Recombination shapes the diversification of the *wtf* meiotic drivers

Yan Wang[1], Hao Xu[1], Qinliu He[1], Zhiwei Wu[1], Zhen Gong[1,*], Guan-Zhu Han[1,*]

[1]College of Life Sciences, Nanjing Normal University, Nanjing 210023, China

[*]Corresponding authors.

E-mail: guanzhu@njnu.edu.cn (G.-Z.H.) or gongzhen@nnu.edu.cn (Z.G.)

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Abstract

Meiotic drivers are selfish genetic elements that distort fair segregation. The *wtf* genes are poison-antidote meiotic drivers that are experiencing rapid diversification in fission yeasts. However, gene duplication alone is insufficient to drive the diversification of *wtf* genes, given the poison encoded by a newly duplicated *wtf* gene can be detoxified by the antidote encoded by the original *wtf* gene. Here, we analyze the evolution of *wtf* genes across 21 strains of *Schizosaccharomyces pombe*. Knocking out each of 25 *wtf* genes in *S. pombe* strain 972h- separately does not attenuate the yeast growth, indicating that the *wtf* genes might be largely neutral to their carriers in asexual life cycle. Interestingly, *wtf* genes underwent recurrent and intricate recombination. As proof-of-principle, we generate a novel meiotic driver through artificial recombination between *wtf* drivers, and its encoded poison cannot be detoxified by the antidotes encoded by their parental *wtf* genes but can be detoxified by its own antidote. Therefore, we propose that recombination can generate new meiotic drivers and thus shape the diversification of the *wtf* drivers.

Introduction

During meiosis, the two alleles at a gene locus are separated into gametes, and each gamete has an equal chance of receiving either allele. This fundamental principle of inheritance, known as Mendel's law of segregation (Abbott and Fairbanks, 2016), holds across most genetic loci in most sexual species. However, meiotic drivers, a class of selfish genetic elements, subvert fair segregation during gametogenesis and are transmitted to more than one-half (even to all) of the functional gametes produced by a heterozygote (Sandler and Novitski, 1957; Lyttle, 1991; Hurst and Werren, 2002; Bravo Nunez et al., 2018b). Meiotic drivers can spread in a population even when they impose fitness costs on their hosts (Crow, 1991; Lindholm et al., 2016). However, the spread of a meiotic driver can be thwarted by the costs imposed on its carriers or by its genetic suppressors (Lindholm et al., 2016).

The fission yeast *wtf* (*with Tf Long Terminal Repeats*) gene family provides an excellent model to study how meiotic drivers act and evolve (Hu et al., 2017; Nuckolls et al., 2017). Many *wtf* genes are autonomous one-gene poison-antidote meiotic drivers that encode both a spore-killing poison (short isoform) and an antidote to the poison (long isoform) using alternative transcriptional initiation (Hu et al., 2017; Nuckolls et al., 2017; Nuckolls et al., 2022). To archieve meiotic drive, all spores are exposed to the poison, whereas only those that inherit *wtf* express the antidote and are rescued (Hu et al., 2017; Nuckolls et al., 2017; Nuckolls et al., 2022). Some other *wtf* genes can act as drive suppressors (Bravo Nunez et al., 2018a; Bravo Nunez et al., 2020a). The poison and the antidote differ only in their N-terminal cytosolic tails containing PY (Leu/Pro-Pro-X-Tyr) motifs. PY motif-dependent ubiquitination promotes the transport of the antidote and the poison (physically interacted with the antidote) from the trans-Golgi network to the endosome, thereby preventing toxicity (Zheng et al., 2023).

The *wtf* gene family is experiencing rapid diversification: the *Schizosaccharomyces pombe* reference genome encodes 25 *wtf* genes, some of which are pseudogenes. The copy numbers of *wtf* genes vary greatly among different *S. pombe* strains, and frequent nonallelic gene conversion occurs between *wtf* genes (Hu et al., 2017; Nuckolls et al., 2017; Eickbush et al., 2019). However, these finding are based on a limited number of strains, and the pattern and extent of recombination in the *wtf* genes remain to be fully explored. Moreover, *wtf* driver genes are present in the last common ancestor (LCA) of the fission yeasts *S. pombe*, *S. octosporus*, *S. osmophilus*, and *S.*

cryophilus, indicating that *wtf* genes have likely maintained the capacity to drive for more than 100 million years (De Carvalho et al., 2022). These fission yeast species carry varying numbers of *wtf* genes, ranging from 5 to 83 (De Carvalho et al., 2022). Yet, it remains perplexing how *wtf* genes achieved such diversification. On one hand, gene duplication can give birth to new *wtf* gene copies. On the other hand, a newly duplicated *wtf* gene might not drive because the poison produced by the newly duplicated *wtf* gene can be detoxified by the original *wtf* gene. Like newly duplicated genes, the most probable fate of a new *wtf* duplicate is pseudogenization (Lynch, 2007; Innan and Kondrashov, 2010). Thus, the vast majority of new *wtf* duplicates experience an early exit from the population, most probably never reaching fixation (Lynch, 2007; Innan and Kondrashov, 2010). To become a new driver, the new *wtf* copy should evolve coupling new poison and new antidote to the new poison through mutations. But the fate-changing mutations are likely to be rare. It follows that gene duplication might be insufficient to drive the diversification of *wtf* genes.

In this study, we analyzed the diversity and evolution of *wtf* genes in the genomes of 21 strains of *S. pombe* that were sequenced using long-read sequencing approaches (Tusso et al., 2022). Through knocking out each of 25 *wtf* genes in *S. pombe* laboratory strain 972h-, no significant attenuated growth was observed, indicating *wtf* genes might be not deleterious in the asexual life cycle. We found that recurrent recombination occurred among *wtf* genes. We generated a novel meiotic driver through artificial recombination between *wtf* drivers, and its encoded poison cannot be detoxified by the antidotes encoded by their parental *wtf* genes but can be detoxified by its own antidote. Therefore, we propose that recombination can generate *wtf* driver with new poisons and might shape the diversification of *wtf* genes.

Results

Diversity and evolution of *wtf* genes in fission yeasts

First, we analyzed the diversity and distribution of *wtf* genes in fission yeasts. The *S. pombe* reference genome (strain 972h-) encodes a total of 25 *wtf* genes. For these 25 *wtf* genes, the number of exons varies from 3 to 6 (Figure 1A) (Bowen et al., 2003). To investigate the relationship among exons from different *wtf* genes, we grouped these *wtf* exons into clusters based on nucleotide

identity of 0.50 (Figure 1B and C). The *wtf* exons were grouped into 10 clusters with >2 members, and 12 exons exist as singletons in the similarity network (Figure 1B and C; Supplementary file 1c). Only exon 1 of all the *25 wtf* genes group together in a cluster, indicating that the first exons are well conserved among the *wtf* genes. No other exon is conserved among all the 25 *wtf* genes. Therefore, the evolution of the *wtf* gene structures appear to be highly dynamic.

We next identified *wtf* genes in 21 strains of *S. pombe* that were sequenced using long-read sequencing approaches (Supplementary file 1b) (Tusso et al., 2022). The copy number of *wtf* genes varies among different *S. pombe* strains, ranging from 24 (strain JB879) to 37 (strain JB1206) (Figure 1D). Synteny analyses show that the *wtf* genes are present in 20 genetic loci (Figure 1D). Multiple *wtf* genes were present in 13 *wtf* loci. Within 20 *wtf* loci, at least one *wtf* gene is present in all of or nearly all of the 21 *S. pombe* strains, suggesting that these 20 *wtf* loci might have originated before the LCA of the 21 *S. pombe* strains. Putative *wtf* pseudogenes are prevalent in many *wtf* loci among the 21 *S. pombe* strains, indicating frequent pseudogenization occurred in the *wtf* genes. These results indicate that *wtf* copy number variation is prevalent among *S. pombe* strains.

We performed phylogenetic analyses of the *wtf* genes from 21 *S. pombe* strains and three other fission yeast species (*S. octosporus*, *S. cryophilus*, and *S. osmophilus*) (Figure 1E; Supplementary file 1d). The *wtf* genes of *S. pombe* form a monophyletic group. Orthologs of *wtf14*, *wtf7*, *wtf11*, and *wtf15* form monophyletic groups, whereas orthologs of other *wtf* genes show complex phylogenetic mixing, indicating complex recombination might have occurred among these *wtf* genes (Figure 1E) (Eickbush et al., 2019). Moreover, the *wtf* genes with 6 exons (including the known functional drivers *wtf4* and *wtf23*) cluster together, and exhibit a ladder-like phylogeny, which might be generated by continual selection driven by antidotes (like the ladder-like phylogeny of influenza A viruses H1N1 and H3N2, which is shaped by continual immune selection (Grenfell et al., 2004; Bedford et al., 2011)). Based on phylogenetic relationship, we divided the *wtf* genes of 21 *S. pombe* strains into eight groups, namely groups 1 to 8, among which groups 5 to 8 include orthologs of *wtf14*, *wtf7*, *wtf11*, and *wtf15*, respectively (Figure 1E). Exon 2 ATG codons (exon-2-ATG) and in-frame ATG within intron 1 and near the start of exon 2 (intron-1-ATG) of *wtf* genes can encode the start of poison protein isoforms (Hu et al., 2017). We found that most of exon-2-ATG and intron-1-ATG are present within group 1 *wtf* genes (Figure 1E). A majority of the *wtf* genes are flanked by solo-LTRs (Bowen et al., 2003) (Figure 1E). However,

115 the solo-LTRs flanking the *wtf* genes do not cluster together but form many distinct groups,
116 suggesting that solo-LTRs were inserted nearby the *wtf* genes multiple times (Supplementary file
117 1a). Together, our results reveal the rapid diversification and turnover of *wtf* genes in a single
118 fission yeast species.

119

120 **No attenuated growth of fission yeast without *wtf* genes**

121 To explore the effect of *wtf* genes on the fitness of fission yeast, we knocked out each of the
122 25 *wtf* genes in the *S. pombe* laboratory strain 972h- using a method based on homologous
123 recombination (Figure 2A). A total of 25 *wtf* knockout strains (*Δwtf1* to *Δwtf25*) were generated.
124 We used spot assay to evaluate the effect of *wtf* gene knockout on the yeast growth, and no growth
125 defect was observed for all the 25 *wtf* knockout strains (Figure 2B). Therefore, our experiment
126 suggests that the *wtf* genes might be largely neutral to the fitness of their carriers in the asexual life
127 cycle at least in normal growth condition.

128

129 **Recurrent recombination in *wtf* genes**

130 Given complex phylogenetic mixing observed among *wtf* genes (Figure 1E), we tested whether
131 recombination occurred. We detected signals of recombination in the 25 *wtf* genes of the *S. pombe*
132 reference genome ($p = 0$) and in the *wtf* genes of the 21 *S. pombe* strains ($p = 0$) using pairwise
133 homoplasy index (HPI) test. Split network analysis also supports the frequent occurrence of
134 recombination in the 25 *wtf* genes of the *S. pombe* reference genome (Figure 3A) and in the *wtf*
135 genes of the 21 strains of *S. pombe* (Figure 3B). In contrast, no recombination signal was tested
136 for groups 5 to 8 using HPI test ($p = 1$ for group 5, $p = 1$ for group 6, $p = 0.53$ for group 7, and p
137 $= 1$ for group 8). We estimated recombination rates of the full-length *wtf* sequences, the first exons,
138 and the *wtf* sequences without the first exons for *wtf* groups 1 to 4. We found that the recombination
139 rate of group 1 *wtf* was highest among the four *wtf* groups (Figure 3C). For group 1, breakpoints
140 are dispersed across the *wtf* sequences (Figure 3D). These lines of evidence suggest that *wtf* genes
141 underwent recurrent and intricate recombination.

142

143 **Generation of a new driver gene through artificial recombination**

144 Given gene duplication alone might be insufficient to shape the diversification of *wtf* genes,
145 we hypothesize that recombination between *wtf* genes can generate new meiotic drives. To test this,
146 we constructed four chimeric *wtf* genes through recombination among known functional meiotic
147 drivers (*wtf23*) and an artificially generated meiotic driver (*wtf18*).

148 We used a proved *Saccharomyces cerevisiae* system to test the activity of poison and antidote
149 proteins encoded by *wtf* genes (Nuckolls et al., 2020). As expected, the expression of the poison
150 proteins (Wtf23^{poison}) encoded by *wtf23* genes caused the yeast growth arrest (Figure 4A). The
151 attenuated growth was alleviated, when the corresponding antidote proteins (Wtf23^{antidote}) were
152 expressed (Figure 4A). We also experimentally analyzed *wtf18* gene, which was known to encode
153 only long (antidote-like) transcripts and probably act as a suppressor (Bravo Nunez et al., 2018a).
154 We artificially introduced an in-frame ATG codon right before the start of exon 2, generating
155 *wtf18$^{poison/-0M}$*. The expression of *wtf18$^{poison/-0M}$* resulted in the yeast growth arrest, suggesting its
156 product, Wtf18$^{poison/-0M}$, is indeed a poison protein (Figure 4B). When co-expressing *wtf18^{antidote}*
157 and *wtf18$^{poison/-0M}$*, the attenuated yeast growth was rescued (Figure 4B), indicating that Wtf18^{antidote}
158 can ameliorate the toxicity of Wtf18$^{poison/-0M}$. Therefore, the introduction of an ATG codon to *wtf18*
159 leads to the generation of a putative meiotic driver.

160 We then constructed four chimeric *wtf* genes through artificial recombination between *wtf23*
161 and *wtf18*, including *wtfC1* (possessing exons 1-2 of *wtf23* and exons 3-6 of *wtf18*), *wtfC2*
162 (possessing exons 1-3 of *wtf23* and exons 4-6 of *wtf18*), *wtfC3* (possessing exons 1-4 of *wtf23* and
163 exons 5-6 of *wtf18*), and *wtfC4* (possessing exons 1-5 of *wtf23* and exon 6 of *wtf18*). The
164 expression of the short isoforms of *wtfC1*, *wtfC2*, *wtfC3*, and *wtfC4* resulted in yeast growth arrest,
165 revealing their toxicity (Figure 4C-F). However, the antidote of *wtfC1* and *wtfC3* cannot detoxify
166 the corresponding chimeric toxins (Figure 4C and E). Interestingly, we generated a putative novel
167 meiotic driver, namely *wtfC4*. Our results show that *wtfC4* encodes a functional poison
168 (WtfC4^{poison}) (Figure 4F). The poison can be detoxified by its own long isoforms (dubbed as
169 *wtfC4^{antidote}*), but cannot be detoxified by the antidote proteins of their parental genes (Figure 4F).
170 Taken together, we generated a new meiotic driver through artificial recombination between pre-
171 existing *wtf* genes.

172

173 **Discussion**

174 In this study, we analyzed the diversity and evolution of *wtf* genes in fission yeasts. The copy
175 number of the *wtf* gene varies among different *S. pombe* strains, revealing rapid diversification and
176 turnover of the *wtf* genes within a single fission yeast species. We detected signals of recurrent and
177 intricate recombination among *wtf* genes as previously reports with limited genomes (Hu et al.,
178 2017; Nuckolls et al., 2017; Eickbush et al., 2019; De Carvalho et al., 2022). We hypothesize that
179 recombination between *wtf* genes can produce new *wtf* genes with new poisons and the antidotes
180 to new poisons. These new *wtf* genes can then driver through populations. As proof-of-principle,
181 we generated a chimeric *wtf* gene that represents a new meiotic driver. The encoded poison of the
182 newly generated meiotic driver can be detoxified by its own long isoforms, but cannot be
183 detoxified by the antidote proteins of their parental genes. However, the other three chimeric *wtf*
184 genes did not show this property, possibly because the last exon might be crucial for antidote
185 function. Indeed, our recombination breakpoint analyses (Figure 3D) reveal substantial
186 recombination might have occurred in the last exon. Together, our results indicate that
187 recombination is likely to drive the rapid diversification of *wtf* gene in fission yeasts.

188 Most of the known meiotic drivers impose costs on their carriers due to direct effects of the
189 driver on survival or fertility, production of a biased sex ratio, or via deleterious mutations linked
190 to the driver (Price and Wedell, 2008; Larracuente et al., 2012; Sutter and Lindholm, 2015;
191 Fishman et al., 2015; Lindholm et al., 2016; Zanders and Unckless, 2019). In outcrossing between
192 individuals from distinct yeast lineages, *wtf* drivers can provide a selective advantage to atypical
193 spores, such as aneuploids and diploids (Bravo Nunez et al., 2020b). In this study, we assessed the
194 effects of the *wtf* genes on the growth of fission yeast during the asexual life cycle through
195 knocking out each of the 25 *wtf* genes in *S. pombe* laboratory strain 972h- separately. We did not
196 observe obvious attenuated growth for these *wtf* knockout strains, indicating *wtf* genes are largely
197 neutral to the fitness of their carriers during the asexual life cycle at least in the normal growth
198 setting. It should be noted that the spot assay used in this study detect large differences in fitness
199 between wide type and *wtf* knockout strains. Nevertheless, it is likely that *wtf* genes evolve mainly
200 in a neutral manner during the asexual life cycle, which explains the presence of high proportion
201 of pseudogenes in *wtf* gene repertoire. Moreover, asexual reproduction is much more frequent than
202 sexual reproduction for yeasts (Tsai et al., 2008). Therefore, even if fate-changing mutations that
203 simultaneously produce new poison and the antidote to new poison occur, the most probable fate

204 of a new *wtf* gene generated by gene duplication is pseudogenization and removed from the
205 population.

206 Gene duplication gives rise to new *wtf* genes. However, the newly generated *wtf* gene can be
207 detoxified by the original *wtf* gene and thus cannot drive through its host population, when the
208 original *wtf* is fixed in the population (Figure 5). Therefore, most, if not all, of *wtf* gene duplicates
209 experience early exist from the host population. When recombination occurs between two pre-
210 existing *wtf* genes, chimeric *wtf* gene with new poison and the antidote to new poison can be
211 generated as this study shows. Then, the *wtf* gene with new driver property can spread in its host
212 population, even reaching fixation (Figure 5). During asexual life cycle, *wtf* genes evolve mainly
213 under genetic drift, and thus can accumulate disruptive mutations, leading to their
214 pseudogenization. Taken together, our study highlights the significance of recombination in
215 shaping the diversification of *wtf* genes.

216

217 # Methods

218 **Identification of the *wtf* genes**

219 We used the blastn algorithm to identify *wtf* genes within 21 *S. pombe* strains with 25 *wtf*
220 genes from *S. pombe* reference genome as the queries and an *e*-cutoff value of 10^{-5}. The identified
221 *wtf* genes were annotated based on the *wtf* genes of reference genome. The sequence identity
222 among the exons of *wtf* genes was calculated using BioAider version 1.334 (Zhou et al., 2020).
223 Exons was then clustered based on the nucleotide identity using igraph package version 2.0.1.1
224 (Csardi et al., 2006; Csardi et al., 2024). We extended 1000 bp flanking each *wtf* gene to establish
225 their syntenic relationships.

226

227 **Phylogenetic analysis**

228 The coding sequences of *wtf* genes of 21 *S. pombe* strains and three other fission yeast species
229 (*S. octosporus*, *S. cryophilus*, and *S. osmophilus*) were aligned using MAFFT version 7 (Katoh and
230 Standley, 2013). To clarify the relationship of 21 *S. pombe* strains, 30 genes were randomly
231 selected and concatenated using Phylosuite version 1.2.1 (Zhang et al., 2020). All the phylogenetic
232 analyses in this study were performed using the maximum likelihood (ML) method implemented

233 in IQ-TREE version 2 (Minh et al., 2020). The best-fit substitution model was selected using the
234 ModelFinder algorithm (Kalyaanamoorthy et al., 2017). Node supports were assessed using the
235 ultrafast bootstrap approximation (UFBoot) method with 1,000 replicates (Hoang et al., 2018).
236 Solo-LTRs were identified using the blast algorithm and aligned using MAFFT version 7 (Katoh
237 and Standley, 2013). Phylogenetic analysis was performed using the approximate maximum
238 likelihood method implemented in FastTree version 2.1.1 (Price et al., 2010).

239

240 **Recombination analysis**

241 Split networks of *wtf* genes were generated using the neighborhood network analysis
242 implemented in Splittree4 (Huson et al., 2006). Pairwise homoplasy index (PHI) test was
243 performed using SplitsTree4 (Huson et al., 2006). Potential breakpoints were detected using 3SEQ
244 (Lam et al., 2018). The recombination rate was estimated using FastEPRR package version 2.0
245 (Gao et al., 2016).

246

247 **Generation of *wtf* knockout strains**

248 The *wtf* knockout (*Δwtf*) strains generated in this study were derived from *S. pombe* strain
249 972h-. We constructed substitution cassettes for each of the 25 *wtf* genes of the *S. pombe* reference
250 genome. Substitution cassettes contain a kanMX resistance marker and two homologous sequences
251 flanking the target *wtf* genes (Moreno et al., 1991; García-Ríos et al., 2014). Substitution cassettes
252 were transformation into *S. pombe* (strain 972h-) through the lithium acetate-based method
253 (Moreno et al., 1991; García-Ríos et al., 2014). *wtf* gene knockout strains were selected for kanMX
254 resistance and were verified by PCR.

255

256 **Plasmid construction**

257 Total RNA of fission yeast was extracted and reverse transcribed into cDNA. Coding
258 sequences of *wtf23*^{antidote}, *wtf23*^{poison}, and *wtf18*^{antidote} were amplified using the corresponding
259 primers (Supplementary file 1e). *wtf18*$^{poison/-M0}$ was generated using *wtf18*^{antidote} as the template and
260 the primer with an artificially introduced ATG (Supplementary file 1e). We generated *wtfC1*
261 through recombining exons 1-2 of *wtf23* and exons 3-6 of *wtf18*, generated *wtfC2* through

262 recombining exons 1-3 of *wtf23* and exons 4-6 of *wtf18*, generated *wtfC3* through recombining
263 exons 1-4 of *wtf23* and exons 5-6 of *wtf18*, and generated *wtfC4* through recombining exons 1-5
264 of *wtf23* and exon 6 of *wtf18*. These *wtf* and *wtfC* genes were then cloned into the GAL1/10 dual
265 expression plasmid Gal_HF. Plasmids were first transformed into *Escherichia coli* and verified by
266 PCR and sequencing. Plasmids were then transformed into *S. cerevisiae* (strain S288C) using the
267 lithium acetate-based method. Yeast transformants were selected for kanMX resistance and were
268 verified by PCR.

269

270 **Spot assay**

271 The yeast strains were cultured in YPD liquid medium at 30°C with shaking at 200 rpm. The
272 overnight cultures were transferred to fresh YPD liquid medium and grown to an OD_{600} value of
273 ~3. Cells were collected by centrifugation, and the OD_{600} was adjusted to 3. Subsequently, the
274 strains were continuously diluted by a 10-fold gradient to 10^{-5}, generating five stock suspensions.
275 The five stock suspensions were used for spot assays. We plated 1.5μL of each stock suspension
276 on the surface of YPD and YPG solid media. The plates were incubated at 30°C, and the growth
277 of colonies were observed.

278

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282

283 ## Data Availability

284 No new data were generated in support of this research.

285

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389

390 ## Figure Legends

391 **Figure 1. The diversity and evolution of *wtf* genes in fission yeasts.** (**A**) The gene structures of
392 the *wtf* genes in the *S. pombe* reference genome. Rectangles and lines represent exons and introns,
393 respectively. Rectangles with the same color indicate exons that share >50% sequence identity. (**B**)
394 The similarity network of exons of the *wtf* genes in the *S. pombe* reference genome. Sequences
395 that share an identity of >50% form a cluster. The colors of *wtf* exons correspond to these in panel
396 (**A**). The exons for each cluster were listed in the Supplementary file 1c. (**C**) The heatmap of
397 nucleotide identity among exons of the *wtf* genes in *S. pombe* reference genome. (**D**) The
398 distribution *wtf* genes in 21 *S. pombe* strains. *wtf* genes were present in 25 genetic loci. Filled
399 circles and empty circles represent the presence or absence of *wtf* genes, respectively. Red
400 pentagons indicate putative *wtf* pseudogenes. The size of circles in purple indicates the number of
401 *wtf* genes. The relationship among 21 *S. pombe* strains was inferred based on phylogenetic analysis
402 of 30 randomly selected genes. (**E**) Phylogenetic relationship of *wtf* genes in 21 *S. pombe* strains.
403 The phylogenetic tree is reconstructed using the maximum likelihood method. Filled circles in
404 blue indicate *wtf* genes in *S. pombe* reference genome. The number of exons, the presence of solo-
405 LTRs, the putative pseudogene status, the presence of intron-1-ATG codon, the presence of exon-
406 2-ATG codon is shown near the corresponding *wtf* gene. *wtf* genes from other fission yeast species
407 were collapsed into a triangle.

408

409 **Figure 2. The effect of *wtf* gene on the growth of *S. pombe*.** (**A**) Generation of *wtf* knockout
410 (*Δwtf*) strains based on the homologous recombination method. Substitution cassette contains a
411 kanMX resistance marker and two homologous sequences flanking the target *wtf* genes. (**B**) Spot
412 assay of *Δwtf* strains. The strains were continuously diluted by a 10-fold gradient to 10^{-5}, and five
413 stock suspensions were spotted onto the YE (Yeast extract) liquid medium.

414

415 **Figure 3. Recombination analysis of *wtf* genes.** (**A**) Split tree of 25 *wtf* genes of the *S. pombe*
416 reference genome. (**B**) Split tree of *wtf* genes from 21 *S. pombe* strains. The *wtf* genes of the *S.*
417 *pombe* reference genome are labelled. (**C**) Recombination rates of *wtf* genes that belong to groups
418 1 to 4. Recombination rates were estimated for the full-length *wtf* sequences, the first exons, and

419 the *wtf* sequences without the first exons. (**D**) Breakpoints detected for *wtf* genes of group1. The
420 exons of *wtf4*, as a gene position reference, are shown.

421

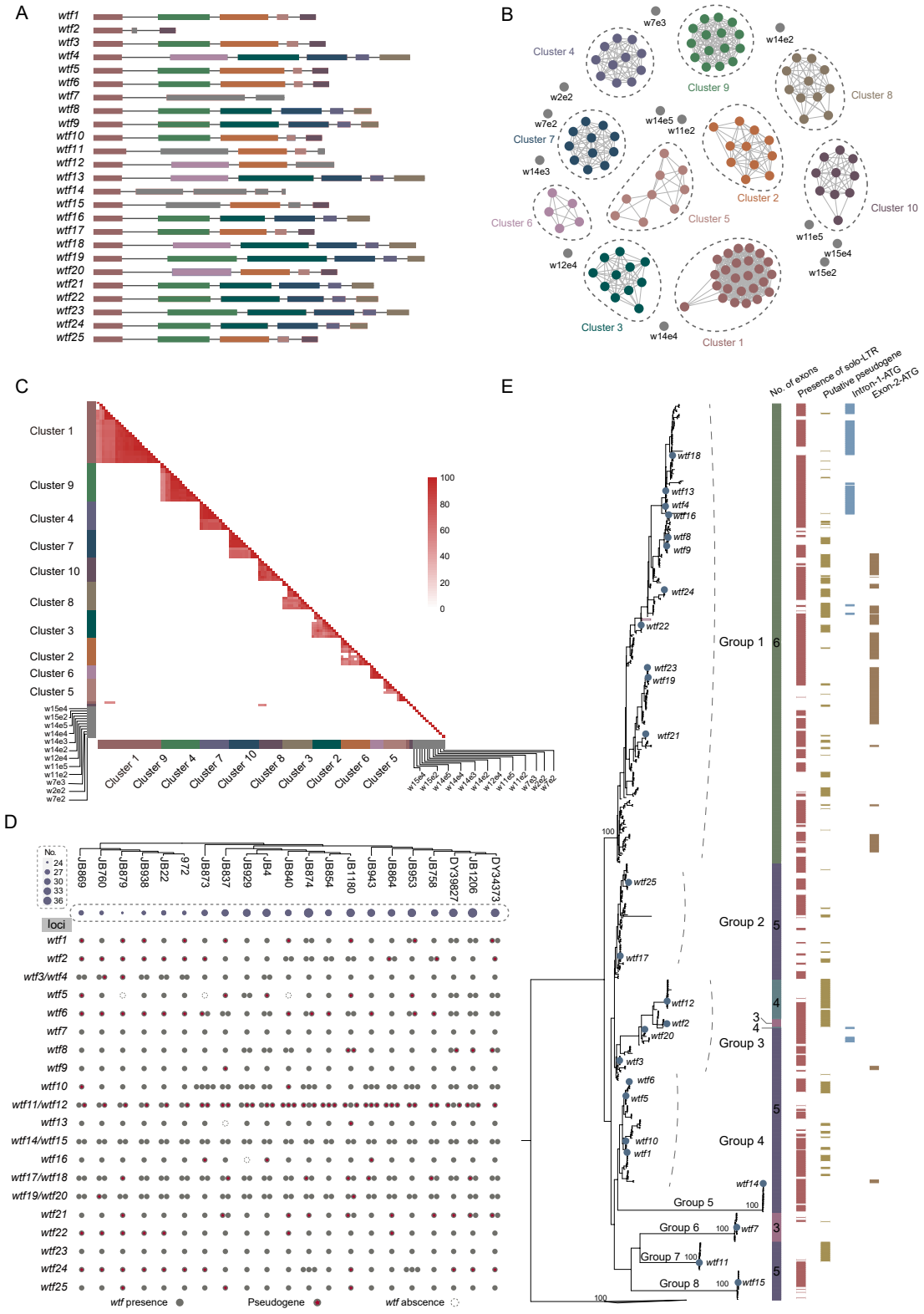
422 **Figure 4. Poison and antidote activity of *wtf* genes and chimeric *wtf* genes.** *wtf23* and *wtf18* are
423 highlighted in green and red, respectively. Rectangles represent exons. The start codon ATG is
424 shown. For *wtf18*, an in-frame ATG codon was introduced right before the start of exon 2,
425 generating $wtf18^{poison/-0M}$. Spot assay of yeast transformed with the short isoforms (encoding
426 poison-like proteins) and the long isoforms (encoding antidote-like proteins) of *wtf23* (**A**) and
427 *wtf18* (**B**). (**C** to **F**) Four chimeric *wtf* genes were generated through artificial recombination,
428 namely *wtfC1* (with exons 1-2 of *wtf23* and exons 3-6 of *wtf18*), *wtfC2* (with exons 1-3 of *wtf23*
429 and exons 4-6 of *wtf18*), *wtfC3* (with exons 1-4 of *wtf23* and exons 5-6 of *wtf18*), and *wtfC4* (with
430 exons 1-5 of *wtf23* and exon 6 of *wtf18*). Spot assay of yeast transformed with the short isoforms
431 (encoding poison-like proteins) and the long isoforms (encoding antidote-like proteins) of *wtfC1*
432 (**C**), *wtfC2* (**D**), *wtfC3* (**E**), and *wtfC4* (**F**) are shown.

433

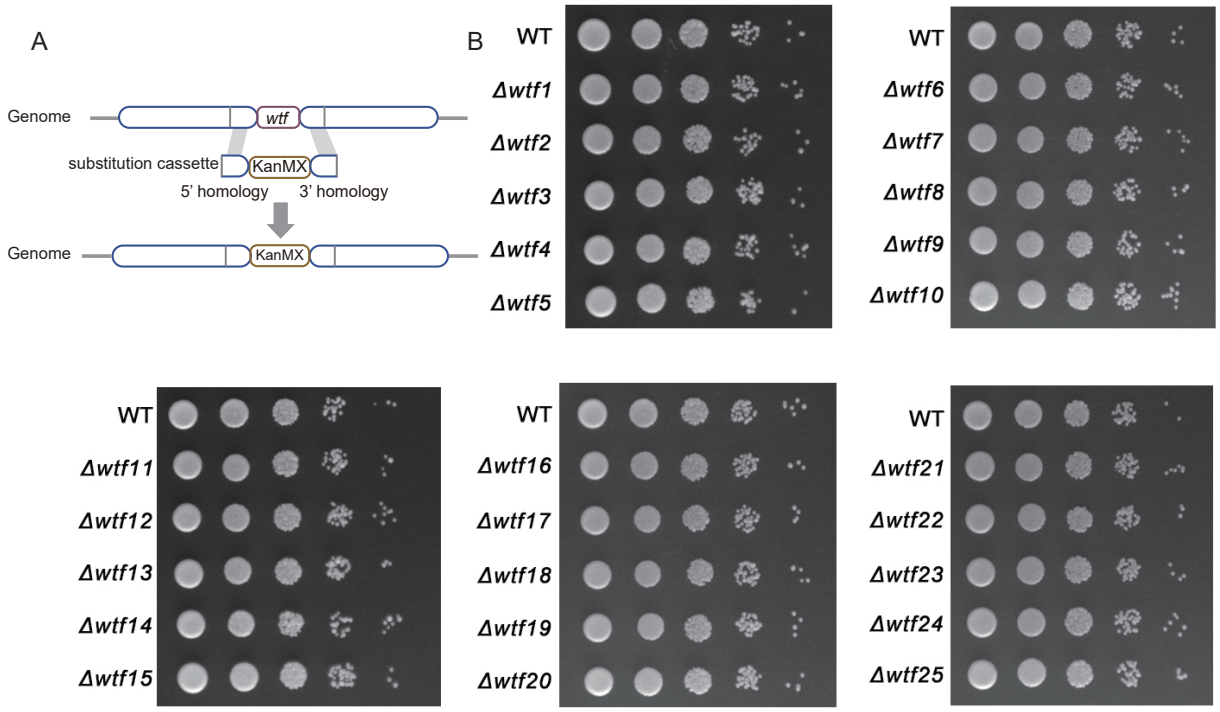
434 **Figure 5. The evolutionary mechanisms of *wtf* genes.** (**A**) Mechanism of *wtf* meiotic driver. *wtf*
435 genes are transcribed into two transcripts, namely long and short isoforms. The long and short
436 isoforms encode antidote and poison, respectively. All spores are exposed to the poison, whereas
437 only those that inherit *wtf* genes express the antidote and are rescued. Spores without the *wtf* allele
438 are destroyed, resulting in the drive of *wtf*. (**B**) Duplication is insufficient to drive the
439 diversification of *wtf* genes. Gene duplication of *wtfA* gives rise to a new *wtf* gene, $wtfA^{dup}$.
440 However, $wtfA^{dup}$ gene can be detoxified by the original *wtfA* gene and thus cannot drive through
441 its host population. (**C**) When recombination occurs between two parental *wtfB* and *wtfC* genes,
442 generating chimeric *wtfD* gene. *wtfD* encodes new poison that cannot be detoxified by their
443 parental antidote and the antidote to new poison. The *wtfD* gene then can spread in its host
444 population.

445

Figure 1


A
wtf1
wtf2
wtf3
wtf4
wtf5
wtf6
wtf7
wtf8
wtf9
wtf10
wtf11
wtf12
wtf13
wtf14
wtf15
wtf16
wtf17
wtf18
wtf19
wtf20
wtf21
wtf22
wtf23
wtf24
wtf25
B
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8
Cluster 9
Cluster 10
C
Cluster 1
Cluster 9
Cluster 4
Cluster 7
Cluster 10
Cluster 8
Cluster 3
Cluster 2
Cluster 6
Cluster 5
100
80
60
40
20
0
D
No.
24
27
30
33
36
JB869
JB760
JB879
JB938
JB22
972
JB873
JB837
JB929
JB4
JB840
JB874
JB854
JB1180
JB943
JB864
JB953
JB758
DY39827
JB1206
DY34373
loci
wtf presence
Pseudogene
wtf abscence
E
No. of exons
Presence of solo-LTR
Putative pseudogene
Intron-1-ATG
Exon-2-ATG
Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7
Group 8
100

448 **Figure 2**



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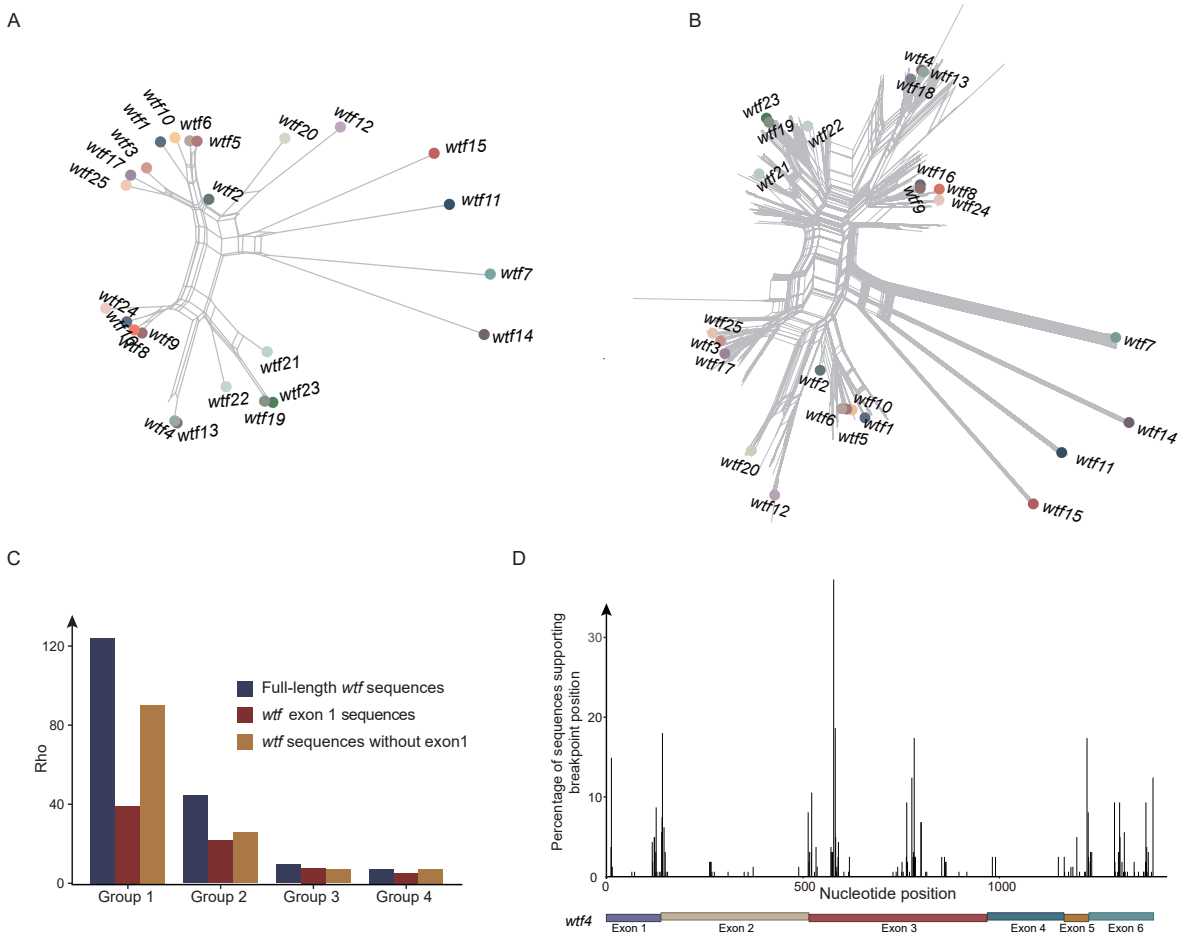

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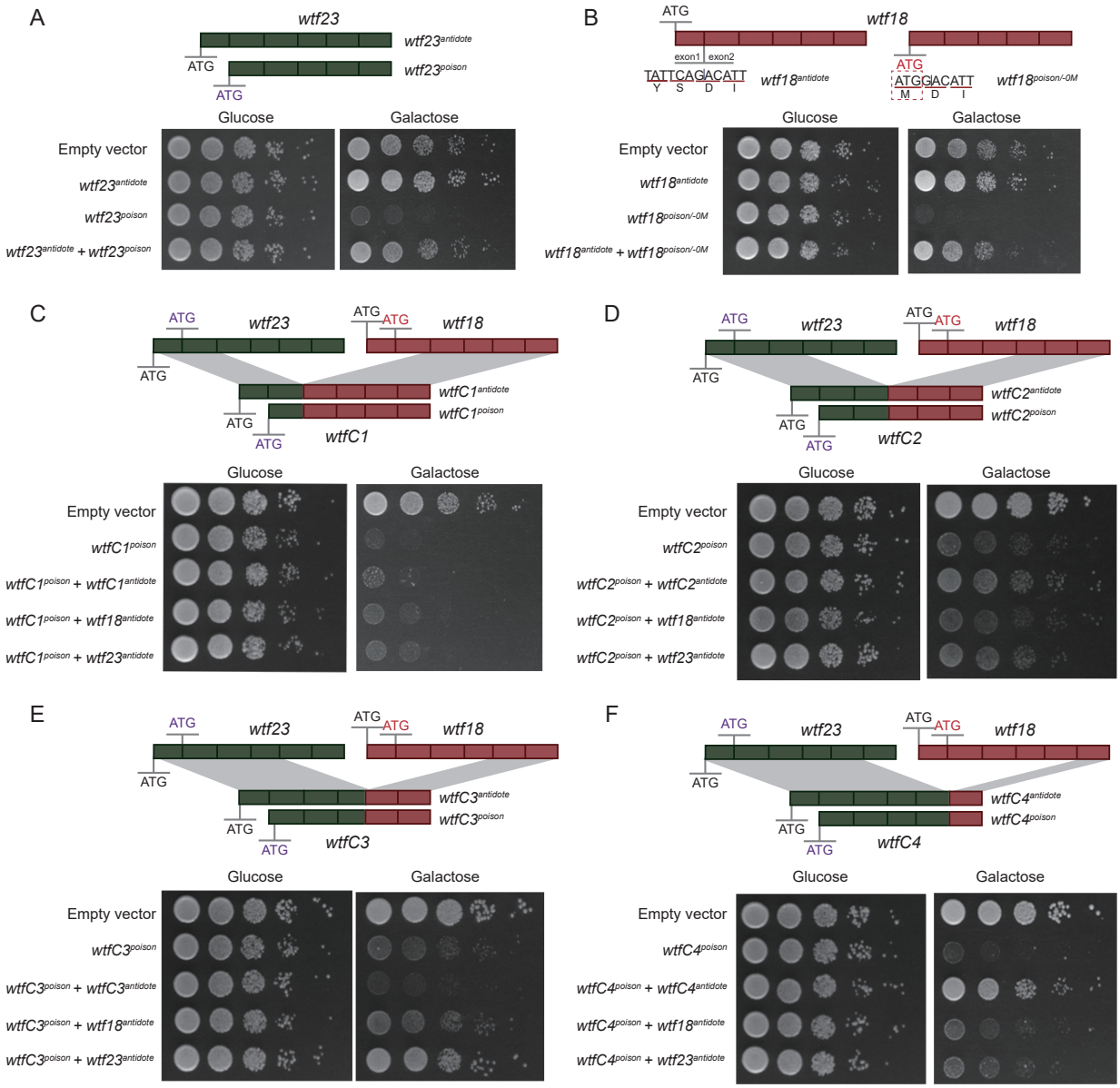

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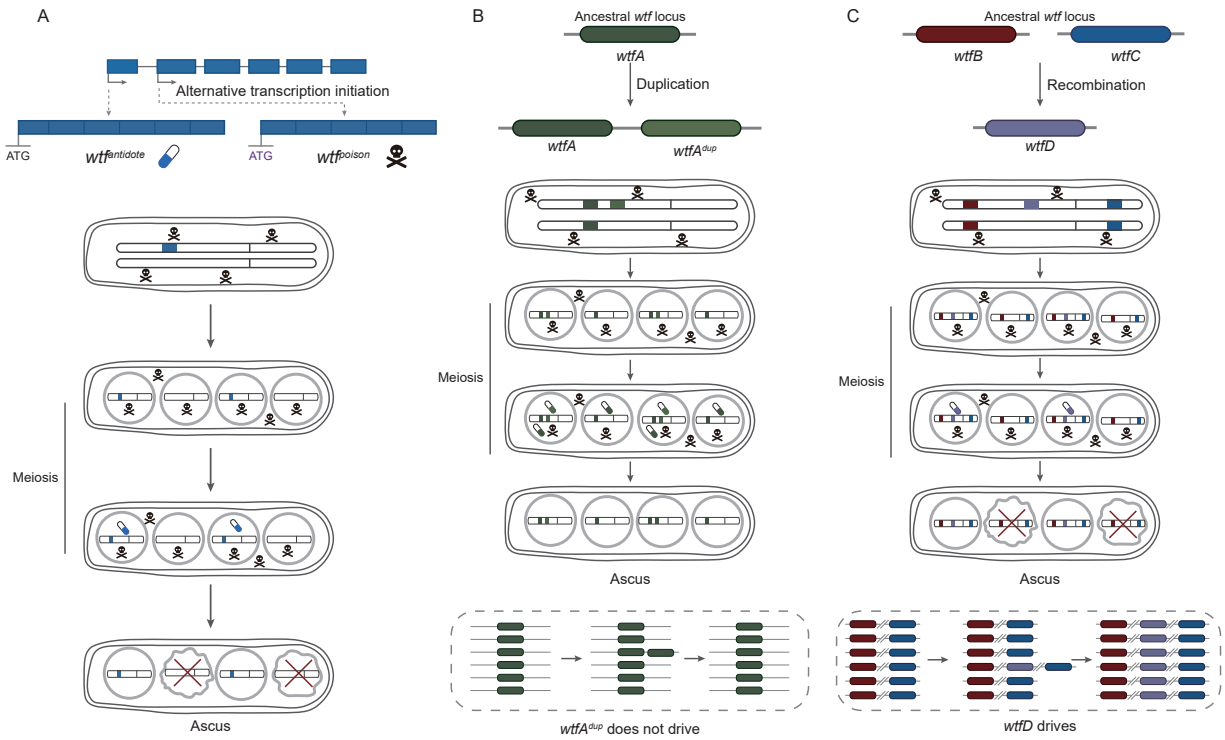
454 **Figure 5**



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