A genome-wide resource of cell cycle and cell shape genes of fission yeast

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Summary

To identify near complete sets of genes required for the cell cycle and cell shape we have visually screened a genome-wide gene deletion library of 4843 fission yeast deletion mutants (95.7% of total protein encoding genes) for their effects on these processes. A total of 513 genes have been identified as being required for cell cycle progression, 276 of which have not been previously described as cell cycle genes. Deletions of a further 333 genes lead to specific alterations in cell shape and another 524 genes result in generally misshapen cells. Here we provide the first eukaryotic resource of gene deletions, which describes a near genome-wide set of genes required for the cell cycle and cell shape.

Key words or phrases

- Genome-wide gene deletion resource
- Cell cycle
- Cell shape
- Fission yeast
Introduction

Understanding how cells reproduce and how they generate their shape are two major goals in eukaryotic cell biology. These two processes are related because during the cell cycle cells duplicate cellular components and reproduce their cell structure in space to generate two daughter cells. A key function of the cell cycle is to ensure accurate replication and segregation of the genome, because errors in genetic transmission can cause mutations and chromosomal rearrangements that may lead to cell death or disease. Failure to accurately reproduce and maintain cell shape can disrupt tissue architecture or influence cell motility and may also lead to cell death or disease. Given the importance of these two processes for cell biology, we have generated a genome-wide resource, cataloguing the genes that when deleted disrupt the cell cycle or cell shape in the fission yeast *Schizosaccharomyces pombe*. This is the first such resource that qualitatively describes a near complete set of genes required for these processes in a eukaryotic organism.

Fission yeast is very amenable for investigating the cell cycle and cell shape [1-3] and has been used extensively for cell cycle studies for many years [4]. It is a rod-shaped, unicellular eukaryote that grows by apical extension and divides by medial fission and septation. This regular cell shape has made fission yeast a very useful organism to identify genes involved in the cell cycle and the generation and maintenance of cell shape. Mutants can easily be identified by visually screening for cells that divide at a longer or shorter length compared to wild type (cell cycle defect), or that do not have a rod-shape (cell shape defect). A long cell phenotype is generated if cells are blocked or delayed in cell cycle progression because they continue to grow but fail to divide and thus become elongated. However not all genes required for the cell cycle show a long cell phenotype when deleted. For example
genes encoding checkpoint proteins that are not required during a normal cell cycle may have a wildtype deletion phenotype and genes required for mitosis often arrest with a more irregular, non elongated cell shape [5]. The long cell phenotype is easily identified by visual screening and is definitive for cells blocked or delayed in cell cycle progression through the G1, S, G2 and cytokinesis phases of the cell cycle [6]. We have therefore focussed on identifying all genes with this phenotype when deleted, to determine the majority of genes required for progression through interphase or cytokinesis in fission yeast.

Genes important for the generation of cell shape are also easily identified because mutants deleted for these genes lose the normal rod-shape. These cells exhibit a range of phenotypes including rounded or stubby, curved, branched, skittle shaped or more generally misshapen [2, 3]. The penetrance of these mutant phenotypes can be quite variable; in some cases the majority of cells having the same altered cell shape, whilst in others the phenotype is of lower penetrance with fewer of the cells exhibiting the phenotype.

Fission yeast currently has 5059 annotated protein coding genes and a genome-wide deletion collection has been constructed with 4836 genes deleted [7]. In this study we have systematically visually screened the deletion collection to identify genes required during interphase of the cell cycle, cytokinesis, and for cell shape. This resource complements and extends earlier studies using gene deletions in budding yeast [8, 9] and RNAi based gene knockdowns in Metazoa [10-15]. Although the budding yeast gene deletion collection has been extensively investigated it has not been subjected to a systematic screen for cell cycle genes such as that carried out here, whilst metazoan RNAi cell cycle studies have shown only limited reproducibility [12]. Given that 3397 (67.14%) of the fission yeast protein coding genes have
identifiable orthologues in Metazoa (http://www.pombase.org) the genome-wide resource provided here will help to identify cell cycle and cell shape genes in other eukaryotes including humans.

**Results**

**Screening of the haploid deletion mutants**

We have microscopically examined and described the deletion phenotypes of 4843 haploid gene deletion mutants of both essential and non essential genes, after sporulating diploid heterozygous deletion mutants and germinating haploid spores on rich medium plates (see Phenotype analysis of the genome-wide set of gene deletions in Methods for details of the screen and Table S1 column G, Table S2, Table S3). Mutants were classified to one of 11 cell shape phenotypes together with 3 additional categories, namely: wild type (WT); arrested as normal spores (Spores); or arrested as normal germinated spores (Germination) (Figure 1, Figure 2A, Table 1A, Table S1 column H), making 14 phenotype categories in total (see Phenotype analysis of the genome-wide set of gene deletions in Methods). These phenotypes were mapped to terms in the Fission Yeast Phenotype Ontology (www.berkeleybop.org/obo/ontology/FYPO) (Table S4) and the gene list for each category can be found in Table S5A-N. All 4843 deletions were assigned to only one of these 14 categories for analysis. The variation in the phenotype for mutants in a particular category can be found in the phenotype description Table S1 column G. For example some long mutants may be slightly curved, and T-shaped cells were observed in a subset of curved cells.

Gene Ontology (GO) term enrichment of biological processes and cellular components for these 14 phenotype categories is shown in Table 2 and Table 3A, 3B
(see Table S6A-N and Table S7A-N for the complete results). There are 643/4843 genes with no GO process annotation. Of these 643 ‘unknowns’ 574 (89.2%) have a WT deletion phenotype. This means that most genes showing one or more of the 13 other deletion phenotypes are assigned a biological process either by inference from other organisms or because they have been partially characterized in fission yeast. However, their cellular shape is often not part of that characterization.

To identify genes required for the cell cycle and cell shape we focussed on the 11 cell shape categories. These phenotypes and their relevance for the cell cycle and cell shape are described in the following sections. To demonstrate the use of this resource we describe a more detailed cytological analysis of a subset of mutants altered in cell shape. In addition we screened the library of haploid viable deletion mutants for hydroxyurea (HU) sensitivity and identified new genes implicated in the DNA checkpoint preventing entry into mitosis when DNA replication is incomplete or DNA is damaged.

**Gene deletion mutants with an elongated cell phenotype**

A long cell phenotype identifies cells blocked in progression through interphase of the cell cycle or cytokinesis (see Introduction). Gene deletion mutants with this phenotype were assigned to three categories, **Long high penetrance (Long HP)** (346/4843), **Long low penetrance (Long LP)** (136/4843) and **Long branched (Long Br)** (31/4843) (Figure 1, Table 1A, Table S4, Table S5G-I). These three categories totalled 513 genes required for cell cycle progression, and we conclude that 10% of fission yeast genes are required directly or indirectly for progression through interphase or cytokinesis (513/4843). 467 genes had a strong elongated deletion phenotype and a further 46 genes were included that were only weakly elongated
when deleted (see **Phenotype analysis of the genome-wide set of gene deletions** in Methods). Of the 513 genes in the **Long** categories 66.5% (341 genes) were essential for viability compared to 26.2% for all genes, and 85.57% (439 genes) were conserved in human. This indicates that many of the genes identified in this study are likely to be important for understanding the cell cycle in other more complex eukaryotes.

All three **Long** categories were enriched for nuclear localisation (Table 3A). The **Long Br** set was enriched for cytokinesis and transcription and included genes encoding subunits of the RNA polymerase II holoenzyme, Mediator and SAGA (Table 2, Table 3B). As expected the **Long HP** and **Long LP** sets were both overrepresented for genes involved in DNA metabolism and the regulation of mitotic cell cycle (Table 2). The **Long HP** set was also enriched for processes and complexes involved in mRNA metabolism (particularly splicing), RNA biogenesis, transcription and DNA replication initiation and 6/6 genes encoding subunits of the MCM complex (see Table 3b and legend for details). In contrast the **Long LP** set was enriched for chromosome segregation and for genes encoding subunits of kinetochore complexes including Mis6-SIM4 (8/14 subunits), Ndc-Mis-Spc (6/10 subunits), Condensin (4/5 subunits) and APC (8/13 subunits) (Table 2, Table 3B). These differences in enrichment indicate that **Long HP** is a good classifier for genes involved in progress through interphase whilst **Long LP** is more specific for genes associated with progress through mitosis (Table 2 footnotes 7,8). Mutants that block in mitosis usually display an irregular less defined cell shape but are not elongated, whereas an elongated phenotype is characteristic of an interphase block, so one possibility is that the **Long LP** gene set is enriched for a subset of mitotic genes that are also required for interphase progression, with some cells arresting in mitosis and other cells in
interphase. Together these three Long phenotype categories (Long HP, Long LP and Long Br) define gene sets important for progression through interphase or cytokinesis. The Long LP gene set is in addition enriched for a set of mitotic genes, which may also be required during interphase.

Cell cycle mutants without a long phenotype

Not all genes previously known to be required during interphase have an elongated phenotype when deleted. We found that DNA replication genes cdc23, ssb1, pol1, cdc18, cdt1, rad4 and four genes encoding core subunits of the RFC (rfc2, rfc3, rfc4 and rfc5), were all annotated to the Missshapen essential set (Miss E) (Table 1A, Table S5D). Some of these genes are known to be required for both DNA replication and the DNA checkpoint and mutant strains enter mitosis with incompletely replicated DNA [16-20]. The Miss E category also contains genes required for mitosis, for example, the kinetochore protein Nnf1 and pericentrin Pcp1. In total 57 genes were annotated to one or more cell cycle process (Table 2, Table S6D). We conclude that the Miss E phenotypic class will be a good starting point to screen for further genes required for both DNA replication and the DNA checkpoint as well as for genes required during mitosis.

Another group of previously known cell cycle genes exhibit a wee phenotype, with viable cells dividing at a shorter length and a smaller cell volume than wild type cells. A partial gene deletion library of viable haploid mutants has already been screened and 18 wee mutants identified [21]. In this study we identified by visual examination 25 genes with a small cell deletion phenotype; this Small set consisted of 11 non-essential genes and 14 essential genes (Table S5M). The 11 non essential genes included 9 of the 18 previously identified wee genes and were mainly those
with a stronger wee deletion phenotype [21]. The two additional genes encode a predicted 26S proteasome non-ATPase regulatory subunit (SPCC18.17c), which Navarro and Nurse (pers. comm.) found to be shorter but also wider and so divided at a wild type cell volume, and a mitochondrial inheritance GTPase (dml1), which is potentially a new wee gene. The 14 essential genes were enriched for tRNA metabolism (Table 2 for summary and Table S6M, Table S7M for details), including genes encoding subunits of the RNase P and MRP (Mitochondrial RNase P), which have roles in RNA processing [22], tRNA 2'-O-ribose methyltransferase, tRNA specific adenosine deaminase (2/2 subunits) and the tRNA-specific splicing endonuclease (2/4 subunits). It is possible that these non viable small mutants, like other similar small size viable mutants identified in budding yeast that affect growth [23], may only indirectly affect cell cycle progression.

**New cell cycle genes**

Our genome-wide screen has identified 513 genes with a long cell deletion phenotype and thus required for the cell cycle in fission yeast. Previously 158 fission yeast genes that generate elongated cells when deleted have been reported and annotated in PomBase ([http://www.pombase.org/](http://www.pombase.org/)) [24]. To validate this qualitative visual approach to identify new cell cycle genes we compared these 158 genes (Table S8A) with the 513 fission yeast cell cycle genes from this screen (Table S5G-I) and found that 147 of the 158 genes were also identified in our screen (Figure 2B, Table S9). The 366 genes not previously reported as elongated when deleted included 90 genes with an existing cell cycle GO annotation and 276 genes with no previously known cell cycle role (Table S8B, Table S8C). The majority of these 276 genes (230/276) are annotated to GO processes which have previously been linked to the
cell cycle, suggesting that the 276 genes are true positives and identify new cell cycle genes. The 230 new genes involved in cell cycle related processes included genes required for ribosome biogenesis, splicing and nucleotide metabolism (Table 1B).

For example 7 genes (dfr1, adkl, hpt1, dea2, dut1, dcd1, tmp1) are concerned with various nucleotide metabolism pathways. Two previously identified cell cycle genes, budding yeast CDC8 (tmp1 orthologue) [25], and the fission yeast cdc22 (ribonucleotide reductase) [26] are also required for nucleotide metabolism. Given the cell cycle role of these two genes, the other genes identified here may also be important for maintaining the nucleotide levels needed for cell cycle progression.

The remaining 46 genes include 17 genes unstudied in any organism (9 of which are conserved in humans), and 29 genes that have existing GO annotations to processes or pathways not previously linked to the cell cycle. Of these, 13 genes are involved in a number of different metabolic pathways including amino acid, carbohydrate and phospholipid metabolism. We investigated whether any of these genes had genetic or physical interactions with genes implicated in the cell cycle using the BioGRID Interaction database [27]. Several genes showed such interactions (Table S10). For example, a predicted pyruvate decarboxylase SPAC1F8.07c interacts with a wee gene zfs1 [21, 28]. It is possible that these 13 metabolic cell cycle genes may act as regulatory links between small molecule biosynthesis pathways and the cell cycle.

**Comparison with a human cell cycle gene set**

To examine the overlap between cell cycle genes in fission yeast and human we identified a set of 521 human genes proposed to be involved in the cell cycle [12], and which have a fission yeast orthologue (Table S11A, Experimental procedures).
The 521 fission yeast orthologues of these human genes were compared to 614 fission yeast genes with an existing mitotic cell cycle annotation, including all genes so far annotated to the mitotic cell cycle either by inference or experiment (Table S11B, Methods, http://www.pombase.org/). There were 113 genes common to both gene sets (Figure 2C, Table S11C). We also compared the 521 gene set with the 276 new cell cycle genes from this study and identified a further 43 genes in common (Figure 2C, Table S11D). Therefore in total, 156 of the 521 conserved genes were involved in the cell cycle in both organisms (29.9%), a similar level to other inter-species comparisons, human/worm at 36% and human/fly at 38% [12, 14, 29, 30]. Possible reasons why these inter-species comparisons in a variety of studies show such a low overlap are considered in the Discussion.

Cell cycle checkpoint genes

To identify new DNA checkpoint genes we screened 2983 viable gene deletion mutants for those that failed to block the cell cycle in the presence of the ribonucleotide reductase inhibitor hydroxyurea (HU) ([31-33]. We further screened these HU sensitive mutants for those with a cut phenotype [34], where cells fail to block cell cycle progress and enter mitosis generating chromosome segregation defects. We identified 132 mutants sensitive to at least 5 mM HU (Methods, Table S12). In the presence of HU, deletion mutants of 8 genes had greater than 60% cells with a cut phenotype (Table S12 Column O). Of these, deletion mutants of *hus1*, *rad1*, *rad3*, *rad26*, *rad17* and *rad9*, which have been previously been shown to be required for establishment of the DNA checkpoint, did not elongate prior to entering mitosis [33]. Deletion mutants of the remaining two genes (*ddb1*, *lem2*) initially became elongated but eventually entered mitosis and displayed a cut phenotype
(Figure 3A). Ddb1 is necessary for stabilizing DNA replication forks and is involved in regulating the replication checkpoint kinase Cds1 [35], and a lem2 mutant has previously been shown to be sensitive to HU [36]. Mutants of a further 24 genes showed a lower level of cut cells (between 20-60%) after 10 hours in HU (Table S12 column P). Of these, 9 genes (nup132, nup40, did4, spc34, vps24, ubr1, mde4, utp16 and ers1) are newly identified as HU sensitive genes with a cut phenotype.

In this study we have identified one new S phase checkpoint gene, lem2, which has a strong cut deletion phenotype, and 9 genes with a lower penetrance cut deletion phenotype, which may influence maintenance of the S phase checkpoint (Figure 3B).

**Cell Shape mutants**

Cell shape mutants other than those with a long cell phenotype have been used to identify genes important for generating the normal rod-shape of a fission yeast cell. Previous studies in fission yeast have identified orb mutants that are spherical because cells fail to grow in a polarized manner, ban (banana) mutants that have a curved or bent cell phenotype because cells no longer orientate the growth zones at 180° along the long axis of the cell, and tea (tip elongation aberrant) mutants, which form a new growth zone in the wrong place often at 90° to the long axis of the cell [2, 3]. Other cell shape mutants included bottle or skittle shaped cells and more generally misshapen cells [37]. We screened the deletion mutants for shape defects using the following 7 categories to describe the mutant phenotypes (see Figure 1 and Table S5D-F, J-L, N for gene lists). i) **Rounded**, which includes the typical orb mutants and also mutants that are more rounded than wild type but not completely spherical. ii) **Stubby**, which look shorter and wider than wild type but are mainly rod-shaped.

These two categories showed a degree of overlap, with some mutants showing both
phenotypes. iii) **Curved**, which includes the ban mutants and the tea mutants. During vegetative growth tea mutants have only a low level of T shaped cells with most cells having a curved phenotype. iv) **Skittle**, one end of cell the is wider than the other end. A total of 333 genes (6.9% of total genes) showed these specific alterations in cell shape when deleted and are thus important for the generation of normal cell shape. A further less well-defined group called **Misshapen** showed an ill-defined potato-like shape or a mixture of other shapes. These fell into three further subgroups: v) viable misshapen mutants, (Miss V), vi) viable misshapen mutants, which have a weak phenotype, (Miss weak V) and vii) essential misshapen mutants (Miss E). There were 524 genes with these more general misshapen deletion phenotypes. In total 857/4843 genes (17.7%) showed altered cell shape when deleted (Table 1A) and 668 of these are conserved in humans (77.9%). No additional cell shape phenotypes were identified compared with earlier work, suggesting that there may only be a restricted number of shapes that a fission yeast cell can adopt.

The Rounded, Stubby and Curved sets are all enriched for genes implicated in cell polarity and for localisation at the cell tip (Table 2, Table 3A), Rounded and Stubby sets for cell wall organization, and the Stubby set for cytokinesis and actin cytoskeleton organization (Table 2 footnote 11). All 14 genes in the Curved set annotated to the cytoskeleton organization category are involved in microtubule cytoskeleton organization (see Table 2 footnote 12) and are also enriched at the cell tip (Table 3A). Therefore, we predict that unknown genes with a curved deletion phenotype when deleted are likely to be involved in microtubule related processes. Similarly the stubby phenotype is likely to be associated with genes that affect actin processes. Genes which generate a skittle phenotype when deleted were enriched for mitochondrial organization; nearly 50% (118/241) of the total genes annotated to
mitochondrion organization were in the **Skittle** category. We found that 19 genes were required for mitochondrial tRNA metabolism (Table 2) and 61 genes for the mitochondrial ribosome, suggesting that mitochondrial translation underlies the skittle phenotype. The **Miss E** category was enriched for genes required for lipid metabolism (35 genes), 15 of which are involved in GPI anchor biosynthesis (Table 2, footnote 1, Table S6D). GPI anchor proteins affect cell wall integrity and loss of these proteins can result in a misshapen cell phenotype [38]. In higher eukaryotes GPI anchor proteins have been implicated in the sorting of membrane proteins important for cell polarization [39] and so in fission yeast GPI anchor proteins could also be implicated in cell polarization.

To investigate whether gene deletions that cause cell shape changes also have defects in the cytoskeleton, bipolar growth pattern or the cell wall, we analysed 54 previously uncharacterised viable cell shape mutants. These were a subset of 352 non-essential genes in the **Miss V**, **Miss weak V**, **Rounded**, **Stubby**, **Curved** and **Skittle** categories (Table 1A). We found that a total of 35 strains had defects in the cytoskeleton and/or the cell wall, bipolar growth or cell separation defects and included 26 mutants with actin or microtubule defects (see Figure 4, Table S13 and Supplementary Material 2 for details). These results indicate that further screening of viable deletion mutants, even those with a weak phenotype, will be a useful approach to identify and characterise genes involved in the cytoskeleton.

**Discussion**

We have visually screened 4843 gene deletion mutants in fission yeast, 95.7% of all protein coding genes, and have identified near genome-wide sets of the genes required for the cell cycle and cell shape, the first systematic description for a
eukaryote. The long cell phenotype in fission yeast defines cells blocked in cell cycle progression during interphase or cytokinesis and so is an effective way to identify genes required for these stages of the cell cycle. GO enrichment analysis has shown that the Long HP and Long Br categories were enriched for genes previously identified as being required during interphase or cytokinesis respectively. The Long LP set included genes previously known to be required during mitosis and we suggest that these genes may represent a subgroup of mitotic genes that have additional roles during interphase. Several genes required for the cell cycle were found in the Miss E gene set suggesting that further analysis of this set may also identify new genes required during interphase or mitosis.

We identified 513 cell cycle genes in total, 276 of which were not previously known to have a role in the cell cycle. Of these new genes, 230 were annotated to GO processes previously implicated in the cell cycle thus identifying new links between the cell cycle and these processes. These included genes required for nucleocytoplasmic transport, mRNA metabolic process (specifically splicing), ribosome biogenesis, and nucleotide metabolism (Table 1B). There were 46 new cell cycle genes not annotated to a process previously associated with the cell cycle, 13 of which are involved in small molecule metabolism. Frequently, only one or two genes were identified for a specific metabolic pathway. For example, *ect1*, is predicted to encode ethanolamine-phosphate cytidylyltransferase, which is rate limiting for synthesis of CDP-ethanolamine, an important step in phospholipid biosynthesis [40]. We speculate that these genes may encode proteins linking different aspects of metabolism, such as metabolite levels or flux, to the cell cycle.

Studies using RNAi in metazoan organisms have identified sets of genes required for cell cycle progression. However these overlap only to a limited extent
between various intra and inter species comparisons in a range from 10% to 38% [12].

Our analysis comparing cell cycle genes in fission yeast and human identified 156/521 orthologous gene pairs (29.9%) involved in the cell cycle in both organisms; a similar percentage overlap to that found in other inter-species studies. A possible reason for the rather limited overlap of cell cycle genes in a wide range of studies may be because gene knockdowns using RNAi can result in varying levels of gene product and thus more variation in cellular phenotype. Our analysis is based on gene deletions that generally eliminate the entire gene function, thus reducing phenotypic variability. Inter species comparisons may also be limited because different phenotypes may be used in different organisms to infer a specific cell cycle defect, and these are not always directly comparable. A cell cycle defect during interphase in fission yeast produces an easily identifiable highly consistent long cell phenotype, which can be used to reliably identify interphase cell cycle genes. Many of the cell cycle genes identified in fission yeast have a conserved cell cycle role in other eukaryotes and so the Long cell cycle gene set identified in this study is likely to be useful to uncover additional cell cycle genes conserved across species.

To catalogue genes required to generate and maintain the correct cell shape we identified categories of deletion mutants with specific cell shape defects. GO analysis showed that genes within these groups could be used to identify genes implicated in the actin cytoskeleton (Stubby), the microtubule cytoskeleton (Curved) and mitochondrial function (Skittle). The Skittle category suggests that there is an uncharacterised mechanism influencing cell shape, which is affected by defects in mitochondrial organization and translation. The distribution of mitochondria in a cell is dependent on microtubules [41] and binding of mitochondria to microtubules can moderate microtubule dynamics [42], raising the possibility that defective
mitochondria may affect microtubules thus leading to cell shape changes. In humans a number of diseases including deafness and muscle pathologies are linked to defects in mitochondrial protein synthesis [43]. The link we have identified in this study between mitochondria and cell shape, suggests that cell shape changes could be an underlying cause of some of these pathologies in humans. We also showed that some genes from the Miss weak V set, although only exhibiting a mild shape change when deleted, have cytoskeletal defects. This suggests that genes with this deletion phenotype will be a good source of new genes affecting the actin and microtubule cytoskeleton.

A limited range of defined cell shapes were identified in the genome-wide gene deletion screen. It appears that only a restricted range of cell shapes is possible for the fission yeast cell, perhaps reflecting topological constraints in cellular organisation, related to the cell wall or the cytoskeleton for example. Future comparisons with other organisms that have been screened for cell shape defects [10, 11, 13] will help identify the genes and processes required to generate, or maintain eukaryotic cell shape.

Our work provides the first near genome-wide sets of gene deletions that influence the eukaryotic cell cycle and cell shape. This qualitative classification of genes according to cell shape phenotypes, based on deletion mutants, provides a resource that will be a good starting point for further studies in fission yeast and for the identification of equivalent gene functions in other eukaryotic organisms.

**Materials and Methods**

**Phenotype analysis of the genome-wide set of gene deletions**
The 4843 deletion strain collection used for this analysis consists of 4825 strains described by Kim et al [7] plus 18 additional gene deletion mutants as shown in Table S2. Changes in gene dispensability from Kim et al [7] for 9 reconstructed strains and 12 re-analysed strains are shown in Table S3. All growth conditions and media were used as described by Moreno, Klar and Nurse [44] unless otherwise stated. Spores were generated as described for gene dispensability analysis [7]. All strains were coded and a blind analysis was conducted. Between two and four isolates for each heterozygous diploid deletion mutant were independently sporulated. A visual examination of the phenotypes of both deletion G418 resistant spores and WT G418 sensitive spores following free spore analysis was carried out after one and two days following plating on non selective YES plates at 25°C and 32°C. The presence of both G418 sensitive and resistant spores allowed a comparison of the deletion mutant with wild type. Any phenotypic differences from WT that could be detected by eye were described as the putative deletion phenotype. After two days colonies were replica plated on to YES plates containing G418 (Sigma) at 25°C and 32°C to confirm the gene deletion phenotype by linkage to the G418 resistant phenotype.

The final deletion phenotype categories for a genome-wide set of genes were generated as follows, a Gene Ontology analysis of the Long group was compared with a GO analysis of the subdivisions Long HP, Long LP and Long Br. These subdivisions formed biologically significant subgroups of the Long group and so these 3 categories were used for further analysis. The same type of GO analysis was used for the Rounded and Stubby groups as these formed biologically distinct categories although there is also overlap between the phenotypes of these 2 categories. The Misshapen group was divided into essential and non essential genes because viable mutants may be more useful for identifying genes required for cell
shape where as the misshapen phenotype observed in the essential group may be less specific for a cell shape defect given that the cells are dying or dead. The remaining categories **WT, Spores, Germination, Skittle, Curved** and **Small** could not be usefully further subdivided by their phenotype.

To estimate the minimal cell length increase detectable we measured the cell length of 34 viable gene deletion mutants described as long after visual screening on plates and which had not previously been implicated in the cell cycle (Table S1 column J). We could detect cells at least 10% or longer compared to wild type (~15.6 µm) and so a 10% or greater increase in cell length was used as the criteria for a long phenotype. The cut off between high penetrance and low penetrance was 30% long cells. This was estimated using inviable mutants that formed microcolonies showing a mixture of long and wild type/short cells. For these mutants 30% or less of the cells had a long phenotype.

To validate this approach we compared the 513 genes from the three **Long** categories with 158 cell cycle genes reported in Pombase as long. We identified 147/158 (93%) of these genes suggesting that the remainder of the genes in our **Long** category are also likely to be involved in the cell cycle. Furthermore only 46/513 were not annotated to a GO category previously linked to the cell cycle.

Cells showing different cell shape defects were photographed using a Zeiss Axioskop microscope with a CF plan X50/0.55 objective and a Panasonic DMC-LX2 camera. Spores from representative strains were plated on to YES solid medium and allowed to germinate or form small colonies before being photographed.

**Screen for new DNA checkpoint genes**
The growth of 2983 viable deletion mutants from Bioneer version 1 were screened on YES agar plates for 24 – 48 hours either in the presence of 2.75, 5.5 mM HU or without HU and scored on a scale of strong (+++), medium (++), weak (+) or no sensitivity, depending on their ability to grow on different HU concentrations compared to no HU (see Table S12). To check whether the 132 HU sensitive mutants were also involved in the DNA checkpoint preventing mitosis, cells were grown in liquid cultures with 11 mM HU and screened for a cut phenotype using DAPI (4’, 6-diamidino-2-phenylindole) to visualize the nucleus.

**Cell length measurements**

Cells were grown to mid exponential growth (2x10^6-1x10^7 cells/ml) in YES liquid medium at 32°C (or 25°C where appropriate) and photographed using a Zeiss Axioplan microscope with X100 objective and a COHU CCD camera. Cell lengths of 30 septated cells were measured using ImageJ.

**Phenotypic analysis and cytoskeleton analysis of viable shape mutants**

For the initial characterization, cells were grown at 18°C, 25.5°C, 29°C and 34°C on minimal and YES medium plates and cell morphology analysed by DIC microscopy. For further characterization, strains showing morphological defects were grown in liquid rich media at 25°C to mid-log phase or in the conditions at which each strain showed the strongest phenotype by DIC microscopy. Septa and pattern of cell growth was visualized with 35 µg/ml calcofluor staining (fluorescent brightener; Sigma). For WT cells the septation index was 15% (n=500 cells) and 30.1% of cells showed monopolar growth (n= 300 cells). Nuclei were visualized with 0.2 µg/ml DAPI (Sigma) or 100 µm/ml IP (Sigma) staining. Actin staining was as described by
Pelham and Chang [45] using AlexaFluor 488-phalloidin (Molecular Probes). For anti-tubulin immunofluorescence, cells were fixed in methanol at -80°C and further processed as described by Hagan and Hyams [46]. Primary antibodies were anti-tubulin ([TAT-1] 1:80 dilution) followed by Alexa 488 goat anti-mouse secondary antibody (Molecular Probes). Microscopy was performed at 23–25°C, either with an Axioplan 2 microscope, (Carl Zeiss, Inc.), equipped with a CoolsnapHQ camera (Roper Scientific) or with a Leica TCS SL confocal microscope. Data were acquired using the X100 objective taking 7 z-sections with 0.5 μm spacing.

**Bioinformatic Analysis**

1.1 Identification of genes already implicated in mitotic cell cycle processes

To identify genes involved in the mitotic cell cycle in fission yeast we used fission yeast GO data from 26th September 2011 ([http://www.pombase.org/](http://www.pombase.org/)) and selected the set of protein coding genes annotated to:

- GO:0000278 mitotic cell cycle
- GO:0000910 cytokinesis
- GO:0006261 DNA-dependent DNA replication
- GO:0000075 cell cycle checkpoint

Minor adjustments were made to this dataset to remove 3 known false positives (SPAC343.17c, SPBC19F8.02, SPAC5D6.08c) and add 3 known false negatives (SPAC23H4.11c, SPAC26A3.03c SPAC23H4.18c). The complete list is provided in Table S11B.

1.2 GO enrichment analysis

GO enrichments were performed using GO term finder
(http://go.princeton.edu/cgi-bin/GOTermFinder with ontology and annotations from 26th September 2011). Threshold P values of 0.001, 0.01 and 0.1 were used to identify specific enrichments. Bonferroni correction was used.

GO slim categories presented in Table 2 and Table 3 refer to the GO IDs in Table S14. All GO terms and P values for each phenotype set are provided in Table S6, Table S7. Genes where number of annotations = 1 were obtained using GO term Mapper http://go.princeton.edu/cgi-bin/GOTermMapper.

The background set was the 4843 gene set used this study.

1.3 Comparison with human cell cycle genes

A list of 1351 human cell cycle genes was extracted from a study by Kittler et al [12] and mapped to current Ensembl IDs (79 of the human identifiers had been retired and were no longer linked to extant genes). The remainder were mapped to 521 fission yeast orthologs using Ensembl Compara 10/11/2011 (cite:http://genome.cshlp.org/content/19/2/327.long)

Acknowledgements

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Figure Legends

Figure 1 Cell Shape categories
Examples of the 11 major cell shape phenotype categories described in this study. For each deletion mutant cells are shown as observed during the screen. Definitions of each phenotype can be found in Table S4. Scale bar (shown in WT) =10 µm.

Figure 2 Distribution of cell shape genes
A. Distribution of 1395 genes with a cell shape deletion phenotype amongst the 11 cell shape categories.
B. Overlap of Long gene set identified in this study (513 genes, green circle) with a set of previously published genes with a long deletion phenotype (158 genes, red circle). For further details see Table S8.
C. Overlap between 1) 521 fission yeast orthologues of human genes with an RNAi cell cycle phenotype (blue circle), 2) 614 genes with a mitotic cell cycle annotation in fission yeast (pink circle) and 276 new cell cycle genes from this study (orange circle). For further details see Tables S8 and Table S11

Figure 3 New HU sensitive cut genes
A. lem2 deletion mutant cells stained with DAPI after growing for 8-10 hours in the presence of HU. Examples of anucleated cells can be seen (white arrow) and cells with unequally segregated chromatin (red arrow).
B. New checkpoint genes identified in this study.

Figure 4 Cytological analysis of novel cell shape mutants
Examples of viable mutants from 4 cell shape phenotype categories analysed for defects in the cytoskeleton, growth pattern or cell wall. A. Wild type, B. \textit{meu2}Δ, C. \textit{yaf9}Δ, D. \textit{spc2}Δ, E. \textit{tlg2}Δ. DIC = Differential Interference Contrast, CF = Calcofluor used to stain the cell wall and septum. For details see Supplementary Material 2 and Table S13.

**Table Legends**

**Table 1**

**A. Summary of phenotype categories**

14 broad phenotype categories were used for analysis and the number and dispensability of genes in the different categories is shown. Each gene is classified by a single phenotype category based on the most penetrant or strongest deletion phenotype. Cells were only described as wildtype (\textbf{WT}) when no other phenotype was observed. The classifier for each gene can be found in Table S1 column H and gene dispensability in Table S1 column I. All genes are included only once and cell phenotype terms from Table S4 that are not included as separate phenotypes are subsets of one of the above categories eg a T shaped mutant is included in the \textbf{Curved} category as curved is the most penetrant phenotype for this type of cell shape mutant.

**B. GO process annotations for 276 novel cell cycle genes**

These 276 genes were not previously known to be involved in the cell cycle in fission yeast. 230 genes are annotated to other GO processes that have accepted links to the cell cycle in fission yeast and 29 genes had a GO process annotation not related to the cell cycle. Only 17 genes were of completely unknown function.
Table 2 GO cellular processes for all phenotype categories

Key for Table 2, and Table 3A, B

<table>
<thead>
<tr>
<th>Key</th>
<th>Description</th>
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</thead>
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<tr>
<td>enriched P &lt;=0.001</td>
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<td>moderately enriched P &lt;=0.01</td>
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<td>weakly enriched P &lt;=0.1</td>
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<tr>
<td>number of genes is 0</td>
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</table>

A summary of the GO analysis to identify genes annotated to cellular processes enriched within particular phenotype categories. The enrichment results were mapped to “GO slim” (high level) terms covering most biological processes observed in fission yeast to give a broad view of the ontology content of the genome-wide gene deletion dataset. For details see Methods, Table S6A-N, Table S14. The total data set is 4843 genes. Footnote numbers are shown in brackets.

Footnotes

1. Includes 15/29 genes annotated to GPI anchor biosynthesis, a descendent of lipid metabolism (P=1.75e-08).

2. Includes 10/41 genes annotated to attachment of spindle to microtubules, a descendent of chromosome segregation (P=0.001).

3. Includes 11/26 genes annotated to histone deacetylation, a descendent of transcription (P=0.00054).

4. Includes 53/118 genes annotated to nuclear mRNA splicing, via spliceosome, a descendent of mRNA metabolism (P =3.7e-27).

5. Includes 5/6 subunits of the elongator complex involved in tRNA wobble uridine modification.
6. Includes 11/25 genes annotated to the septation initiation signaling cascade (P=0.00013), and 5/15 genes annotated to the stress activated protein. kinase signaling cascade, 4/19 genes annotated to TOR signalling and 3/17 genes annotated to cAMP mediated signalling (none enriched) all descendents of signalling.

7. Includes 27/79 genes annotated to regulation of interphase, a descendent of regulation of the mitotic cell cycle (P=1.23e-09).

8. Includes 18/67 genes annotated to regulation of mitosis (P=4.88e-11) and 6/27 genes annotated to attachment of spindle microtubules to kinetochore (P= 0.0351), descendents of regulation of the mitotic cell cycle.

9. Includes 13/115 genes annotated to microtubule cytoskeleton, a descendent of cytoskeleton organization (P=0.00706).

10. Includes 4/9 genes annotated to Cdc42 signal transduction, a descendent of signalling (P= 0.006).

11. Includes 7/52 genes annotated to actin cytoskeleton organization, a descendent of cytoskeleton organization (P=8.09e-05).

12. Includes 13/115 genes annotated to microtubule cytoskeleton organization, a descendent of cytoskeleton organization (P=2.12e-08) and 5/5 genes annotated to gamma tubulin complex localization a descendent of microtubule cytoskeleton organization (P=3.11e-08).

13. Includes 3/11 genes annotated to carbon catabolite repression of transcription, a descendent of signalling (P= 0.00484).

14. All 19 genes involved in mitochondrial tRNA metabolism.

Table 3
**GO cellular components and complexes for all phenotype categories**

**A.** Summary of the GO analysis for cellular components enriched within particular phenotype categories. For details see Experimental procedures, Table S7A-N and Table S14. For further details and key see Table 2 legend. Footnote numbers are shown in brackets.

**Footnotes**

1. Includes 164/193 genes annotated to plasma membrane (P=1.74e-09) and 661/914 genes annotated to intrinsic to membrane (P=5.45e-09), both descendents of membrane.

2. Includes 7/8 subunits of chaperonin containing T-complex (P=1.29e-07) and 4/5 subunits of eukaryotic translation initiation factor 2B complex (P=0.001).

3. Includes 5/8 subunits of COP I coated vesicle membrane (P=0.001).

4. Includes 8/14 subunits of vacuolar proton-transporting V-type ATPase complex (P=7.26e-06).

5. See Table 2C for breakdown of nuclear complexes.

6. Includes 5/8 subunits of Arp2/3 protein complex (P=0.00922).

7. Includes 5/7 subunits of oligosaccharyltransferase complex (P=0.003), 3/3 subunits of glycosylphosphatidylinositol-N-acetylglucosaminyltransferase (GPI-GnT) (P=0.05068), 6/11 subunits of TRAPP complex (P=0.00417) and 4/4 subunits GARP complex (P=0.00313).

8. Includes 2/4 subunits of AP-1 adaptor complex (P=0.02077).

9. Includes 12/81 genes annotated to cytosolic large ribosomal subunit (P=0.00936), a descendent of ribosome.

10. Includes 5/6 subunits of Elongator holoenzyme complex (P=0.002) and 5/5 subunits of RNA cap binding complex (P=0.0004).
11. Includes 32/211 genes annotated to spindle pole body (P=0.006), a descendent of microtubule cytoskeleton.
12. Includes 20/211 genes annotated to spindle pole body (P=0.00014), a descendent of microtubule cytoskeleton.
13. Includes 2/4 subunits of mitochondrial sorting and assembly machinery complex (P=0.22496).
14. Includes 2/2 subunits of eRF1 methyltransferase complex (P=0.03840).
15. Includes 4/8 subunits of mannosyltransferase complex (P=0.00081).
16. Includes 8/194 genes annotated to ER membrane (P=0.15), a descendent of Golgi/ER.
17. Includes 4/10 genes annotated to equatorial MTOC (P=0.00015) and 8/51 genes annotated to spindle pole body (P=0.1), descendents of microtubule cytoskeleton.
18. Includes 2/2 subunits of tea1 cell end complex (P=0.008).
19. Includes 4/211 genes annotated to spindle pole body (P=1), a descendent of microtubule cytoskeleton.
20. All 61 genes encode subunits of mitochondrial ribosome (61/70 subunits P=3.98e-88).

B. A summary of the GO analysis for nuclear complexes enriched within the 3 Long phenotype categories. For details see Materials and Methods and Table S14. For further details and key see Table 2 legend. Footnote numbers are shown in brackets

Footnotes
1. Includes 13/26 subunits of U4/U6 x U5 tri-snRNP complex (P=9.18e-07), 6/7 subunits of U6 snRNP (P=0.00017) and 6/6 subunits U2 snRNP (P=0.0747).


3. Includes 4/15 subunits of U1 snRNP (P=0.07660), 3/6 subunits of t-UTP complex (P=0.04886) and 6/7 subunits of U6 SnRNP (P=0.00017).

4. All 5 genes encode subunits of holo TFIIH complex (5/10 subunits P=1.08e-07).

References


Figure 3

(a)

(b)

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Table 1

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(b)

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<td>MS L (μM)</td>
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<td>Total (μM)</td>
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Supplementary Material 1

Table S1 General information for 4843 fission yeast deletion mutants screened in this study

For general information see column headings. Classification of genes using the phenotype terms WT, Spores, Germination, Misshapen essential, Misshapen viable, Misshapen weak viable, Long high penetrance, Long low penetrance, Long branched, Rounded, Stubby, Curved, Small and Skittle, shown in Column H. For a description of each phenotype term see Table S4. Each gene was classified by a single phenotype category based on the most penetrant or strongest deletion phenotype (Column H). Cells were only described as wild type (WT) when no other phenotype was observed. A description of the deletion phenotype for each gene (column G), using controlled vocabulary, demonstrates the variation in the deletion phenotype observed for genes annotated to one particular phenotype category. Using the Excel filter function column G can also be used to select for genes with any of the phenotypes that were not analysed further see Table S4 Terms for other phenotypes observed. Cell length measurements for weak long genes are shown in Column J.
### Table S2 Dispensability of 18 genes not previously included in the set of genome-wide gene deletions

<table>
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<tr>
<th>Cosmid ID Newly constructed deletions</th>
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<th>Published phenotype</th>
<th>Dispensability this study</th>
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<td>SPCC320.04c</td>
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<td>V</td>
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Genes shown here include 6 genes with newly constructed gene deletion mutants and 12 genes that were not analysed by Kim et al 2010 [1] for gene dispensability as they were less than 40% deleted.
### Table S3 Gene dispensability changes from Kim et al 2010

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<td>V</td>
<td>E</td>
<td>Re-analysed - same as pub</td>
<td></td>
</tr>
<tr>
<td>SPCC1739.07</td>
<td>V</td>
<td>E</td>
<td>V</td>
<td>Deletion remade confirmed different to pub</td>
<td></td>
</tr>
</tbody>
</table>

3 gene deletion strains have been reconstructed as they had a different phenotype to previously published data. We find that 2 are still different to published data and one is now the same as previously published. Deletion mutants of 6 other genes that were non-essential (V) were remade and these genes are now essential (E). 12 gene deletion mutants were re-analysed and found to have a different dispensability, 7 of these now have the same phenotype as previously published deletion mutants.

Pub = previously published independently of the genome wide gene deletion project
The cell phenotypes terms used to describe each gene deletion mutant with a brief description of the phenotype. A definition of each phenotype can be found using the FYPO ID at [www.berkeleybop.org/obo/ontology/FYPO](http://www.berkeleybop.org/obo/ontology/FYPO), [http://www.pombase.org/](http://www.pombase.org/).

In this study phenotype terms may describe the phenotype of spores, germinated spores or cells. A more detailed description of the phenotype of each deletion mutant

<table>
<thead>
<tr>
<th>Cell Phenotype term</th>
<th>Cell Phenotype term description</th>
<th>FYPO identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terms used for analysis</td>
<td>Normal cell morphology (wild type)</td>
<td>0000672</td>
</tr>
<tr>
<td>Spores</td>
<td>Normal spore morphology most fail to germinate</td>
<td>0000306</td>
</tr>
<tr>
<td>Germination</td>
<td>Normal germinated spore morphology most fail to divide</td>
<td>0000310</td>
</tr>
<tr>
<td>Misshapen E</td>
<td>Uniformly lumpy or a range of different shapes cells/spores are inviable</td>
<td>0015111</td>
</tr>
<tr>
<td>Misshapen V</td>
<td>Uniformly lumpy or a range of different shapes. Cells are viable</td>
<td>001510</td>
</tr>
<tr>
<td>Misshapen weak V</td>
<td>Uniformly lumpy or a range of different shapes. Phenotype is weak, cells are viable</td>
<td>001510</td>
</tr>
<tr>
<td>Long HP</td>
<td>Distance between cell ends is longer than normal in greater than 20% of cells</td>
<td>0011122</td>
</tr>
<tr>
<td>Long LP</td>
<td>Distance between cell ends is longer than normal in &lt;20% of cells</td>
<td>0011122</td>
</tr>
<tr>
<td>Long Br</td>
<td>Distance between cell ends is longer than normal and cells branch next to septum at right angles to the long axis of cell</td>
<td>001512</td>
</tr>
<tr>
<td>Rounded</td>
<td>Spherical or oval shaped cell</td>
<td>0000946</td>
</tr>
<tr>
<td>Stubby</td>
<td>Shorter and wider than normal</td>
<td>0000024</td>
</tr>
<tr>
<td>Curved</td>
<td>Cell ends not apposed at 180° along long cell axis</td>
<td>0000016</td>
</tr>
<tr>
<td>Small</td>
<td>Lower cell volume than normal</td>
<td>0000645</td>
</tr>
<tr>
<td>Skittle</td>
<td>Tapered at one end</td>
<td>0000014</td>
</tr>
<tr>
<td>Terms for other phenotypes observed</td>
<td>Cell wall integrity compromised causing cytoplasm to be released</td>
<td>0000647</td>
</tr>
<tr>
<td>Septated</td>
<td>Increased number of septated cells compared to normal or multiseptated or misplaced septum</td>
<td>0000650, 0000117</td>
</tr>
<tr>
<td>Swollen</td>
<td>Increased in all dimensions compared to normal</td>
<td>0000025</td>
</tr>
<tr>
<td>T-shaped</td>
<td>Single branch at right angles to the long axis of cell</td>
<td>0000013</td>
</tr>
<tr>
<td>Vacuolated</td>
<td>Wild type cells with abnormal vacuolated appearance</td>
<td>001581</td>
</tr>
</tbody>
</table>
is in Table S1 column G. Deletion mutants with a cell phenotype of interest can be selected for using Table S1 column H for the phenotype, column I for gene dispensability and column A for systematic ID of the deleted gene. As well as the 14 major cell phenotypes see Terms used for analysis, some deletion mutants had more than one phenotype eg. SPAC16C9.05 which is described as long, curved. These genes were assigned to the most penetrant deletion phenotype. Some deletion phenotypes that were observed were not studied further as independent phenotype categories (see Terms for other phenotypes observed). Any genes showing these additional phenotypes were also assigned to one of the 14 major phenotypes. For those interested in these phenotypes the genes can be selected for using Table S1 column G and selecting for the appropriate term shown in Terms for other phenotypes observed.

**Table S5 A-N Cell Phenotype category gene lists**

Gene lists for the 14 phenotype categories

A Wildtype

B Spores

C Germination

D Misshapen essential

E Misshapen viable

F Misshapen weak viable

G Long high penetrance

H Long low penetrance

I Long branched

J Rounded
Table S6 A-N GO processes for genes in each cell phenotype category
For the GO analysis the following parameters were used

Aspect P

P-value cutoff 1

Calculate FDR Yes

Regulation links followed Yes

Bonferroni correction Yes

Uploaded gene list phenotype category_names_only_number of genes (see A-N below)

Annotation file gene_association.GeneDB_Spombe

Uploaded background file back_ground_set_names_only_4843

Evidence codes used IEA (3515), IEP (422), IGI (1231), NAS (1631), IPI (1175), ND (2139), IC (1446), ISS (10811), RCA (722), IMP (2916), IDA (9671), TAS (1093)

A Wildtype

B Spores

C Germination

D Misshapen essential

E Misshapen viable

F Misshapen weak viable
G Long high penetrance
H Long low penetrance
I Long branched
J Rounded
K Stubby
L Curved
M Small
N Skittle

Table S7A-N GO component for genes in each cell phenotype category
For the GO analysis the following parameters were used

Aspect C
P-value cutoff 1
Calculate FDR Yes
Regulation links followed Yes
Bonferroni correction Yes

Uploaded gene list phenotype category_names_only_number of genes (see A –N below)

Annotation file gene_association.GeneDB_Spombe

Uploaded background file back_ground_set_names_only_4843

Evidence codes used IEA (3515), IEP (422), IGI (1231), NAS (1631), IPI (1175), ND (2139), IC (1446), ISS (10811), RCA (722), IMP (2916), IDA (9671), TAS (1093)

A Wildtype
B Spores
C Germination
Table S8 New cell cycle genes in fission yeast

Gene lists for the comparison of 513 genes with a long cell deletion phenotype with 158 genes previously published as having a long cell deletion phenotype.

A 158 genes previously published as long when deleted

B 90 genes identified in this study which have a previous GO cell cycle annotation

C 276 new cell cycle genes from this study
Eleven genes previously published as having a long cell phenotype when deleted were not identified in our 513 Long gene set. 9 genes also have a GO cell cycle annotation and 2 genes are previously published as having a long cell deletion phenotype but currently have no GO cell cycle annotation. Several of these mutants are gene disruptions rather than deletions, which may account for the different phenotypes. In

<table>
<thead>
<tr>
<th>Systematic ID</th>
<th>Gene name</th>
<th>Function</th>
<th>Published phenotype</th>
<th>Phenotype this study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GO cell cycle annotation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAC1486.04c</td>
<td>alm1</td>
<td>Medial ring protein Alm1</td>
<td>Deletion. High penetrant long cells</td>
<td>Curved and is also described as Long weak</td>
</tr>
<tr>
<td>SPAC16A10.04</td>
<td>rho4</td>
<td>Rho family GTPase Rhö4</td>
<td>Deletion. Septation defect and long cells</td>
<td>WT only 50% deleted</td>
</tr>
<tr>
<td>SPBC1709.05</td>
<td>sks2</td>
<td>Heat shock protein, ribosome associated molecular chaperone Sks2</td>
<td>Disruption High penetrant long cells</td>
<td>WT</td>
</tr>
<tr>
<td>SPBC1734.14c</td>
<td>suc1</td>
<td>Cyclin-dependent protein kinase regulatory subunit Suc1</td>
<td>Disruption. Low penetrance long cells</td>
<td>Miss E</td>
</tr>
<tr>
<td>SPBC1A4.05</td>
<td>blt1</td>
<td>Regulation of the G2/M transition Blt1</td>
<td>Deletion High penetrant long cells</td>
<td>WT only 76% deleted</td>
</tr>
<tr>
<td>SPBC31F10.13c</td>
<td>hip1</td>
<td>Hira protein, histone chaperone Hip1</td>
<td>Deletion. High penetrant long cells</td>
<td>WT</td>
</tr>
<tr>
<td>SPBC646.13</td>
<td>sds23</td>
<td>Inducer of sexual development Sds23 Moc1</td>
<td>Deletion. Long cells at high and low temperature</td>
<td>WT</td>
</tr>
<tr>
<td>SPBC660.13c</td>
<td>ssb1</td>
<td>DNA replication factor A subunit Ssb1</td>
<td>Deletion. Low penetrant long cells</td>
<td>Miss E</td>
</tr>
<tr>
<td>SPBC6B1.09c</td>
<td>nbs1</td>
<td>Mre11 complex subunit</td>
<td>Deletion. High penetrant long cells</td>
<td>WT only 66% deleted</td>
</tr>
<tr>
<td><strong>No GO cell cycle annotation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAC1006.03c</td>
<td>red1</td>
<td>RNA elimination defective protein Red1</td>
<td>Deletion. Low penetrant long cells</td>
<td>Miss V</td>
</tr>
<tr>
<td>SPAC1F3.07c</td>
<td>rsc58</td>
<td>RSC complex subunit Rsc58</td>
<td>Deletion. Low penetrant long cells</td>
<td>Miss weak V</td>
</tr>
</tbody>
</table>
others the long phenotype is low penetrance and may have been missed by us. For 3 genes blt1, rho4 and nbs1 it may be that our strains are incorrect as they are not complete gene deletions (see TableS1, column D. For the hip1 gene the phenotype is different between our strain and the previously published strain.

**Table S10  New cell cycle genes involved in small molecule metabolism**

BioGrid analysis to identify genetic or physical interactions of new cell cycle genes not previously known to be involved in the cell cycle. For interacting genes we have shown the systematic ID and the gene name where known.

**Footnotes**

1. Rate limiting step in synthesis of CDP-ethanolamine.

2. Catalytic subunit of serine palmitoyltransferase (SPT), which catalyzes the commitment step in the synthesis of sphingolipids.

3. Converts sphingosine-1-phosphate to sphingosine. Sphingosine-1-phosphate is a cofactor of the human E3 ubiquitin ligase TRAF1 [2] and is important for pro survival processes.

4. slm9 implicated in the mitotic control [3].

5. zfs1 suppressor of cdc16 [4] and identified as a wee gene in fission yeast [5].

6. Both gpd1 and tdh1 encode glyceraldehyde-3-phosphate dehydrogenase and act in different metabolic pathways but both interact with the stress response pathway.

7. Cyk3, transglutaminase-like superfamily, required for cytokinesis in fission yeast.

8. Plays a central role as a glucosyl donor in cellular metabolic pathways.

**Table S11 Comparison with human cell cycle gene orthologues**
Gene lists from the comparison of 521 orthologues implicated in the cell cycle in humans, 614 genes with a GO cell cycle annotation in fission yeast and 276 new cell cycle genes from this study.

A. Human cell cycle gene orthologues - 521 genes

B. Genes with GO cell cycle annotation in fission yeast - 614 genes

C. Genes identified as cell cycle genes in humans and fission yeast - 113 genes

D. New cell cycle genes from this study identified as cell cycle genes in humans - 43 genes

**Table S12 HU sensitivity screen**

Analysis of 132 genes identified in a screen of 2983 gene deletion mutants for HU sensitivity and cut phenotype. 132 HU sensitive genes were identified (Column C) by sensitivity to different HU concentrations, strong (+++), medium (++), weak (+).

These genes were then tested for a cut phenotype in the presence of 11mM HU Columns D-K. Column L, previously reported HU sensitivity in budding yeast, Column M, previously reported HU sensitivity in fission yeast. Further information about each gene shown to have a cut phenotype when deleted is shown in Columns N-P. Column N genes previously reported as cut, Column O genes showing greater than 60% CUT, Column P showing 20-60% CUT.

**Table S13 A summary of the analysis of 54 gene deletion mutants with a shape defect**

Summary of the analysis of a set of morphology mutants previously uncharacterized for actin cytoskeleton, microtubule cytoskeleton, cell wall, cell growth pattern and septation. Mutants are grouped according to their deletion phenotype on plates.
Previously published gene without cytoskeleton characterization

Gene not previously published, new morphology gene

Weak morphology defect with no evidence of actin, microtubule, growth pattern or cytokinesis/cell wall defects

MT = microtubules, ts = temperature sensitive, pen = penetrance. YES = Yeast extract agar adenine, leucine, histidine and uracil

For details see Supplementary Material 2 **Analysis of a set of viable deletion mutants with a cell shape defect.**

+ actin cytoskeleton, microtubule cytoskeleton, cell wall, cell growth pattern or septation defect

- no actin cytoskeleton, microtubule cytoskeleton, cell wall, cell growth pattern or septation defect

**Table S14 Mappings for GO processes**

Mappings for biological processes, locations and complexes used in Table 2, Table 3A and Table 3B. * significant enrichments reported for either term.

**Supplementary Material 2**

**Analysis of a set of viable deletion mutants with a cell shape defect**

We analysed 54 viable morphology mutants and 35/54 (64.8%) strains showed some type of defect (cytoskeleton defects, cytokinesis defects or monopolar pattern of growth). Immunofluorescence analysis of the cytoskeleton in these morphological mutants shows that 26/35 (74.3%) strains had actin or microtubule cytoskeleton defects. The remaining 9/35 (25.7%) strains, show either monopolar pattern of growth
as judged by calcofluor staining, cell wall defects (cell lysis) or defects in cell-cell separation (accumulation of septated cells/ septation index greater than WT).

Among the genes whose deletions show cytoskeleton defects, cytokinesis defects or monopolar pattern of growth, 15/35 (42.8%) have not been previously studied and a further 20/35 (57.2%) strains, have been previously studied but the morphological defects have not been characterized. Mutants have been categorized according to their phenotype on plates.

Details can be found our website at

http://www.london-research-institute.org.uk/research/paul-nurse-jacqueline-hayles/resources/paper-supplementary

References


