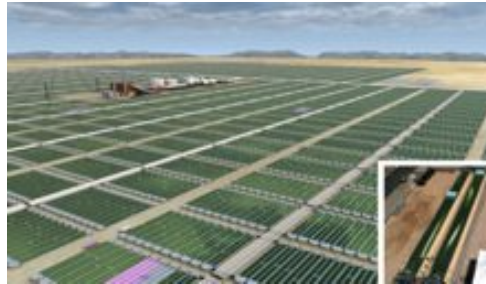


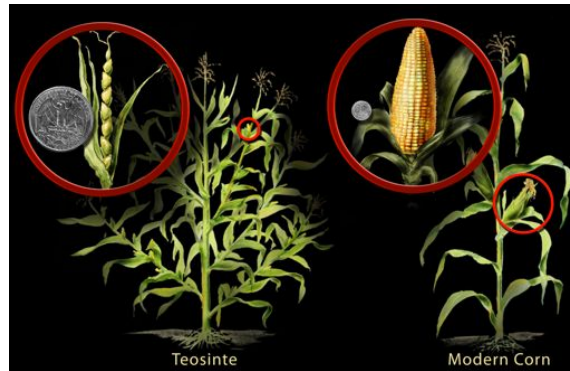
Reproduction of microalgae:
coming down to the basis



Su-Chiung Fang

How can we make algae as a
energy crop?

It takes thousand years to domesticate of maize from teosinte



Nicolle Rager Fuller, National Science Foundation

How do we speed up the processes?

What can be done to make algae-based biofuel economical ?

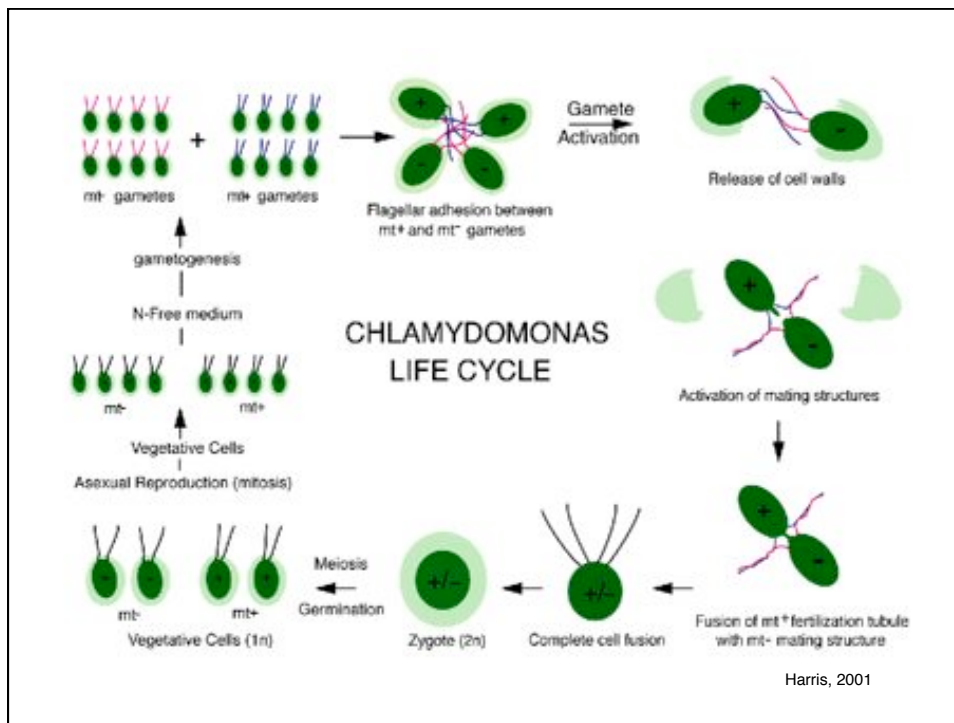
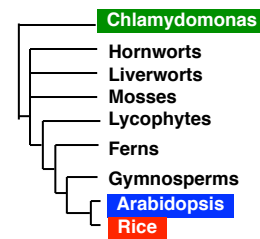
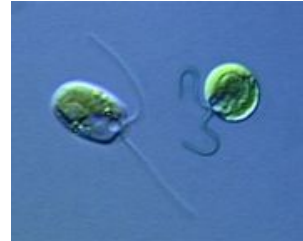
- Efficient methods to recover algal biomass from the diluted broths
- Extract algal oil from moist biomass pastes without the need of drying
- Effective way to increase algal biomass production capacity
- Amenable genetic and metabolic engineering protocol
 - Improve growth rate
 - Enhance photosynthetic efficiency and biomass yield
 - Elevate oil content
 - Reduce or eliminate contamination
 - Improve temperature tolerance

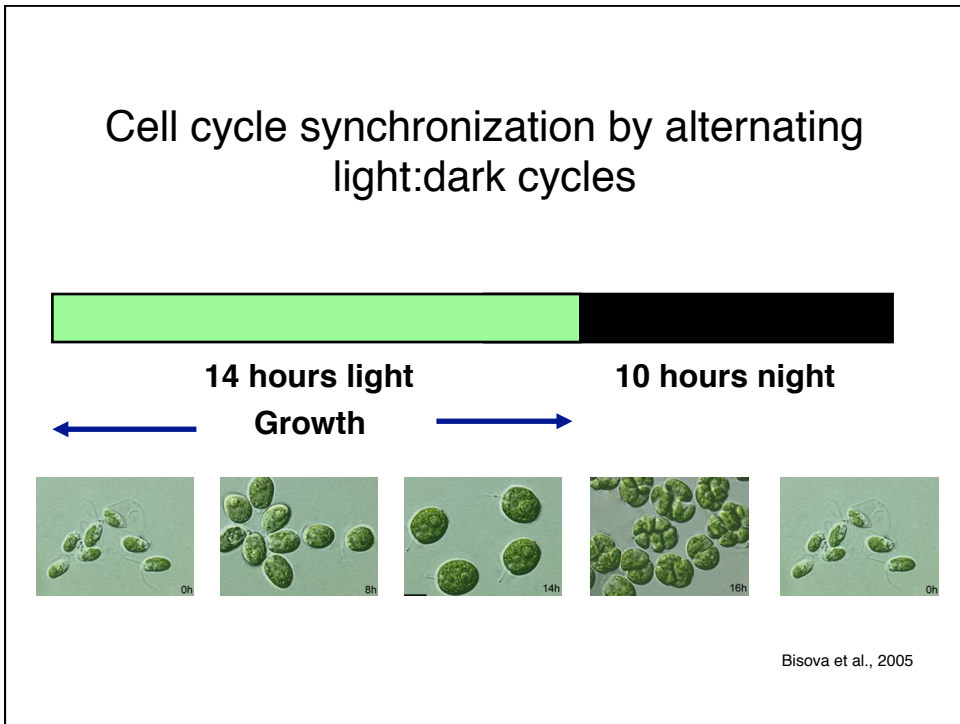
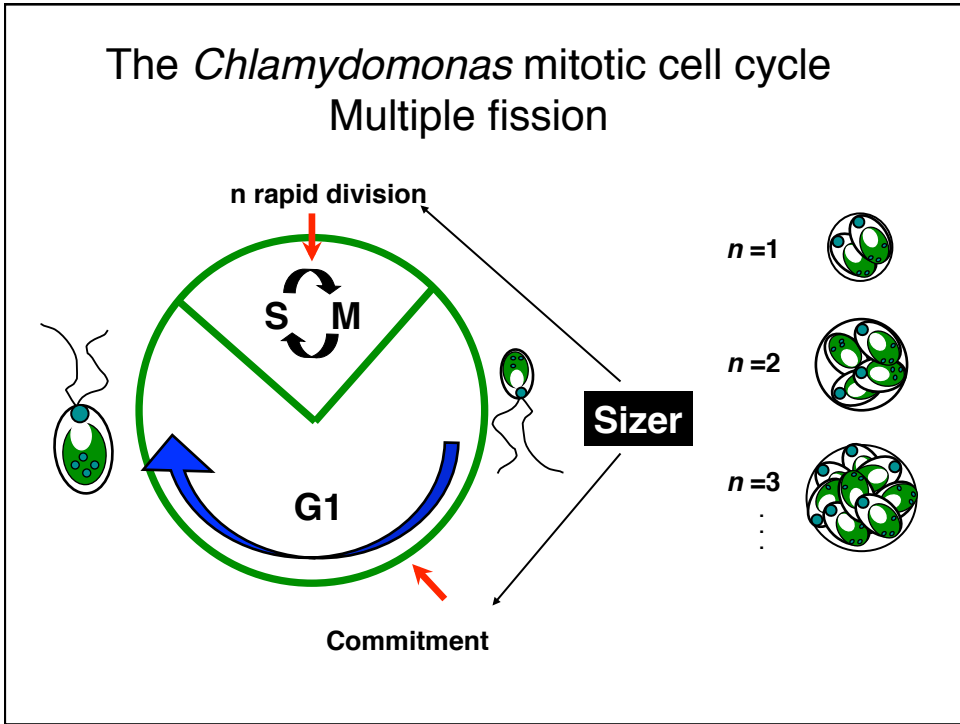
A functional model system for algae

- Short lifecycle
- Simple growth requirements
- Tractable genetics
- Can be efficiently transformed or genetically altered—it can be used to rapidly gain the knowledge about basic biology necessary for applied biology.

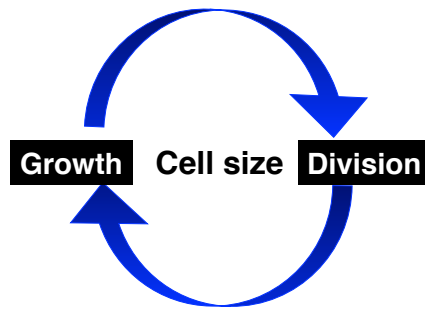
Chlamydomonas reinhardtii

- Unicellular green alga, related to land plant
- Sequenced haploid genome of ~125 MB
- Molecular and genetic tools
 - Nuclear and Organellar Transformation
 - Tetrad Analysis
 - RNAi
 - Reporters
 - Regulated Promoters
 - Microarray



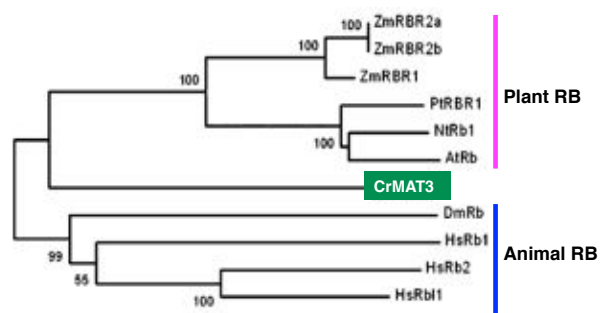
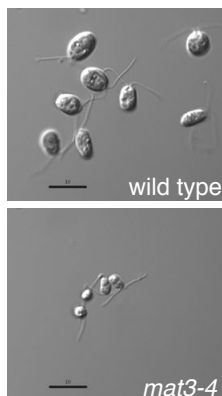


Cell size control requires the balance of growth and division

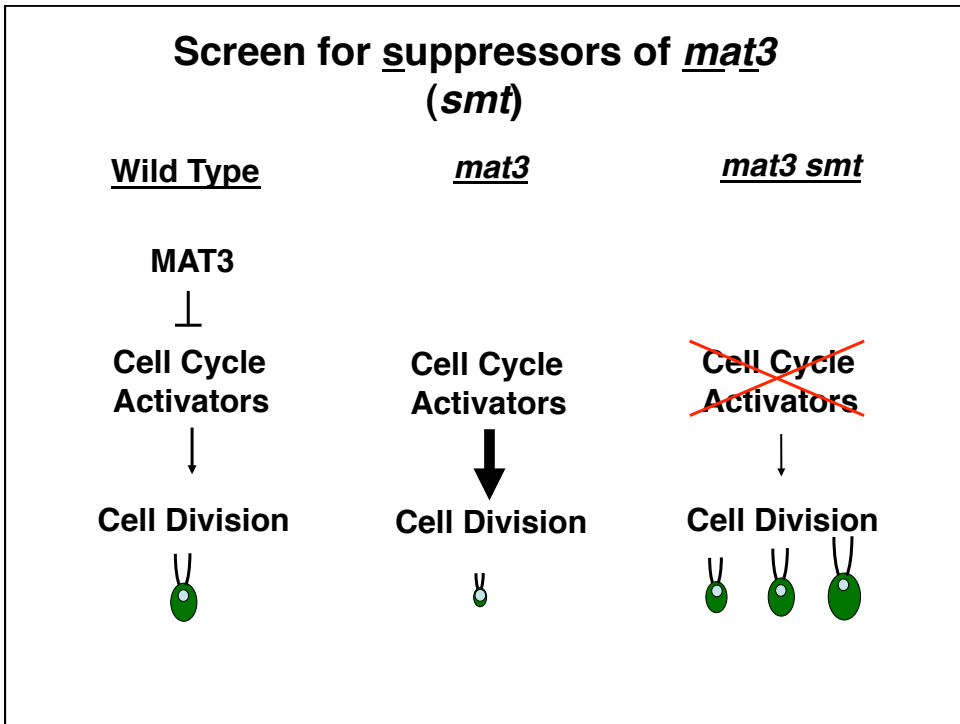
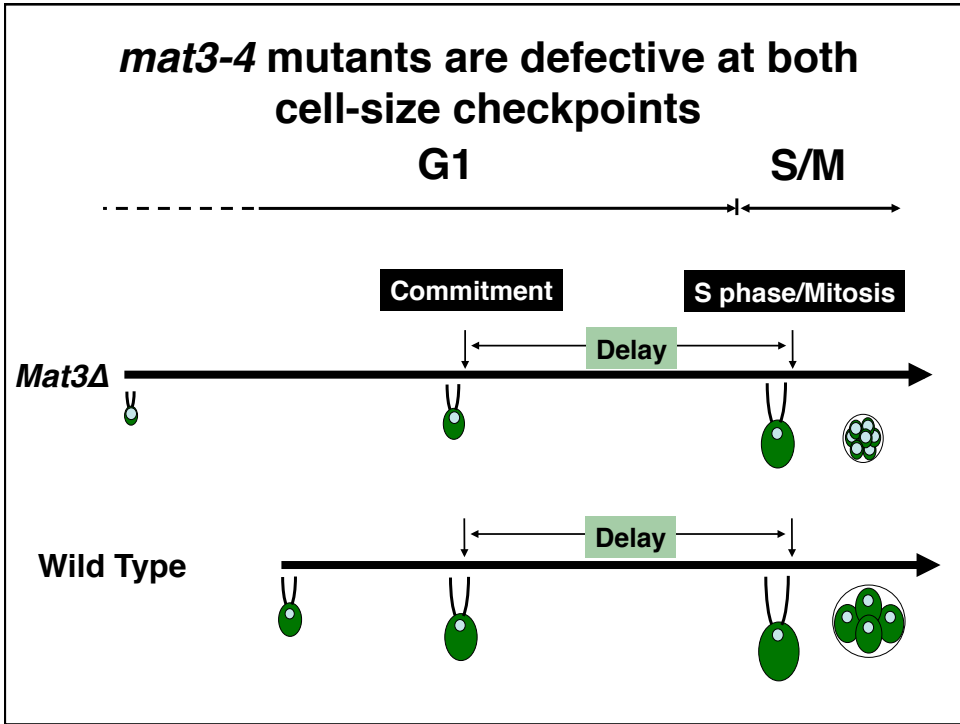


What is the underlying genetic network of size control mechanism?


MAT3 encodes the Chlamydomonas homolog of the retinoblastoma protein (RB)



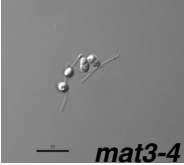
Umen and Goodenough, 2001; Bisova et al., 2005



19 suppressors of *mat3-4* (*smt*)



wt


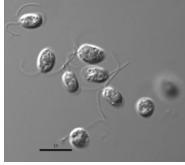
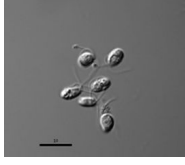


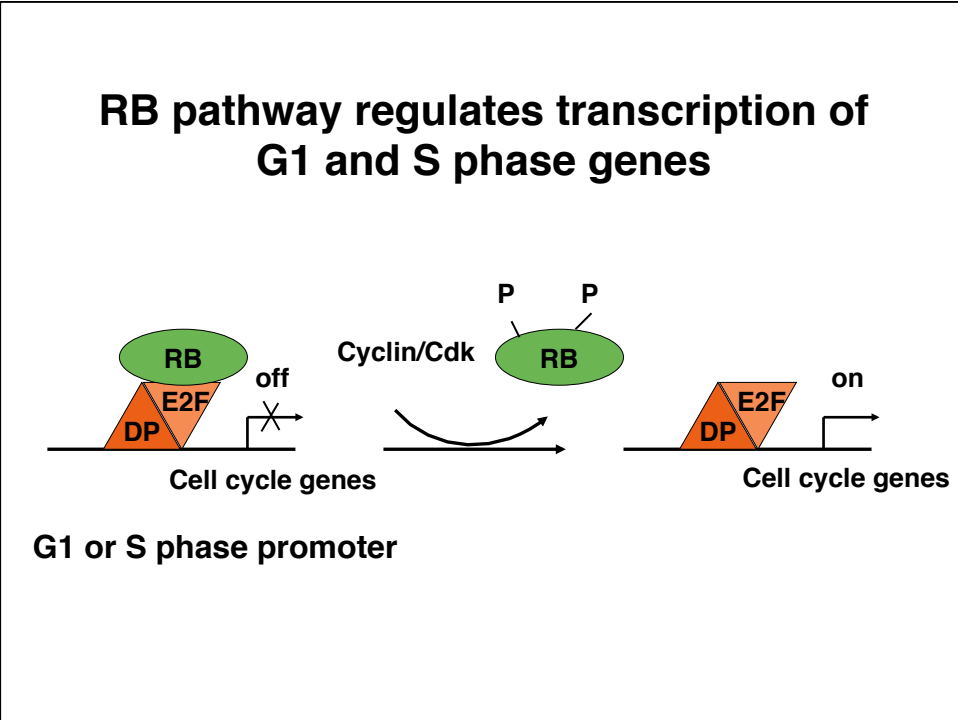
mat3-4

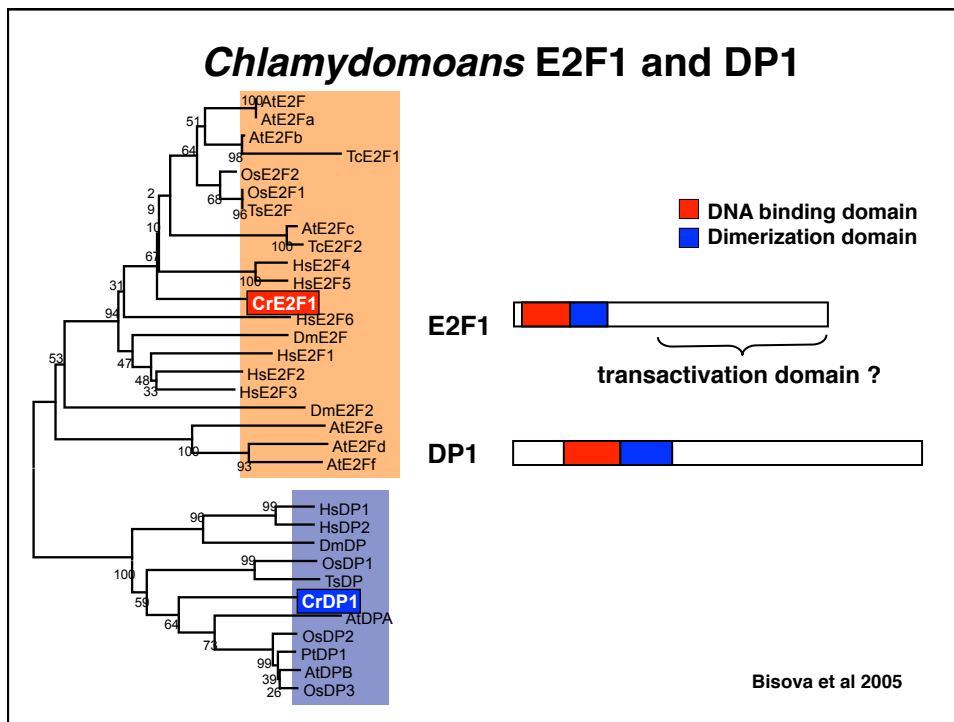
Class I: larger than wt
DP1 (12)

Class II: close to wt
E2F1 (3)

**Class III: larger than *mat3-4*,
smaller than wt**
SMT7, SMT14, SMT15, SMT16



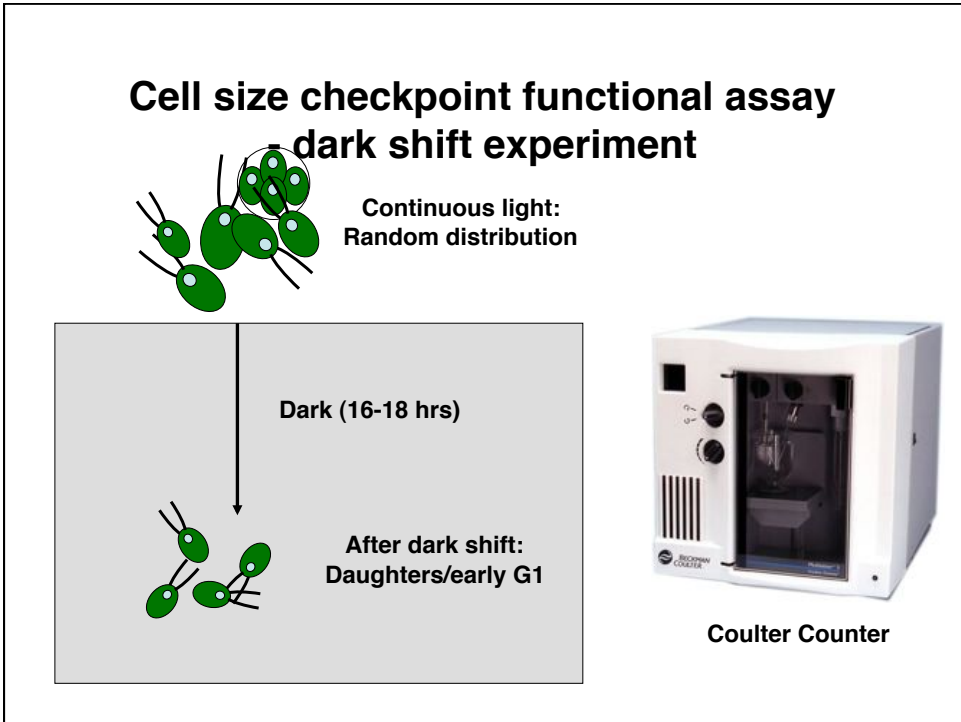
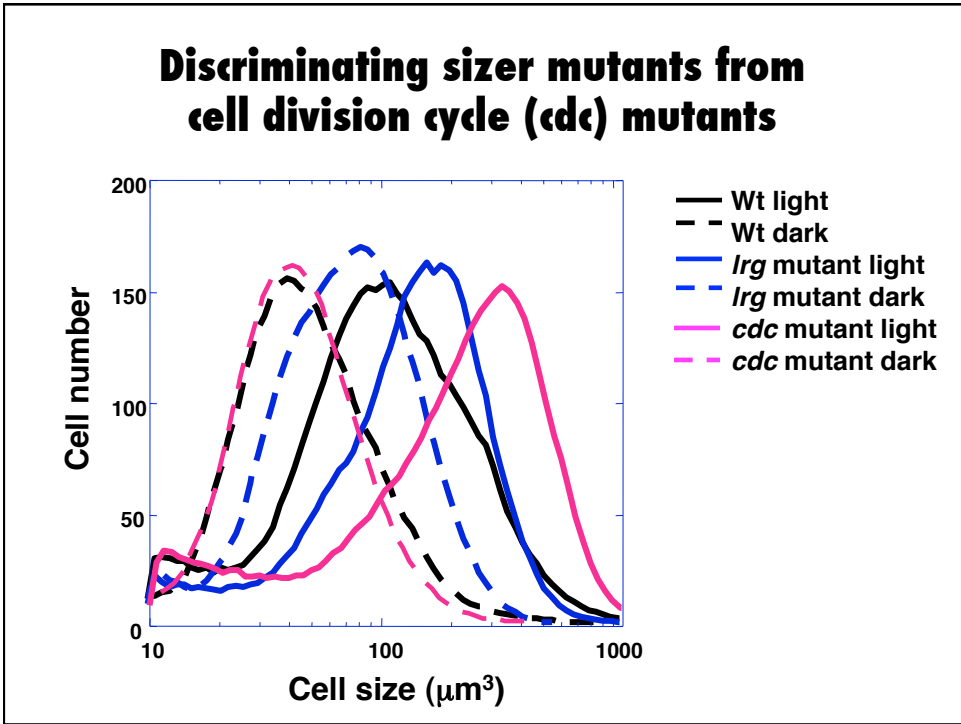


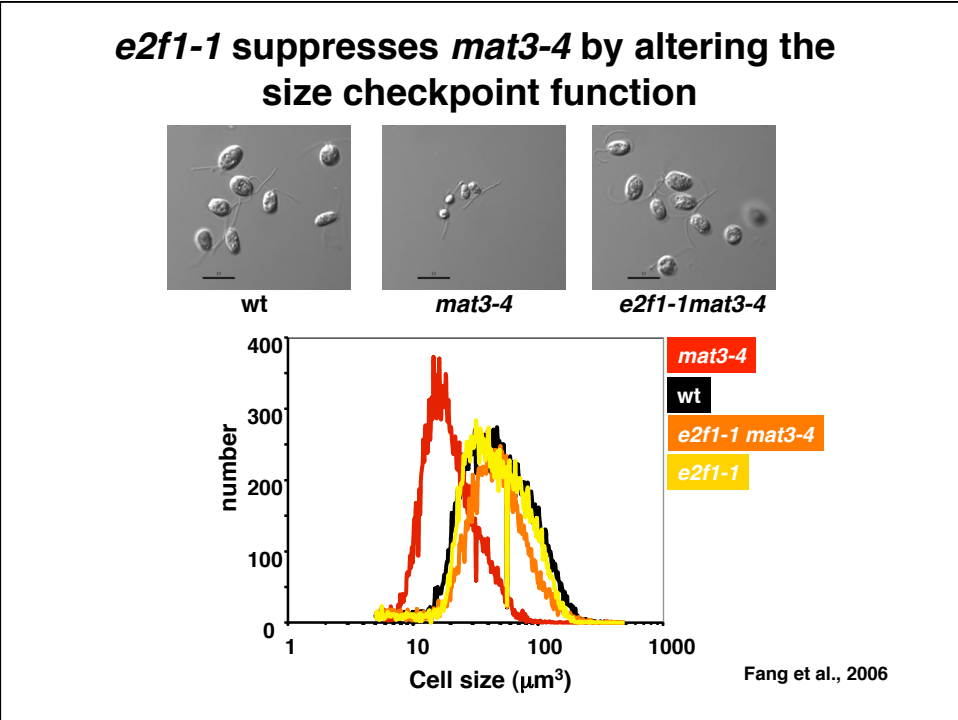
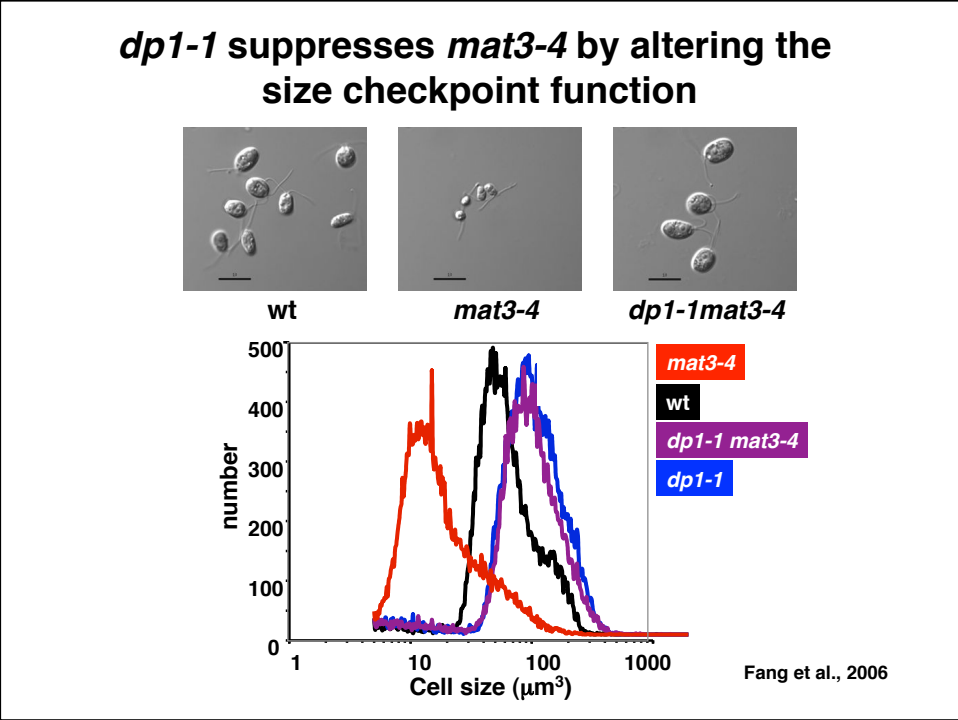
How does *dp1-1* and *e2f1-1* suppress *mat3*?

Two potential mechanisms:

Slow down the cell cycle

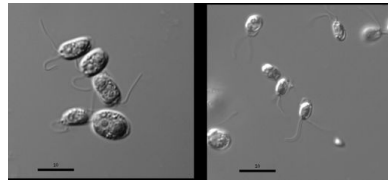
Alter the cell size checkpoint function



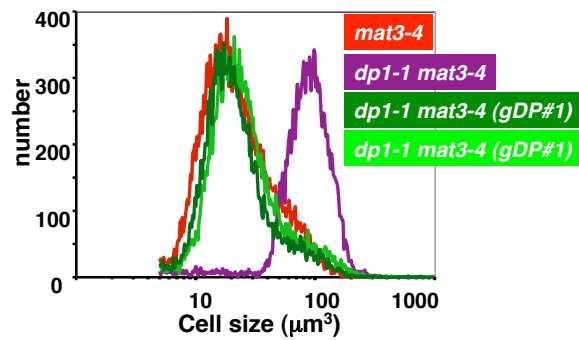


Genomic DP1 complementation

(*dp1-1 mat3-4* + *DP1* = *mat3-4*)



dp1-1mat3-4 *dp1-1mat3-4 (gDP1#1)*

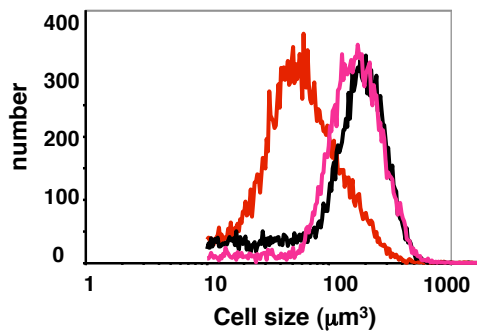
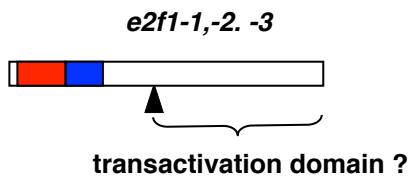


number

Cell size (μm^3)

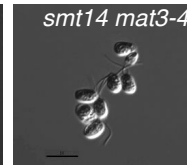
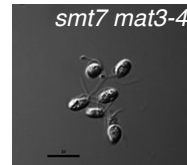
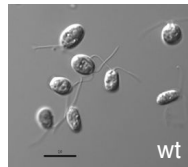
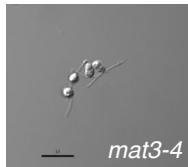
mat3-4
dp1-1 mat3-4
dp1-1 mat3-4 (gDP1#1)
dp1-1 mat3-4 (gDP1#1)

e2f1-1 mutant is dominant



mat3-4 mat3-5/E2F1 E2F1
mat3-4 mat3-5/e2f1-1 E2F1
MAT3 MAT3 /E2F1 E2F1

19 suppressors of *mat3-4*



Class I: larger than wt

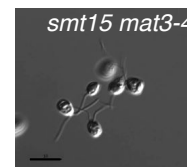
DP1 (12)

Class II: close to wt

E2F1 (3)

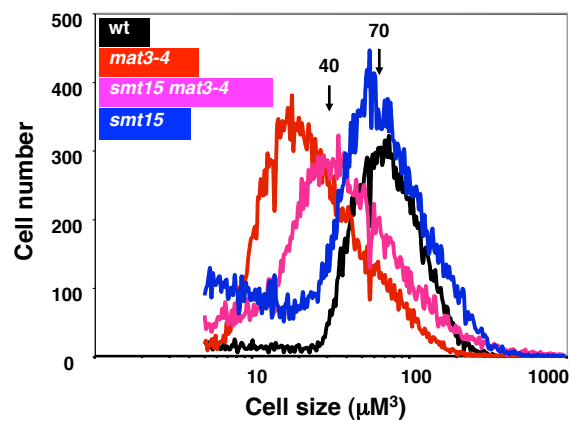
**Class III: larger than *mat3-4*,
smaller than wt**

SMT7, *SMT14*, *SMT15*, *SMT16*

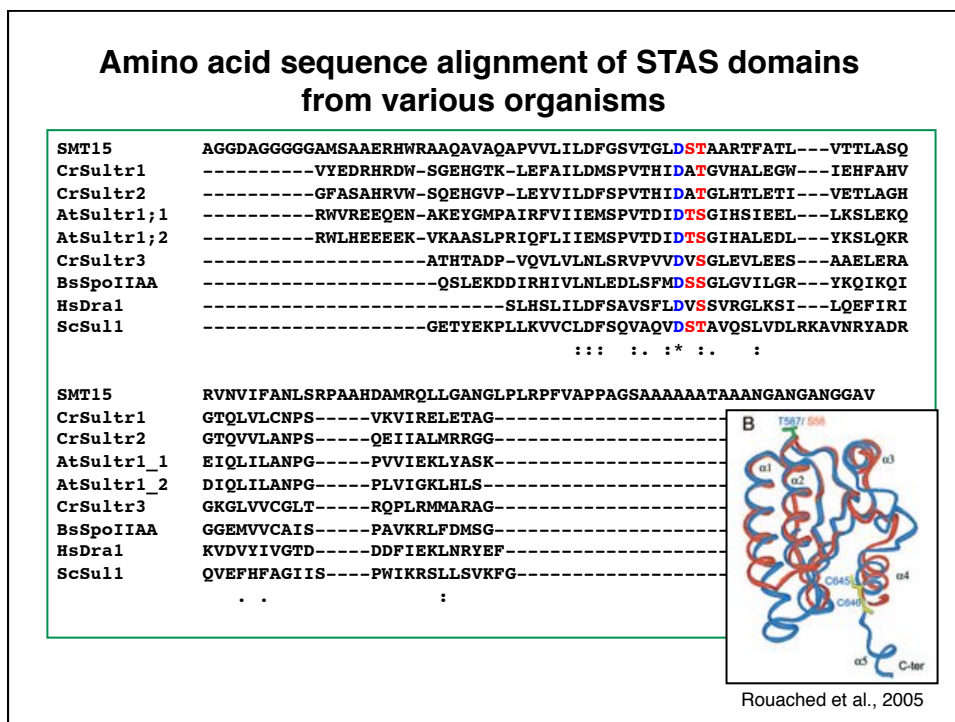
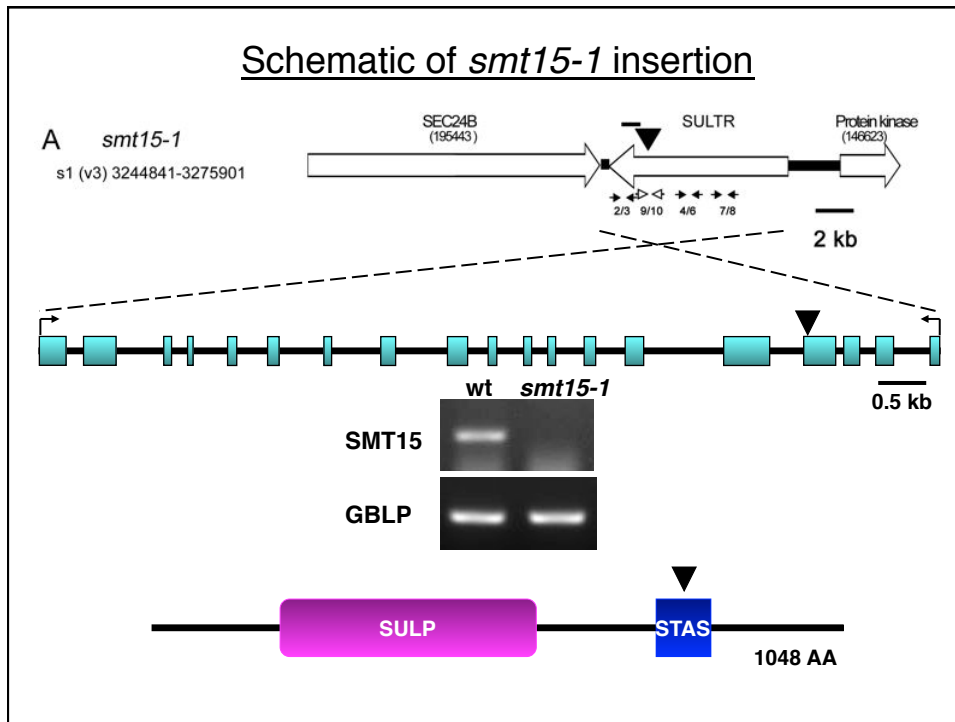


Fang and Umen, 2008

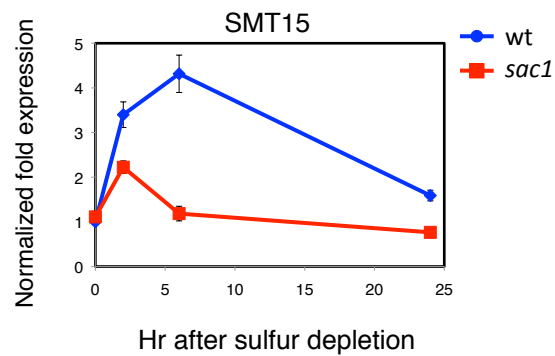
Size distribution of *smt15-1* and *smt15-1 mat3-4*



Genotype	Daughter cell size (μm^3)	Commitment cell size (μm^3)	Doubling time (hrs)
Wild type	65 ± 2.7	200 ± 5	5.1 ± 0.3
<i>smt15-1</i>	51 ± 4.1	159 ± 11	8.3 ± 0.5
<i>mat3-4</i>	21 ± 1.4	103 ± 10	7.3 ± 0.2
<i>smt15-1 mat3-4</i>	37 ± 2.1	179 ± 13	9.7 ± 0.6



SMT15 mRNA is up-regulated by sulfur depletion



Fact sheet of *smt15*:

- *smt15-1* is a small size mutant with reduced Commitment size threshold
- *smt15-1* suppress *mat3-4* by increasing the Commitment size threshold and decrease the number of cell division
- It is paradoxical that SMT15 is both positive and negative regulator of the cell cycle
- *smt15-1* has a simple insertion in a locus that encodes a potential sulfate transporter
- Part of SAC response is affected in *smt15-1* mutant
- *smt15-1* single mutant has growth defect and can not be synchronized by alternating L/D cycle

Summary:

- The genetic architecture of RB/E2F/DP pathway is conserved in *Chlamydomonas*
- MAT3/RB is a negative regulator of the cell cycle
- E2F1 and DP1 are positive regulators of the cell cycle
- *SMT7*, *SMT14*, *SMT15*, and *SMT16* are novel targets of the RB/MAT3
- *Chlamydomonas* is an excellent model for studies on cell growth and cell division

Acknowledgment

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