Project Title

One step further into the mystery of inheritance: Meiotic chromosome segregation in yeast

Supervisors:

Dr. Yoshinori Watanabe (University of Tokyo, Japan)
Dr. Prakash Arumugam (University of Warwick, UK)

Summer 2011 Misa Ogura

THE UNIVERSITY OF WARWICK

IATL Institute for Advanced Teaching and Learning
The space where collaborative learning happens

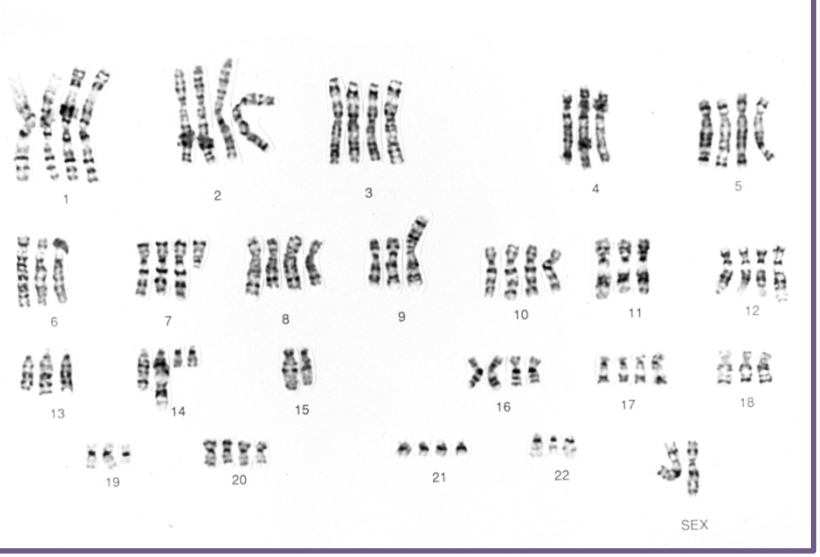
Why is study of chromosome dynamics important?

Chromosome missegregation

Aneuploidy: an abnormality in number of chromosomes

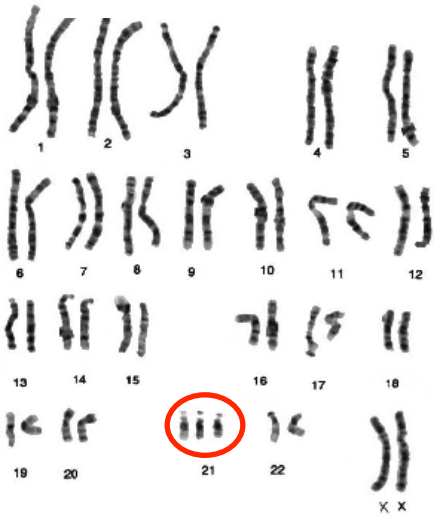
→ hallmark of **cancer cells**
→ primary cause for some **genetic diseases**

Cancer



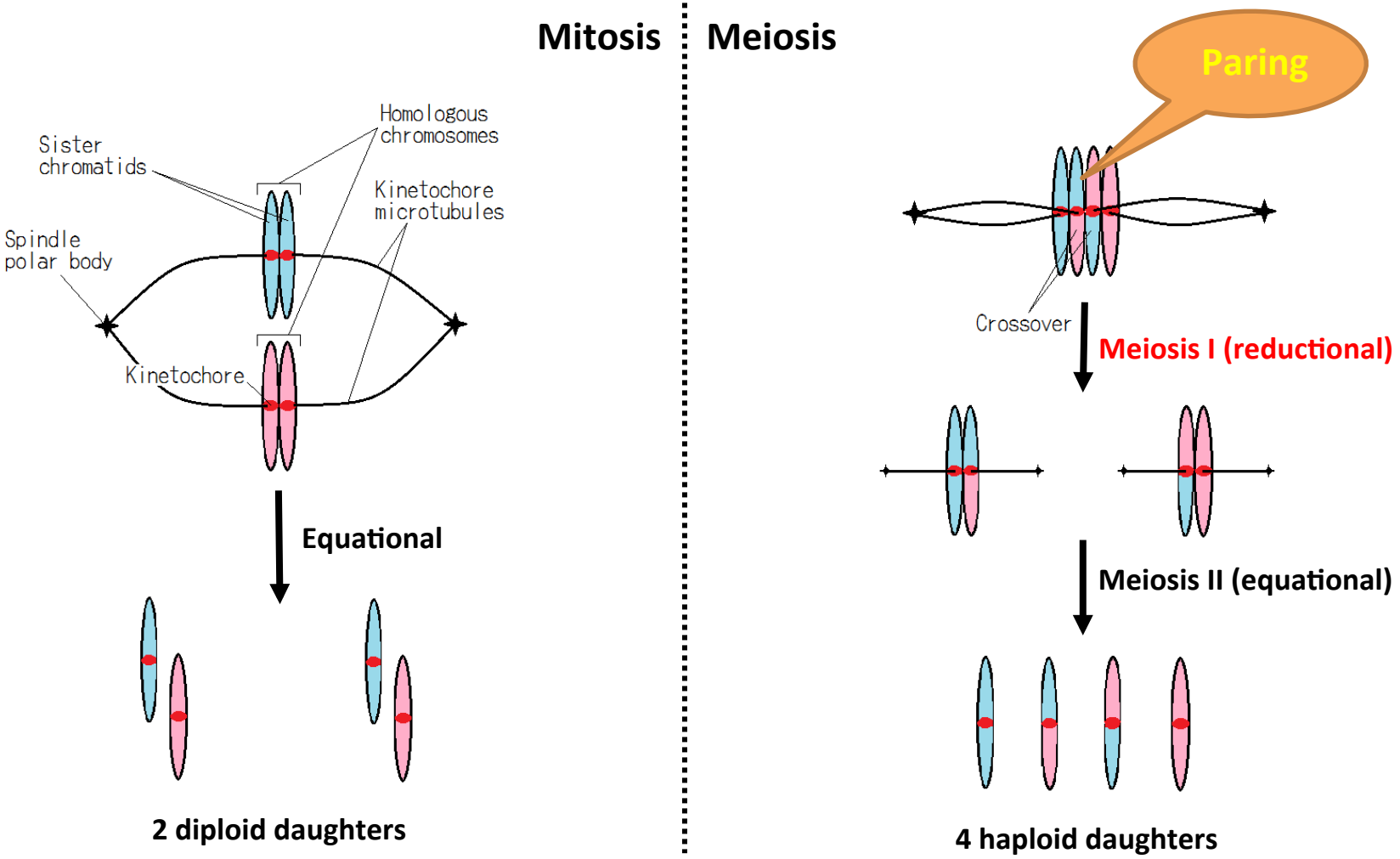
from Hillman *et al. BMC Cancer.* 2007

Down syndrome

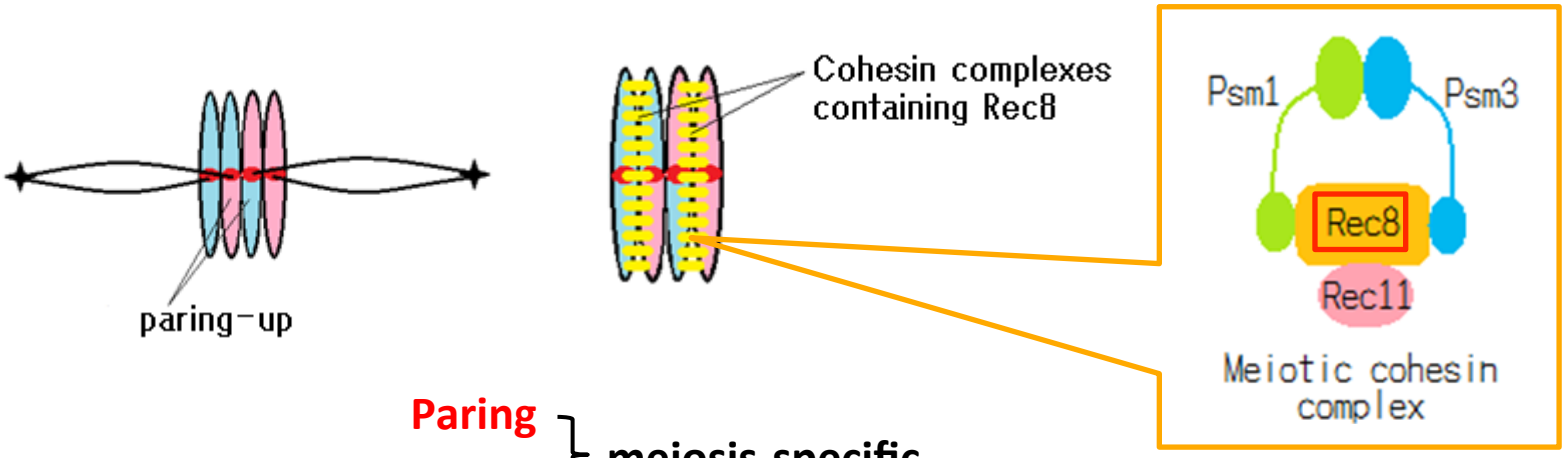


from http://www.ucl.ac.uk/~ucbhjow/bmsi/bmsi_7.html

What is unique in meiosis?



Aim of the project

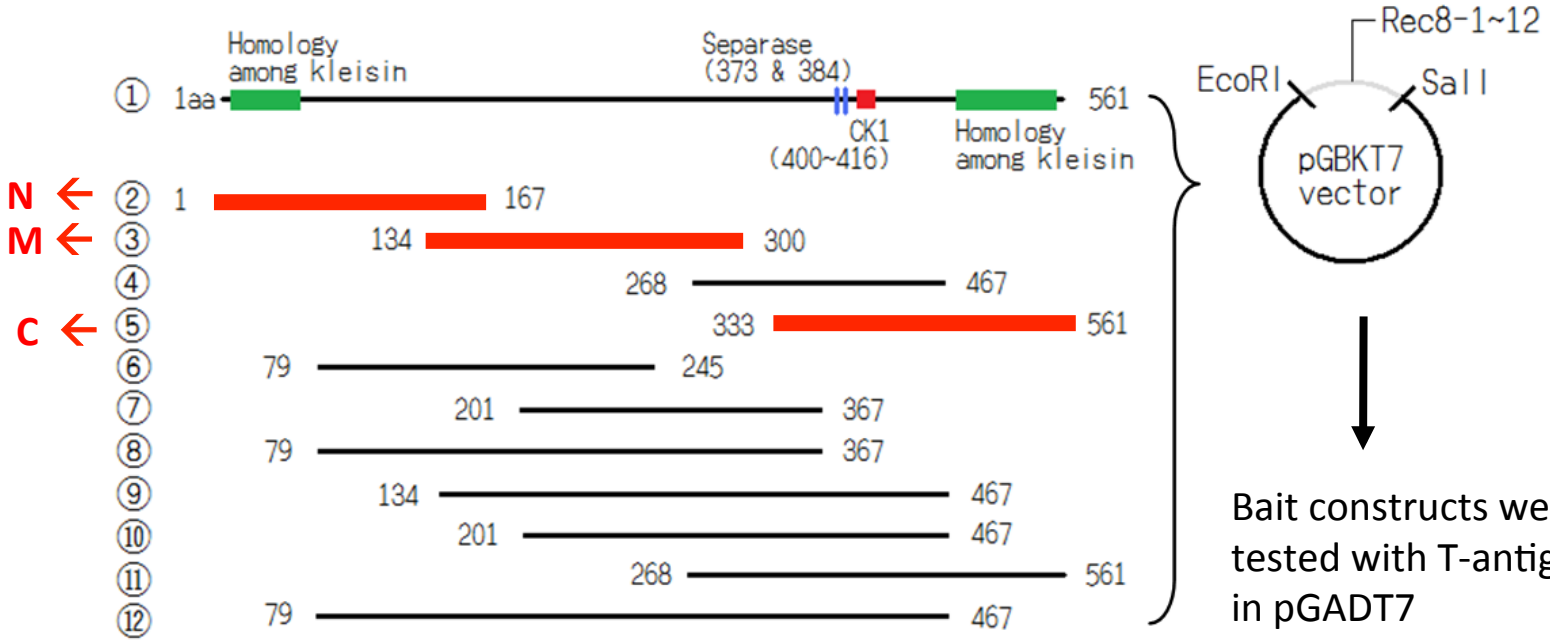


Paring
Rec8 } **meiosis-specific**

To find out proteins that directly interact with Rec8, through genetic screening using the yeast 2 hybrid ststem

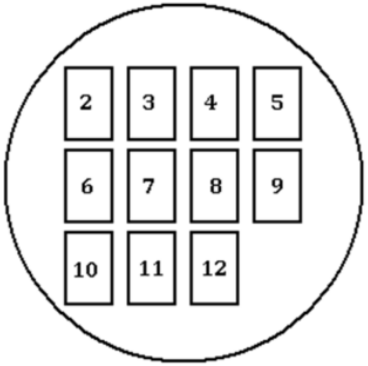
crucial roles in paring- up of homologous chromosomes at prophase I

Results

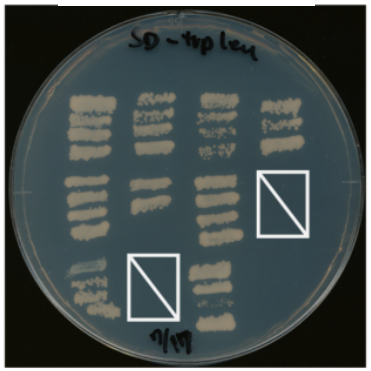


Bait constructs were tested with T-antigen in pGADT7

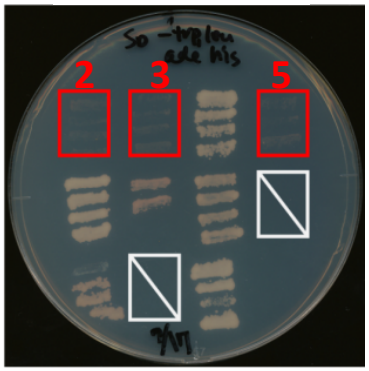
Background check



-LW



-LWHA



no transformants

Low background (red box)

Rec8-2… N fragment

Rec8-3… M fragment

Rec8-5… C fragment

Results (continued)

Library cDNA transformation

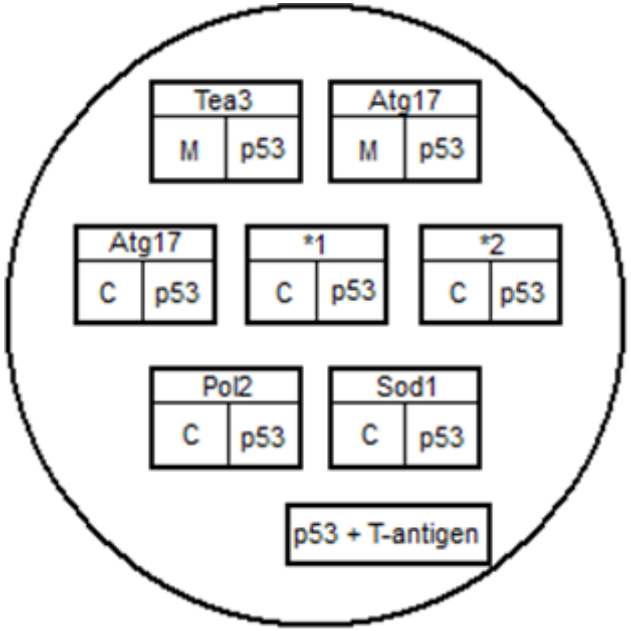
Yeast strain: AH109
Bait: Rec8 N, M, and C fragments
Library: pombe pVP16 patI 4.5hr
Library size: 2.5 x 10^6
Screening size: N: 1.9 x 10^6
M: 1.8 x 10^6
C: 2.2 x 10^6

Screening condition	Efficiency (colonies/plate)		
	N	M	C
-H	too many	too many	too many
-HA	**4.3 x 10^4**	**4.0 x 10^4**	**4.9 x 10^4**
-HA + 1mM 3AT	0	0	0
-HA + 5mM 3AT	0	0	0
-HA + 10mM 3AT	0	0	0

Positive colonies: N: 0, M: 3 , C: 24

Description (interaction with M fragment)	Frequency (3)
Autophagy-related protein Atg17	2
Tip elongation aberrant protein Tea3	1
Description (interaction with C fragment)	**Frequency (24)**
Autophagy-related protein Atg17	20
Siderophore-ion biosynthesis protein…*1	1
Fructose 1,6 –bisphosphate aldolase…*2	1
DNA pol epsilon catalytic subunit Pol2	1
Superoxide dismutase Sod1	1

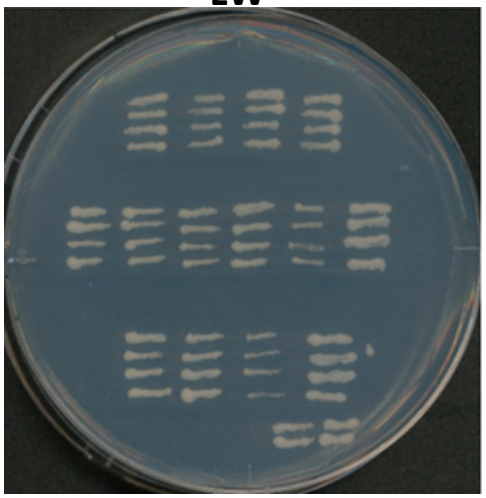
Results (continued)



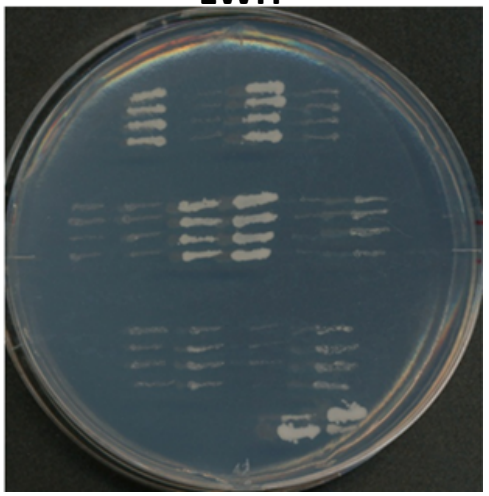
Reproducibility for the following interactions was confirmed:

1. **Rec8-M and Tea3**
2. **Rec8-M and Atg17**

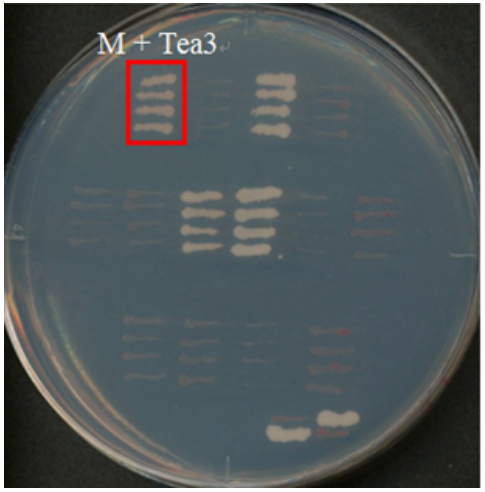
-LW



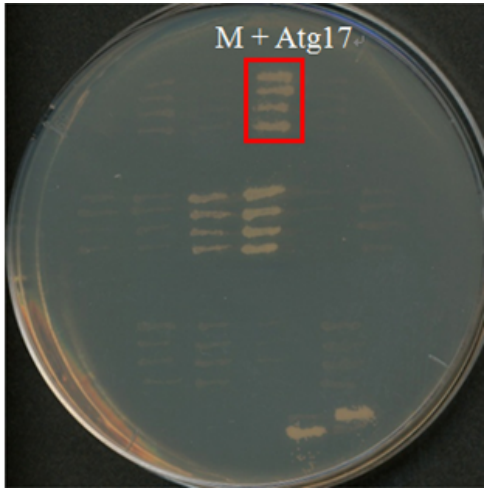
-LWH



-LWHA

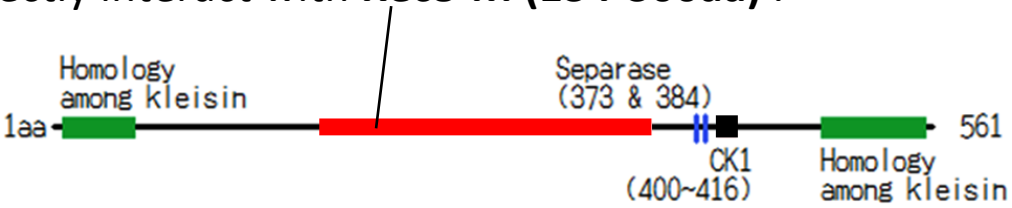


-LWHA + 1mM 3AT



Conclusion

Tea3 and **Atg17** directly interact with **Rec8-M (134-300aa)** .



Future tasks

I. At least one more round of screening is required for each of N, M and C fragments to cover the library size.

II. Nature of the relationship between Rec8 and Tea3/Atg17 is unknown.
→ Other tests have to be applied for further characterisation.

III. The library used was 4.5hrs after the release from pat1 arrest.
→ Libraries taken at other time points can be used for more thorough screening.

Aknowledgements

My special thanks goes to Dr. Watanabe and Dr. Arumugam, who kindly agreed to be supervisors for this project, the Institute for Advanced Teaching and Learning, which provided me funding, and all the members at the Watanabe lab who helped me in every aspects of various experiments.