The transcriptional program of meiosis and sporulation in fission yeast

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Sexual reproduction requires meiosis to produce haploid gametes, which in turn can fuse to regenerate a diploid organism. We have studied the transcriptional program that drives this developmental process in *Schizosaccharomyces pombe* using DNA microarrays. Here we show that hundreds of genes are regulated in successive waves of transcription that correlate with major biological events of meiosis and sporulation. Each wave is associated with specific promoter motifs. Clusters of neighboring genes (mostly close to telomeres) are co-expressed early in the process, which reflects a more global control of these genes. We find that two Atf-like transcription factors are essential for the expression of late genes and formation of spores, and identify dozens of potential Atf target genes. Comparison with the meiotic program of the distantly related *Saccharomyces cerevisiae* reveals an unexpectedly small shared meiotic transcriptome, suggesting that the transcriptional regulation of meiosis evolved independently in both species.

Diploid cells of the fission yeast *S. pombe* undergo sexual differentiation under conditions of nutritional stress, notably nitrogen starvation. The meiotic divisions generate four haploid cells that develop into spores (Fig. 1), specialized cells that are highly resistant to environmental stress[1]. We have used DNA microarrays[2] to follow the meiotic expression of 99.3% of the roughly 4,900 known and predicted genes of fission yeast[3]. Our data provide a comprehensive insight into the transcriptional program that drives differentiation in this model organism.

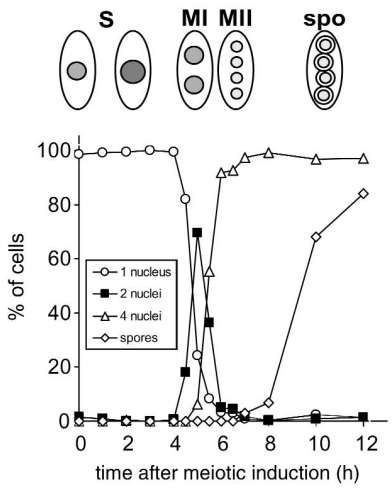

Fig. 1 Meiotic landmarks. Sexual differentiation culminates in the overlapping processes of meiosis and sporulation. In *pat1*-driven meiosis, premeiotic S phase starts about 2 h after inactivation of Pat1p (not shown) and is accompanied by chromosome pairing and recombination. The first meiotic division (MI) takes place at 5 h, and the second one (MII) at about 5.5 h. The forespore membrane, which is the precursor of the spore plasma membrane, is formed between 5.5 and 6.5 h (not shown). Mature spores (spo) appear at 10–12 h.

Good synchrony is essential to achieving high temporal resolution of expression profiles. We therefore used thermosensitive mutants of the meiotic inhibitor *Pat1p* (refs 4,5) in our studies. Diploid *pat1* cells were arrested in G1 by removing nitrogen, and synchronous meiosis was induced by a temperature shift to inactivate Pat1p (Fig. 1). We carried out four independent experiments with *pat1* and confirmed the results by following meiosis and sporulation in a wildtype diploid strain. In addition, we carried out control experiments to discard artifacts caused by the temperature shift. We validated our results by comparison with published data on the expression of meiotic genes (Web Table A online).

Starvation and progression through meiosis led to marked changes in gene expression (Fig. 2*a*). Almost 2,000 genes were significantly upregulated at least twofold (Methods) as compared with their expression in vegetative cells, and more than 700 genes were induced more than fivefold. Hundreds of genes also showed significant downregulation. Overall, more than 50% of the genome is regulated. We used *k*-means clustering to classify the upregulated genes according to their expression profiles (Fig. 2*b*). Given the extent of the changes, we concentrated on those genes whose expression was induced at least fourfold as compared with vegetative cells (1,033 genes). We defined four temporal classes that coincided with major biological processes of sexual differentiation: response to nutritional changes (starvation- or pheromone-induced genes), premeiotic S phase and recombination (early), meiotic divisions (middle) and spore formation (late).

The group of starvation- or pheromone-induced genes included about 250 genes that were upregulated on removal of nitrogen. The behaviors of some of these genes were altered as a consequence of the *pat1* mutation or the temperature shift. We therefore classified them according to their expression profiles during wildtype meiosis into three subgroups: transient, continuous and delayed. The group of about 100 genes that were expressed transiently is rich in transporters and proteins related to nitrogen metabolism (Web Table B online). In several cases, there is induction of genes encoding transporters for an alternative nitrogen source (such as allantoin) and enzymes required for its metabolism. Genes of the 'continuous group' (~70; Web Table C online) include those encoding regulators of the mating response (*ste7*, *gpa1*) as well as about 20 genes induced in several stress conditions (D. Chen, personal communication).

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The 'delayed' genes (~40; Web Table D online) include most genes known to be required for pheromone signaling and control of meiotic entry[1]: genes encoding pheromone and its receptors, mating-type genes (*matMi*, *matPi*, *matMc*, *matPc*), and genes encoding transcriptional (*ste11*) and meiotic regulators (*mei2* and *pat1*). The gene *fus1* (ref. 6), which is required for cell fusion after mating, showed a similar expression profile. The transcription factor Ste11p[7] regulates the expression of sexual differentiation genes. Accordingly, we found an enrichment of sequences related to the Ste11p-binding site (TR box) in the promoters of the 'delayed' genes (Web Table H online).

The group of early genes contains about 100 genes that are induced before the meiotic divisions (Web Table E online). It includes genes involved in the main events that take place at this stage of meiosis: S phase, recombination (Fig. 2*c*) and establishment of chromosome cohesion. The gene *dhc1*, which encodes the heavy chain of cytoplasmic dynein, is required for nuclear movement and chromosome pairing during meiotic prophase[8]. A gene encoding an additional dynein subunit (SPBP35G2.01C) and one similar to *S. cerevisiae* genes required for nuclear movement (SPBC1620.04C) were also upregulated. Genes for three cell-cycle regulators are included in this cluster: *cig2*, encoding an S phase–activating cyclin; *spd1*, encoding an S-phase-delaying protein; and SPCC1620.04c, encoding a putative anaphase-promoting complex (APC) regulator of the *CDC20/fizzy* family. The upstream sequences of the early genes are enriched in the motif ACGCGT (Web Table H online). This motif, called the MluI box, has been identified in the promoters of S phase and recombination genes[9,10]. Transcription of these genes is probably regulated by Cdc10p, Res2p and Rep1p[11–14], all of which are induced at this period of meiosis (Fig. 3*e*).

The group of middle genes (561) shows a peak that coincides with the meiotic divisions (Web Table F online). The group contains genes required for regulation and progression through mitosis, such as genes encoding cell-cycle regulators (*cdc25*, encoding a mitotic activator, and the cyclin gene *cdc13*), components of the condensin complex (five genes), kinesins (*klp5*, *klp6*), components of the spindle pole body (*sad1*, *cut12*) and kinases (*plo1*, *ark1* and *fin1*, of the Polo, Aurora and NimA families, respectively). This suggests that nuclear divisions during mitosis and meiosis share key regulators. Progression through mitotic anaphase requires the APC. The transcripts of all ten components of the APC are strongly induced during the meiotic divisions (Fig. 2*d*). Meiosis-specific regulators of the APC exist in both budding and fission yeast[15–17].

Several middle genes have functions in secretion or cell morphogenesis that may be required for formation of the forespore membrane (which will become the spore plasma membrane). The genes *spo3* and *psy1* (ref. 18), which encode components of the membrane traffic machinery and are required for forespore membrane synthesis, were also induced. We also identified 30 other genes encoding proteins related to membrane traffic and secretion, including vesicle and target SNAREs (SNAP receptors), components of the SNAP (soluble NSF (*N*-ethylmaleimide-sensitive fusion) attachment protein) complex, and Rab proteins and their regulators. Most of these genes have not been studied. Six middle genes are required for normal cell shape: the kinase genes *orb5*, *ssp1* and *kin1*; the formin gene *for3*; and the GTPase genes *ras1* and *cdc42*. Their roles in meiosis have not been examined, but their expression patterns suggest that they have functions in spore formation in addition to their known role in cell morphogenesis. Finally, four septins are strongly induced. Septins are involved in forespore formation in *S. cerevisiae*[19].

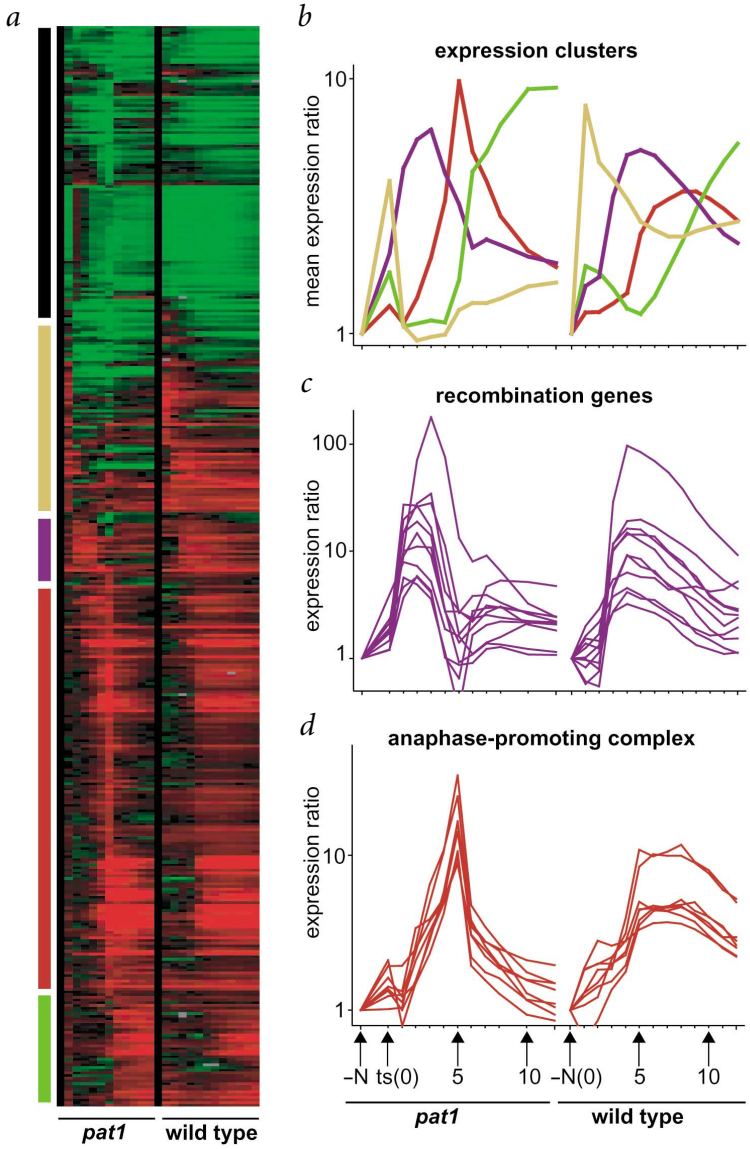

Fig. 2 Transcriptional program of meiosis and sporulation. ***a***, Expression patterns of downregulated (green) or upregulated (red) genes during meiosis and sporulation. Columns represent experimental time points and rows represent genes. The brightness of the colors is proportional to the amount of regulation. The bars on the side show the approximate position of the main expression clusters, colored as in *b*. ***b***, Average expression profiles of four main clusters: nitrogen-starvation response (yellow), early (blue), middle (red) and late (green). In *pat1*-driven meiosis (left), cells are synchronized in G1 by nitrogen removal (–N) and enter meiosis by temperature shift at time 0 (ts). In wildtype meiosis (right), cells enter meiosis when nitrogen is removed (–N, time 0). ***c***, Recombination genes (*rec6*, *rec7*, *rec8*, *rec10*, *rec11*, *rec12*, *rec15*, *meu13*, *dmc1*, *mus81*, *rad51*) show a typical early induction pattern. ***d***, Genes encoding components of the APC (*apc1*, *apc5*, *nuc2*, *cut4*, *cut20*, *cut23*, SPBP23A10.04, SPAC343.03) show a middle induction pattern. In *b–d*, the *y* axis represents the ratio of mRNA levels to those in vegetative cells (log scale) and the *x* axis shows hours after meiotic induction.

The induction of middle genes required the Mei4p transcription factor (436 middle genes were expressed at <30% of the wildtype levels in a *mei4* mutant), and about 90% of the Mei4p target genes[20,21] are included in this group (J.M., unpublished data). In agreement with this, more than 50% of middle genes have motifs that are similar to the Mei4p-binding site in the 500-bp region upstream of their coding sequence (the FLEX sequence[20]; Web Table H online). The gene *mei4* itself, which activates its own transcription[20,21], was strongly induced (Fig. 3*e*).

Genes in the late group (133) were induced after the meiotic divisions and their expression remained high until the completion of sporulation (Web Table G online). During this period, spores mature and acquire resistance to environmental stress. Consistent with this, the cluster contains 28 genes that are upregulated under several stress conditions (D. Chen, personal communication). Two genes involved in cell-cycle regulation (*wee1*, encoding a *cdc2* inhibitor, and *ste9*, encoding an APC regulator) may function in maintaining the dormant state of the spore or in controlling germination. Several genes related to cell-wall synthesis may be important for the development of the specialized spore wall. Finally, components of the cyclic AMP pathway, which regulates entry into meiosis[1], were induced at late stages of meiosis. The role of this pathway in spore formation or germination has not been examined.

No transcription factors or DNA motifs had been known to be involved in the regulation of late genes. We found that late-gene promoters were enriched in sequences related to binding sites of Atf transcription factors (Web Table H online), which have conserved roles in the regulation of stress responses. The genes *atf21* and *atf31*, encoding two transcription factors of this family[22,23], were induced with a middle pattern, which suggested that they might regulate the expression of late genes. We constructed deletion mutants and examined their phenotypes. Conjugation and meiotic divisions proceeded normally, but spore formation was defective (Fig. 3*a*). We used microarrays to identify potential targets of Atf21p and Atf31p (Fig. 3*b*,*c*). Atf21p and Atf31p were required for the expression of overlapping sets of genes, as all of the genes affected in *atf31Δ* mutants were also affected in *atf21Δ* mutants (Fig. 3*b*). All of the potential targets were upregulated in late meiosis after induction of *atf21* and *atf31* (Fig. 3*d*). We estimate that Atf21p and Atf31p regulate about 55% of the late genes, including most of those related to stress (22 out of 28). By contrast, Atf21p and Atf31p are not required for the expression of stress genes induced as part of the nitrogen-starvation response.

Sexual differentiation in fission yeast involves a complex transcriptional program, in which more than 1,000 genes are strongly induced. Transcriptional regulation occurs in successive waves, each under the control of different transcription factors. All known meiotic transcriptional regulators were themselves induced (Fig. 3*e*), which suggests that the meiotic program is driven by a cascade of transcription factors, with those that function early activating later ones. Eleven uncharacterized transcriptional regulators were induced at least tenfold during various stages of the process, and many others showed smaller increases (Fig. 3*f* and Web Table I online). These may contribute to the implementation of the transcriptional program, and our analysis of *atf21* and *atf31* supports this idea. A comprehensive approach, combining genetics and genomics, should allow the dissection of the transcriptional network.

Notably, we also found cases of correlation between physical position on the chromosome and expression profile (Fig. 3*g*). Five chromosomal regions are highly enriched in genes induced by

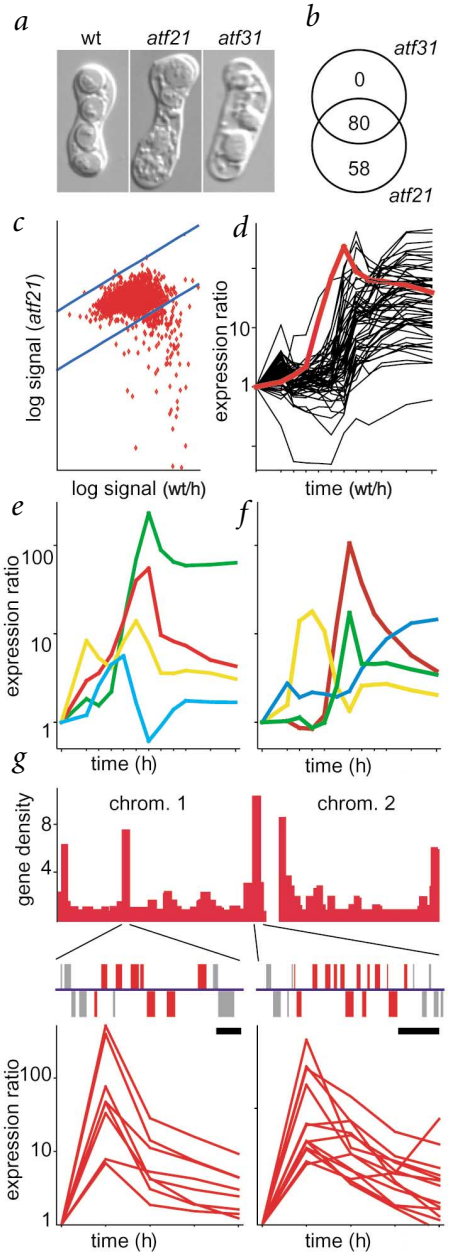

Fig. 3 Transcriptional regulation during meiosis. ***a***, Spores of wild type (wt), *atf21Δ* and *atf31Δ*. ***b***, Overlap between *atf21*- and *atf31*-dependent genes. ***c***, Comparison of gene expression levels between wildtype and *atf21* meiotic cells. Genes outside the blue lines show more than a twofold difference in expression and are potential Atf21p targets. ***d***, Transcriptional profiles of *atf21*- (red) and *atf21*-dependent genes (black). ***e***, Examples of transcriptional regulators with a role in meiosis: *rep1* (yellow), *res2* (blue), *mei4* (red) and *atf21* (green). ***f***, Examples of uncharacterized transcriptional regulators that are induced during meiosis: zinc-finger-containing SPBC1718.02 (yellow) and SPBC1105.14 (blue), copper-fist transcription factor SPCC584.02 (red) and TFIID component SPAC12G12.05c (green). Axes in *d*–*f* are as in Fig. 2. ***g***, Correlation between chromosomal position and expression pattern. Top, density of genes induced by nitrogen starvation along chromosomes I and II. Middle, physical position of genes induced by nitrogen starvation (red boxes) or not upregulated (gray boxes) for two clusters on chromosome I. Genes above the blue line are transcribed from left to right, those below from right to left. Scale bars represent 10,000 base pairs. Bottom, expression profiles of red genes shown in the physical map (wildtype meiosis, as in Fig. 2). The clusters include genes from SPAC1002.18 to SPAC1399.01c (left) and SPACC29B12 to SPAC1039.11c (right).

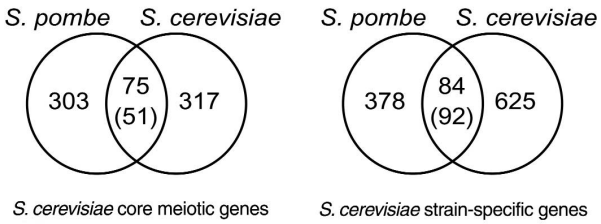

Fig. 4 Overlap between the meiotic transcriptomes of budding and fission yeast. The 378 meiotically induced fission-yeast genes with budding-yeast orthologs were compared to the 'core meiotic genes' (induced in two different strains, left) or 'strain-specific genes' (induced in one strain but not both, right) of budding yeast. Genes induced by nitrogen starvation were not used in this comparison. Numbers in parentheses represent the overlap expected by chance, given the sizes of the gene sets considered and the total number of 2,894 genes with orthologs.

nitrogen starvation (in one case, the cluster contains 14 contiguous genes with similar profiles). These genes are not transcribed in the same orientation and are therefore unlikely to be part of an operon. The co-regulated clusters may reflect transcriptional control at a more global level, possibly by regulation of chromatin architecture. Four of these gene clusters are close to the telomeres of chromosomes I and II, near transcriptionally silenced regions[24]. This raises the possibility that activation of silenced regions has a role in the transcriptional response to nitrogen starvation.

The transcriptional program of meiosis and sporulation in the budding yeast *S. cerevisiae* has been studied using microarrays[25,26]. About 915 genes are induced in both yeast strains that have been studied, whereas nearly 1,400 genes are strain specific[26]. We compared the first group, representing the budding yeast 'core' meiotic genes, against our data and used the strain-specific genes as a control. We determined whether orthologs of genes induced in fission yeast were also upregulated in budding yeast. Of 378 such genes, 75 were induced in both budding yeast strains (Fig. 4). This is a small enrichment (about 1.5 times the number expected by chance), but it is highly significant ($P < 3 \times 10^{-5}$). By contrast, the overlap with strain-specific genes was not significant (Fig. 4). These genes define a core meiotic transcriptome that is shared between the two eukaryotes and is enriched in cell-cycle regulators and genes required for recombination and chromosome cohesion (Web Table J online). The first group includes B-type cyclins (encoded by *S. pombe cdc13* and *cig2*, *S. cerevisiae CLB1*, *CLB3–CLB6*), polo kinase (encoded by *plo1/CDC5*) and a phosphatase (encoded by *flp1/CDC14*). Genes encoding seven components of the APC are also induced in both yeasts, as are all members of the *CDC20/fizzy* family of APC regulators (three in budding yeast, five in fission yeast). The recombination/cohesion group contains *rec7/REC114*, *rec8/REC8*, *dmc1/DMC1*, *smc3/SMC3* and *meu13/HOP2*. Although the overall process of meiosis and sporulation is similar in budding and fission yeast, there is little conservation of the regulatory machinery[1]. This fact, together with the limited number of genes in the core meiotic transcriptome, raises the possibility that the regulation of meiosis was either very simple or absent in the common ancestor of both yeasts. Mechanisms of meiotic control (including transcriptional regulation) might have evolved later to make the process more efficient.

Methods

Strains and culture conditions. We used standard media and methods[27]. We induced *pat1*-driven meiosis as follows: *pat1-114/pat1-114 ade6-M210/ade6-M216* h^+/h^+ cells were grown in minimal medium containing 2% glucose (EMM) plus 0.5% NH_4Cl, and then resuspended in EMM without NH_4Cl (EMM-N) and incubated for 14 h at 25 °C. Meiosis was started by shifting the cells to 34 °C in the presence of 0.05% NH_4Cl. Controls were carried out by nitrogen starving *ade6-M210/ade6-M216* h^+/h^+ cells as above, and then resuspending them in EMM-N or EMM (0.05% NH_4Cl) at 34 °C. We induced wildtype meiosis in *ade6-M210/ade6-M216* h^+/h^- diploid cells by incubating them in minimal medium containing 0.5% glucose without NH_4Cl at 30 °C. We deleted *atf21* and *atf31* in an *h90* background using the one-step PCR method[28]. Meiosis in *atf21* and *atf31* mutants was induced as for the wildtype diploid cells. The *atf21/atf31* cells were incubated at 28 °C and samples were taken after 15 h. Forespore membrane formation was measured by the accumulation of the spo3GFP marker[18].

Microarray experiments. We produced DNA microarrays by spotting PCR products corresponding to 99.3% of known and predicted *S. pombe* open reading frames onto glass slides. Detailed protocols for array production and analysis will be published elsewhere. RNA was isolated by phenol extraction and purified using RNeasy (Qiagen). We prepared cDNA probes using Superscript (Invitrogen).

Data acquisition and analysis. Microarrays were scanned with a Genepix 4000B scanner and analyzed with Genepix software (Axon Instruments). Clustering and visualization was done with GeneSpring (Silicon Genetics), Cluster and Treeview[29]. We determined statistical significance using SAM (significance analysis for microarrays)[30]. The false discovery rate was adjusted to less than 0.1%. Gene annotations were taken from the GeneDB database at The Wellcome Trust Sanger Institute.

Quantification of gene density along chromosomes. We determined the proportion of genes induced by nitrogen starvation for a running window of 20 consecutive genes along each chromosome. To assess the significance of enrichment, we calculated for each window the probability of getting the observed results by chance (using the hypergeometric distribution). We transformed the data to $-\log_{10}$(probability) values to facilitate visualization.

Probe sequences and the complete processed data set are available from our website.

ArrayExpress accession numbers. E-SNGR-2 to E-SNGR-7 (raw data).

URLs. The authors' website (for probe sequences and the complete processed data set), http://www.sanger.ac.uk/PostGenomics/S_pombe/; ArrayExpress (raw data), http://www.ebi.ac.uk/arrayexpress/; GeneDB, http://www.genedb.org.

Note: Supplementary information is available on the Nature Genetics website.

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Competing interests statement

The authors declare that they have no competing financial interests.

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