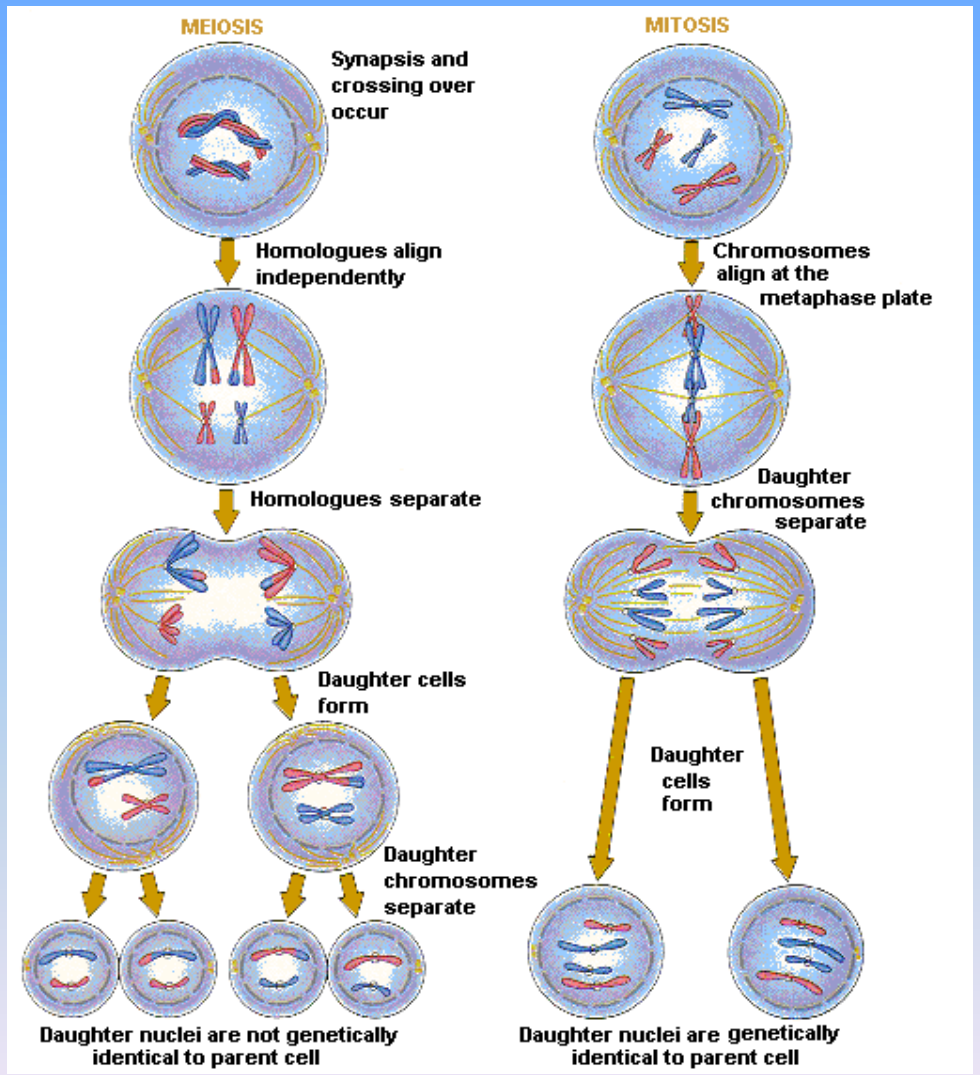
From meiosis to biological rhythmicity: how a study of a mutant's phenotype led to the modeling of biological oscillations

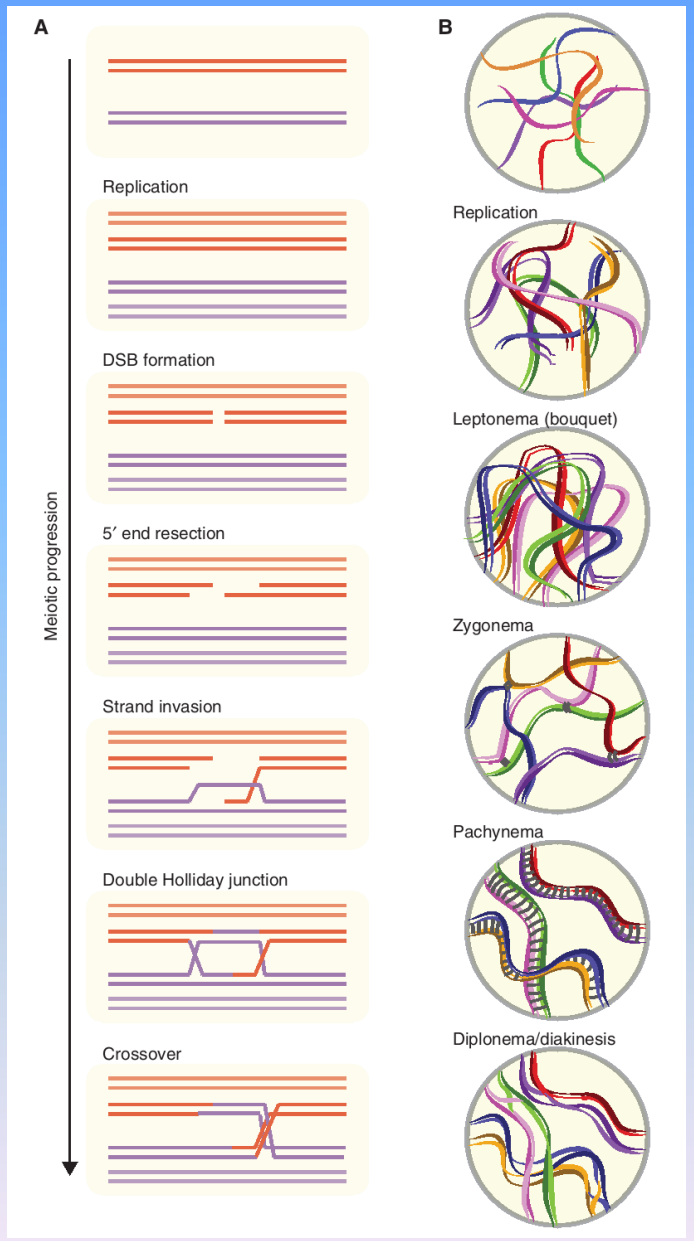
Ming Yang
Department of Botany
Oklahoma State University

Main points of presentation

- The meiotic cell cycle and the SCF complexes
- Meiotic defects in *ask1-1* and discrete spindle elongation
- Slow diffusion underlies rhythmic microtubule assembly
- Short reaction pauses and a negative feedback loop as sufficient conditions for generating sustained long oscillations in biological systems

Meiosis vs. mitosis





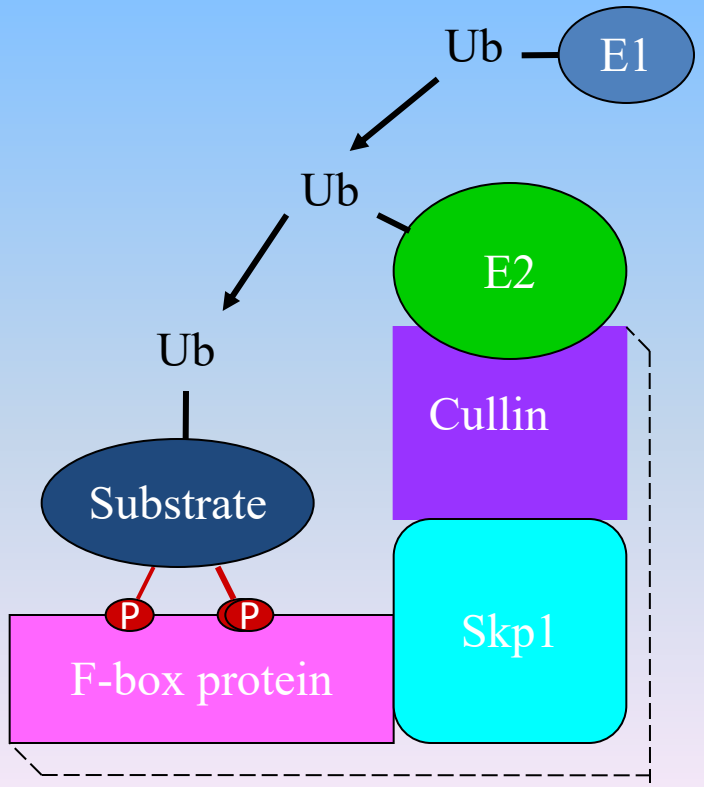
← S phase and G_2 phase

Prophase I

Subramanian and Houchwagen, Cold Spring Harb Prospet Biol, 6: a016675, 2014

SCFs control degradation of specific proteins

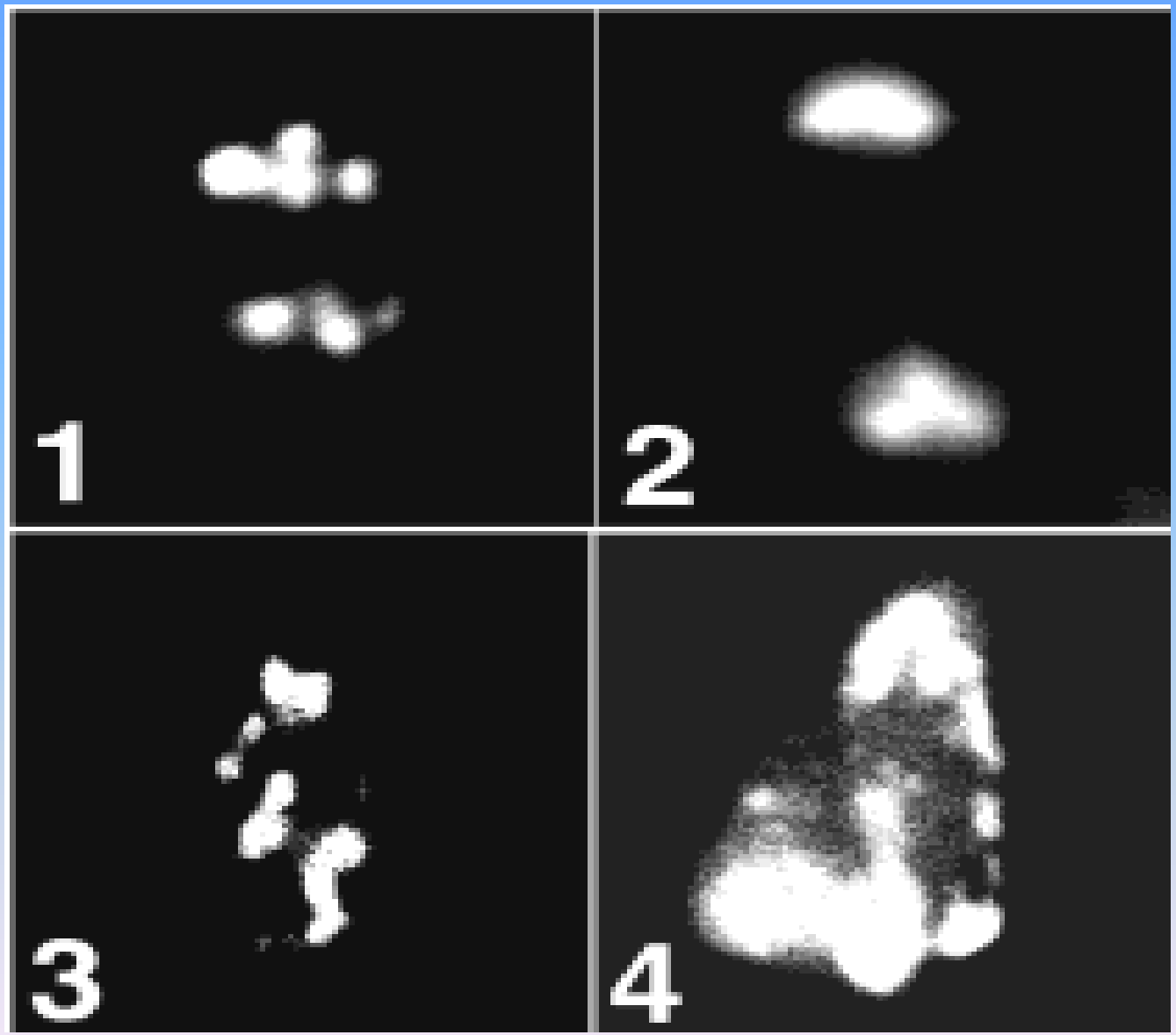
- SCFs (Skp1-Cullin-F-box protein) are ubiquitin ligases.
- ASK1 is ARABIDOPSIS SKP1-LIKE1, the major homolog of SKP1.

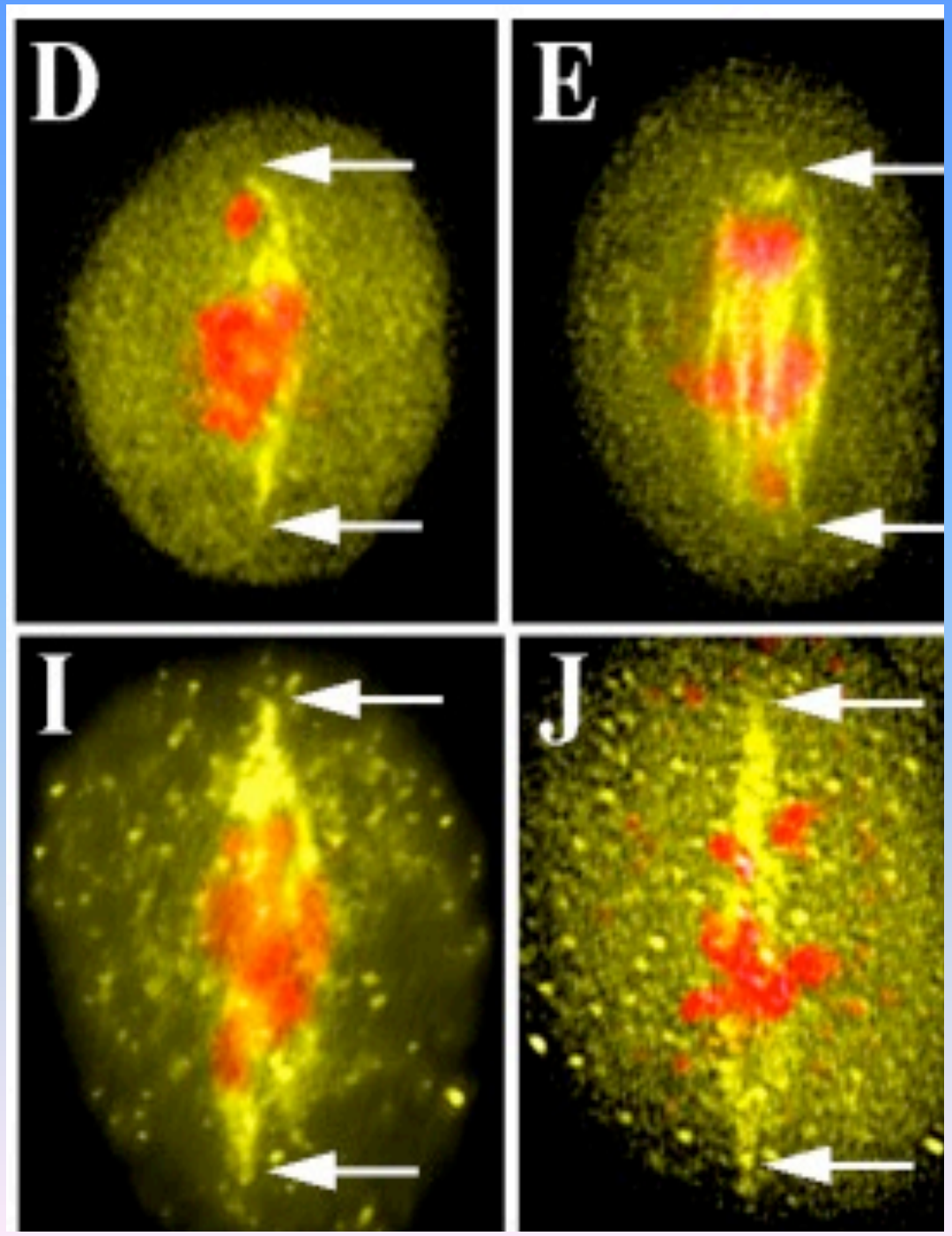


ask1-1 has multiple defects

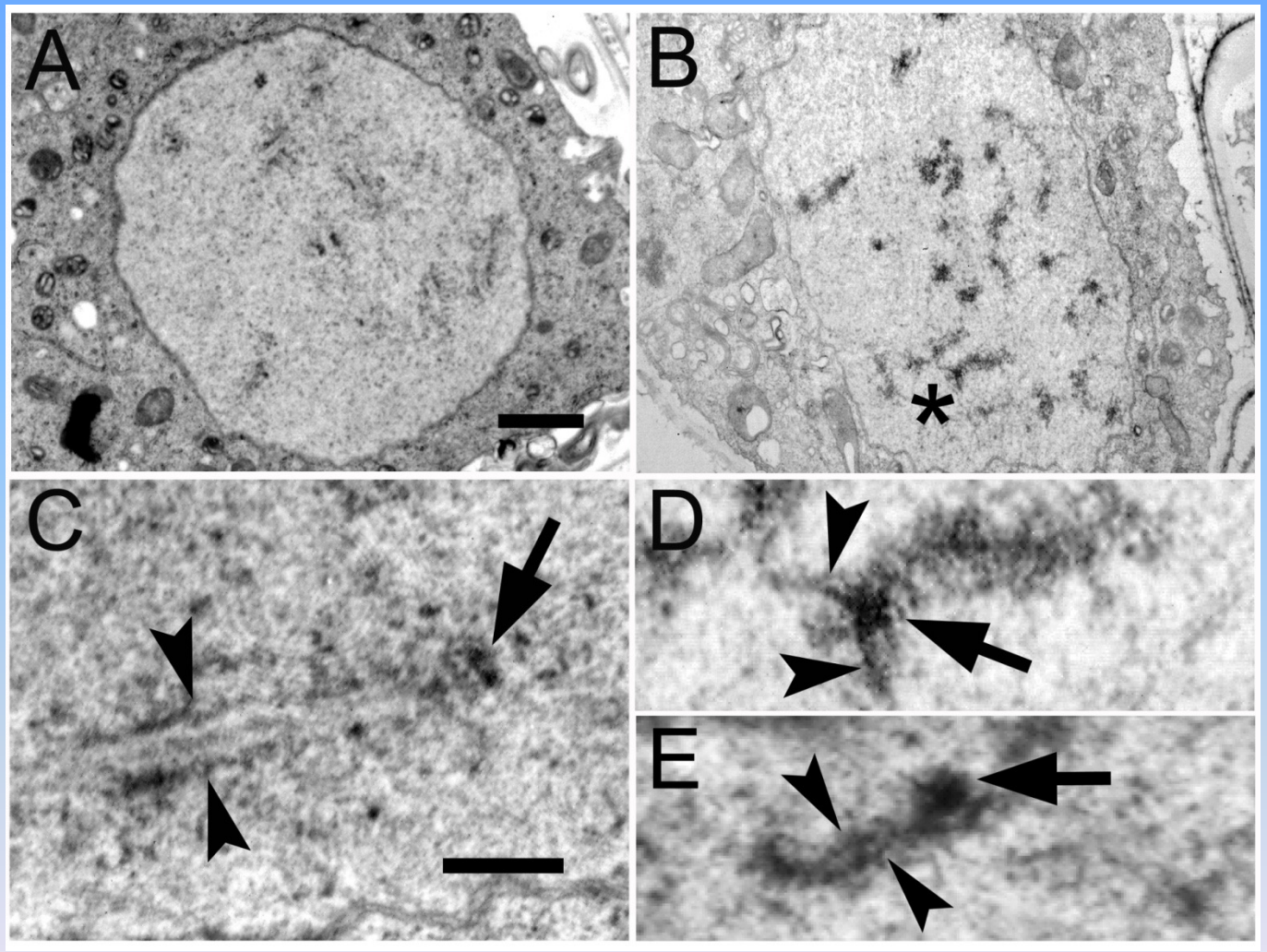
WT

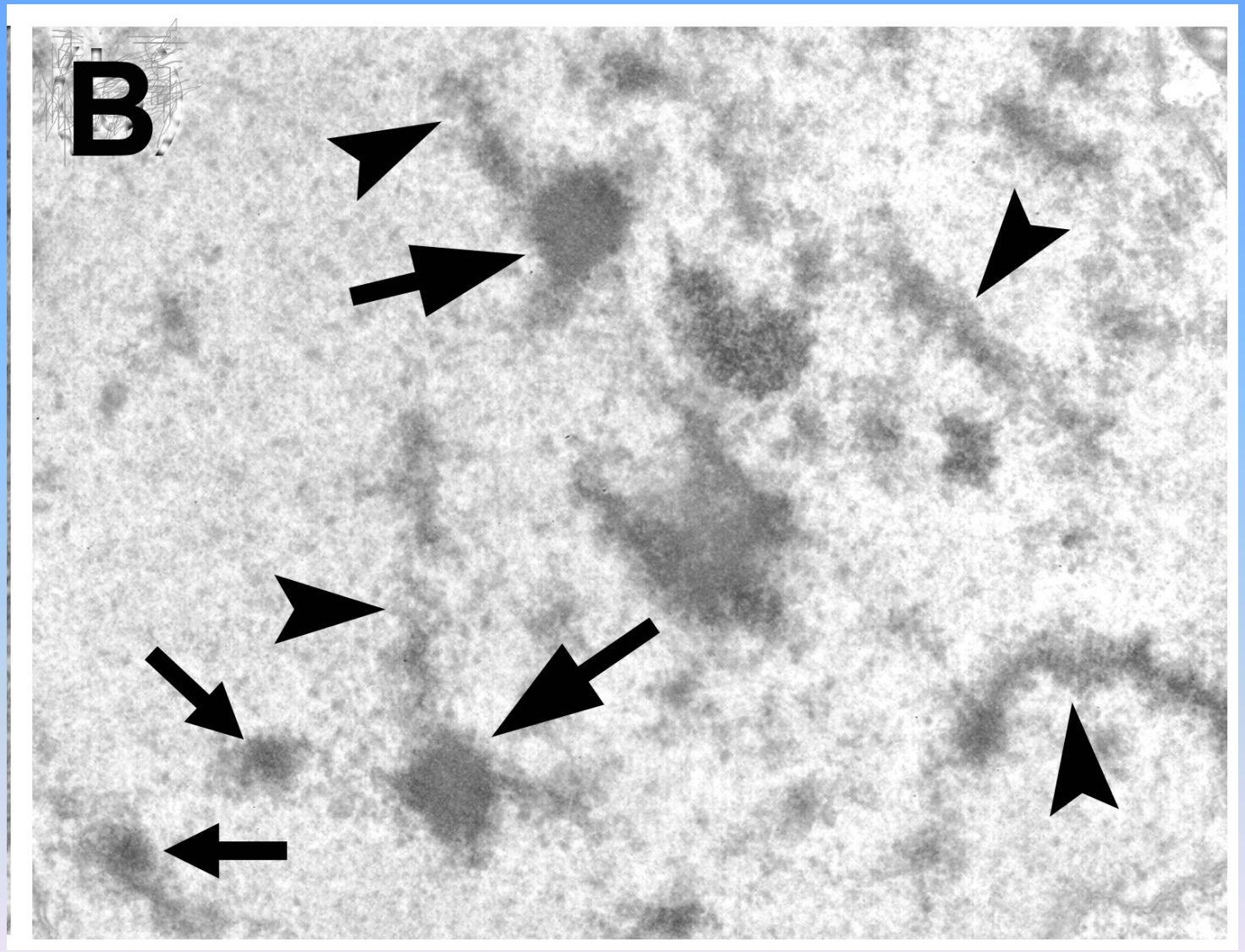
ask1-1




D
E
I
J

ask1-1 is asynaptic with many electron-dense foci

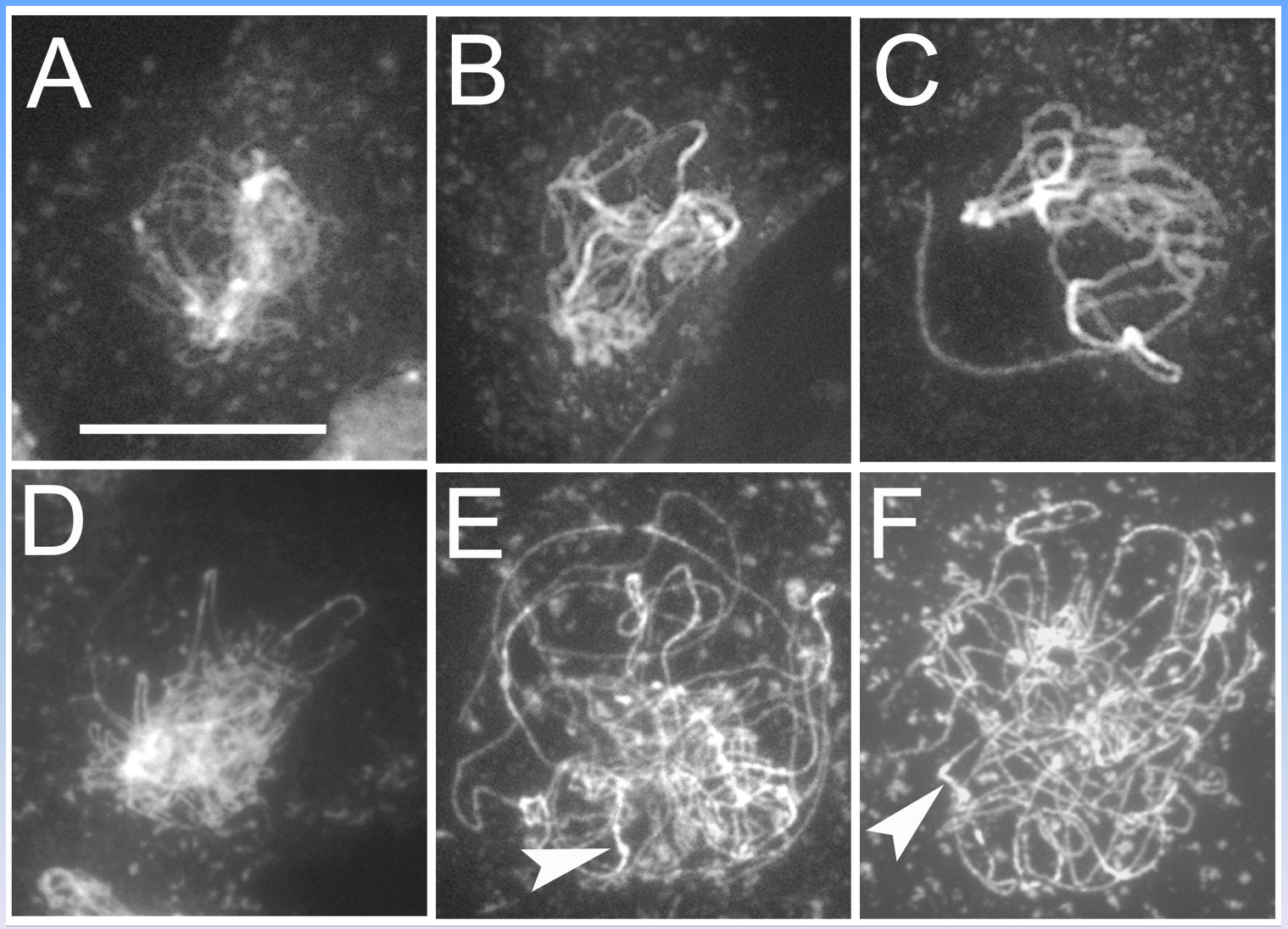



B

Chromosome spread

WT

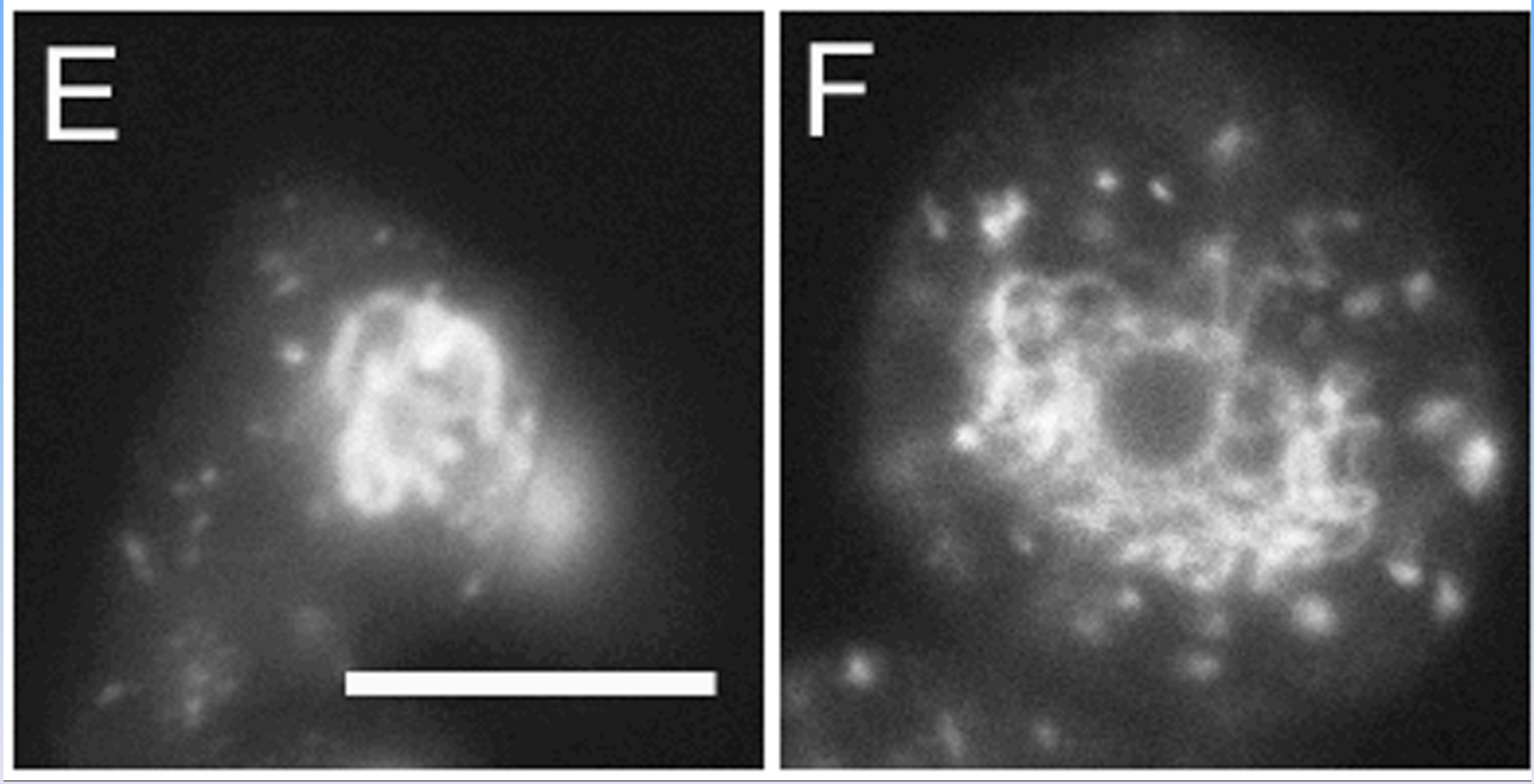
ask1-1



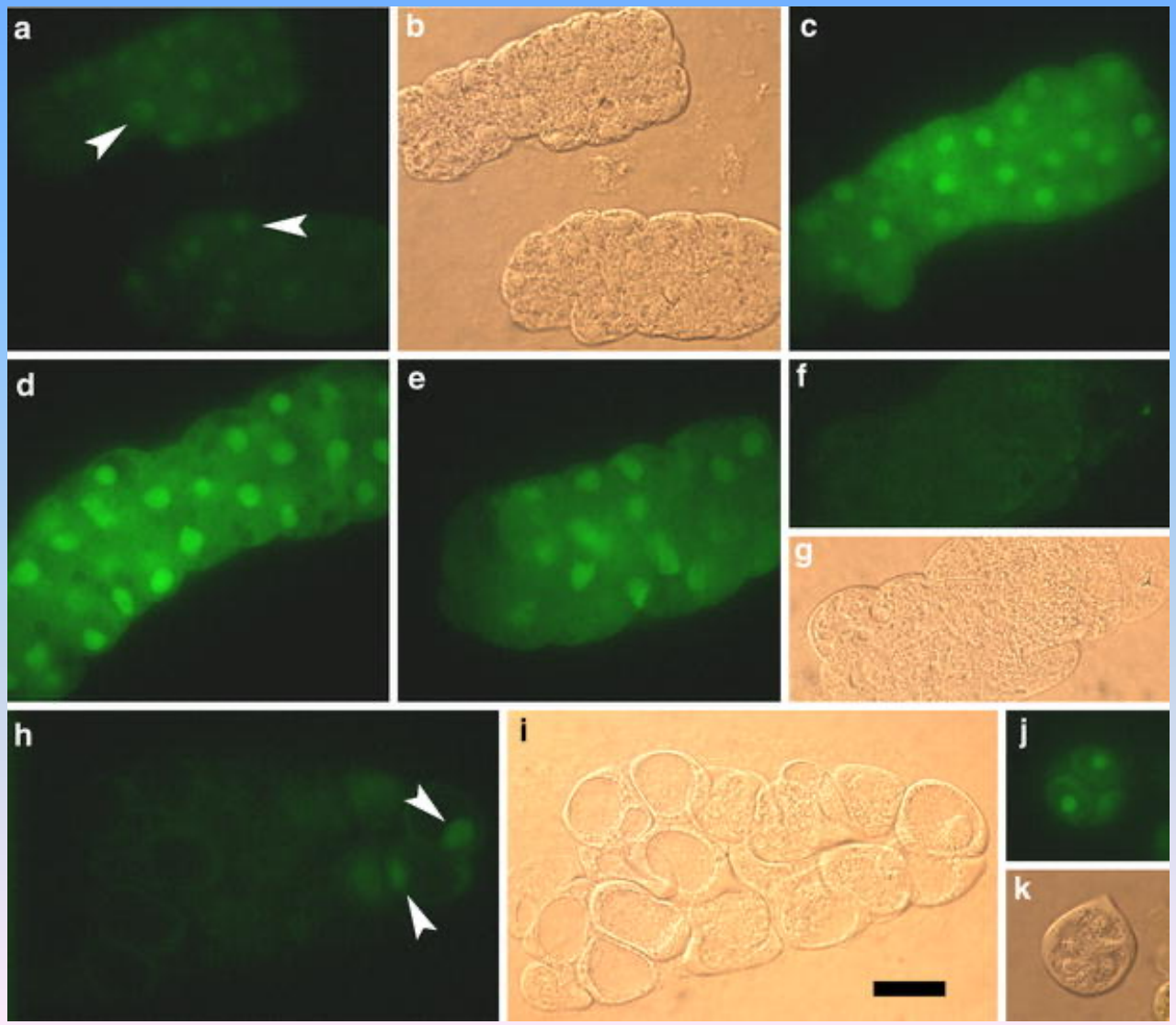
Meiocytes at pachytene (WT) or comparable stage (*ask1-1*)

WT

ask1-1



ASK1-GFP signal in meiocytes at different stages of male meiosis



Summary of meiotic defects in *ask1-1*

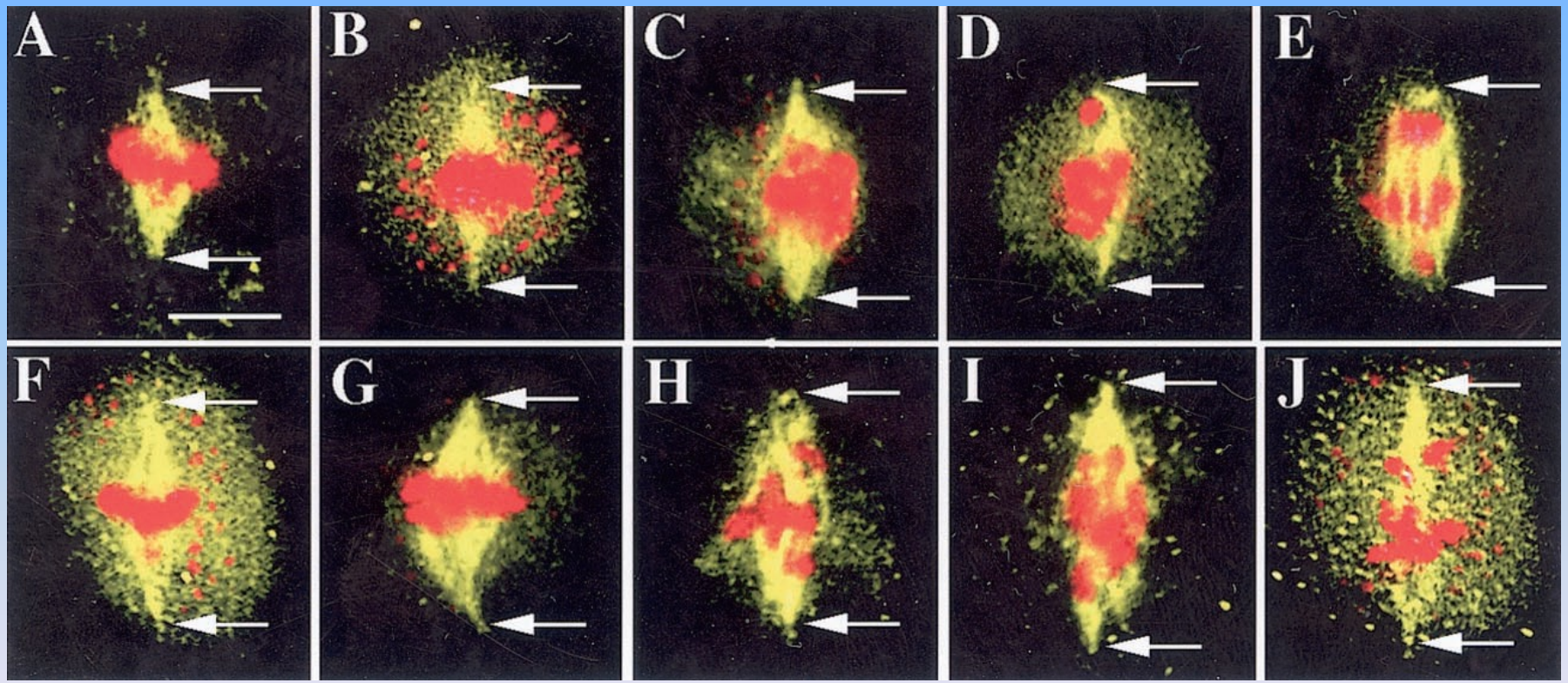
- Male meiotic products consist of 2-7 spores.
- Chromosomes entangle, non-disjoin, and are stretched by the anaphase-I spindle.
- Chromosomes are asynaptic in prophase I.
- Anaphase spindles can be either longer or shorter than those of the WT.
- Prophase-I meiocytes likely contain unresolved recombination intermediates.

Similar meiotic defects have been found in fission yeast only recently by Okamoto et al. (2012), except that the pairing of homologous chromosomes have not been explored in yeast.

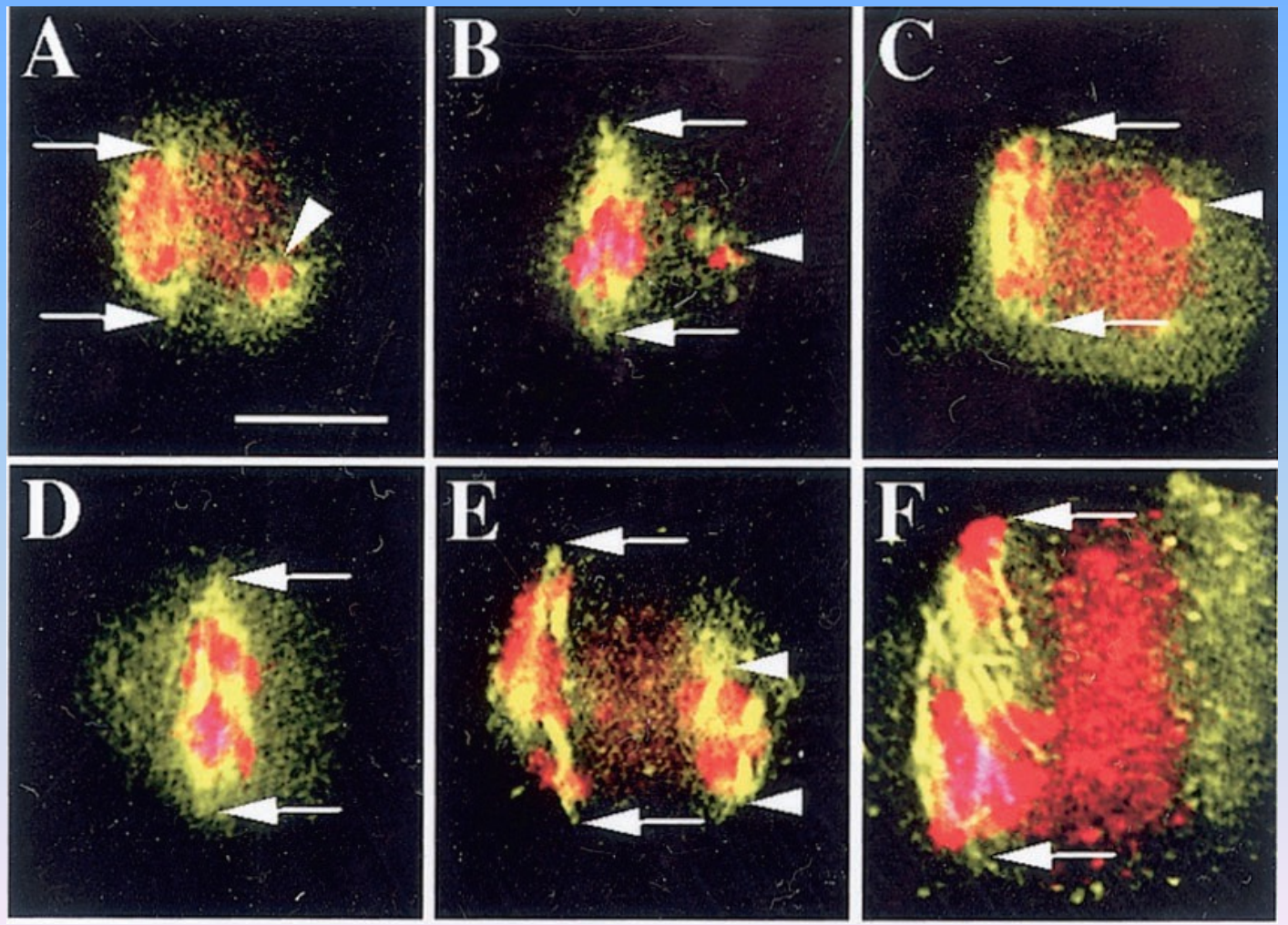
The similarities between Arabidopsis and fission yeast *skp1* mutants suggest the existence of a conserved pathway involving Skp1 in meiosis because Skp1 is not expected to directly regulate recombination and chromosome behavior.

But the above investigation took an unexpected turn…

Spindle elongation in *ask1-1* male meiosis I



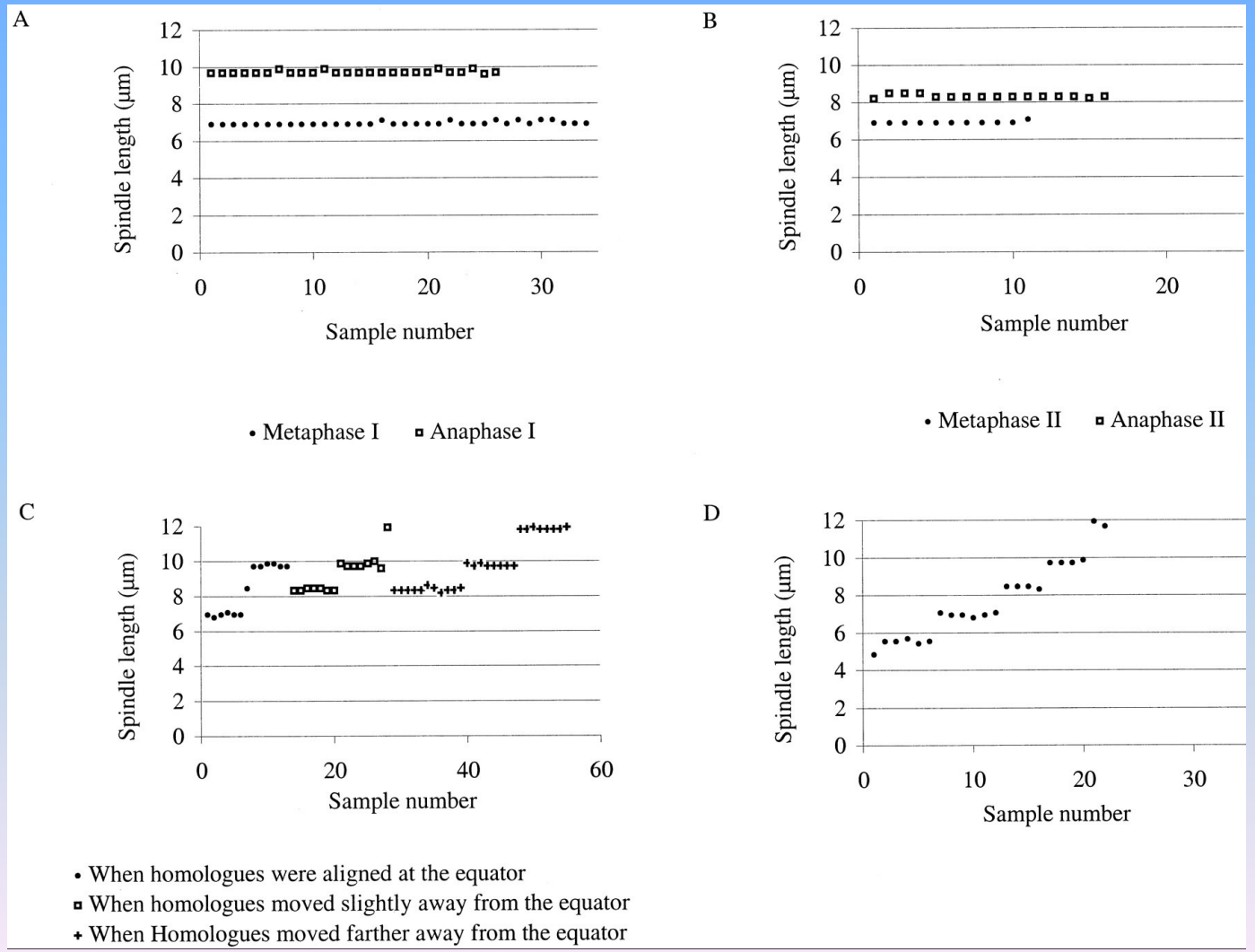
Spindle elongation in *ask1-1* male meiosis II



Distribution of WT and *ask1-1* spindle lengths in meiosis I and meiosis II

WT

ask1-1



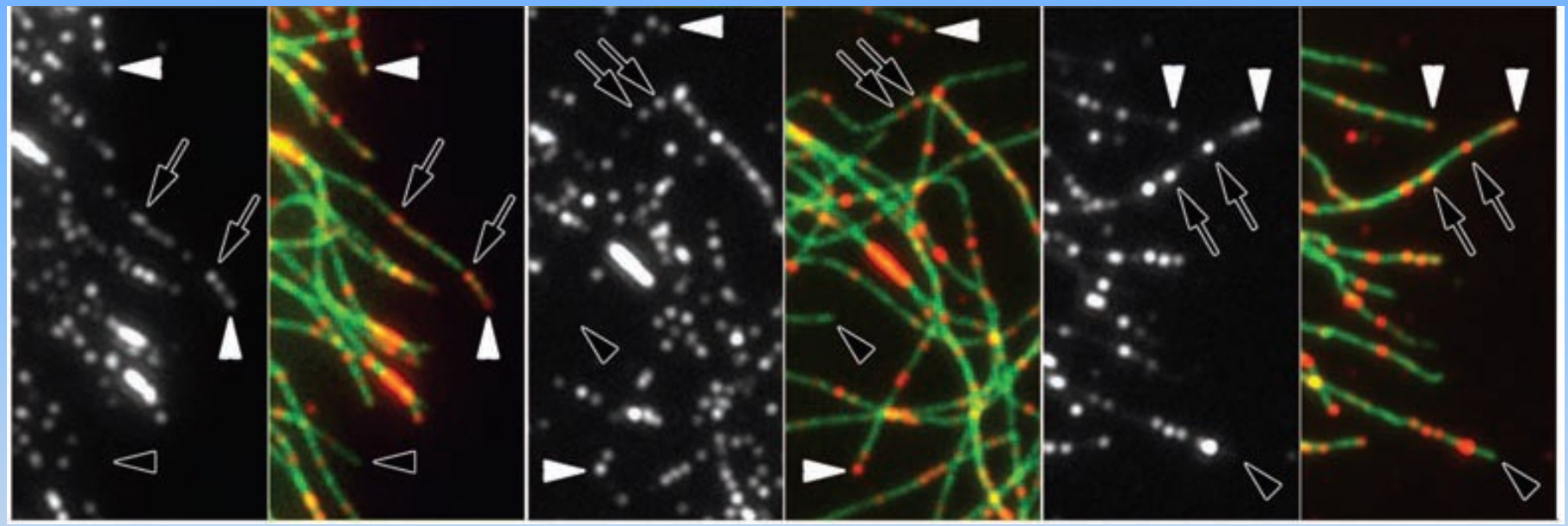
Spindle length differences in four other organisms

Organism	Length	Length Difference
S. cerevisiae (Winey et al., 1995)	L1 0.7 ± 0.1 (n = 4) L2 1.4 ± 0.1 (n = 6)	L2 - L1 = 0.7
F. capucina (Tippit et al., 1978)	L1 1.3 ± 0.1 (n = 2) L2 2.6 ± 0.1 (n = 25)	L2 - L1 = 1.3
Slime mold (Moens, 1976)	L1 2.1 ± 0.1 (n = 3) L2 4.9 ± 0.3 (n = 6)	L2 - L1 = 2.8
Rat kangaroo (PtK1 cells; Armstrong and Snyder, 1989)	L1 13.2 (n = 5) L2 17.4 (n = 5)	L2 - L1 = 4.2
Rat kangaroo (PtK1 cells; Snyder et al., 1986)	L1 12.2 (n = 6) L2 16.4 (n = 6)	L2 - L1 = 4.2

Spindles seem to elongate by multiples of 0.7 μm, but why?

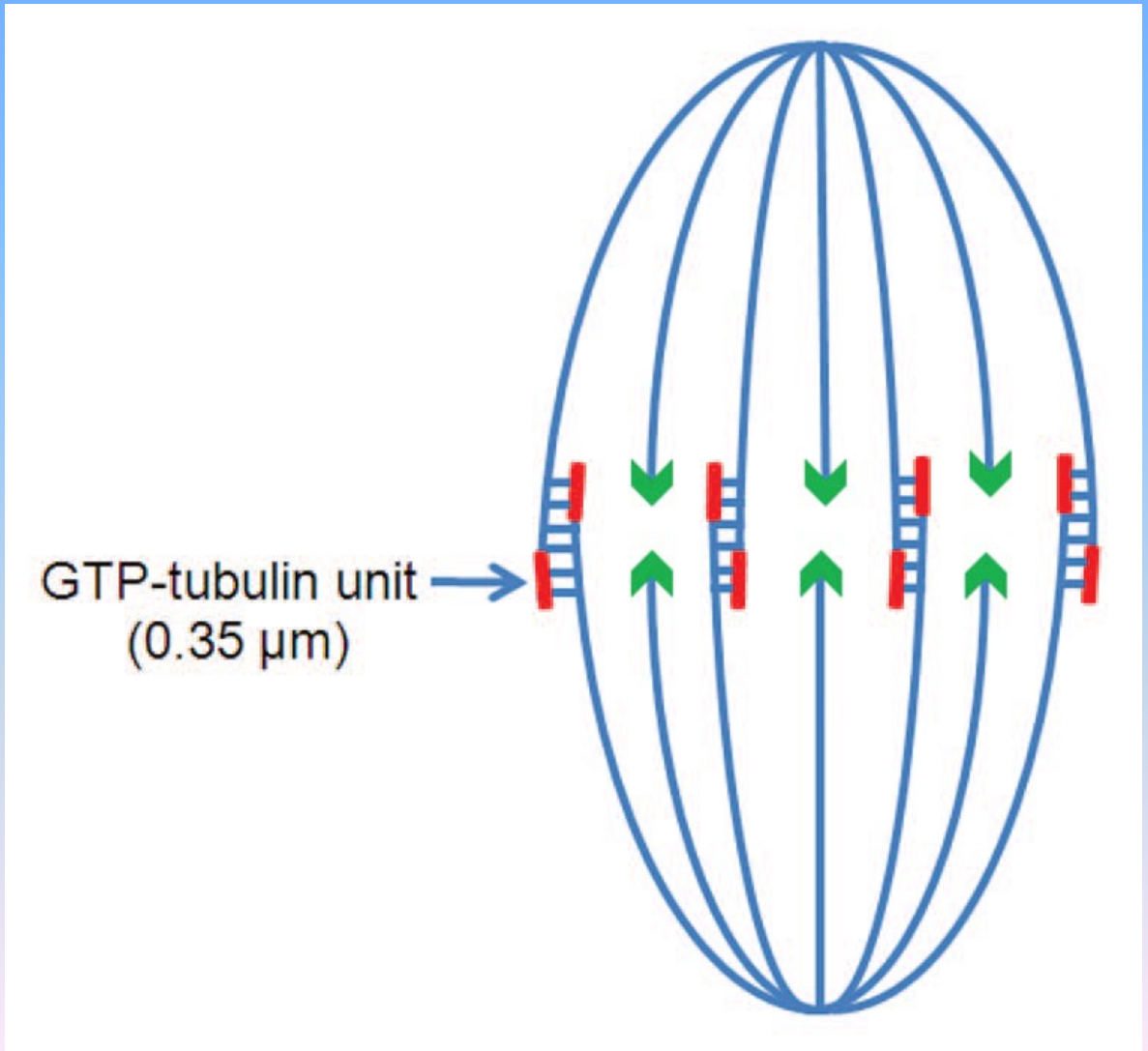
Years passed without any progress as to the answer

Discrete lengths of GTP-tubulin segments on human microtubules



(Dimitrov et al., Science, 322: 1353-56, 2008)

A model for discrete spindle elongation

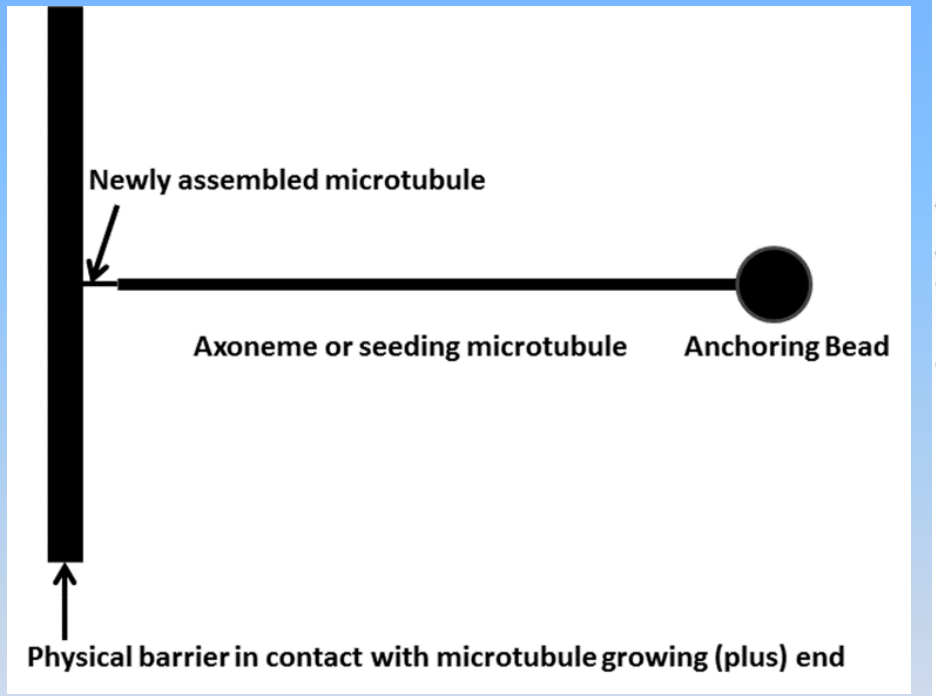


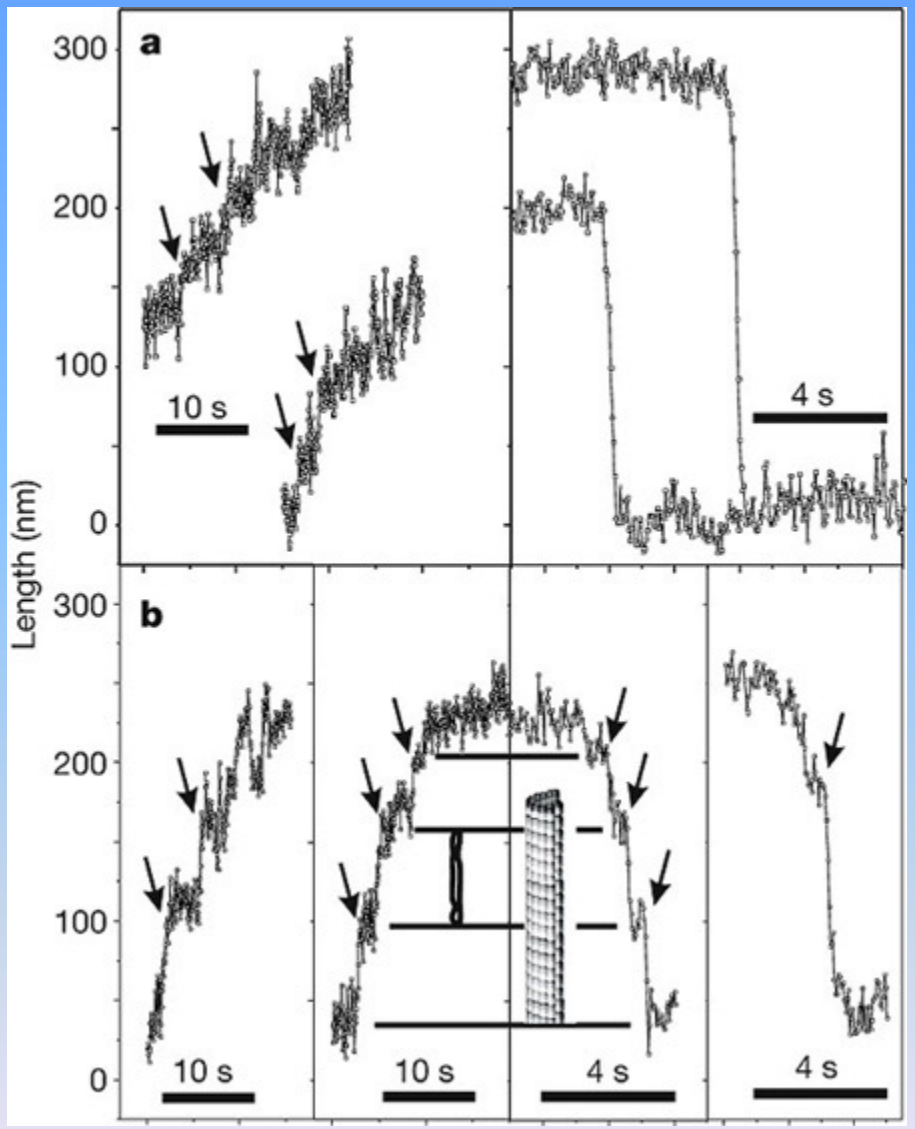
Years passed, still no satisfying answer to the question

Until I saw the following papers

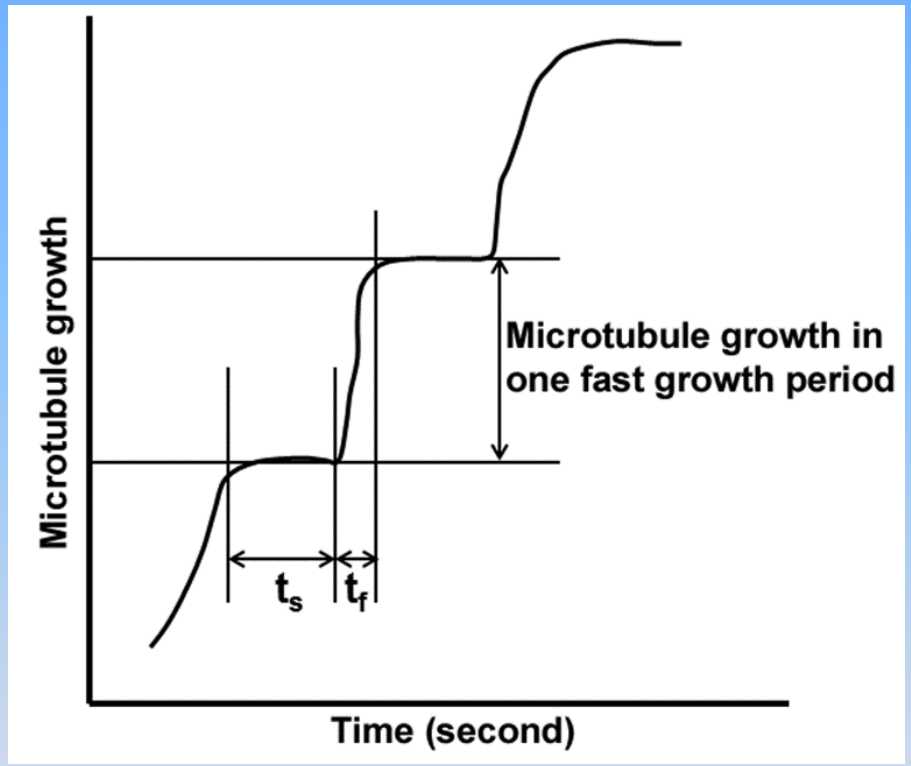
Kerssemakers et al., Assembly dynamics of microtubules at molecular resolution, *Nature*, 442: 709-712, 2006.

Schek et al., Microtubule assembly dynamics at the nanoscale, *Current Biology*, 17: 1445-1455, 2007.





(Kerssemakers, et al., 2006)



$t_d = t_f + t_s$

$t_d \approx x^2/2D$

Average durations (in second) of t_f, t_s, and t_d in *in vitro* microtubule assembly

Mean t_f ± standard error	Mean t_s ± standard error	Mean t_d	Seed for microtubule assembly
0.55 ± 0.09 (n = 5)	3.85 ± 0.57 (n = 17)	4.4	Axoneme - XMAP215
0.63 ± 0.11 (n = 6)	2.33 ± 0.37 (n = 11)	2.96	Axoneme + XMAP215
0.44 ± 0.04 (n = 8)	0.54 ± 0.08 (n = 6)	0.98	Microtubule fragments

The flux of tubulin during the fast growth period can be expressed as the following according to Fick's First Law

$$J = -D(\partial C/\partial X) = -D[(C_0 - C_c)/L]$$

$$J_{-xmap215} = -D[(C_0 - C_{c\text{-}xmap215})/L_{-xmap215}] = [39/(6.022x10^{23})]/(at_{f\text{-}xamp215})$$

$$J_{+xmap215} = -D[(C_0 - C_{c+xmap215})/L_{+xmap215}] = [78/(6.022x10^{23})]/(at_{f+xamp215})$$

The calculated $t_{d+xmap215}/ t_{d\text{-}xmap215} = x_{+xmap215}^2/x_{-xmap215}^2 = 0.5$

For comparison, the experimental $t_{d+xmap215}/ t_{d\text{-}xmap215} = 2.96/4.4 = 0.67$

By the same principle

$J_{Schek} = -D_{Schek}(5 - 2)/L_{Schek} = [39/(6.022x10^{23})]/(at_{fSchek})$

It was then calculated that $D_{Schek} \approx 2.8D_{-xmap215}$

According to the descriptions of the two papers, it is clear that $D_{Schek} > D_{-xmap215}$

- The previous calculations support the idea that repetitions of a temporary disruption of the tubulin gradient followed by reestablishment of the gradient manifest into a rhythmic microtubule assembly behavior.

- A hemisphere with a radius R of the length of the average diffusion distance during the time of t_f and the assembly site as the center is deemed a relevant space in which the disruption takes place.

Then, if the tubulin concentration within the hemisphere is reduced to the tubulin critical concentration, the number of consumed tubulin dimers, N, is

$$N = (2/3)\pi R^3[(C_{edge} - C_{center})/2](6.022x10^{23}) \approx (2/3)\pi R^3[(R/x)(C_0 - C_c)/2](6.022x10^{23}),$$

$$\text{or } N \approx 2.96(6.022x10^{23})t_f^{2}t_d^{-1/2}D^{3/2}(C_0 - C_c)$$

Calculated
(assuming D = 0.07 $\mu m^2/s$ in Kerssemakers et al.
and D = 0.07x2.8 $\mu m^2/s$ in Schek et al.)

$N_{-xmap215} \approx 40$

$N_{+xmap215} \approx 80$

$N_{Schek} \approx 91$

Experimental

$N_{-xmap215} \approx 39$

$N_{+xmap215} \approx 78$

$N_{Schek} \approx 39$

Conclusions

- This study demonstrates that a small diffusion coefficient of a reactant can lead to rhythmic behavior of the reaction in a heterogeneous reaction system.

- This study also suggests that chemical reactions in biological systems in general are discrete.

How discrete chemical reactions affect biological system behavior?

To answer the above question, we examined how periodic short pauses (several seconds/pause) affect the behavior of a non-linear system with a negative feedback loop described by the following ordinary differential equations.

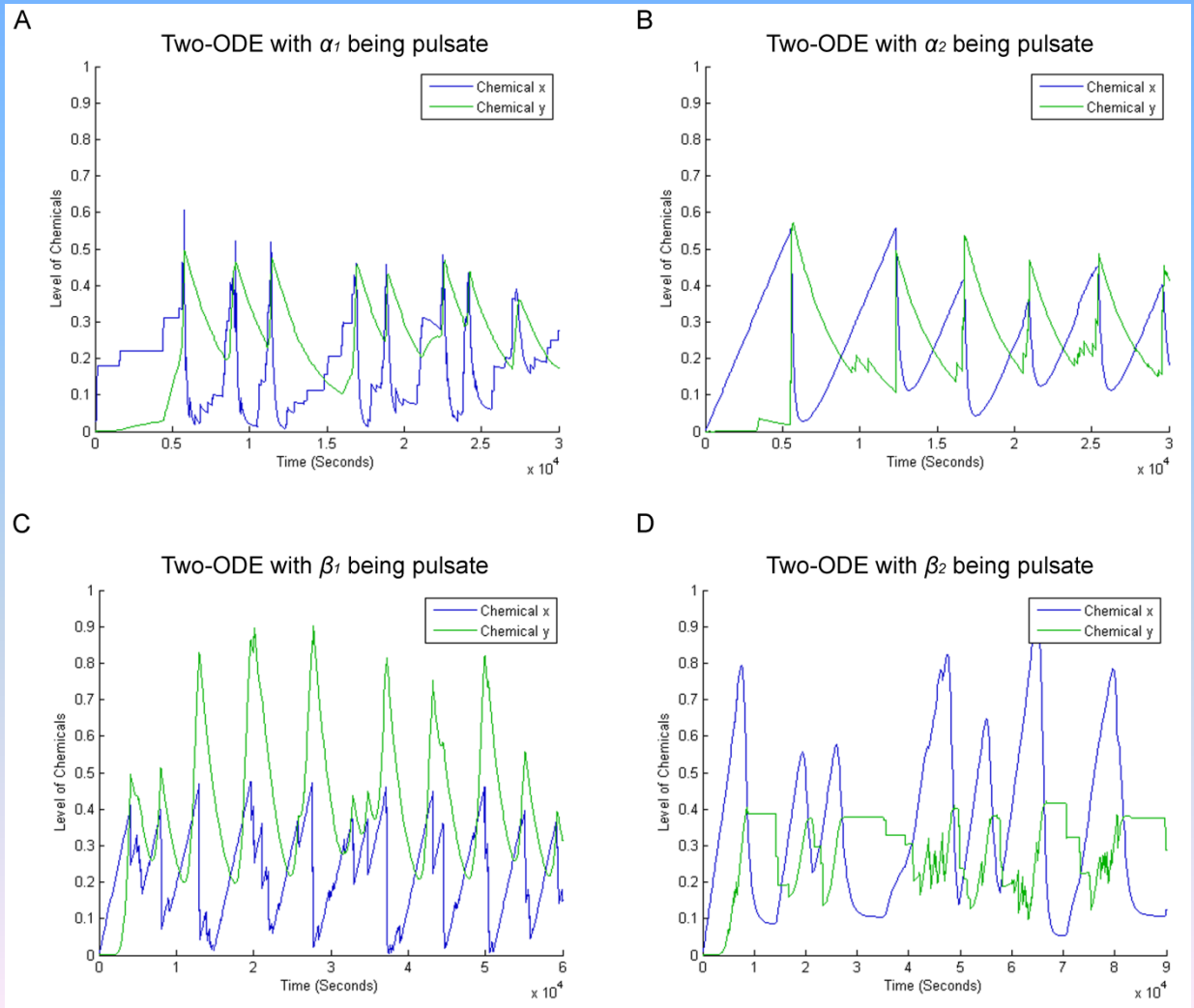
$$\frac{dx}{dt} = \alpha_1 - \beta_1 x \frac{y^{n_1}}{K_1^{n_1} + y^{n_1}}, \qquad \textbf{[1]}$$

$$\frac{dy}{dt} = \alpha_2 (1 - y) \frac{x^{n_2}}{K_2^{n_2} + x^{n_2}} - \beta_2 y. \qquad \textbf{[2]}$$

Equation for reactions with short pauses

$$\frac{dP}{dt} = \theta \left[\frac{t_f}{t_d} + \sum_{k=1}^{m-1} \frac{2m}{(\pi k)^2} \sin \frac{\pi k}{m} \sin\left(\frac{\pi k t_f}{t_d}\right) \cos\left(\frac{2\pi k}{t_d}\left(t - \frac{t_f}{2}\right)\right) \right]$$

The system undergo sustained oscillations with periods in hours



Further expansion of the model may produce a simple unified model that can account for different types of oscillations with a range of periods.

Acknowledgments

Oklahoma State University
Yixing Wang
Genqing Liang
Josh McLoud
Steve Hartson

Cold Spring Harbor Lab
Hong Ma
Yi Hu
Muhammad Lodhi
Richard Mccombie

University of Louisiana at Lafayette
Roy Brown

South China Agricultural University
Hong Wu

University of Pennsylvania
Louis Yang

Funding

National Institute of General Medical Sciences, NIH
Oklahoma State University
Oklahoma Center for the Advancement of Science and Technology
National Science Foundation Bridge to the Doctorate Fellowship