Consequences of loss of function of a1 & a2 (usually leads to sterility)

STE = wt genes
ste = mutant genes

1. Loss of α1 results in sterility because of the lack of expression of α-specific genes

2. Loss of α2 allows expression of both a-specific & α–specific gene banks with result that antagonism -> sterility

3. Inactivation of both α1 and α2 renders α cells a-like because α-specific genes are off and a-specific genes are on.
(alf phenotype - for a-like faker)

4. Loss of a1 makes no difference as a-specific genes remain on

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Switching rules in homothallic strains (HO strains)

1. Switch occurs in pairs of cells

2. Only "experienced" mother* cells switch

3. "Experienced" cells switch at least 50% of the time at minimum (usually 80-90% of time)**

*An "experienced" cell is one that has been a mother (previously produced a daughter)

** Switching occurs only at a ~ once/106 cell divisions/ho strains

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Switching rules suggest

1. Switching is not random: rather cells are directed to pick as a donor gene for the mating-type switch gene of the opposite type than the one already in the MAT locus.

2. switching occurs prior to or at the time of MAT DNA synthesis, and then the genetic switch is replicated and passed to both resulting cells (if occurred after DNA synthesis then only one of the pair would be switched)

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Nature of cassettes*

1. Core region of unique, transcribed information that defines each mating type
   Silenced by Ya = 642 bp } idiomorphic sequences
   Sir/Mar Repression Yα = 747 bp }

2. Two flanking regions**
   X = 704 bp
   Z-1 = 239 bp

** These are common to all three loci, *HML*, *HMR* & *MAT*
** Provide sequence homology which facilitates the recombination that results in exchange of the y regions

3. *MAT* and *HML* share 2 additional loci
   W & Z-2 ~ 723 & 88, respectively

* both loci > 100 kbp away from centromere-linked *MAT* locus near left & right telomeres

MAT <-> *HML* ~ 200 kb
MAT <-> *HMR* ~ 150 kb

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Cell cycle definition

The name given to the repeated events that occur between the formation of a daughter cell by division of its mother cell and the time when the daughter itself divides

In *S. cerevisiae*
Time between daughter separating from mother and daughter producing its own daughter

Early leader in field – Dr. Leland H. Hartwell see R.R. #14 + 15 for example

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Keys to early success

1. Yeast cells of *S. cerevisiae* were amenable to synchronization

2. So-called *c*ell *d*ivision *c*ycle (cdc) mutants were relatively easily derived

3. the insight that the yeast bud and its growth could serve as a major landmark of the progression of a eucaryotic cell through its cell cycle

Initial goal: determine the relationship between DNA synthesis – nuclear division cylcle and the cell growth/cell division cycle.

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Types of synchronization

1. Induction - involves distortion of cycle in way that all cells arrest in one cell cycle stage, after which they are released to divide in syncrony

2. Selection - isolation of selected population from among an asynchronously dividing population, which is at same stage of cell cycle, so when put in fresh medium all cells divide in unison.

Bud formation and cell division -> 2 easily scored landmarks

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ts *cdc* mutants
for functional analysis

Conditional “lethal” strains that have altered alleles in their genomes, which render them unable to complete certain specific events of a normal *c*ell *d*ivision *c*ycle (cdc) at a restrictive condition,* but allow them to grow normally at a permissive condition.**

* usually $37^{o}C$ - $41^{o}C$

** usually $25^{o}C$

* arrest in a single "terminal phenotype"

* most not really lethal as down shift shows cells often still alive – just don’t grow reproductively to -> turbid broth cultures or dense plate colonies compared to wt

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Initial events scored in *cdc* strains

1. Terminal phenotypes at restrictive temperature (37^{o}C) *

2. Whether or not a 1st cycle arrest mutant (arrested in 1st cell cycle after shift to 37^{o}C) <100% increase in cell #.

3. Mutation's execution point

4. What landmark(s)** inhibited (particularly initial defect).

* selection based on little or no growth at ~ 37->41^{o}C and production of a terminal phenotype

**

Bud emergence	}	
DNA synthesis	}	
Cell separation	}	change with deeper study
Nuclear migration	}	
Etc.	}	

study over when gene product identified and function found

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Use of *cdc* mutants for functional mapping

1. Comparisons of the phenotypes of single mutants

2. Comparisons of the terminal phenotypes of two single mutants with that of the corresponding haploid double mutant

3. Use in reciprocal shift experiments

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Functional sequencing with single mutants a

1.* D1 La Lb — Lb is not dependent on La
→/ →/ →

2.* D1 Lb La — La is not dependent on Lb
→ → →

3.** D1 LaLb — La & Lb are interdependent
→ →
La

4.*** D1 → — La & Lb are independent events
→/ Lb
→/

L = landmark
D = initial defect.
*good for 1 & 2 if a & b far apart in timing
**poor for #3
*** very good for #4

Often was done by time lapsing on agar medium.

EXAMPLES

D1 = DNA synthesis
La = mitosis
La^1 = bud emergence
Lb = cytokinesis

D1 La Lb "dependent"
→/ →/ →/

cytokinesis depends on mitosis, which
depends on DNA synthesis

La^1
D1 →/ mitosis dependent upon DNA
→/ La synthesis, but bud emergence
→/ is independent of DNA synthesis.

IN double *cdc* mutants*

Mutant	Initial Defect	Terminal phenotype
cdc 24	BE	
cdc 8	DNA syn	
cdc 24,8		

** unique phenotype

Important to remember that these are haploid double mutants as heterozygous diploids would be like wt because of complementation - however, could construct

cdc 24	*cdc* 8
cdc 24	*cdc* 8

"strains homozygous recessive diploids --> same results"

Landmark events in the cell cycle

earlier, broader definition:

> events which can be monitored by an available assay that provides information about the position of cells within the cell cycle.

more recent, narrower definition:

> events that actually duplicate and segregate cell constituents and produce daughter cells.

RHO-type GTP-Binding proteins, such as Cdc42p

1. Carry prenylation sequences and the addition of lipid moieties to these domains modulates their binding to membranes and may therefore affect their activity

2. Like Ras, all bind guanine guanine nucleotides, being active when bound to GTP and inactive when bound to GDP;

3. All are able to hydrolyze GTP, for which they require a GAP, which acts as a negative modulator;

4. Involve a GEF in their activation, closing the GTPase cycle;

5. Are thought to stimulate actin reorganization in vivo.

A unified theory of cell cycle control

this prevailing theory invokes two central coordinative mechanisms so that cell cycle events occur in the proper order with repsect to each other: e.g. chromosome segregation follows dna replication.

1. a cell cycle clock based on a set of highly conserved serine threonine protein kinases (cdks; *c*yclin-*d*ependent-*k*inases)

2. checkpoint controls, which involve regulatory pathways that monitor the progress of key cell cycle events and delay progression, if those events have not been satisfactorily completed.

The cell cycle clock

the ticking of the clock is manifested as cyclical changes in cdk kinase activities*

these phosphorylations regulate many processes, including even the synthesis, activation levels, and proteolysis of cdk regulators that contribute to the oscillations of cdk activities themselves.

*main cdk is cdc28p,** although there are others

**all cdk are inactive as monomers

*require association with positive regulatory proteins, called cyclins for activity.

**cdc28p levels do not fluctuate, and are produced in excess

Cyclins

diverse family of kinases, all of which have a distinctive "cyclin box" required for binding and activation of cdks.

most, but not all, exhibit periodic accumulation.

main cyclins*

Cln1p-3p	cell cycle control: G1
Clb1p-6p	cell cycle control S, G2 M

*all these activated Cdc28p, leading to posttranslational regulation of Cdc28p activity.

The checkpoint controls

Early studies showed that certain events are linked in "dependent pathways" dna replication → no mitosis

Later studies support the idea of central cell cycle clock based on cyclin/cdk regulators

→paradox*

*How can cells maintain dependency relationships if events are triggered independently by an autonomous clock?

Paradox resolution

Possibility #1: events are mechanistically linked: completion of event #1 produces substrate for event #2.

Possibility #2: events are mechanistically unlinked, but a regulatory pathway ensures that the later event does not begin until earlier one has been completed*

*Finding of mutations and drugs that uncoupled the dependent events and permitted the second event to occur, even when the first event was blocked, provided needed evidence for possibility #2.

Looking back
cdc genes and *cdc* mutant groups

1. *cdc* genes important to the ticking of the clock: *cdc*28, *cdc*4, *cdc*34, *cdc*53, *cdc*16, etc.

2. *cdc* genes important for processes monitored by checkpoint controls: *cdc*2, *cdc*6, *cdc*7, *cdc*8, *cdc*9, etc.

3. *cdc* genes that, if mutated, cause cells to arrest in g1 in ways that mimic effects of extracellular signals: (e.g. *cdc*70 [*gpa*1, *scg*1] and *cdc*72 [nmt] pheromone response) and *cdc*35 [*cyr*1] nutritional-deprivation response.

4. *cdc* genes involved directory in morpho-genesis: *cdc*24, *cdc*42, *cdc*3, *cdc*10; with these, the uniform terminal morphology reflects the role of the gene products in bud formation and cytokinesis and not actual cell cycle arrest**.**
