Neofunctionalization Of Homoeologs Explains Multiple Mechanisms Of Chromosome Pairing Control Associated With the Ph1 Gene Of Polyploid Wheat

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C-banded Karyotype of Wheat (Chinese Spring)

- Allopolyploid (hexaploid)
- $2n=6x=42$
- Genomic size = 16 billion bp
- Various ploidy levels exist
- Tetra- and hexaploid types are cultivated

Gene expression analysis by Virtual Northern + SSCP

<table>
<thead>
<tr>
<th>Number of Genomic Copies</th>
<th>Number of Genes Analyzed</th>
<th>Number of Copies Expressing</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>56</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>76</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>632</td>
<td>254</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>26</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>854</td>
<td>184</td>
</tr>
</tbody>
</table>

Expression profile of wheat genes

87% of the genes showed differential expression of homoeologs
**Ph1 gene prevents single gene transfers from wild relatives**

Chromosome pairing
Metaphase I, meiosis

\( \text{A} \quad \text{B} \quad \text{D} \)

\( \text{Ph1} \)
Strict diploid-like pairing

\( \text{Ph1} \)
Multivalents

Okamoto & Sears, 1958
Riley & Chapman, 1958

**Other pairing regulators**

- **Ph2** on 3DS (Mello-Sampayo et al., 1968; 1967)
- Another suppressor on 3AS (Driscoll, 1972)
- Gene(s) on 5BS promote pairing (Riley et al., 1967) - less effective
- 5DS also promotes pairing better than 5BS (Feldman, 1966; Riley et al., 1966)

**Hypothesized mode of action of the Ph1**

**Pre-synaptic hypothesis**
- Ph1 determines the premeiotic alignment of chromosomes spacially separating homologs and homoeologs thus leading to strict homologous pairing

**Post-synaptic hypothesis**
- Both homologs as well as homoeologs pair during pachytene and the Ph1 gene resolves paired chromosomes into homolog-only pairing

**Multiple functions associated with the Ph1 gene**

- Absence of 5D leads to asynapsis (Riley 1966; Feldman 1966)
- Extra dose of 5D but not of 5A, can partially compensate for the loss of 5B (Riley 1966)
- Regulates chromosome pairing via telomeres (Dr. Moore’s group)
- Decreases the degree and speed of chromosome condensation (Upadhya & Swaminathan, 1967)
- Functions via microtubule-centromere interaction by modulating phosphorylation of tubulin proteins (Feldman 1993).
- Functions during premeiotic mitosis by affecting chromosomal movement towards the poles and consequently, their arrangement in the nucleus (Avivi et al 1982; Feldman 1988)

**Role of 5B in chromosome pairing**

<table>
<thead>
<tr>
<th>Chromosome constitution</th>
<th>Univalents</th>
<th>Bivalents</th>
<th>Trivalents/quadra-divalent</th>
<th>Paired chromosomes of cells %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>33.27</td>
<td>0.37</td>
<td>-</td>
<td>2.18</td>
</tr>
<tr>
<td>Monosomic 5A</td>
<td>32.47</td>
<td>0.77</td>
<td>-</td>
<td>4.53</td>
</tr>
<tr>
<td>Monosomic 5B</td>
<td>16.03</td>
<td>6.07</td>
<td>1.5901.18</td>
<td>44.56</td>
</tr>
<tr>
<td>Monosomic 5D</td>
<td>32.27</td>
<td>0.87</td>
<td>-</td>
<td>5.12</td>
</tr>
</tbody>
</table>

Chromosome pairing: analysis of F1’s of Aegilops cracca X Monosomics for Group 5 chromosomes/CS (Tyankova 2001)

**Inference**
- Essential no effect on homoeologous chromosome pairing
- Increased homoeologous chromosome pairing, multivalents & higher order pairing
- Asynapsis, rare multivalents & frequent interlocking of the bivalents

- F1’s of Monosomics for chromosome V crossed with AADD amphidiploid, Okamoto 1957

**Role of group 5 homoeologs during meiosis**

- **5A**
  - Essentially no effect on homoeologous chromosome pairing

- **5B**
  - Increased homoeologous chromosome pairing, multivalents & higher order pairing

- **5D**
  - Asynapsis, rare multivalents & frequent interlocking of the bivalents

- Weak effect
- Critical for the basic chromosome pairing

- F1’s of Aegilops cracca X Monosomics for Group 5 chromosomes/CS (Tyankova 2001)

**Inference**
- Weaker/ineffective copy
- Regulates diploid-like chromosome pairing
Dosage effect of the Ph1 gene copies

- Four doses of 5A can restore normal pairing in the absence of 5D
- Neither 5A nor 5D can compensate for the loss of 5B
- 5B cannot compensate for the loss of 5D
- 5A is a weaker copy
- 5A and 5D copies share a common function
- 5B copy has a novel Function

Alternate splicing of the 5B copy

The 5B copy proteins:
- Lack highly conserved motif corresponding to the exon I
- Contains a 60bp insertion resulting in a smaller protein
- Multiple functions of the 5B copy

3D structure comparison of the predicted gene proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>5A</th>
<th>5D</th>
<th>5B&lt;sup&gt;alt&lt;/sup&gt;</th>
<th>5B</th>
</tr>
</thead>
<tbody>
<tr>
<td>5B</td>
<td>29%</td>
<td>29%</td>
<td>43%</td>
<td>100%</td>
</tr>
<tr>
<td>5B&lt;sup&gt;alt&lt;/sup&gt;</td>
<td>27%</td>
<td>27%</td>
<td>100%</td>
<td>43%</td>
</tr>
<tr>
<td>5D</td>
<td>13%</td>
<td>100%</td>
<td>27%</td>
<td>29%</td>
</tr>
</tbody>
</table>

Structural differences among the C-Ph1 gene copies

Electrostatic patches

Different electrostatic patches indicate different nucleic acid binding sites

Bhullar et al., 2014, PNAS; Poster no: P0610
Conclusions

- Majority of the wheat genes express from more than one homoeolog
- About 87% of the wheat genes show differential expression pattern among homoeologs
- Novel, polyploid-specific function of the Ph1 gene seems to have evolved due to neofunctionalization of a gene important for the basic function of chromosome pairing
- For the Ph1 gene, neofunctionalization happened due to changes in gene structure, expression, and alternate splicing to create a novel function
- The 5D copy of the gene is perhaps ancestral and the polyploid-specific function of the 5B copy evolved via neofunctionalization
- Most if not all functions associated with the Ph1 gene by various studies are probably controlled by various structural and functional variants of the gene
- Most variants have weaker overlapping functions