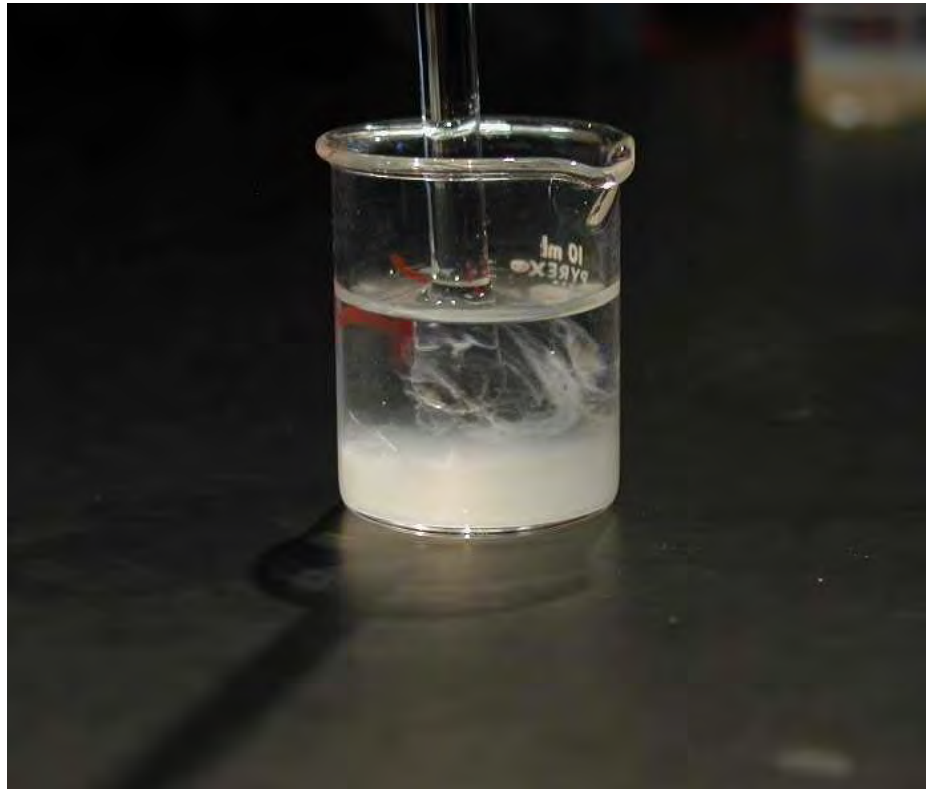
Objective 2.2.8 – RNAi and Entomopathogens

Overarching goals:

- A. To understand basic biology of BMSB
- B. To establish methods for gene-specific pest control & population monitoring

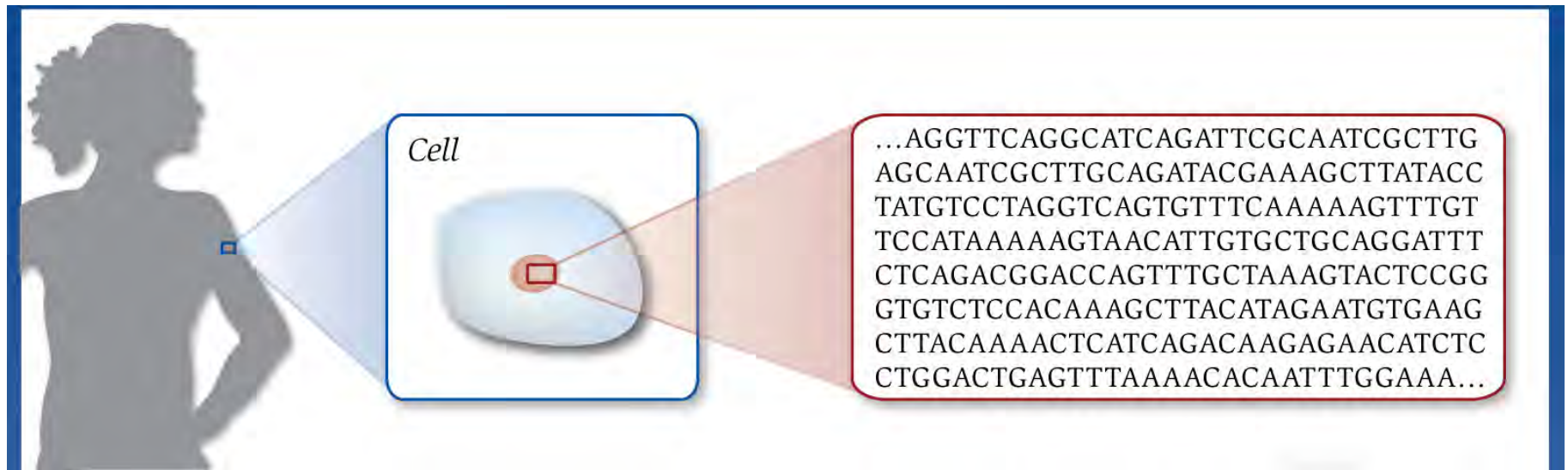


Pure DNA

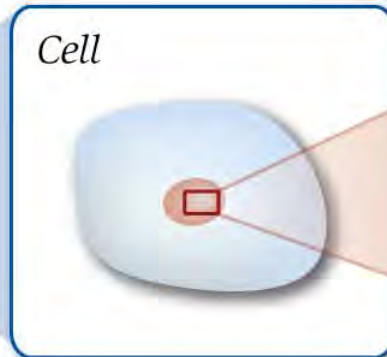


biology.clc.uc.edu

Every cell in our body contains DNA



The human genome contains about 3 billion nucleotides



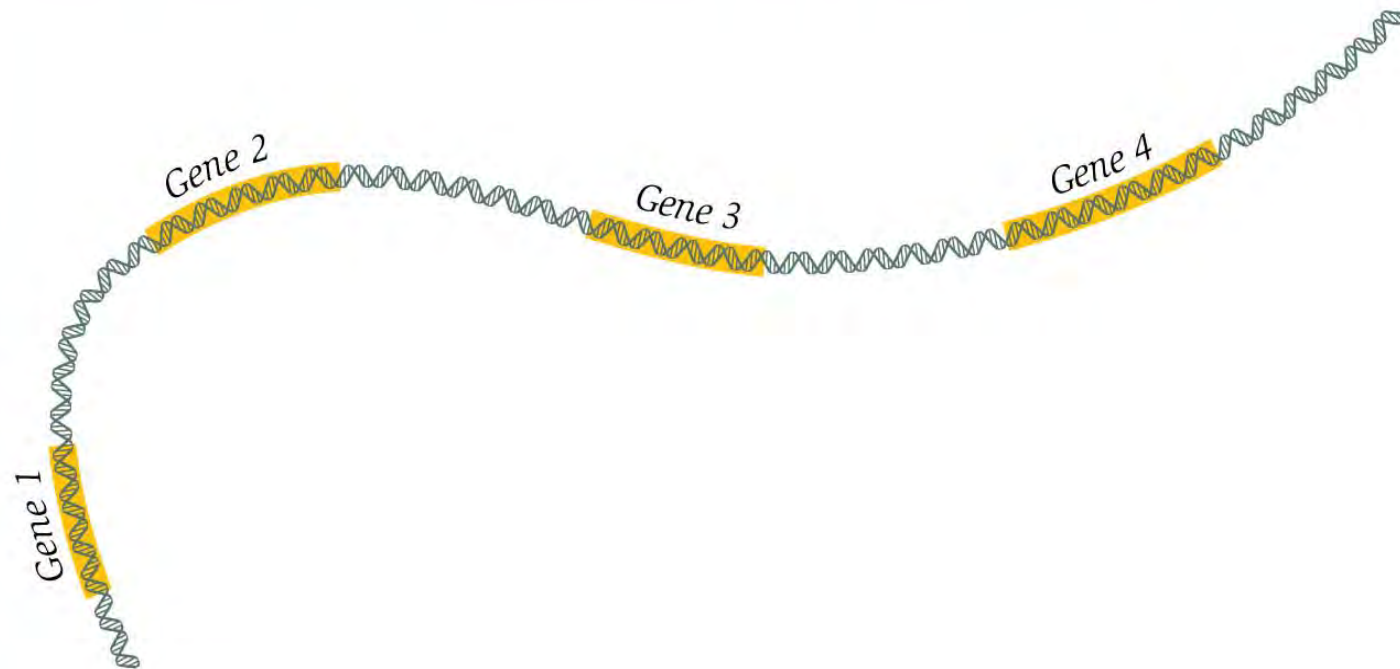
```
...AGG TTCAGGCATCAGATTCGCAATCGCTTG  
AGCAATCGCTTGCAGATACGAAAGCTTATACC  
TATGTCCTAGGTCAGTGTTCAAAAGTTTGT  
TCCATAAAAAGTAACATTGTGCTGCAGGATTT  
CTCAGACGGACCAGTTTGCTAAAGTACTCCGG  
GTGTCTCCACAAAGCTTACATAGAATGTGAAG  
CTTACAAAACATCAGACAAGAGAACATCTC  
CTGGACTGAGTTTAAACACAATTTGGAAA...
```

3 billion nucleotides would fill about 200 1,000-page phone books



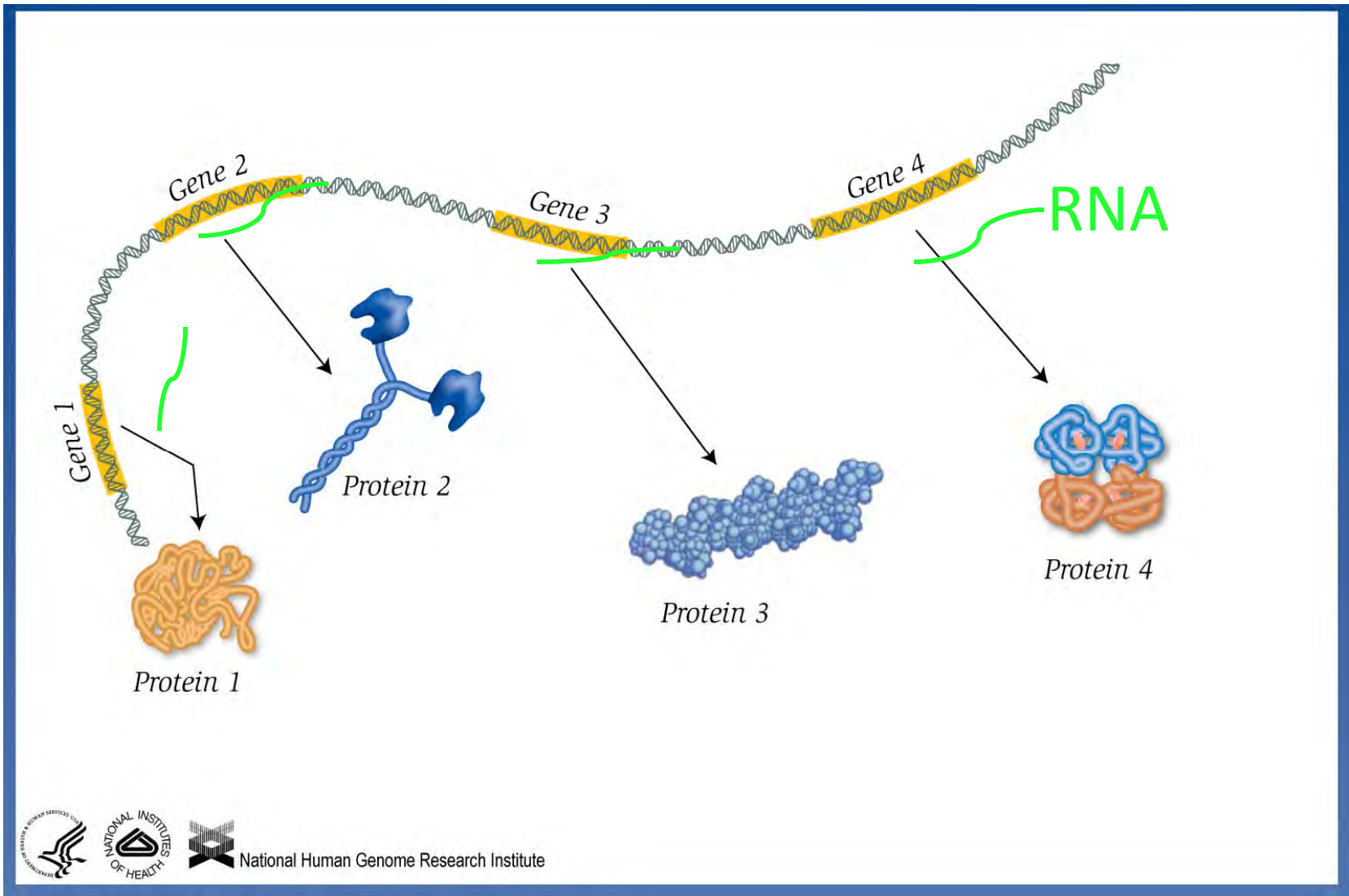
National Human Genome Research Institute

DNA is organized into genes

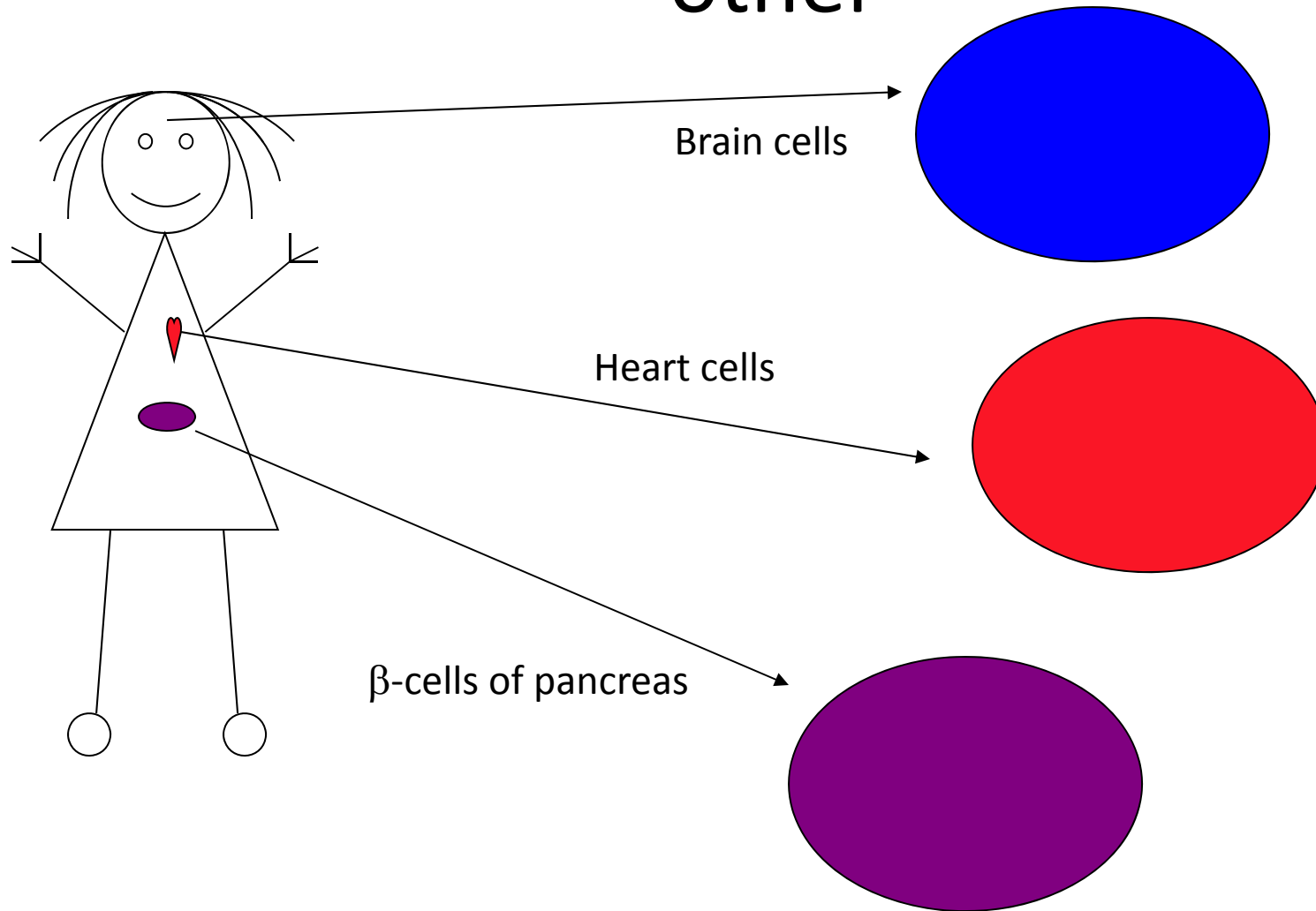


National Human Genome Research Institute

Genes are active when they are expressed: copied to RNA and protein

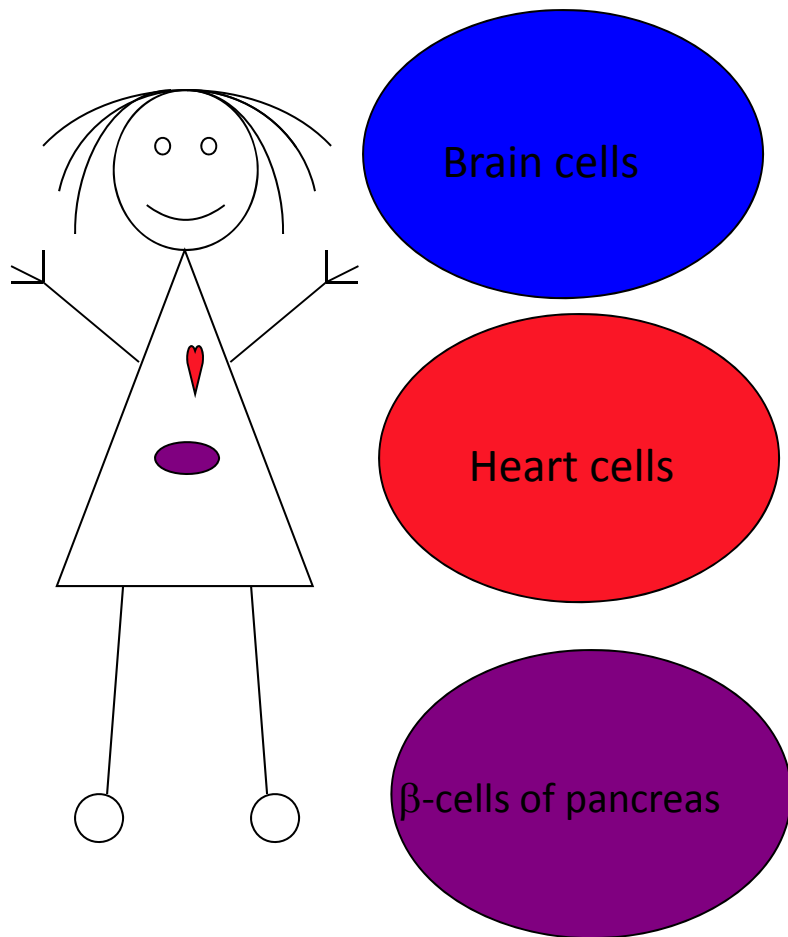


Differential expression of genes makes cells and tissues different from each other



All cells have same DNA

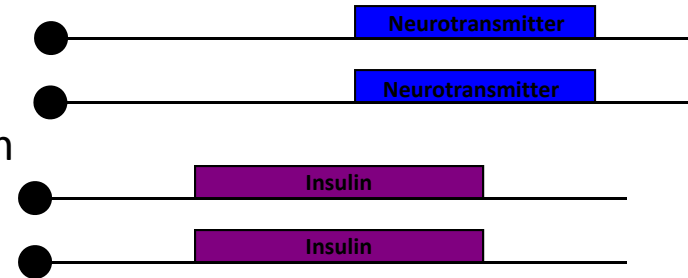
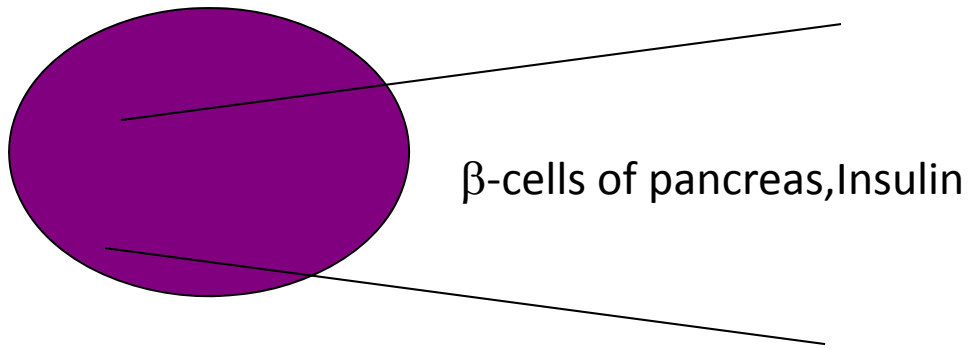
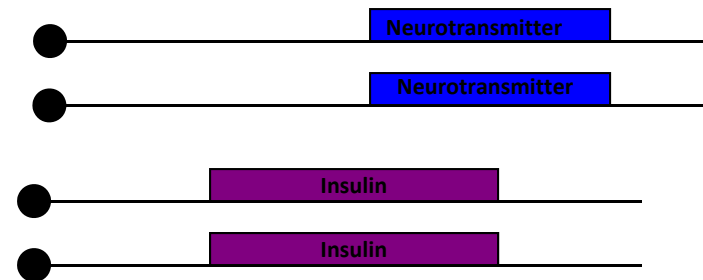
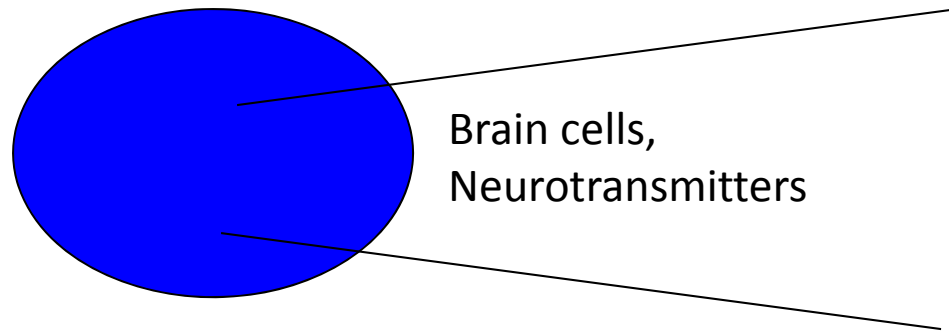
Different cell types *EXPRESS* different gene



- Brain cells express neurotransmitters that send messages along neurons
- β -cells of the pancreas express insulin

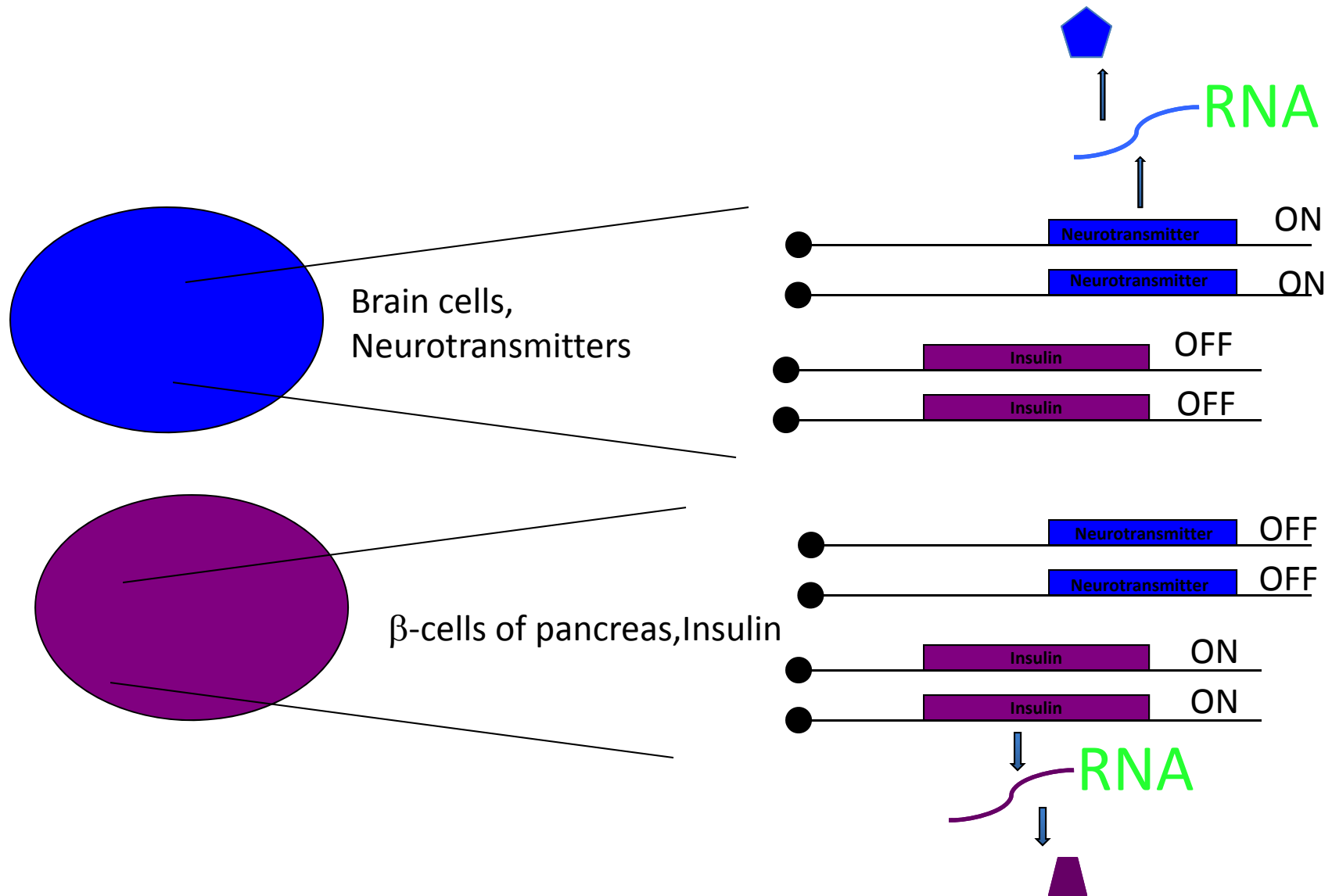
Brain cells express brain-specific gene

Pancreas cells express pancreas-specific gene



Brain cells express brain-specific gene

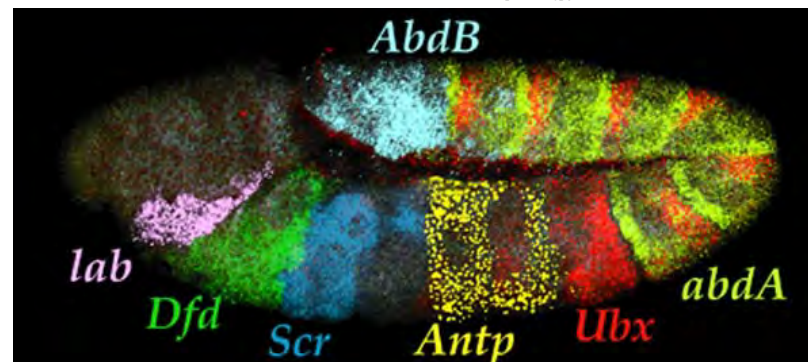
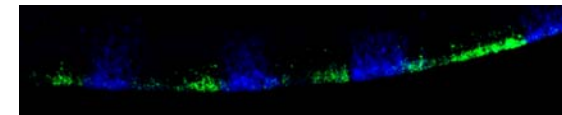
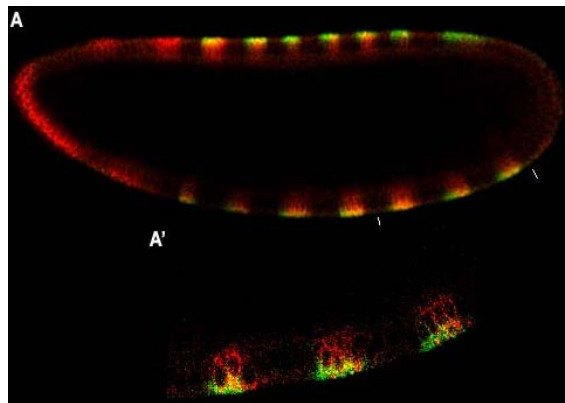
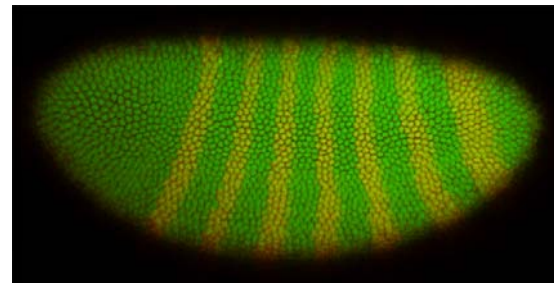
Pancreas cells express pancreas-specific gene



Knowing which cells express particular genes provides clues about function

- “Transcriptome” is the entire assembly of all genes expressed
- Most plants and animals express ~ 10,000-20,000 genes
- Transcripts (RNA) corresponding to specific genes are expressed in specific cells at specific times in the animal life cycle

VISUALIZATION OF GENE EXPRESSION IN *Drosophila melanogaster* (antibody or RNA probe)



Tells us when and where genes can act

Objective 1.5 – Genetic studies of BMSB

To understand BMSB genetics and biological function we must:

1. Identify genes - completed
2. Determine when and where they are expressed- methods established
3. Identify sets of genes useful for basic and applied studies – in progress
4. Determine function of selected genes – Objective 2.2.8



Objective 1.5 – Genetic studies of BMSB

Step 1: Identify all genes expressed by BMSB – Rapid transcriptome sequencing of an invasive pest, the brown marmorated stink bug *Halyomorpha halys*

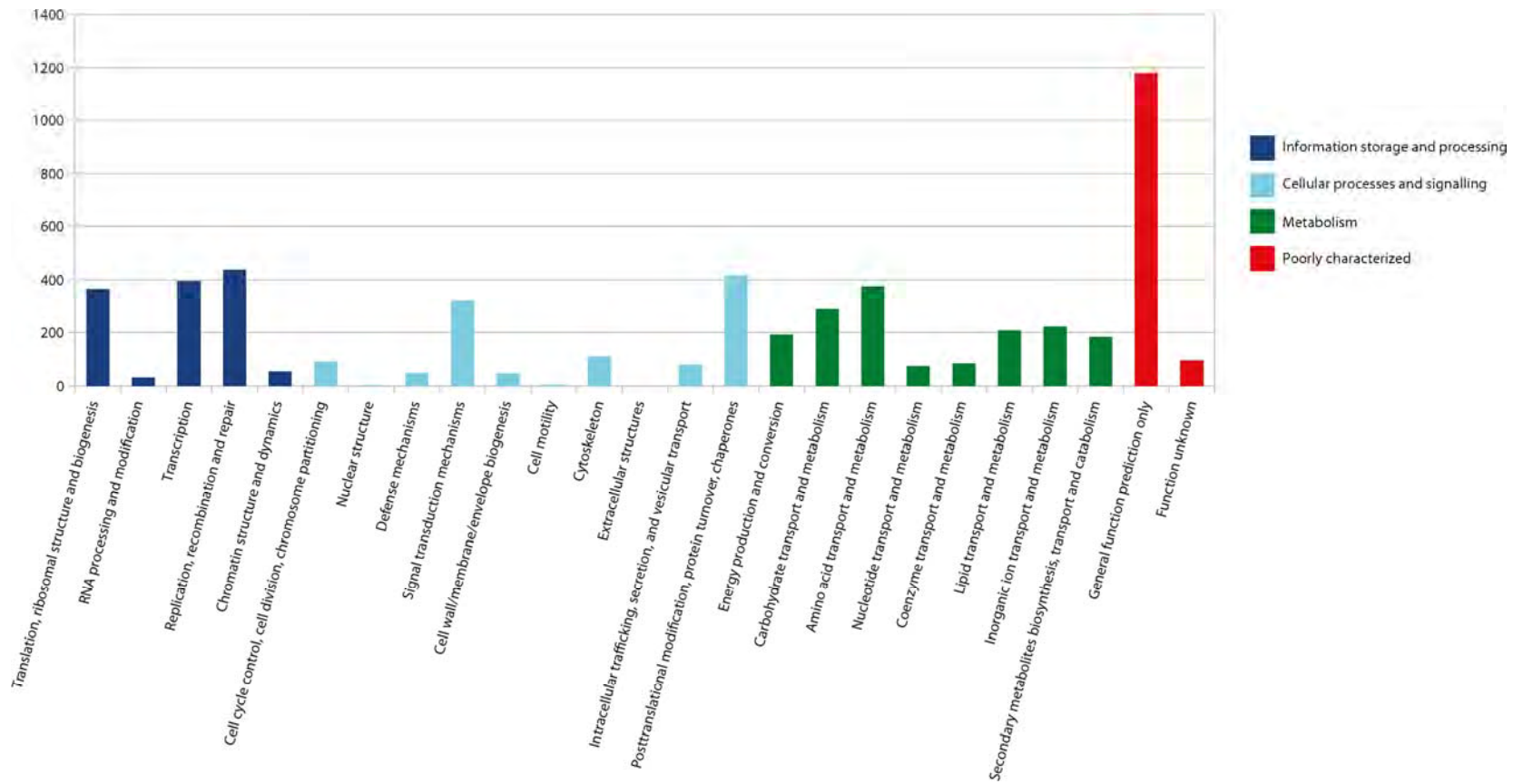
Ioannidis et al. BMC Genomics (2014) 15: 738 Julie Dunning-Hotopp at IGS, Baltimore



- RNA collected from each life stage
- Divided into two pools: pre-adults, adults
- Illumina strand-specific sequencing
- Identified 13,211 transcripts (most not full-length)
- Lateral gene transfer events identified
- Polymorphisms identified in ~1% of positions
in ~86% of transcripts – useful for population monitoring
- Most genes in genome likely identified:
 - *Drosophila* - ~ 14,000 genes
 - Aphids ~ 34,000 genes



All expected gene categories represented in predictions



Detoxification genes differentially

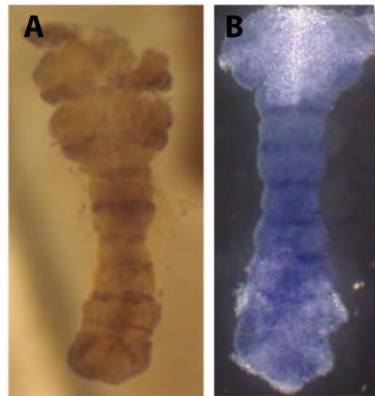
Name	Functional Annotation	Stage Upregulated
comp25785_c0_seq1	Cytochrome P450 gene	Adults
comp26191_c0_seq1	Glutathione S-transferase	Adults
comp15122_c0_seq1	Cytochrome P450 gene	Adults
comp16607_c1_seq2	Cytochrome P450 gene	Adults
comp15049_c0_seq1	Cytochrome P450 gene	Adults
comp15912_c1_seq2	Cytochrome P450 gene	Adults
comp20672_c0_seq4	Cytochrome P450 gene	Adults
comp13685_c1_seq2	Cytochrome P450 gene	Adults
comp40610_c0_seq2	Cytochrome P450 gene	Adults
comp8954_c0_seq1	Glutathione S-transferase	Adults
comp20241_c0_seq1	Cytochrome P450 gene	Adults
comp18070_c0_seq2	Cytochrome P450 (2 ORFs)	Adults
comp11443_c0_seq1	Cytochrome P450 gene	Adults
comp18881_c0_seq1	Cytochrome P450 gene	Adults
comp7095_c0_seq2	Cytochrome P450 gene	Adults
comp14891_c0_seq2	Cytochrome P450 gene	Adults
comp8170_c0_seq1	Cytochrome P450 gene	Adults
comp4236_c0_seq1	Probable cytochrome P450 gene	Adults
comp2339_c0_seq1	Cytochrome P450 gene	Adults
comp6322_c0_seq2	Cytochrome P450 gene	Adults
comp17381_c0_seq6	Cytochrome P450 gene	Adults
comp23582_c1_seq1	Cytochrome P450 gene	Adults
comp4238_c0_seq1	Cytochrome P450 gene	Adults
comp3991_c0_seq3	Glutathione peroxidase (3 ORFs)	Adults
comp8540_c0_seq1	Probable cytochrome P450 gene	Adults
comp6146_c0_seq1	Glutathione S-transferase	Pre-adults
comp11026_c2_seq1	Cytochrome P450 gene	Pre-adults
comp21713_c0_seq1	Cytochrome P450 gene	Pre-adults
comp3892_c0_seq1	Cytochrome P450 gene	Pre-adults
comp18921_c0_seq3	Catalase	Pre-adults
comp25932_c0_seq1	Cytochrome P450 gene	Pre-adults
comp10873_c0_seq1	Probable cytochrome P450 gene	Pre-adults
comp12303_c0_seq1	Cytochrome P450 gene	Pre-adults
comp8344_c0_seq1	Probable cytochrome P450 gene	Pre-adults

Objective 1.5 – Genetic studies of BMSB

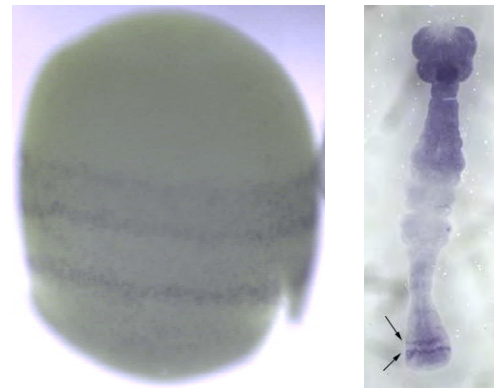
Step 2: Establish techniques to visualize gene expression – Yong Lu

Antibody immunostaining on whole mount BMSB embryos

Gene isolation and in situ hybridization to specific RNA



Hh-En expressed in segment primordia



Hh-eve expressed in stripes



Objective 1.5 – Genetic studies of BMSB

Step 3: Identify sets of genes useful for basic and applied studies – in progress

Classes of genes under analysis:

“Housekeeping genes” vs. Cell-type specific genes

To increase specificity we prefer to look at cell-type specific genes:

Genes regulating fertility & early embryonic development

Genes involved in nutrient sensing & Metabolism – insulin

pathway

Hormone & pheromone pathways

Methods:

Function is predicted based on known function in other insects, usually

Drosophila

Isolate full length gene sequences (5' and 3' RACE)

Determine when and where expressed (RT-PCR and in situ hybridization)

Determine function by knocking down gene expression



Objective 2.2.8 – RNAi as tool to knock down gene expression

To determine utility of RNAi for pest control:

Step 1. Identify genes that generate clear phenotypes after knockdown in related species - completed

Step 2. Test whether RNAi functions in BMSB – completed

Step 3. Choose sets of genes that would be useful for targeting different life stages of BMSB – in progress

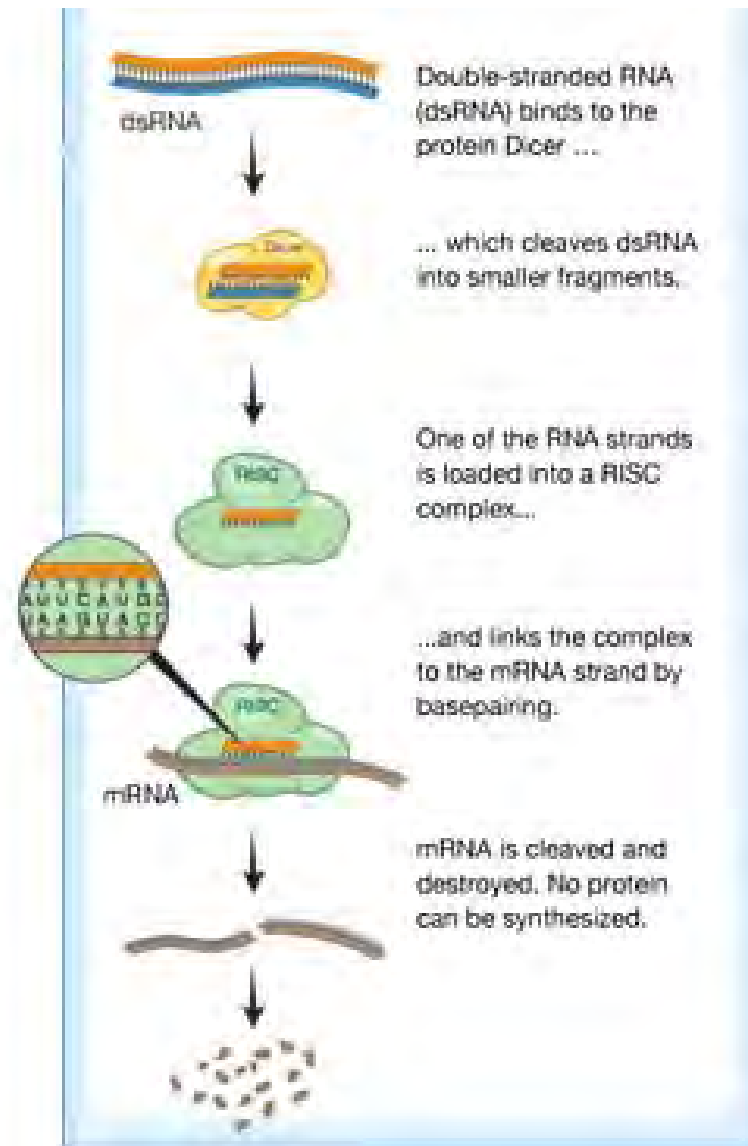
Step 4. Find regions of the genes that are BMSB-specific & test – in progress

Step 5. Test delivery methods - future



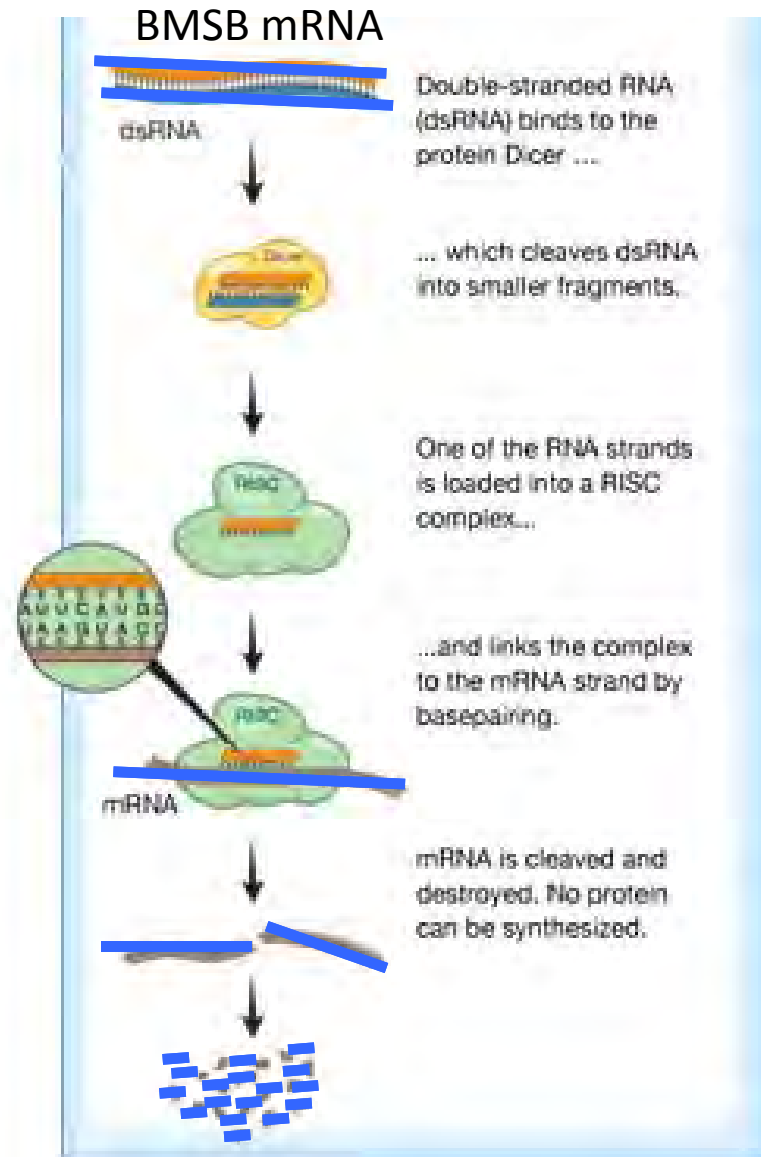
Objective 2.2.8 – RNAi as tool to knock down gene expression

- RNA interference (RNAi) destroys target mRNA
- Can be made highly specific by identifying gene sequences not found in other genes or species

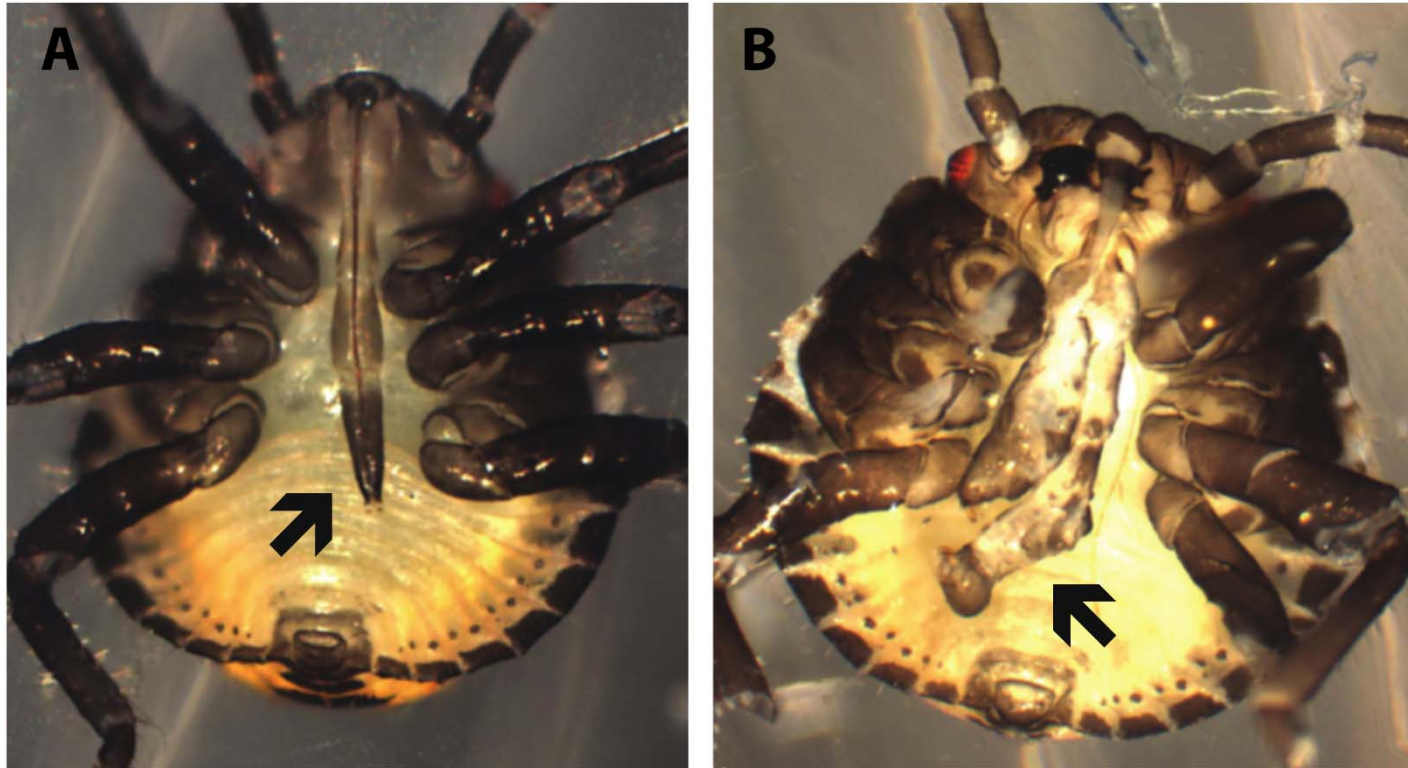


RNAi “tricks” cell to destroy its own RNA

- “Designer” RNAi will be attacked by cell’s anti-viral response
- Enzymes will destroy the mRNA of interest
- Approach target any mRNA for destruction



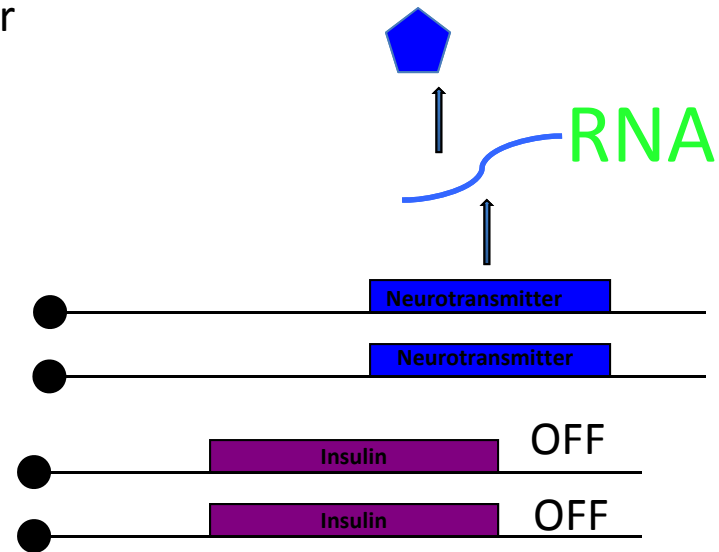
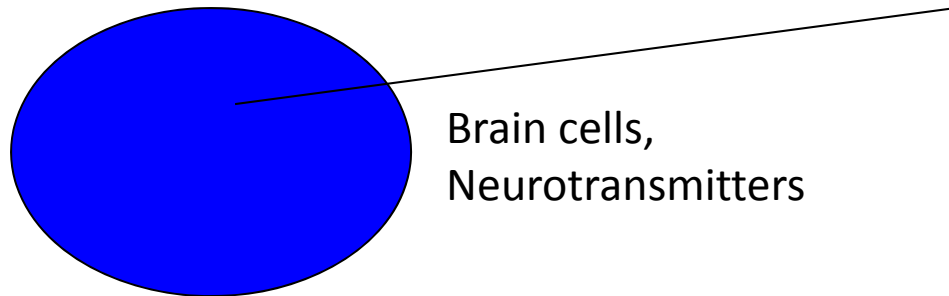
Halyomorpha halys, BMSB, *Scr* pRNAi



- Step 1. Identify genes that generate clear phenotypes after knockdown in related species – *Scr* is a homeotic gene found first in *Drosophila*
- Step 2. Test whether RNAi functions in BMSB – *Scr* knockdown by RNAi results in homeotic transformation of mouthparts

Objective 2.2.8 – RNAi as tool to knock down gene expression

Step 3: Choose sets of genes that would be useful for targeting different life stages of BMSB – in progress
Genes can only be targeted if they are expressed



Step 4. Find regions of the genes that are BMSB-specific & test – in progress

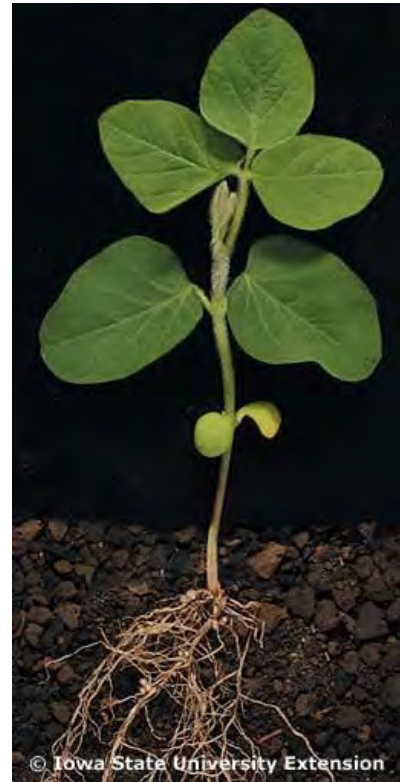


Objective 2.2.8 – RNAi as tool to knock down gene expression

5. Test delivery methods – next step



Delivery by injection effective



Plans: Test delivery by feeding

Genetic studies of BMSB and RNAi

- Identified ~ 13,000 BMSB genes by high throughput transcriptome sequencing
- Polymorphisms useful for population tracking
- Gene categories broad and suggest most genes were identified
- Techniques established for analysis of gene expression
- BMSB is susceptible to RNAi
- Delivery methods need assessment
- Feedback from you on which genes would be of most interest for us to pursue

