Does experimental evolution tell us anything useful?

KITP 2017

Ask questions!

http://catalog.fborfw.com/strips/98/FB112398.gif

And since time expired…

Yeast can evolutionarily learn to predict…

- High salt induces arrest
- Arrest prevents lethal DNA replication
- Two ways of letting more Na^+ in
  - Kill inducers of K^+ entry (K^+/Na^+ balance critical)
  - Alter sugar transporters to admit Na^+

Nichole Wespe

OUTLINE

Time travel (fossils and DNA sequence) extremely limited

Experimental evolution should be asking specific questions

Question 1: How does evolutionary novelty appear?

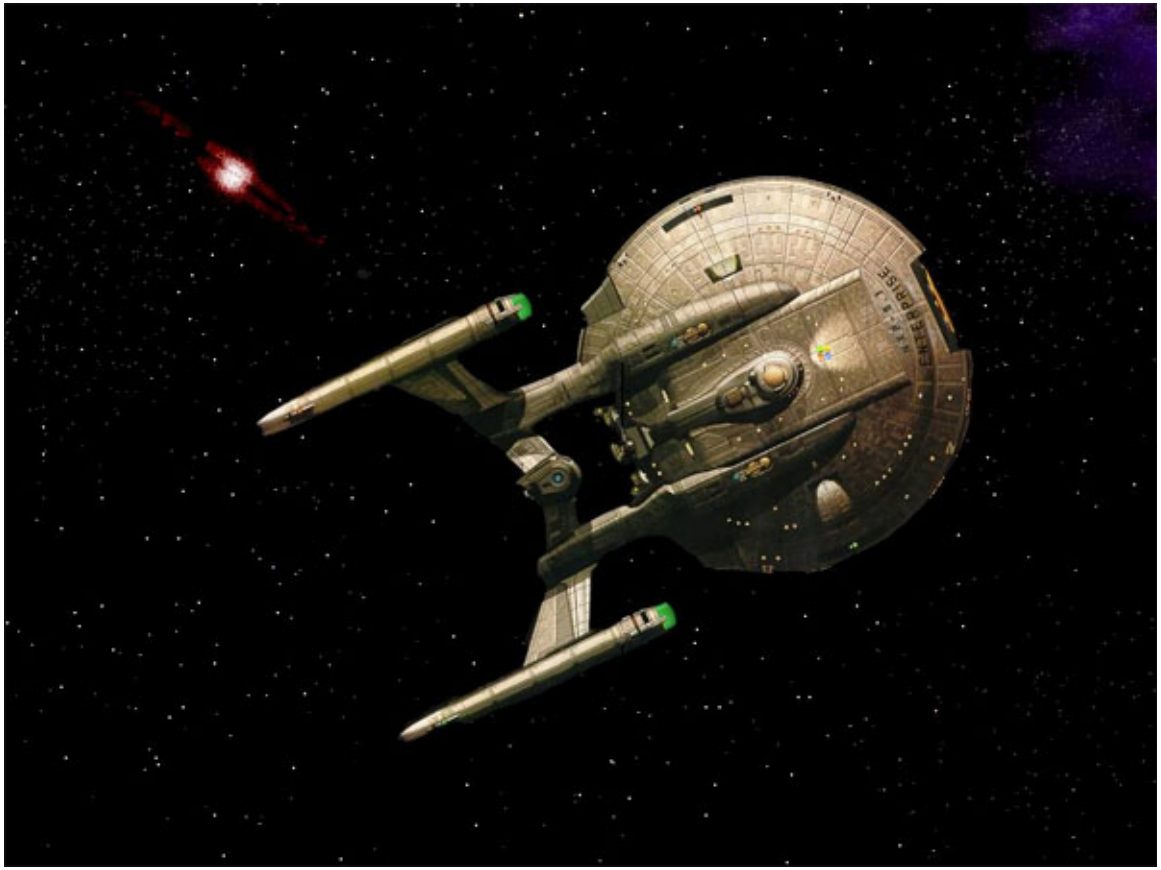
Question 2: How do cells adapt to the loss of beloved proteins?

Question 3: Can evolution mimic Pavlov?

No non-theological purpose

http://www.eso.org/public/images/eso1118a/

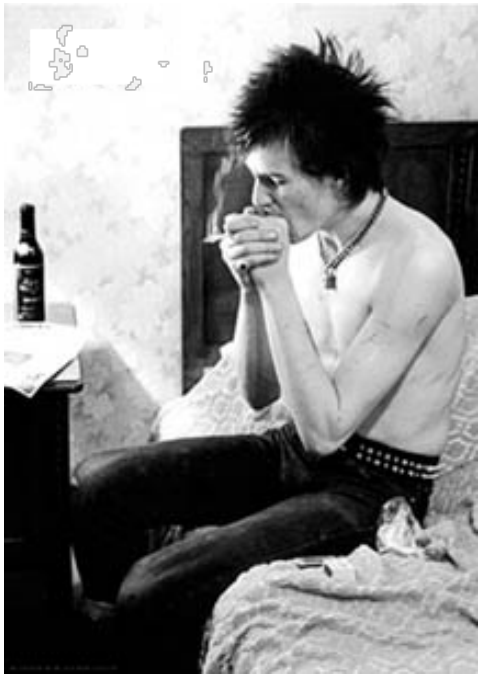
But organisms are...



…vehicles to disperse genes through time and space

Yes, evolution happens, Dorothy

At least 5 mutations



Normal Cell

Cancer Cell

Careful with chromosomes

Careless with chromosomes

Evolution

Depends on

- Inheritance: offspring like parents
- Mutation: genetic variation constantly generated
- Selection: some genotypes leave more progeny

We have trouble understanding it because

- Evolution is dominated by successions of *very rare* events
- Relative probability determines evolutionary path
- Historical inference from fossils & DNA imperfect

What we're missing

Experimental versus “real” evolution

Advantages

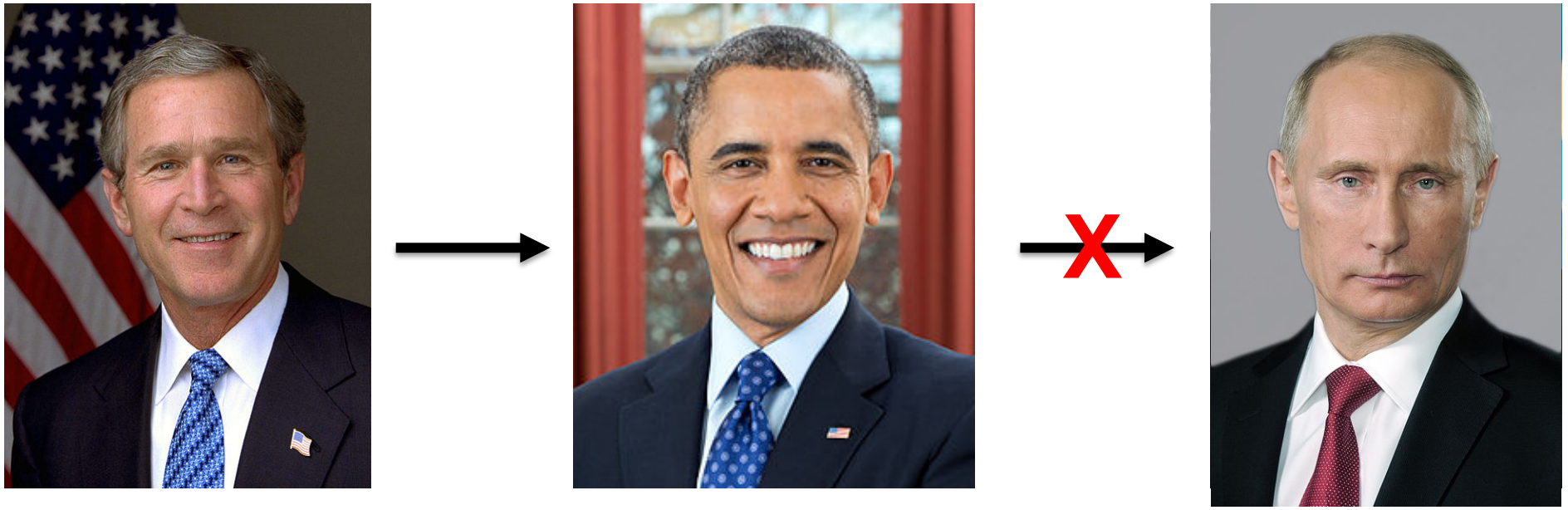
- Starting point known
- Selective pressure designed (not known!)
- Can keep “living fossil” record
- Ancestral and evolved can interbreed
- Multiple, parallel experiments possible

Disadvantages

- Time and population size limited
- Natural environments are much, much more complex
- Unknown relevance to long term, natural evolution

What is novelty and how do you get it?

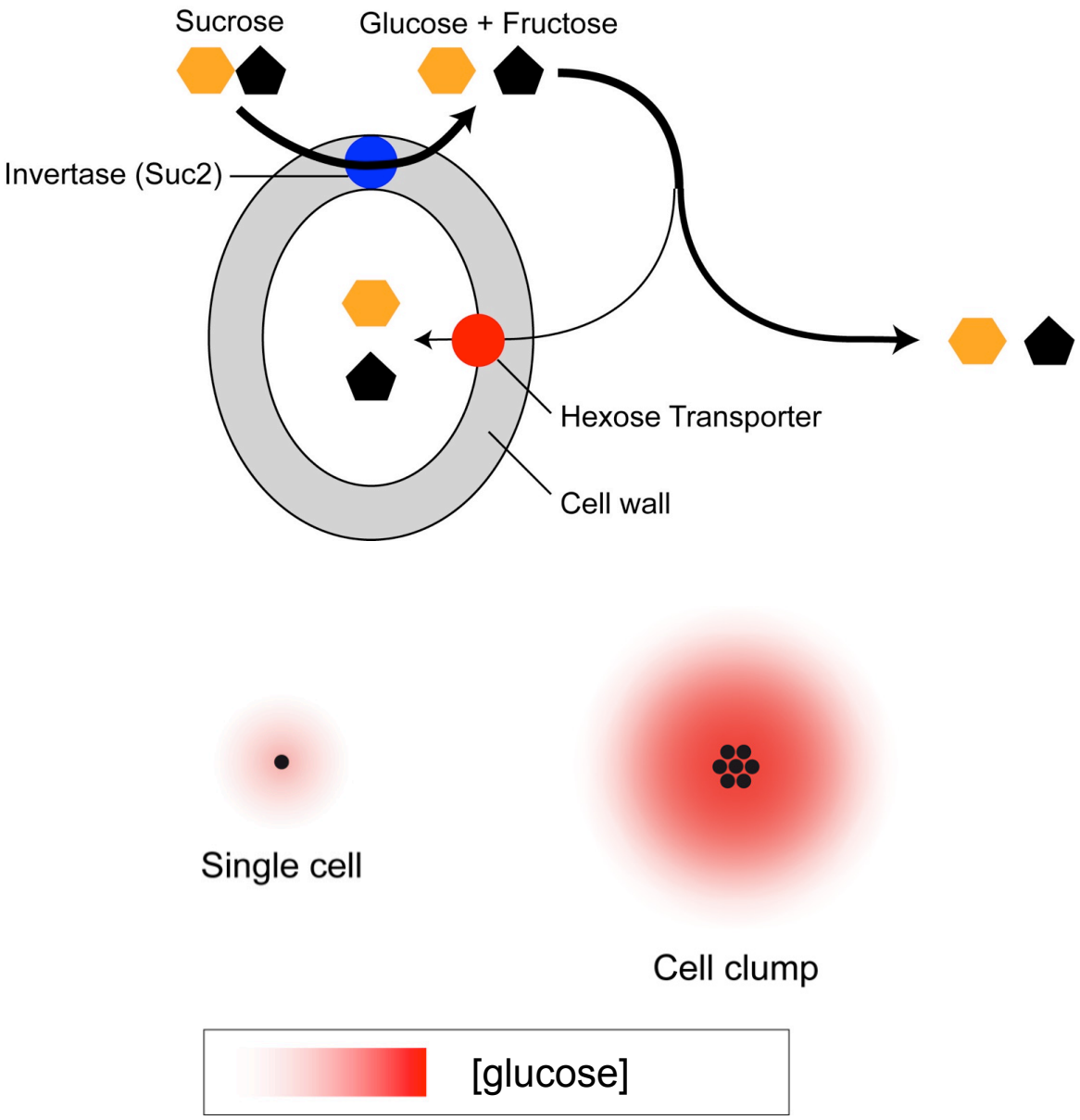
Acquiring a qualitatively new,fitness-increasing property



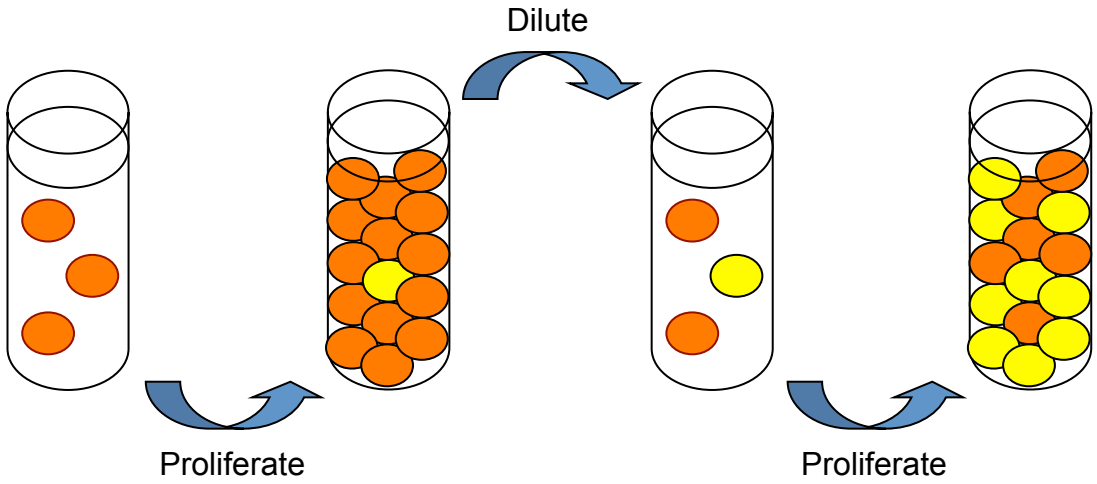
Does nutrient capture select for multicellularity?

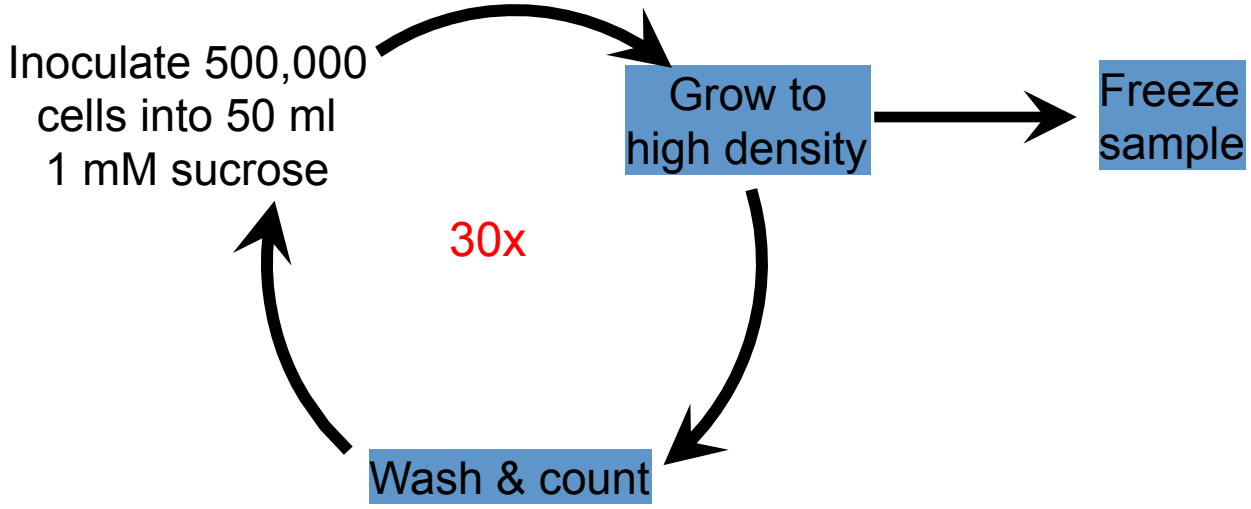
John Koschwanez

Why be multicellular? To utilize public goods?

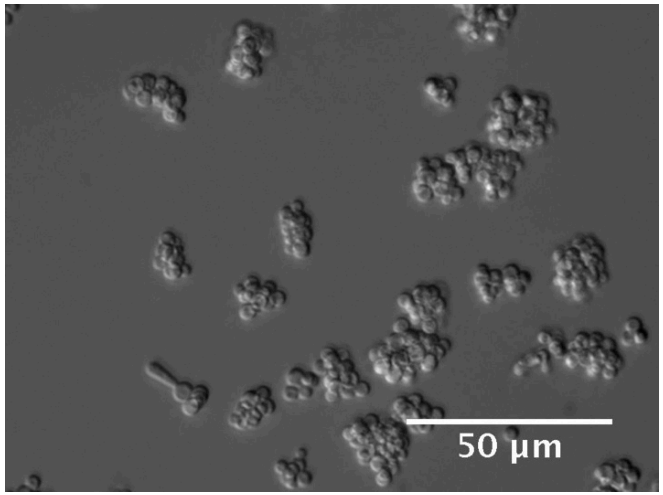


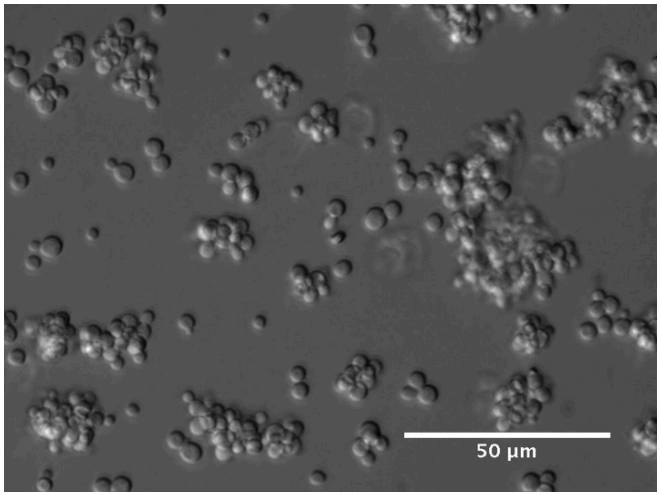
WWED? :Evolving multicellularity

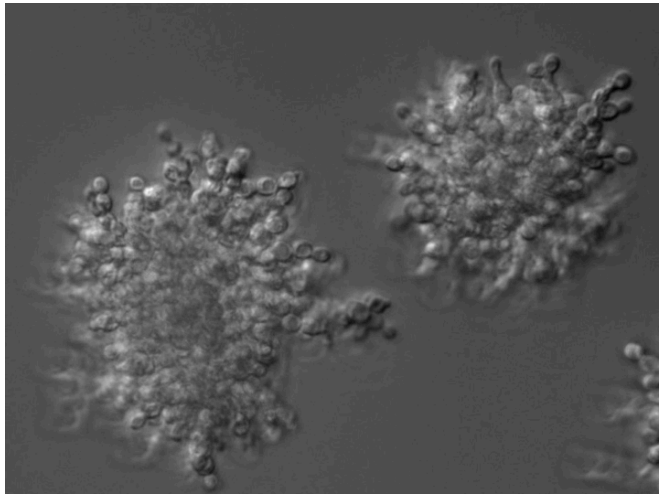


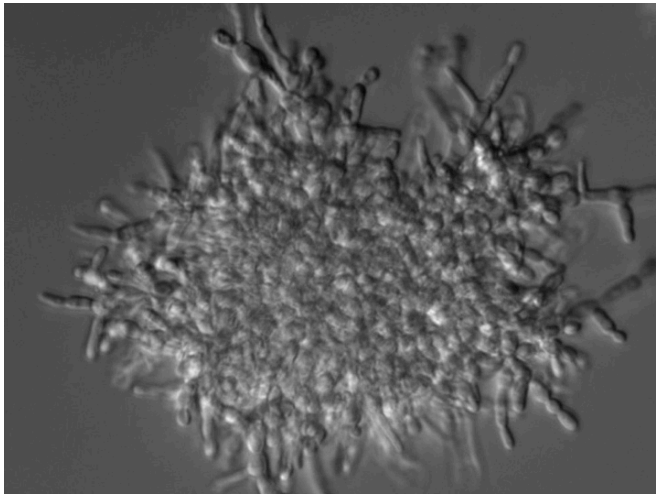


Experimentally evolved multicellularity









Koschwanez et al. *eLife.*, **2**, e00367 (2013)

Who's mutated?

Times mutated	# Genes	Names
7	1	*ACE2*
6	1	*UBR1*
3	2	*RGT1, SNF3,*
2	11	*IRA1,IRA2, etc*
1	39	*many genes!*

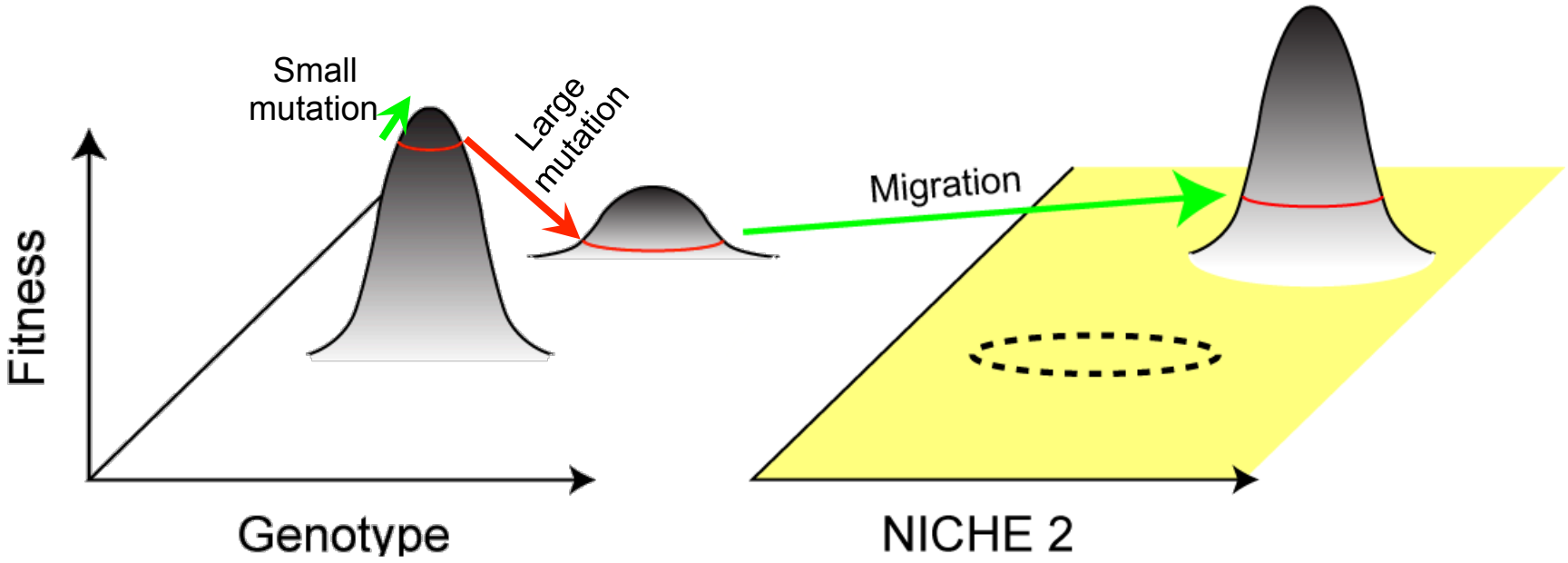
80 mutations: 7 promoter, 19 stop, 12 indel, 42 missense

Most (perhaps all) missense mutations are loss of function

3 frequently mutated pathways

- cAMP signaling (altered go/no go gambling setpoint)
- catabolite repression (reduced glucose addiction)
- mediator complex (complicated, confusing transcription control)

A model for novelty



In a crowded ecosystem

- Niche 2 occupied
- Mutant outcompeted
- Mutant = hopeless monster

In a virgin ecosytem

- Niche 2 empty
- Mutant survives if $w_{abs} > 1$
- Mutant = hopeful monster

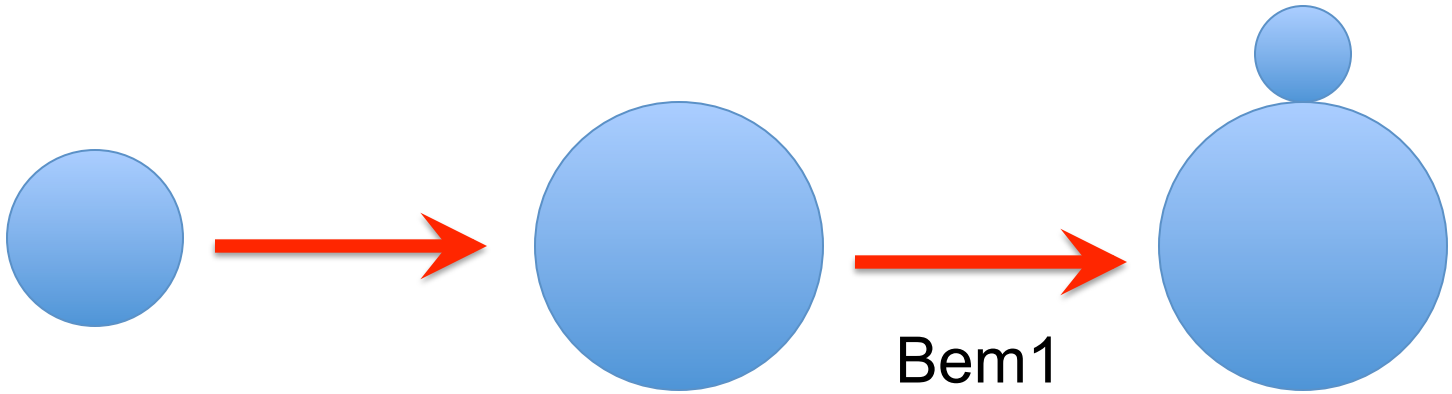
Evolving to live without important genes

Liedewij Laan (TU Delft)

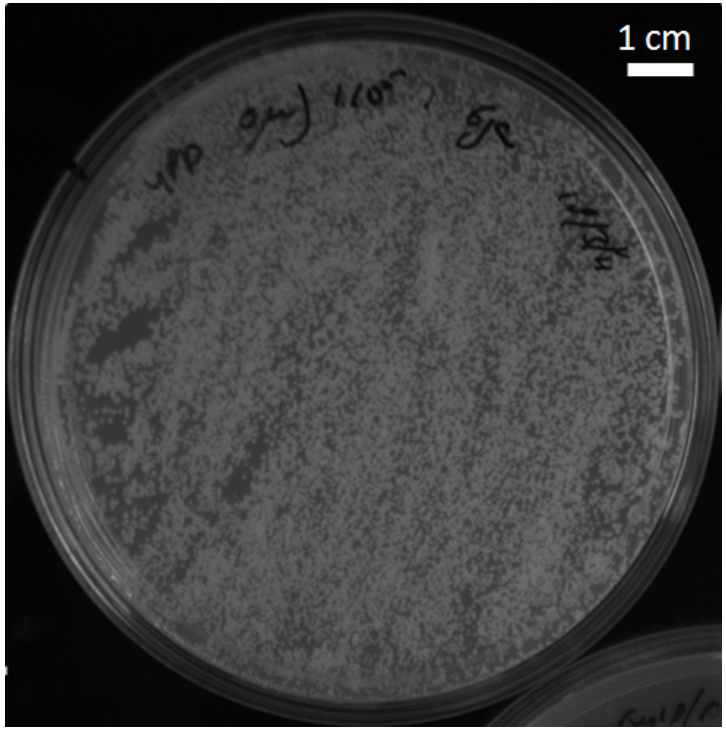
A seriously compromising mutation

If cars evolved

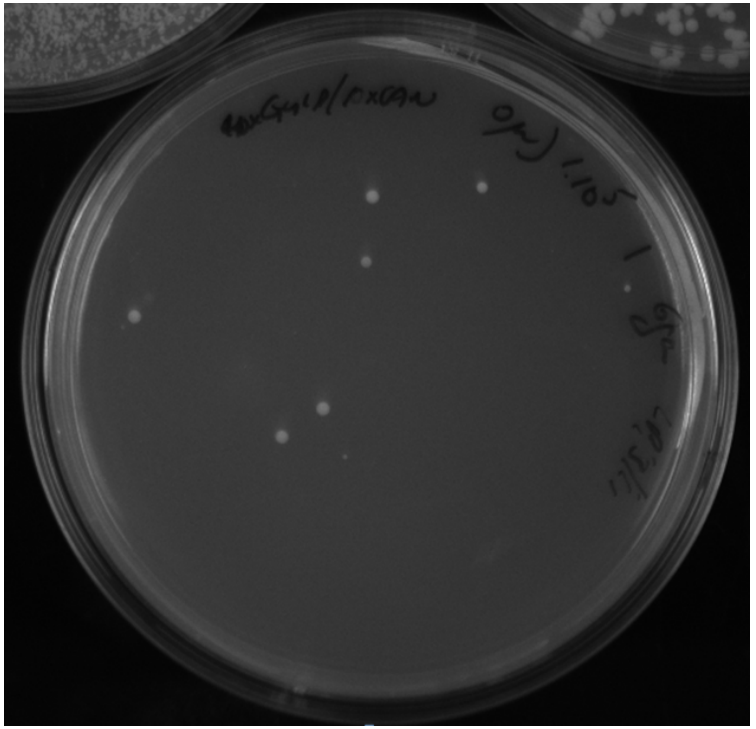
Yeast breaks symmetry to proliferate



Removing Bem1 is very bad

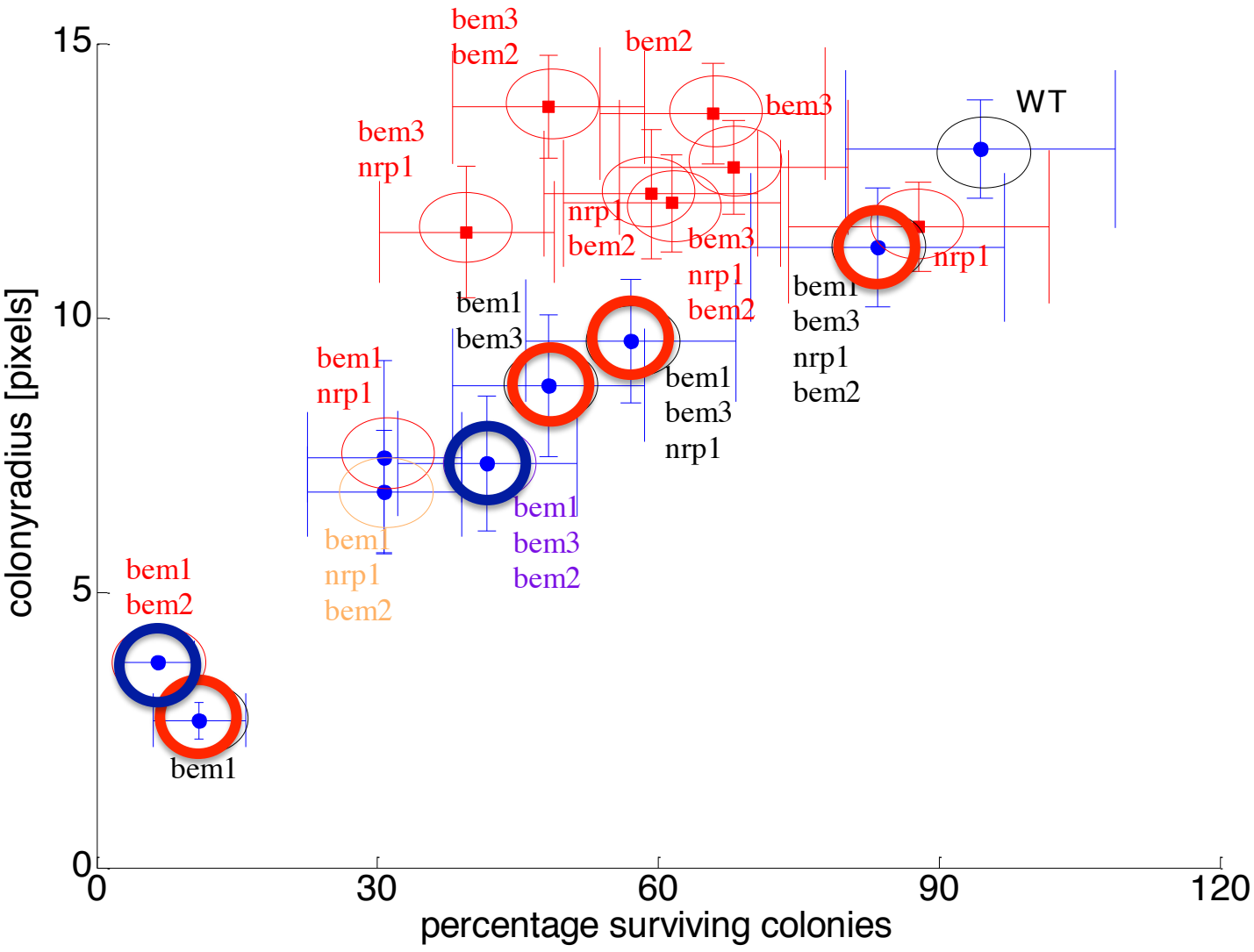


BEM1



bem1Δ

Looking at all combinations explains order of mutations



Previous selections

Multiple, poorly related trajectories

Novelty: Multicellularity (eLife ‘13)

Novelty: Circadian oscillator (eLife ’14)

Single consistent trajectory

Loss important protein: (Bem1) (eLife ‘15)

Loss of function mutations dominate

Are these straws in the wind?

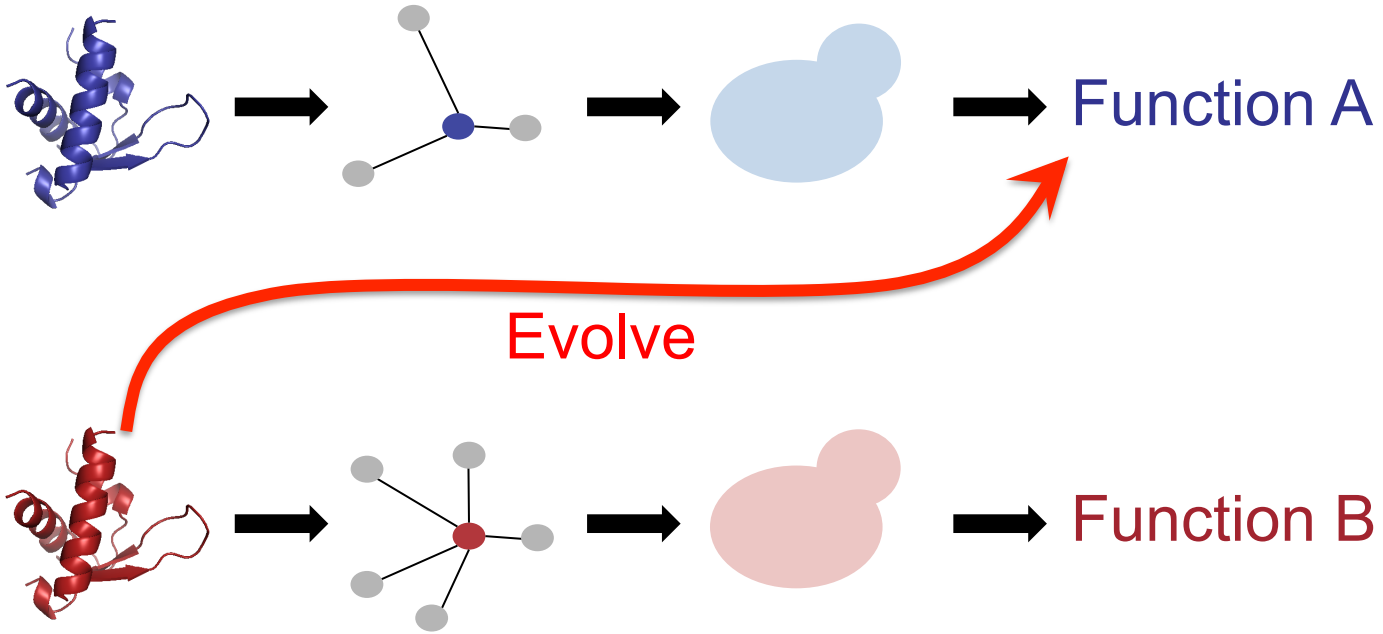
Repair trajectories more reproducible?

Loss of function dominates causal mutations?

Learning to live with the wrong part

Phoebe Hsieh

Paralogs: functionally specialized proteins



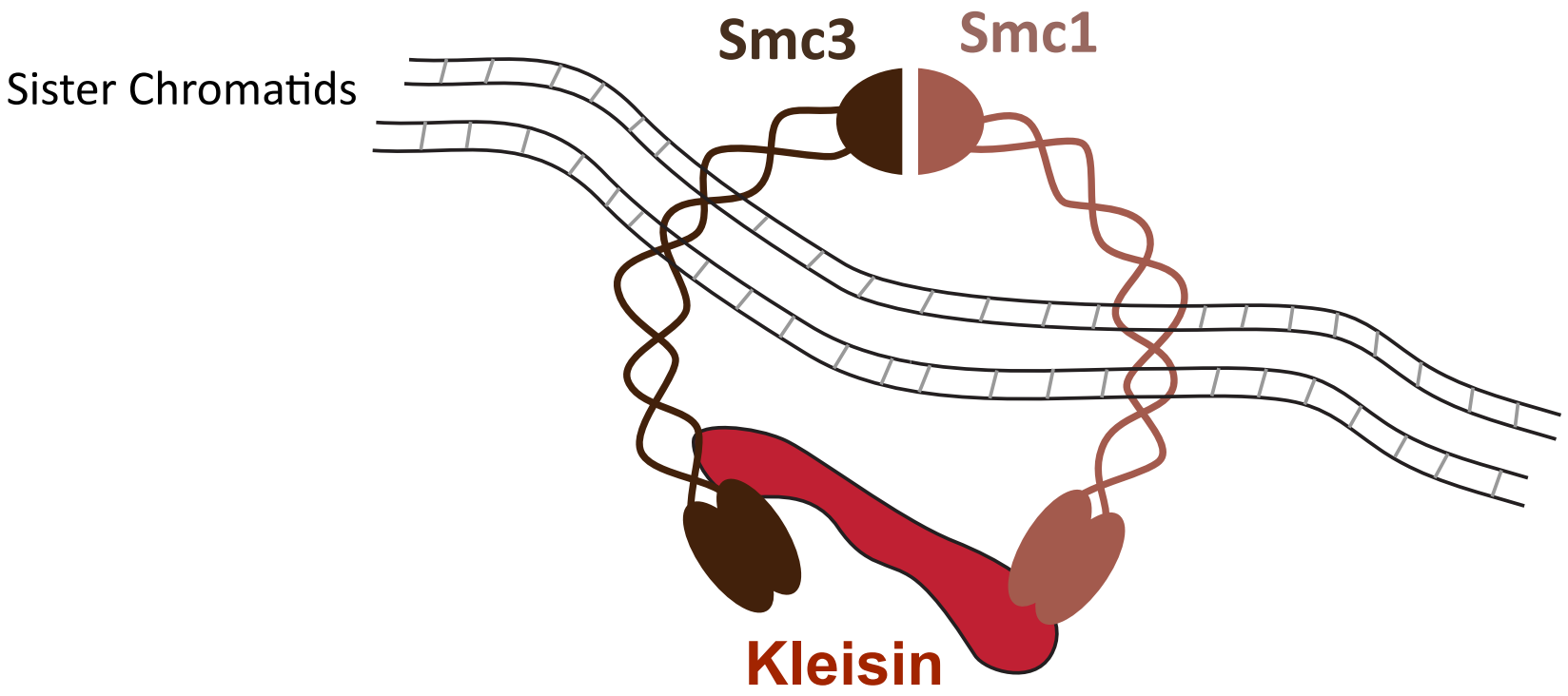
How does evolution substitute red for blue?

Change in protein itself?

Change in usual suspects (its interacting partners)?

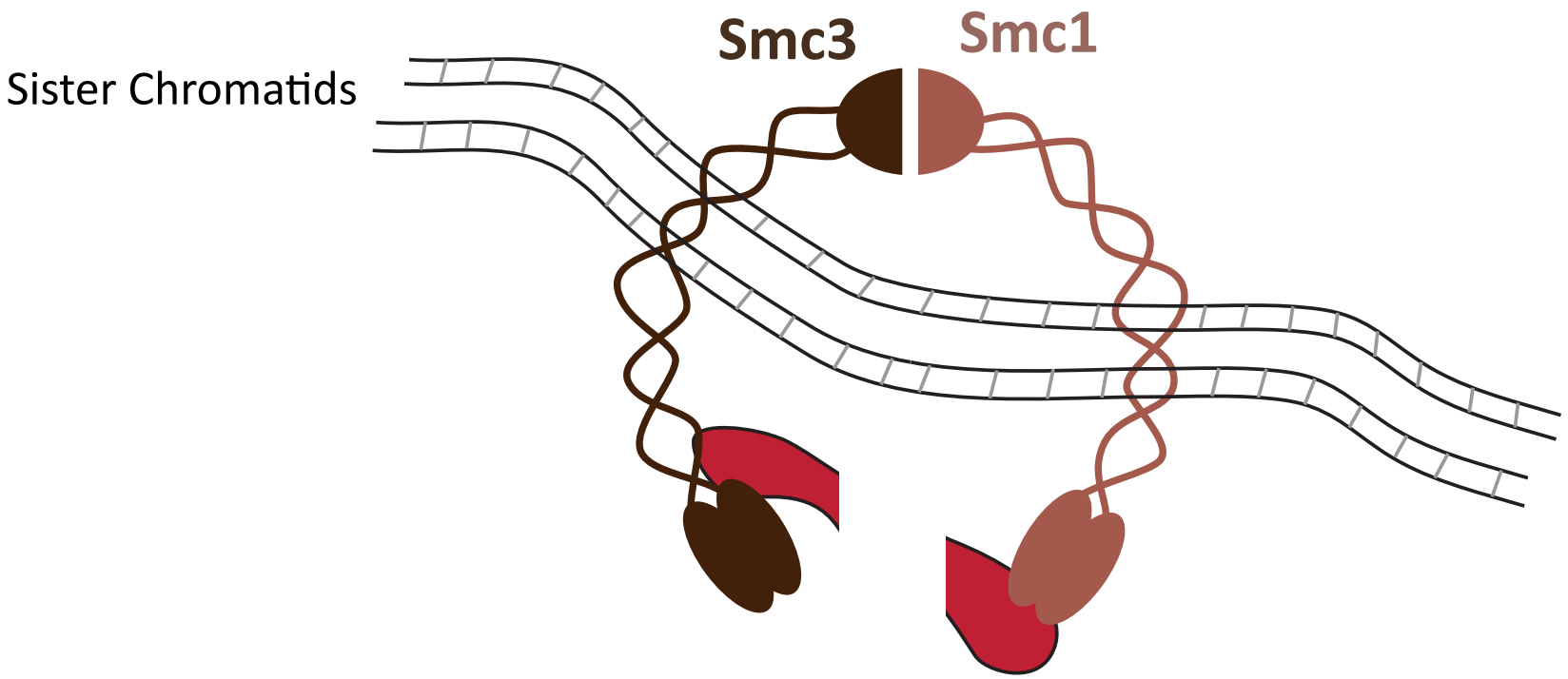
Change in novel suspects ?

Kleisin is a subunit of cohesin complex

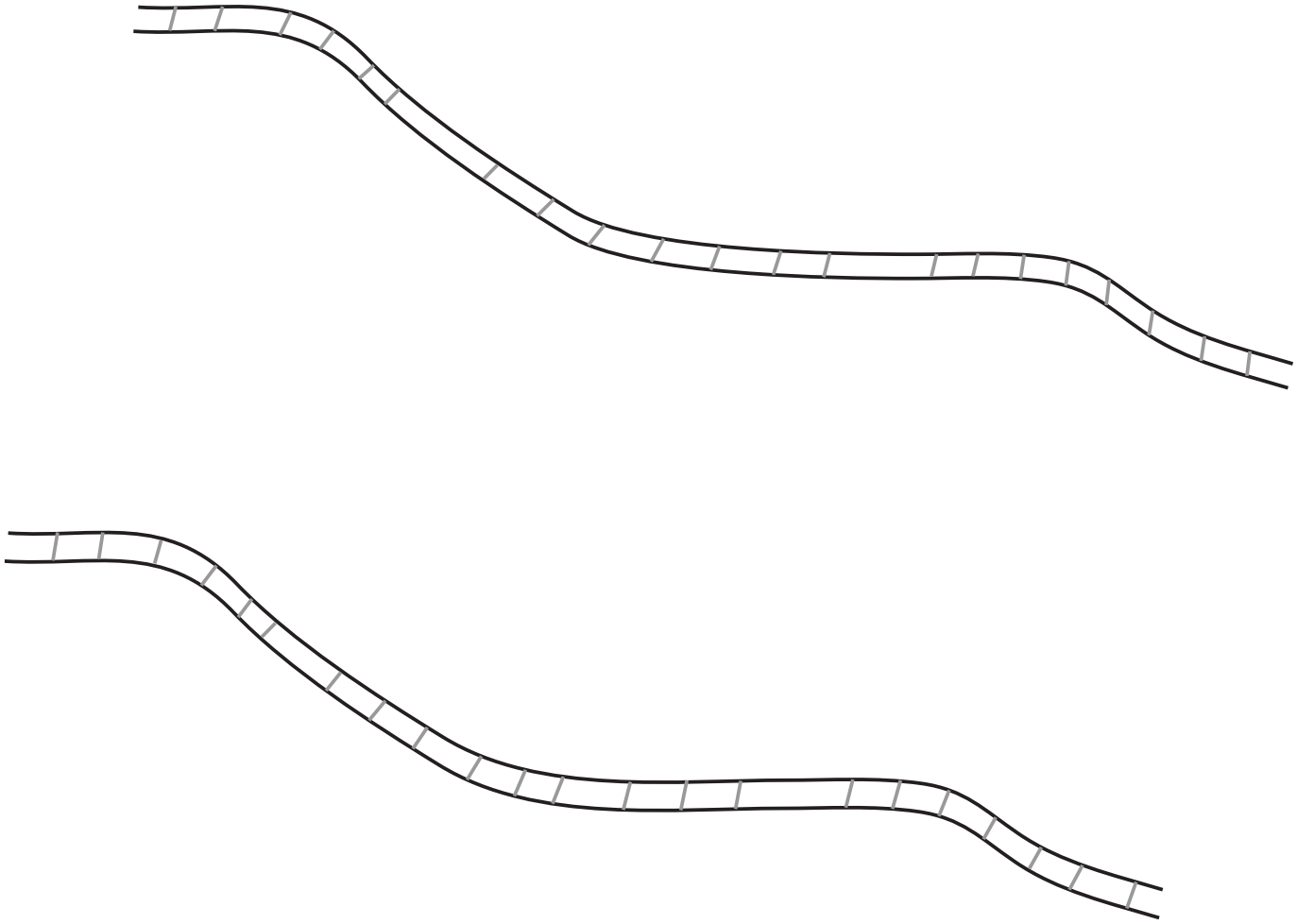


Conserved & essential for eukaryotic chromosome segregation

Cleaving kleisin separates sister chromosomes

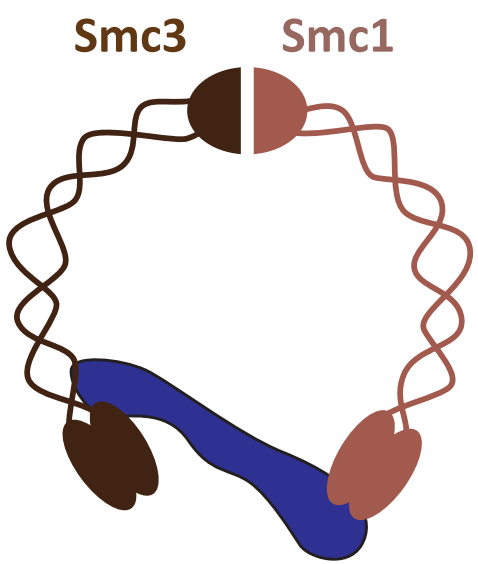


Cleaving kleisin separates sister chromosomes



Mitotic and meiotic kleisin paralogs

Mitotic Cohesin Complex



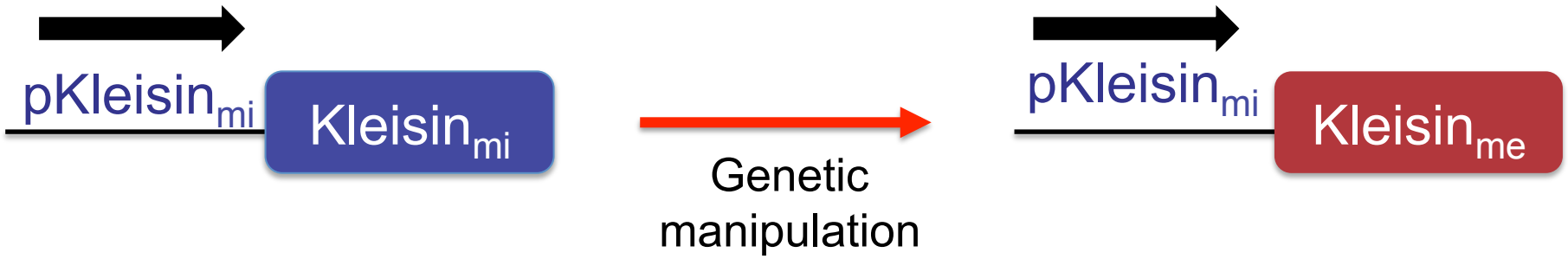
Mitotic Kleisin: Scc1

Meiotic Cohesin Complex

Meiotic Kleisin: Rec8

Proteolysis different to allow different modes of chromosome segregation

Meiotic kleisin is bad for mitotic cells

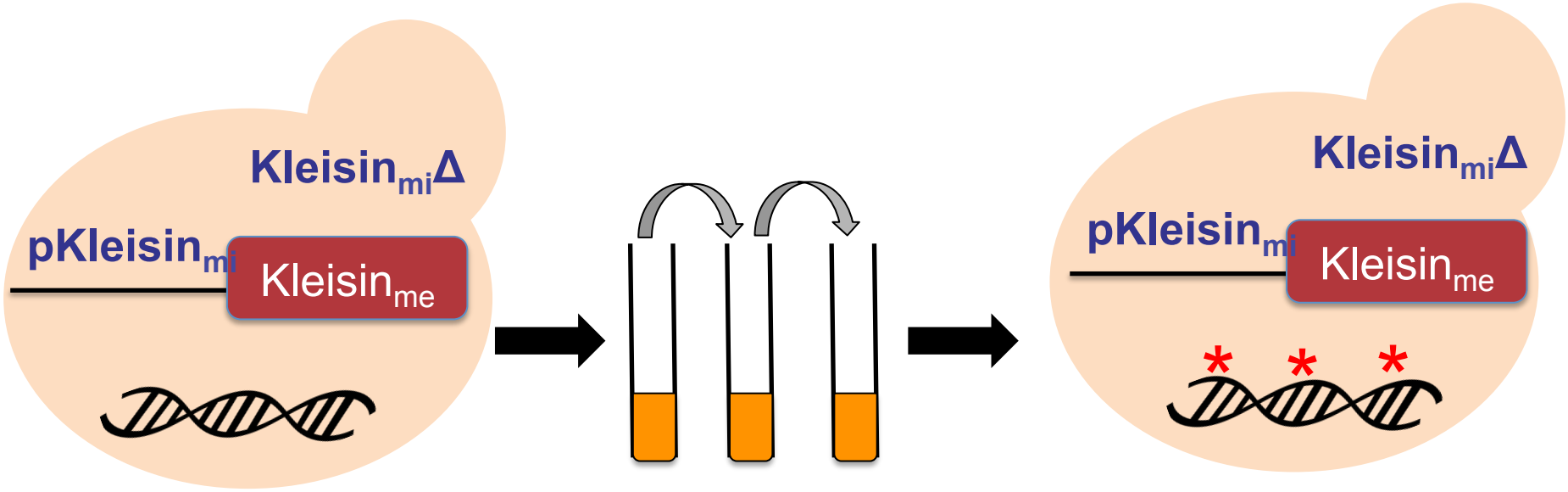


Slow growth

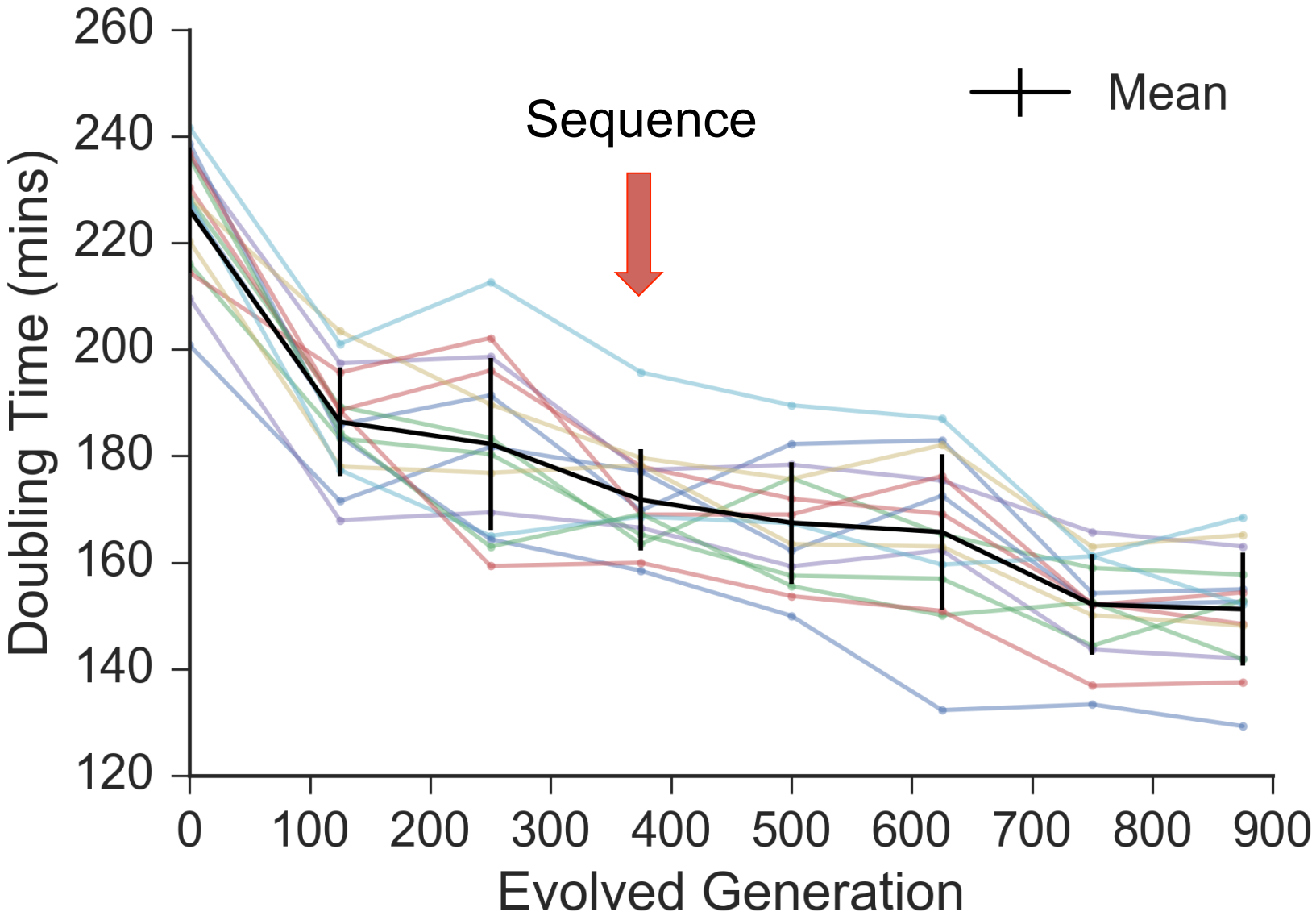
Reduced cohesin binding

Defective sister chromosome cohesion

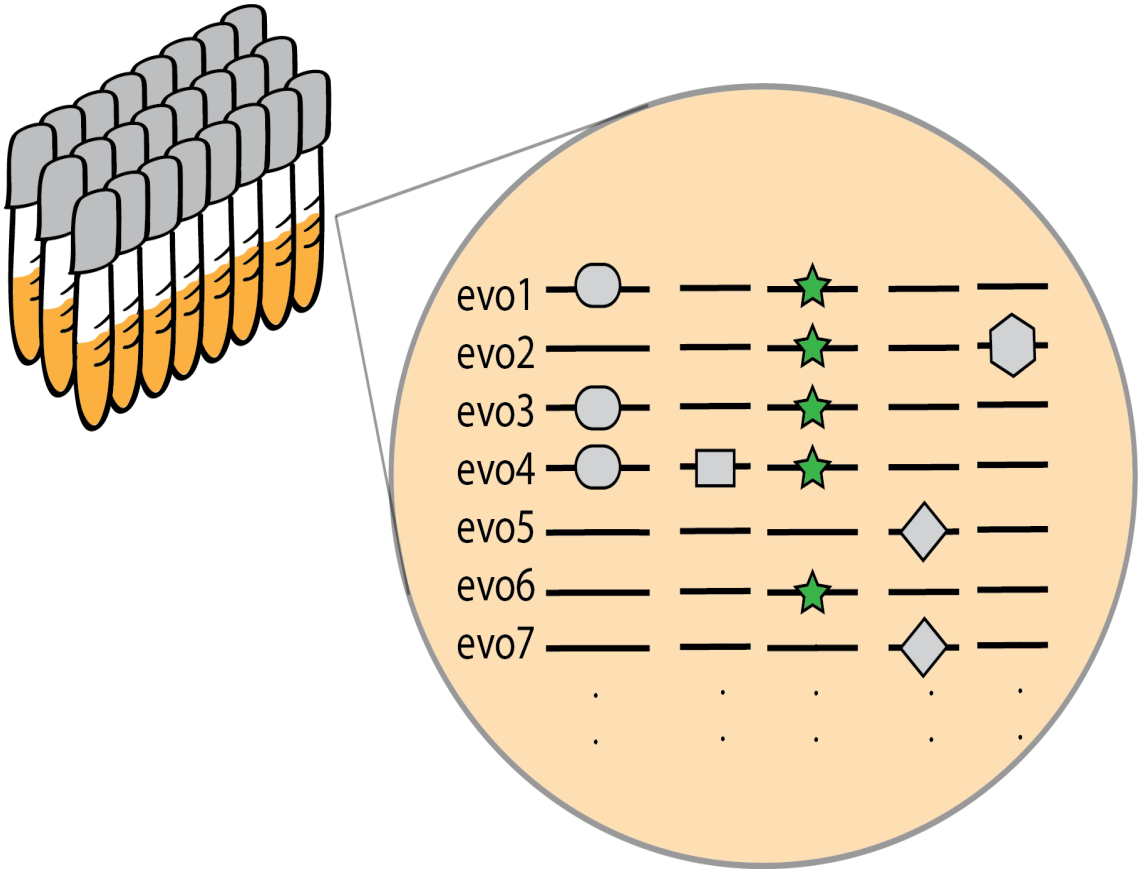
Evolving cells to live with meiotic kleisin



All populations evolve faster proliferation



Sequencing finds putative causative mutations

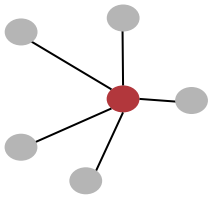


Experiments test their causality!!!

Who are the putative causal mutations?

Usual suspects

No mutation in the Kleisin$_{me}$

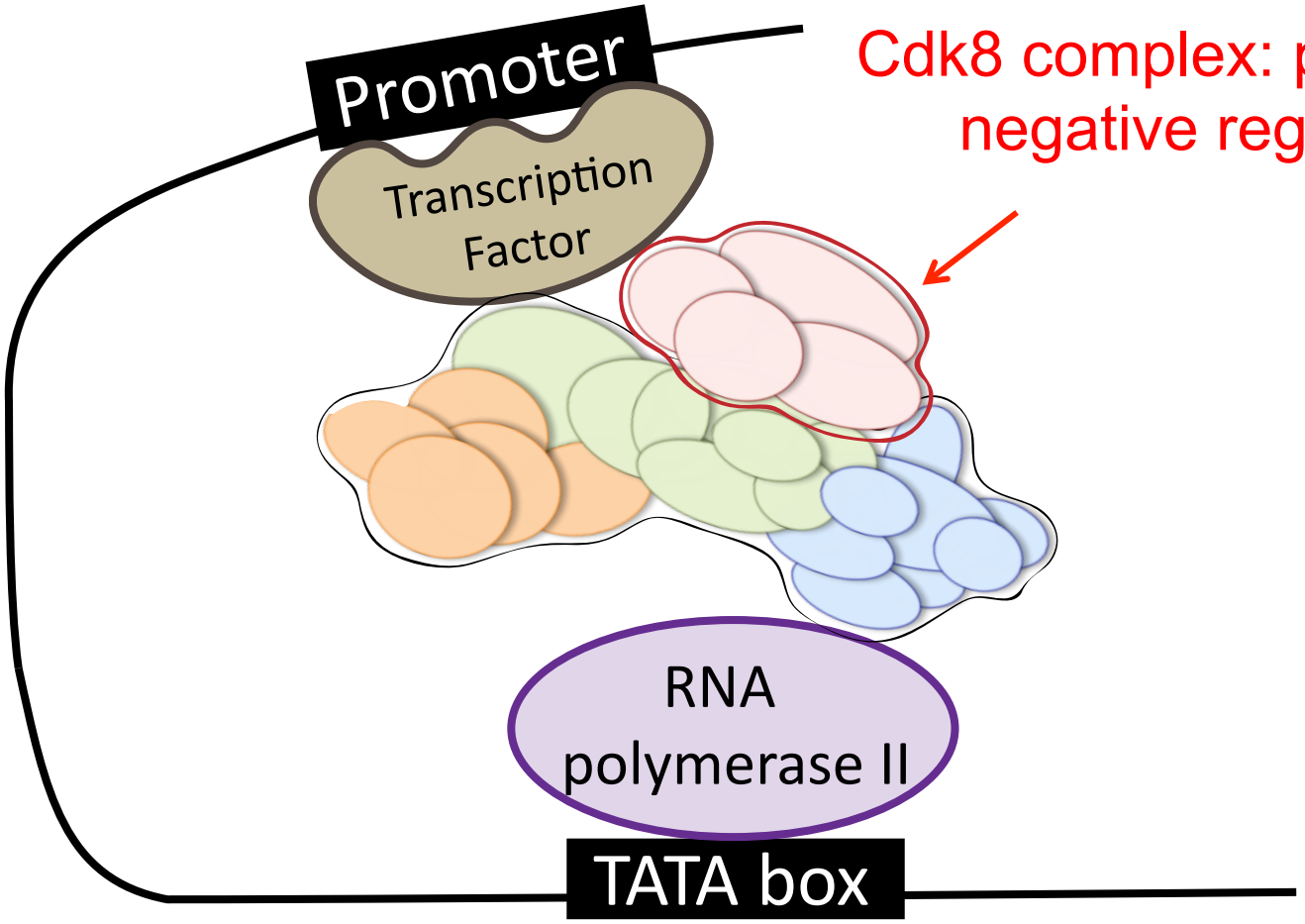


Rare mutations in three kleisin-interacting partners
Cohesin subunit: *SMC1*, *SMC3*
Kleisin protease: *ESP1*

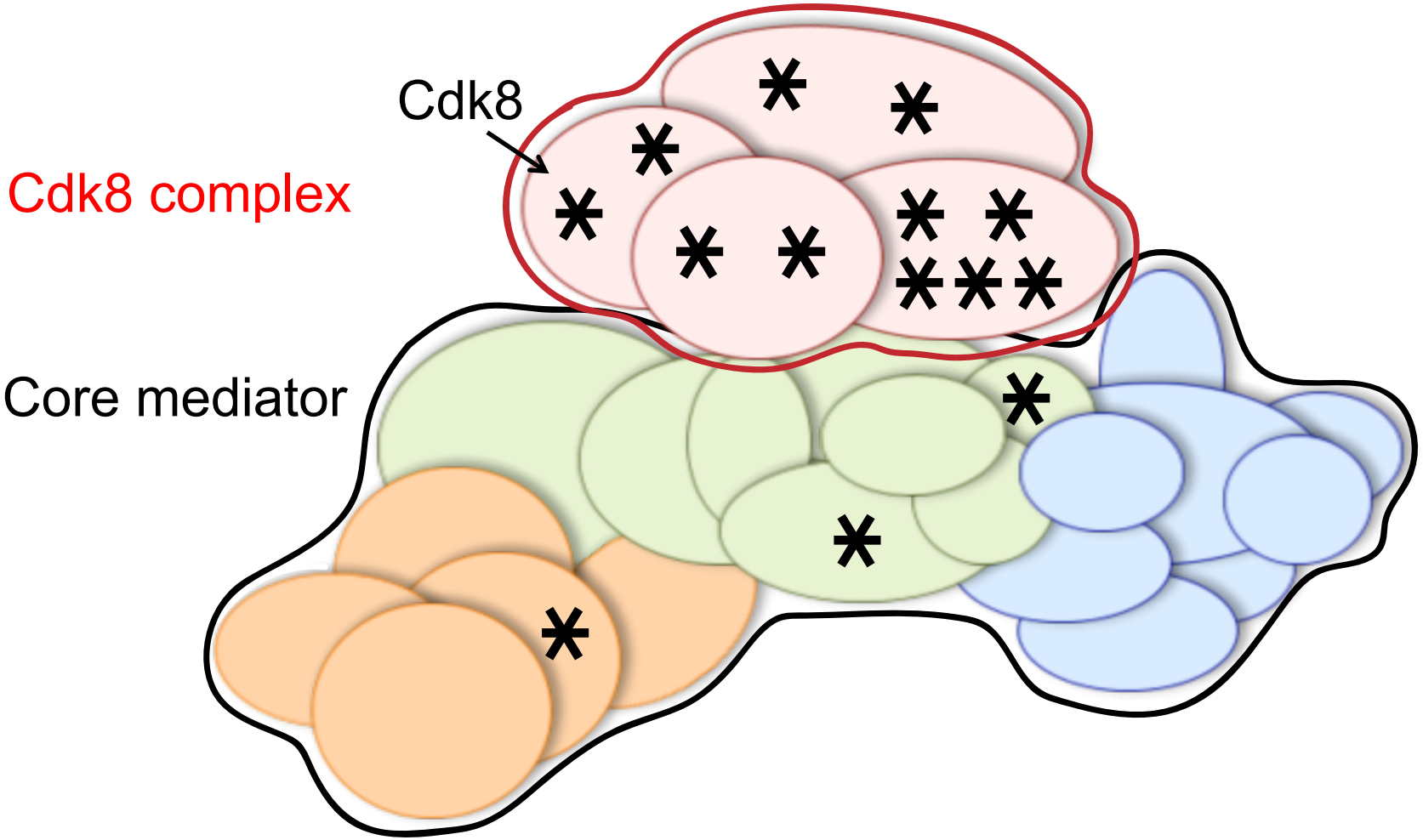
Novel suspects

Transcriptional mediator complex

Mediator links RNA polymerase II and transcription factors

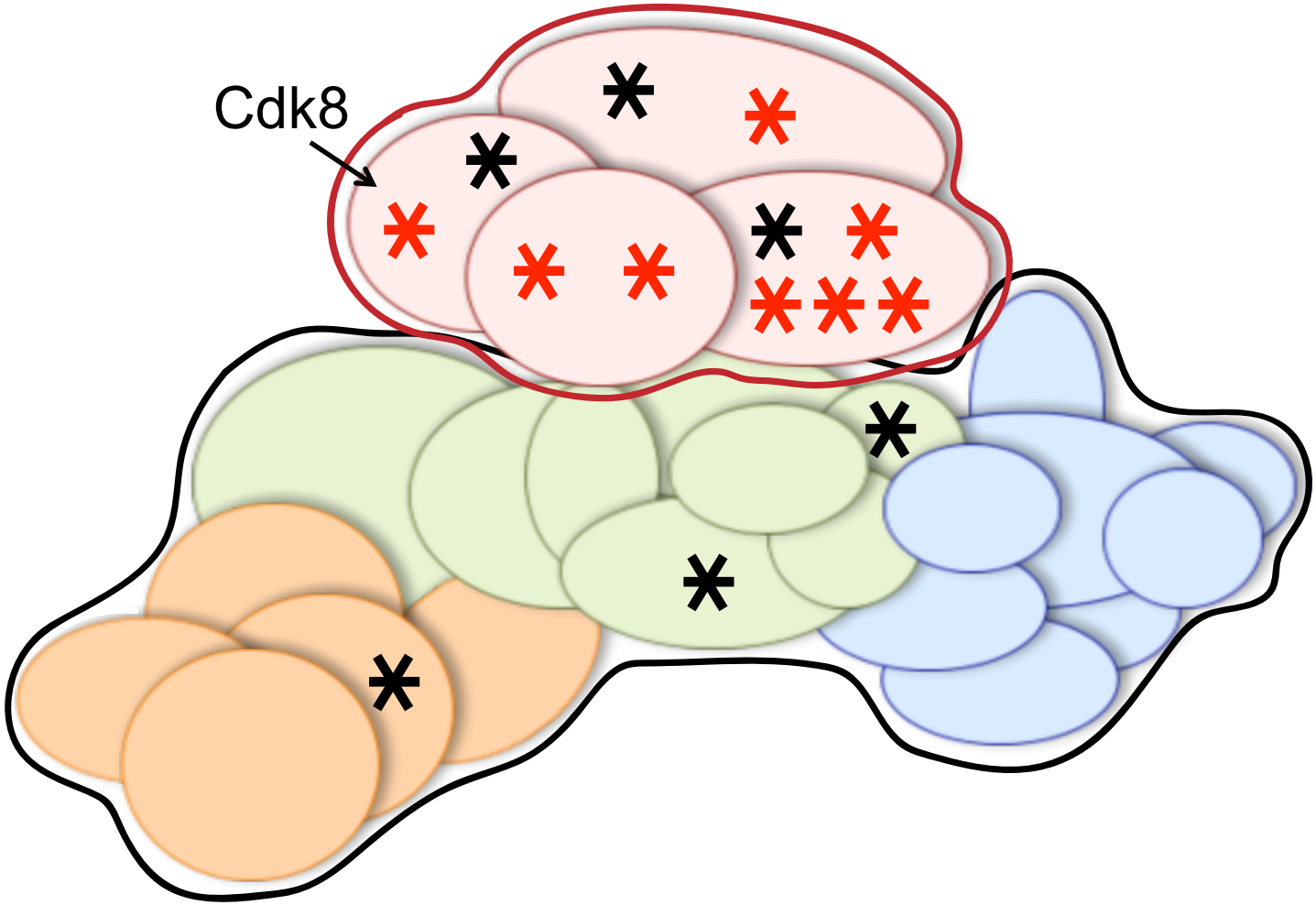


The Cdk8 complex is highly mutated



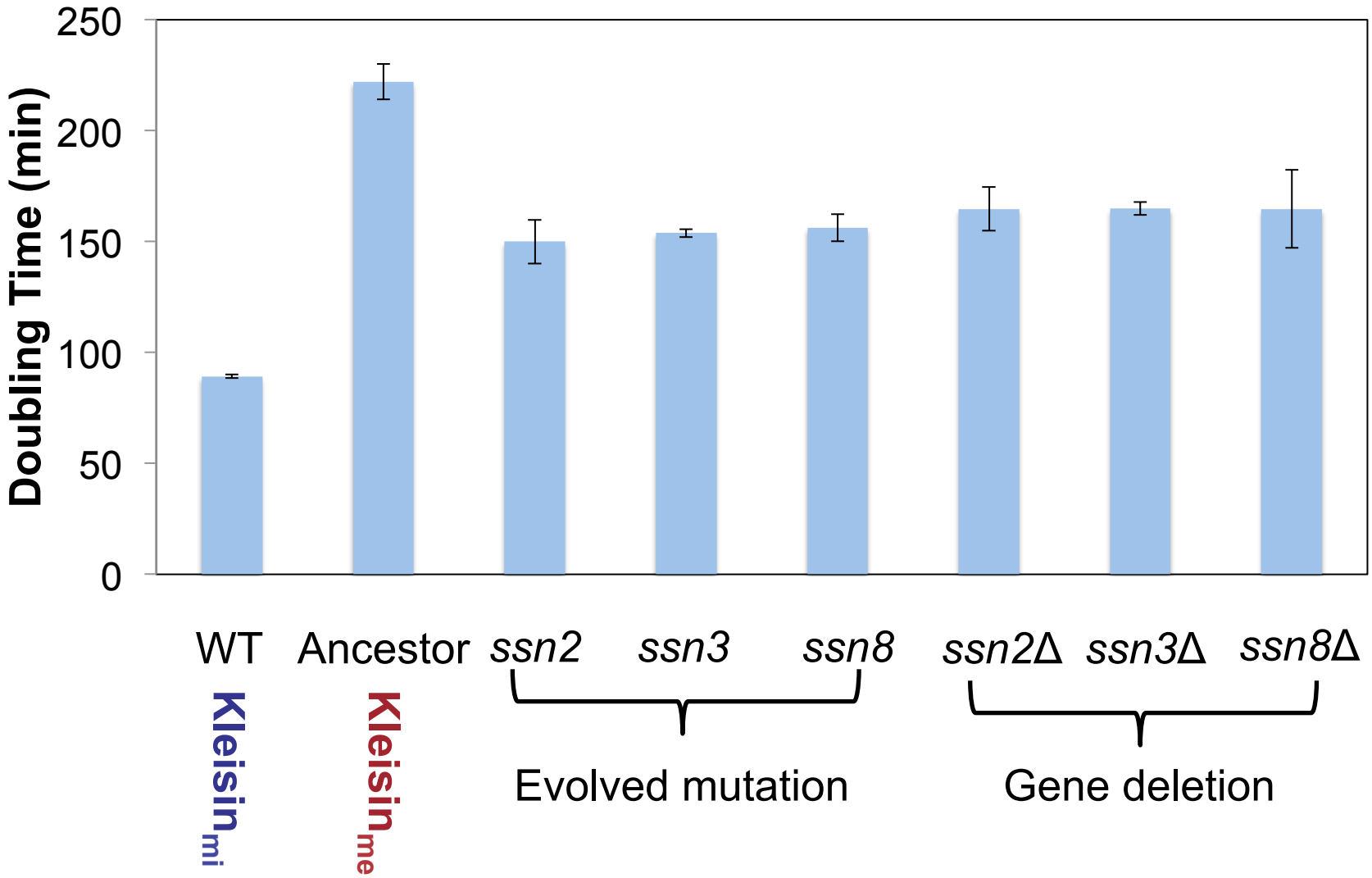
Mediator mutations in 14/15 independent lineages

The Cdk8 complex is highly mutated



Majority of mutations: early stop codon

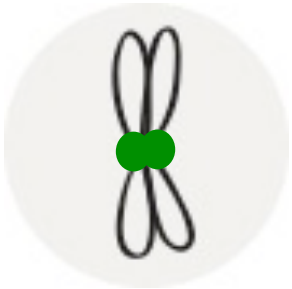
Cdk8 complex mutations kill proteins

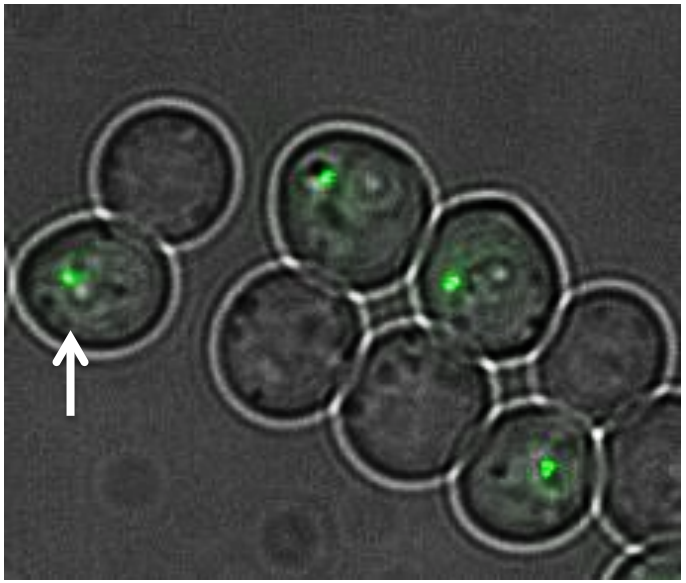


Does loss of Cdk8 function fix sister cohesion?

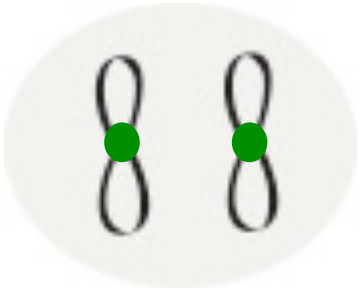
The assay: mark one chromosome with little green dots

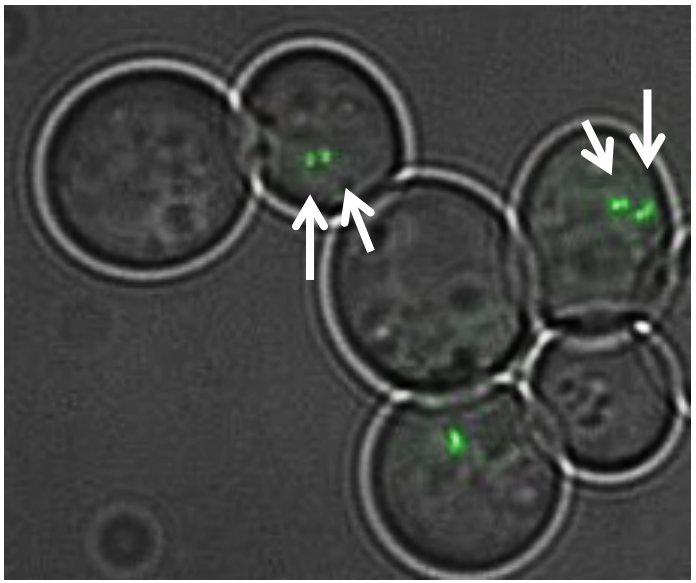
Sister cohesion



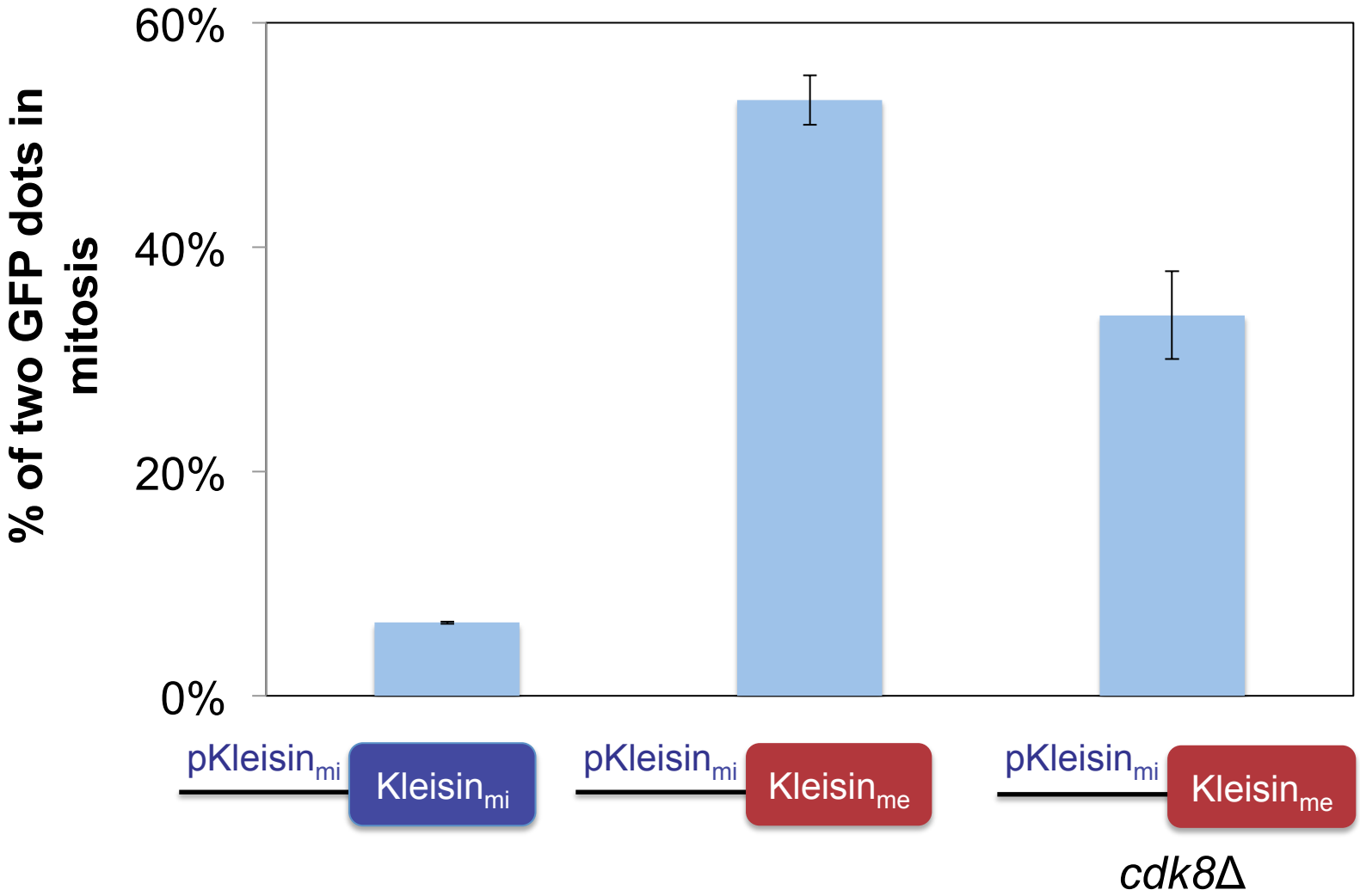


No sister cohesion





Loss of Cdk8 partially fixes cohesion defect



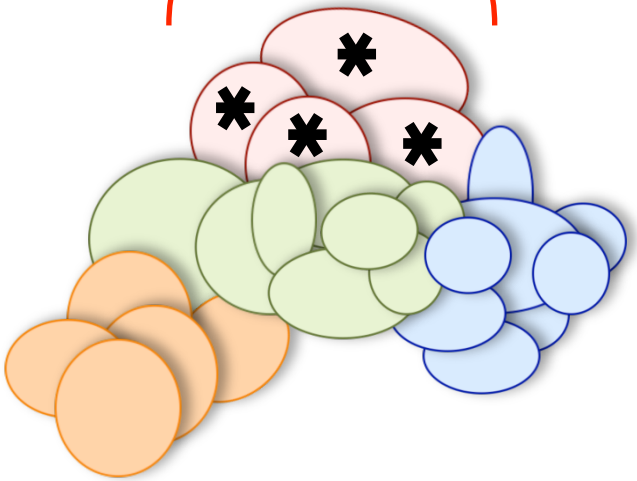
Summary

Yeast cells adapt to use meiotic kleisin in mitosis

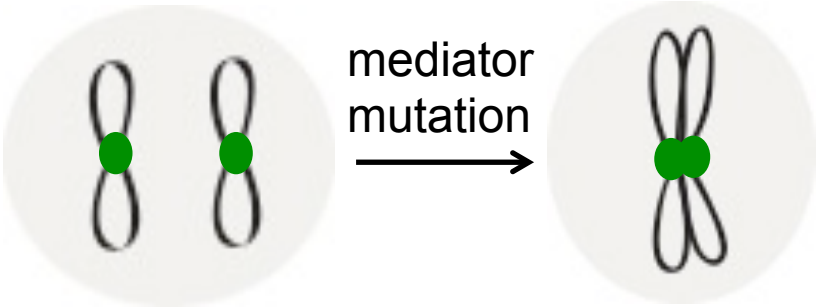
Primary target: transcriptional mediator complex

Killing Cdk8 reduces cohesion defect

Cdk8 complex



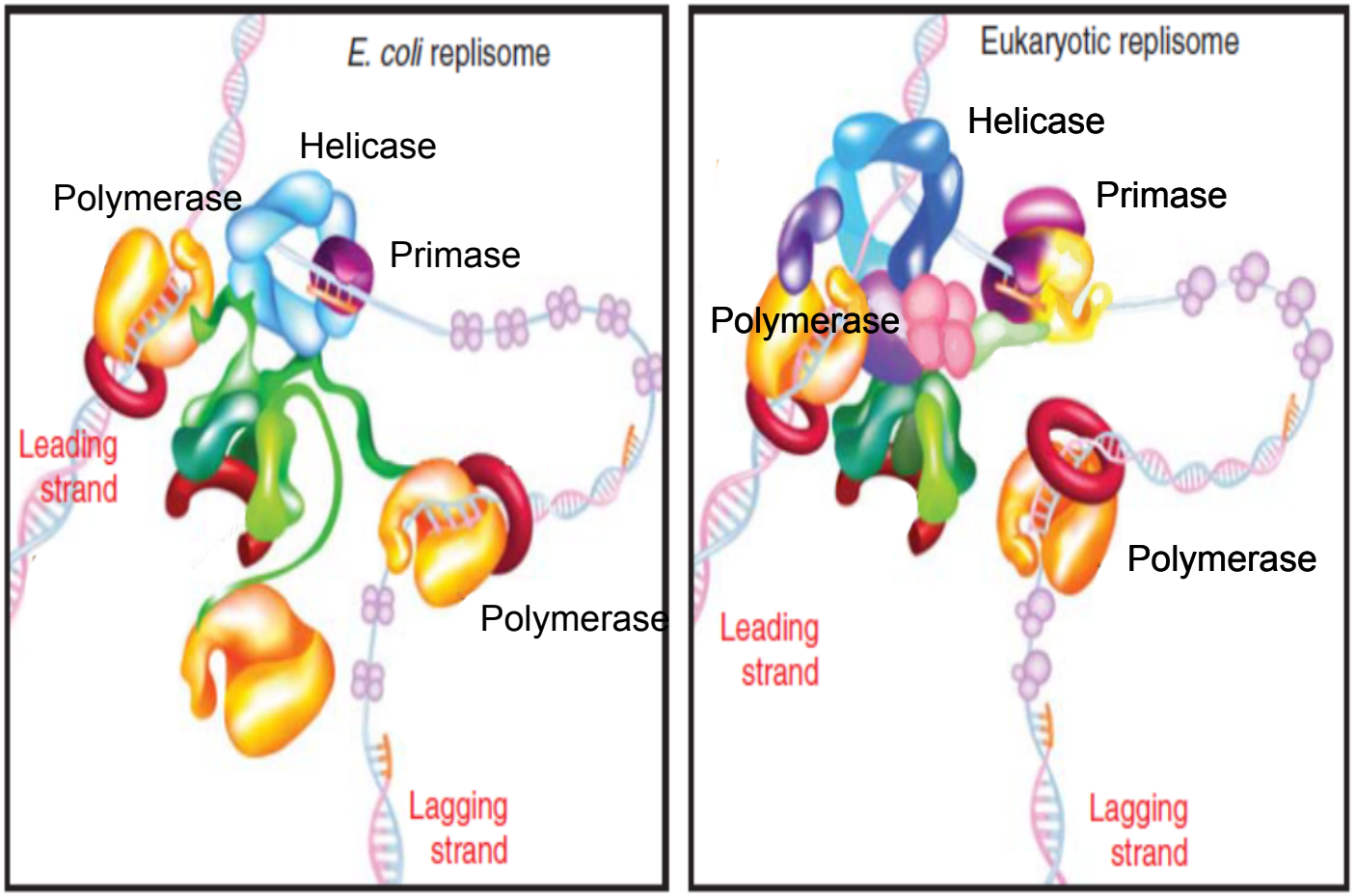
Fix sisters cohesion defect



Evolving to live with unstable replication forks

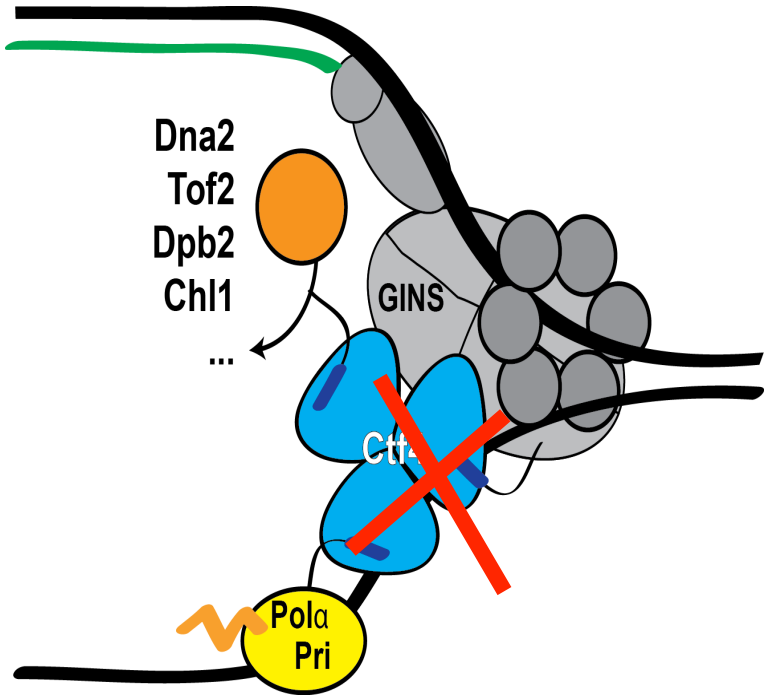
Marco Fumasoni

Organization of bacterial and eukaryotic replisomes



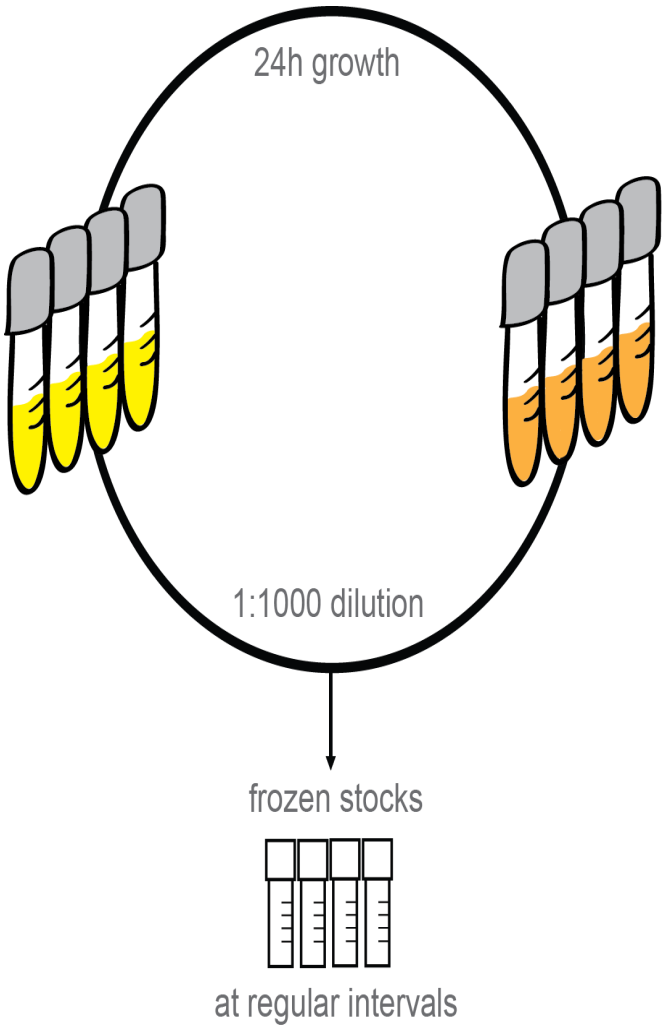
Adapted from O'Donnell et al., 2013

Genetically perturbing the replisome: *ctf4Δ*



- Poor growth (Miles and Formosa, 1992)
- Premature sister chromatid separation (Hanna et al., 2001)
- Defect in Homologous Recombination (Ogiwara et al., 2007)
- Perturbed DNA damage tolerance (Fumasoni et al., 2015)

Experimental evolution



GENOTYPES EVOLVED

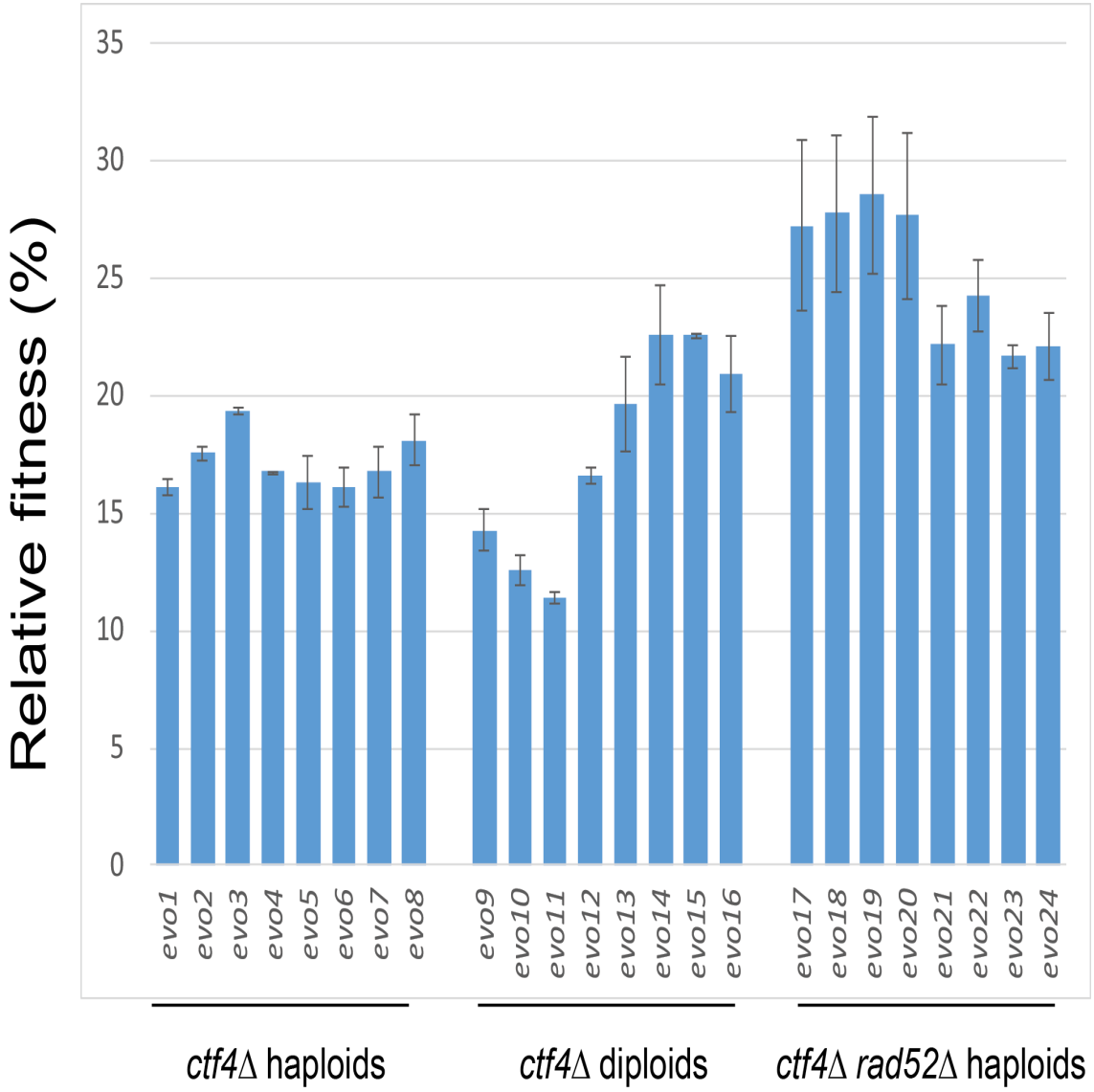
ctf4Δ

ctf4Δ / *ctf4Δ*

ctf4Δ rad52Δ

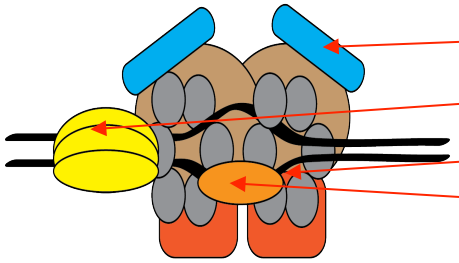
10 ~ Generations per day = 1000 ~ Generations in 4 months

Relative fitness increase over 1000 generations



Mutations affect the replication machinery

Replication origin



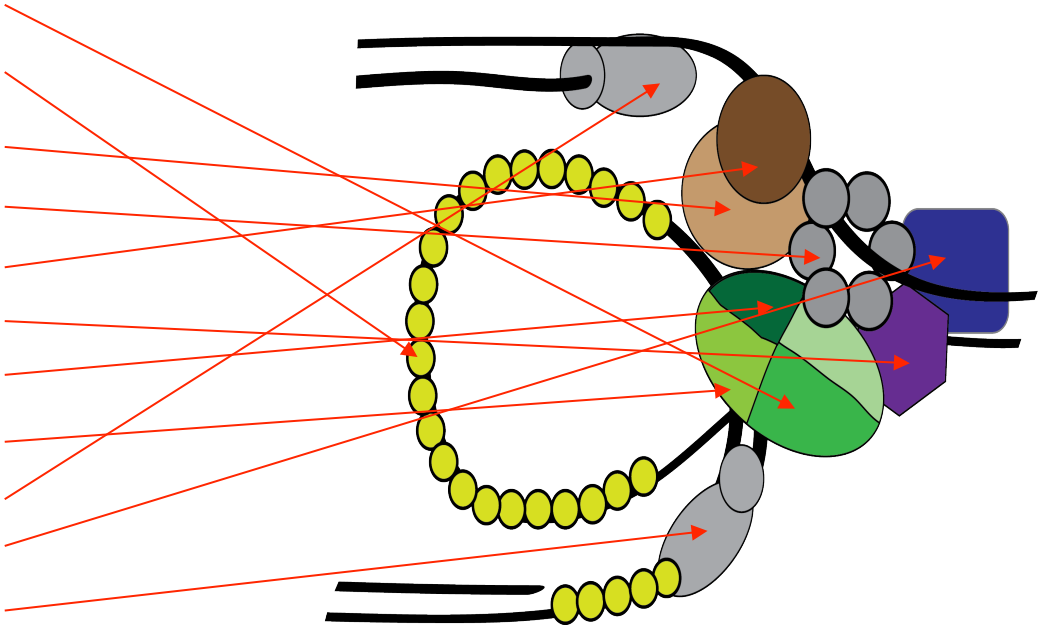
Recurrent gene hits

DPB11 (3)
ORC1 (1)
MCM10 (1)
FKH1 (1)

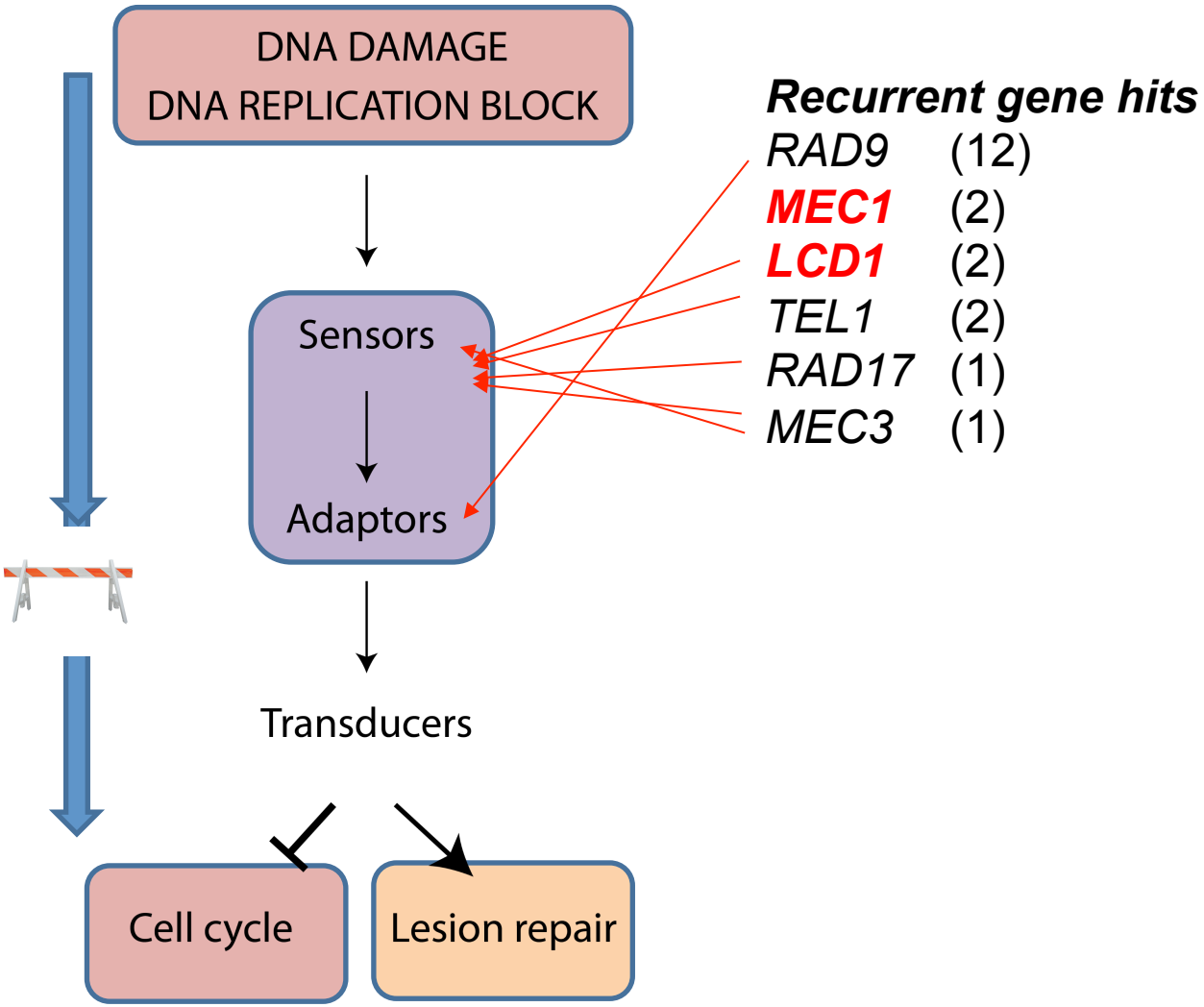
Essential Genes

Replication fork

SLD5 (6)
RFA1 (4)
CDC45 (3)
MCM3 (3)
TOF1 (3)
CSM3 (3)
PSF3 (3)
PSF1 (2)
DPB2 (2)
TOP1 (2)
POL32 (2)

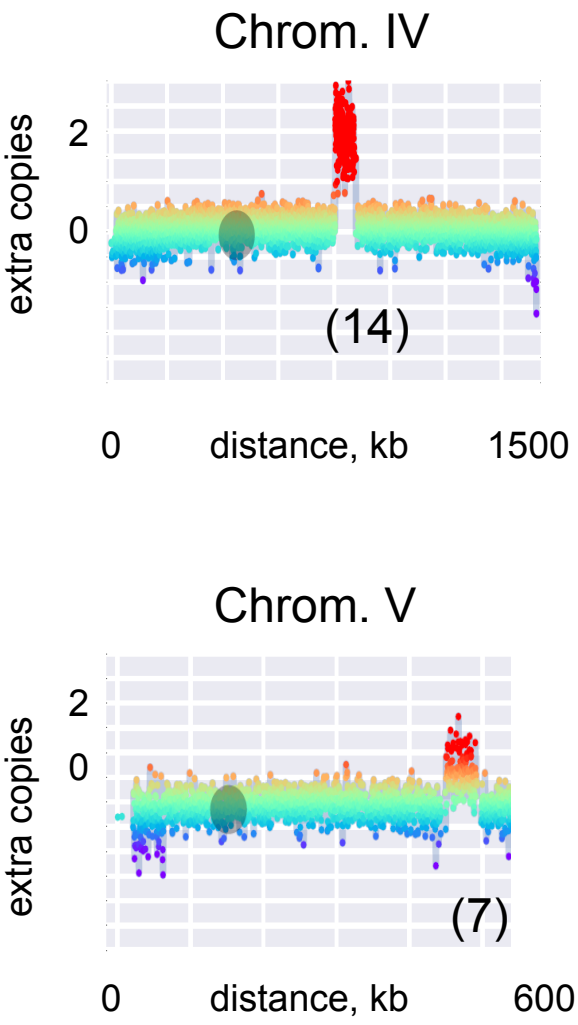


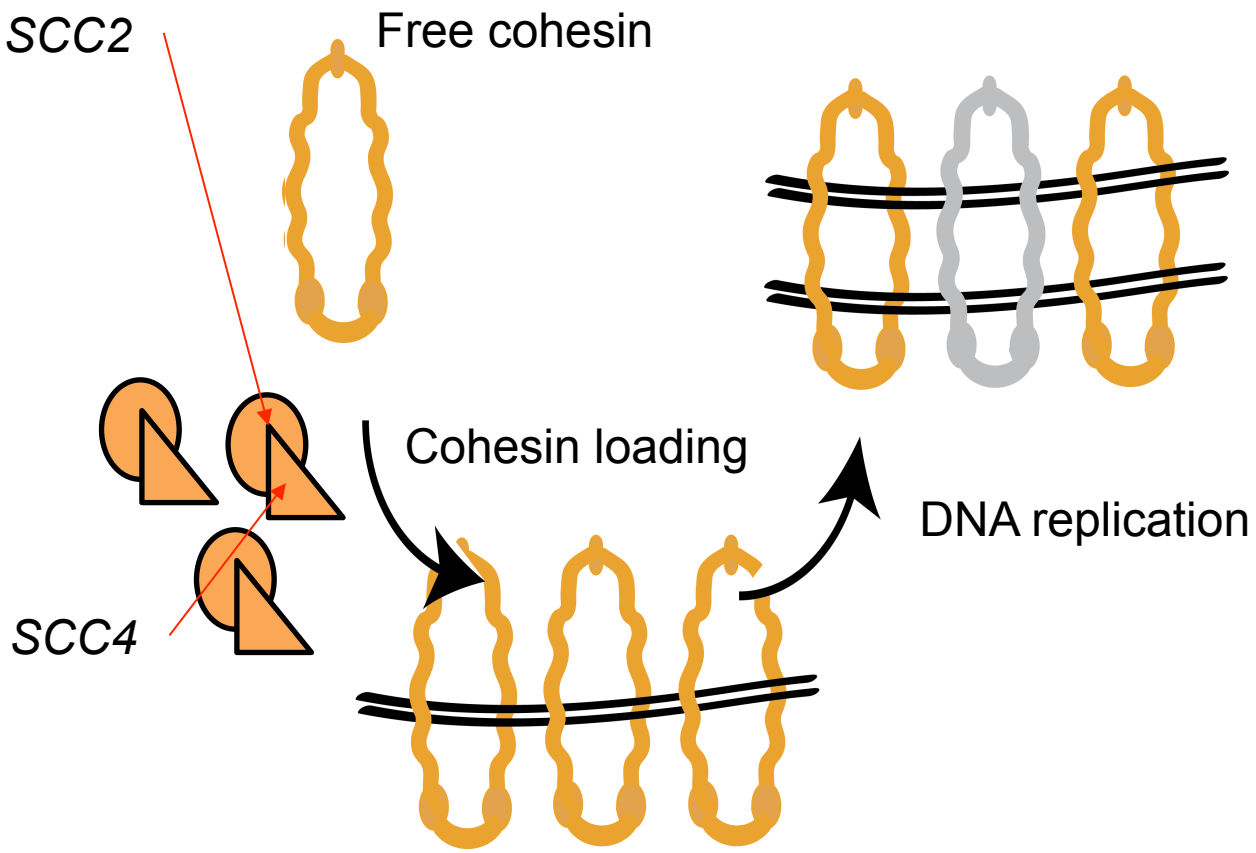
Mutations break the DNA damage checkpoint



These mutations found only in haploids

Recurrent chromosome rearrangements





These mutations found only in recombination^{+} strains

Summary

Cells adapt to perturbed DNA replication

The replisome can accept mutations in essential genes

Other conserved pathways have been turned up or down

Are these straws in the wind?

Repair trajectories more reproducible?

Loss of function dominates causal mutations?

In nature, novelty produced by gene inactivation?