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Susan Wessler's Lecture Part 2:
The Dynamic Genome

Teaching Tools were prepared by Jim Burnette with Susan Wessler.

Contents

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1. Keywords and Terms

Transposable elements, mPing/Ping, genomics, genetics, gene expression, computational biology/bioinformatics, environmental stress

2. Review Questions

1. How do autonomous and non-autonomous elements result in spotted corn kernels? Draw a diagram.
2. Briefly discuss the two classes of transposable elements.
3. What explains the differences in genome sizes among the grasses (maize, rice, barley, etc.) although they have the same number of genes?
4. What are MITEs?
5. What is mPing and where was it found?

6. What are shared vs. unshared mPing insertions?
7. Where does mPing NOT insert?
8. How does mPing affect gene expression?

3. Answers to Review Questions

1. If a TE is inserted in a pigment gene, no pigment can be made. An autonomous element can excise from the gene during seed development resulting in pigment production in those cells. The nonautonomous element can excise only if an autonomous element is also in the genome.
2. Class I elements transpose using an RNA intermediate. The element copy does not move and Class I can be thought of as copy-and-paste elements. Class II elements move by excising from the chromosome and inserting into a new location. They are DNA elements and can be thought of as cut-and-paste elements.
3. The number of TEs contained in each genome. The larger the genome the greater the number of TEs found in it.
4. Miniature Inverted Repeat Transposable Elements. These as short (100-1000 bp) nonautonomous elements found in high copy number in the genome. An example is mPing.
5. mPing is a MITE found in the rice genome. In some strains only 50 copies are found but in others there are hundreds of copies.
6. Shared mPing insertions are those that are found in different strains of rice. Unshared insertions are found in only one strain because mPing is currently transposing in these strains. This created a unique situation where the Wessler lab was able to test the effect of mPing insertions on gene expression.
7. mPing avoids exons and preferentially inserts near the promoters of genes.
8. mPing has been shown to increase the expression of certain genes. In some cases expression was increased after an environmental stress.

4. Discussion Questions

1. Explain in greater detail what MITEs are?

2. How are TEs tools of evolution?

3. How do you identify an active MITE?

4. How does amplification not kill the host? Where are the elements?

5. Does amplification benefit the host?

6. Does mPing insertion provide stress inducible expression?

5. Answers to Discussion Questions

1. Mites are:
    a. DNA elements
    b. Non-autonomous
    c. Small
    d. Some are deletion derivatives of autonomous TEs others are not related to any known TEs
    e. Most abundant TE in and near genes
    f. Do not kill the host and may be beneficial
    g. Some MITES have regulatory sequences such as promoter or transcription factor binding sites that may result in altered gene expression
    h. Most alleles caused by MITEs are old, that is there are multiple mutations between alleles may be contribution to expression differences.

2. Discussion points
    a. McClintock's genome shock hypothesis
    b. Abundance of TEs in the genome
    c. Limitations of studying individual elements on gene expression
    d. Need a system in which TEs are in the process of amplifying
    e. "Genes sitting in a sea of TEs"
    f. MITEs often located introns and near genes
    g. Need organism where TEs are creating new alleles.

3. You can identify an active MITE by:
    a. Star diagrams
    b. Study genomics. Genetics is too limited.
    c. Rice genome is useful

d. Genome small
e. Completely sequenced. Allows a computational approach
f. Use computer programs to find TEs
g. Multiple copies that are identical or almost identical.
h. Ning Jiang found mPing, an element with 51 identical copies in Nipponbare rice strain
i. Validation of mPing
j. Using rice cell culture where TEs had been shown to previously move around
k. Using transposon display Jiang validated the computational results.
l. Ping is the autonomous element for mPing.
m. Four rice strains were found that had over 500 copies of mPing. These were independent bursts.
n. mPing is still moving as demonstrated by finding new insertions in subsequent generations.

4. Discussion points:

    a. High throughput sequencing used to identify the location of the mPings
    b. Shared vs unshared is very important.
    c. MITEs prefer to insert into single copy sequences on the chromosomes.
    d. mPing prefers to not insert into exon sequences
    e. mPing inserts preferentially within 1 kb of the transcription start site.

5. To measure any effect on gene expression, alleles were identified that had an mPing in EG4 that was absent in Nipponbare. Using microarrays the following was found:

    a. 78% No difference
    b. For 111/156 remaining there was a difference with mostly up regulation of gene expression. Majority of these were upstream of the gene.
    c. Microarray analysis was confirmed with qPCR
    d. Problem: NB has only 50, EG4 has 1000. So there is a problem that expression changes could be due to the high number of elements and not just one specific one.
    e. Overcome this problem by looking at expression in the other high copy strains such as A123. A123 has 1000s of insertions but expression is similar to NB. A157 has the insertion and expression is high like EG4.

6. mPing insertion does provide stress inducible expression in some cases:

    a. Rice was stressed with cold, salt, and dry. For some alleles yes, genes were up regulated during different stresses.
    b. Upstream insertions and intron insertions cause stress induced expression
    c. These are dominant alleles. This is important if a new allele could be adaptive.

6. Explain or Teach These Concepts to a Friend

1. What is the definition of a genome? Of Genomics?
2. Using a genetics textbook teach a friend about how recombinant inbred lines are created.

7. Research the Literature on Your Own

1. How are microarrays used to measure gene expression?
2. What is RNAseq. How could this technology be used to measure gene expression instead of microarrays?
3. Are there other examples in the literature of a dominant allele conferring a selective advantage to a crop plant?

8. Papers for Journal Club

1. Jiang N, Bao Z, Zhang X, Hirochika H, Eddy S R, McCouch S R, and Wessler S R (2003). An active DNA transposon family in rice. Nature. 421: 163-167.
2. Naito K, Cho E, Yang G, Campbell M A, Yano K, Okumoto Y, Tanisaka T and Wessler S R (2006). Dramatic amplification of a rice transposable element during recent domestication. Proc. Natl. Acad. Sci. USA. 103: 17620 - 17625.
3. Naito K, Zhang F, Tsukiyama T, Saito H, Hancock CN, Richardson AO, Okumoto Y, Tanisaka T, Wessler SR. (2009). Unexpected consequences of a sudden and massive transposon amplification on rice gene expression. Nature. 461(7267): 1130-4.
4. These three papers are the basis for the iBioseminar talk. The 2003 paper describes identifying mPing as an active element in rice. The 2006 paper discusses the high copy number strains of rice. The final paper shows the effect of mPing on gene expression.