

7.06 Assignment - Spring 2013

2/20/13

EXPERIMENTAL SET UP INFORMATION

Drug Treatments

1. Nocodazole: induces cell cycle arrest in metaphase (at the G2/M transition?)

Name: Nocodazole

Concentration: 15 µg/mL

Duration: 120 minutes

[check on duration - other information says to grow the cells for 2 generation times (approx. 4 hours?) (@ 30 °C). But is 2 hours sufficient?]

reference: Amberg, Burke and Strathern, Cold Spring Harbor Protocols, "Inducing Yeast Cell Synchrony: Nocodazole Arrest". Adapted from *Methods in Yeast Genetics*, 2005 edition by Amberg, Burke and Strathern.

2. Hydroxyurea: induces a cell cycle arrest during S phase

Name: Hydroxyurea

Concentration: 200 mM

Duration: 120 minutes

3. Alpha factor: used to synchronize cells in G1

Name: Alpha Factor

Concentration: 2 µg/mL

Duration: 120 minutes

[Additional note: to release alpha factor block, add pronase (0.1 µg/mL), which digests the alpha-factor.]

4. Phosphatase

Name: Protein Phosphatase 1 (PP1)

Concentration: Either have '1 unit' as the only option or have no concentration drop down menu --> I like the idea that they have to add one 1unit.

Duration: 30 minutes

[NEB PP1: <https://www.neb.com/products/p0754-protein-phosphatase-1-pp1#tabselect0>]

Strains

1. Name: Wild Type

- expresses the wild-type phenotype at the permissive temperature (30 °C)
- expresses the wild-type phenotype at the restrictive temperature (37 °C)

2. Name: Mutant 1

The mutant phenotype of the Mutant 1 strain is a block during mitosis that occurs after the Metaphase-anaphase transition. We can tell that there is a block either at G2/M or during M phase because of the build-up of cells with 2C content. However, by western blotting we observe that securin is cleaved, indicating that the cells have passed the metaphase-anaphase transition.

Mutant 1 is a temperature sensitive conditional mutant:

- expresses the wild-type phenotype at the permissive temperature (30 °C)

- expresses the mutant phenotype at the restrictive temperature (37 °C)

3. Name: Mutant 2

The mutant phenotype of the Mutant 2 strain is a block during S phase. We can tell that there is a block during S phase due to the prevalence of cells between 1C and 2C content by flow cytometry.

Mutant 2 is a temperature sensitive conditional mutant:

- expresses the wild-type phenotype at the permissive temperature (30 °C)
- expresses the mutant phenotype at the restrictive temperature (37 °C)

Temperature

- Restrictive Temperature: 37 °C
- Permissive Temperature: 30 °C

WESTERN BLOTTING CONDITIONS

Primary Antibodies:

rabbit anti-cdt1
 rabbit anti-cdk1
 mouse anti-cyclin B
 rabbit anti-securin
 rabbit anti-cdk2
 mouse anti-cyclin E
 rabbit anti-rad21
 rabbit anti-pgk1

Secondary Antibodies:

rabbit anti-mouse
 goat anti-rabbit

Western blotting results:

Protein sizes (kDa)

Cdt1: 65

Cdk1: 34, 35, 36 [34 = unphosphorylated form, 35 = 1 phosphorylation, 36 = 2 phosphorylation]

Cyclin B: 58

Securin: 41.8

Cdk2: 33, 34, 35 [33 = unphosphorylated form, 34 = 1 phosphorylation, 35 = 2 phosphorylation]

Cyclin E: 48

Rad21: [yeast]: 68 (whole protein), 29.5, 19.9 (Notes to myself: fragments: N term: 18.9, middle: 29.5, C term: 19.9)

Pgk1: 45

See separate picture that illustrates the band appearance under specific conditions.

Notes:

Alison's notes to herself [Feel free to ignore!]:

Protein	Molecular Weight (kDa)	Source	Notes	Link
Cdt1	65	Rabbit anti-human		http://www.cellsignal.com/products/3386.html
Cdk1 (cdc2)	34	Rabbit anti-human, mouse, rat	May cross-react with CDK2 and CDK3	http://www.cellsignal.com/products/9112.html
	34	mouse anti-human, monkey	Does not cross-react with other cdks	http://www.cellsignal.com/products/9116.html
CDK1 - phospho T14	36	rabbit anti-mouse, rat, human	recognizes Cdk1, phospho T14/Y15 (expected molecular weight= 32 kDa, but detects band around 36 kDa)	http://www.abcam.com/cdk1-phospho-t14-antibody-ab4823.html
CDK1 - phospho Y15	35	rabbit anti-mouse, rat, human	predicted molecular weight = 34 kDa, but detects at 35 kDa	http://www.abcam.com/cdk1-phospho-y15-antibody-ab47594.html
Cyclin B (Cyclin B1)	58	rabbit anti-human, mouse, rat, monkey, hamster		http://www.cellsignal.com/products/4138.html
Securin	41.8	rabbit anti-human, mouse, rat	gene name PDS1	http://www.abcam.com/Securin-antibody-ab26273.html
Cdk2	33	rabbit anti-human, mouse,	CDK2 has inhibitory phosphorylation on Thr14 and Tyr15.	http://www.cellsignal.com/products/2546.html

		rat monkey	To be activated, cdk2 needs to be dephosphorylated at these two residues by cdc25 phosphatase to be activated and needs to be phosphorylated at Thr160.	
cdk2-PhosphoThr160	33	rabbit anti-human, mouse, rat	recognizes phosphorylated cdk2 @ Thr160	http://www.cellsignal.com/products/2561.html
cdk2 - Phospho Y15	33	rabbit anti-mouse, human	predicted M.W. = 34 but recognizes at 33	http://www.abcam.com/cdk2-phospho-y15-antibody-epr2233y-ab76146.html
Cdk2 - Phospho T14	34	rabbit anti-human, hamster	predicted M.W. = 34 and recognizes at 34	http://www.abcam.com/cdk2-phospho-t14-antibody-ep2234y-ab68265.html
Cyclin E (Cyclin E2)	48	rabbit anti-human		http://www.cellsignal.com/products/4132.html
Cyclin E (Cyclin E1)	48-56	mouse anti-human, mouse, rat, hamster, monkey	Recognizes endogenous levels of total cyclin E1 protein. It does not cross-react with cyclin E2	http://www.cellsignal.com/products/4129.html
Rad21 cohesin	68	rabbit anti-human, mouse, rat, monkey	recognizes endogenous levels of total rad21 protein	http://www.cellsignal.com/products/4321.html
PGK1 (loading control)	45	rabbit anti-mouse, rat, human, yeast	Hannah recommends using this protein as a loading control for cell cycle experiments	http://www.abcam.com/pgk1-antibody-ab154613.html

Cohesin is a complex of 4 proteins: Scc1, Scc3, Smc1, and Smc3. When Scc1 binds to both Smc proteins, then a closed ring structure is made. When it binds to one of the Smc proteins, then there is an open ring.

Separase cleaves cohesin (between Smc1 and Smc3) to cleave the ring and remove cohesin from the chromosomes, allowing anaphase to proceed.

Protein sizes from antibody websites:

Smc1: 145 kDa <http://www.cellsignal.com/products/6892.html>

Smc3: 140 kDa <http://www.cellsignal.com/products/5696.html>

Scc1 (Rad21): 130 kDa

Scc3:

Yeast homologs:

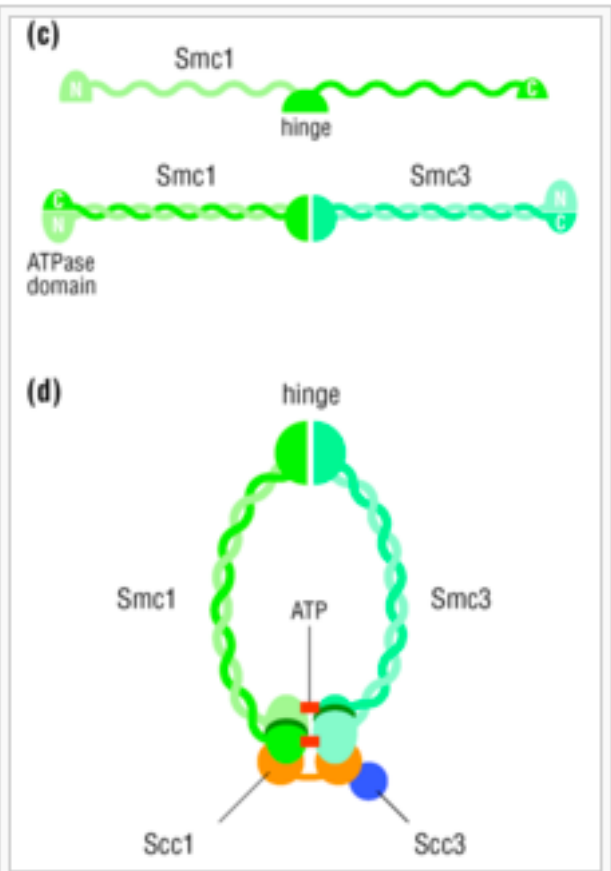
Smc1: 141 kDa

Smc3: 141 kDa

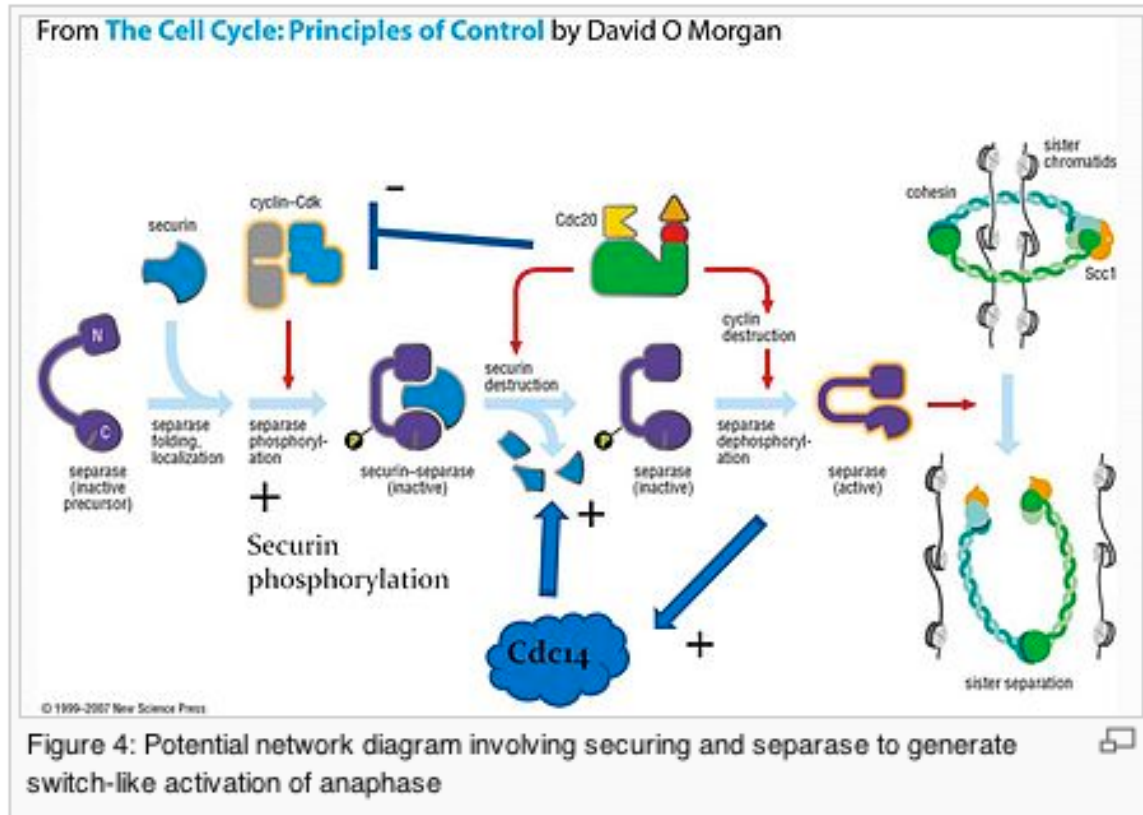
Scc1: 63.3 kDa

Scc3: 133 kDa

Images from Wikipedia:



The yeast cohesin complex consists of specialized proteins, including Scc1.^[4]



1. Human cohesin gets cleaved in 2 places → 3 fragments as reported in Science 293 (5533): 1320-1323.
2. Nature Cell Biology 12: 185-192 (2010).

FLOW CYTOMETRY CONDITIONS

Cell treatment

- Fixed is the only option.
- Have 'Live' visible, but opaque.

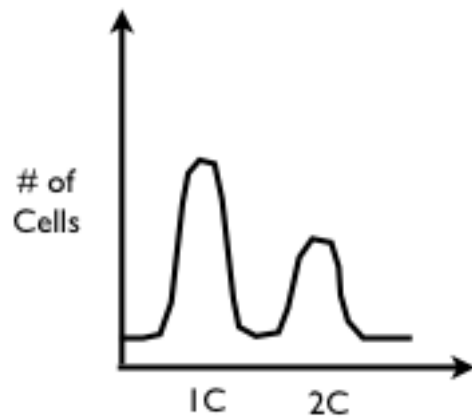
DNA content treatment

Name: PI

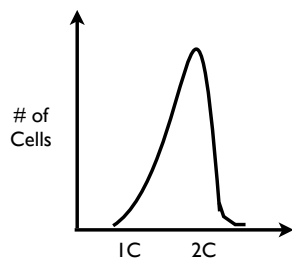
PI (Propidium Iodide) is a dye that is used to quantitatively assess DNA content.

Flow Cytometry Results:

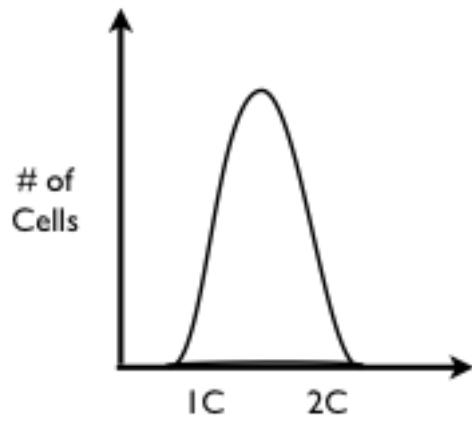
1. Wild-type strain at 30 °C, Wild-type strain at 37 °C, Mutant 1 Strain at 30 °C, Mutant 2 Strain at 30 °C:



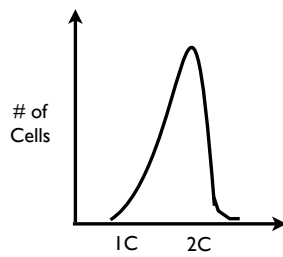
2. Mutant 1 strain at 37 °C, Mutant 1 strain at 37 °C + Nocodazole:



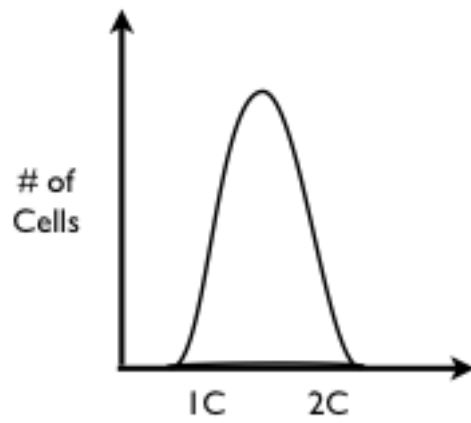
3. Mutant 2 strain at 37 °C, Mutant 2 strain @ 37 C + Hydroxyurea:



3. Nocodazole



4. Hydroxyurea, Mutant 1 Strain @ 37 + Hydroxyurea:



5. Alpha-Factor, Alpha factor treatment of the Mutant 1 or Mutant 2 strains @ 37:

