CHAPTER 1

Introduction to Fission Yeast as a Model System

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Here, we briefly outline the history of fission yeast, its life cycle, and aspects of its biology that make it a useful model organism for studying problems of eukaryotic molecular and cell biology.

INTRODUCTION

Over the last half century, the fission yeast *Schizosaccharomyces pombe* has been used increasingly as a model organism for investigating eukaryotic cellular and molecular processes. This has been driven mainly by the development of new investigative methodologies and the fact that several biological processes conserved in *S. pombe* and in other eukaryotes have been lost or adapted in the model budding yeast *Saccharomyces cerevisiae*. *S. pombe* has been used as a laboratory organism since the 1950s, but until the early 1970s, only approximately 15 laboratories throughout the world worked with fission yeast, publishing approximately 20 papers each year. Now there are more than 300 fission yeast laboratories worldwide, with more than 400 papers published each year (Fig. 1A). The amount of new information about fission yeast has also significantly increased over the past 10 years (as indicated by the number of annotations per paper; see Fig. 1B and http://www.pombase.org/).

In this Introduction, we provide a short history of fission yeast as a model organism, and to give a sense of the type of work that is now possible with this model eukaryote, we summarize some of the specific cellular and molecular processes that have been studied more extensively in fission yeast. We refer to other contributions in this collection for more detail and for descriptions of protocols covering different techniques and procedures that can be used to study this organism.

HISTORY

S. pombe was initially observed and isolated by Saare and colleagues from contaminated millet beer, which had been delayed on the journey from East Africa to Germany. A pure culture of *S. pombe* was isolated by Ziedler, under the supervision of Paul Lindner who was the first person to describe it in detail, in the early 1890s. Lindner named it *Schizosaccharomyces* to distinguish it from budding yeast (because it reproduces vegetatively by means of fission) and *pombe* after the Swahili word for beer (Lindner 1893). A translation of this report is available at http://www.bcf.usc.edu/~forsburg/. Lindner's illustrations showed fission yeast to be a single-celled rod-shaped organism that formed ascospores and was able to undergo filamentous growth.

S. pombe has proven to be an excellent laboratory model organism. But does it make good beer? Well, the travel writer Tim Cahill (Cahill 1996) has tasted *pombe*, and this is what he thought about it.

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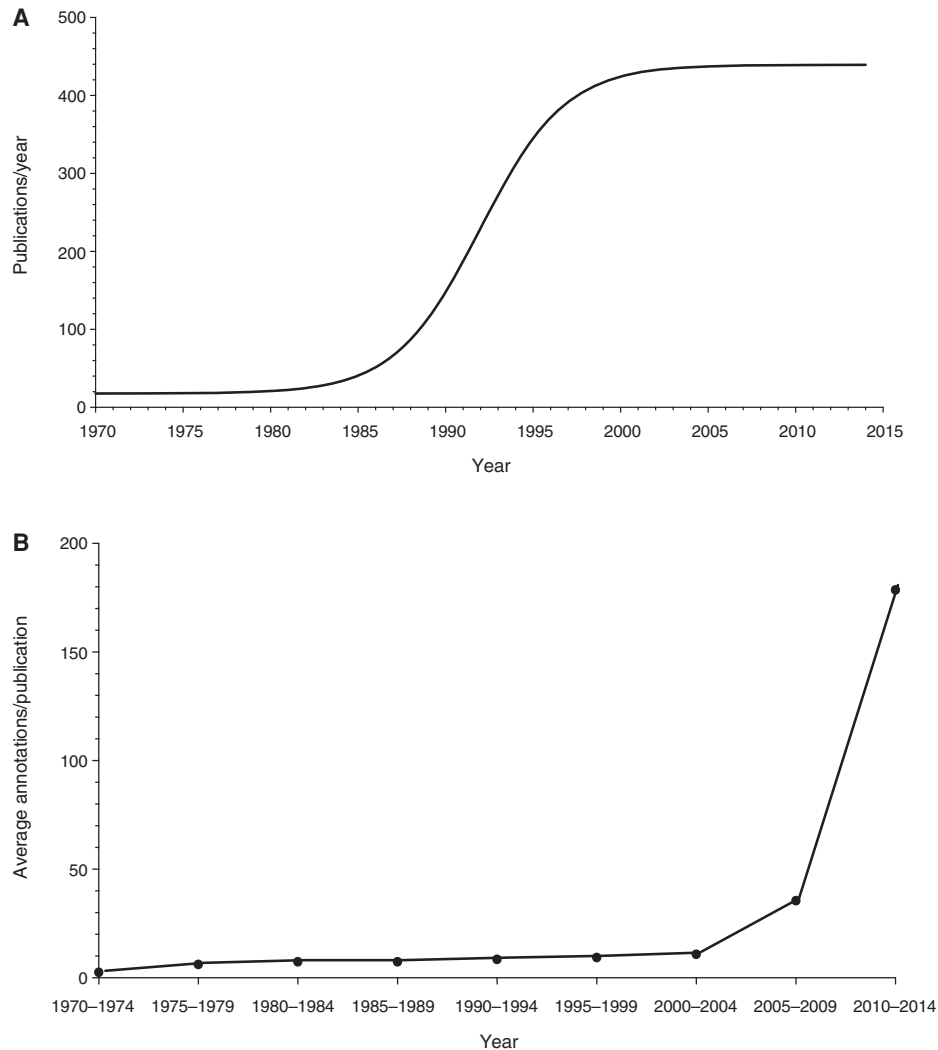
FIGURE 1. Numbers of fission yeast publications and annotations in the past 45 yr. (*A*) Number of fission yeast publications per year (not cumulative) since 1970. (*B*) Average number of annotations extracted per paper per 5-yr period since 1970 (using the same annotation procedures). An annotation is a piece or unit of information that connects a gene to a defined term from an ontology such as Gene Ontology (GO) and that includes supporting evidence attributed to a source (preferably, and usually, a published paper).

> In central Africa, under the Virunga volcanoes, people make a kind of banana beer they call *pombe* that is served in one liter brown glass bottles that once contained beer. *Pombe* simply means beer in Swahili, but I was cautioned about this banana variety: don't pour it into a glass, said the brewer himself; you don't want to actually see it.
>
> The *pombe* is best drunk with a wooden straw. This is because the fermenting bananas leave a thick layer of black sludge on the glass. I've since learned that, in the final brewing process, the beer can be filtered through a fine cloth. I'm thinking that my brewer may have found that process superfluous.

In the interests of science, Paul Nurse decided to visit a jungle brewery in Uganda, near the Virunga volcanoes, to taste *pombe* for himself. He trekked into the jungle and located an open-air banana brewery in a village near the Virunga foothills. He found the beer to be certainly particulate but not black. The taste was sweet and very alcoholic but definitely drinkable. However, the distilled version looked decidedly safer. So draw your own conclusions.

The establishment of fission yeast as an experimental laboratory organism came with Urs Leupold who, as his PhD project in the 1940s, developed *S. pombe* for genetic analysis. The strains he derived

are not from the Lindner strain and are thought to be from a strain deposited in the yeast collection in Delft, the Netherlands in 1924, under the name *S. pombe* var. *liquefaciens*. Osterwalder had isolated this strain from rancid wine at the Federal Experimental Station of Vini- and Horticulture in Wädenswil, Switzerland.

Leupold isolated two homothallic strains, h^{90} (968) and h^{40} (which has since been lost), and two heterothallic strains with opposite mating types, h^- and h^+, thus allowing the development of classical genetic procedures (Leupold 1950). There are several heterothallic strains with different genomic configurations at the mating type locus, but the heterothallic strains commonly used in the laboratory are h^{+N} (975) and h^{-S} (972) (Egel 1989). From the 1950s until the 1970s, genetic studies were carried out in several laboratories including those of Urs Leupold, Jürg Kohli, and Peter Munz in Bern working mainly on genetics (Munz and Leupold 1970; Kohli et al. 1977) and Murdoch Mitchison, in Edinburgh, studying cell biology, particularly the cell cycle (Mitchison 1957). Mitchison chose fission yeast because it was rod-shaped and grew by tip elongation so that the length of the cells reflected cell cycle progression, with the longest cells about to enter mitosis and shortest cells having just septated at the beginning of the cell cycle. The two fields of genetics and cell biology were brought together in Murdoch Mitchison's laboratory in the mid-1970s, by a group of postdocs and graduate students including Peter Fantes, Kim Nasmyth, Paul Nurse, and Pierre Thuriaux who used fission yeast genetics to study the cell cycle. Other laboratories working early on with fission yeast included Chris Bostock, Colin Clarke, Richard Egel, Herbert Gutz, Brian Johnson, Anwar Nasim, Carl Robinow, and Eva Streiblova. A little later fission yeast work started in Japan with Chikashi Shimoda, Masayuki Yamamoto, and Mitsuhiro Yanagida.

Fission yeast is a member of a small clade currently consisting of only four *Schizosaccharomyces* species, *S. pombe*, *S. japonicus*, *S. cryophilus*, and *S. octosporus*. All four species have been sequenced, and *S. japonicus*, in particular, is now being developed as a second model organism (see Furuya and Niki 2009 and Chapter 18 Introduction: *Schizosaccharomyces japonicus*: A Distinct Dimorphic Yeast among the Fission Yeast [Aoki et al. 2016]). An understanding of the evolutionary history of *Schizosaccharomyces* is a current topic of research with comparative studies between these different species (Rhind et al. 2011) and between wild *S. pombe* strains collected from around the world, particularly from vineyards and distilleries (Brown et al. 2011; Jeffares et al. 2015). The global dispersal of *S. pombe* strains is thought to have started around 340 BCE and was probably associated with the spreading of technologies such as brewing. It reached the New World sometime between 1422 and 1752 CE, which is coincident with, and likely to be the result of, the European colonization of the Americas (Jeffares et al. 2015). *Schizosaccharomyces* is thought to have diverged from budding yeast hundreds of million years ago and, at the molecular level, is as closely related to humans as it is to budding yeast (Hedges 2002).

LIFE CYCLE

Fission yeast is a rod-shaped unicellular eukaryote that is ~7–14 μm in length and ~4 μm wide. It grows by tip elongation and divides by medial fission. It has a rapid life cycle with a generation time in vegetative growth of 2–4 h at 36°C–25°C in complex and minimal media (see Chapter 2 Introduction: Growth and the Environment of *Schizosaccharomyces pombe* [Petersen and Russell 2016]). Cells continue to undergo mitotic cell cycles until nutrients become limiting and, as growth ceases, they enter the stationary phase (see Chapter 12 Introduction: Analysis of the *Schizosaccharomyces pombe* Cell Cycle [Hagan et al. 2016]). In appropriate conditions fission yeast cells produce mating pheromones; h^+ cells produce P factor and h^- cells produce M factor. If nitrogen levels become limiting, cells accumulate in G_1 and, in response to mating pheromones, cells of the opposite mating type conjugate and undergo nuclear fusion to form a diploid zygote. This zygote proceeds to premeiotic S phase followed by two successive meiotic nuclear divisions to form four haploid spores (see Chapter 11 Introduction: Analysis of *Schizosaccharomyces pombe* Meiosis [Yamashita et al. 2016]). If nutrients are restored, these spores will germinate and re-enter the mitotic cell cycle. *S. pombe* is normally a haploid organism, however, at the diploid zygote stage, if cells are not committed to the meiotic programme and nitrogen levels are increased, they reenter vegetative growth as diploids (Fig. 2C). Thus *S. pombe* can be studied either as a haploid or a diploid organism.

WHY IS *S. pombe* A GOOD MODEL EUKARYOTE?

S. pombe has excellent classical and molecular genetics, which has enabled the isolation of many mutants to study specific cell and molecular biological processes. In Chapter 3 Introduction: Genetics Analysis of *Schizosaccharomyces pombe* (Ekwall and Thon 2016), Karl Ekwall and Geneviève Thon introduce the basic classical genetic protocols and describe how these are used to isolate mutants in processes of interest, and in Chapter 4 Introduction: Molecular Genetic Tools and Techniques in Fission Yeast (Murray et al. 2016), Johanne Murray and colleagues describe the basic molecular genetic techniques available for research. *S. pombe* was the fourth eukaryote to be sequenced, and its 14-Mb genome currently has 5059 protein-coding genes annotated, of which 67% are conserved in humans (http://www.pombase.org) (Wood et al. 2002). The sequencing and annotation of the *S. pombe* genome paved the way for the development of a range of methodologies for genome-wide analyses in fission yeast (see Chapter 16 Introduction: High-Throughput Quantitative Genetic Interaction Mapping in the Fission Yeast *Schizosaccharomyces pombe* [Roguev et al. 2016], Chapter 7 Introduction: SILAC Technology in Fission Yeast [Maček et al. 2016], and Chapter 6 Introduction: Elementary Protein Analysis in *Schizosaccharomyces pombe* [Grallert and Hagan 2016]).

  S. pombe has a typical eukaryotic cell cycle, shows a highly polarized growth pattern, and has a defined shape, features that make it very useful for studying processes such as the mitotic and meiotic cell cycles, cell shape, and cellular growth. It can also be used for the study of developmental switches in response to environmental cues such as those that control the transition between the vegetative and sexual life cycles. Although of a similar size to the budding yeast genome, the *S. pombe* genome consists of only three chromosomes of 5.6, 4.8, and 3.6 Mb, respectively, for Chromosomes I, II, and III compared with the 16 chromosomes in budding yeast. Genome organization in *S. pombe* shows several features also found in higher eukaryotes: Compared with budding yeast, the three chromosomes show more extensive chromosome condensation during mitosis (Robinow 1977; Hiraoka et al. 1984; Petrova et al. 2013); the telomeres, centromeres, and origins of replication are more similar to complex eukaryotes than is the case for budding yeast (Wood et al. 2002); and heterochromatin can be formed by both RNAi-dependent and RNAi-independent pathways (see Reyes-Turcu and Grewal (2012) and Chapter 14 Introduction: Analysis of Heterochromatin in *Schizosaccharomyces pombe* [Cam and Whitehall 2016]). The process of splicing also appears to be more similar to splicing in human cells. Nearly 50% of fission yeast genes have at least one intron, and in total there are 5300 introns in 2510 protein-coding genes (http://www.pombase.org/status/statistics). By comparison, in budding yeast there are only 344 genes with a total of 376 introns in the genome as a whole (www.yeastgenome.org/).

Mitotic Cell Cycle

The *S. pombe* mitotic cell cycle is separated into the G_1, S, G_2, and M phases (Fig. 2A; see Chapter 12 Introduction: Analysis of the *Schizosaccharomyces pombe* Cell Cycle [Hagan et al. 2016]). Nuclear division is normally followed by cell division generated by the formation of a septum to give two equally sized daughter cells, each containing a complete copy of the genome. There are two major controls regulating progress through the cell cycle, the G_1–S transition and the G_2–M transition. The highly conserved cyclin-dependent serine/threonine protein kinase CDK1 (Cdc2 in fission yeast), which forms a complex with a regulatory cyclin subunit, is required at both these control points for the timing and size at which cells undergo these transitions. During the fission yeast cell cycle, the major size control occurs at the G_2–M transition, and the G_1–S control is cryptic, as newly divided cells are of greater cell size than required to undergo the G_1–S transition. However, mutants that enter mitosis and divide at a small cell size to generate newborn cells of smaller than the size requirement for the G_1–S transition have a longer G_1 phase before entering S phase (Fig. 2A,B; Nurse and Thuriaux 1980).

  Cdc2 activity appears at the G_1–S transition, reaches a peak as cells enter mitosis, and is reduced to a low level during anaphase and G_1. Cdc2 forms a complex with a number of different cyclins (Puc1,

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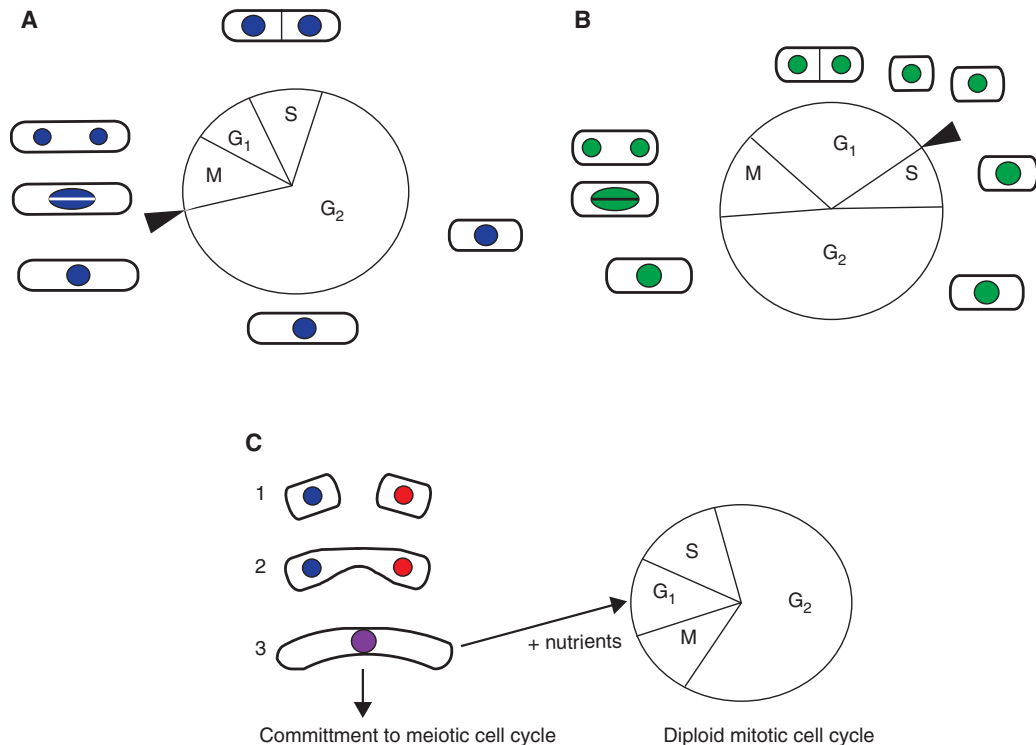
FIGURE 2. The *S. pombe* mitotic cell cycle. (*A*) A wild-type haploid mitotic cell cycle. In wild-type cells, the G_1–S transition control is normally cryptic. Cells are of greater cell size than the size requirement for the G_1–S transition after cell division and thus spend minimal time in G_1. Progress through the cell cycle is regulated at the major control point indicated by the black arrowhead. (*B*) A *wee* mutant mitotic cell cycle. Cells that enter mitosis at a small cell size (*wee* mutants) may be below the size requirement for the G_1–S transition after cell division. These small cells need to grow to reach the size requirement for the G_1–S transition before they can enter S phase; therefore, they spend more time in G_1 and a shorter time in G_2 compared with wild-type cells. Progress through the cell cycle is regulated at the major control point, which is indicated by the black arrowhead. (*C*) The formation of diploids. This occurs in three stages: (*1*) cells of opposite mating types arrest in G_1 under conditions of low nitrogen; (*2*) cells conjugate in response to mating pheromones; and (*3*) the nuclei of the mating cells fuse to form a diploid zygotic ascus. If nutrients are provided at this stage, the diploid zygote returns to the mitotic cell cycle as a diploid h$^+$/h$^-$ cell. If nutrients are not increased and the *mei2* gene is activated, the zygote becomes committed to the meiotic cell cycle.

Cig1, Cig2, and Cdc13) at different stages of the cell cycle. Cdc13 (cyclin B) is the only essential cyclin in fission yeast and cells with only the Cdc2–Cdc13 complex present and lacking the other cyclins can undergo an apparently normal cell cycle, suggesting that, rather than cyclin-dependent substrate specificity, a properly regulated pattern of Cdc2 kinase activity is all that is required to bring about normal progression through the cell cycle (Fisher and Nurse 1996; Coudreuse and Nurse 2010). The activity of the Cdc2–Cdc13 complex during the cell cycle is inhibited by Cdc2 tyrosine15 (Y15) phosphorylation brought about mainly by the Wee1 kinase (Gould and Nurse 1989). A low Cdc2 kinase activity is sufficient to bring about S phase, whereas high levels are required to undergo mitosis and cell division. At G_2–M, Y15 dephosphorylation by the phosphatase Cdc25 dramatically increases the kinase activity of Cdc2–Cdc13, and cells enter mitosis (Moser and Russell 2000; Morgan 2006).

As cells enter mitosis interphase microtubules are disassembled (Hagan and Hyams 1988; Sato and Toda 2010), the duplicated spindle pole body (SPB) inserts into the nuclear membrane (Ding et al. 1997), and daughter SPBs separate to generate a mitotic spindle. Partially condensed chromosomes become aligned on the spindle through attachment at the kinetochore (Hiraoka et al. 1984; Sanchez-Perez et al. 2005; Tang and Toda 2013), and an actinomyosin band forms over the nucleus defining the future site of cytokinesis (Lee et al. 2012). Once correctly aligned, sister chromatids segregate to opposite poles of the elongating spindle, followed by spindle breakdown (Watanabe 2010; Hsu and

Toda 2011). The nuclear envelope remains intact during mitosis, and the nucleus elongates and divides to form two daughter nuclei (McCully and Robinow 1971; Ding et al. 1997). The actinomyosin band forms a ring at the cell center between the two daughter nuclei, and as it contracts a septum is formed, which is then degraded to bring about cytokinesis and cell separation (Krapp and Simanis 2008; Gupta et al. 2014; Stachowiak et al. 2014). The inactivation of Cdc2 at the metaphase anaphase transition allows both exit from mitosis and the formation of prereplicative complexes required for the initiation of DNA replication (Stern and Nurse 1996; Dischinger et al. 2008). As Cdc2 kinase activity starts to rise again, cells undergo the G_1–S transition and DNA replication is initiated, starting the cell cycle again.

Growth and Morphology

Fission yeast cells normally grow until they reach a length of ~14 μm before undergoing the G_2–M transition. The size at which cells enter mitosis is modulated by environmental cues such as nutrient levels and stress (Fantes and Nurse 1977). In poor nutrient conditions, cells divide at a smaller size than in nutrient-rich media. This size modulation occurs through the MAP (mitogen-activated protein) kinase and TOR (target of rapamycin) signaling pathways acting on Cdc25 (Hartmuth and Petersen 2009) (see Chapter 2 Introduction: Growth and the Environment of *Schizosaccharomyces pombe* [Petersen and Russell 2016]). Cell size at mitosis is also affected by the Pom1 pathway, which acts through Wee1, bringing about Cdc2 Y15 phosphorylation to prevent entry into mitosis (Young and Fantes 1987; Martin and Berthelot-Grosjean 2009; Moseley et al. 2009). There is cross talk between these two pathways, and controls acting independently of Cdc2Y15 phosphorylation also affect the timing of entry into mitosis (Navarro and Nurse 2012).

Growth at the cell ends is also regulated during the cell cycle. As cells enter mitosis, cell growth ceases as actin is delocalized from the growing ends and becomes localized at the cell center (Marks et al. 1986). Actin in newly divided daughter cells becomes relocated to the end that was present in the mother cell (old end), and growth is initiated here. Later in the cell cycle, actin also becomes localized at the new end, formed at septation, resulting in initiation of growth at this site; this is known as new-end takeoff (NETO) (Mitchison and Nurse 1985). During a normal cell cycle, NETO is dependent on attainment of both a certain cell size and DNA replication. The significance of this growth pattern is unclear but may, for example, be important for linking the DNA replication checkpoint and micro-tubule-dependent polarized growth (Kume et al. 2011), ensuring both daughter cells obtain one previously growing end or to prevent h[90] sister cells mating with each other (Merlini et al. 2013). Fission yeast cells mate in G_1 when they only have one growth zone at the old end and thus are more likely to mate with another cell rather than with their sister.

Because it is rod shaped and has this highly polarized growth pattern, *S. pombe* is an excellent model for studying cell morphology. The identification of a range of shape mutants and the development of excellent imaging techniques has been instrumental to our understanding of how cell shape is generated and maintained (see Chapter 8 Introduction: Electron Microscopy of Fission Yeast [McIntosh et al. 2016], Chapter 9 Introduction: Fixed Cell Imaging of *Schizosaccharomyces pombe* [Hagan and Bagley 2016], and Chapter 10 Introduction: Live Cell Imaging in Fission Yeast [Mulvihill 2016]). Many of the shape mutants have defects in microtubule organization and the positioning and size of growth zones (Chang and Martin 2009; Piel and Tran 2009; Hayles et al. 2013; Mishra et al. 2014). The interphase microtubule cytoskeleton consists of three to four bundles of microtubules orientated with the plus ends toward the cell tips and the minus ends overlapping at the cell center (Foethke et al. 2009). These microtubule bundles are important for transporting factors to the cell ends to ensure growth along the long axis of the cell, thus maintaining a rod shape (Brunner and Nurse 2000; Terenna et al. 2008; Chang and Martin 2009). Defects in the cell wall structure can also lead to cell shape defects (Calonge et al. 2000), suggesting that the cell wall and the cytoskeleton interact to determine the overall shape of the cell. For information about the cell wall structure and reliable methods for rapid analysis of cell wall polymers, see Chapter 15 Introduction: Fission Yeast Cell Wall Analysis (Pérez and Ribas 2016).

Mating and the Meiotic Cell Cycle

Fission yeast is also an excellent model for meiosis, and the transition from mitotic to meiotic cycles is a straightforward example of cellular differentiation in a highly malleable model system. The decision to enter the mitotic cell cycle is determined at a point of commitment in G_1, and once cells enter mitotic S phase they cannot normally undergo meiosis until they have completed that mitotic cell cycle and reentered G_1 (Nurse and Bissett 1981). During vegetative growth, Mmi1 degrades meiotic-specific transcripts and the Pat1 kinase phosphorylates and inactivates Mei2, an RNA-binding protein and a key component of the switch to the meiotic cell cycle (Yamamoto 2010). In nitrogen-limiting conditions, cells become arrested in G_1 and cells of the opposite mating type conjugate to form a diploid zygote, known as a zygotic ascus (Merlini et al. 2013). An h^+/h^- diploid cell after nitrogen starvation can proceed directly to meiosis without conjugation, forming an azygotic ascus. In both situations the two Mat1 mating type loci, Mat1-Pc, Pi, and Mat1-Mc, are expressed in the same cell, allowing expression of Mei3, which inhibits Pat1 (see Fig. 1 in Chapter 11 Introduction: Analysis of *Schizosaccharomyces pombe* Meiosis [Yamashita et al. 2016]). This allows the accumulation of active, dephosphorylated Mei2, which inhibits Mmi1, thus allowing meiotic-specific transcripts to accumulate, and cells become committed to the meiotic programme (see Chapter 11 Introduction: Analysis of *Schizosaccharomyces pombe* Meiosis [Yamashita et al. 2016] and references therein).

Many of the genes required for mitosis are also required for meiosis, suggesting that the meiotic cell cycle is a modified mitotic cell cycle (Gutiérrez-Escribano 2015). Premeiotic S phase is followed by the first meiotic division (MI) and during prophase of MI, homologous chromosome pairing and recombination occur. This stage is characterized by an oscillatory horsetail movement of the chromosomes, led by attachment, via the telomeres, to the SPB, which is the fission yeast equivalent of the centrosome (Robinow 1977, 1978; Chikashige et al. 2014). The telomere bouquet, as this arrangement is known, is important for promoting chromosome pairing and recombination and for subsequent formation of the meiotic spindle (Tomita et al. 2013). A similar telomere arrangement is also found in other eukaryotes including humans (Shibuya et al. 2014). Like other eukaryotes MI is a reductional division, and there is no intervening S phase before the second meiotic division (MII), which is the more mitotic-like nuclear division (see Fig. 3 in Chapter 11 Introduction: Analysis of *Schizosaccharomyces pombe* Meiosis [Yamashita et al. 2016]). The meiotic pathway is completed when the four haploid daughter nuclei become enclosed in the spore wall to form four haploid spores.

Stationary Phase

Stationary phase in fission yeast has often been compared with the G_0 stage during the cell cycle of multicellular eukaryotes. However, there are differences between the two cellular states. Stationary phase cells (quiescence) have highly reduced metabolic activity (Su et al. 1996), whereas G_0 cells are still metabolically active. *S. pombe* cells can enter and exit stationary phase from any phase of the mitotic cell cycle (e.g., when nitrogen is limiting cells arrest in G_1), and if glucose is limiting cells arrest in G_2. When the level of nitrogen or glucose is increased, cells exit the stationary phase and reenter the cell cycle in G_1 or G_2, respectively (Costello et al. 1986; Wei et al. 1993). Genes required for a number of different processes have been identified that are necessary for both normal proliferation and entry into stationary phase in response to nitrogen starvation (Sajiki et al. 2009). Recent metabolomic analyses have identified the early changes that occur in response to limiting nitrogen (see Sajiki et al. 2013). For discussion of techniques for metabolomics analysis in *S. pombe*, see Chapter 17 Introduction: Metabolic Analysis of *Schizosaccharmyces pombe*: Sample Preparation, Detection, and Data Interpretation (Pluskal and Yanagida 2016).

Splicing

Splicing in *S. pombe* has a number of similarities to splicing in higher eukaryotes, including the presence of SR-like proteins, important for alternative splicing (Biamonti et al. 2014) and the requirement for the essential splicing factor U2AF (Sridharan et al. 2011). Around 50% of genes contain one or more introns in fission yeast; an average intron is 81-bp long with a mode of 48 bp and they

tend to be found at the 5′ end of genes (Wood et al. 2002) with a branch site consensus sequence of [C/T]TAA[T/C] (Bitton et al. 2014). Although no alternative splicing has yet been observed in fission yeast, because of the high number of genes that contain introns, and the presence of genes encoding splicing factors such as SR-like proteins, it is possible that alternative splicing could occur in *S. pombe* (Awan et al. 2013; Bitton et al. 2014). For an overview of methods used to analyze mRNAs, including methods to investigate splicing, see Chapter 5 Introduction: Analysis of RNA Metabolism in Fission Yeast (Wise and Nielsen 2016).

Chromosome Structure

Telomeres

As discussed above, *S. pombe* chromatin has a number of features common to mammalian cells that are absent or much modified in budding yeast. Telomeric DNA is ~300 bp in length and is composed of a double-stranded stretch of repeats with the consensus sequence $TTAC(A)GG(G_{1-4})$ followed by a 5′ single stranded overhang of the G-rich strand. The sequence-specific proteins that bind to the double and single stranded repeats, Taz1 and Pot1, respectively, as well as associated proteins, are conserved in humans (Nandakumar and Cech 2013). The telomeres of Chromosomes I and II are flanked proximally by subtelomeric regions of about 20–40 kb and contain three repetitive regions STE1, STE2, and STE3, whereas the subtelomeric regions of Chromosome III are replaced with ~1.2 Mb of rDNA distributed between the two chromosome arms. *S. pombe* can survive in the absence of telomeres mainly by the formation of circular chromosomes (Almeida and Godinho Ferreira 2013) and at a lower frequency by the addition of repetitive sequences to linear chromosome ends (Jain et al. 2010).

Centromeres

Centromeres in *S. pombe* are considerably larger and more complex than those of budding yeast, and, like centromeres of higher eukaryotes, contain many repetitive sequences. Centromeres of chromosomes I, II, and III are ~35 kb, ~65 kb, and ~110 kb in length, respectively, and consist of a central core (CEN) of ~4 kb containing a region of ~1.4 kb, which has ~48% identity between all three centromeres. The CEN regions are flanked by *imr1R* and *imr1L* (**i**nner **m**ost **r**epeats), and a varying number of *dg* and *dh* sequences in the outer repeat elements (*otr*) (Niwa et al. 1989; Wood et al. 2002). Assembly of heterochromatin at the centromere repetitive sequences is directed by the RNAi machinery (see below) and is important for the incorporation of a centromere-specific histone H3 variant, CENP-A^{cnp1}. CENP-A incorporation is required for assembly of the kinetochore and thus for the primary function of the centromere (French and Straight 2013).

Origins of Replication

In fission yeast, origins of replication are also larger and more complex than the defined origins of replication found in budding yeast. *S. pombe* origins are between 0.5 and 3 kb in length and there is no obvious consensus sequence other than AT richness (~75% or greater) and a preferential localization within intergenic regions. Results from several studies using different methodologies have given broadly similar results about origin number and efficiency during the cell cycle. As a general estimate there appear to be about 1000–1200 origins in fission yeast with only a subset of these firing in any one cell cycle. There are about 400–500 efficient origins, with 100–200 of these firing once in approximately one to two cell cycles, and 300–400 firing once in approximately two to five cell cycles. In addition there are about 500–800 weaker, less efficient origins, firing once in approximately only five to 20 cell cycles (Segurado et al. 2003; Heichinger et al. 2006; Hayashi et al. 2007; Wu and Nurse 2009; Daigaku et al. 2015; Kaykov and Nurse 2015).

The genome-wide pattern of usage for the three polymerases required for DNA replication has been analyzed using Pu-seq (polymerase usage sequencing). This has shown that polymerase Polα primase, which initiates replication at origins, is rapidly replaced by the leading strand polymerase Polε and the lagging strand polymerase Polδ. Although the general distribution of Polδ and Polε on

the leading and lagging strands is maintained across the genome, Pu-seq also revealed more subtle changes in individual replicons where leading strand synthesis could be initiated by Polδ before being replaced by Polε (Daigaku et al. 2015).

DNA combing has been used to look at single DNA molecules and this has shown that at S phase, onset origins fire randomly and sparsely throughout the chromosomes. Later in S phase, clusters of fired origins appear and form the basis of nuclear replication foci. The rate of origin firing peaks just before mid S phase, and toward the end of S phase nearly all the available origins fire within the unreplicated regions. These data suggest that origin firing is mostly random and not part of a deterministic program (Kaykov and Nurse 2015). Origin selection is dependent on the recruitment of replication factors, and the temporal order of origin firing is the result of competition for recruitment of ORC (origin recognition complex) proteins during mitosis and the formation of prereplicative complexes during G_1, with efficient origins being more successful. The efficiency of origin firing is dependent on the binding of factors including Cdc45, a component of the preinitiation complex, with efficient origins more able to bind Cdc45 than inefficient origins (Patel et al. 2008; Wu and Nurse 2014). For discussion of methods to study DNA replication, see Chapter 13 Introduction: Analysis of DNA Metabolism in Fission Yeast (Antequera and Humphrey 2016).

Chromatin Modification

Chromatin modification in fission yeast shows a high degree of conservation with that found in higher eukaryotes (see Chapter 14 Introduction: Analysis of Heterochromatin in *Schizosaccharomyces pombe* [Cam and Whitehall 2016]). Assembly of heterochromatin occurs by posttranslational modification of histones and a common feature of these regions of heterochromatin is the presence of repetitive elements. The RNAi pathway is present in *S. pombe* but not in *S. cerevisiae* and is necessary for the assembly of heterochromatin in specialized regions of the genome, ensuring centromere function, mating type silencing, and silencing of repetitive elements (Zofall and Grewal 2006). There are three highly conserved components of the RNAi pathway, Dicer (Dcr1 RNAase), Agonaute (Ago1 DNA binding protein), and the RNA-directed RNA polymerase Rbp1. Briefly, Dicer slices double stranded RNA transcribed from repetitive regions to form small interfering RNAs (siRNAs), which are amplified by Rdp1, These siRNAs bind to Argonaute and guide chromatin remodeling complexes to the complementary genomic site. Here Clr4 brings about histone H3 methylation at K9, creating binding sites for the HP1 family of chromodomain proteins (Chp1, Chp2, and Swi6) to establish heterochromatin. Heterochromatin can spread across large domains, brought about in part by oligomerization of Swi6 (Grewal 2010).

CONCLUDING REMARKS

In this introduction we have briefly outlined the history of fission yeast, its life cycle, and aspects of its biology that make it a useful model organism for studying problems of eukaryotic molecular and cell biology. The protocols in this collection will be useful for both researchers new to fission yeast and those already working with the organism. In addition to the continued development of new protocols there are excellent resources currently available for fission yeast researchers such as Pombase (http://www.pombase.org/), genome-wide gene deletion collections (http://us.bioneer.com/products/spombe/spombeoverview.aspx), a plasmid library of YFP (yellow fluorescent protein)-tagged ORFs (open reading frames) that are accessible for Gateway cloning (http://www.riken.jp/SPD), and various laboratory webpages that provide useful resources—for example, those of Susan Forsburg's laboratory at the University of Southern California (http://www-bcf.usc.edu/~forsburg/) and Jürg Bähler's laboratory at UCL (http://www.bahlerlab.info/resources/). "pombelist" is an active and engaging online discussion forum (https://listserver.ebi.ac.uk/mailman/listinfo/pombelist). It is likely that future research in fission yeast will generate new techniques and protocols and continue to contribute to our understanding of eukaryotic cellular processes.

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