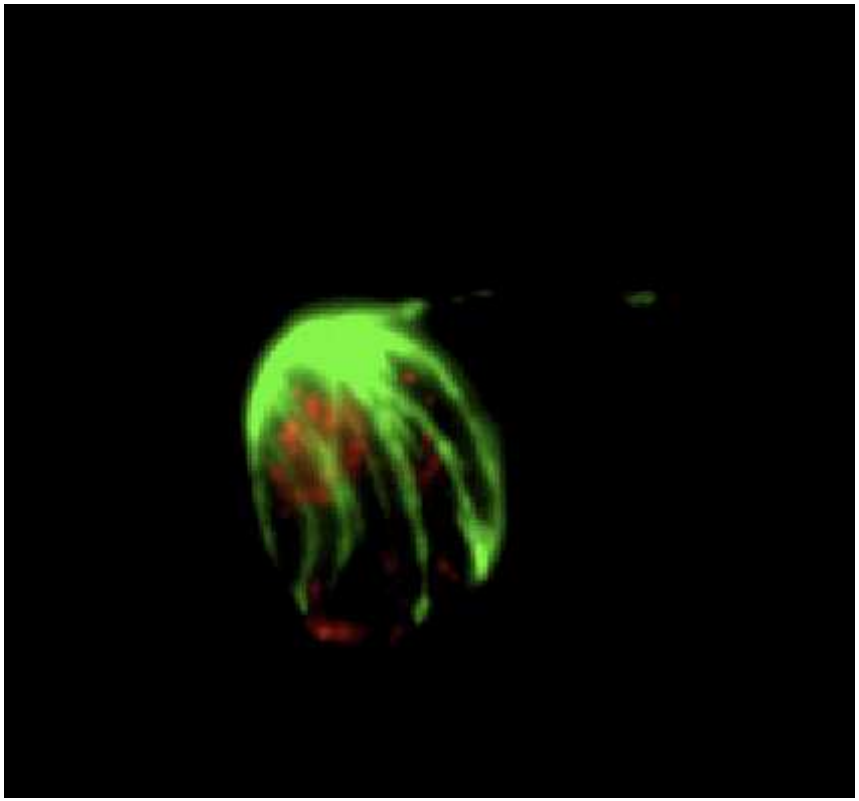
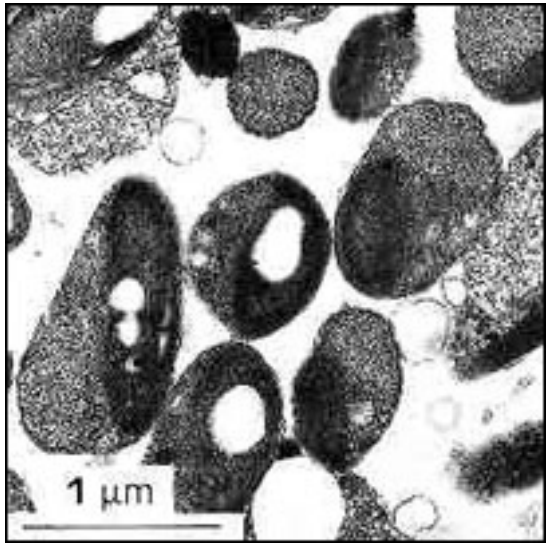
Genetic dissection of the Retinoblastoma tumor suppressor pathway: a Chlamydomonas approach



Su-Chiung Fang

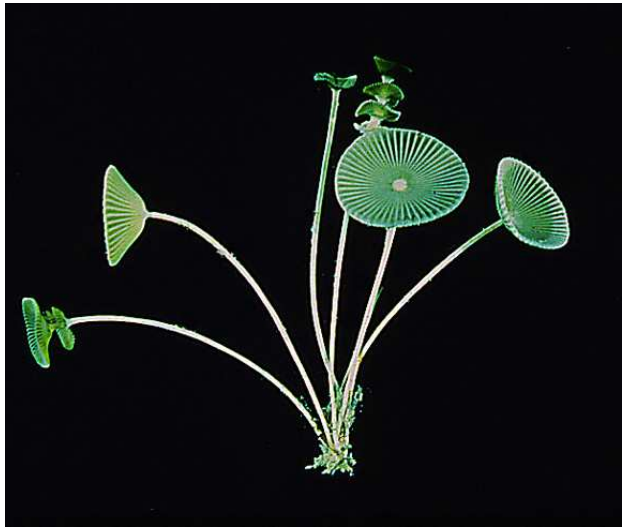
Cell size variation

Ostreococcus



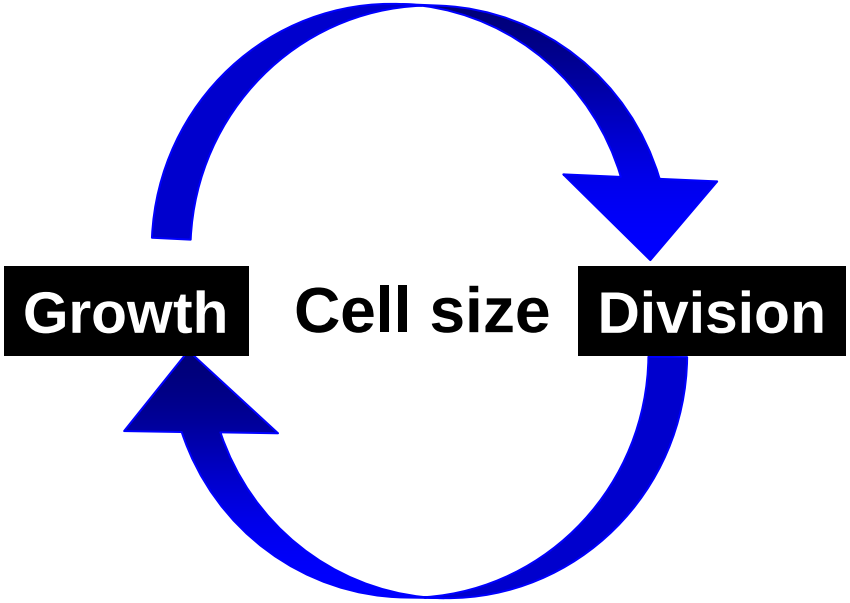
1 µM

Acetabularia



5 cM

Cell size control requires the balance of growth and division



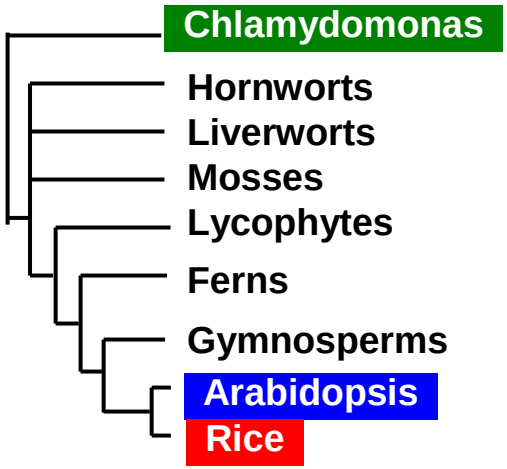
What is the underlying genetic network of size control mechanism?

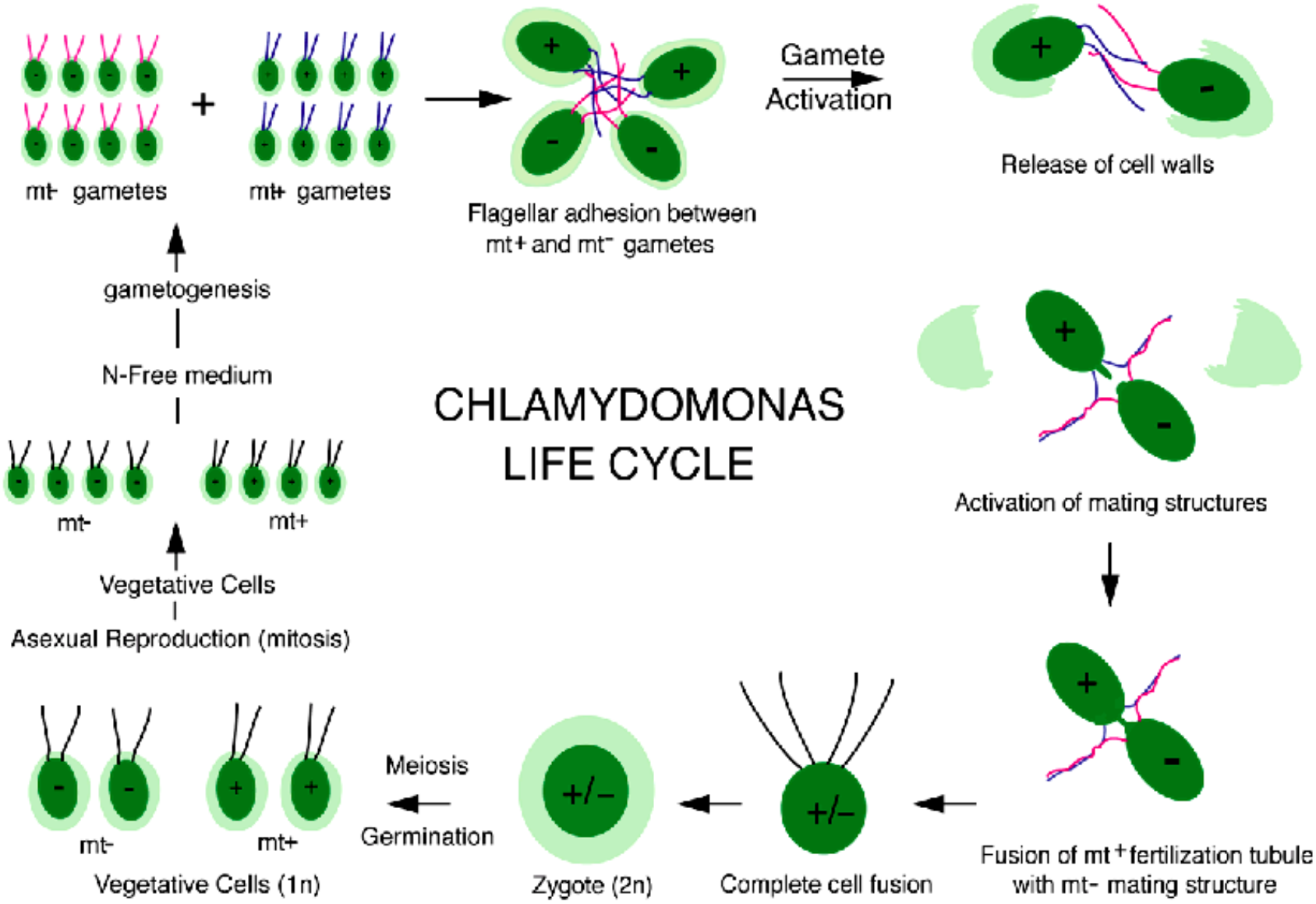
Chlamydomonas reinhardtii

- **Unicellular green alga, related to land plant**

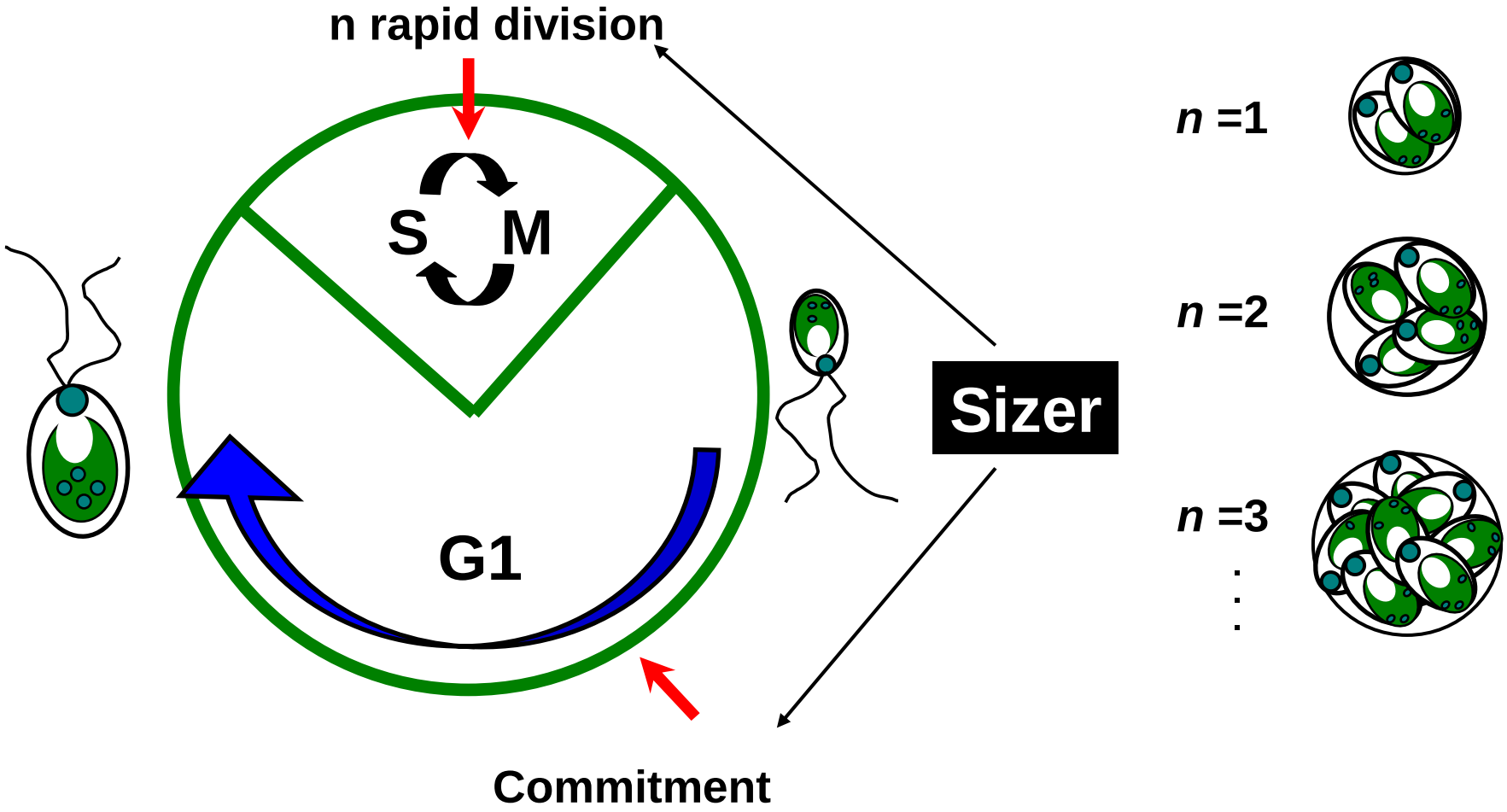
- **Sequenced haploid genome of ~125 MB**

- **Molecular and genetic tools**
  - **Nuclear and Organellar Transformation**
  - **Tetrad Analysis**
  - **RNAi**
  - **Reporters**
  - **Regulated Promoters**
  - **Microarray**




CHLAMYDOMONAS
LIFE CYCLE
mt- gametes
mt+ gametes
Flagellar adhesion between
mt+ and mt- gametes
Gamete
Activation
Release of cell walls
gametogenesis
N-Free medium
mt-
mt+
Vegetative Cells
Asexual Reproduction (mitosis)
Activation of mating structures
Meiosis
Germination
+/–
Zygote (2n)
Complete cell fusion
Fusion of mt+ fertilization tubule
with mt- mating structure
mt-
mt+
Vegetative Cells (1n)

The *Chlamydomonas* mitotic cell cycle



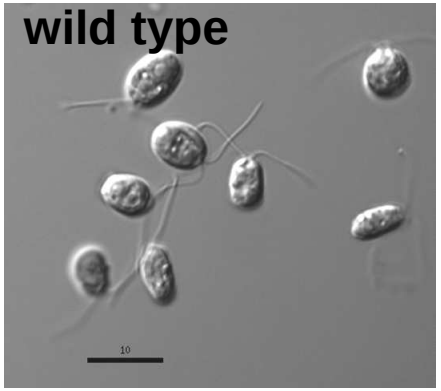
Cell cycle synchronization by alternating light:dark cycles

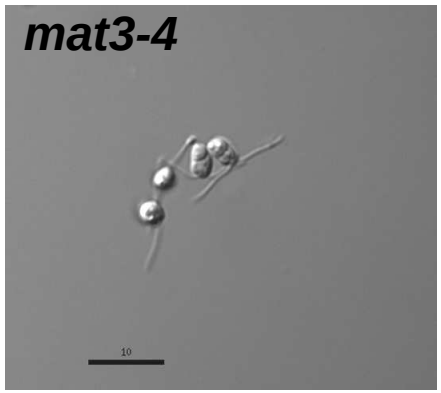
14 hours light

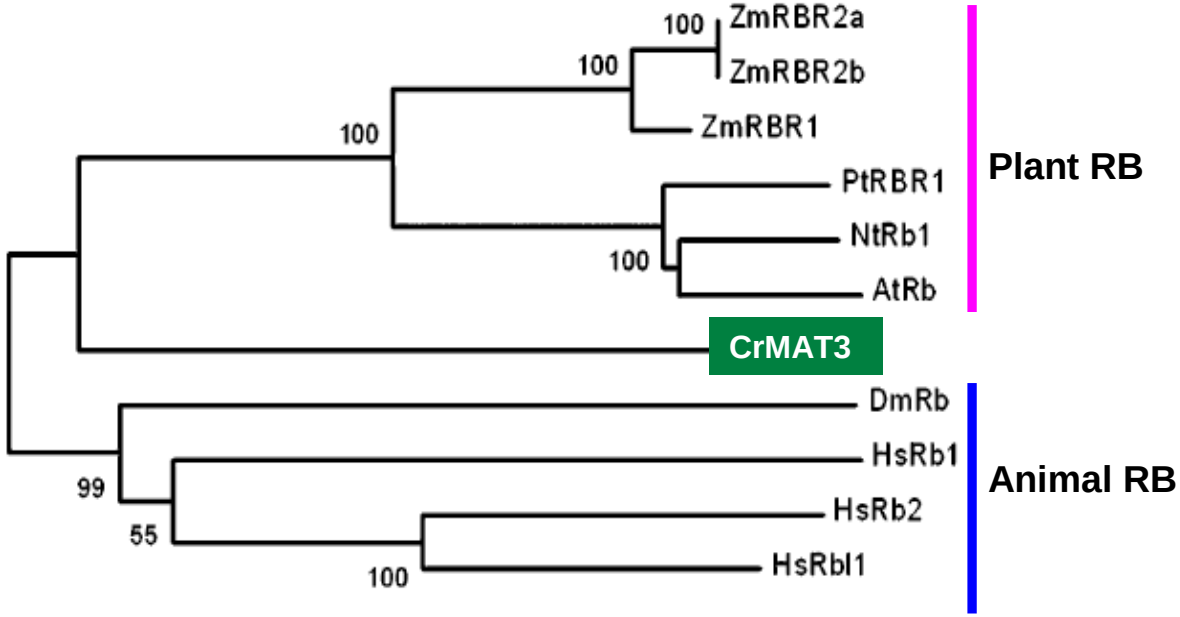
10 hours night

Growth

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

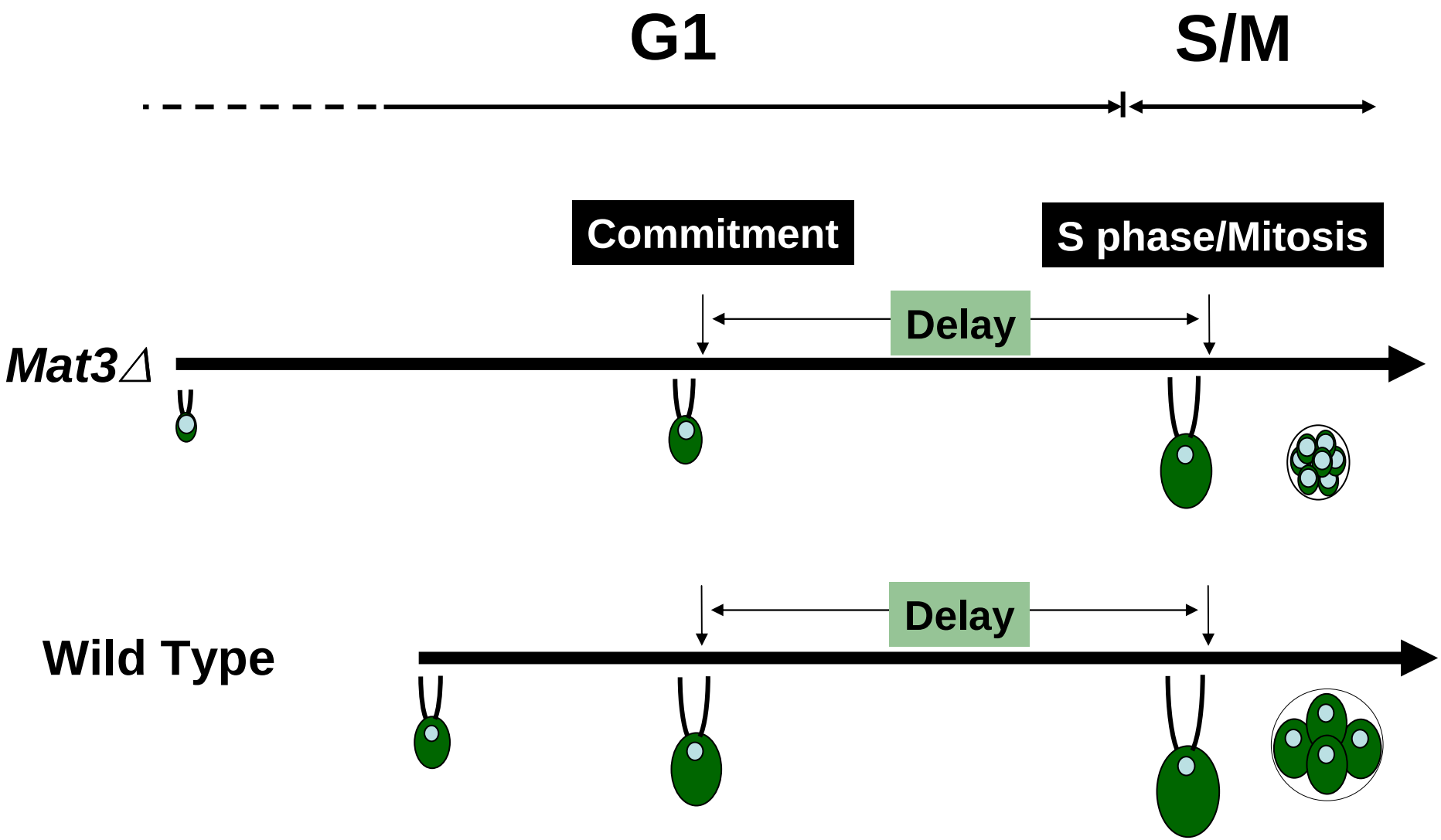






Umen and Goodenough, 2001; Bisova et al., 2005

mat3-4 mutants are defective at both cell-size checkpoints



Screen for suppressors of *mat3* (*smt*)

Wild Type

MAT3

⊥

Cell Cycle Activators

↓

Cell Division

mat3

Cell Cycle Activators

⬇

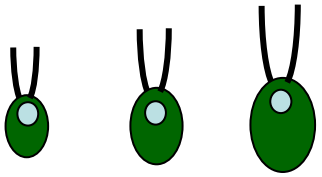
Cell Division

mat3 smt

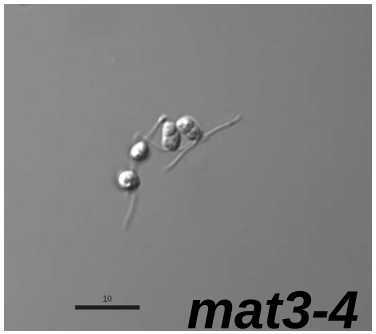
~~**Cell Cycle Activators**~~

↓

Cell Division

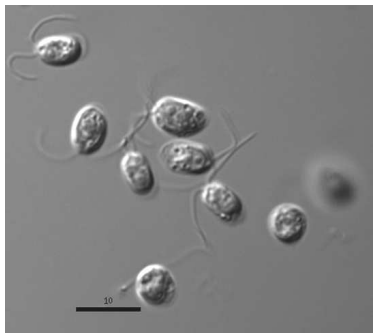


19 suppressors of *mat3-4 (smt)*

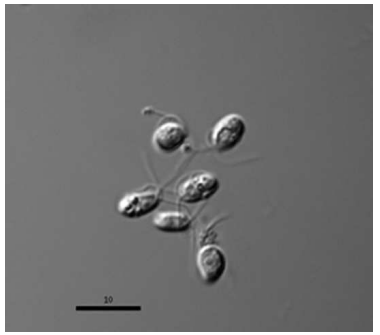


Class I: larger than wt
***DP1* (12)**

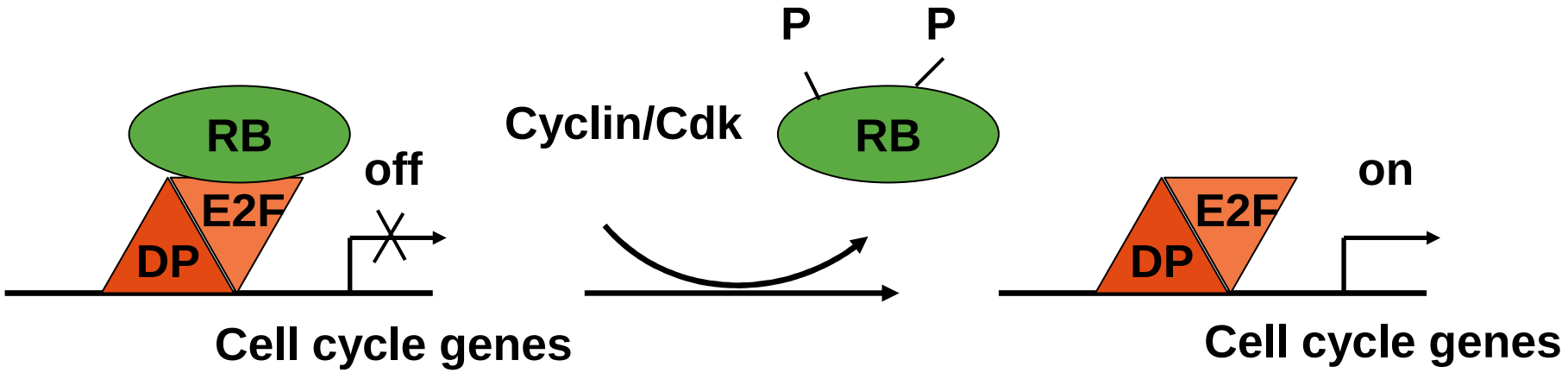
Class II: close to wt
***E2F1* (3)**



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

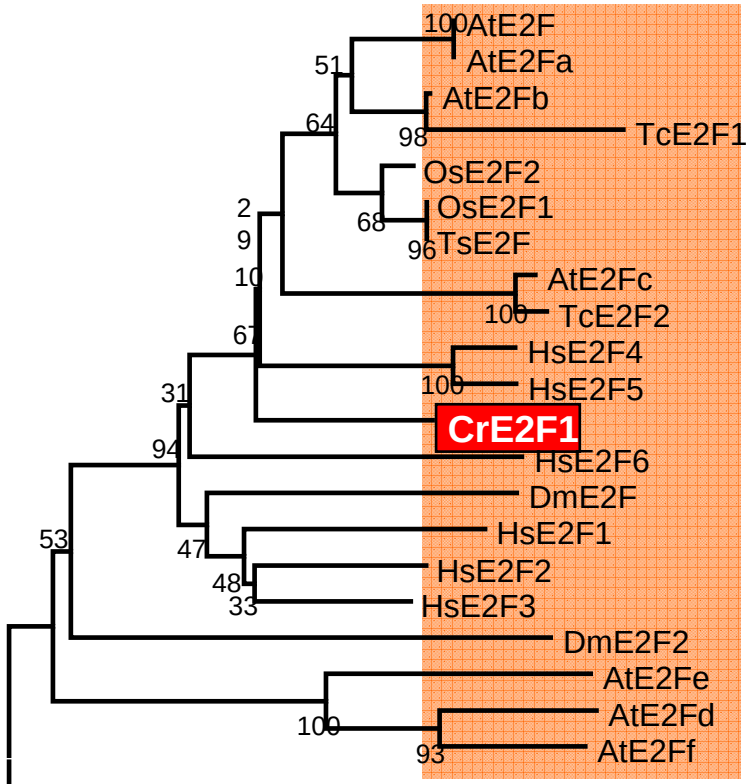


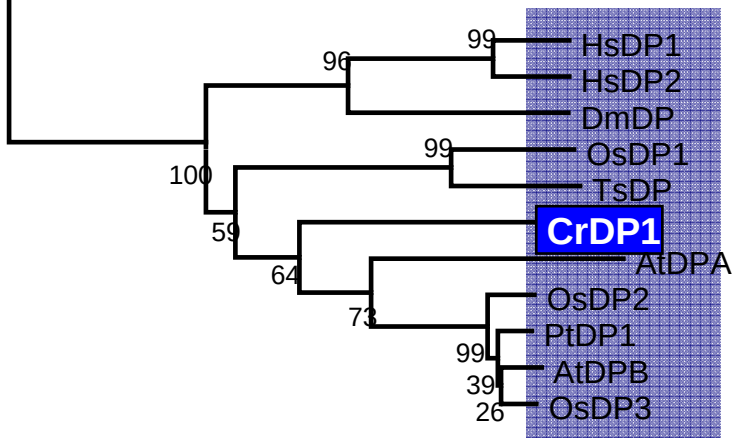
RB pathway regulates transcription of G1 and S phase genes

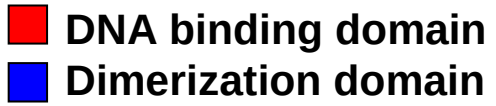


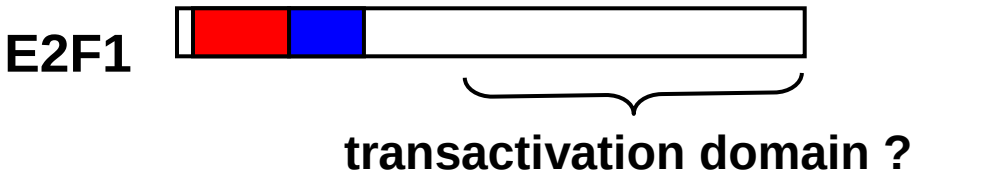
G1 or S phase promoter

Chlamydomoans E2F1 and DP1









Bisova et al 2005

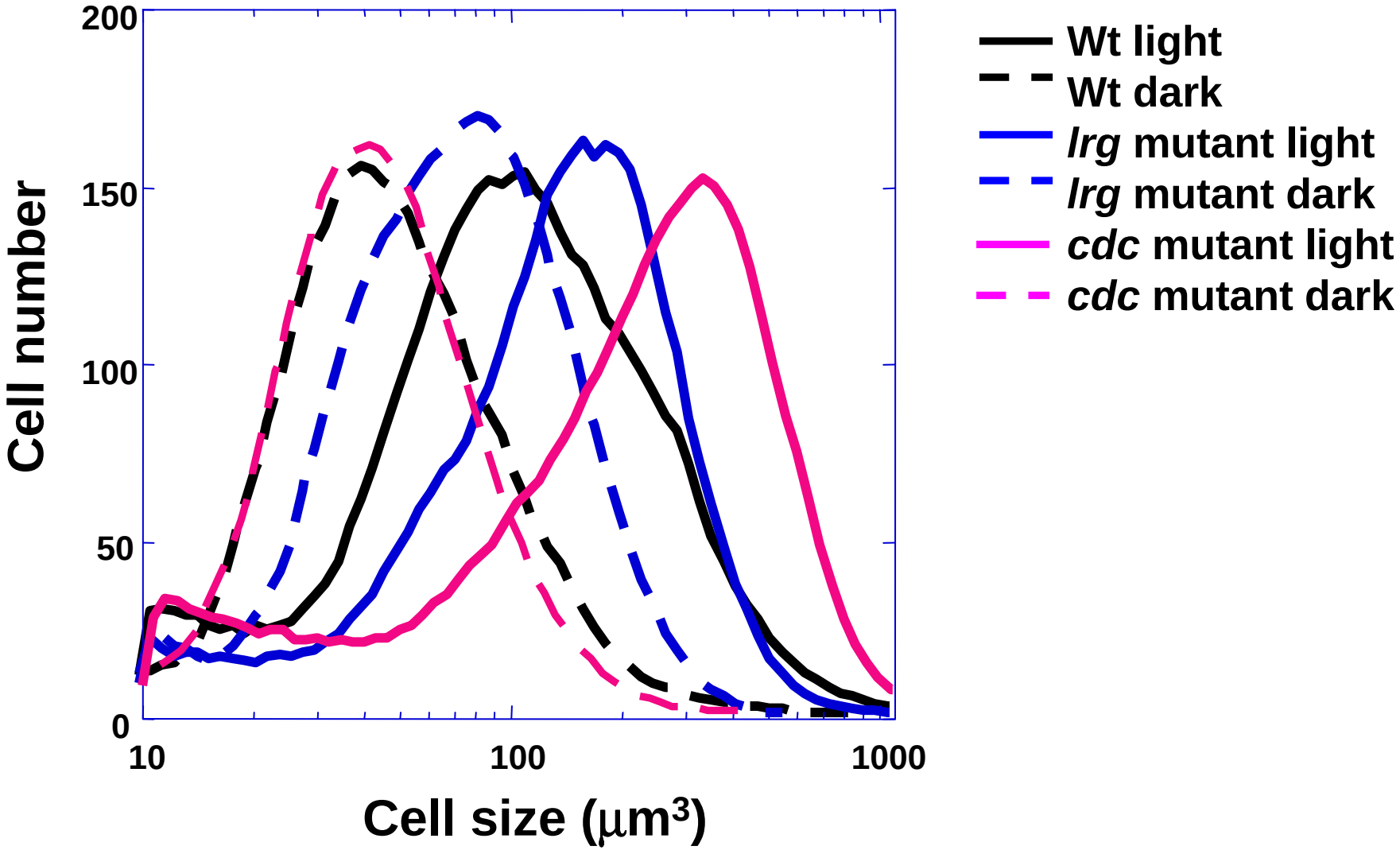
How does *dp1-1* and *e2f1-1* suppress *mat3*?

Two potential mechanisms:

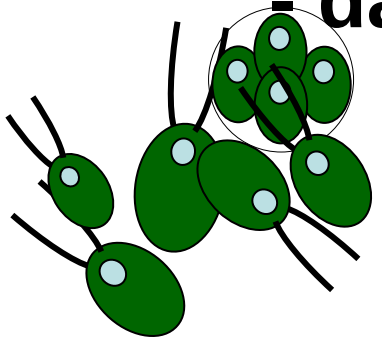
Slow down the cell cycle

Alter the cell size checkpoint function

Discriminating sizer mutants from cell division cycle (cdc) mutants

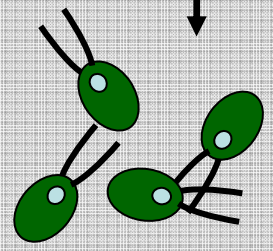


Cell size checkpoint functional assay - dark shift experiment



Continuous light:
Random distribution

Dark (16-18 hrs)

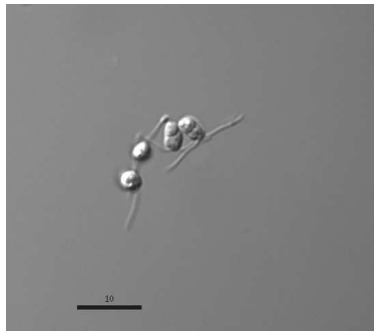


After dark shift:
Daughters/early G1

Coulter Counter

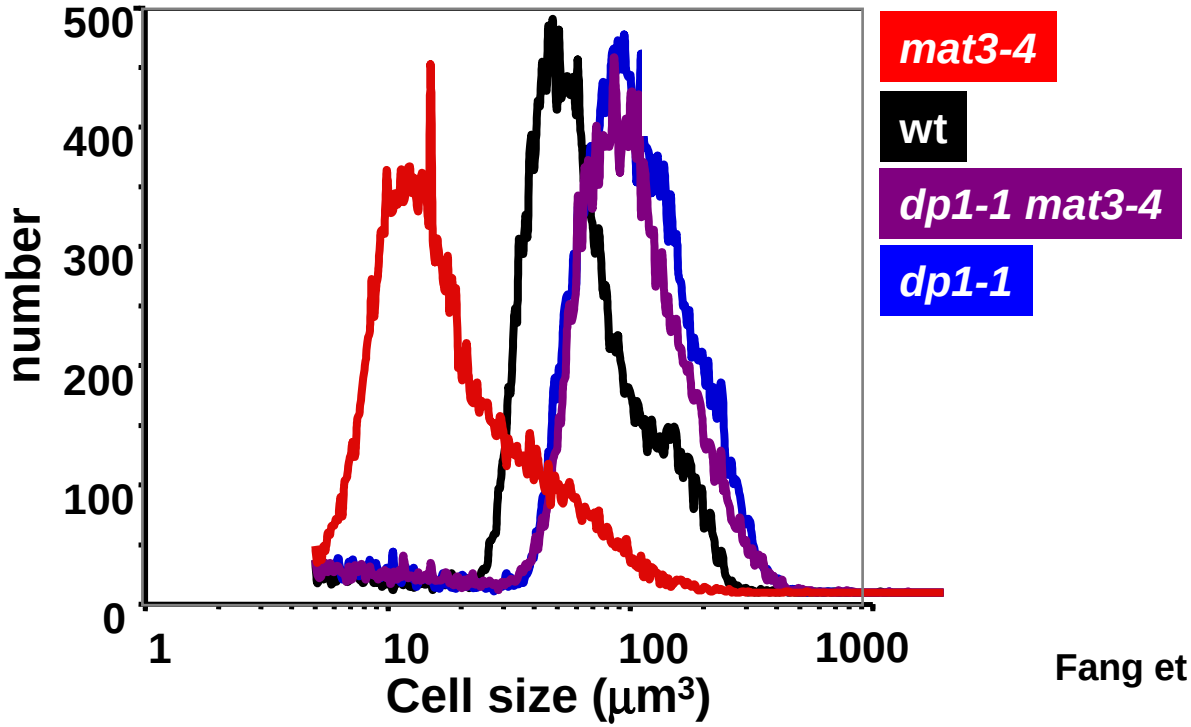
dp1-1 suppresses *mat3-4* by altering the size checkpoint function

wt



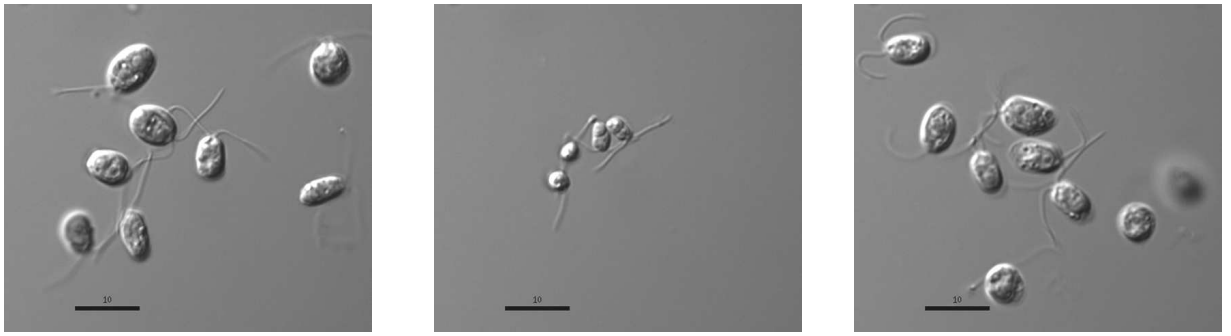
mat3-4

dp1-1mat3-4

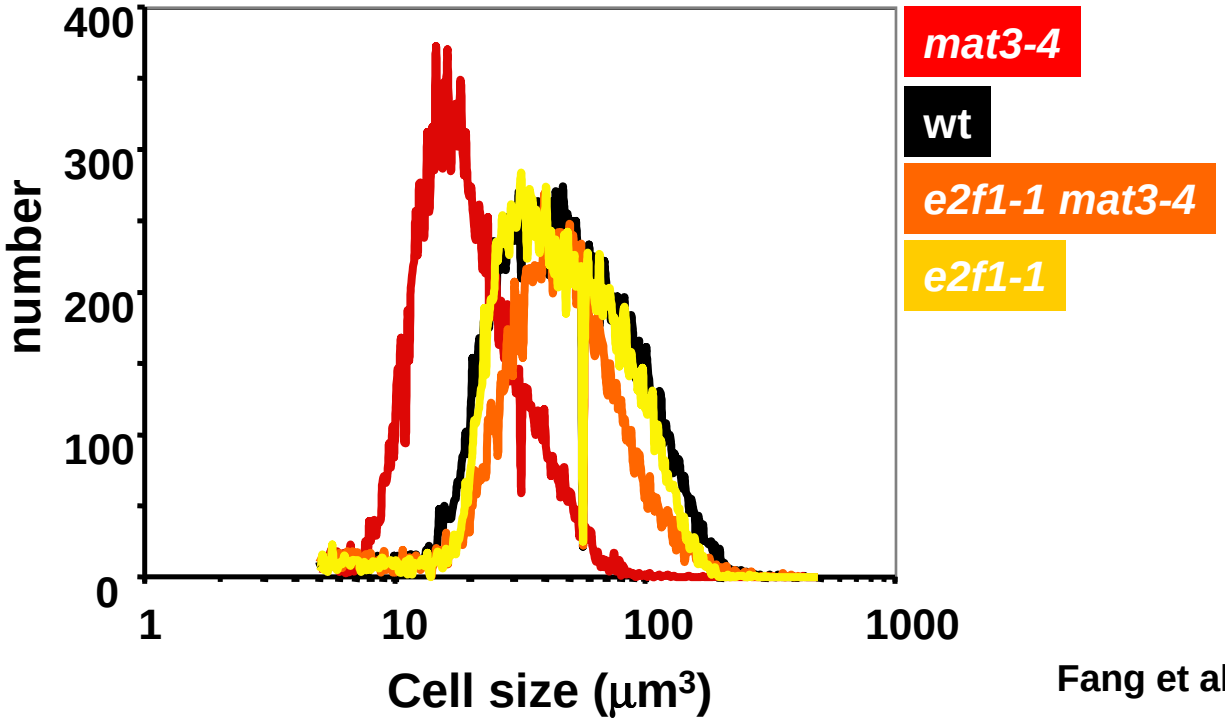


Fang et al., 2006

e2f1-1 suppresses *mat3-4* by altering the size checkpoint function



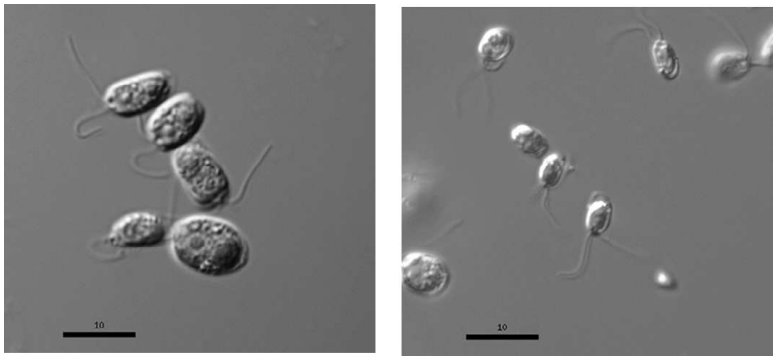
wt *mat3-4* *e2f1-1mat3-4*



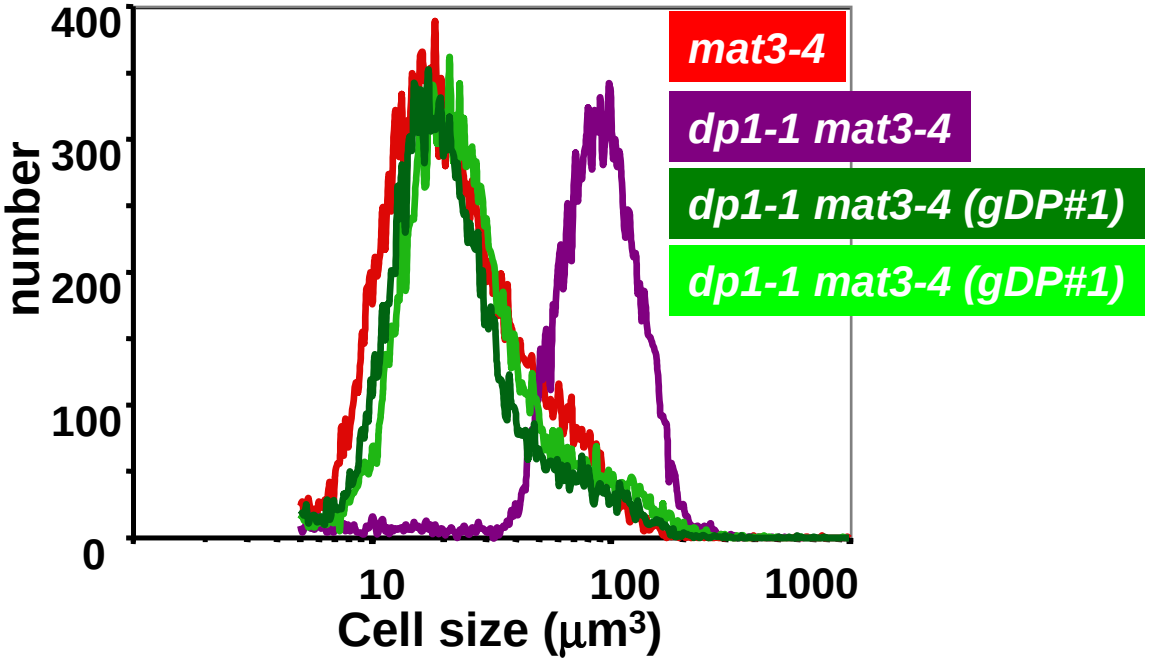
Fang et al., 2006

Genomic DP1 complementation

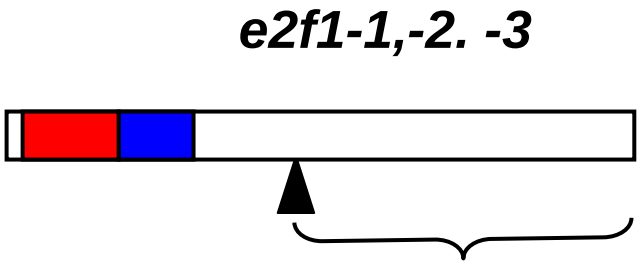
(*dp1-1 mat3-4 + DP1 = mat3-4*)



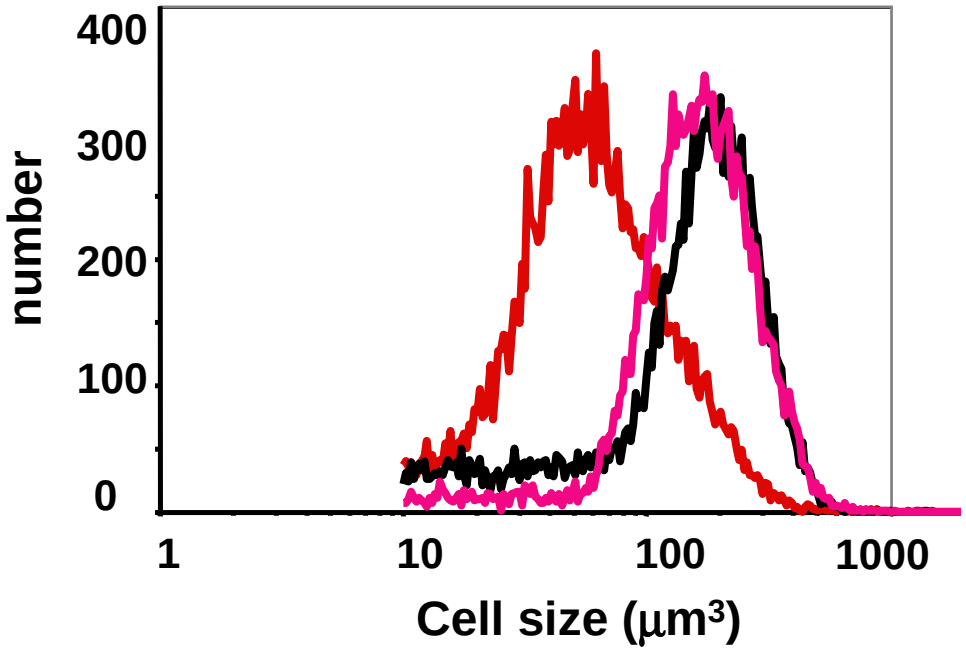
dp1-1mat3-4 *dp1-1mat3-4 (gDP1#1)*



e2f1-1 mutant is dominant



transactivation domain ?



mat3-4 mat3-5/E2F1 E2F1

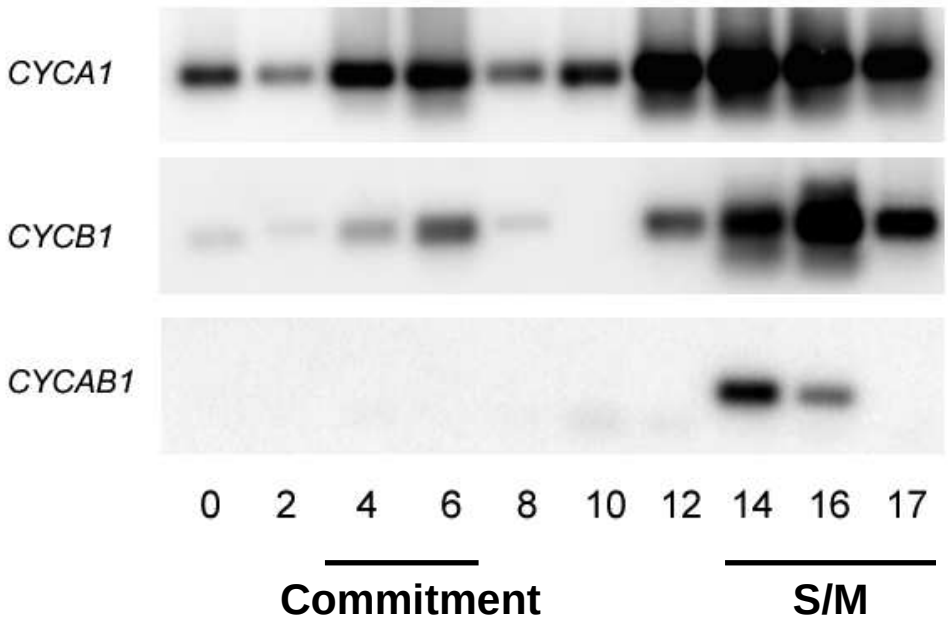
mat3-4 mat3-5/e2f1-1 E2F1

MAT3 MAT3 /E2F1 E2F1

Summary:

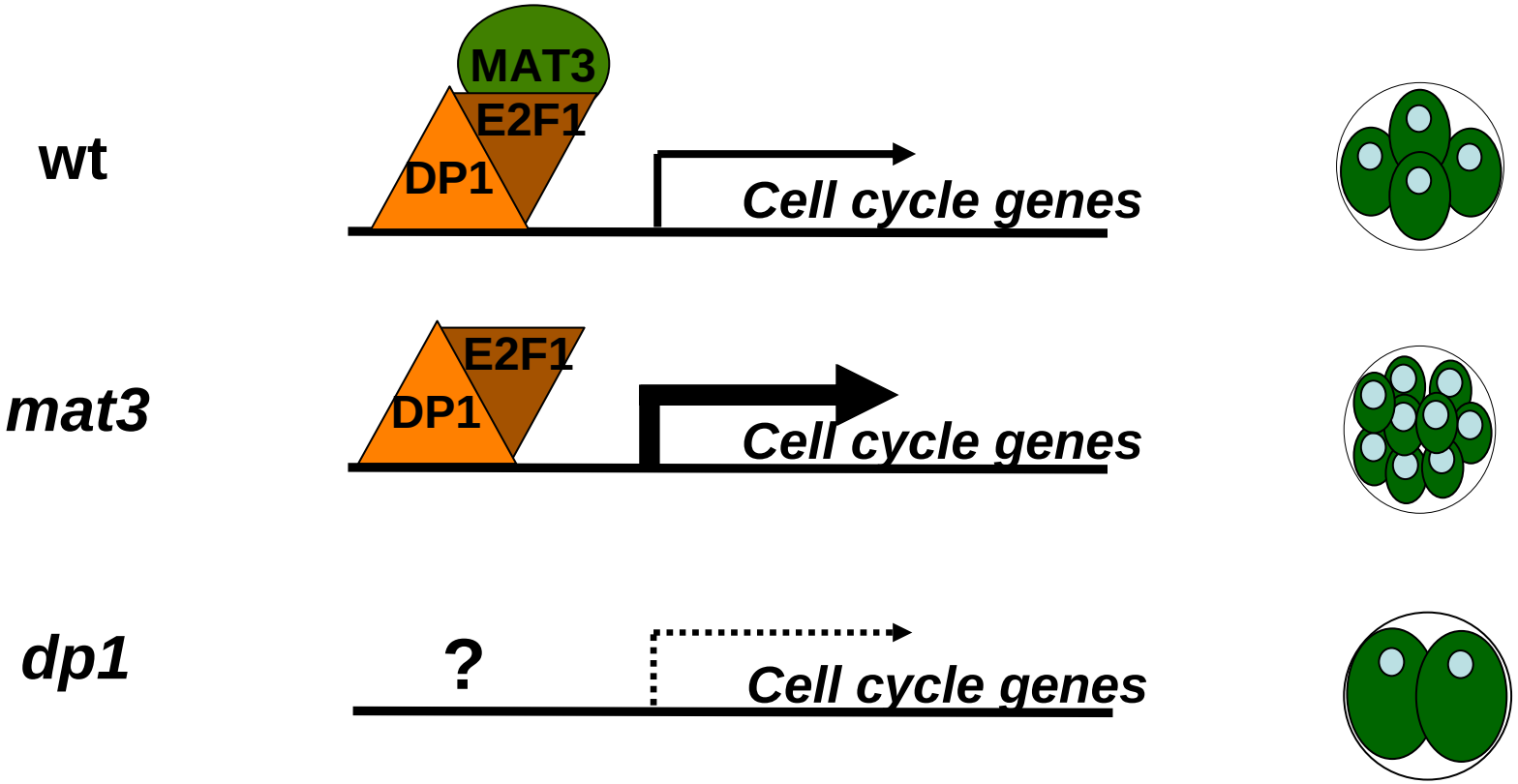
Mutations in *E2F1* or *DP1* suppress *mat3-4* by affecting size checkpoint function

Periodic transcription of the cell cycle genes

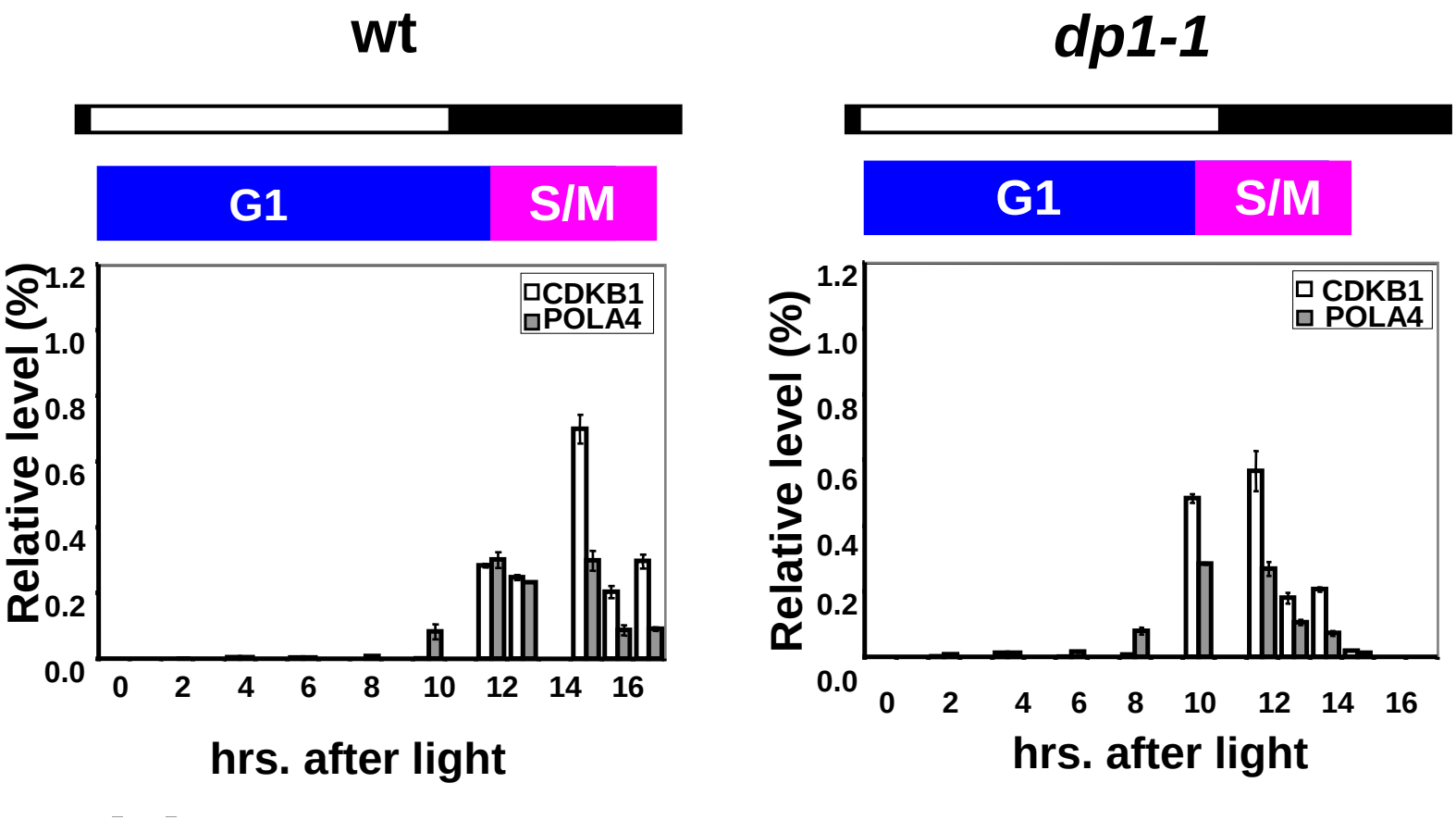


Bisova et al., 2005

Transcriptional Model for MAT3 and DP1 and in Cell Cycle Control



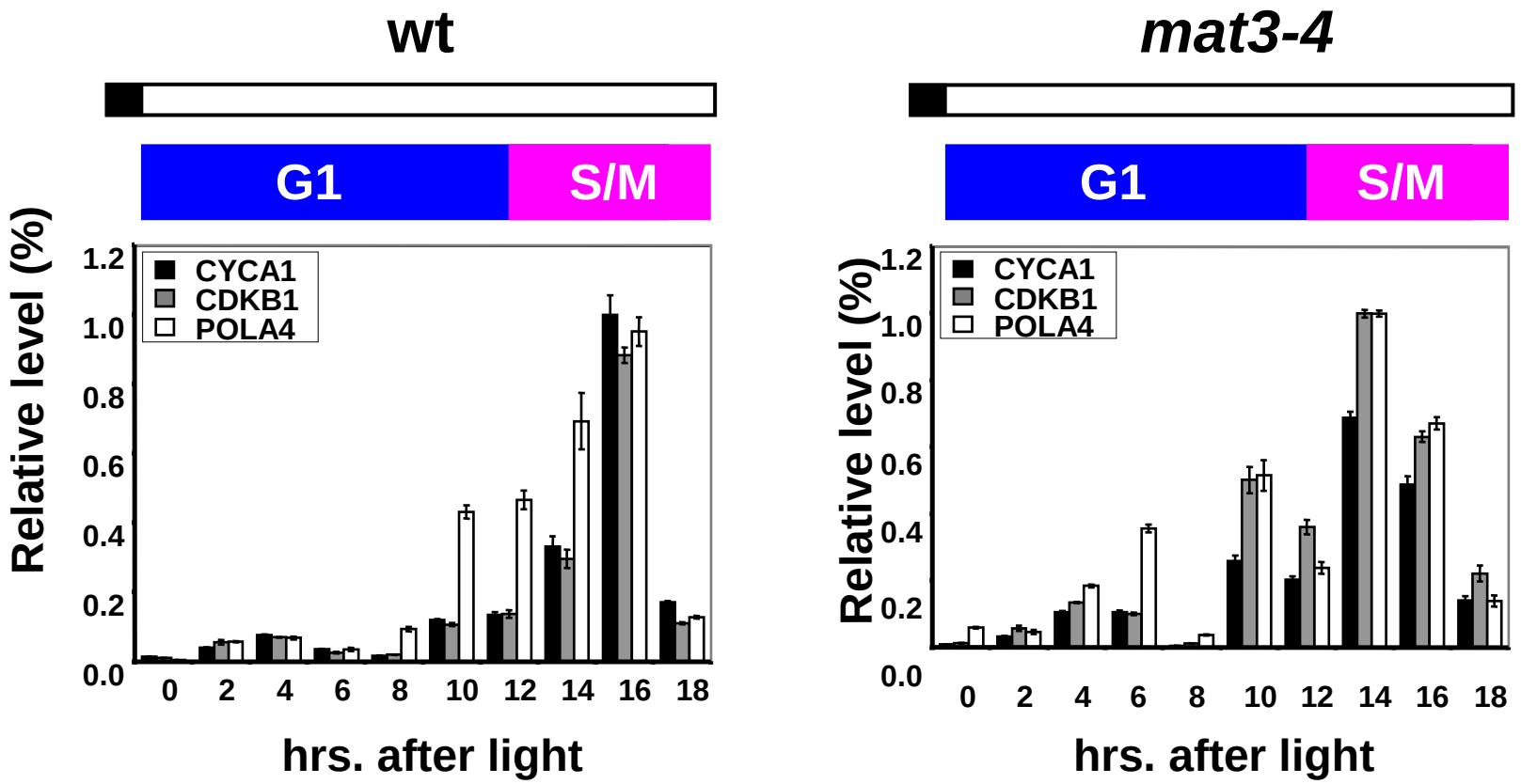
Normal induction of cell cycle genes in *dp1-1*



qRT-PCR analyses

Fang et al., 2006

Normal induction of cell cycle genes in *mat3-4*

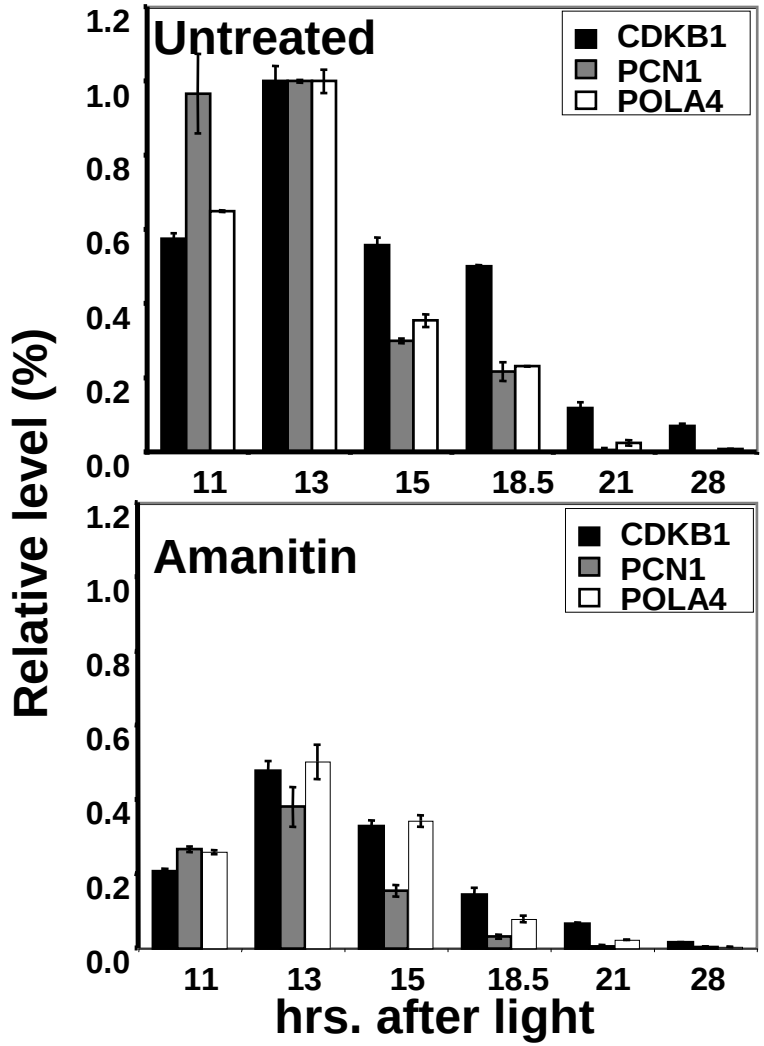


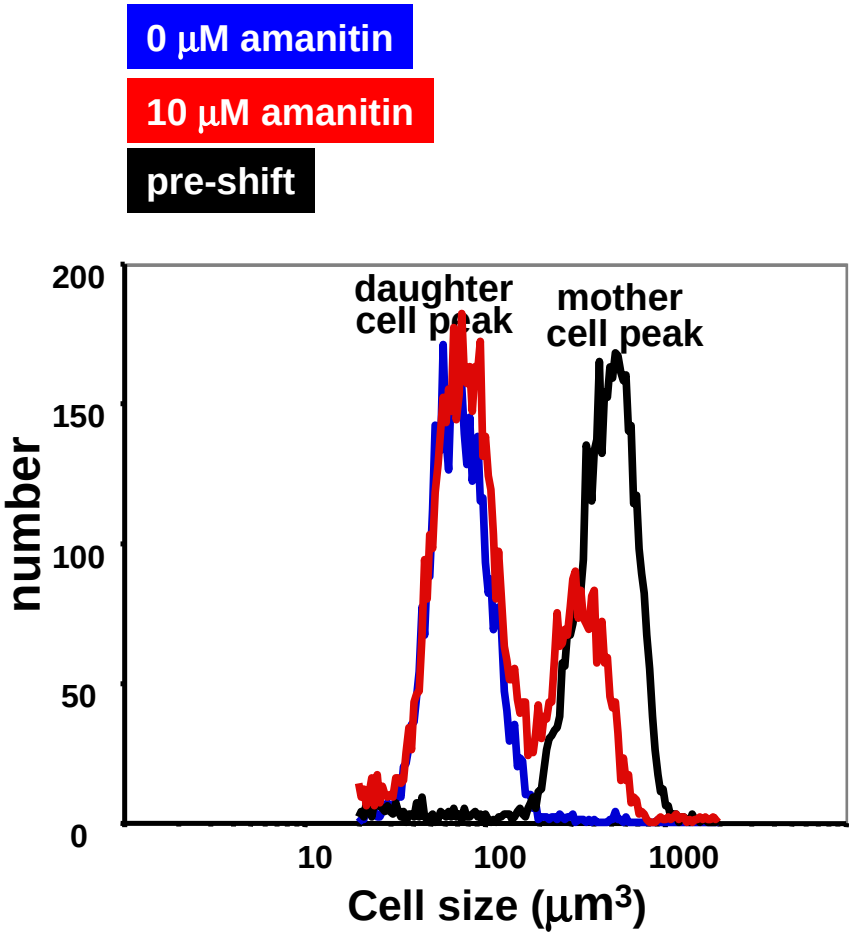
qRT-PCR analyses

Fang et al., 2006

Is periodic transcription of the cell cycle genes important for size checkpoint control?

Blocking cell cycle transcription does not alter daughter cell size





Fang et al., 2006

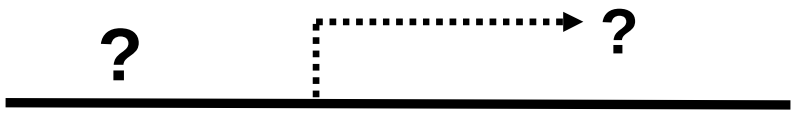
Summary:

- **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**
- **No periodic transcriptional defect**
- ***SMT7*, *SMT14*, *SMT15*, and *SMT16* are novel targets of the RB/MAT3**
- ***Chlamydomonas* is an excellent model to dissect the RB pathway**

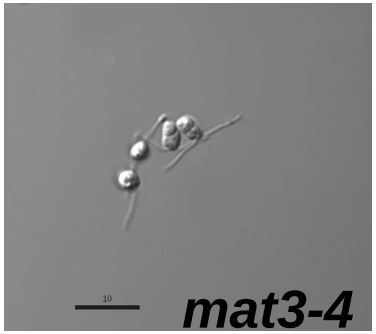
- **Are there transcriptional targets?**
- **What are the downstream effectors?**

mat3

dp1



19 suppressors of *mat3-4*



Class I: larger than wt

DP1 (12)

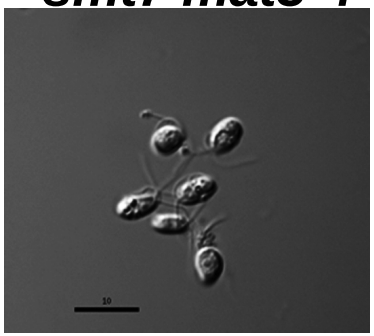
Class II: close to wt

E2F1 (3)

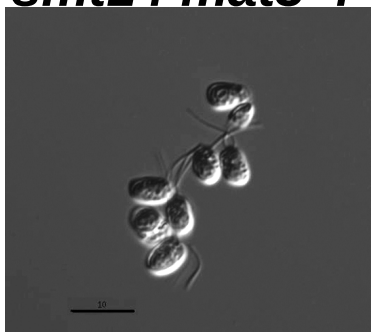
Class III: larger than *mat3-4*, smaller than wt

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

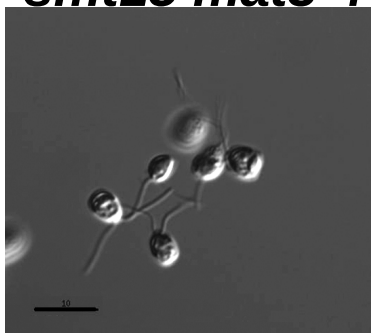
smt7 mat3-4



smt14 mat3-4



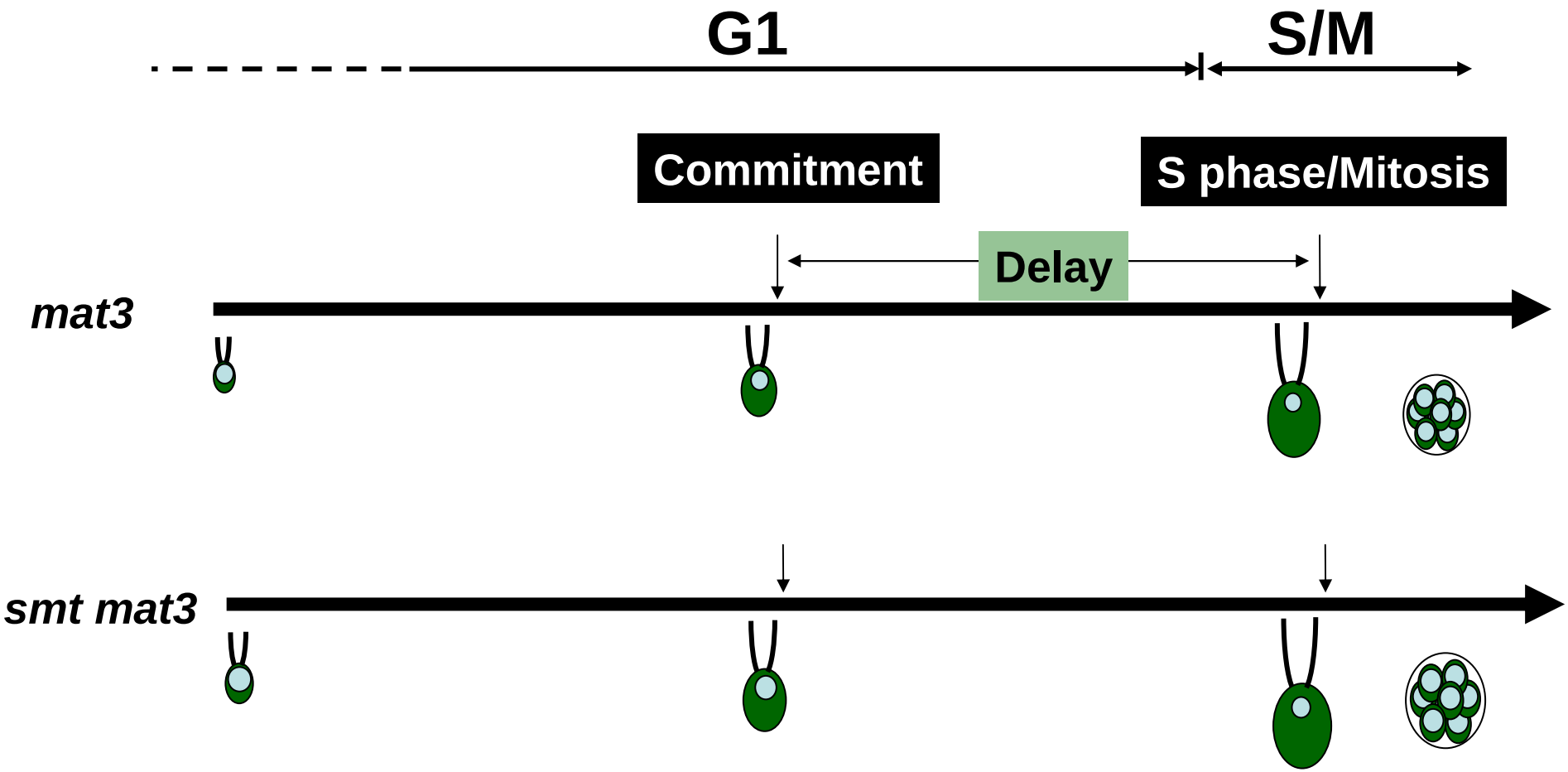
smt15 mat3-4



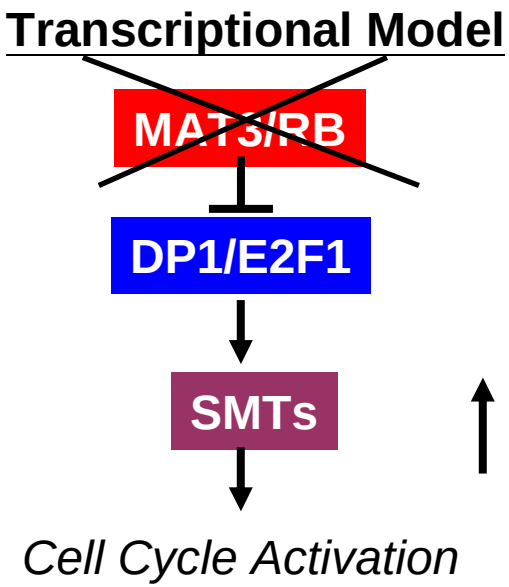
smt16 mat3-4

Fang and Umen, 2008

Class III *smt* mutants suppress *mat3-4* by affecting both cell-size checkpoints



	Candidate gene model	Mutation	Induction in *mat3* (qRT-PCR)
SMT7	SUMO peptidase, novel protein, thioredoxin-related protein	~ 19 Kb deletion	no
SMT15	Putative sulfate transporter	Insertion	no
SMT16	Putative eukaryotic initiation factor 4B	Insertion	NT
SMT14	No known gene model	Insertion	no

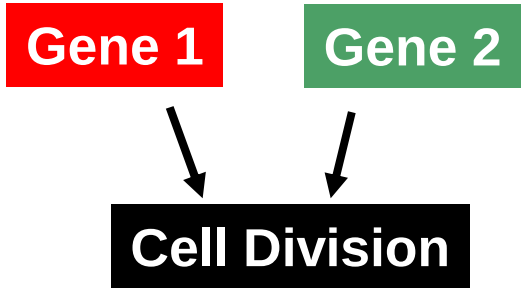


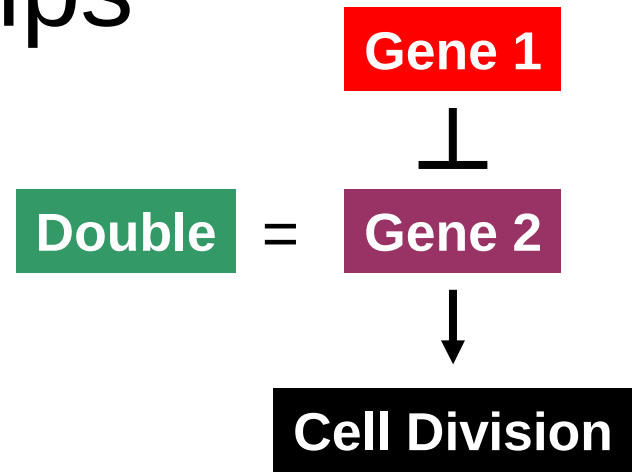
Class III SMTs are not transcriptional targets of the RB pathway

Do *smt* mutants interact to each other?

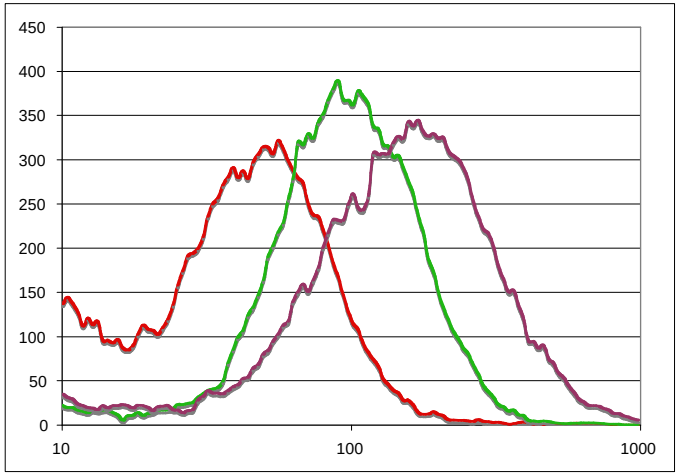
- Do class III smts interact with *dp1*?
- Do class III smts interact with each other?

Possible relationships

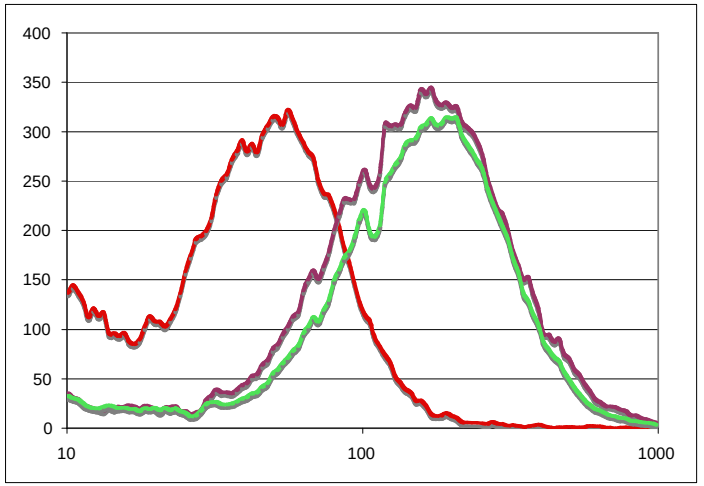




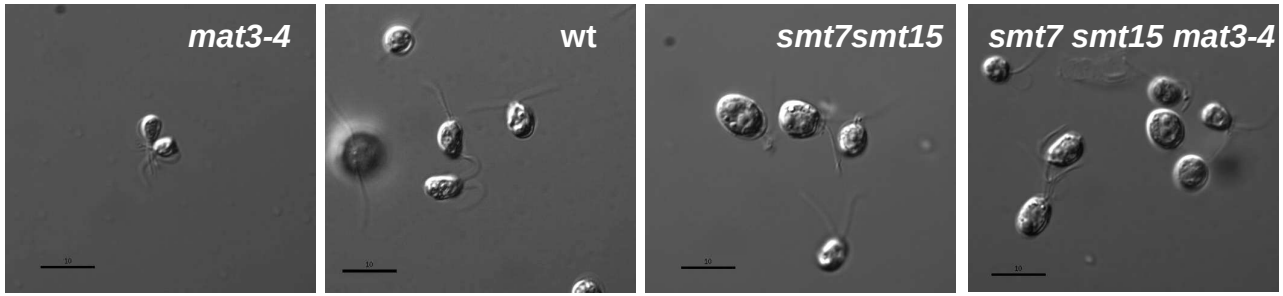
Additive

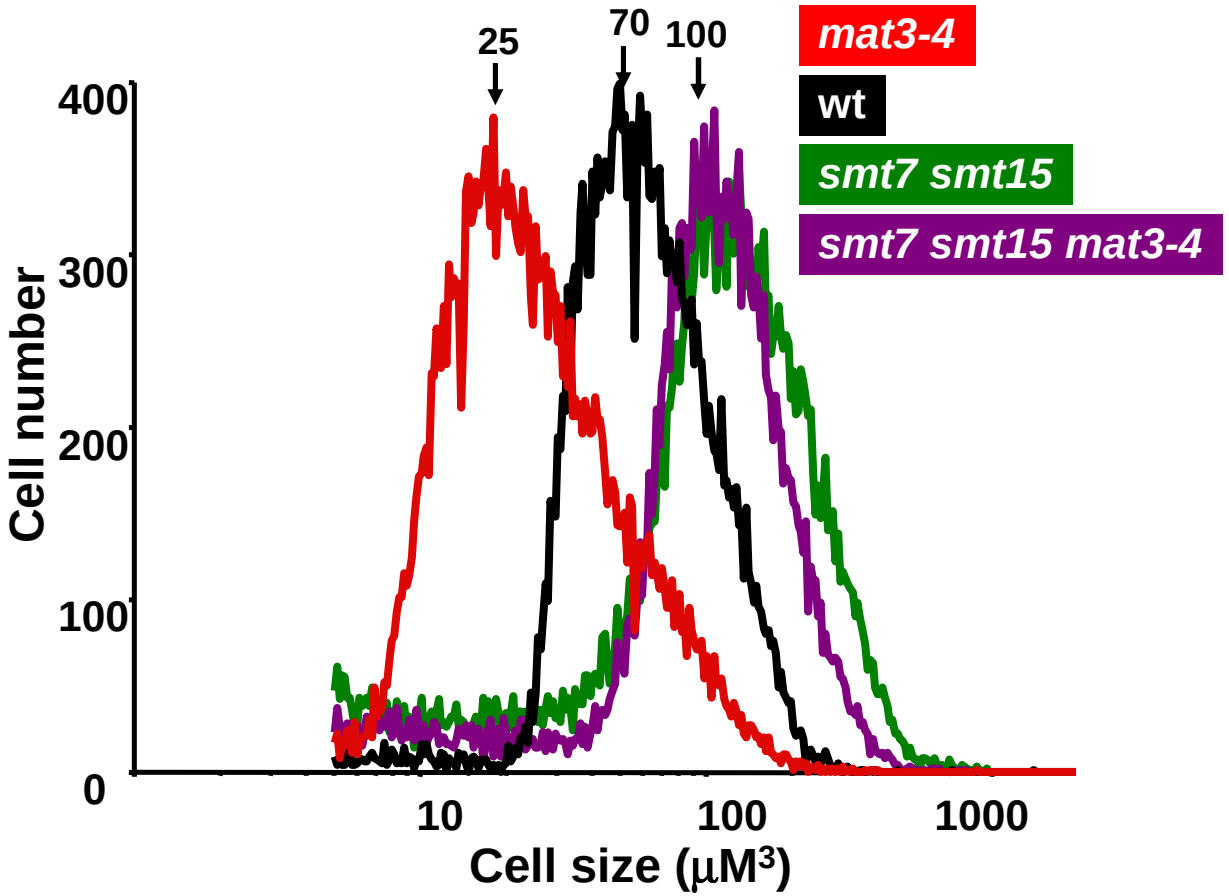


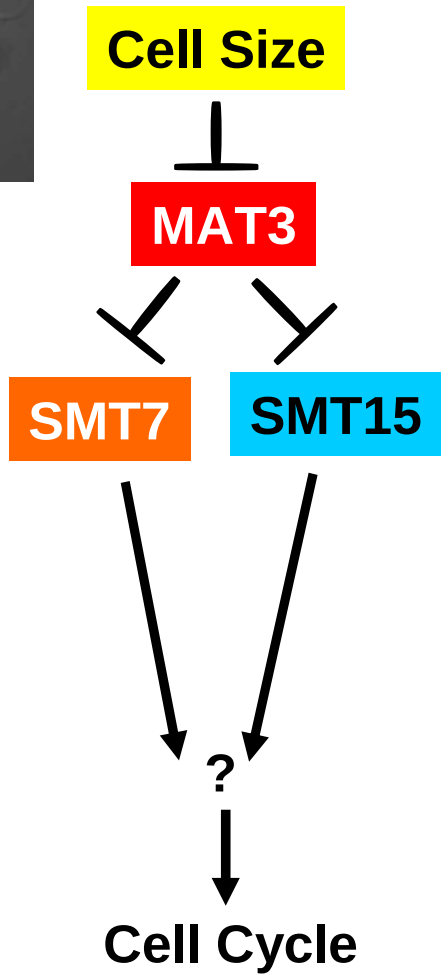
Epistatic



smt7-1 smt15-1 makes cells bigger







Fang and Umen, 2008

Fact sheet of smt7-1:

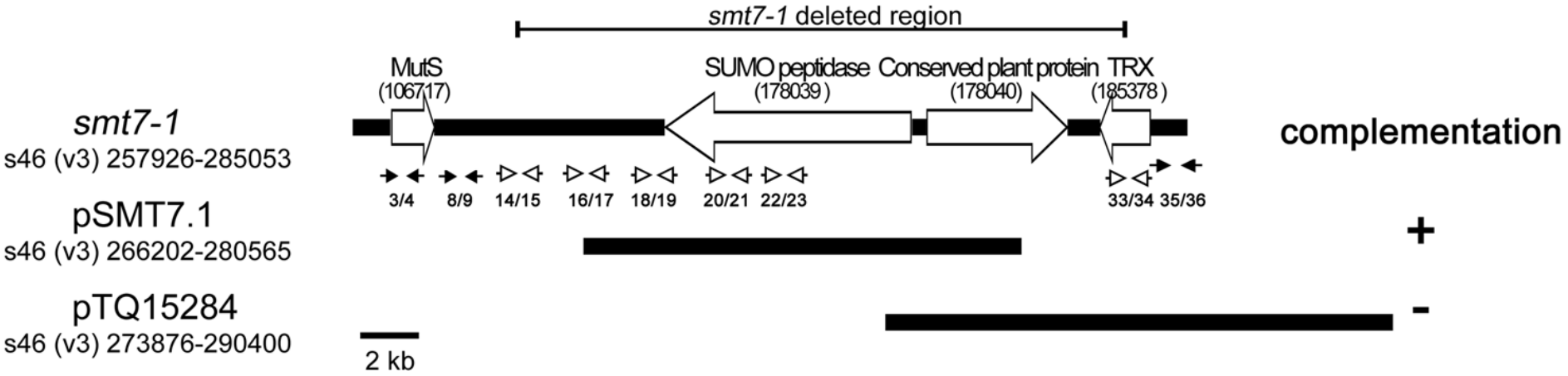
- ***smt7-1*** **single mutant does not have obvious size phenotype**

- ***smt7-1*** **single mutant has defective Commitment size threshold and can not be synchronized by alternating L/D cycle**

- ***smt7-1*** **suppress** ***mat3-4*** **by affecting both size control checkpoints**

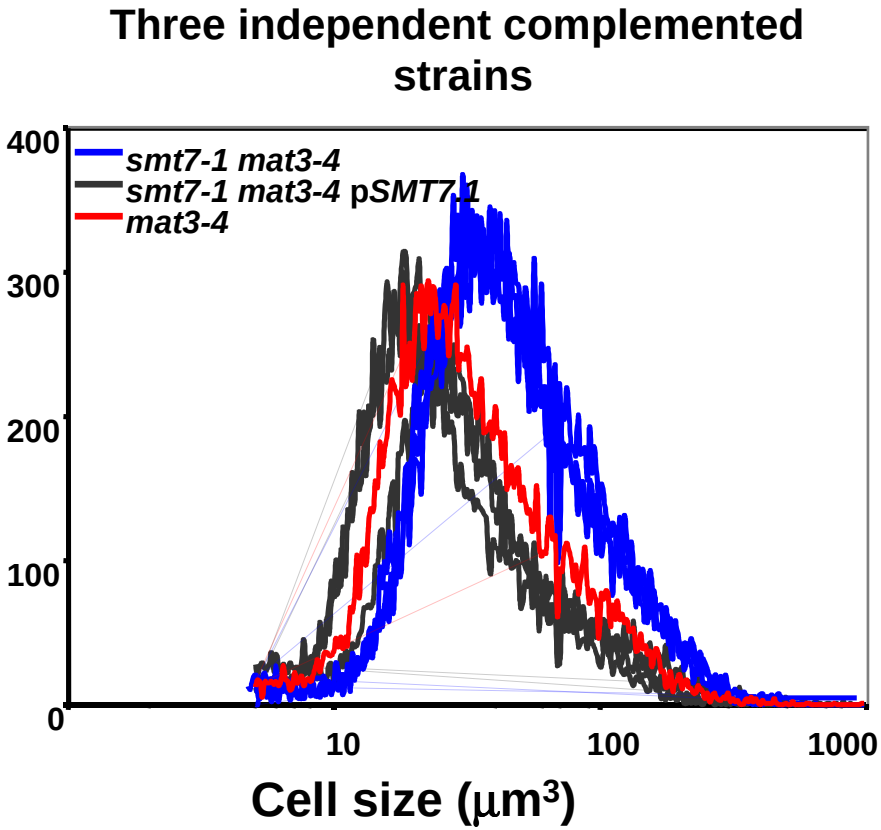
- ***smt7-1*** **has ~ 19 kb deletion that encompasses three gene models: a SUMO-specific peptidase, a conserved plant protein with unknown function, and a thioredoxin-related protein**

Schematic of *smt7-1* deletion



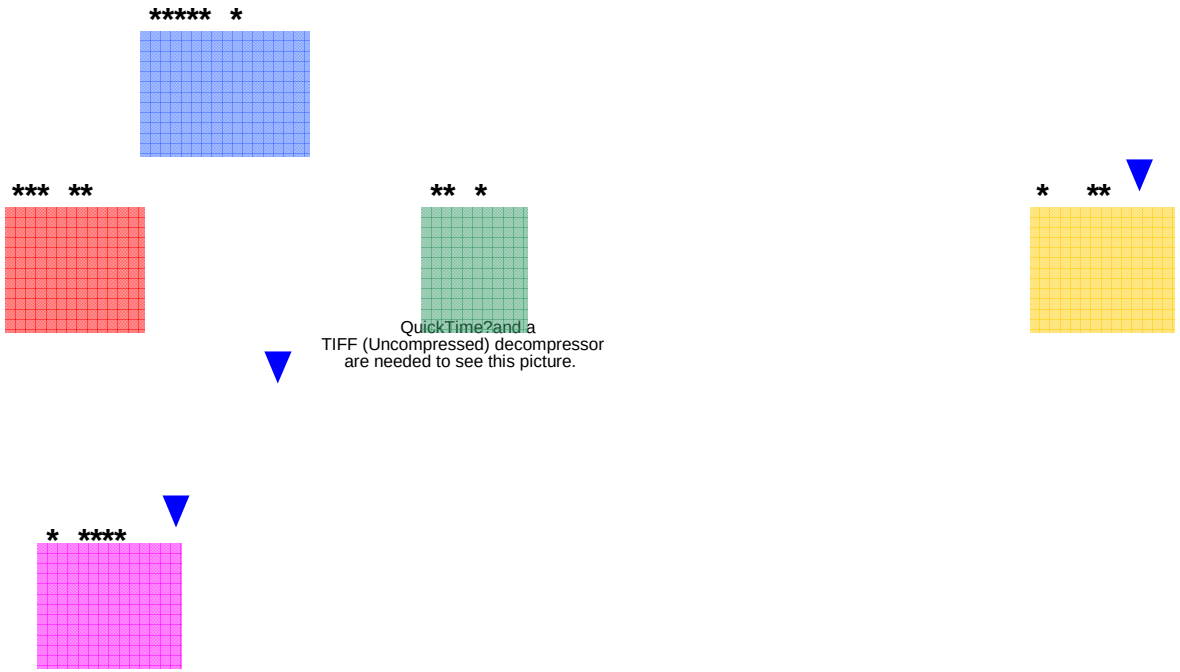
Fang and Umen, 2008

SMT7 is a small ubiquitin-like modifier (SUMO)-specific peptidase

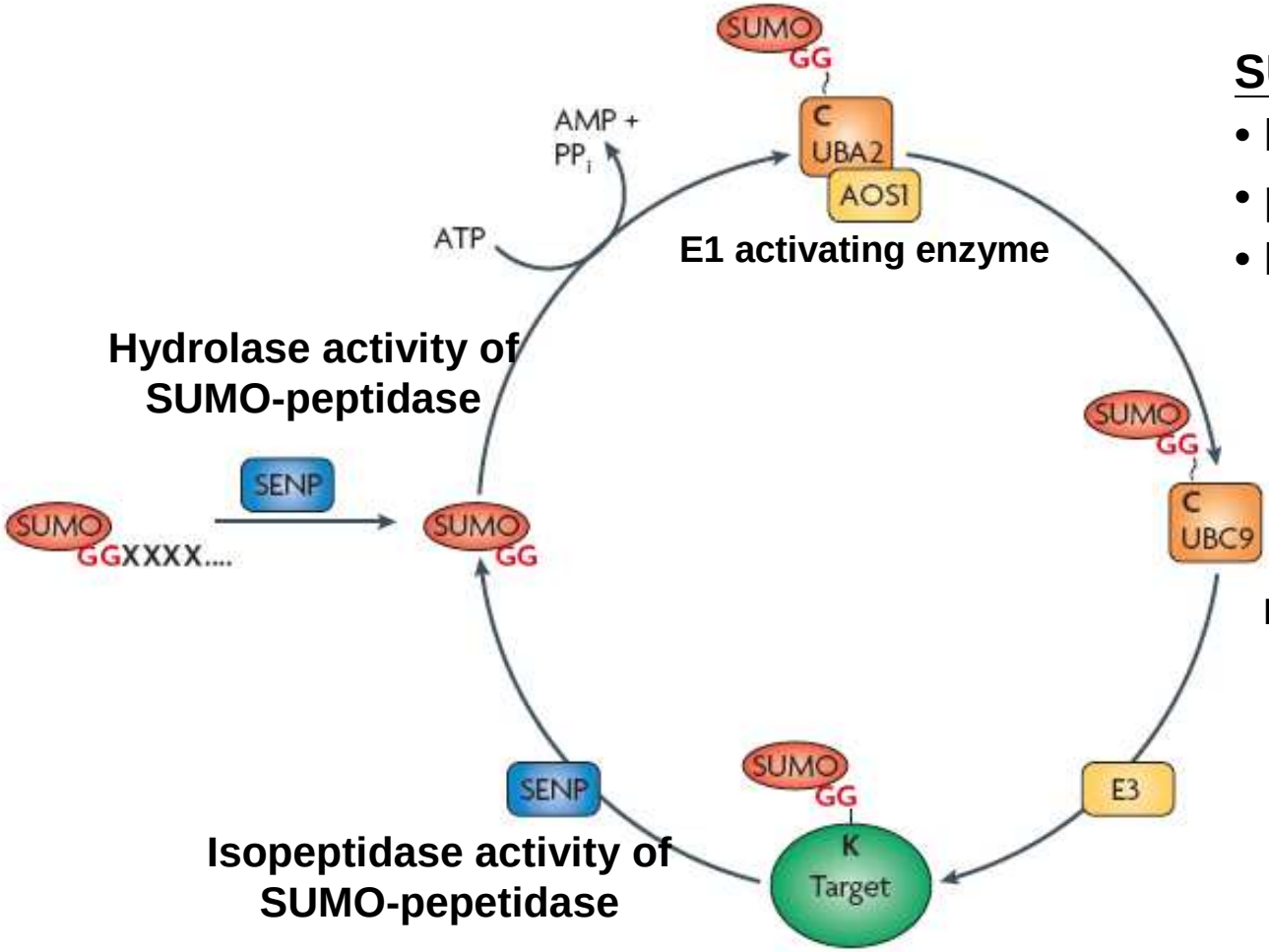


Fang and Umen, 2008

ClustalW alignment of SUMO peptidases from various organisms



The mechanism of reversible sumoylation

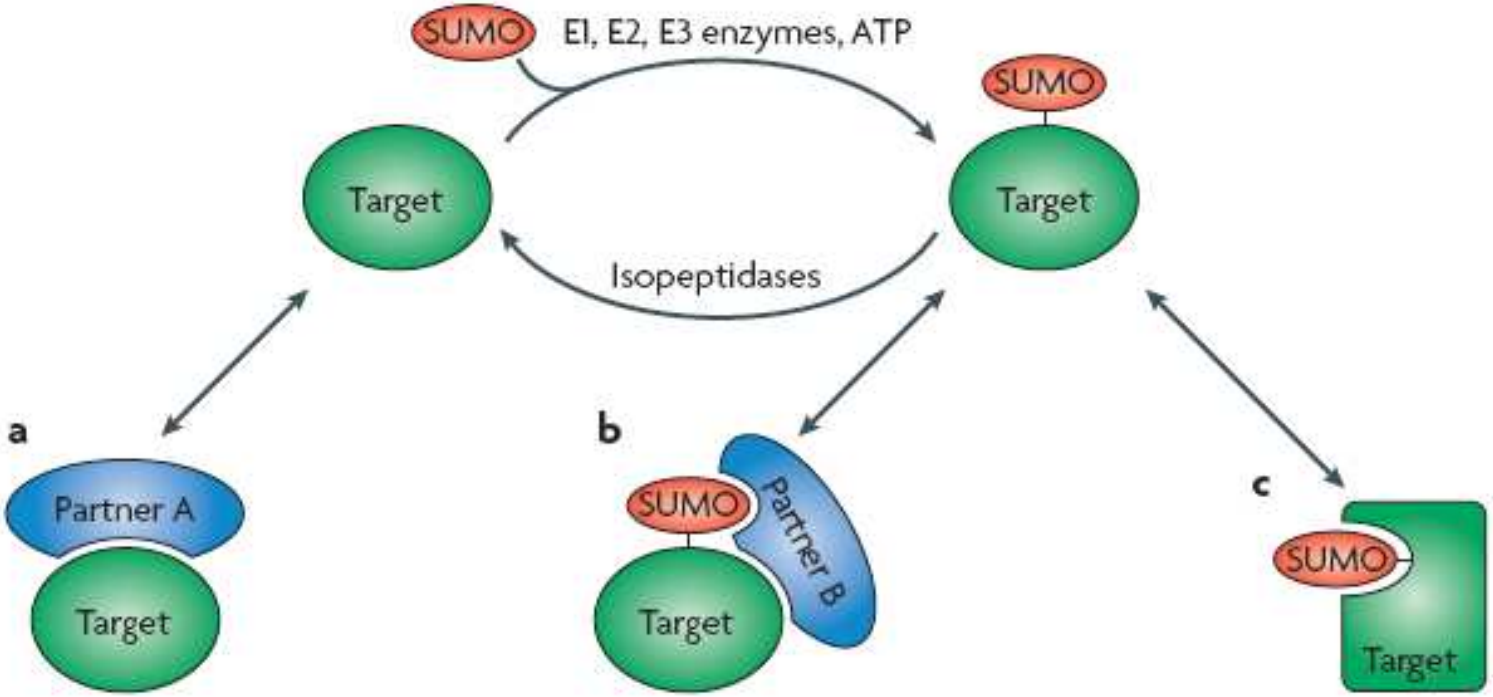


SUMOylation

- **Protein localization**
- **protein stability**
- **Protein activity**

Geiss-Friedlander and Melchior, 2007

Molecular consequences of sumoylation



- **ZNF76/TATA binding protein**
- **E2-25K/ubiquitin E1 enzyme**

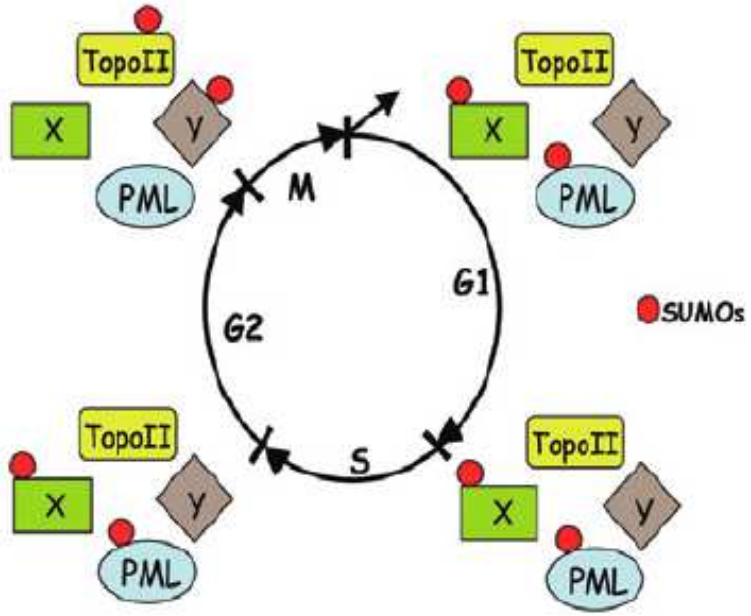
- **RanGAP1/RanBP2**
- **P300/HDAC6**
- **PCNA/DNA helicase Srs2**

- **Thymine DNA glycosylase**

Geiss-Friedlander and Melchior, 2007

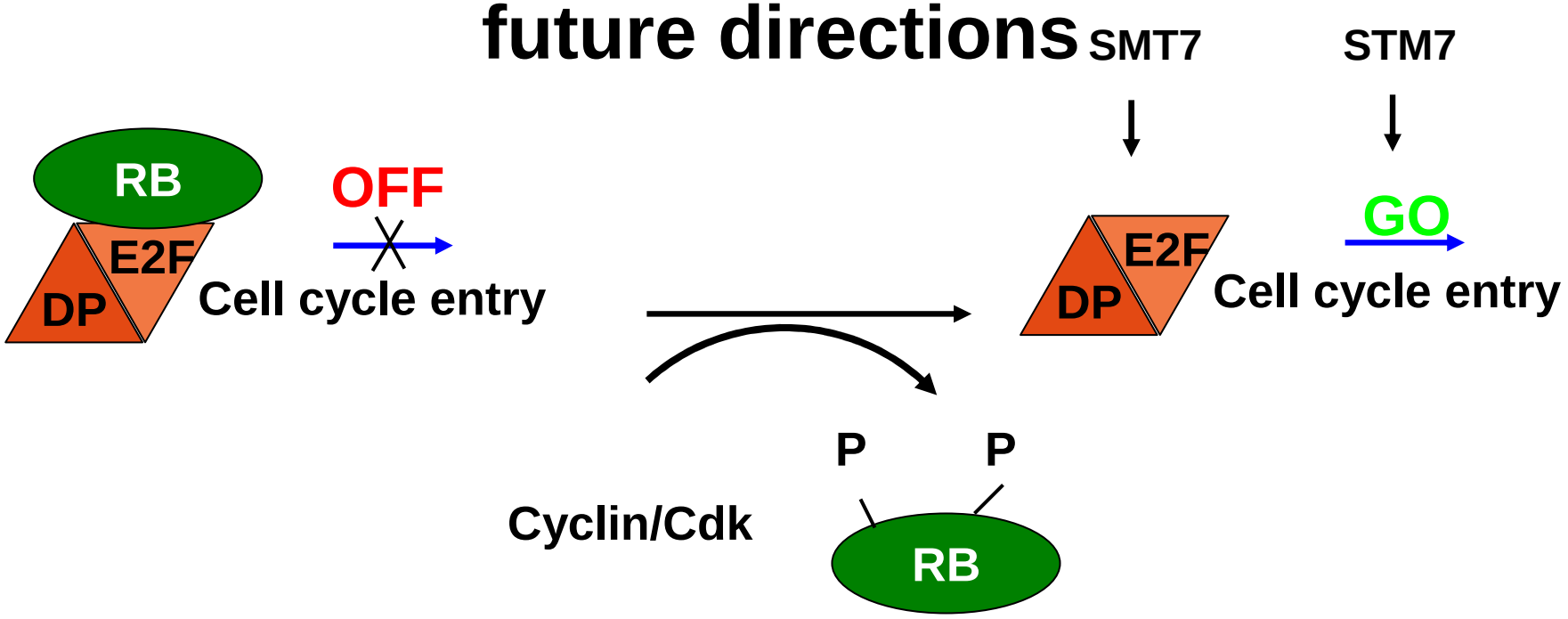
SUMO-specific peptidases and cell cycle:

- **Yeast Ulp1 is required for cell cycle progression**
- **HeLa cells depleted of SENP5 by RNAi failed to proliferate**
- **Orderly cycles of SUMOylation and deSUMOylation regulate progression through the cell cycle.**



Di Bocco and Gill, 2006

SMT7 working model and future directions



- Determine the functional roles of SMT7 as a SUMO peptidase
- Isolate the targets of SMT7 and examine how sumoylation of these targets affects cell cycle progression

Fact sheet of smt15-1:

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

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