

# Meiotic cell cycle progression and its connection to genome evolution

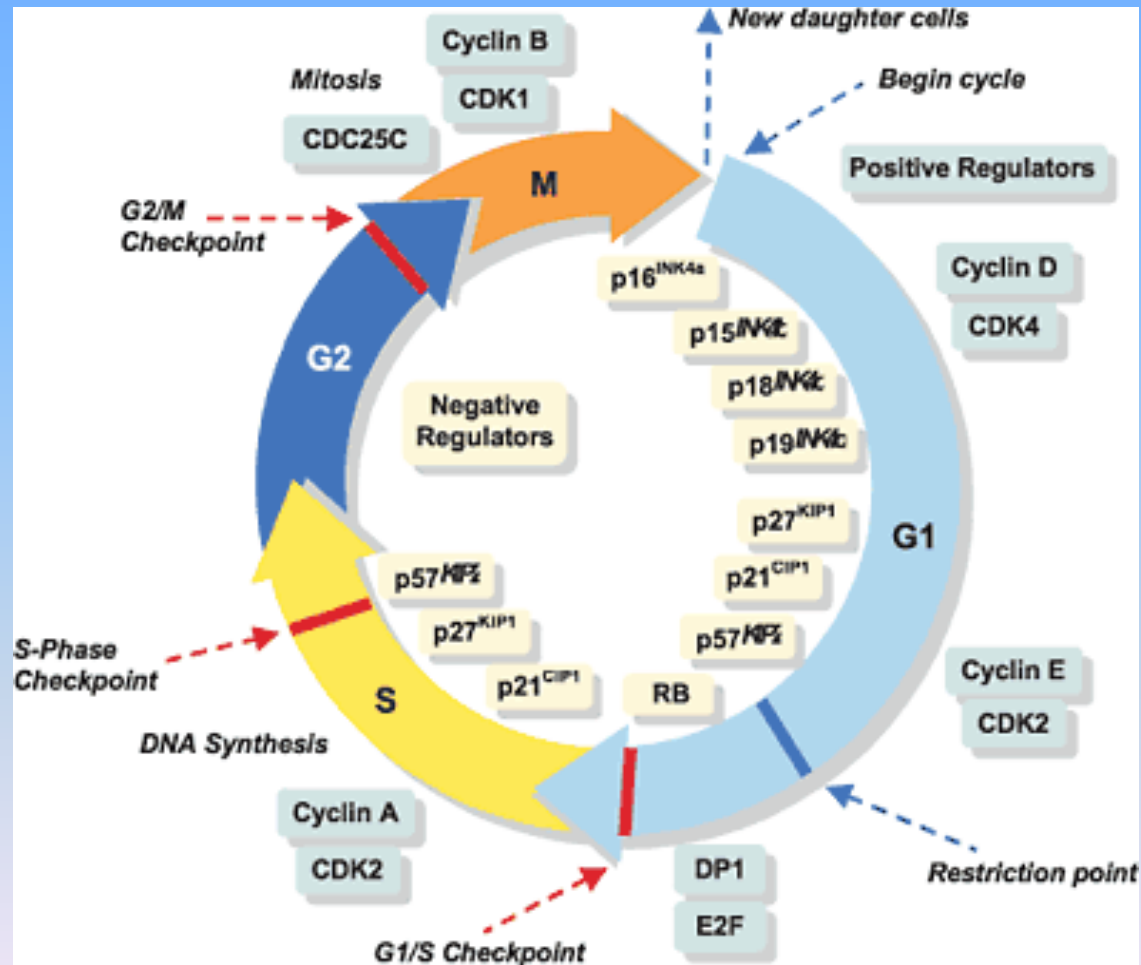
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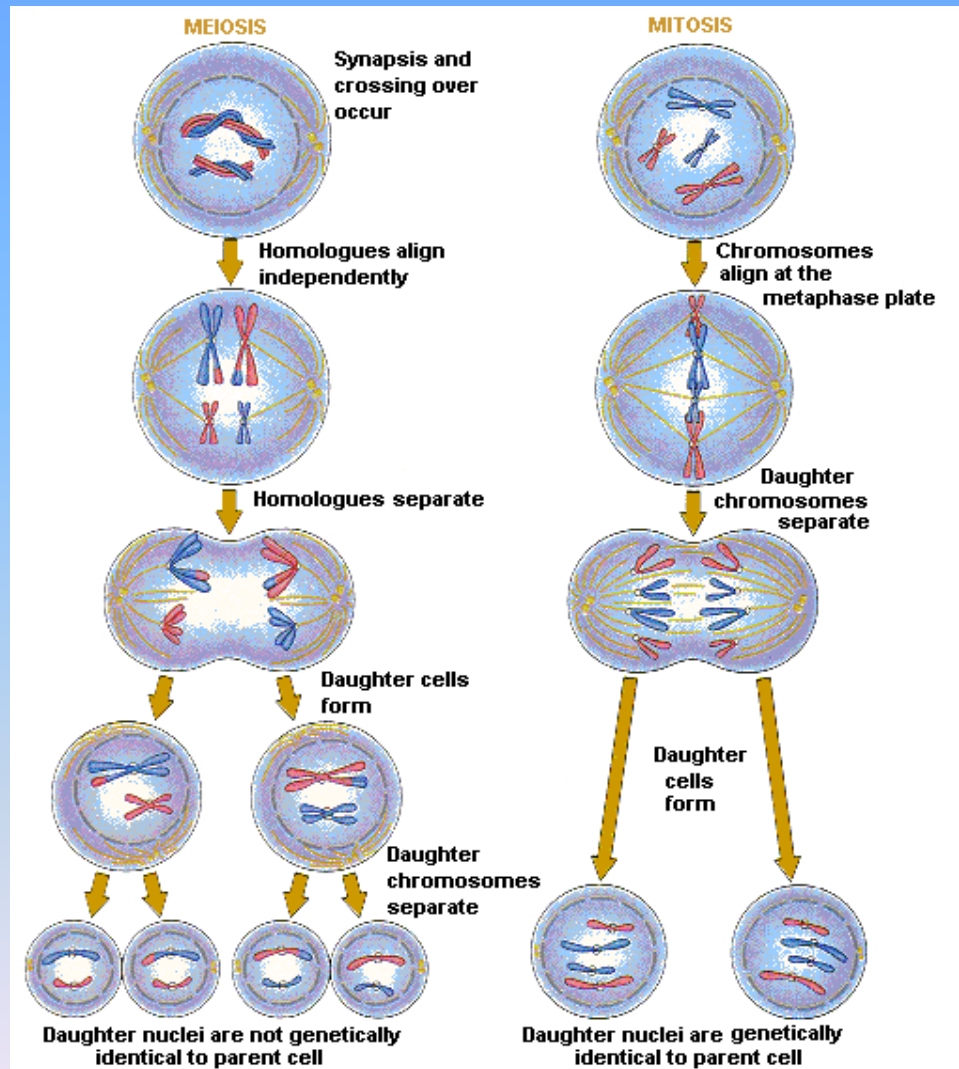
## **Main points of presentation**

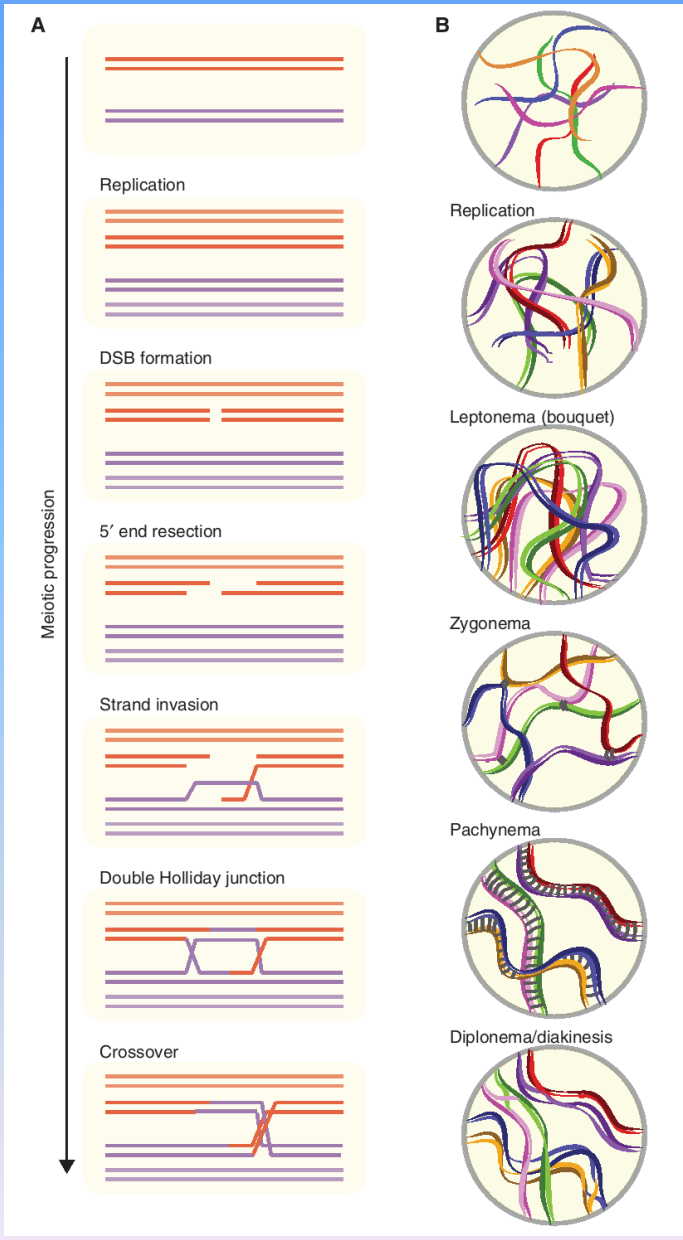
- **The meiotic cell cycle**
- **The meiotic checkpoint network (MCN)**
- **Our work on meiotic cell cycle progression in Arabidopsis**
- **Consequences of defective cell cycle progression in meiosis**
- **How unreduced gametes may affect genome evolution**

# Different cyclins oscillate once in a cell cycle with CYCAs expressed from S phase to G2 or M phase



# Meiosis vs. mitosis





← S phase and G<sub>2</sub> phase

Prophase I

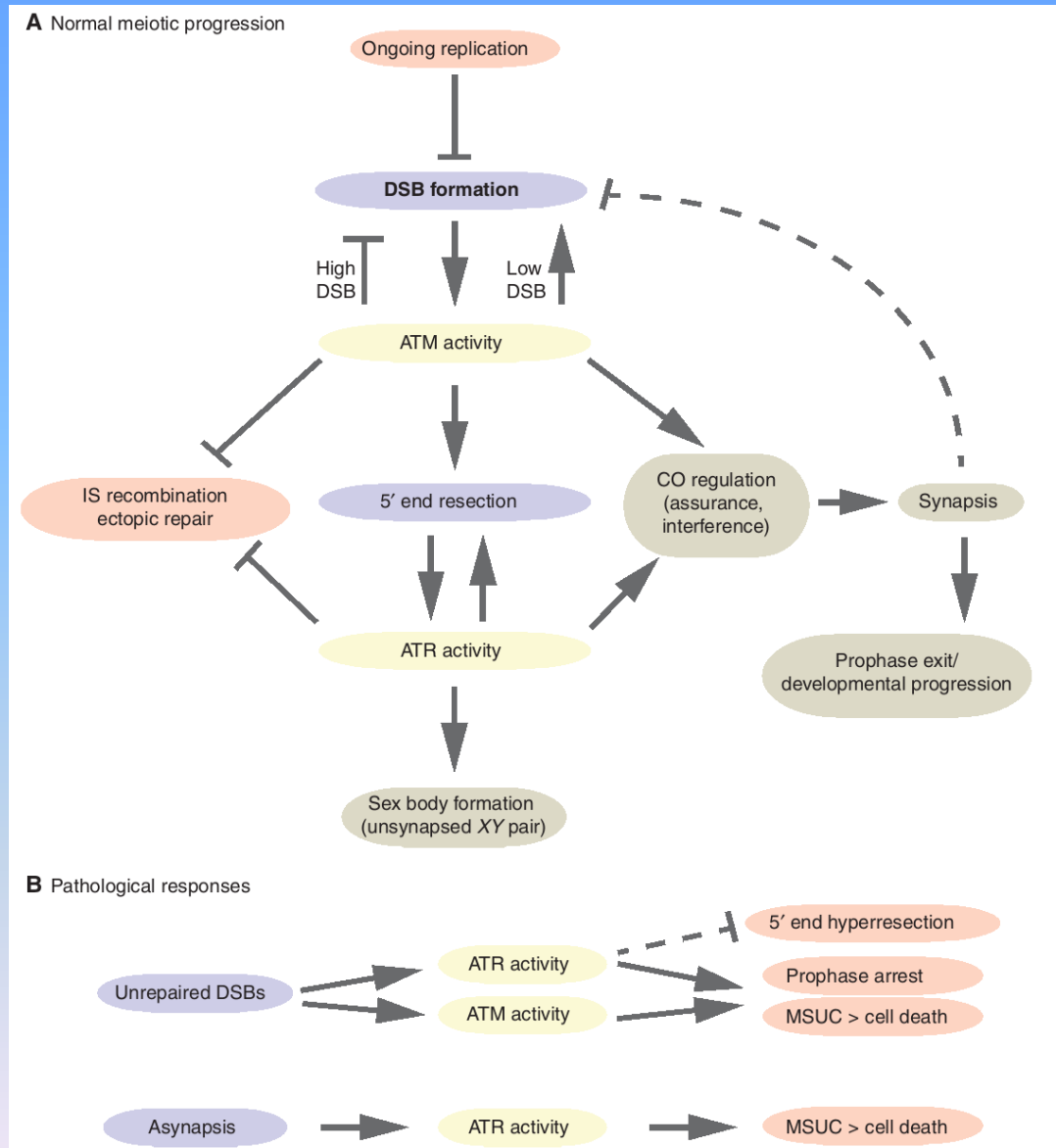
## **Outcomes of defective meiotic cell cycle progression**

- **Animals:** cell death without completion of meiotic cell cycle progression
- **Yeasts:** cell cycle arrest or switching to mitosis if conditions are right
- **Plants:** delayed cell cycle progression but progressing through meiosis

## **Meiotic cell cycle progression is regulated by the meiotic checkpoint network (MCN)**

- **MCN consists of a smaller number of proteins that are highly connected**
- **MCN functions in both normal meiotic cell cycle progression and in response to defects in prophase I**
- **The key components of the network are evolutionarily conserved**
- **Different outcomes of defective meiotic cell cycle progression suggest variation in the architecture of the MCN in different organisms**
- **Currently little is known about the components and architecture of the MCN in plants**

# Dependent relationships established by the MCN





**Table 1.** MCN proteins and their homologs

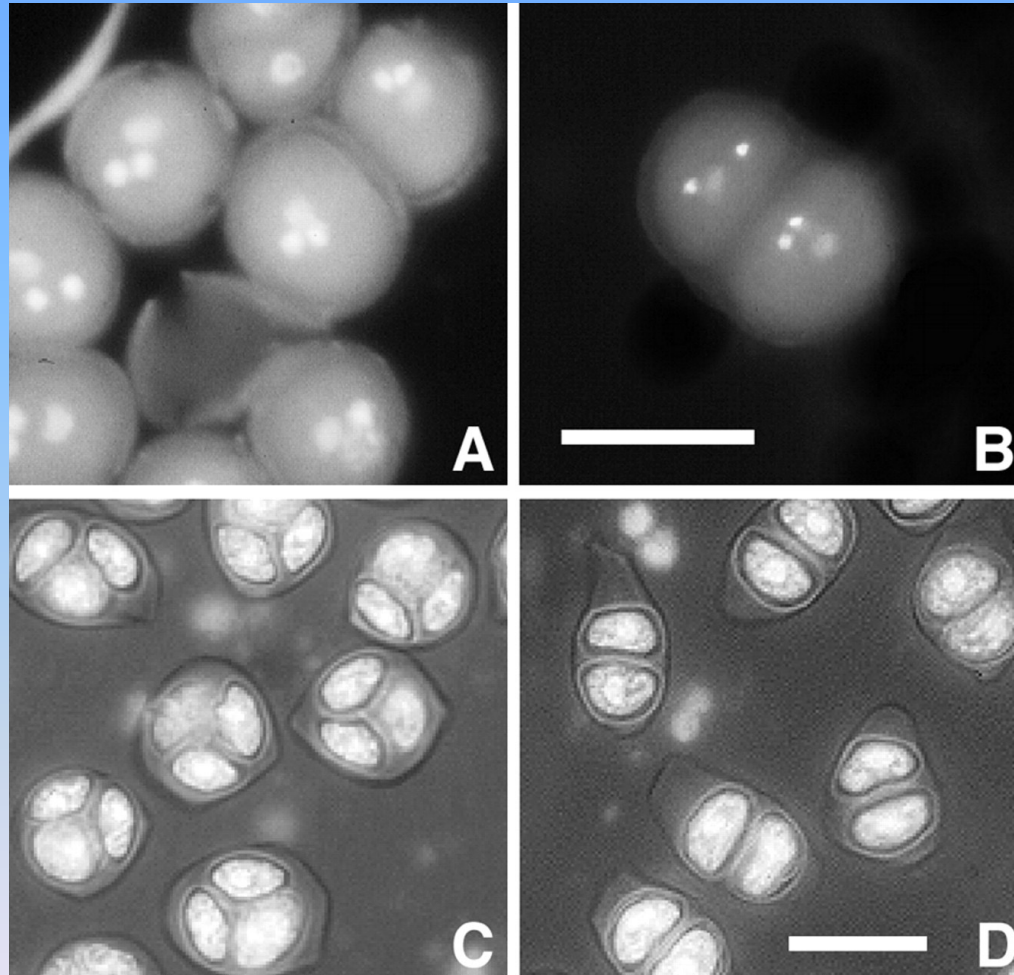
Mammals	<i>S. cerevisiae</i>	<i>C. elegans</i>	<i>S. pombe</i>	<i>Drosophila</i>	<i>Arabidopsis</i>	Function
<b>Signaling proteins</b>						
<b>ATR</b>	<b>Mec1</b>	<b>ATL-1</b>	<b>Rad3p</b>	<b>Mei-41</b>	<b>ATR</b>	PI3 <sup>a</sup> kinase-like kinase
<b>ATM</b>	<b>Tel1</b>	<b>ATM-1</b>	Tel1p	<b>Atm</b>	<b>ATM</b>	PI3 kinase-like kinase
<b>RAD9A,</b> <b>RAD9B</b>	<b>Ddc1</b>	<b>HPR-9</b>	<b>Rad9p</b>	<b>Rad9A,</b> Rad9B	–	PCNA <sup>b</sup> -like clamp (9-1-1 complex)
<b>RAD1</b>	<b>Rad17</b>	<b>MRT-2</b>	<b>Rad1p</b>	Rad1		PCNA-like clamp (9-1-1 complex)
<b>HUS1</b> HUS1B	<b>Mec3</b>	<b>HUS-1</b>	Hus1p	<b>Hus1</b>	–	PCNA-like clamp (9-1-1 complex)
<b>CHK1</b>	(Chk1)	<b>CHK-1</b>	Chk1p	(Grp)	–	Protein kinase
<b>CHK2</b>	<b>Rad53</b> <b>Mek1</b>	<b>CHK-2</b>	<b>Cds1p</b> <b>Mek1p</b>	<b>Mnk</b>	–	Protein kinase with FHA <sup>c</sup> domain
Several	<b>Cdc5</b>	<b>PLK-2</b>	Plo1p	<b>Polo</b>	–	Protein kinase
<b>HORMAD1</b> <b>HORMAD2</b>	<b>Hop1</b>	<b>HTP-1</b> <b>HTP-2</b> HIM-3	Hop1	–	ASY1	Chromosomal HORMA-domain proteins
<b>SYCP3</b>	<b>Red1?</b>	<b>HTP-3?</b>	Rec10?	C(2)M?	ASY3?	Chromosome axis component
Several	<b>Sir2</b>	<b>SIR-2</b>	Sir2p	<b>Sir2</b>	SRT1 SRT2	NAD-dependent deacetylase
<b>TRIP13</b>	<b>Pch2</b>	<b>PCH-2</b>	–	<b>Pch2</b>	–	AAA <sup>+</sup> -ATPase <sup>d</sup>

## **Our work on meiotic cell cycle progression in Arabidopsis**

- **The function of the ARABIDOPSIS SKP1-LIKE1 (ASK1)**
- **The function of the TARDY ASYNCHRONOUS MEIOSIS (TAM)**

**How was the work started?**

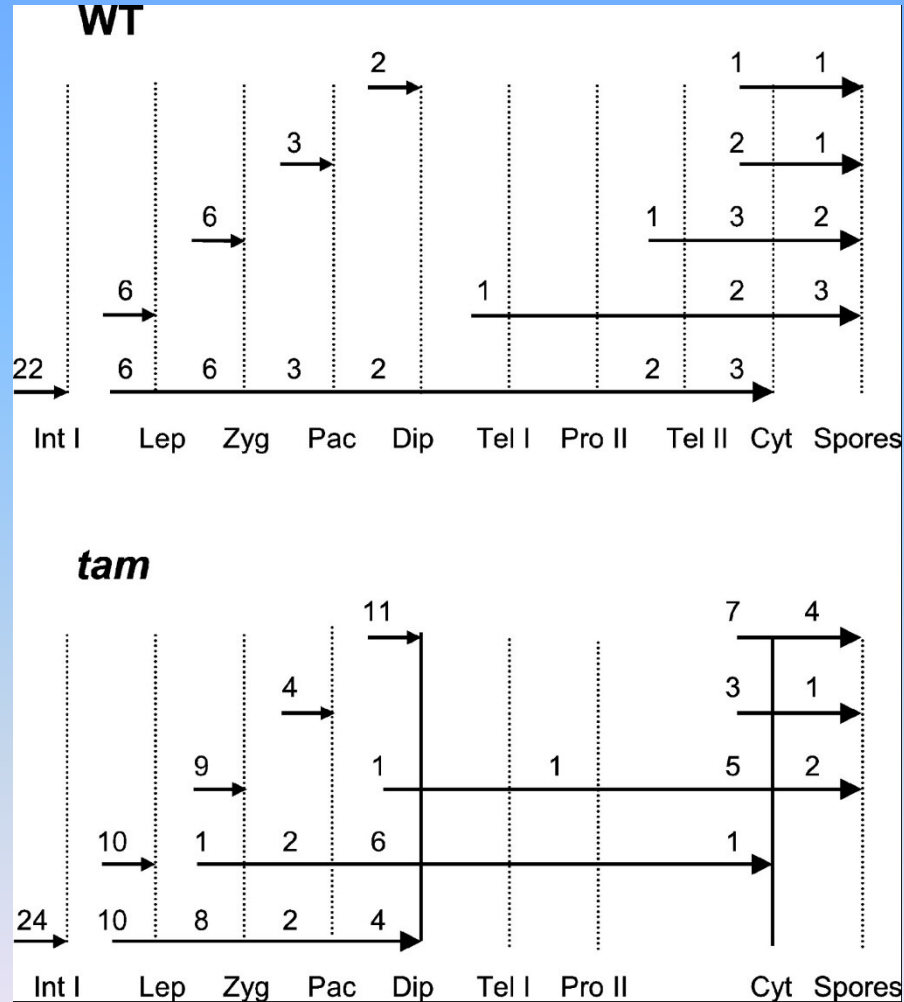
# Formation of double gametophytes and dyad meiotic products in *tam-1*



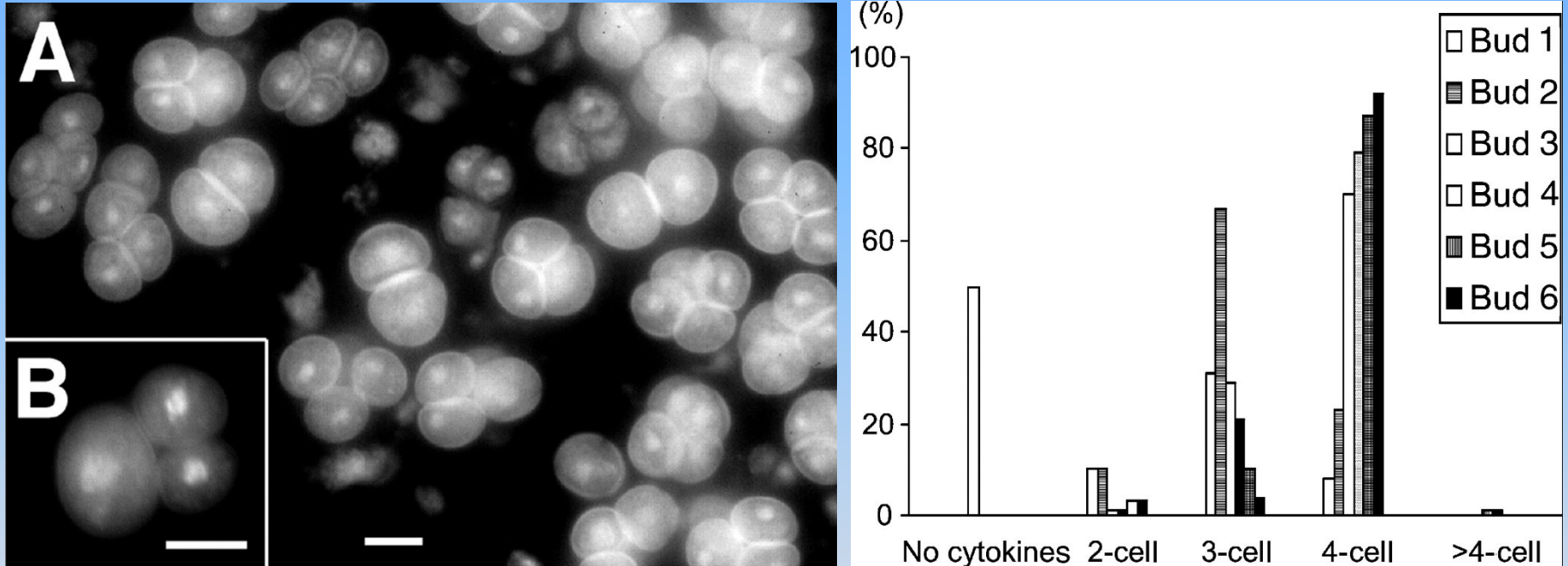
**Question:**

**Do dyads result from precocious cytokinesis or slower cell cycle progression?**

# Comparison of meiotic stages in two consecutive buds in both WT and *tam-1* plants

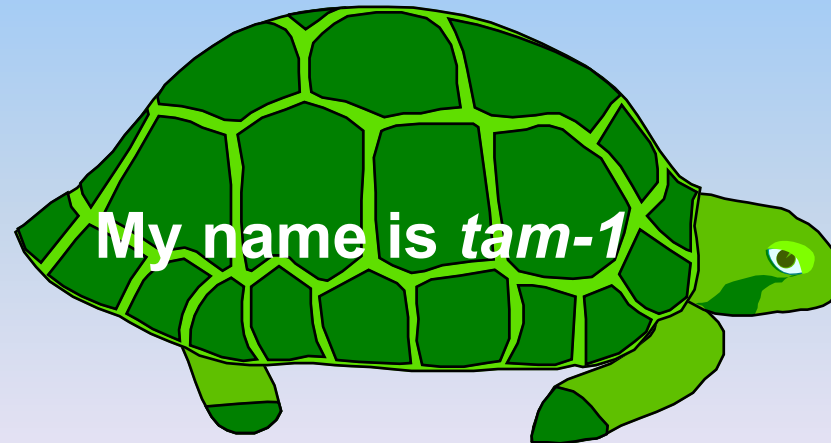


# Dynamics in number of microspores per meiotic product during development



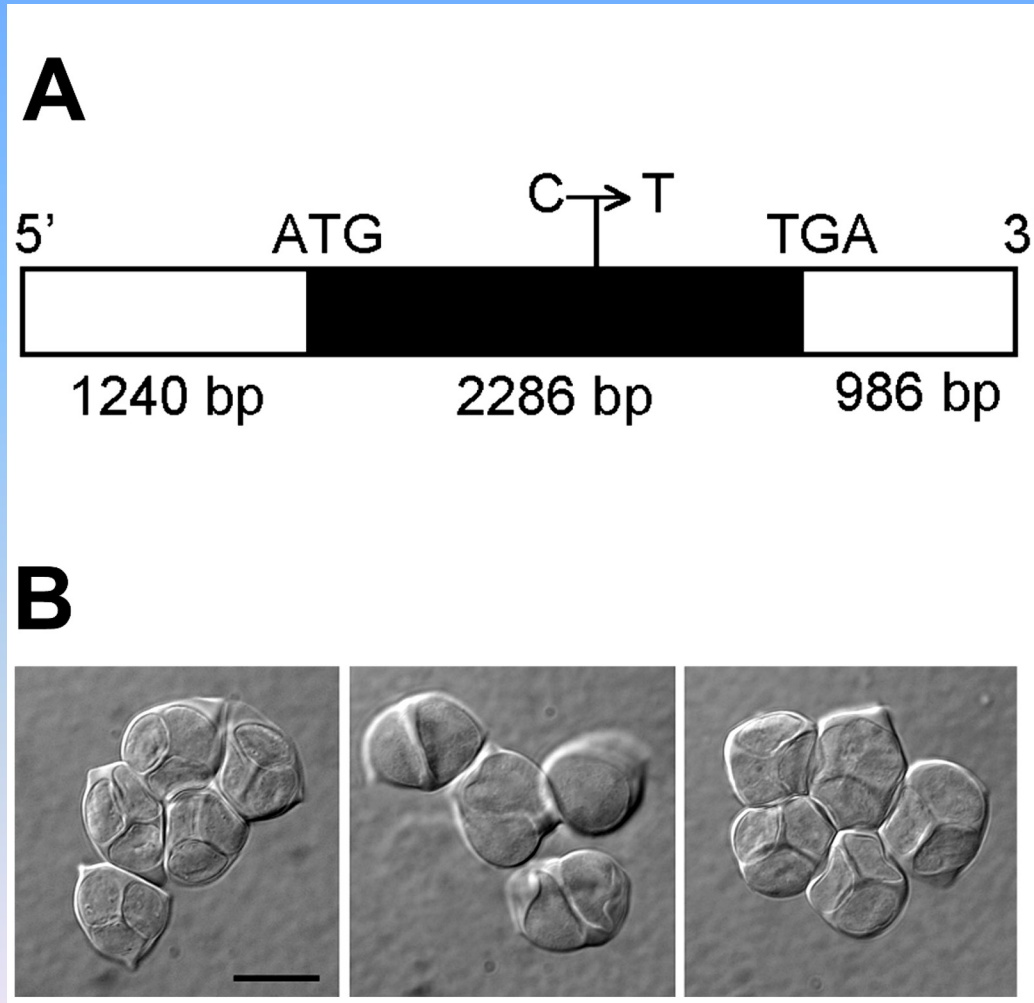
## Conclusion

Male meiotic cell cycle progression in *tam-1* is slower than in the WT; TAM is required for the normal pace of cell cycle progression during male meiosis

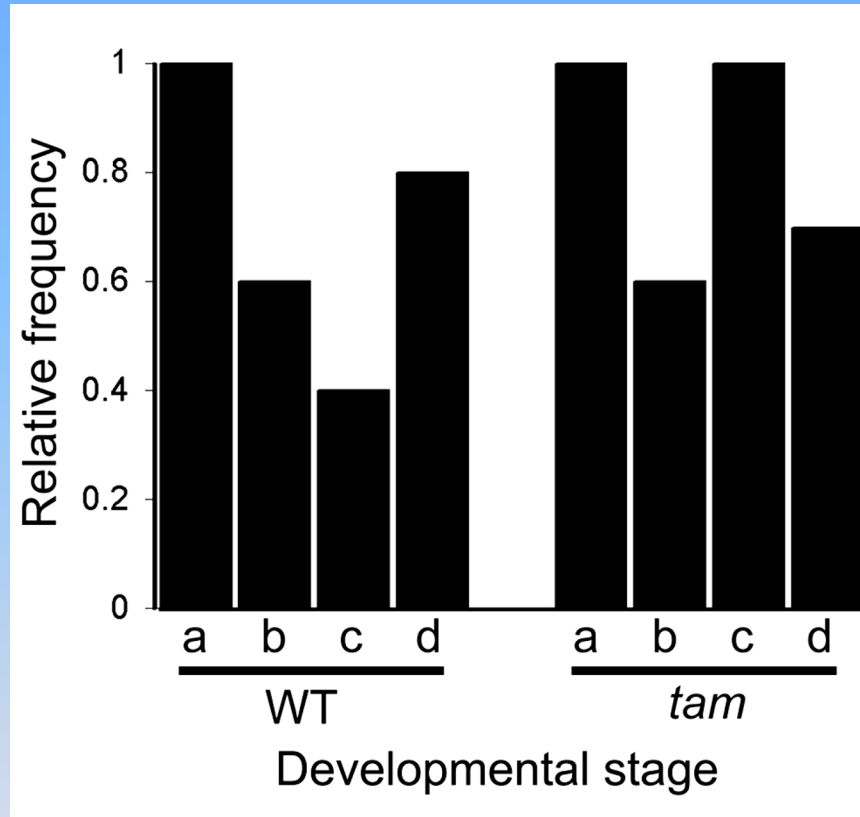




# Identification of *TAM* by map-based cloning: *CYCA1;2*, an A-type cyclin, complemented *tam-1* meiotic defect

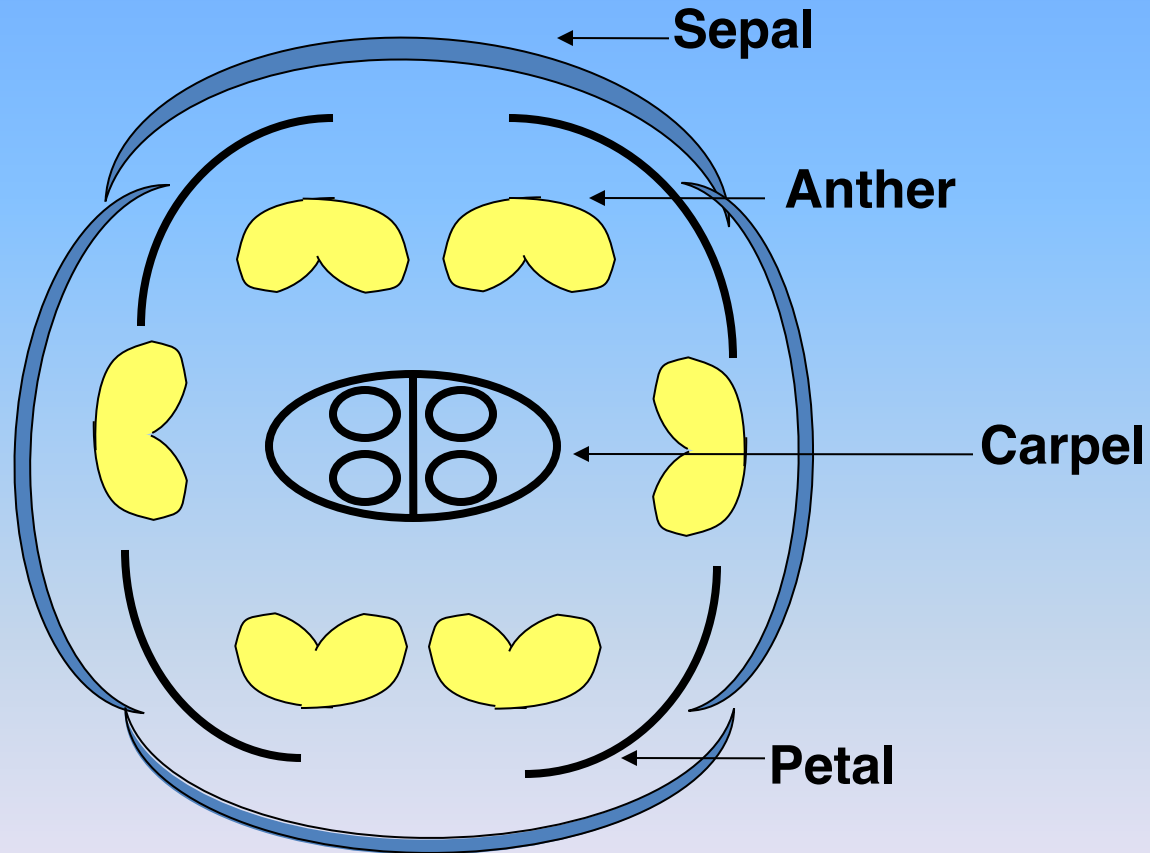


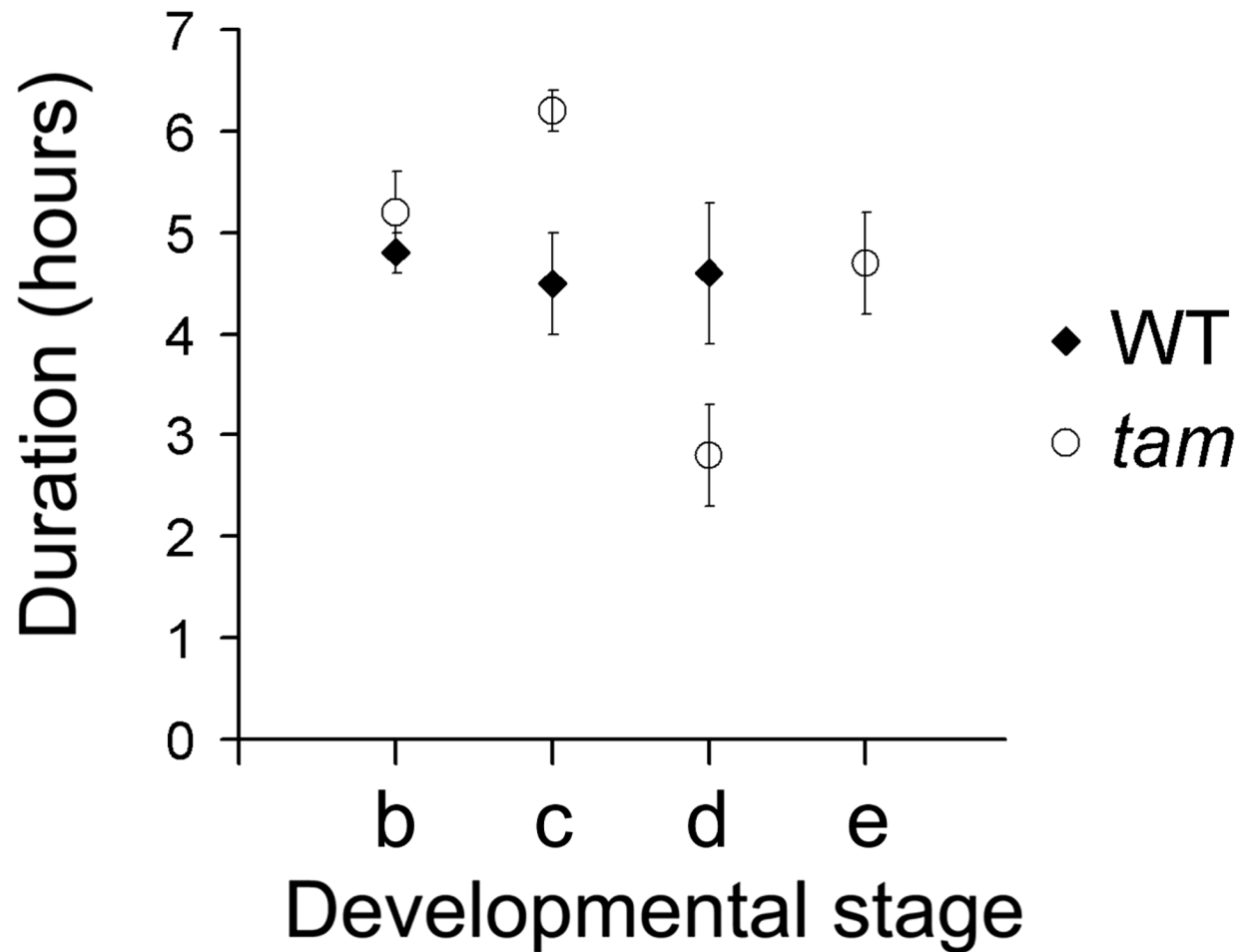
# Further characterization of *tam-1* revealed that TAM primarily functions in pachytene



a, leptotene; b, zygotene; c, pachytene; d in WT, diplotene to early tetrads; d in *tam*, diplotene to early dyads

**Examine stages in two anthers in the same bud  
at two time points**



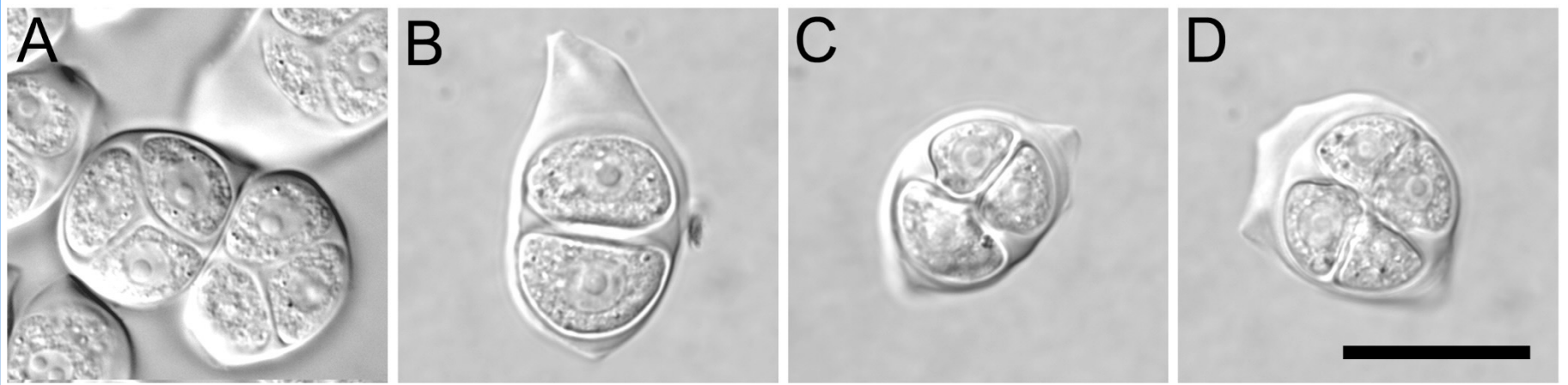


b, zygotene; c, pachytene; d in WT, diplotene to early tetrads; d in *tam*, diplotene to early dyads; e, early dyads to early tetrads

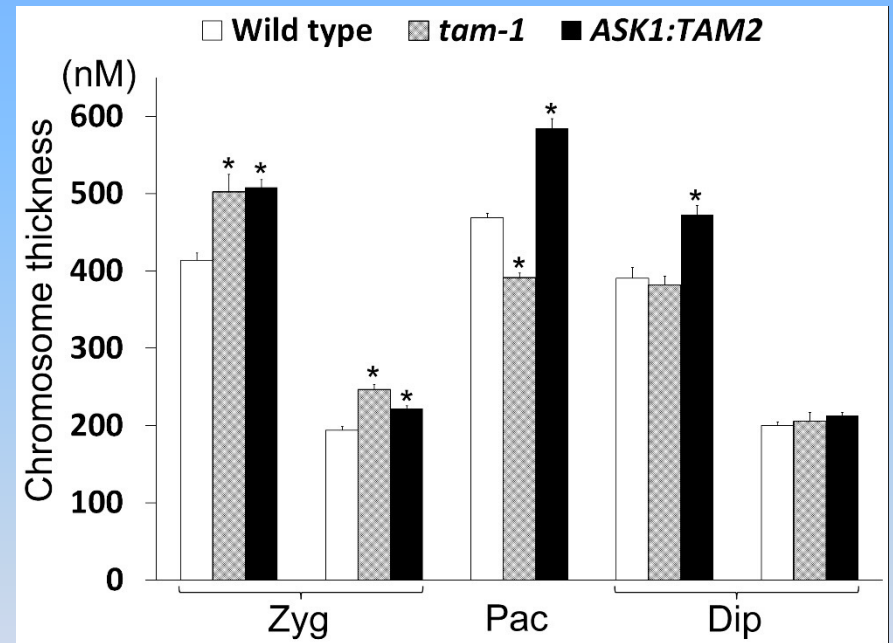
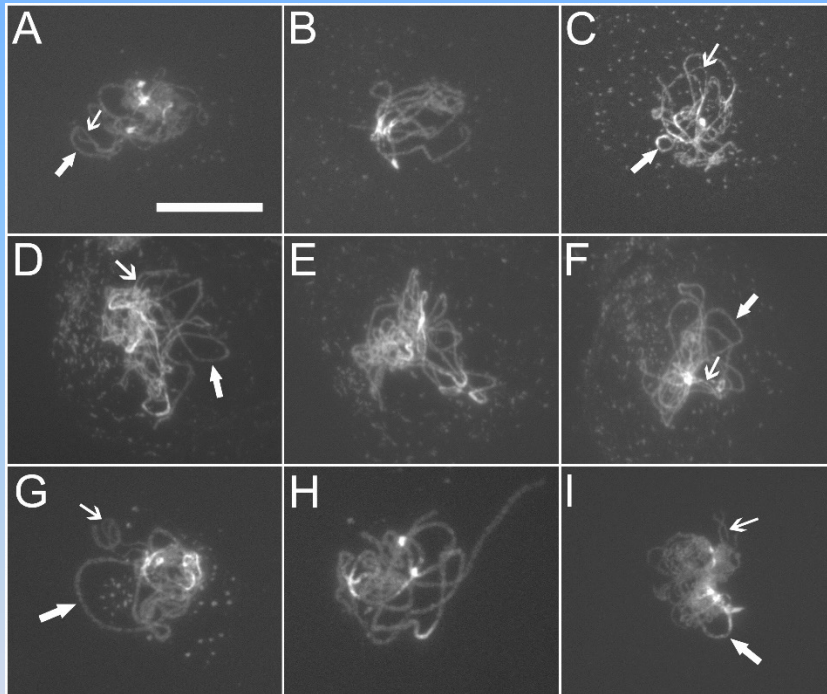
## **Conclusion**

**TAM regulates the progression of pachytene and meiosis II.**

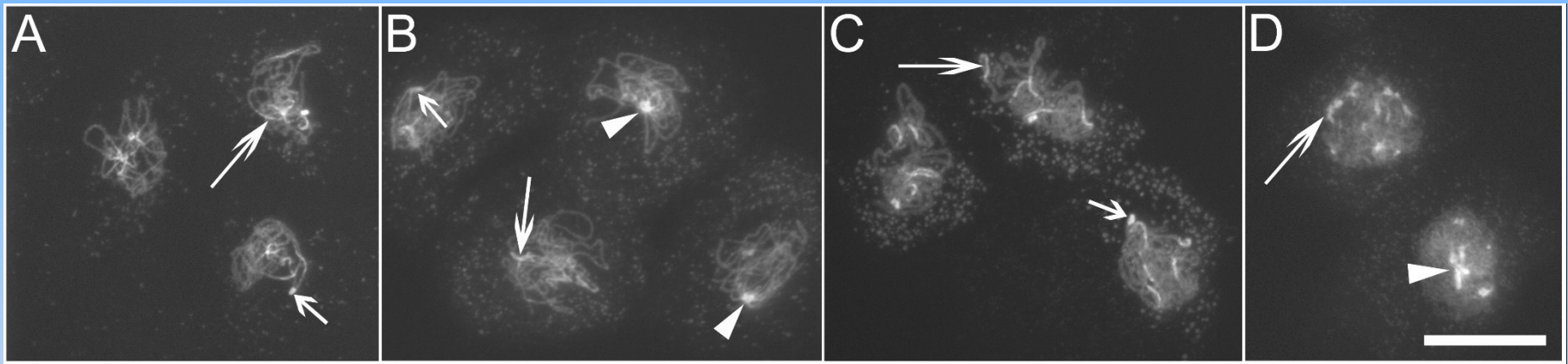
**Overexpression of *TAM* results in the production of the same meiotic products as in the loss-of-function *tam* mutants**



# Chromosome thread thickness is differentially affected in *TAM*-overexpression lines and *tam* mutants

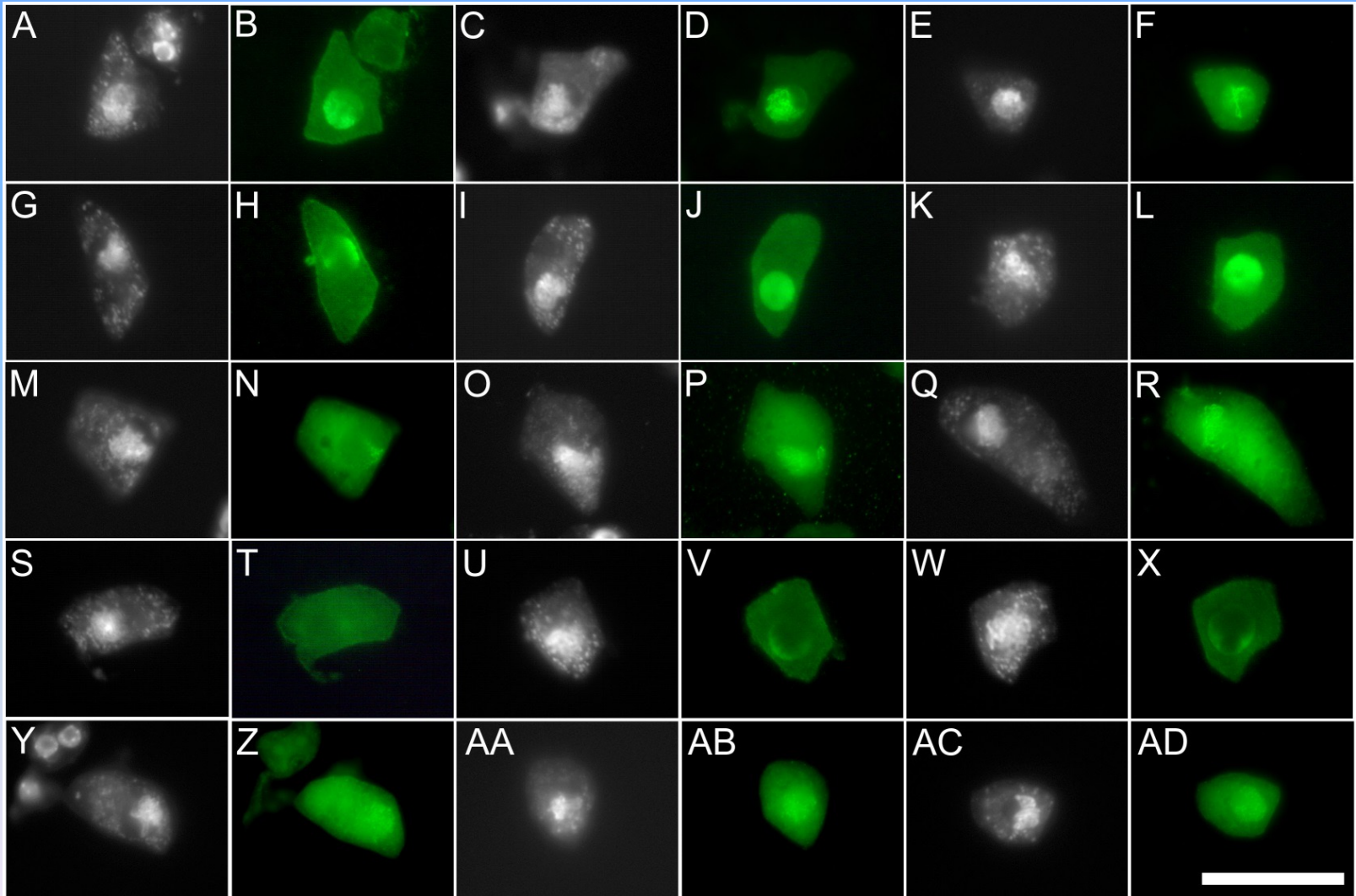


**Pericentromeric heterochromatin regions tend to cluster in *tam* mutants but not in *TAM*-overexpression lines**





# ASY1 subcellular localization is differentially affected in *tam* mutants and *TAM*-overexpression lines



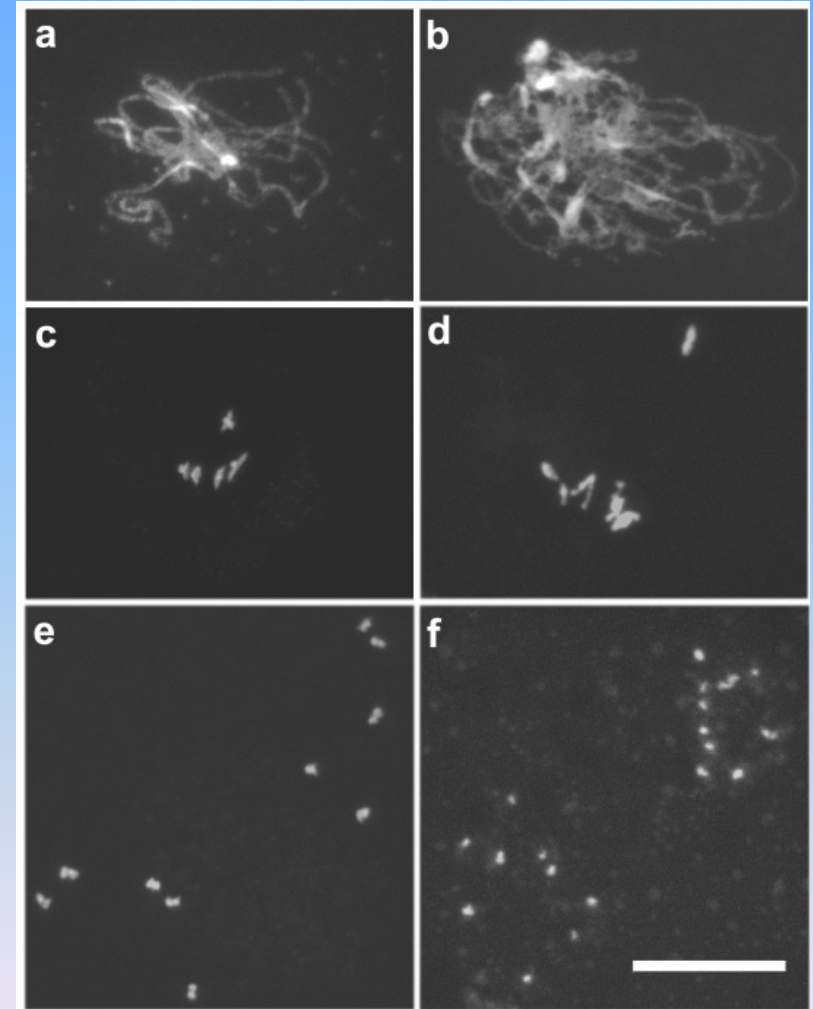
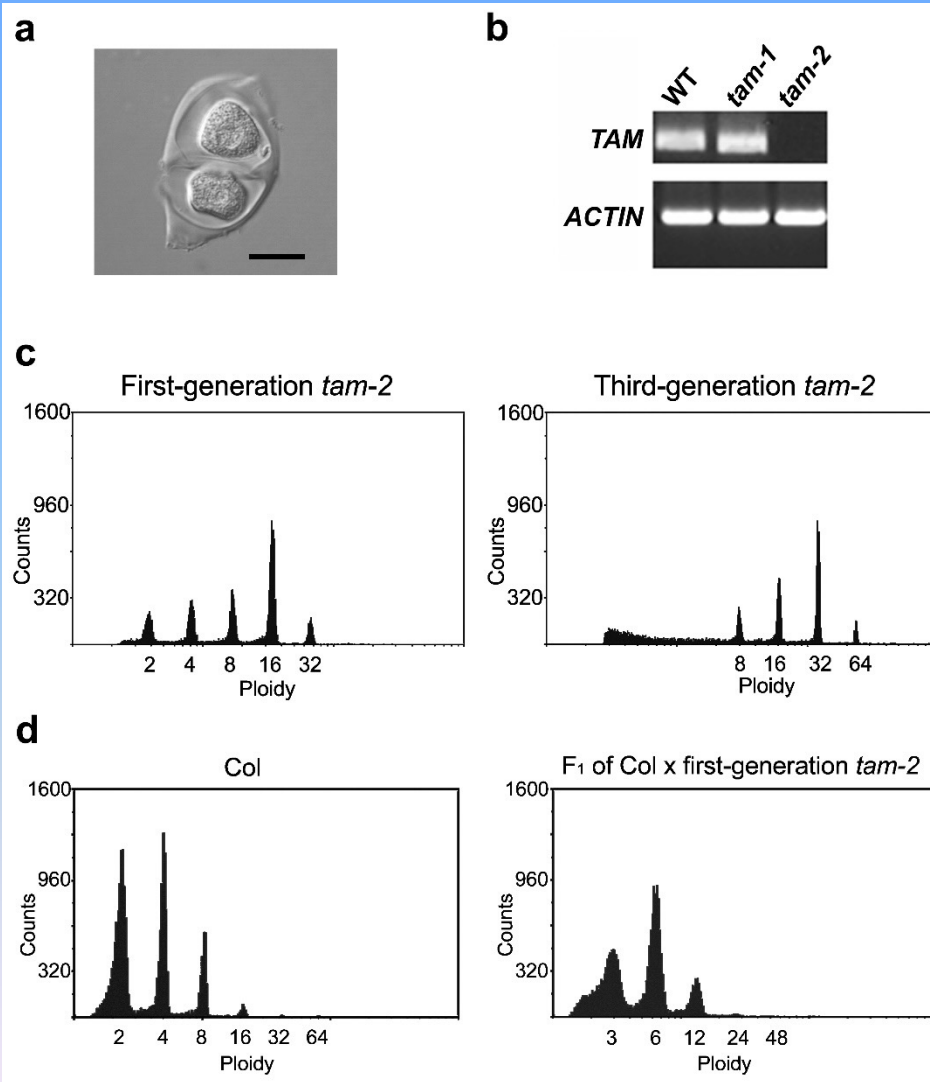
## Hypothesis

**Either the different meiotic defects or a common defect such as missing *ASY1* on the chromosomal axes triggers the MCN in the *tam* mutants and *TAM*-overexpression lines, leading to the production of the same abnormal meiotic products.**

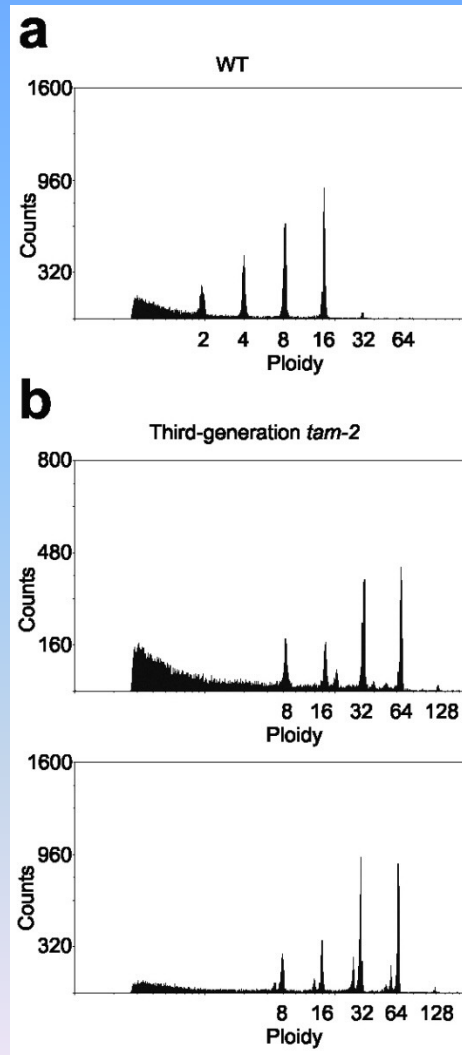
## Arabidopsis mutants in which the MCN is potentially activated based on the presence of meiotic products of dyads and triads

Protein	Function	Dyads	Triads	Tetrads	Polyads
ASK1	Ubiquitin ligase/cell cycle progression, synapsis	Yes	Yes	Yes	Yes
DMC1	Single-strand DNA invasion	Yes	Yes	Yes	Yes
DYAD/SWI1	Novel protein/meiotic progression	Yes	Yes	Yes	No
SDS	Cyclin/meiotic progression	Yes	Yes	Yes	Yes
TAM/CYCA1;2	Cyclin/meiotic progression	Yes	Yes	Yes	Yes
AESP	Separase/against nonhomologous centromere aggregation	Yes	Yes	Yes	Yes
DUET/MMD1	PHD finger protein/meiotic progression	Yes	?	No	No
OSD1	APC inhibitor	Yes	Yes	Yes	No
AtPS1	Forkhead –associated domain/spindle orientation in meiosis II	Yes	Yes	No	No
TOP3a	DNA topoisomerase IIIa/resolution of Holliday junctions	Yes	No	No	No
RMI1	Resolution of Holliday junctions	Yes	No	No	No

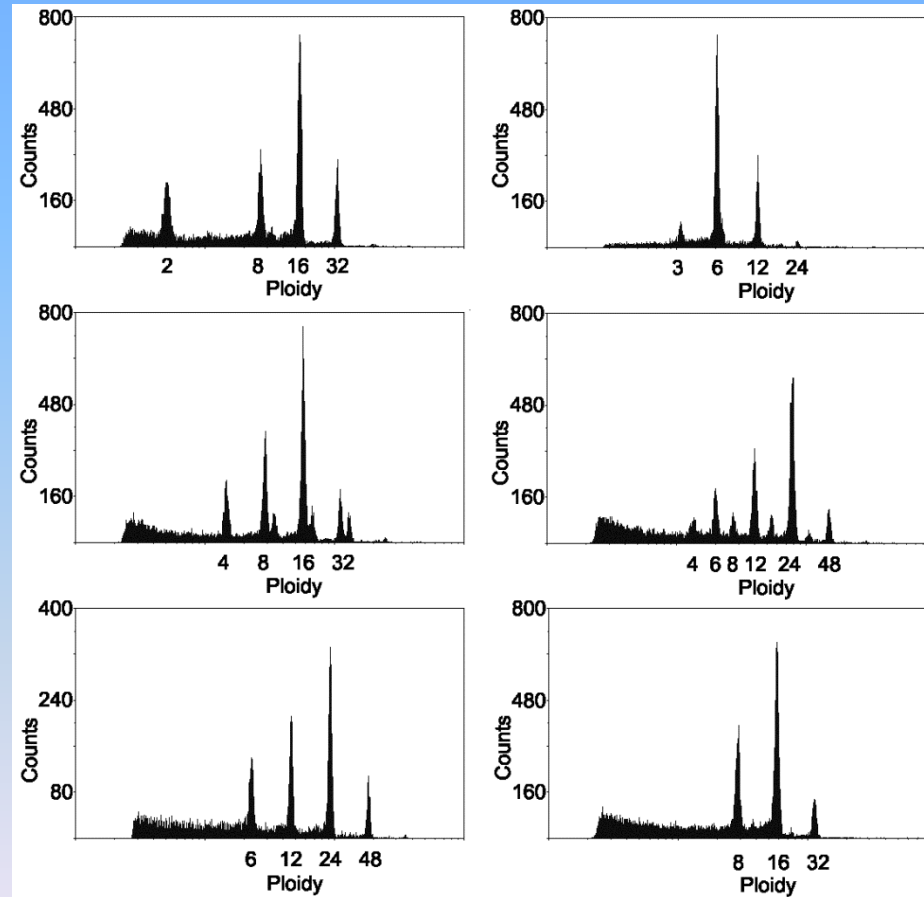
# The *tam-2* null allele produces unreduced gametes that lead to genome duplication in the next generation



# Evidence for genomic instability in the third generation of *tam-2*



# Evidence for dramatic ploidy reductions and genomic instability in the fourth generation of *tam-2*

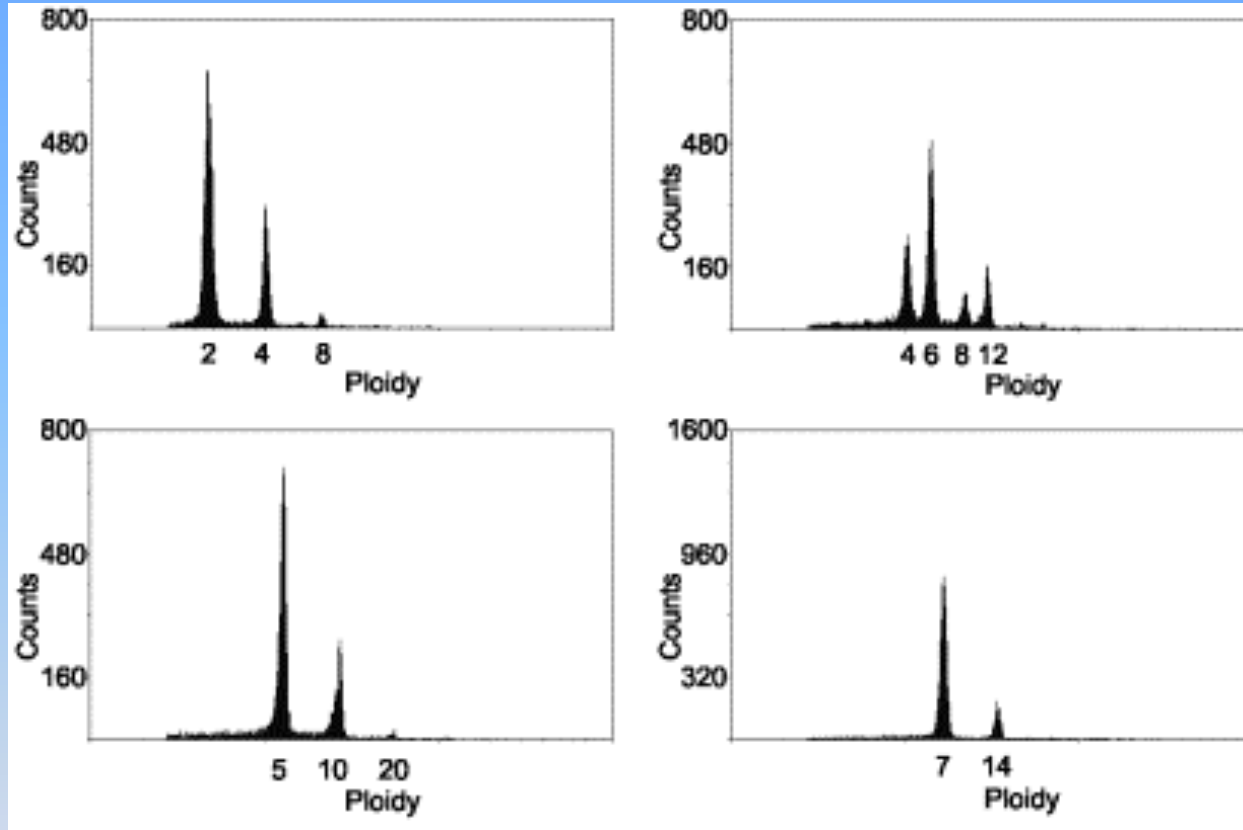


# Plants with different baseline ploidy levels in the fourth generation of *tam-2*.

Ploidy Plant family	2x	3x	4x	6x	7x	8x
1 (n=8)	1		2	4	1	
2 (n=7)		1	1	2	1	2
3 (n=7)			2	5		
4 (n=6)					1	5
5 (n=12)		1	1	2	6	2

**n is the number of plants examined.**

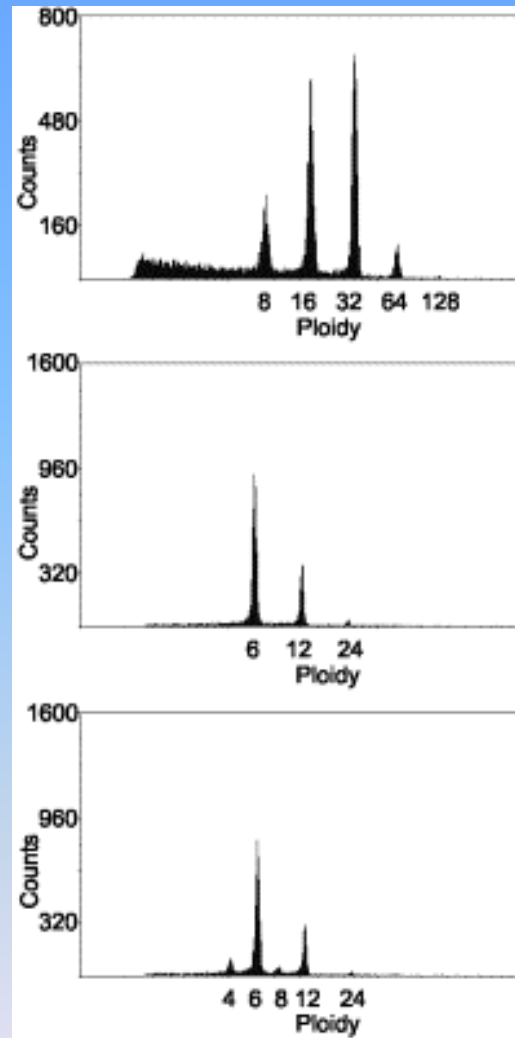
## An artificial hexaploid line also exhibits genomic instability



Upper left: diploid wild type. Others: plants from an artificial hexaploid line.

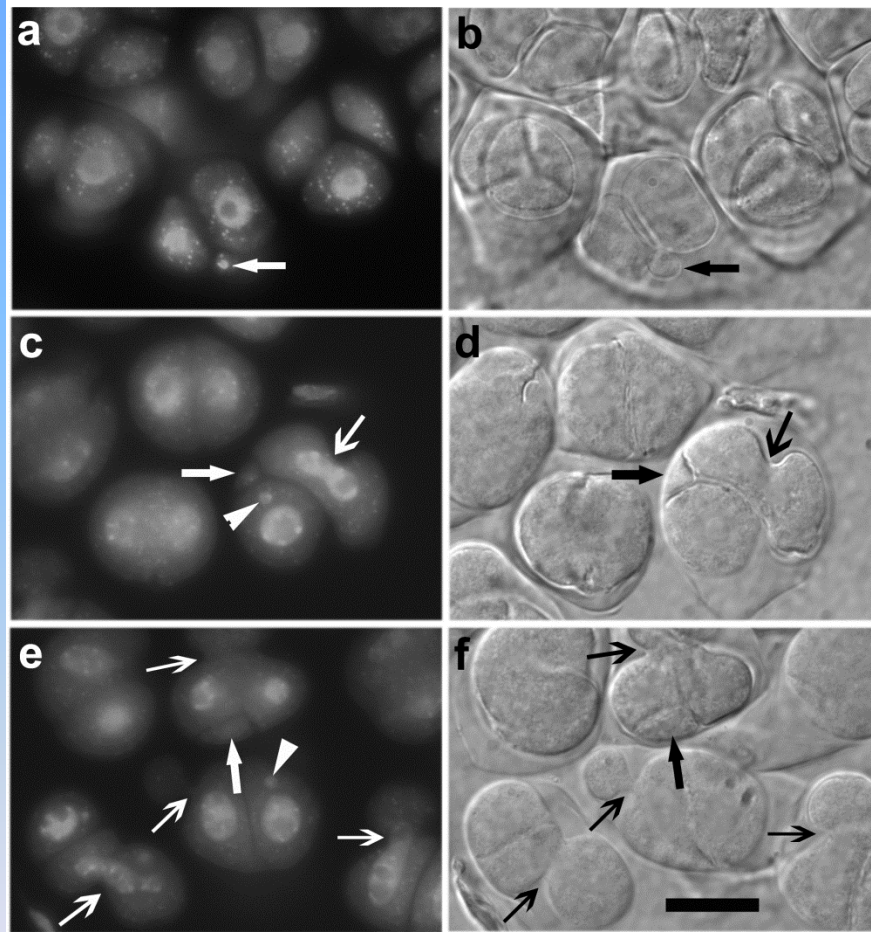


# Progeny from an artificial octaploid line also exhibits genomic instability

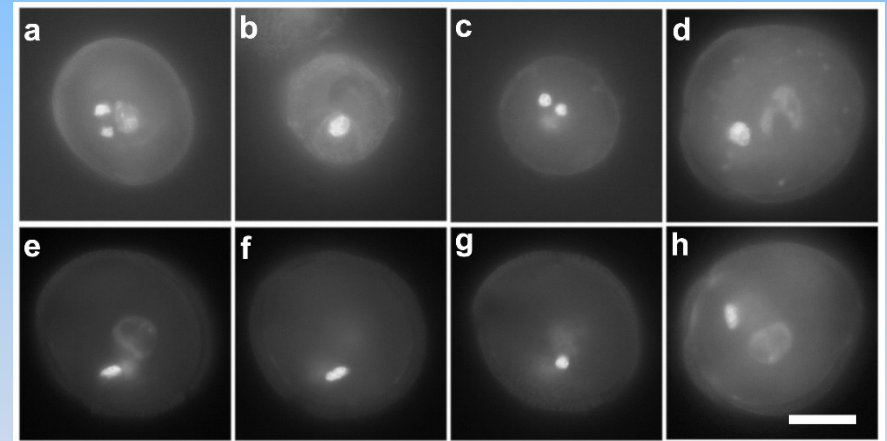


Uppermost: octaploid parent. Other two: progenies from the parent

# Evidence for an unusual mechanism for the dramatic ploidy reduction



Chromosome missegregation and spore budding in male meiosis. (a, b) Artificial octaploid, showing tetrads with one containing a small extra cell with DNA (arrows). (c–f) Dyads in the third-generation *tam-2*, containing small extra cells (thick arrows), spore budding (thin arrows), and micronuclei (arrowheads).



Nuclear size and number in pollen. (a) Artificial octaploid. (b) Second-generation *tam-2*. (d–h) All from a third-generation *tam-2*. (e–g) Different focal planes of the same pollen grain.

**MCN may be activated by multiple mechanisms to generate unreduced gametes in nature**

- **Mutations such as *tam*—autopolyploidy**
- **Hybridization between distant species—allopolyploidy**
- **Environmental factors such as low temperature--autopolyploidy**

## **Characteristics of octaploid plants that possibly contribute to genome evolution**

- **Octaploid plants produce progenies of dramatically reduced ploidy and possibly greatly altered genome composition**
- **Greatly reduced fertility of octaploid plants and their progeny are subject to out-crossing**
- **Genome changes will be either lost due to sterility or fixed in just a few generations**

## **Acknowledgements**

### **OSU**

Yixing Wang  
Ajay Jha  
Brian Hercyk

### **The Samuel Roberts Noble Foundation**

Hwa-Soo Shin  
Rujin Chen

### **PGEC**

Sophia Chen  
Jean-Louis Magnard  
Michele Leary  
Sheila McCormick

### **The John Innes Center**

John Doonan

### **University of Birmingham**

Chris Franklin

## **Funding**

USDA

University of California

The Samuel Roberts Noble Foundation

Oklahoma State University

Underwood Fellowship, BBSRC, UK

Oklahoma Center for the Advancement of Science and Technology