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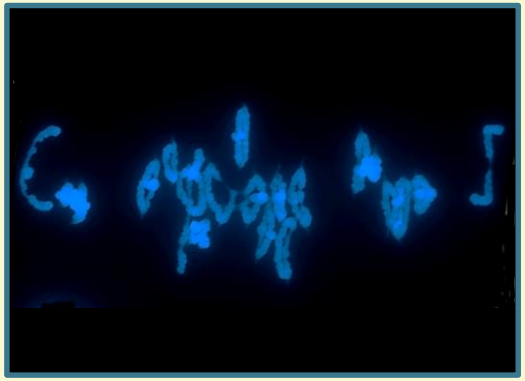
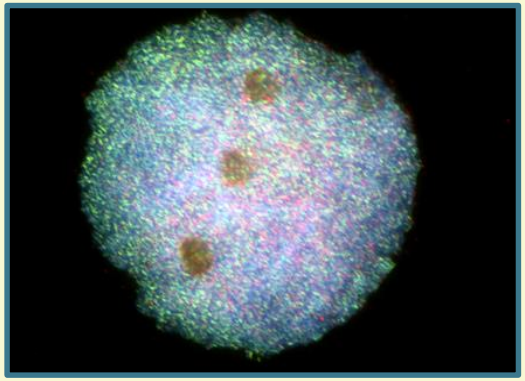
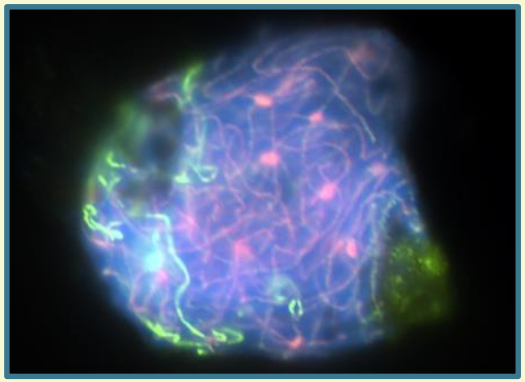
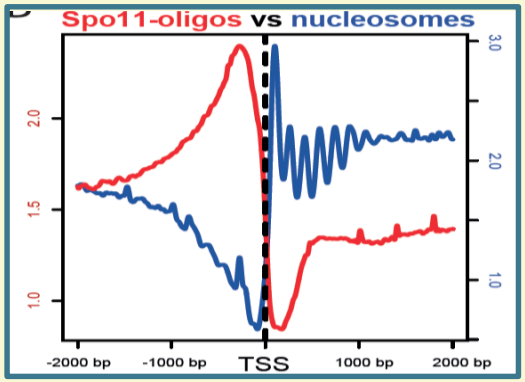
BBSRC bioscience for the future

Releasing Natural Variation in Bread Wheat by Modulating Meiotic Crossovers









James Higgins
(WGIN trait coordinator for Recombination)

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Background

- Genetic crossing over occurs during meiosis
- There is a skewed bias of crossovers (COs) towards the ends of chromosomes in wheat
- Creates linkage-drag so that separation of beneficial traits with detrimental traits is difficult
- We have put together a team with expertise in wheat genomics and meiotic recombination to modulate the frequency and distribution of COs

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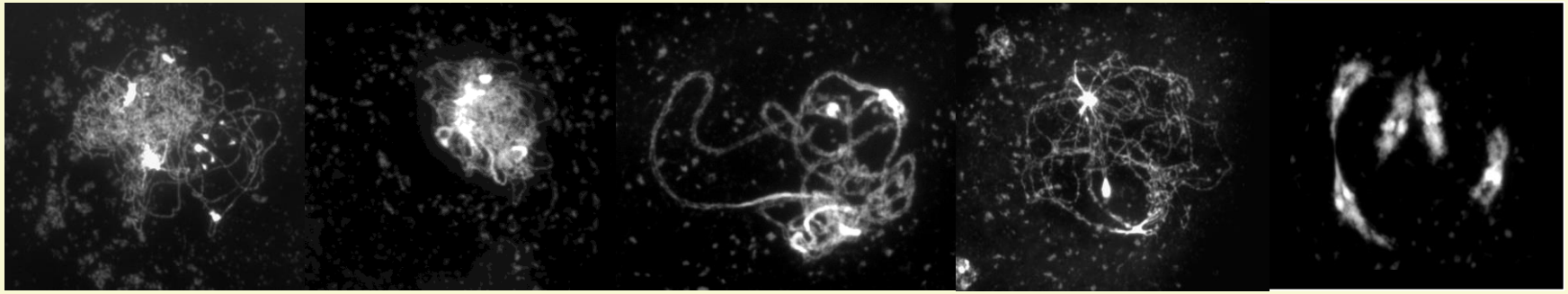
Meiosis is required to halve the number of chromosomes during sexual reproduction

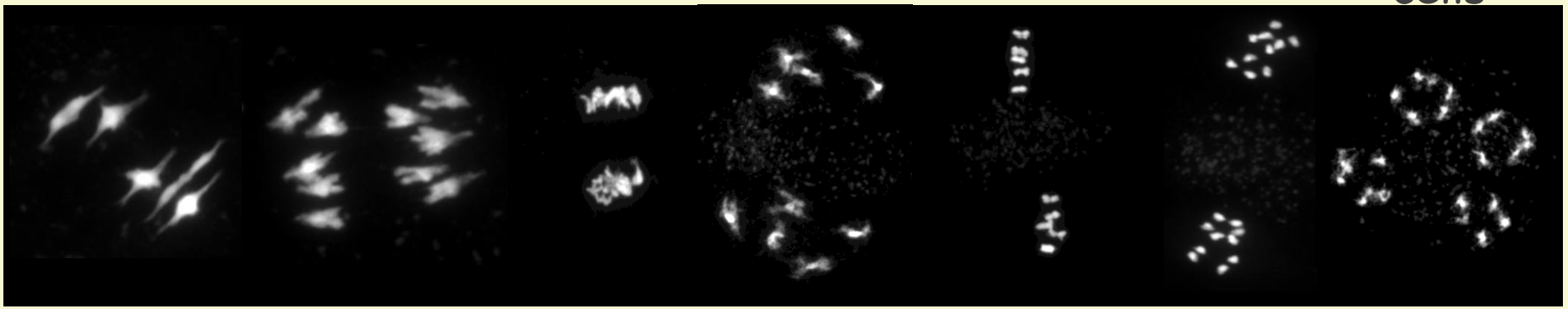
Prophase I



leptotene zygotene pachytene diplotene diakinesis

1st/2nd division

4 haploid cells



metaphase I anaphase I telophase I dyad metaphase II anaphase II tetrad

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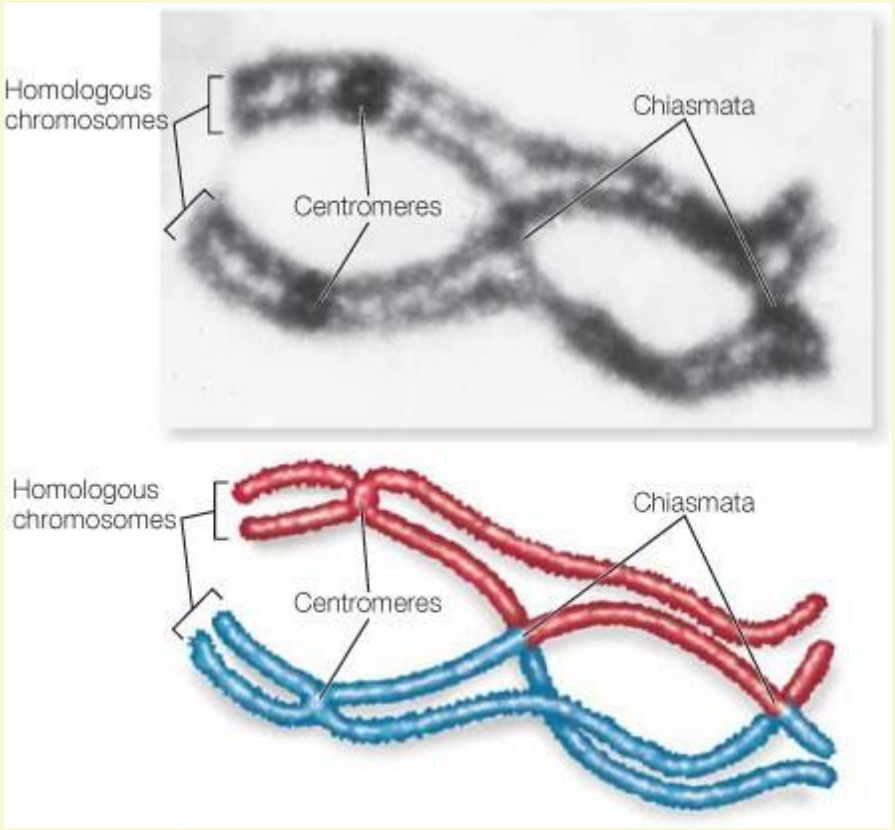
Meiosis is characterised by homologous recombination

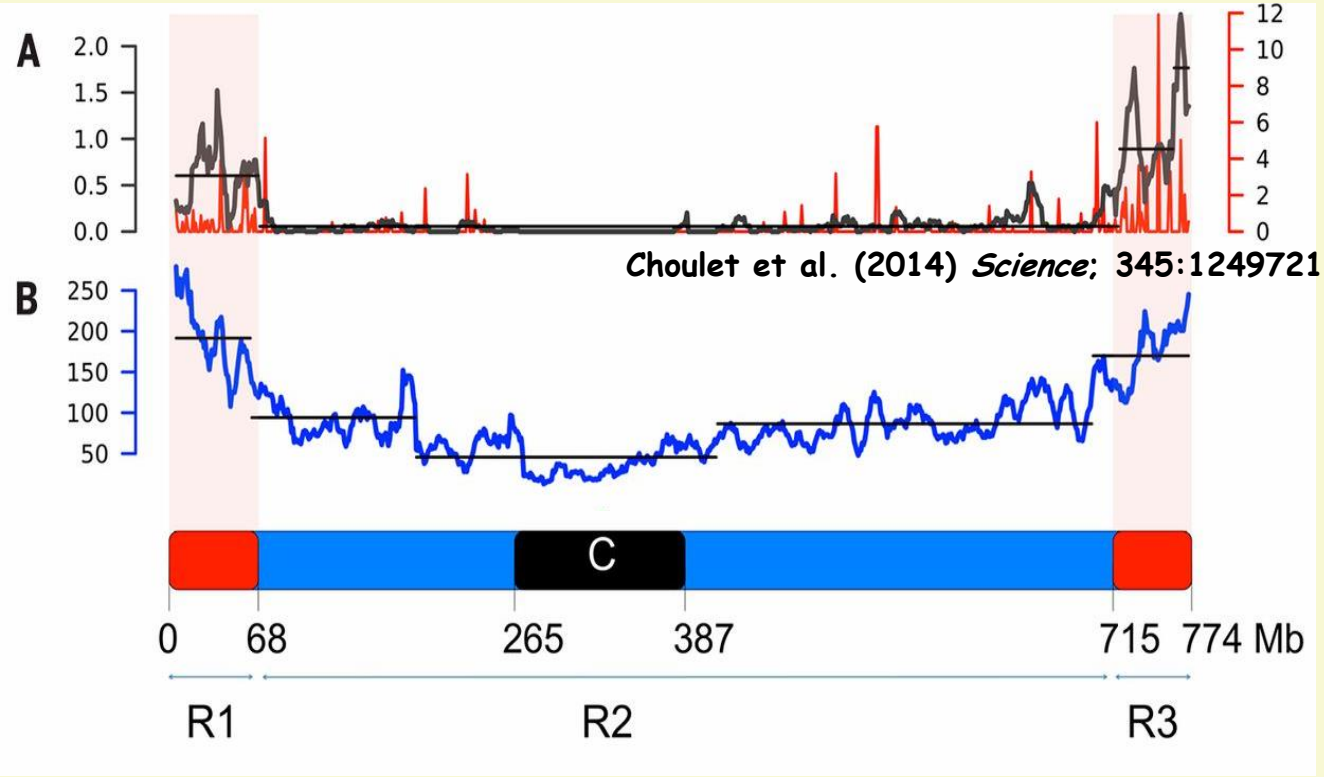


- Chiasmata are physical sites of genetic COs
- Ensure accurate chromosome segregation
- Promotes genetic variation between generations

CO distribution is highly skewed on wheat Chr.3B

CO rate

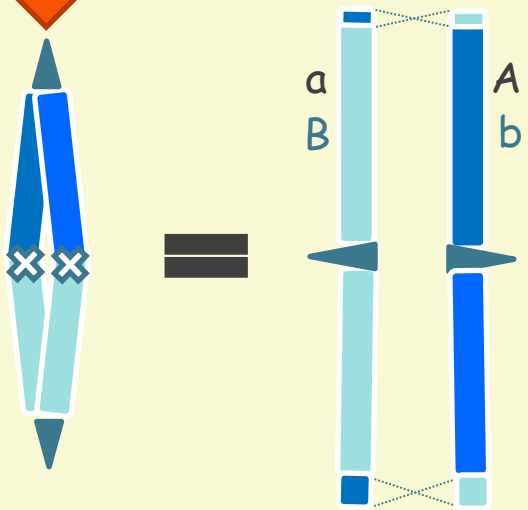
Gene content



- All COs occurred in ~13% of the chromosome
- No significant correlation was observed between recombination rate and gene content, coding DNA, or TE content

CO distribution is highly skewed in wheat chromosomes

- 21 pairs of homologous chromosomes



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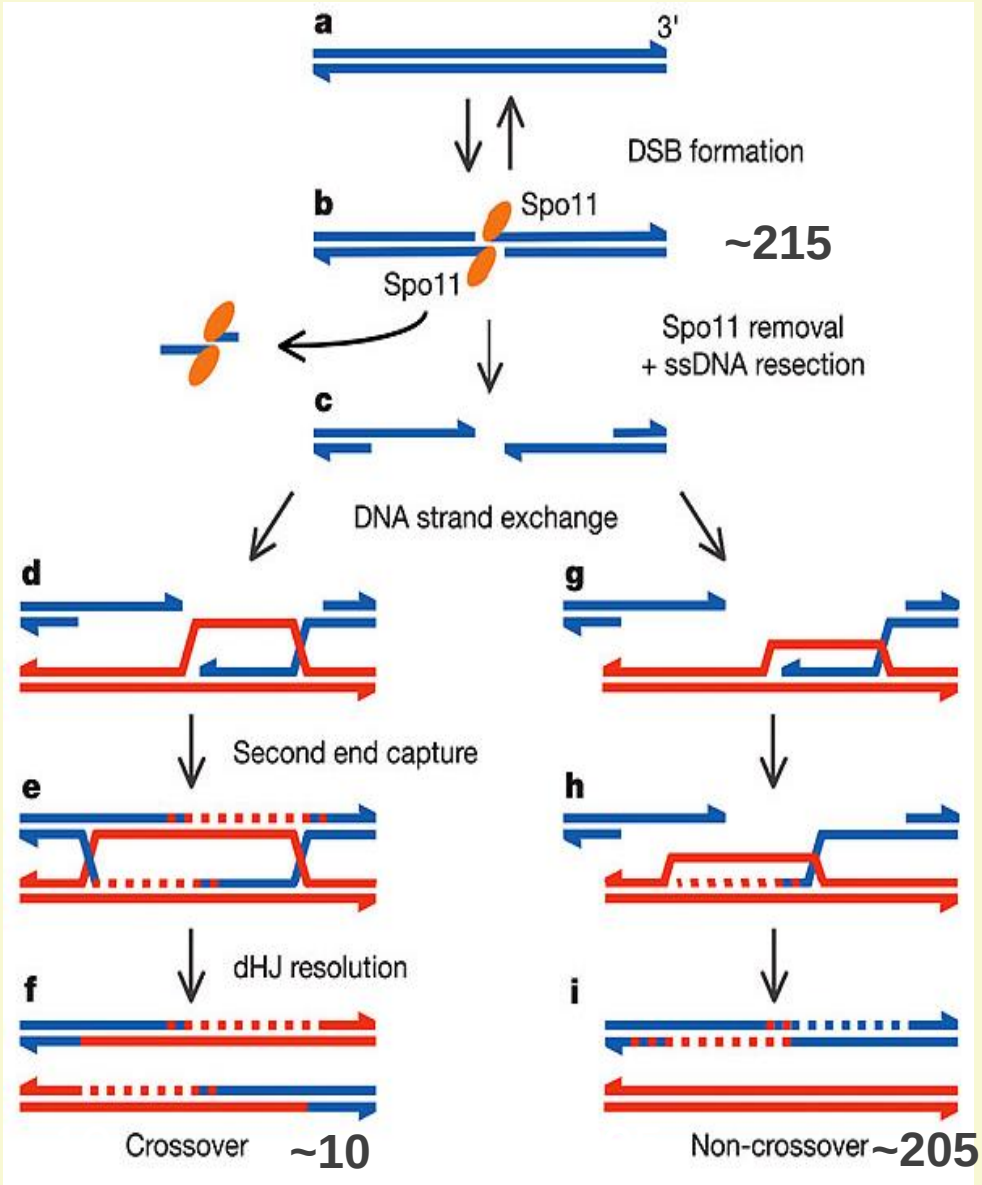
How is crossover formation controlled?

Meiotic recombination in Arabidopsis

Early recombination
SPO11-1/2
PRD3
NBS1
RAD50
MRE11
ATM/ATR
DMC1
RAD51

Late recombination
MSH4
MSH5
FANCM
MUS81
RECQ4A/B
MER3 (RCK)
SHOC1
PTD
RPA1a
HEI10
MLH3
MLH1



Structural proteins
ASY1
REC8
ASY3
ZYP1

Chromatin
γH2A.X
H2A.Z
H3K4me3?
MET1

Cell cycle
RBR
CDKA
CDKG
SDS1

CO regulators
PCH2
FIDG1
MPSS1

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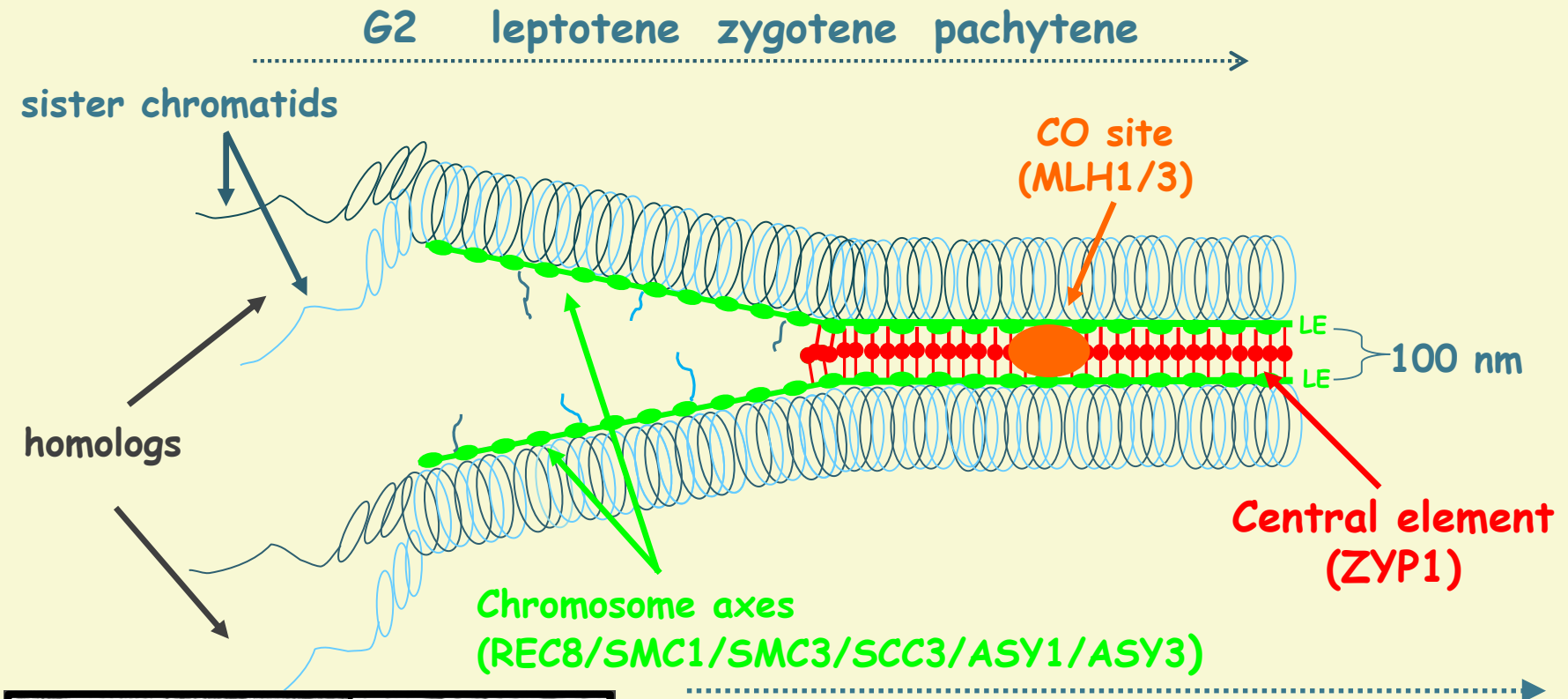
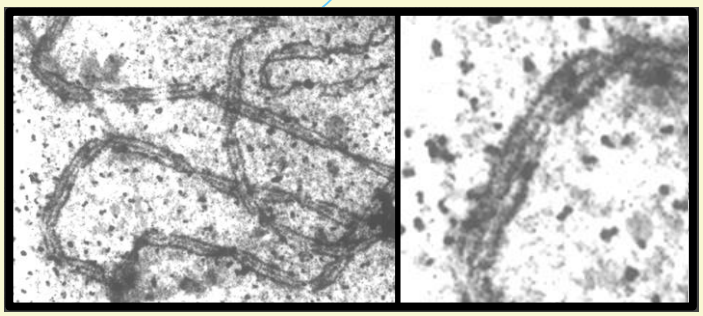
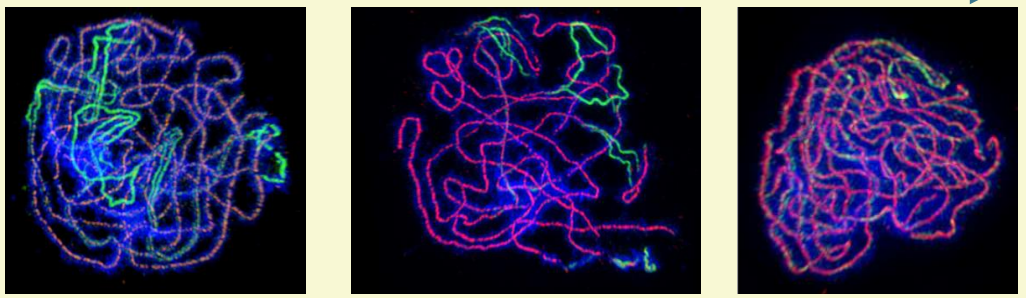
The Arabidopsis synaptonemal complex





SC silver stained



Immunolocalization - ASY1 ZYP1 DAPI

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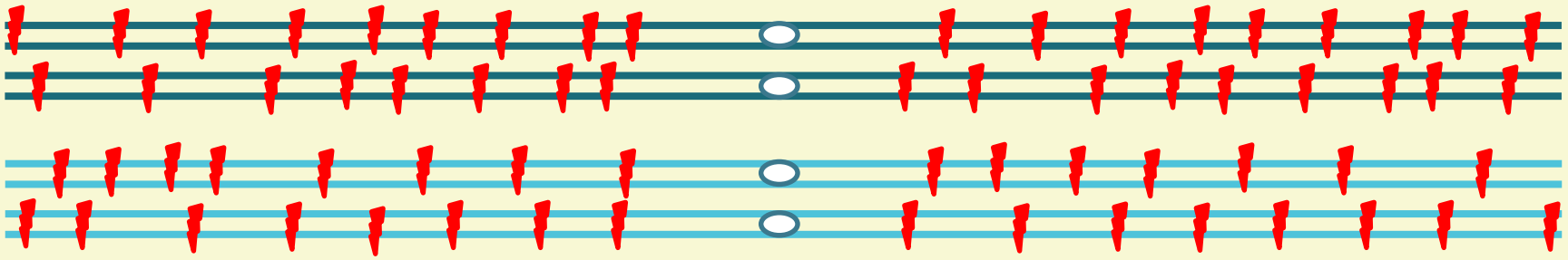
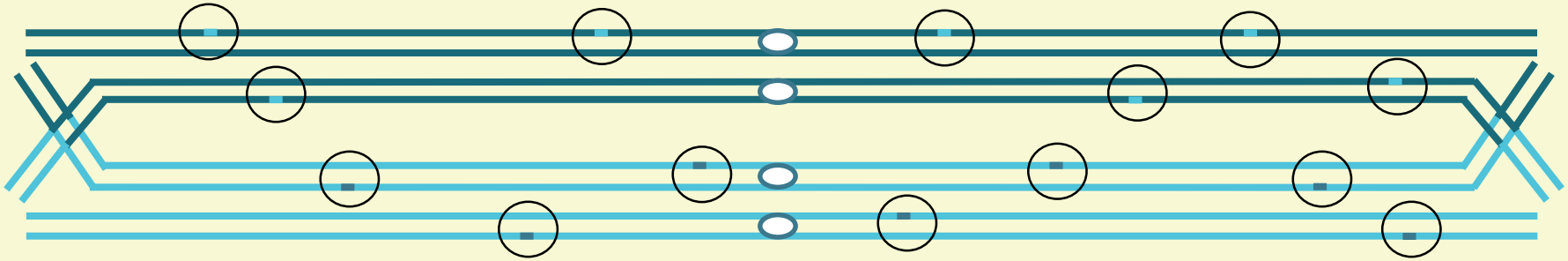
Crossover control in barley

Homologous recombination underpins genetic exchange



~ 66 DSBs/4 sister chromatids

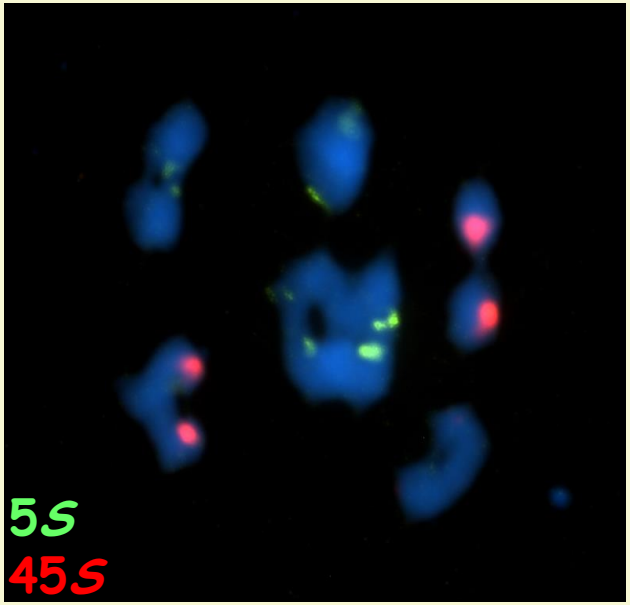
homologous recombinational repair



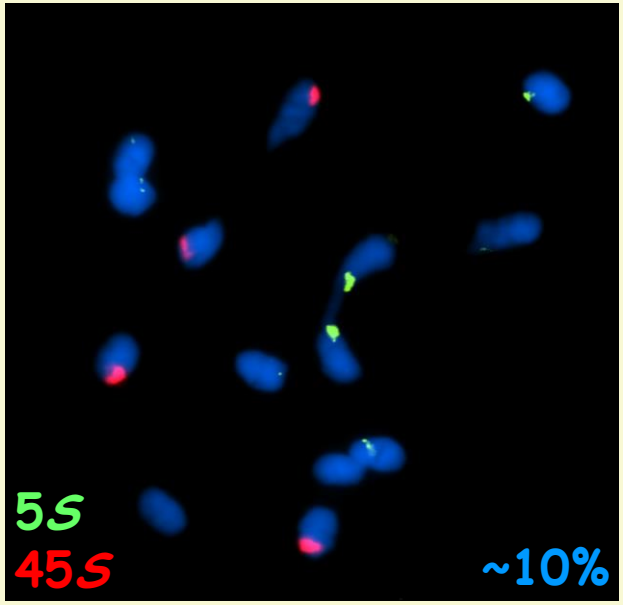
~14 ZYP1 initiation sites (expect ~150bp gene conversion)
~1-3 crossovers per homolog pair

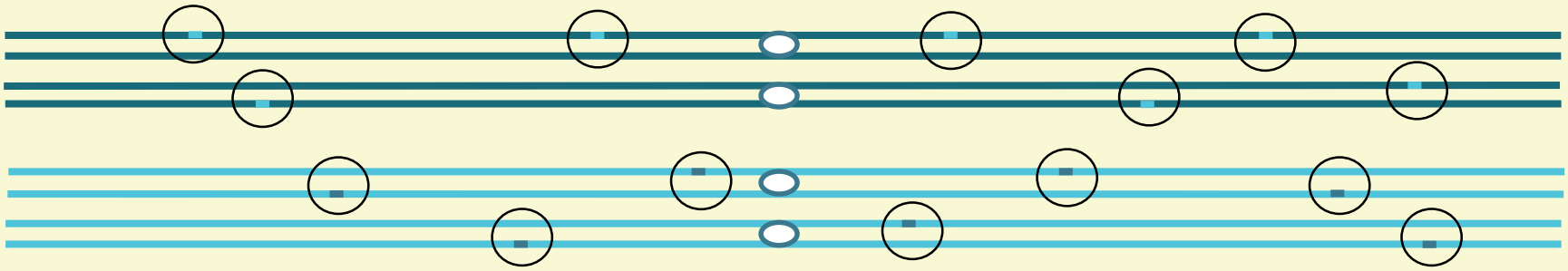
Altering CO distribution with *ZYP1* RNA interference

Control



ZYP1 RNAi





(Barakate *et al.* 2014, *The Plant Cell*)

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Timing of the meiotic program is critical for CO formation in barley

The Plant Cell, Vol. 24: 4096–4109, October 2012, www.plantcell.org

Spatiotemporal Asymmetry of the Meiotic Program Underlies the Predominantly Distal Distribution of Meiotic Crossovers in Barley[W]

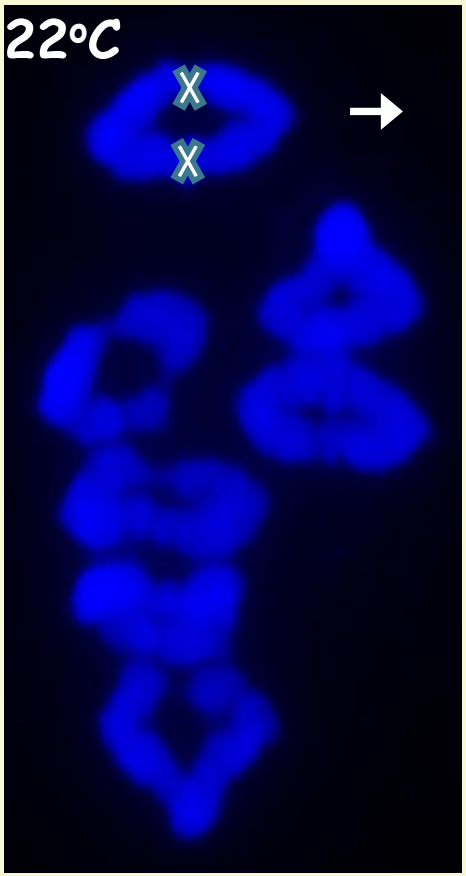
James D. Higgins,[a] Ruth M. Perry,[a] Abdellah Barakate,[b] Luke Ramsay,[c] Robbie Waugh,[c] Claire Halpin,[b] Susan J. Armstrong,[a] and F. Chris H. Franklin[a,1]

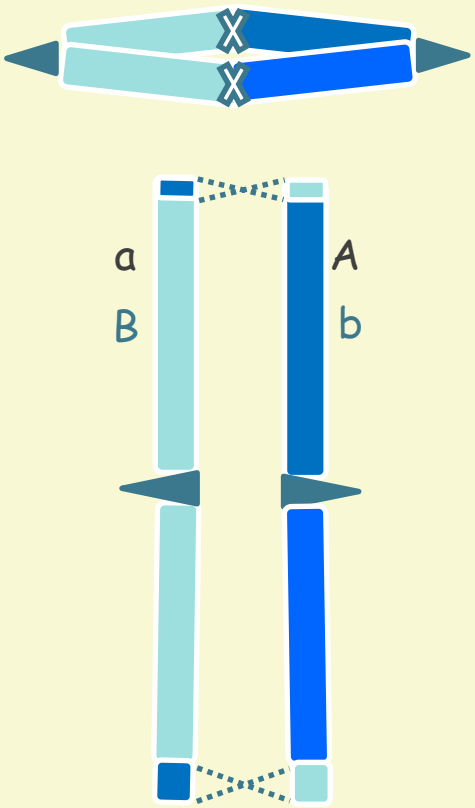
[a] School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom
[b] Division of Plant Sciences at James Hutton Institute, University of Dundee, Invergowrie, Dundee DD1 5EH, Scotland
[c] James Hutton Institute, Invergowrie, Dundee DD2 5DA, Scotland

Meiosis involves reciprocal exchange of genetic information between homologous chromosomes to generate new allelic combinations. In cereals, the distribution of genetic crossovers, cytologically visible as chiasmata, is skewed toward the distal regions of the chromosomes. However, many genes are known to lie within interstitial/proximal regions of low recombination, creating a limitation for breeders. We investigated the factors underlying the pattern of chiasma formation in barley (*Hordeum vulgare*) and show that chiasma distribution reflects polarization in the spatiotemporal initiation of recombination, chromosome pairing, and synapsis. Consequently, meiotic progression in distal chromosomal regions occurs in coordination with the chromatin cycles that are a conserved feature of the meiotic program. Recombination initiation in interstitial and proximal regions occurs later than distal events, is not coordinated with the cycles, and rarely progresses to form chiasmata. Early recombination initiation is spatially associated with early replicating, euchromatic DNA, which is predominately found in distal regions. We demonstrate that a modest temperature shift is sufficient to alter meiotic progression in relation to the chromosome cycles. The polarization of the meiotic processes is reduced and is accompanied by a shift in chiasma distribution with an increase in interstitial and proximal chiasmata, suggesting a potential route to modify recombination in cereals.

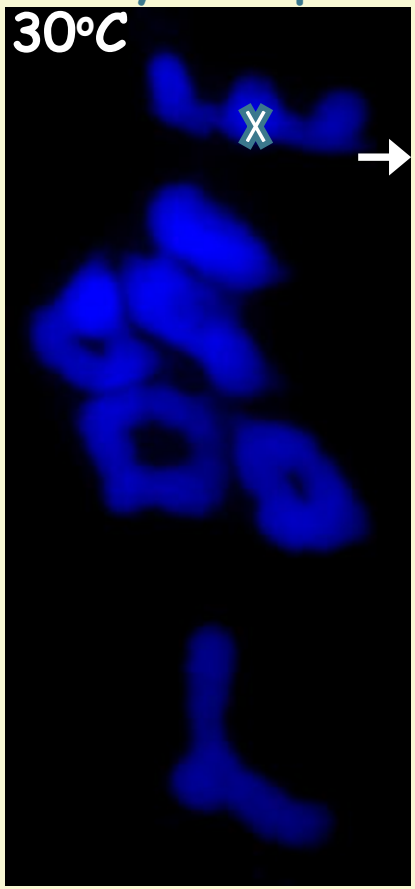
Elevated temperatures alter CO distribution

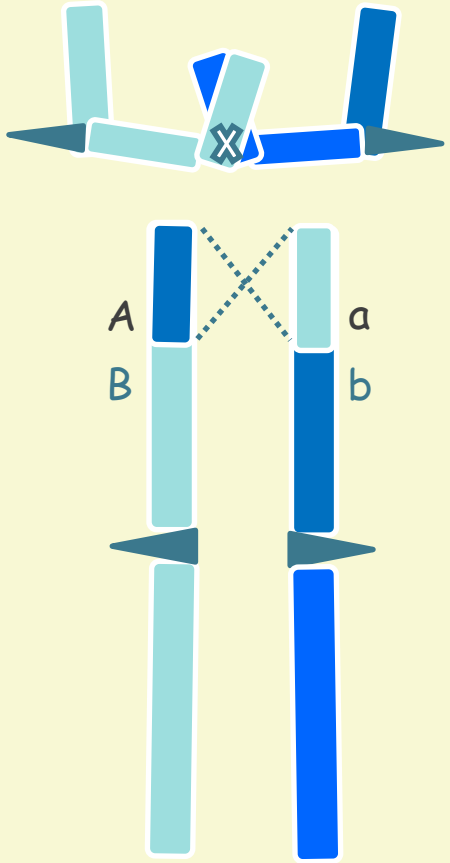
Barley metaphase I





Barley metaphase I





- increases speed of heterochromatic DNA replication
- meiotic events more synchronous

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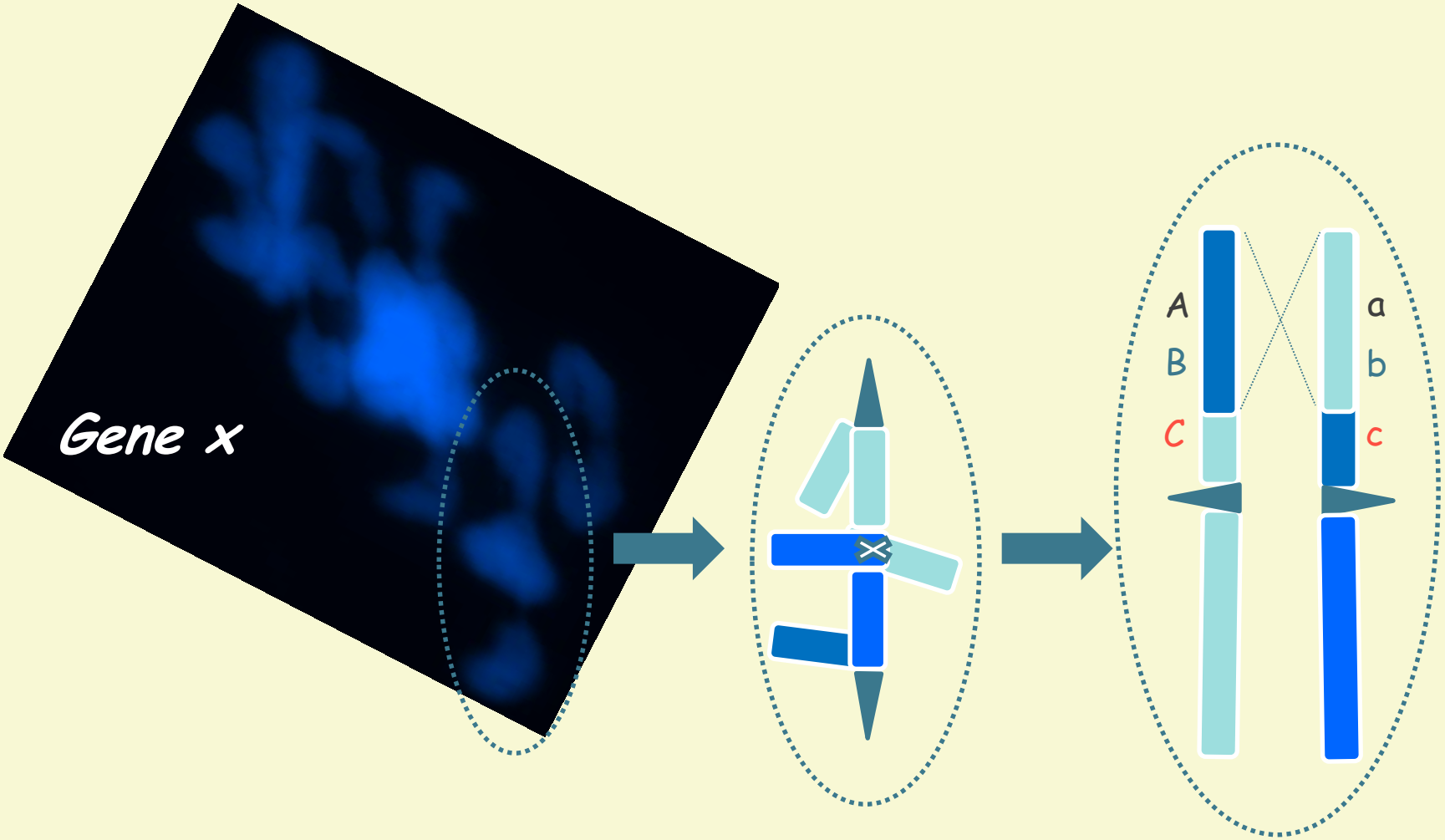
Altering CO distribution in barley



(Higgins and Barakate, unpublished)

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How can we use this knowledge to modulate CO formation in wheat?

The wheat LoLa recombination team

Lead PI: Keith Edwards (Bristol)
Co-PI: James Higgins (Leicester)
Co-PI: Chris Franklin & Eugenio Sanchez-Moran (Birmingham)
Co-PI: Ian Henderson (Cambridge)
Co-PI: Cristobal Uauy (JIC)

- **Work Package 1: Factors influencing the distal bias of COs in bread wheat** (Lead CF; participants UoBi, UoL, UoC)
- **Work Package 2: Manipulation of CO distribution and frequency** (Lead JH; Participants UoL, UoBi, UoBr, JIC, KWS, RAGT)
- **Work Package 3: A synthetic biology approach to target recombination in wheat** (Lead IH; participants UoC, UoL, UoBi, UoBr, JIC)
- **Work Package 4: Application** (Lead CU; participants JIC, UoBr, UoL, UoBi, UoC, KWS, RAGT)
- **Work Package 5: Programme management, dissemination and training** (Lead KE; participants JIC, UoBr, UoL, UoBi, UoC, KWS, RAGT)

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Work package 2

Aims:

- To increase COs in wheat 'cold' chromosomal regions for application in WP4
- Verify CO modulation using state-of-the-art cytological and molecular techniques
- To understand the mechanism behind modulated COs

Target genes:

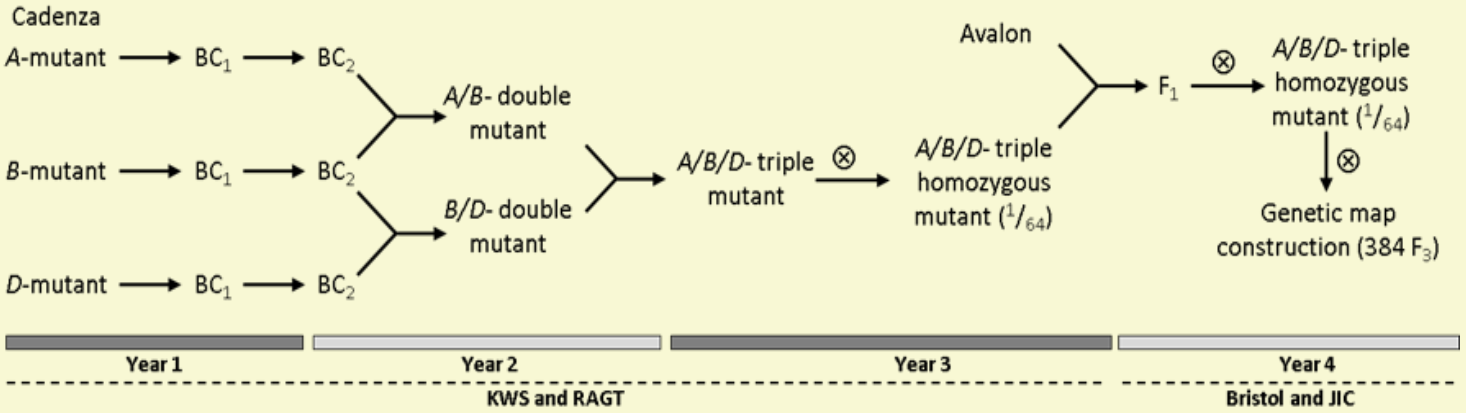
- DNA repair
- Chromatin modifiers
- CO modulators

Approaches:

- Target Induced Local Lesion (TILLing)
- CRISPR/Cas
- Virus induced gene silencing (VIGS)
- Heat stress

Progress: Generating material

TILLing lines



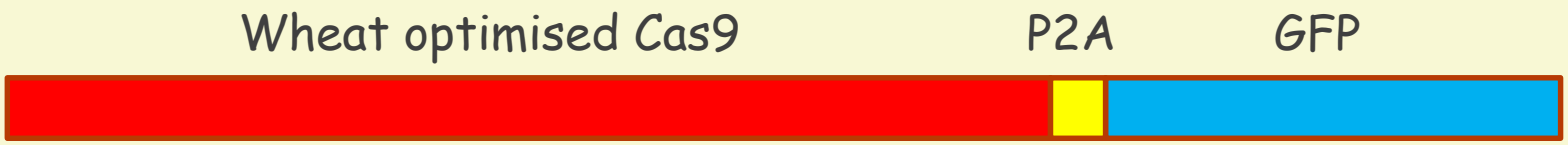
Kronos (tetraploid): fixing material for double knockouts of homologous genes

VIGS

- Testing constructs in collaboration with Kostya Kanyuka (RRES)

CRISPR/Cas

Keith Edwards (Bristol), Caroline Sparks & Alison Huttly (RRES)



- Successful transient assays in protoplasts and immature embryos
- 4/28 transgenic plants showed evidence of gene editing

1. All three copies of *Spo11* (including one plant which appears to carry edits in all three copies)
2. Two copies of *Fancm (A and B genome)*
3. The 3B copy of *Ga20 oxidase*

- All plants where fertile and all set seed
- The ability to mix large numbers of guide RNAs will reduce the cost of generating edited lines for a range of genes

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Summary

- In wheat COs are limited in number and distribution
- Transfer knowledge and tools from Arabidopsis and barley to wheat
- Multi-pronged approach with leading UK expertise to alter COs
- Unlock genetic diversity in wheat

Acknowledgements

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Mark Winfield
Sacha Przewieslik-Allen

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Ian Henderson
Wei Jiang

R·A·G·T

Chris Burt

KWS

Ed Byrne
Nikki Kettles

John Innes Centre
Unlocking Nature's Diversity

Cristobal Uauy
James Simmonds