Topic Introduction

Analysis of the *Schizosaccharomyces pombe* Cell Cycle

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Schizosaccharomyces pombe cells are rod shaped, and they grow by tip elongation. Growth ceases during mitosis and cell division; therefore, the length of a septated cell is a direct measure of the timing of mitotic commitment, and the length of a wild-type cell is an indicator of its position in the cell cycle. A large number of documented stage-specific changes can be used as landmarks to characterize cell cycle progression under specific experimental conditions. Conditional mutations can permanently or transiently block the cell cycle at almost any stage. Large, synchronously dividing cell populations, essential for the biochemical analysis of cell cycle events, can be generated by induction synchrony (arrest-release of a cell cycle mutant) or selection synchrony (centrifugal elutriation or lactose-gradient centrifugation). *Schizosaccharomyces pombe* cell cycle studies routinely combine particular markers, mutants, and synchronization procedures to manipulate the cycle. We describe these techniques and list key landmarks in the fission yeast mitotic cell division cycle.

ORGANIZATION AND ANALYSIS OF THE FISSION YEAST CELL CYCLE

In a typical eukaryotic mitotic cell division cycle, genome duplication in S phase is separated from its segregation in mitosis by an intervening gap phase called G₂. S phase is preceded by another gap phase called G₁. A point in G₁ phase, called START in yeasts and equivalent to the restriction point in mammalian cells, marks commitment to the cell cycle (Hartwell 1974; Pardee 1974). Cells can execute alternative fates such as conjugation or sexual differentiation between the exit of the previous cycle and transition to START. Once cells have passed START, they are committed and must complete the ongoing cell cycle before they can undergo these alternative fates. The second major decision point that determines the rate of progression through the cell division cycle governs the timing of commitment to mitosis (M phase) at the G₂/M boundary, which is the major control point in fission yeast (Nurse 1975, 1985). Under most laboratory conditions, fission yeast cells have already passed the size threshold to transit START before division. As a consequence, the next round of DNA replication is initiated before completion of the cytokinesis of the ongoing cell cycle (Mitchison and Creanor 1971a). Thus, the first control point a newly divided cell encounters is the G₂/M control, making this transition the rate-limiting step in most fission yeast cell cycles (Nurse 1975, 1985).

Growth Medium

One of the key features that makes fission yeast such a powerful system for cell cycle analysis is its ability to grow in defined synthetic media (Mitchison 1970) (see Introduction: **Growth and**

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the Environment of *Schizosaccharomyces pombe* [Petersen and Russell 2016]). Every experimental condition is therefore reproducible between experiments and laboratories. For cell cycle analysis, the medium of choice has traditionally been EMM2 (Nurse 1975); however, switching the nitrogen source from ammonium chloride to sodium glutamate to make EMMG is a useful way to expose and study how the reliance on particular component parts in the cell cycle control program changes to generate a different hierarchy of controls in different environments (Fantes and Nurse 1977). Growth in EMMG medium, which uses the poor-quality nitrogen source proline, resets START control to a size that exceeds cell size at birth. Consequently, the smallest cells in EMMG cultures are in G₁ and must grow before they can pass START (Fantes and Nurse 1977). Finally, the complete removal of nitrogen from a logarithmically dividing culture invokes a starvation response resulting in a rapid round of division followed by cell cycle arrest in G₁ before START (Egel and Egel-Mitani 1974). Cell division is not particularly synchronous when nitrogen is returned to the starved G₁ cells (see Protocol: Synchronization of S Phase in *Schizosaccharomyces pombe* Cells by Transient Exposure to M-Factor Pheromone [Nielsen 2016]); however, this asynchrony is not an issue if a phenotype can be followed by live cell imaging during the recovery from arrest. A nitrogen-starved G₁ arrest is also a useful way to delay cell cycle progression while a gene is induced or repressed by manipulation of *nmt1*-derived promoters and is a particularly effective way to block the cell cycle to allow time for slow-folding fluorescent proteins to mature into their active state to monitor the partitioning of old versus new organelles (Grallert et al. 2004).

Uniform Cell Size at Division

A second attribute that makes fission yeast such a powerful system for cell cycle studies is the uniformity of cell size at division in defined conditions (Mitchison 1970; Nurse 1975, 1985). Consequently, a cell's size is a direct indication of its position in the mitotic cell division cycle and the isolation of small cells from an asynchronous culture, by either centrifugal elutriation (see Protocol: Cell Cycle Synchronization of *Schizosaccharomyces pombe* by Centrifugal Elutriation of Small Cells [Hagan et al. 2016a]) or lactose gradient density centrifugation (see Protocol: Cell Cycle Synchronization of *Schizosaccharomyces pombe* by Lactose Gradient Centrifugation to Isolate Small Cells [Hagan et al. 2016b]), will generate a population of cells that progresses synchronously through the cell cycle. Furthermore, because cells cease elongation after entry into mitosis, the length of a septated cell is a direct indication of the timing of mitotic commitment (Mitchison 1970; Nurse 1975; Mitchison and Nurse 1985).

CELL DIVISION CYCLE (*cdc*) MUTANTS

Because cell division cycle (*cdc*) mutants (Nurse et al. 1976a; Nasmyth and Nurse 1981) remain key tools in the analysis and manipulation of the cell cycle, it is important to consider some of the basic concepts they define.

Execution Point Analysis

The original *cdc* screens identified conditional mutants in which cell division was blocked but growth continued after shifting from 25°C to 35°C. Once linkage analysis had assigned all mutations to genes, asynchronous cultures were shifted to the restrictive temperature and cell number followed to determine the transition/execution point for each gene (the point in the cell cycle at which the gene product is no longer required for completion of the cell cycle [Hartwell 1974; Nurse et al. 1976a]). When a *cdc* gene product acts toward the end of the cell cycle, most cells will not have reached this execution point at the time of shift, but will do so shortly thereafter, and the cell number stops increasing very soon after the shift. In contrast, when a gene product acts early in the cycle, most cells will have already completed the gene function at the shift and so will undergo one round of division before reaching the execution point, and cell number continues to increase for some time after the temperature shift.

There are a number of key points to consider in this context. First, if the *cdc* mutation alters the cell cycle profile at the permissive temperature, subsequent execution point analysis will not be mapped onto a wild-type cell cycle. Second, multiple alleles should be used to determine an execution point because a *cdc* phenotype means only that there is insufficient gene function to support division. The mutant protein may not have lost all, or even the majority, of its activity. In reality, most alleles are hypomorphs that are not fully functional at the permissive temperature and/or retain different levels of residual function. For example, although Cdc18 is an essential component of the replisome that is required for DNA replication, the execution point of the *cdc18-K46* allele is mid-G₂, rather than the −0.1 of a cycle in early S phase when it acts (Nasmyth and Nurse 1981). Third, the cycle arrest may arise from a checkpoint-dependent arrest in a later cell cycle stage. Thus, the G₂/M execution points of the DNA replication mutants *cdc27-K3* and *cdc1-7* advance by one-third of the cell cycle when the DNA damage checkpoint response is compromised in *wee1* mutants (Fantes 1983).

Genetic Manipulation to Generate Cultures Enriched for Distinct Cell Cycle Stages

Inactivation of *cdc* mutations by a change in conditions, typically temperature, offers a powerful way to generate cultures that are highly enriched for one particular stage of the cell cycle (Table 1). However, when designing experiments, it is important to remember that START of the next cell cycle is passed before cytokinesis is complete (Mitchison and Creanor 1971a; Nurse 1975, 1985). The execution point of *cdc10* mutations in the START transcription factor is therefore −0.1 of the cell cycle (Nurse et al. 1976a). Consequently, every cell in a temperature-sensitive *cdc10* mutant culture will divide once before cell cycle progression is arrested. This means that, in practical terms, it will take an entire generation time for cells to accumulate at START, such that a *cdc10-ts* culture in EMM2 must be held for 6 h at 36°C to generate a population of pre-START cells. As S phase immediately follows START, the same rationale applies to the addition of hydroxyurea (HU) to halt DNA replication. This contrasts with the 4.25 h used to accumulate cells at the G₂/M boundary with *cdc25-22* in EMM2.

Drug Control of Cell Cycle Progression and Reciprocal Shift Experiments

Cell cycle manipulation by the addition of drugs such as the DNA synthesis inhibitor HU, the MAP kinase inhibitor SP600125, or the microtubule poison methyl 2-benzimidazolecarbamate (MBC), or by the addition of ATP analogs to strains harboring an analog sensitive kinase, are powerful complements to *cdc* mutant manipulations (Mitchison 1974; Fantes 1982; Umeson et al. 1983; Dischinger et al. 2008; Hartmuth and Petersen 2009; Grallert et al. 2012; Aoi et al. 2014). Furthermore, the removal of a DNA synthesis inhibitor such as deoxyadenosine or HU from an arrested population can provide moderate synchronization of mitotic progression (Mitchison and Creanor 1971b; Petersen and Hagan 2003).

Induction Synchrony and Reciprocal Shift Analysis

For some *cdc* mutants, the function of the gene product is restored rapidly on return to the permissive condition. The most widely used application of the reversibility of *cdc* mutant arrest is “induction synchronization” (Mitchison and Creanor 1971b). Most commonly, *nda3-KM311* or *cdc25-22* mutants are released from an arrest to synchronize progression through mitosis and S phase (see Protocol: Synchronizing Progression of *Schizosaccharomyces pombe* Cells from Prophase through Mitosis and into S Phase with *nda3-KM311* Arrest Release [Grallert et al. 2016] and Protocol: Synchronizing Progression of *Schizosaccharomyces pombe* Cells from G₂ through Repeated Rounds of Mitosis and S Phase with *cdc25-22* Arrest Release [Hagan et al. 2016c]) (Uemura et al. 1987; Moreno et al. 1989; Tay et al. 2013). The reversibility of some mutations is also exploited in reciprocal shift experiments in which the cycle is sent from arrest at one point directly into a different arrest at another point, such as a shift from a *cdc* arrest to a drug arrest. Indeed, a simple way to determine whether a cell that has an intact DNA replication checkpoint has completed S phase is to place it onto plates containing HU and ask whether it has divided one and a half generation times later

TABLE 1. Common genetic approaches to manipulate cell cycle progression

Genotype	Genetic manipulation	Cell cycle position	Notes	References
<i>cdc10-V50</i> <i>cdc10-M17</i>	ts^- R LOF	Prestart, conjugation competent	Cells must be incubated for 6 h at restrictive temperature <i>cdc10-V50</i> is a tight allele <i>cdc10-M17</i> is used for arrest release induction synchronization Induction synchronization with <i>cdc10-M17</i> should be used with great caution as there are significant differences in the subsequent DNA damage responses to suggest that replication is abnormal (see Protocol: Synchronization of S Phase in <i>Schizosaccharomyces pombe</i> Cells by Transient Exposure to M-Factor Pheromone [Nielsen 2016]) Also known as <i>cdc30</i> Failure to ligate replicated DNA generates a late S phase defect	Marks et al. 1992; Verkade and O'Connell 1998
<i>orp1-4</i> <i>cdc17-K42</i>	ts^- LOF ts^- R LOF	Replication defective Late S phase replication checkpoint arrest at G ₂ /M boundary		Grallert and Nurse 1996 Nasmyth 1977
<i>pREP1rum1⁺</i>	OP	Repeated S phase without intervening M phase	pREP1-based, <i>nmt1⁺</i> driven overproduction	Moreno and Nurse 1994
<i>cdc13-Δ</i>	Δ LOF	Repeated S phase without intervening M phase	Important to induce plasmid loss from <i>cdc13-Δ</i> pcdc13 in EMM2-N for 17 h before adding nitrogen to see phenotype	Hayles et al. 1994
<i>cdc25-22</i>	ts^- R LOF	G ₂ /M boundary	Moderate mitotic commitment defect at 25°C means cell size at 25°C in EMM2 is ~16 μm	Thuriaux et al. 1980; Hagan and Hyams 1988; Moreno et al. 1989
<i>cdc25-22</i> <i>wee1-as8</i>	ts^- as ⁻ R LOF	G ₂ arrest at 36°C	Widely used in induction synchronization of mitosis Analog addition to inhibit Wee1-as8 gives highly synchronous mitotic and S phase progression at 36°C	Tay et al. 2013
<i>cdc2-33</i>	ts^- R LOF	25% start 75% G ₂ /M boundary	Very useful for using with temperature-sensitive mitotic or S phase mutants Very penetrant allele Useful for transient arrest release approach if a second mutation of interest has synthetic genetic interactions with <i>cdc25-22</i> If small G ₂ cells are isolated before shift, 100% G ₂ arrest Useful at 25°C, but temperature-sensitive at higher temperatures	Nurse et al. 1976a; Nurse and Bissett 1981; King and Hyams 1982; Grallert et al. 2015
<i>cdc2-as</i>	ts^- as ⁻ R LOF	Depends on [analog]	Low levels of analog G ₂ /M arrest High levels of analog START arrest Dominant/recessive not determined Useful for enriching for "mitotic" modifications of a protein of interest for mass spectrometry 30'-1 h after shift to 36°C	Dischinger et al. 2008
<i>cdc2-17</i> <i>wee1-50</i> <i>cdc2-3w</i>	ts^- LOF ts^- R LOF ts^- D GOF	Arrest at G ₂ /M boundary Constant mitotic commitment in a "mitotic catastrophe"		MacNeill et al. 1991 Russell and Nurse 1987
<i>wis1-DD</i> <i>nda3-KM311</i>	D GOF cs^- R LOF	Abbreviated G ₂ No microtubules, so no spindle formation SAC-dependent mitotic arrest (prophase/metaphase)	Hyperactive Sty1 MAP kinase signaling Tightest arrest requires 18°C in EMM2 or 20°C in YES Very useful for reciprocal shift analysis with <i>ts^-</i> mutants (Many genes can mutate to promote microtubule stability. Consequently, <i>nda3-KM311</i> strains quickly accumulate suppressors that compromise the penetrance of the block, so always work with a strain that had been freshly isolated from -80°C and never store in at 4°C or passage strains in the incubator. Backcross if the arrest weakens.) <i>ark1-as3</i> inhibition of the checkpoint in <i>nda3-KM311</i> to promote mitotic exit without spindle formation	Shiozaki et al. 1998 Hiraoka et al. 1984; Uemura et al. 1987
<i>nda3-KM311</i> <i>ark1-as3</i>	cs^- R LOF as ⁻ R LOF	SAC recovery		Vanoosthuysse and Hardwick 2009

(continued)

Table 1. Continued

Genotype	Genetic manipulation	Cell cycle position	Notes	References
<i>nda3-KM311 + CBZ 36°C</i>	cs ⁻ R LOF drug	Lack of microtubules maintained on shift to 36°C	Useful to assess ability of ts ⁻ mutants to maintain checkpoint	Murone and Simanis 1996
<i>cut7-24</i>	ts ⁻ R LOF	No spindle formation (prophase/metaphase)	Tight recessive allele	Hagan and Yanagida 1990
<i>plo1-ts41</i>	ts ⁻ R LOF	No spindle formation (prophase/metaphase)	Very penetrant loss of function mutation in polo kinase	Grallert et al. 2013
<i>cut4-533 cut9-665</i>	ts ⁻ R LOF	No polo activity Metaphase arrest No SAC activity	Loss of function in APC/C Double mutant tighter than either single alone	Hirano et al. 1986; Samejima and Yanagida 1994; Yamashita et al. 1996; Heitz et al. 2001 Petrova et al. 2013
<i>nmix1slp1⁺</i>	SO	Metaphase arrest No substrate delivery to APC/C	Challenging to combine with other mutations as spore viability is very low	
<i>pREP1mad2⁺</i>	OP	Metaphase arrest hyperactive SAC	Multicopy <i>nmt1⁺</i> driven, overproduction	He et al. 1997
<i>mts3-1</i>	ts ⁻ R LOF	Metaphase arrest	Proteosome deficiency in cyclin destruction blocks metaphase anaphase transition	Gordon et al. 1993
<i>cut8-563</i>	ts ⁻ R LOF	Metaphase arrest	Not very penetrant Proteosome location defect that severely compromises the metaphase-anaphase transition	Tatebe and Yanagida 2000
<i>spg1-B8 cdc7-A20</i>	ts ⁻ R LOF	No SIN	Individually these SIN mutants are mildly hypomorphic; together they give a very robust arrest	Fankhauser and Simanis 1994; Schmidt et al. 1997; Tanaka et al. 2001
<i>cdc16-116</i>	ts ⁻ R LOF	Hyperactive SIN	Very useful for dissociating SIN activation from mitosis according to Cerutti and Simanis (1999)	Minet et al. 1981; Cerutti and Simanis 1999
<i>cdc16::ura4⁺ cdc7-A20</i>	ts ⁻ / cs ⁻ , LOF	"Tunable" SIN	Cells viable at 32°C, but hyperactive SIN when temperature is dropped to 25°C or less, and no SIN at 36°C	Fournier et al. 2001
<i>cdc15-140</i>	ts ⁻ , R, LOF	Cytokinesis checkpoint active	Cells arrest transiently (1 cell cycle) binucleated	Nurse et al. 1976b; Liu et al. 2000;
<i>cdc3-6</i>			No septum in 15, 3, and 12 give disperse septum deposition Wee1-dependent checkpoint	Utzig et al. 2000
<i>cdc12-112</i>			β-glucan synthase mutation that activates the cytokinesis checkpoint to arrest cells in interphase: S-phase is completed, but the next mitosis is blocked	Le Goff et al. 1999; Liu et al. 2000
<i>cps1-191</i>	ts ⁻ R LOF	Cytokinesis checkpoint active	F-actin ring persists; spindle is disassembled Lacks an anilin related core structural component of actin ring	
<i>mid1-Δ</i>	Δ LOF	Misplaced and misshapen septum		Sohrmann et al. 1996
<i>plo1-ts8</i>	ts ⁻ R LOF	Misplaced and misshapen septum	Very poorly penetrant <i>plo1</i> allele Normal spindle formation; misplaced septum at 36°C	MacIver et al. 2003a
<i>mto1-Δ</i>	Δ LOF		No postanaphase array of microtubules	Venkatram et al. 2004; Samejima et al. 2005

APC/C: anaphase promoting complex/cyclosome; as⁻: analog sensitive; cs⁻: cold sensitive; D: dominant; EMM2: Edinburgh Minimal Medium 2; EMM2-N: EMM2 without nitrogen; GOF: gain of function; LOF: loss of function; OP: overproduction; R: recessive; SIN: septum initiation network; SO: shut off; ts⁻: temperature sensitive; Δ: deletion.

TABLE 2. Cell cycle markers for fixed cell microscopy

Event	Marker	Application	References
DNA	Histone H2A-RFP	<i>hta 1⁺</i> tagged at integrated at <i>leu1</i> locus	Nakazawa et al. 2011
DNA replication	Mik1-mycHis	Nuclear fluorescence identifies S phase cells	Christensen et al. 2000
DNA replication	Mcm4-GFP, Cdt1-YFP	Need to extract soluble pools of protein before fixation	Kearsey et al. 2000; Ralph et al. 2006
DNA replication	Edu (5-ethynyl-2'-deoxyuridine)	Need to express human equilibrative nucleoside transporter 1 (hENT1) in host strain. The Click-iT Edu Alexa-Fluor kit currently offers the most convenient detection approach	Sivakumar et al. 2004; Coudreuse and Nurse 2010
Late G ₂	Calcofluor White/Blankophor/Fluorescent Brightener 28	Activation of growth at new cell end: NETO	Mitchison and Nurse 1985
Late G ₂	Plo1 antibodies and tag	Plo1 recruitment to late G ₂ SPB	Mulvihill et al. 1999; Grallert et al. 2013
Late G ₂	Commercial MPM2 monoclonal antibody	Plo1 kinase activation on G ₂ SPB	MacIver et al. 2003b
Mitotic commitment	Cut3-3HA6His	Nuclear import of Cut3 is controlled by Cdc2-Cdc13 activation	Sutani et al. 1999
Mitotic commitment	Commercial Histone H3 S10 phospho-specific antibody	Histone H3 phosphorylation by Aurora kinase Ark1 is a very early mitotic event	Petersen et al. 2001
Spindle formation	TAT1 anti-alpha tubulin antibodies	Spindle microtubules	Hagan and Hyams 1988; Hagan and Yanagida 1990
Spindle formation	Sad1 antibodies or tag	SPB separation	Hagan and Yanagida 1995
Spindle formation	Cut12 antibodies or tag	SPB separation	Bridge et al. 1998
Spindle formation	Sid4-HA	SPB separation	Chang and Gould 2000
Metaphase anaphase transition	Cdc13 antibodies	Loss of Cdc13 signal	Alia et al. 1990
Metaphase anaphase transition	Cut2-HA	Loss of Cut2 nuclear fluorescence	Funabiki et al. 1996
Metaphase anaphase transition	Cnp1-HA	Centromere splitting	Takahashi et al. 2000
Spindle assembly checkpoint activation	Bub1-6HA	Tag partially compromises SAC activity	Bernard et al. 1998
Anaphase B SIN activity	Cdc7-HA	Asymmetric SPB association in anaphase B	Sohrmann et al. 1998
Early cytokinesis	Phalloidin and antibodies	Actin ring formation	Marks and Hyams 1985; Marks et al. 1986
Late cytokinesis	MPM2	8% fixation reveals phosphorylation of actin ring components	MacIver et al. 2003b
Septation	Calcofluor White/Blankophor/Fluorescent Brightener 28		Mitchison and Nurse 1985
DNA	DAPI	2.5% glutaraldehyde fixation reveals detail of chromosome structure better than formaldehyde-based approaches	Toda et al. 1981
Nucleolus	Propidium iodide		
Nucleolus	Nuc1 antibodies	RNA polymerase I large subunit	Hirano et al. 1989

NETO: new-end takeoff (activation of the dormant growth zone in G₂ phase [Mitchison and Nurse 1985]); SAC: spindle assembly checkpoint; SPB: spindle pole body.

TABLE 3. Cell cycle markers for live cell microscopy

Structure	Marker	Application/Comment	References
Chromatin	Histone H3-mRFP, Histone H3-CFP	Tagging at native <i>hht1</i> ⁺ locus	Tomita and Cooper 2007
DNA	Hoescht33258	More permeable than the 33342 that was used by Chikashige et al. (1994) The short wavelength required for excitation can cause damage	Chikashige et al. 1994
Late G ₂	Plb1-GFP	Recruited to SPB in late G ₂	Bähler et al. 1998; Grallert et al. 2013
Mitotic commitment	Cnd2-GFP or Cut3-GFP	Nuclear accumulation of condensin is controlled by Cdc2/Cyclin B activation	Sutani et al. 1999; Aono et al. 2002
Mitotic commitment	Atb2-GFP Atb2-RFP, Atb2-Cherry	Tubulin distribution reveals spindle formation	Unsworth et al. 2008; Snaith et al. 2010
Spindle formation	Sid4-CFP, Sid4-GFP, Sid4-tdTom	SPB separation	Tomlin et al. 2002; Grallert et al. 2013
Spindle formation	Sad1-DsRed, Sad1-CFP	SPB separation	Chikashige and Hiraoka 2001; Tanaka et al. 2005
SAC activation	Mad3-GFP, Mad2-YFP, Mad2-GFP	Kinetochore fluorescence	Garcia et al. 2002; Ikui et al. 2002; Millband and Hardwick 2002
SAC activation	Bub1-GFP	Kinetochore fluorescence	Toyoda et al. 2002; Yamaguchi et al. 2003
Metaphase anaphase transition	Cut2-GFP, Cdc13-GFP	APC/C-dependent loss of nuclear fluorescence	Tatebe and Yanagida 2000; Decottingnies et al. 2001
Metaphase/anaphase transition	Centromeric LacO-GFP	Centromere splitting	Nabeshima et al. 1998
Metaphase/anaphase transition	Cnp1-GFP Cnp1-Cherry	256 LacO repeats inserted at <i>lys1</i> ⁺ 30 kb from <i>cenI</i> LacI-NLS-GFP integrated at <i>his7</i> ⁺	Takahashi et al. 2000; Alvarez-Tabares et al. 2007
Anaphase B spindle mid-zone	Klp9-GFP	Centromere splitting	Fu et al. 2009
Anaphase B	Nsk1-GFP	Anaphase B mid-zone	Buttrick et al. 2011; Chen et al. 2011
Anaphase B SIN activity	Cdc7-GFP	Associates with interface between kinetochores and SPBs on Cdc13 destruction	Sohrmann et al. 1998
Nucleolus	Gar2-GFP	Asymmetry of SPB signal	Win et al. 2006
Telophase/mitotic exit	Atb2-GFP Atb2-RFP, Atb2-Cherry	Splitting of nucleolus	Unsworth et al. 2008; Snaith et al. 2010
Telophase/mitotic exit	Mto2-YFP, Mto2-GFP, Mto1-CFP	Appearance of postanaphase arrays	Savin et al. 2004; Samejima et al. 2005
Contractile actin ring	pnmt41-GFP CHD	Formation of EMTOC Only a small window after induction in which to perform experiments, as higher expression of GFP-CHD stabilizes actin structures	Karagiannis et al. 2005; Martin and Chang 2006; Wu et al. 2006
Contractile actin ring	LifeAct	F-actin	Huang et al. 2012
Contractile actin ring	utrophin (Utr)-CH	F-actin	Huang et al. 2012
Contractile actin ring	Rlc1-GFP	Good signal at all temperatures Tendency to form aggregates at 36 °C	Le Goff et al. 2000; Naqvi et al. 2000
Contractile actin ring	Mid1-GFP	Hard to see nuclear fluorescence at higher temperatures; best viewed at 19 °C Ring fluorescence is more robust	Paoletti and Chang 2000
Late cytokinesis	Mid2-GFP		Berlin et al. 2003; Tasto et al. 2003
Late cytokinesis	Septin	Spn1	Wu et al. 2003

APC/C: anaphase promoting complex/cyclosome; EMTOC: equatorial microtubule organizing center (Heitz et al. 2001); SPB: spindle pole body.

TABLE 4. Cell cycle markers: Landmark changes

Molecule	Cell cycle phase	Change	Comments	References
Mik1-mycHis	S phase	Destruction	Protein levels peak in S phase	Christensen et al. 2000
Cdc18	G ₁ -S	Phosphorylation	Target of Cdc10 transcription factor	Jallepalli et al. 1997
Cdc11-HA	M-CK	Phosphorylation	Peaks after anaphase onset	Krapp et al. 2001
Histone H2A mRNA	S-phase	Peaks	Sharp S-phase peak	White et al. 1986
<i>cdc18⁺</i> mRNA	G ₁ -S	Peaks	Sharp S-phase peak	Kelly et al. 1993
<i>cdc15⁺</i> mRNA	M-G ₁	Peaks	Sharp early M peak	Fankhauser et al. 1995; Utzig et al. 2000
Cdc15p	M-G ₁	Dephosphorylation	After M entry, rephosphorylated at ring contraction	Fankhauser et al. 1995; Utzig et al. 2000
<i>plo1⁺</i> mRNA	M-G ₁	Peaks	Early M peak	Anderson et al. 2002
Mid1	Mitosis	Phosphorylation	High when a spindle is present	Sohrmann et al. 1996
Cut9-HA	Early mitosis	Phosphorylated from mitotic commitment to anaphase		Tay et al. 2013
Cdc13	Early mitosis	Destruction at metaphase anaphase transition	Destruction incomplete	Booher et al. 1989; Hayles and Nurse 1995
Histone H3	Early mitosis	Phosphorylation on serine 10	Commercial antibody available	Petersen et al. 2001
Histone H1 kinase activity	Early mitosis	Enhanced from entry to anaphase	Addition of Histone H1 to crude cell extracts	Moreno et al. 1989
Histone H1 kinase activity	Early mitosis	Enhanced from entry to anaphase	p13 Sepharose precipitated Cdc2-Cyclin B complexes	Booher et al. 1989

(Broek et al. 1991). If S phase was complete at the time of plating, it will have divided and then arrested; if not, it will still be a single cell.

Pheromone Response

Advances in peptide synthesis have permitted the development of a highly effective protocol for releasing cells from a G₁ phase arrest induced by exposure to M factor (see Protocol: **Synchronization of S phase in *Schizosaccharomyces pombe* Cells by Transient Exposure to M-Factor Pheromone** [Nielsen 2016]).

It is important to remember that induction synchronization, selection synchronization by centrifugation, and release from M factor arrest are each associated with their own artifacts. When identical results are obtained with different induction or selection synchronization procedures, they probably reflect genuine changes resulting from cell cycle progression or its controls.

TABLE 5. Molecules whose levels do not change with cell cycle progression

Molecule	Molecular mass (kDa)	Antibody	Notes	References
Cdc2	34	PN24 or commercial monoclonal antibody (e.g., ab5467 from Abcam)		Lee and Nurse 1987; Hayles and Nurse 1995
α -tubulins (Nda2, Atb2)	50	TAT-1	The most widely used loading control High protein levels make it unreliable for accurate assessment of fine changes	Woods et al. 1992
Plo1	75	HN184		Mulvihill et al. 1999
Sid2	78			Grallert et al. 2012
Cdc10	87			Simanis and Nurse 1989
Plo1-GFP	110	HN184 or any GFP antibody		Mulvihill et al. 1999

CELL CYCLE MARKERS

An accurate map is key to negotiating any unknown territory. Key landmarks on the fission yeast cell cycle map for fixed and live cell imaging are listed in Tables 2 and 3, while reference molecules for studying changes at the molecular level are listed in Tables 4 and 5. An accurate measure of the timing and efficiency of DNA replication can be gauged by measuring DNA content using the method described in Protocol: **Analyzing *Schizosaccharomyces pombe* DNA Content by Flow Cytometry** (Boye et al. 2016).

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