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1 **Proteomic analysis of meiosis and characterization of novel short open reading**
2 **frames in the fission yeast *Schizosaccharomyces pombe*.**
3

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25

26 **Abstract**

27 Meiosis is the process by which haploid gametes are produced from diploid precursor
28 cells. We used stable isotope labeling by amino acids in cell culture (SILAC) to
29 characterize the meiotic proteome in the fission yeast *Schizosaccharomyces pombe*.
30 We compared relative levels of proteins extracted from cells harvested around meiosis
31 I with those of meiosis II, and proteins from premeiotic S phase with the interval
32 between meiotic divisions, when S phase is absent. Our proteome datasets revealed
33 peptides corresponding to short open reading frames (sORFs) that have been
34 previously identified by ribosome profiling as new translated regions. We verified
35 expression of selected sORFs by Western blotting and analyzed the phenotype of
36 deletion mutants. Our data provide a resource for studying meiosis that may help
37 understand differences between meiosis I and meiosis II and how S phase is
38 suppressed between the two meiotic divisions.
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40
41 **Keywords:** meiosis, fission yeast *Schizosaccharomyces pombe*, SILAC, short open
42 reading frames
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51 **Introduction**

52

53 Sexual reproduction depends on meiosis, a process that generates haploid gametes
54 from a diploid precursor cell. The fission yeast *Schizosaccharomyces pombe* is a
55 useful model organism for studying meiosis. One of the advantages of using fission
56 yeast is that highly synchronous meiosis can be induced by inactivation of the Pat1
57 protein kinase [1-4]. Moreover, a broad spectrum of genomic and proteomic tools is
58 available. Progression of meiosis is accompanied by complex changes of gene
59 expression [5]. These changes in fission yeast meiosis have been studied by various
60 approaches including transcriptional profiling using DNA microarrays and ribosome
61 profiling to investigate the translational landscape [6, 7].
62 A comprehensive study analyzing changes of the *S. pombe* meiotic proteome using
63 stable isotope labeling by amino acids in cell culture (SILAC) was published during
64 the course of our work [8]. Krapp et al. quantified 3268 proteins throughout fission
65 yeast meiosis induced by the inactivation of a temperature-sensitive allele of the Pat1
66 kinase (*pat1-114*) and found that the levels of 880 proteins changed at least 2-fold.
67 Their study revealed a high degree of post-transcriptional regulation of protein levels
68 and a global switch from anabolic to catabolic processes during meiosis [8].
69 In our current work, we performed SILAC based quantitative analysis of the *S. pombe*
70 proteome during meiosis. In addition to *pat1-114*-induced meiosis, we used an
71 improved synchronization protocol based on chemical inactivation of an ATP analog–
72 sensitive form of the Pat1 kinase (*pat1-as2*), which eliminates negative effects of the
73 higher temperature needed to inactivate the Pat1-114 kinase. We not only analyzed
74 standard proteins, but also proteins encoded by short open reading frames (sORFs),
75 which are usually defined as proteins smaller than 100 amino acids. Such sORFs were

76 often ignored during genome annotations to minimize false positive ORFs [9-11].
77 However, recent analyses have revealed numerous examples of proteins encoded by
78 sORFs that have important cellular functions [12, 13]. We searched our SILAC based
79 mass-spectrometry data and found peptides corresponding to novel sORFs that have
80 been previously identified by ribosome profiling. We verified expression of selected
81 sORFs by Western blot analysis and performed phenotypical characterization of
82 deletion mutants. Finally, we discuss gene organization at the corresponding genomic
83 regions and relevant refinements in the annotation of the fission yeast genome.
84
85
86 **Results and discussion**
87
88 **SILAC based analysis of the meiotic proteome**
89 There are several important differences between meiosis I (MI) and meiosis II (MII).
90 Chiasma formation, mono-orientation of sister kinetochores, and protection of
91 centromeric cohesion are key aspects of MI chromosomes that are absent during MII.
92 In addition, MI is preceded by an S phase during which DNA is replicated but there is
93 no S phase between MI and MII [14, 15]. Quantitative comparison of the proteomes
94 of various meiotic stages allows identification of proteins whose levels are
95 differentially regulated. Such proteins that are specifically present or absent during a
96 particular stage of meiosis may be important regulators of meiosis-specific processes.
97 To identify such regulators, we used SILAC based proteome analysis to compare
98 relative levels of proteins present during various stages of meiosis. SILAC labeling
99 combined with high-resolution mass spectrometry is one of the key methods for
100 quantitative proteomics [16] that is also available for fission yeast [8, 17].

101 We used SILAC based proteome analysis in synchronous meiotic cultures induced by
102 inactivation of a temperature-sensitive allele *pat1-114* to compare relative levels of
103 proteins present during premeiotic S phase (meiS) with the interval between meiotic
104 nuclear divisions (MI-II), when S phase is absent (Figure 1, Table S1). Although this
105 synchronization protocol based on the inactivation of a temperature-sensitive allele of
106 the Pat1 kinase (*pat1-114*) has been widely used to study meiosis in the fission yeast *S.*
107 *pombe*, it is not ideal for studying meiotic divisions because of chromosome
108 missegregation defect [18, 19]. Previous studies showed that *pat1-114*-induced
109 meiosis differs from wild-type meiosis in some aspects, such as chromosome
110 segregation. Whereas in wild-type cells sister centromeres segregate to the same pole
111 in anaphase I, in meiosis induced by inactivation of Pat1-114 by elevated temperature
112 sister centromeres segregate to the same pole very inefficiently in anaphase I cells [18,
113 19]. In order to overcome this obstacle, we have developed a synchronization protocol
114 based on *pat1-as2*. Chemical inactivation of an ATP analog–sensitive form of the
115 Pat1 kinase (*pat1-as2*) by adding the ATP analog 1-NM-PP1 allows the induction of
116 synchronous meiosis without the need of elevated temperature. In *pat1-as2*-induced
117 meiosis, chromosomes segregate with higher fidelity and spore viability is higher than
118 in *pat1-114* meiosis [2-4]. We used *pat1-as2*-induced meiotic cultures to compare
119 relative levels of proteins extracted from cells harvested around MI with those of MII
120 (Figure 1, Table S1).

121 To exclude possible isotope effects of the heavy $^{13}C_6$ lysine and differences between
122 batches of labeled amino acids, we performed experimental replicates with reversed
123 labels. Unlabeled meiS was analyzed with heavy lysine labeled MI-MII transition
124 (meiS (L) + MI-MII (H)) and heavy lysine labeled meiS was analyzed with unlabeled
125 MI-MII transition (meiS (H) + MI-MII (L)). Similarly, unlabeled MI was analyzed

126 with heavy lysine labeled MII (MI (L) + MII (H)) and heavy lysine labeled MI was
127 analyzed with unlabeled MII (MI (H) + MII (L)) (Table S1). Normalized ratios
128 (heavy/light) of peptides corresponding to selected proteins involved in DNA
129 replication and chromosome segregation are shown in Table 1.

130

131 **Verification of proteins encoded by sORFs identified by ribosome profiling**

132 Ribosome profiling of *S. pombe* diploid cells undergoing meiosis identified 373
133 sORFs encoding short proteins that were at least 30 amino acids long that have not
134 been previously described (Table S2) [7]. These included short proteins in ncRNAs,
135 unannotated regions and 5'-UTRs (encoded in uORFs). We searched our SILAC
136 based mass-spectrometry results for peptides corresponding to these 373 novel sORFs
137 identified by ribosome profiling and found unique peptides corresponding to nine
138 sORFs (Figure 2A, Figure S1, Table S3).

139 Next, we constructed strains expressing C-terminally TAP-tagged ORF18274,
140 ORF33564, ORF96155, ORF30606, ORF30707 and ORF692. Western blot analyses
141 revealed bands of expected sizes but also additional bands (Figure 2B, Figure S2,
142 Figure S3). Further experiments are needed to clarify what these additional bands
143 represent. While all six TAP-tagged proteins were detected in meiotic extracts,
144 ORF30606-TAP, ORF30707-TAP and ORF692-TAP were present also in extracts
145 from vegetative cells (Figure 2B, Figure S2, Figure S3). ORF18274-TAP (Prl46-TAP)
146 and ORF692-TAP (Prl3-TAP) were independently constructed and detected by
147 Western blotting by Duncan and Mata [7]. However, we noticed that ORF18274-TAP
148 is larger than originally described [7]. This is probably due to extended N-terminus,
149 which starts already before the beginning of the non-coding RNA *prl46*.

150 During the course of this work, there were changes in annotations of three sORFs [11].
151 *ORF35915* has been annotated as the second exon of the *ina17* gene [20] and
152 *ORF96155* as the first exon of the *mug62* gene [7]. Detailed analysis of the sequence
153 variants identified an indel error that affected the gene structure annotation of *pta1*,
154 whose coding sequence was extended at the 3'-end and included *ORF142944* [21].
155 Thus, it is likely that *ORF35915*, *ORF142944* and *ORF96155* do not encode
156 independent short proteins but they are part of larger genes (Figure S1).
157
158 **Phenotypical characterization of sORFs deletion mutants**
159 We analyzed the consequences of deleting *ORF18274*, *ORF35915*, *ORF142944*,
160 *ORF30707* and *ORF692*. In a haploid *S. pombe* strain, we were able to delete
161 *ORF18274*, *ORF35915*, *ORF30707*, *ORF692* but not *ORF142944*. Tetrad analysis of
162 a diploid strain heterozygous for *ORF142944* deletion showed that spores carrying
163 *ORF142944* deletion germinated but did not form colonies (data not shown). This
164 result is consistent with the finding that *ORF142944* is part of the *pta1* gene, which is
165 essential for cell growth [10, 21].
166 We next analyzed phenotypes of *ORF18274Δ*, *ORF35915Δ*, *ORF30707Δ* and
167 *ORF692Δ* deletion strains. Mutant vegetative cells showed no apparent growth
168 defects or altered cell morphology (data not shown). The growth of mutant cells was
169 similar to wild type in the presence of DNA damaging agents such as methyl
170 methanesulfonate, camptothecin, hydroxyurea, zeocin and menadione (Figure 3A).
171 Chromosome segregation in mutant cells, as scored by GFP labeled centromere of
172 chromosome I, was similar to wild type during both mitosis and meiosis (data not
173 shown). Spore viability in mutant strains was similar to wild type, suggesting that
174 there is no major meiotic defect in mutant cells (Figure 3B and 3C). We conclude that

175 *ORF18274*, *ORF35915*, *ORF30707* and *ORF692* are dispensable for vegetative
176 growth under all tested conditions and production of viable spores.
177
178 Taken together, we performed SILAC based quantitative analysis of the *S. pombe*
179 proteome during meiosis. Our results provide a resource for studying meiosis that
180 may help understand differences between MI and MII and how S phase is suppressed
181 between the two meiotic divisions. Our meiotic proteome datasets revealed unique
182 peptides corresponding to only nine sORFs, out of 373 sORFs that have been
183 previously identified by ribosome profiling as new translated regions. It is possible
184 that more of these short proteins are present in meiotic and/or vegetative *S. pombe*
185 cells, however their reliable detection will require more sensitive analyses including
186 enrichment for short proteins before the mass-spectrometry analysis. Our results are
187 consistent with previous findings that *ORF35915*, *ORF142944* and *ORF96155* do not
188 encode independent short proteins but they are part of larger genes. They are also
189 consistent with the notion that *ORF21231, ORF18274, ORF33564, ORF30606,*
190 *ORF30707* and *ORF692* encode short proteins. However, we cannot exclude the
191 possibility that these six sORFs are also part of larger genes. While we detected
192 ORF21231 only by mass-spectrometry, ORF18274, ORF33564, ORF30606,
193 ORF30707 and ORF692 were detected by both mass-spectrometry and Western
194 blotting. The role of these short proteins remains unknown. Future experiments
195 should include detailed analyses of mutant phenotypes and sensitive *in silico* searches
196 to assess possible conservation of identified sORFs during evolution. Identification of
197 sORFs that encode proteins and deciphering their roles are important future goals
198 arising from our current work and other proteomic and ribosome profiling studies.
199

200 **Materials and methods**

201

202 **Strain construction**

203 We constructed TAP-tagging plasmids containing long regions homologous to the
204 target gene according to our protocol described in Cipak et al. [22] for all nine sORFs
205 (ORF18274, ORF21231, ORF33564, ORF35915, ORF142944, ORF96155,
206 ORF30606, ORF30707 and ORF692). We transformed these plasmids into a haploid
207 *S. pombe* strain JG12017 and verified successful tagging in yeast transformants by
208 PCR. We constructed strains expressing C-terminally TAP-tagged ORF18274,
209 ORF33564, ORF96155, ORF30606, ORF30707 and ORF692 but not ORF142944,
210 where no yeast transformants were obtained. Western blot analyses revealed bands of
211 expected sizes but also additional bands (Figure 2B, Figure S2, Figure S3). No bands
212 were observed in extracts prepared from strains carrying ORF21231-TAP and
213 ORF35915-TAP (data not shown).
214 Genotypes of strains and the figures and tables in which each was used are in Table
215 S4. Genes were deleted as described in Gregan et al. [23]. Spore viability was
216 determined as described in Phadnis et al. [24].

217

218 **Meiotic synchronization and SILAC labeling**

219 Diploid *S. pombe* strains carrying temperature sensitive *pat1-114* (JG16328) or ATP
220 analog-sensitive *pat1-as2* (JG16419) were incubated at 25°C over-night in EMM2
221 liquid medium (3.0 g/l potassium hydrogen phthalate, 2.2 g/l Na_2HPO_4, 5.0 g/l
222 NH_4Cl, 1.0% (w/v) glucose, 75 mg/l lysine, supplemented with salts, vitamins and
223 minerals) [2, 3]. The cells were collected by centrifugation, diluted in fresh EMM2
224 medium supplemented with 75 mg/l lysine (light sample) or 75 mg/l heavy lysine

225 (heavy sample) into OD_{600} = 0.0375 and grown at 25°C until OD_{600} = 0.5 – 0.6. After
226 centrifugation the cells were washed 3 times with deionized water, resuspended in
227 EMM2-N medium (3.0 g/l potassium hydrogen phthalate, 2.2 g/l Na_2HPO_4, 1.0%
228 (w/v) glucose, supplemented with salts, vitamins and minerals) and incubated at 25°C
229 for 12 h to arrest the cells in G_1 phase. Arrested cells were centrifuged and
230 resuspended in the same volume of EMM2 medium supplemented with 75 mg/l lysine
231 (light sample) or 75 mg/l heavy lysine (heavy lysine labeled sample). Meiosis was
232 induced by shifting the cells to 34°C (*pat1-114*) or by adding 1-NM-PP1 (Toronto
233 Research Chemicals) to 25 μM and incubated at 25°C (*pat1-as2*). Unlabeled and
234 heavy lysine labeled cells from various stages of meiosis were collected by filtration
235 through 0.45 μm membrane disc filter (Pall Corporation). Cells were frozen in liquid
236 nitrogen and disrupted by Cryogenic Grinder (6775 Freezer/Mill Cryogenic Grinder,
237 SPEX SamplePrep).
238 Heavy lysine was purchased from Cambridge Isotope Laboratories (U-13C6, CLM-
239 2247-0.25), TRIzole Reagent from Invitrogen (15596-026, 100 ml), GN-6 Metricel
240 MCE Membrane Disc Filters from Pall Corporation (66265, 47 mm, plain, sterile) and
241 Magnetic Filter Funnels from Pall Corporation (4242, 47 mm, 300 mL capacity).
242

243 **Protein extraction**

244 Yeast powders from light sample (0.1 g) and heavy lysine labeled sample (0.1 g)
245 isolated from particular stages of meiosis were mixed and resuspended in 6 ml of
246 TRIzol reagent. Proteins were extracted by vigorous shaking at 4°C for 15 min. The
247 sample was centrifuged at 12000g for 15 min at 4°C to remove insoluble material.
248 Supernatant was incubated for 5 min at RT, extracted with chloroform (ratio TRIzol
249 to chloroform was 5:1) and centrifuged at 12000g for 15 min at 4°C. Organic phase

250 containing DNA and proteins was collected and DNA was precipitated by mixing the
251 supernatant with 1.8 ml 100% ethanol and centrifuged at 2000g for 5 min at 4°C.
252 Phenol-ethanol supernatant was collected and mixed with 9 ml of isopropanol to
253 precipitate the proteins. The proteins were collected by centrifugation at 12000g for
254 10 min at 4°C and washed 3 times by 0.3 M guanidine hydrochloride in 95% ethanol
255 and 1 time in 95% ethanol. Vacuum dried proteins were dissolved in 1 ml of 8 M urea
256 supplemented with 0.5 M NH_4HCO_3 for 1 h at RT.
257
258 **Mass spectrometry analysis**
259 Protein extracts were reduced with DTT and then alkylated with iodoacetamide.
260 Protein solution was diluted with water to 6M urea and then digested with LysC
261 (Wako) at 1:30 ratio at 37°C overnight. The digests were desalted and lyophilized,
262 then dissolved and chromatographically separated on a strong cationic exchanger
263 (SCX) with a mixed salt- and pH-gradient in 15% acetonitrile (ACN). Up to 70
264 fractions were collected and ACN was removed by sample concentration in the speed
265 vac. The peptide fractions were separated in a second dimension on a C18 column on
266 a nano HPLC (Dionex, Thermo Scientific) applying 1 hour gradient. Eluting peptides
267 were analysed on a QExactive Orbitrap (Thermo Scientific) in a data-dependent
268 mode. The 12 most intense peptides in the survey scan recorded at 70000 resolution at
269 200 m/z, were subjected to CID fragmentation with 30% collision energy. CID
270 spectra were recorded at 17500 resolution and an AGC target value of 5E4. The MS
271 data were searched with MaxQuant 1.4. [25] against the *S. pombe* reference database
272 (https://www.pombase.org, 2013-03-19) and the sequences of the sORFs (TableS2)
273 with the following settings: LysC specificity, carabamidomethylation on Cys as fixed,
274 oxidation of methionin and acetylation of protein N-termini as variable modification.

275 The SILAC quan node was selected with $^{13}C_6$ lysine as the heavy label. All other
276 parameters were set to default. Results were filtered on protein and peptide level for a
277 1% FDR.
278
279 **Western blot analysis**
280 Proteins were separated by electrophoresis through 12% polyacrylamide gels
281 containing SDS (0.1%) and transferred to a PVDF membrane (Immobilon-P
282 membrane with 0.45μm pore size from Millipore). The membrane was blocked with
283 2% (w/v) milk-PBS-T (phosphate buffer saline buffer with 0.1% (v/v) Tween-20) and
284 probed with antibodies. TAP-tagged proteins were detected using rabbit
285 antiperoxidase antibody linked to peroxidase (PAP, Dako; 1:10000 dilution) in 0.1%
286 PBS-T (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 0.1% (v/v)
287 Tween-20). Tubulin was detected using mouse-anti-α-tubulin antibody (Sigma-
288 Aldrich T5168; 1:10000 dilution) and rabbit anti-mouse IgG-HRP secondary antibody
289 (Santa Cruz Biotechnology; 1:5000 dilution) in 2% (w/v) milk PBS-T.
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Disclosure statement

No potential conflict of interest was reported by the authors.

Author contributions

B.H., J.K., S.B.P., L.C. and D.A. performed experiments. C.D., J.M., G.A., A.S., Z.B., S.B.P., L.C. and J.G. analysed the data and prepared the manuscript.

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325 **Figure Legends**

326

327 **Figure 1. Flowchart of the SILAC based proteome analysis and progression of**
328 ***pat1*-induced meiosis.**

329 (A) Unlabeled (+ light lysine) and heavy lysine (+ heavy lysine) labeled diploid *S.*
330 *pombe* cells were arrested by nitrogen starvation and meiosis was induced by shifting
331 the cells to 34°C (*pat1-114*) or by adding 1-NM-PP1 (*pat1-as2*). Unlabeled and heavy
332 lysine labeled cells from various stages of meiosis were mixed in equal amounts and
333 protein extracts were prepared. After digestion with lysC protease, peptides were
334 fractionated by strong cation exchange (SCX) and analyzed by mass spectrometry
335 (LC-MS/MS).

336 (B) Meiotic cells as described in (A) were fixed, stained with DAPI and nuclei were
337 counted in 100 cells per time point. Shown are the fractions of cells that contained one
338 nucleus (1n), two nuclei (2n) or more than two nuclei (3n or more) at the indicated
339 time points after meiosis induction (hours). MeiS, MI-MII, MI and MII indicate when
340 cells were harvested for the SILAC based proteome analysis.

341

342 **Figure 2. Verification of proteins encoded by novel sORFs identified by ribosome**
343 **profiling.**

344 (A) List of sORFs for which corresponding peptides were identified by mass-
345 spectrometry.

346 (B) *pat1-114* cells expressing indicated TAP-tagged proteins were arrested by
347 nitrogen starvation and released into meiosis at 34°C (Figure S2). Cells were
348 harvested at the indicated time points (hours) after meiosis induction and protein
349 extracts were analyzed by Western blotting. Protein extracts were also prepared from

350 cycling vegetative cells (Mit). As a positive control (PC), protein extracts were
351 prepared from a pool of cells expressing TAP tag alone, harvested at 4, 5 and 6 hours
352 after meiosis induction. As a negative control (NC), protein extracts were prepared
353 from a pool of wild-type cells, harvested 2-6 hours after meiosis induction. TAP tag
354 was detected using rabbit antiperoxidase antibody linked to peroxidase and tubulin
355 was detected using mouse-anti-α-tubulin antibody. Molecular weight marker (kDa) is
356 indicated on the left. Additional Western blots are shown in Figure S3.
357

358 **Figure 3. *ORF18274Δ*, *ORF35915Δ*, *ORF30707Δ* and *ORF692Δ* cells are not**
359 **sensitive to DNA damaging agents and produce viable spores.**
360 (A) Cells were grown on YES medium for one day, diluted and spotted onto YES
361 plates containing the indicated amounts of menadione (MD), methyl
362 methanesulfonate (MMS), camptothecin (CPT), hydroxyurea (HU) or zeocin (ZEO).
363 Plates were incubated for 3 days at 32°C. *pds5Δ* was used as a control that is known
364 to be sensitive to DNA damaging agents.
365 (B) Spore viability of the indicated strains was measured by tetrad dissection in two
366 independent experiments. 80 tetrads were dissected for each strain.
367 (C) Spore viability of the indicated strains was measured by random spore analysis.
368 100000 spores were plated per plate, incubated for 30 hours at 32°C and 200
369 spores/colonies were scored in at least two independent experiments. Microcolonies
370 consisting of up to four cells and colonies containing more than four cells were
371 scored.
372
373
374

375 **Supplementary Figure Legends**

376

377 **Figure S1. Annotated chromosomal regions with studied sORFs.**

378

379 **Figure S2. Progression of *pat1-114* cells expressing indicated TAP-tagged**
380 **proteins into meiosis.**

381 *pat1-114* cells expressing indicated TAP-tagged proteins were arrested by nitrogen
382 starvation and released into meiosis at 34°C. Fixed cells were stained with DAPI and
383 nuclei were counted in 100 cells per time point. Shown are the fractions of cells that
384 contained one nucleus (1n), two nuclei (2n) or more than two nuclei (3n or more) at
385 the indicated time points after meiosis induction (hours). The progression of meiosis
386 in all tested mutant strains was similar to that in wild-type cells.

387

388 **Figure S3. Western blot analysis of ORF692-TAP.**

389 Protein extracts were prepared and analyzed by Western blotting as described in
390 Figure 2B.

391

392 **Table S1. Quantitative proteomic analysis using SILAC.**

393

394 **Table S2. sORFs (longer than 90 bp) identified by ribosome profiling.**

395

396 **Table S3. Peptides corresponding to sORFs.**

397

398 **Table S4. *S. pombe* strains used in this study.**
399

400

401 **References**

402

403 1. Bahler, J., Schuchert, P., Grimm, C., and Kohli, J. (1991). Synchronized
404 meiosis and recombination in fission yeast: observations with pat1-114 diploid
405 cells. Curr Genet *19*, 445-451.
406 2. Cipak, L., Polakova, S., Hyppa, R.W., Smith, G.R., and Gregan, J. (2014).
407 Synchronized fission yeast meiosis using an ATP analog-sensitive Pat1
408 protein kinase. Nat Protoc *9*, 223-231.
409 3. Cipak, L., Hyppa, R.W., Smith, G.R., and Gregan, J. (2012). ATP analog-
410 sensitive Pat1 protein kinase for synchronous fission yeast meiosis at
411 physiological temperature. Cell Cycle *11*, 1626-1633.
412 4. Guerra-Moreno, A., Alves-Rodrigues, I., Hidalgo, E., and Ayte, J. (2012).
413 Chemical genetic induction of meiosis in Schizosaccharomyces pombe. Cell
414 Cycle *11*, 1621-1625.
415 5. Otto, G.M., and Brar, G.A. (2018). Seq-ing answers: uncovering the
416 unexpected in global gene regulation. Curr Genet *64*, 1183-1188.
417 6. Mata, J., Lyne, R., Burns, G., and Bahler, J. (2002). The transcriptional
418 program of meiosis and sporulation in fission yeast. Nat Genet *32*, 143-147.
419 7. Duncan, C.D., and Mata, J. (2014). The translational landscape of fission-
420 yeast meiosis and sporulation. Nat Struct Mol Biol *21*, 641-647.
421 8. Krapp, A., Hamelin, R., Armand, F., Chiappe, D., Krapp, L., Cano, E.,
422 Moniatte, M., and Simanis, V. (2019). Analysis of the S. pombe Meiotic
423 Proteome Reveals a Switch from Anabolic to Catabolic Processes and
424 Extensive Post-transcriptional Regulation. Cell Rep *26*, 1044-1058 e1045.
425 9. Spirek, M., Benko, Z., Carnecka, M., Rumpf, C., Cipak, L., Batova, M.,
426 Marova, I., Nam, M., Kim, D.U., Park, H.O., et al. (2010). S. pombe genome
427 deletion project: an update. Cell Cycle *9*, 2399-2402.
428 10. Kim, D.U., Hayles, J., Kim, D., Wood, V., Park, H.O., Won, M., Yoo, H.S.,
429 Duhig, T., Nam, M., Palmer, G., et al. (2010). Analysis of a genome-wide set
430 of gene deletions in the fission yeast Schizosaccharomyces pombe. Nat
431 Biotechnol *28*, 617-623.
432 11. Lock, A., Rutherford, K., Harris, M.A., Hayles, J., Oliver, S.G., Bahler, J., and
433 Wood, V. (2019). PomBase 2018: user-driven reimplementation of the fission
434 yeast database provides rapid and intuitive access to diverse, interconnected
435 information. Nucleic Acids Res *47*, D821-D827.
436 12. Hollerer, I., Higdon, A., and Brar, G.A. (2018). Strategies and Challenges in
437 Identifying Function for Thousands of sORF-Encoded Peptides in Meiosis.
438 Proteomics *18*, e1700274.
439 13. Herberg, S., Gert, K.R., Schleiffer, A., and Pauli, A. (2018). The Ly6/uPAR
440 protein Bouncer is necessary and sufficient for species-specific fertilization.
441 Science *361*, 1029-1033.
442 14. Marston, A.L., and Amon, A. (2004). Meiosis: cell-cycle controls shuffle and
443 deal. Nat Rev Mol Cell Biol *5*, 983-997.
444 15. Hua, H., Namdar, M., Ganier, O., Gregan, J., Mechali, M., and Kearsey, S.E.
445 (2013). Sequential steps in DNA replication are inhibited to ensure reduction
446 of ploidy in meiosis. Mol Biol Cell *24*, 578-587.
447 16. Ong, S.E., Blagoev, B., Kratchmarova, I., Kristensen, D.B., Steen, H., Pandey,
448 A., and Mann, M. (2002). Stable isotope labeling by amino acids in cell
449 culture, SILAC, as a simple and accurate approach to expression proteomics.
450 Mol Cell Proteomics *1*, 376-386.

451 17. Macek, B., Carpy, A., Koch, A., Bicho, C.C., Borek, W.E., Hauf, S., and
452 Sawin, K.E. (2017). Stable Isotope Labeling by Amino Acids in Cell Culture
453 (SILAC) Technology in Fission Yeast. Cold Spring Harb Protoc *2017*, pdb
454 top079814.
455 18. Yamamoto, A., and Hiraoka, Y. (2003). Monopolar spindle attachment of
456 sister chromatids is ensured by two distinct mechanisms at the first meiotic
457 division in fission yeast. Embo J *22*, 2284-2296.
458 19. Kakui, Y., Sato, M., Tanaka, K., and Yamamoto, M. (2011). A novel fission
459 yeast mei4 mutant that allows efficient synchronization of telomere dispersal
460 and the first meiotic division. Yeast *28*, 467-479.
461 20. Jimenez, J., Duncan, C.D., Gallardo, M., Mata, J., and Perez-Pulido, A.J.
462 (2015). AnABlast: a new in silico strategy for the genome-wide search of
463 novel genes and fossil regions. DNA Res *22*, 439-449.
464 21. Hu, W., Suo, F., and Du, L.L. (2015). Bulk Segregant Analysis Reveals the
465 Genetic Basis of a Natural Trait Variation in Fission Yeast. Genome Biol Evol
466 *7*, 3496-3510.
467 22. Cipak, L., Spirek, M., Novatchkova, M., Chen, Z., Rumpf, C., Lugmayr, W.,
468 Mechtler, K., Ammerer, G., Csaszar, E., and Gregan, J. (2009). An improved
469 strategy for tandem affinity purification-tagging of Schizosaccharomyces
470 pombe genes. Proteomics *9*, 4825-4828.
471 23. Gregan, J., Rabitsch, P.K., Rumpf, C., Novatchkova, M., Schleiffer, A., and
472 Nasmyth, K. (2006). High-throughput knockout screen in fission yeast. Nat
473 Protoc *1*, 2457-2464.
474 24. Phadnis, N., Cipak, L., Polakova, S., Hyppa, R.W., Cipakova, I., Anrather, D.,
475 Karvaiova, L., Mechtler, K., Smith, G.R., and Gregan, J. (2015). Casein
476 Kinase 1 and Phosphorylation of Cohesin Subunit Rec11 (SA3) Promote
477 Meiotic Recombination through Linear Element Formation. PLoS Genet *11*,
478 e1005225.
479 25. Cox, J., and Mann, M. (2008). MaxQuant enables high peptide identification
480 rates, individualized p.p.b.-range mass accuracies and proteome-wide protein
481 quantification. Nat Biotechnol *26*, 1367-1372.
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