Fingerprinting fission yeast: polymorphic markers for molecular genetic analysis of *Schizosaccharomyces pombe* strains

Ann-Marie Patch and Stephen J. Aves

Correspondence

Stephen Aves

S.J.Aves@exeter.ac.uk

School of Biosciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, UK

The fission yeast *Schizosaccharomyces pombe* is widely used as a model eukaryote for cell and molecular studies but little is known of natural genetic variation in this species. In order to obtain informative molecular markers, imperfect tandem repeats, identified through bioinformatic methods, were tested for length polymorphism in six wild-type strains of *Sch. pombe* isolated from different substrates and geographical locations in Africa, America, Asia and Europe. Of 26 loci tested, 21 were multi-allelic, consistent with tandem repeat copy number variation. Eleven of these polymorphic tandem repeats are in regions encoding intracellular proteins. Most of the protein-coding repeats are not sited within structured domains but have non-regular predicted structure; one has a repeat unit length corresponding to integer turns of a predicted amphipathic α -helix secondary structure, suggesting that this repeat may be tolerated because copy number mutations change α -helix length but not orientation within the protein structure. In contrast to the differences observed between natural isolates of *Sch. pombe*, genetic strains were found to be essentially isogenic: only two polymorphic loci were detected out of 26 minisatellites and five microsatellites tested in 16 strains, including a hypervariable microsatellite in the *med15* gene. The polymorphic tandem repeat markers identified in this study will prove useful for DNA fingerprinting and molecular analysis of natural genetic variation in *Sch. pombe* isolates.

Received 23 August 2006

Revised 9 November 2006

Accepted 20 November 2006

INTRODUCTION

The fission yeast *Schizosaccharomyces pombe* has been widely exploited as a model eukaryote for genetic, cell and molecular biological studies, particularly relating to mating type and the cell cycle (Egel, 2004). *Sch. pombe* was one of the first eukaryotes to have its genome completely sequenced and annotated (Wood *et al.*, 2002) and it has fully entered the post-genomic age, with a wide range of molecular genetic, genomic and functional genomic techniques available to study its genome and its proteome, which at 4912 predicted proteins is one of the smallest for a free-living eukaryote (Sunnerhagen, 2002; Forsburg & Rhind, 2006; Marguerat *et al.*, 2006). *Sch. pombe* and two other fission yeast species of the same genus form a distinct group of ascomycete fungi which are phenotypically and phylogenetically highly divergent from budding yeasts such as *Saccharomyces cerevisiae* or *Candida albicans* (Kurtzman & Robnett, 1998; Sipiczki, 2000). Differing estimates put the

divergence of *Sch. pombe* and *Sac. cerevisiae* at 330–420 or 1070–1220 million years ago and, when compared with calculations for the divergence of fungi and metazoa at between 1000–1100 or 1600 million years ago, indicate a long evolutionary separation (Sipiczki, 2000; Heckman *et al.*, 2001). This large evolutionary divergence between fission and budding yeasts makes *Sch. pombe* a particularly valuable model organism alongside *Sac. cerevisiae*, as comparisons between these species can be very informative for extrapolation to other eukaryotes (Forsburg, 1999).

As a model organism, *Sch. pombe* has the great advantage that all genetic strains and mutants derive from a single isolate, and are therefore assumed to be isogenic. Genetic strains were derived by Leupold (1950) from a *Sch. pombe* strain isolated in 1924 from grape juice originating from Southern France, and originally designated *Sch. liquefaciens* (Osterwalder, 1924). This *Sch. pombe* wild-type strain is just one of many dozens isolated from substrates ranging from fruit juice and molasses to fermented beverages from all inhabited continents of the world, particularly in the decades following the original description of this species isolated from East African millet beer (the word ‘pombe’ is Swahili for beer; Lindner, 1893). In contrast to the concentration of research on genetic strains of *Sch. pombe*,

Abbreviations: CBS, Centraalbureau voor Schimmelcultures; CTD, C-terminal domain; indels, insertions or deletions; NCYC, National Collection of Yeast Cultures.

Three supplementary figures are available with the online version of this paper.

relatively few studies have been carried out on natural variants of this fission yeast species, and very few indeed using molecular genetic techniques. Study and sequencing of budding yeasts, such as the *Saccharomyces sensu stricto* group, has demonstrated the valuable insights that can be gained from the molecular analysis of natural variation in yeasts (Kellis *et al.*, 2003).

The variability of tandem repeats has led to their widespread use as genetic markers for mapping, population and DNA fingerprinting studies (Calafell *et al.*, 1998). Tandem repeats are approximate or exact head-to-tail copies of a nucleotide sequence and are often classified by repeat unit size as microsatellites or minisatellites. They occur in both protein-coding and intergenic regions of eukaryotic genomes from yeast to humans (Vergnaud & Denoeud, 2000; Tóth *et al.*, 2000; Katti *et al.*, 2001) and there is accumulating evidence that they may serve functional roles within coding sequences and as regulatory elements or mutational hotspots (Kashi *et al.*, 1997; Fondon & Garner, 2004; Verstrepen *et al.*, 2005; Bowen & Wheals, 2006). Tandem repeats frequently exhibit copy number polymorphisms caused by mechanisms such as DNA replication slippage and asymmetric recombination (Debrauwere *et al.*, 1997; Chambers & MacAvoy, 2000; Richard & Pâques, 2000; Viguera *et al.*, 2001) and in humans, expansions of microsatellites (tandem repeats with a short repeat unit length) have been associated with genetic diseases including neurodegenerative disorders such as fragile X syndrome and Huntington's disease (Sutherland & Richards, 1995; Mitas, 1997; Tóth *et al.*, 2000; Boby *et al.*, 2005).

In this study we screened the *Sch. pombe* genome for tandem repeats that can be used as markers for genetically fingerprinting this species of fission yeast. We demonstrate the usefulness of these markers for exploring the similarities and differences between *Sch. pombe* isolates from different geographical locations, and we investigate the extent of tandem repeat polymorphism within genetic strains of *Sch. pombe*.

METHODS

Strains. *Sch. pombe* wild-type strains representing different geographical isolates were obtained from the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands, and two control Leupold (1950) strains were obtained from the National Collection of Yeast Cultures (NCYC), Norwich, UK. All isolates were sequenced at the rDNA ITS locus to confirm that they were strains of *Sch. pombe*. Genetic strains were obtained from the *Sch. pombe* community. Details of all strains are shown in Table 1.

Selection of tandem repeat markers and primer design.

Potential fingerprinting targets were selected from a comprehensive analysis of the whole *Sch. pombe* genome sequence (Wood *et al.*, 2002) for perfect and approximate tandem repeats, performed using Tandem Repeats Finder (Benson, 1999; A.-M. Patch, & S. J. Aves unpublished). Minisatellite and microsatellite tandem repeats were selected as candidates for fingerprinting from protein-coding and non-coding regions using the following criteria: $\geq 60\%$ matches of repeat sequences to the consensus, $\leq 16\%$ insertions or deletions

(indels), an overall estimated fragment length between 175 and 600 bp, and, where possible, ≥ 4 repeated copies. Minisatellites were defined as having repeat units of ≥ 7 bp. These criteria were chosen to provide tandem repeat alleles that could potentially be resolved by agarose gel electrophoresis. Excluded from this group were any tandem repeats that crossed exon boundaries ($< 2\%$ of total).

Primer sequences were selected from sequences flanking the target tandem repeats and separated by at least 20 nt each side. Optimal primers were designed using the web-accessible version of Primer3 (Rozen & Skaletsky, 2000) with ideal T_m set at 57°C , ideal primer length of 21 bp, and other default parameters. All primer pairs were assessed for primer-primer interactions using the web-accessible 'Net Primer' program (<http://www.premierbiosoft.com/netprimer/index.html>). Oligonucleotide primers were synthesized by MWG Biotech and details of these are given in Table 2.

DNA preparation and PCR amplification. *Sch. pombe* strains were cultured in YE medium and DNA was extracted according to the protocol of Moreno *et al.* (1991) but using 2 mg lyticase ml^{-1} (Sigma-Aldrich) in place of Zymolyase-20T for cell lysis. PCR reactions were in 50 μl containing 30 ng DNA, 1.5 mM MgCl_2 , 200 μM of each dNTP, 20 pmol of each primer, 1 unit *Taq* polymerase and $1 \times$ *Taq* buffer (Promega or New England Biolabs). PCR reactions had an initial denaturation step of 2 min at 94°C followed by 30 cycles of 20 s denaturation at 94°C , 30 s annealing at 57°C and 1 min 15 s elongation at 72°C ; there was a final extension step at 72°C for 5 min. PCR products were fractionated in 2% agarose TBE gels alongside 100 bp ladder (New England Biolabs).

Sequencing and data analysis. PCR products were sequenced directly after cleaning by ethanol precipitation; 2 ng template was used in a 10 μl reaction also containing 2 μl BigDye Terminator (Applied Biosystems), 2 μl HalfTerm buffer (Genpak), and 10 pmol primer. Reactions had an initial denaturing step of 1 min at 70°C followed by 25 cycles of 10 s denaturation at 96°C , 5 s annealing at 50°C , 4 min elongation at 60°C , and a final step at 60°C for 5 min. Products were ethanol precipitated and sequenced on an ABI3100 automated sequencer. Electropherograms were analysed using the freeware version of CHROMAS (Technelysium) and compared with the genomic template using BLASTN. Sequence alignments were produced with CLUSTALW (Thompson *et al.*, 1994). Protein structure prediction was carried out using the web-accessible software PredictProtein (Rost *et al.*, 2003) and PSIPRED (McGuffin *et al.*, 2000). Predictions of intrinsically unstructured/disordered protein domains were confirmed by two independent disorder prediction software tools DISOPRED2 and vSL2 (Ward *et al.*, 2004; Peng *et al.*, 2006).

A phenetic unrooted dendrogram was constructed, based on a distance matrix obtained by counting the number of differences between corresponding alleles, using neighbour-joining cluster analysis performed with PHYLIP (Phylogeny Inference Package) version 3.6 (Felsenstein, 2005).

RESULTS

Minisatellite markers enable DNA fingerprinting of *Sch. pombe* isolates

In order to determine informative markers for DNA fingerprinting of *Sch. pombe* strains, a subset of tandem repeats identified bioinformatically in the sequenced *Sch. pombe* genome was selected (see Methods). The screening criteria were devised so that polymorphisms could be readily identified using standard agarose gels, ensuring that total amplicon lengths exceeded 175 bp. High internal conserva-

Table 1. *Sch. pombe* strains

Strain*	Genotype	Obtained from†	Original isolate	Substrate	Country of origin
972	h^-	NCYC 1827	Osterwalder (formerly <i>Sch. liquefaciens</i>)	Grape juice	France
975	h^+	NCYC 1828	Osterwalder (formerly <i>Sch. liquefaciens</i>)	Grape juice	France
CBS356	Wild-type	CBS	<i>Sch. pombe</i> Lindner type strain		
CBS357	Wild-type	CBS	P. Greg (formerly <i>Sch. mellacei</i>)	Cane-sugar molasses for rum making	Jamaica
CBS2775	Wild-type	CBS	K. Sakaguchi (formerly <i>Sch. pombe</i> var. <i>ogasawaraensis</i>)	Fermenting molasses	Japan
CBS5557	Wild-type	CBS	Rankine & Fornachon (formerly <i>Sch. malidevorans</i>)	Listan grapes	Spain
CBS5682	Wild-type	CBS	J. P. van der Walt	Sorghum beer	Swaziland, Southern Africa
SJA148	h^-	R. Egel	Osterwalder	Grape juice	France
SJA167	h^- <i>arg4-55</i>	P. Thuriaux	Osterwalder	Grape juice	France
SJA175	h^- <i>arg5-189</i>	P. Thuriaux	Osterwalder	Grape juice	France
SJA216	h^- <i>nda4-108 leu1-32</i>	M. Yanagida	Osterwalder	Grape juice	France
SJA237	h^- <i>cut5-580 leu1-32</i>	M. Yanagida	Osterwalder	Grape juice	France
SJA273	h^- <i>nda3-KM311</i>	M. Yanagida	Osterwalder	Grape juice	France
SJA277	h^- <i>cdc23-M30</i>	P. Nurse	Osterwalder	Grape juice	France
SJA272	h^- <i>cdc25-22</i>	P. Nurse	Osterwalder	Grape juice	France
SJA244	h^- <i>cdc23-IE2</i>	P. Nurse	Osterwalder	Grape juice	France
	<i>ade6-704 leu1-32</i>				
SJA122	h^- <i>ura4-294 tps14-5</i>	H. Schmidt	Osterwalder	Grape juice	France
	<i>ade5-36 swi5-39</i>				
SJA120	h^{90} <i>swi5-39</i>	H. Schmidt	Osterwalder	Grape juice	France
SJA238	h^- <i>ade6-M210</i>	H. Schmidt	Osterwalder	Grape juice	France
SJA300	h^- <i>rad3-d::ura4⁺</i>	A. Carr	Osterwalder	Grape juice	France
	<i>ura4-d18 leu1-32</i>				
	<i>ade6-M210</i>				
SJA302	h^+ <i>hsk1-1312 ura4-d18</i>	S. Forsburg	Osterwalder	Grape juice	France
	<i>leu1-32 ade6-M210</i>				

*Strains 972 and 975 are from Leupold (1950); genetic strains are identified by an SJA prefix.

†CBS, Centraalbureau voor Schimmelcultures, The Netherlands; NCYC, National Collection of Yeast Cultures, Norwich, UK; R. Egel, Institute of Molecular Biology, University of Copenhagen, Denmark; P. Thuriaux, CEA/Saclay, Gif-sur-Yvette, France; M. Yanagida, Kyoto University, Japan; P. Nurse, Rockefeller University, New York, USA; H. Schmidt, Institut für Genetik, Technische Universität Braunschweig, Germany; A. Carr, Genome Damage and Stability Centre, University of Sussex, UK; S. Forsburg, University of Southern California, USA.

tion of sequence between repeated units increases the likelihood of polymorphic alleles (Strand *et al.*, 1993); therefore a minimum value of 60% was set for mean percentage identity between repeat copies, and a maximum value of 16% for indels (insertions and deletions); note that all of the selected tandem repeats were imperfect. Tandem repeat targets were selected from both protein-coding and non-coding regions of the genome to allow comparison of tandem repeat polymorphism rates between these. In total 26 minisatellites (Table 2) were chosen which best matched the screening criteria: 15 within protein-coding sequences and 11 within intergenes. Flanking primers were designed for PCR amplification of each of these, ensuring that they were not sited within any residual degenerate copies of the repeats.

The usefulness of the 26 minisatellites as genetic markers was tested on six independently identified *Sch. pombe* isolates. These were chosen to include widespread geographical origins in Africa, Asia, Europe and America, and to represent a diversity of source substrates including molasses, fruit juice and alcoholic beverages (Table 1). The two heterothallic strains 972 h^- and 975 h^+ (Leupold, 1950) were representatives of the Osterwalder (1924) isolate from French grape juice which has given rise to the genetic strains used in virtually all molecular studies on *Sch. pombe*. The other five chosen isolates have been the subject of comparative molecular studies: CBS5557, formerly known as *Sch. malidevorans*, has been used for analysis of tandem repeats in the *cpy1* gene (Ingavale *et al.*, 1998) and, together with the *Sch. pombe* type strain CBS356 (also known as EF1),

Table 2. Tandem repeat characteristics and primer sequences

No.	Position*	Repeat unit length†	Copy no.‡	Fragment length§	Match (%)	Primer sequences	Alleles
1	SPAC13G6.10c	33	4.1	206	71	L: GACAGGAGCCACATAGGATGA R: CCTGTATCAACTCCCAGAGACA	1
2	<i>spt5</i>	9	5.2	241	84	L: AACAGAGTTCTCCCGTGGATT R: CTTTTCTTCTACCGCCACCT	1
3	SPAC56F8.12–SPAC56F8.14c	9	12.7	384	71	L: CAATAGAATCGGGTGAGGTACG R: AATGCTGCTGGTAAAGATATGC	1
4	<i>gar2</i>	21	4.0	229	76	L: AGTCCCGAACCTTCAAAGAAA R: CGCTGGAACTCTCCTTCTTCT	3
5	SPAC16E8.01	21	4.4	466	91	L: GCCACTGGTTATCAACCTTTA R: GAACTTGCACACCAGTCATCT	2
6	<i>cpy1</i>	27	7.6	293	93	L: GAGAACTTTGCATGTTTTGCTC R: TCCTTTCAAACACCATGAGCTT	5
7	<i>cpy1</i>	39	10.8	481	96	L: CTCCTCAAGCTCATGGTGTTT R: ATTTGACGATGAGGATCGAGAG	3
8	<i>fiol1</i> –SPAC1F7.09c	10	6.9	200	81	L: CAACAAGTGACAAGCCATGAAT R: CCTAGGGGTGGAAGTAAGAAGA	2
9	<i>tif471</i>	24	20.5	560	85	L: GGTGCACTTGATTGATTTTCAG R: CAACAGCGTATCCAGGAAAAAG	2
10	SPAC26F1.13c–SPAC26F1.12c	21	2.5	249	75	L: TTGGATCGTTTGAGCCATATT R: GGATTAATCGTTGGGAAAAATCA	2
11	<i>app1</i>	45	12.0	585	80	L: ACCACGGGCTCATTAAGTTGT R: GGCGCAACCATCAGTCATTA	2
12	SPAC11E3.13c–SPAC11E3.14	9	9.2	228	67	L: TGAAAAATGAAAGGAATGCCAAT R: GTGCACGTTTCGTACATCATA	2
13	<i>rlp1</i> –SPBC1685.13	10	10.3	182	60	L: AGGTAGAAGGGATTTCGACAGC R: ACAGGCAGTGGAAGAGAGACA	4
14	SPBC36.03c– <i>cys11</i>	9	13.9	298	74	L: CGATATAGTCGAACATAGCCGTA R: TGGAGGTGAAAAGGACATAGAA	3
15	<i>wis1</i> –SPBC409.08	9	5.2	242	76	L: GACTACATCGGCAAAAACACC R: GCAAGCAAATTACACCTGACC	2
16	<i>rpb1</i>	21	25.5	599	80	L: GATATTCACGTCACCTGCTTA R: CAAAAGCATTGTCATCTTCAC	2
17	SPBC1711.05	54	8.7	571	92	L: CATCCGGAAGTGATCTTCAT R: CTTTCGGAGTCAGAGGAATCAG	3
18	SPBC19C7.10	33	4.8	245	77	L: GACACCTATCAAGCCTTCTCTTC R: CGAACTTCTTTTCCAACCTTCC	1
19	<i>nep1</i>	33	3.9	244	83	L: ATCACCACCATCATCATACA R: TGGACAGAAGGTAATGGCGTA	2
20	<i>sat1</i> – <i>ssn6</i>	9	7.2	296	75	L: GGAGGTAGGAGGAACGCTAGAT R: ATTGGATGTTTAGTTGGCGAGT	6
21	SPBC26H8.09c	33	2.6	595	69	L: GCTCTGAATCAATGCGATGTA R: TTTCGTTTGACACCAACTTTTC	2
22	SPCC330.04c	21 + 33	6.1 + 6	400	94,99	L: AACATCAAGGATTTCTCGAACTA R: GCCTCCAGCTTGGTTTCG	3
23	<i>sap1</i> –SPCC1672.03c	14	7.7	226	72	L: TCTGGGTGAAAGAATGAGCAAT R: GACGTTTGCAGTGTTGAAGAGA	3
24	<i>ent1</i>	48	8.4	489	80	L: AAAGGGTTGTTGGACTCAAATG R: TTTCATCCGTCCCTCAAATACC	1
25	SPCC1739.07–SPCC1739.08c	10	10.6	189	63	L: ATGAAAGAGGAGGAAAAGAGGT R: GAATTGAGGCGAAAGAGAGAGA	2
26	<i>wtf21</i> – <i>amt1</i>	7	9.3	204	66	L: ATGAGAAGGAGGCGGTAATTTT R: TCCACTTCCCTATCTGCTTGTT	2

Table 2. cont.

No.	Position*	Repeat unit length†	Copy no.‡	Fragment length§	Match (%)	Primer sequences	Alleles
27	<i>rpp203</i>	3	17.7	220	80	L: CTGTCGGTATCGAAAGTGAGT R: TCGTCAGATTCTTCTTCTCC	1
28	<i>isp4-rpl26</i>	5	13.2	233	85	L: TTTTATGGAATCAACGTCCAC R: CCCGCTCCAAGATACTAAAA	1
29	<i>med15</i>	3	32.0	240	97	L: CACCAATTCTATGCAAAAAT R: TACCTTGAGGGTTTGGAAAA	5
30	<i>sat1-ssn6</i>	5	11.4	230	88	L: TCGAAACAACATACATCCGTA R: GCTGCACATAAAGGAAAAAGA	1
31	<i>rpp102</i>	3	24.0	176	71	L: TCTTCATCAGACTCCTCTTCTTC R: ATCTTTGCTAAGGCTTTGGAG	1
32	SPCC757.12	6	8.3	244	79	L: TCCTTCTACTTCGATGAGTGC R: ACTACTGTATGCCGTGGAAGT	1

*A single gene/systematic name indicates a coding tandem repeat; two names indicate flanking genes of a non-coding tandem repeat.

†Repeats 27–32 are microsatellites.

‡Copy number in sequenced genome.

§Predicted fragment length (bp).

||Number of alleles observed in six *Sch. pombe* isolates (for minisatellite markers 1–26) or 16 genetic strains (for microsatellite markers 27–32).

for rRNA sequencing studies (Kurtzman & Robnett, 1991, 1998). CBS356, CBS357 and CBS5557 have been the subject of *Schizosaccharomyces* nuclear DNA reassociation studies (Vaughan Martini, 1991), and CBS356, CBS5557 and CBS5682 (also known as CSIR Y-457 and identical to EF3) of mating configuration studies (Schlake & Gutz, 1993). Zimmer *et al.* (1987) analysed mitochondrial genome variation with CBS356, CBS2775 and CBS5682; this last strain has also been the subject of comparative transposon analysis (Levin *et al.*, 1990).

Fig. 1 shows that most (81%) of the bioinformatically identified minisatellites demonstrated polymorphism within these six *Sch. pombe* isolates. Ten of the 11 loci within intergenic sequences and 11 out of 15 loci within protein-coding sequences had more than one allele identified (Fig. 1, Table 2): just five markers were uninformative within the six isolates. There is no significant difference in the frequencies of informative loci between the protein-coding and non-coding minisatellites [$P(\chi^2) > 0.1$]; the frequencies of moderately polymorphic loci containing two to three alleles are similar between these sequence types and each contains one highly polymorphic locus with more than four alleles (Fig. 2). As expected, the 972 h^- and 975 h^+ Leupold strains were indistinguishable with all tandem repeat markers (Fig. 1, lanes 1 and 2).

None of the minisatellite markers contained a perfect tandem repeat sequence (Table 2). Overall, the observed degree of polymorphism (number of alleles) was not correlated with internal percentage match for the tandem repeats ($r^2 = 0.018$, $P = 0.52$), but there was a significant association for those tandem repeats in protein-coding regions ($r^2 = 0.34$, $P = 0.02$). There was no correlation

between number of alleles and the percentage of indels in the tandem repeats ($r^2 = 0.023$, $P = 0.46$).

Analysis of polymorphic minisatellite alleles

The data in Fig. 1 demonstrate that six *Sch. pombe* isolates of different geographical origin can be distinguished by tandem repeat length using minisatellite markers. An unrooted phenetic dendrogram was constructed of the relationships between the *Sch. pombe* isolates, based on a distance matrix of the polymorphic minisatellite alleles. The tree was constructed with the neighbour-joining program of PHYLIP and the input order was randomized to avoid construction bias. The tree obtained (Fig. 3) groups the isolates 972/975 (French) and CBS5557 (Spanish), both of which originate from wine production with grapes. The CBS357 (Jamaican) and CBS2775 (Japanese) isolates were both taken from fermenting molasses: for the Jamaican source this was intended for rum making and originated from cane sugar production. These have been grouped with the isolate from local beer, CBS5682 (South African). The origin of the type strain CBS356 is uncertain but it may derive from the original Lindner strain; Fig. 3 shows that it is clearly distinct from the other five isolates. CBS356 was obtained from the Král Collection, the world's first microbial culture collection, which was established in Prague in 1890, the same year as the shipment of millet beer from East Africa from which Lindner first isolated and described *Sch. pombe* (Lindner, 1893).

The allele size differences in Fig. 1 are consistent with integer differences in copy numbers of tandem repeats, and to check this PCR products were sequenced from different alleles for two polymorphic loci: tandem repeats in genes *rpb1* and

Fig. 1. Genetic fingerprinting of *Sch. pombe* isolates. Images 1–26 show PCR amplification products of the respective minisatellite tandem repeats 1–26 in Table 2 from *Sch. pombe* strains 972 h^- (lane 1), 975 h^+ (lane 2), CBS356 (lane 3), CBS357 (lane 4), CBS2775 (lane 5), CBS5557 (lane 6), and CBS5682 (lane 7). The marker lane (M) contains 100 bp ladder.

Fig. 2. Number of alleles identified in six *Sch. pombe* isolates for minisatellite loci within 15 protein-coding sequences (black bars) and 11 intergenic sequences (white bars).

SPCC330.04c. For both loci the sequences corresponded with the allele sizes observed by gel electrophoresis and confirmed that the alleles differ by integer numbers of repeat units. The *rpb1* gene in *Sch. pombe* isolate CBS356 contains only 23 copies of the 21 bp tandem repeat as compared with 25 copies in the Osterwalder isolate (see supplementary Fig. S1, available with the online version of this paper). The encoded heptapeptide repeat is in the essential C-terminal domain (CTD) of the large subunit of RNA polymerase II; this repeat is known to vary in copy number between species (Stiller & Hall, 2002) but to our knowledge this is the first report of a CTD repeat copy number variation between naturally occurring strains of *Sch. pombe*. A more complex pattern was observed in the three alleles of the SPCC330.04c gene, where the Osterwalder isolate has six repeats of 21 bp followed by six repeats of a 33 bp unit, the CBS356 isolate has three copies of the 21 bp unit and only four of the 33 bp unit whereas CBS2775 has five repeats of the 21 bp unit and eight of the 33 bp unit (see supplementary Fig. S2). This interesting pattern of switching from a repeat unit size of 21 bp to a 33 bp unit is discussed below, but again the sequencing confirmed that the alleles all differed by integer numbers of repeat units. At both loci mutations seem to affect the middle of the tandemly repeated region and this is most evident in the multiple alignment of the SPCC330.04c gene sequences. Within the 21 bp repeated sequence isolate CBS2775 has lost the third unit and CBS356 has lost the third, fourth and fifth units but the first, second and sixth units remain unchanged compared with the Osterwalder strain. Within the 33 bp repeated sequence CBS356 has lost the fourth and fifth repeat units, and isolate CBS2775 has gained two units after repeat five, compared with the Osterwalder isolate. At both loci the tandem repeat sequences for the 972 *h*⁻ or 975 *h*⁺ strains were identical to the published sequence for the 972 *h*⁻ strain (Wood *et al.*, 2002).

Minisatellite and microsatellite polymorphisms in genetic strains of *Sch. pombe*

Virtually all strains used for *Sch. pombe* genetics are derived from homothallic and heterothallic strains obtained by Leupold (1950) from the Osterwalder (1924) isolate, and are assumed to have isogenic backgrounds. The high level of

Fig. 3. Unrooted phenetic tree illustrating relationships between *Sch. pombe* isolates of different geographical origin constructed by the neighbour-joining method based on a distance matrix of polymorphic minisatellite alleles. CBS356 is the type strain of *Sch. pombe* Lindner; 972 is an *h*⁻ genetic strain derived from the Osterwalder isolate formerly named *Sch. liquefaciens*; CBS357 was formerly *Sch. mellacei*; CBS2775 was formerly *Sch. pombe* var. *ogasawaraensis*; CBS5557 was formerly *Sch. malidevorans*.

variation seen with tandem repeat markers provides the opportunity to test this assumption. We therefore extended the tandem repeat marker analysis to a selection of genetic strains obtained from a diversity of laboratories in the *Sch. pombe* community (Table 1), but all ultimately derived from Leupold strains. The strains included mutants defective in DNA replication (*cdc23*, *hsk1*, *nda4*), cell cycle checkpoints (*cut5*, *rad3*), mitosis (*cdc25*, *nda3*) and recombination (*swi5*). In addition to the 26 minisatellite markers described above a further six tandem repeats were also selected with repeat unit sizes of ≤6 bp, i.e. microsatellite markers (Table 2, numbers 27–32). All 32 pairs of primers were applied to each of 16 genetic strains.

No tandem repeat differences were detected between any of the genetic strains with 30 of the 32 markers (data not shown). The two exceptional polymorphic loci were the minisatellite in the *tif471* gene, for which one strain exhibited a shorter allele (supplementary Fig. S3), and the microsatellite marker in the *med15* gene, which gave five alleles in genetic strains (Fig. 4a). The *tif471* gene encodes the translation factor eIF4G scaffold protein that mediates the binding of mRNA and associated proteins to the 43S ribosomal preinitiation complex, overexpression of which causes aberrant *Sch. pombe* cell morphology (Hashemzadeh-Bonehi *et al.*, 2003). Sequencing of both of the *tif471* alleles revealed that three 24 bp repeat units had been lost from the SJA302 strain in comparison to the 972 *h*⁻ strain (supplementary Fig. S3).

The five alleles of *med15* were sequenced and all were found to differ from the 32 repeat units predicted by the published sequence (Fig. 4). The shortest repeated region is found in laboratory strain SJA273, with just over half of the published number of repeated units (17 units) and the longest in strain SJA272 with 40 repeated units; these are consistent with the estimates from gel electrophoresis. This hypervariable

Fig. 4. Polymorphism of the *med15* micro-satellite tandem repeat locus in *Sch. pombe* laboratory strains. (a) PCR amplification products of the tandem repeat locus within the protein-coding sequence of *med15* analysed by agarose gel electrophoresis for laboratory strains: lane 1, 972 *h*⁻; lane 2, 975 *h*⁺; lane 3, SJA148; lane 4, SJA167; lane 5, SJA122; lane 6, SJA272; lane 7, SJA237; lane 8, SJA175; lane 9, SJA277; lane 10, SJA244; lane 11, SJA216; lane 12, SJA273; lane 13, SJA120; lane 14, SJA238; lane 15, SJA300; lane 16, SJA302. (b) Alignment of the published 972 *h*⁻ sequence (Wood *et al.*, 2002) with sequenced PCR products for strains 975 *h*⁺, SJA273, SJA302, SJA216, SJA122, SJA120 and SJA272. The 3 bp repeat units are indicated by arrows.

tandem repeat encodes a homopolymeric stretch of glutamine residues within the predicted *Med15* protein, which is an orthologue of Gal11, a component of the Mediator complex which forms part of the RNA polymerase II holoenzyme (The Wellcome Trust Sanger Institute; www.genedb.org).

DISCUSSION

The popularity of *Sch. pombe* as a model organism has concentrated studies of this species on genetic strains derived by Leupold (1950) from a single wild-type isolate (Osterwalder, 1924). Our data open the door to molecular analysis of natural variation in this fission yeast species by providing 21 minisatellite markers for genetic fingerprinting of different isolates of *Sch. pombe*, and provide evidence of some variation between genetic strains. The minisatellite allele differences can be detected using agarose gels, without recourse to sequencing; therefore the markers can be readily used for distinguishing between strains of *Sch. pombe*. We attempted to extend the analysis to other members of the genus *Schizosaccharomyces* by using the same primers against *Sch. japonicus* and *Sch. octosporus* DNA. However, no reproducible PCR products were obtained for *Sch. octosporus* and only faint bands were visible for *Sch. japonicus* when using very low annealing temperatures, which did not match any of the alleles identified in the *Sch. pombe* isolates (data not shown). Attempts by Ingavale *et al.*

(1998) to amplify the two tandem repeats in the *cpy1* gene of *Sch. pombe* in *Sch. japonicus* and *Sch. octosporus* similarly failed to give detectable products. This failure of *Sch. pombe* primers to anneal to DNA in the other two *Schizosaccharomyces* species indicates that there is significant sequence divergence between the three species within this genus, and is consistent with the conclusion from rRNA sequencing that the three recognized fission yeast species are not closely related (Kurtzman & Robnett, 1991, 1998).

Ingavale *et al.* (1998) analysed tandem repeats in the vacuolar carboxypeptidase Y (*cpy1*) gene of two isolates of *Sch. pombe* and found just one difference between the Osterwalder isolate and the Rankine & Fornachon isolate CBS5557 formerly known as *Sch. malidevorans*. We have confirmed these data and extended them to four more *Sch. pombe* isolates, discovering three and five distinct alleles for the respective RS I and RS II markers in this gene (Fig. 1: gels 7 and 6). In addition we have identified a further 19 minisatellite markers at other loci which are informative for fingerprinting strains of *Sch. pombe*. Only five of the tandem repeats we tested were uninformative (Fig. 2), although it is possible that some of these may show polymorphisms if analysed in other *Sch. pombe* isolates.

All of the minisatellite polymorphic markers were imperfect tandemly repeated sequences and overall there was little correlation between internal conservation of sequence and the number of polymorphic alleles. This supports the

conclusion of Denoeud *et al.* (2003) that measurements of overall internal conservation, used as a polymorphism predictor for microsatellites, are not applicable to minisatellites, presumably owing to the greater complexity of their mutation processes. Our sequence data do indicate that polymorphisms occur at particular internal sites in the tandem repeats where neighbouring units share a high degree of sequence conservation, rather than in the terminal repeat units. However, these internal sites should not be looked at in isolation because they form part of larger repetitive elements, which are important to give a complete picture, particularly in evolutionary studies. Five of our *Sch. pombe* minisatellites exhibited polymorphism despite having less than 70 % sequence match between individual repeat units. This demonstrates the great importance of considering imperfect as well as perfect tandem repeats in any study related to expansion and contraction of such sequences.

Surprisingly, there was no significant difference in the frequency of informative loci between protein-coding and non-coding tandem repeats. This shows that length variations can be tolerated in many *Sch. pombe* proteins. Gene ontology (GO) analysis of the ten polymorphic proteins reveals a wide diversity of biological process and molecular function, but all of the proteins are predicted to be intracellular and four are nuclear. These are therefore very different proteins from the set of serine/threonine-rich repeat-containing cell wall proteins identified in *Sac. cerevisiae* by Verstrepen *et al.* (2005) and Richard & Dujon (2006). All the *Sch. pombe* polymorphic tandem repeats are predicted to be in regions with no discernible 3D structure, and for only the repeats in SPAC16E8.01 (β -sheet/coil), SPCC330.04c and Tif471 (α -helices) are rigid secondary structures predicted. It therefore appears that these length variations mostly correspond to structurally flexible regions of the encoded proteins. The high mutation rates could increase the evolutionary effects of variation on cellular regulatory processes (Tompa, 2003). Our identification of polymorphic protein repeats in *Sch. pombe* can allow further investigation of such elements and comparison to those identified in *Sac. cerevisiae* and other eukaryotes.

The SPCC330.04c protein contains a complex tandem repeat encoded by 21 bp and 33 bp repeat units (supplementary Fig. S2) which is predicted to form an α -helical secondary structure, part of which is markedly amphipathic. The repeat units of 21 bp and 33 bp correspond to two and three α -helical turns respectively and allele sizes are consistent with integer differences in the tandem repeats; therefore this protein could vary between strains simply by differences in the length of the α -helix, not in the relative orientation of its residues or adjoining tertiary structure. This 'integer-helix' model can also explain the pattern of switching from 21 bp to 33 bp repeat units within the one tandem repeat.

Genetic strains of *Sch. pombe*, unlike those of *Sac. cerevisiae*, are all derived from one original isolate and

are therefore assumed to be isogenic. The availability of DNA fingerprinting markers has allowed this hypothesis to be tested. Reassuringly for the *Sch. pombe* community, we found no differences between 16 genetic strains for 30 out of 32 tandem repeats we tested, including six microsatellites. The exceptions were the repeat in the *tif471* gene which in a single genetic strain exhibited the shorter allele also observed in the CBS357 isolate, and the trinucleotide repeat in *med15* which appears to be hypervariable. It is possible that the presence of checkpoint or cell cycle mutations may have influenced the probability of genomic changes, although the DNA replication *hsk1* mutant, which has a length mutation at the *tif471* locus, appears to be in the ancestral state at the *med15* locus. In the *med15* gene five different alleles were detected in the otherwise essentially isogenic 16 genetic strains examined. The reason for the hypervariability of this microsatellite in a gene encoding a predicted RNA polymerase II holoenzyme component remains to be determined. One possibility is that it might lie close to a meiotic recombination hotspot; however, it is > 300 kb from the nearest of the ten meiotic hotspots detected by Steiner & Smith (2005). Testing of this possibility awaits full genomic mapping of meiotic hotspots in *Sch. pombe*. The principal mechanism for expansions and contractions of microsatellites is DNA replication slippage (Ellegren, 2004); therefore this is a more likely explanation for variation at this locus. The finding of an unstable microsatellite in *Sch. pombe* affords the possibility to examine not just the mechanism underlying mutation, but also the particular properties of this locus that confer hypervariability, thereby elucidating mechanisms by which eukaryotic cells normally ensure genome stability at other microsatellite loci.

Few molecular studies have been carried out on natural wild-type variants of *Sch. pombe*. The chromosomal locations and total numbers of Tf1 and Tf2 retrotransposons differ greatly in various isolates of *Sch. pombe* (Levin *et al.*, 1990), as might be expected, although the sequences appear to be highly conserved (Hoff *et al.*, 1998). Size differences have been reported in the mitochondrial genomes of different *Sch. pombe* isolates due to the presence or absence of introns (Zimmer *et al.*, 1987). On the other hand, Takahashi *et al.* (1992) found that centromeres are highly conserved between natural variants of *Sch. pombe*. Some strains would particularly merit further study, for example *Sch. pombe* NCYC 132, which was extensively used for early cell cycle investigations (Mitchison, 1989) and has recently been studied in relation to hyphal growth (Amoah-Buahin *et al.*, 2005), and *Sch. kambucha*, whose mating-type locus has been sequenced and shows around 2 % difference from standard strains (Singh & Klar, 2003). Many scores of wild-type strains of *Sch. pombe* have been isolated, from all inhabited continents of the world and from many types of substrate. The markers described in this study provide a route into population biology and will permit extensive studies of the natural variation of this important model species.

ACKNOWLEDGEMENTS

We thank Matthew Redden, Kirstie Affleck, Charlotte Cymbalista and Daniel Harrison for valuable contributions to the project, and Antony Carr, Richard Egel, Susan Forsburg, Paul Nurse, Henning Schmidt, Pierre Thuriaux and Mitsuhiro Yanagida for *Sch. pombe* strains. A.-M.P. was funded by a studentship from the Biotechnology and Biological Sciences Research Council (BBSRC) of the UK and Kirstie Affleck by a Genetics Society vacation studentship.

REFERENCES

- Amoah-Buahin, E., Bone, N. & Armstrong, J. (2005). Hyphal growth in the fission yeast *Schizosaccharomyces pombe*. *Eukaryot Cell* **4**, 1287–1297.
- Benson, G. (1999). Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res* **27**, 573–580.
- Boby, T., Patch, A.-M. & Aves, S. J. (2005). TRbase: a database relating tandem repeats to disease genes for the human genome. *Bioinformatics* **21**, 811–816.
- Bowen, S. & Wheals, A. E. (2006). Ser/Thr-rich domains are associated with genetic variation and morphogenesis in *Saccharomyces cerevisiae*. *Yeast* **23**, 633–640.
- Calafell, F., Shuster, A., Speed, W. C., Kidd, J. R. & Kidd, K. K. (1998). Short tandem repeat polymorphism evolution in humans. *Eur J Hum Genet* **6**, 38–49.
- Chambers, G. K. & MacAvoy, E. S. (2000). Microsatellites: consensus and controversy. *Comp Biochem Physiol B Biochem Mol Biol* **126**, 455–476.
- Debrauwère, H., Gendrel, C. G., Lechat, S. & Dutreix, M. (1997). Differences and similarities between various tandem repeat sequences: minisatellites and microsatellites. *Biochimie* **79**, 577–586.
- Denoeud, F., Vergnaud, G. & Benson, G. (2003). Predicting human minisatellite polymorphism. *Genome Res* **13**, 856–867.
- Egel, R. (2004). *The Molecular Biology of Schizosaccharomyces pombe: Genetics, Genomics and Beyond*. Berlin: Springer.
- Ellegren, H. (2004). Microsatellites: simple sequences with complex evolution. *Nat Rev Genet* **5**, 435–445.
- Felsenstein, J. (2005). PHYLIP – Phylogeny Inference Package, version 3.6. Distributed by the author. University of Washington, Seattle, USA.
- Fondon, J. W., III & Garner, H. R. (2004). Molecular origins of rapid and continuous morphological evolution. *Proc Natl Acad Sci U S A* **101**, 18058–18063.
- Forsburg, S. L. (1999). The best yeast? *Trends Genet* **15**, 340–344.
- Forsburg, S. L. & Rhind, N. (2006). Basic methods for fission yeast. *Yeast* **23**, 173–183.
- Hashemzadeh-Bonehi, L., Curtis, P. S., Morley, S. J., Thorpe, J. R. & Pain, V. M. (2003). Overproduction of a conserved domain of fission yeast and mammalian translation initiation factor eIF4G causes aberrant cell morphology and results in disruption of the localization of F-actin and the organization of microtubules. *Genes Cells* **8**, 163–178.
- Heckman, D. S., Geiser, D. M., Eidell, B. R., Stauffer, R. L., Kardos, N. L. & Hedges, S. B. (2001). Molecular evidence for the early colonization of land by fungi and plants. *Science* **293**, 1129–1133.
- Hoff, E. F., Levin, H. L. & Boeke, J. D. (1998). *Schizosaccharomyces pombe* retrotransposon Tf2 mobilizes primarily through homologous cDNA recombination. *Mol Cell Biol* **18**, 6839–6852.
- Ingavale, S. S., Kaur, R., Aggarwal, P. & Bachhawat, A. K. (1998). A minisatellite sequence within the propeptide region of the vacuolar carboxypeptidase Y gene of *Schizosaccharomyces pombe*. *J Bacteriol* **180**, 3727–3729.
- Kashi, Y., King, D. & Soller, M. (1997). Simple sequence repeats as a source of quantitative genetic variation. *Trends Genet* **13**, 74–78.
- Katti, M. V., Ranjekar, P. K. & Gupta, V. S. (2001). Differential distribution of simple sequence repeats in eukaryotic genome sequences. *Mol Biol Evol* **18**, 1161–1167.
- Kellis, M., Patterson, N., Endrizzi, M., Birren, B. & Lander, E. S. (2003). Sequencing and comparison of yeast species to identify genes and regulatory elements. *Nature* **423**, 241–254.
- Kurtzman, C. P. & Robnett, C. J. (1991). Phylogenetic relationships among species of *Saccharomyces*, *Schizosaccharomyces*, *Debaryomyces* and *Schwanniomyces* determined from partial ribosomal RNA sequences. *Yeast* **7**, 61–72.
- Kurtzman, C. P. & Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek* **73**, 331–371.
- Leupold, U. (1950). Die Vererbung von Homothallie und Heterothallie bei *Schizosaccharomyces pombe*. *C R Lab Carsberg Sér Physiol* **24**, 381–480.
- Levin, H. L., Weaver, D. C. & Boeke, J. D. (1990). Two related families of retrotransposons from *Schizosaccharomyces pombe*. *Mol Cell Biol* **10**, 6791–6798.
- Lindner, P. (1893). *Schizosaccharomyces pombe* n. sp., ein neuer Gährungsreger. *Wochenschr Brauerei* **10**, 1298–1300.
- Marguerat, S., Jensen, T. S., de Lichtenberg, U., Wilhelm, B. T., Jensen, L. J. & Bähler, J. (2006). The more the merrier: comparative analysis of microarray studies on cell cycle-regulated genes in fission yeast. *Yeast* **23**, 261–277.
- McGuffin, L. J., Bryson, K. & Jones, D. T. (2000). The PSIPRED protein structure prediction server. *Bioinformatics* **16**, 404–405.
- Mitas, M. (1997). Trinucleotide repeats associated with human disease. *Nucleic Acids Res* **25**, 2245–2254.
- Mitchison, J. M. (1989). Cell cycle growth and periodicities. In *Molecular Biology of the Fission Yeast*, pp. 205–242. Edited by A. Nasim, P. Young & B. F. Johnson. San Diego: Academic Press.
- Moreno, S., Klar, A. & Nurse, P. (1991). Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol* **194**, 795–823.
- Osterwalder, A. (1924). *Schizosaccharomyces liquefaciens* n. sp., eine gegen freie schweflige Säure widerstandsfähige Gärhefe. *Mitt Gebiete Lebensmittelunters Hyg* **15**, 5–28.
- Peng, K., Radivojac, P., Vucetic, S., Dunker, A. K. & Obradovic, Z. (2006). Length-dependent prediction of protein intrinsic disorder. *BMC Bioinformatics* **7**, 208.
- Richard, G. F. & Dujon, B. (2006). Molecular evolution of minisatellites in hemiascomycetous yeasts. *Mol Biol Evol* **23**, 189–202.
- Richard, G. F. & Pâques, F. (2000). Mini- and microsatellite expansions: the recombination connection. *EMBO Rep* **1**, 122–126.
- Rost, B., Yachdav, G. & Liu, J. (2003). The PredictProtein Server. *Nucleic Acids Res* **32** (Web Server issue), W321–W326.
- Rozen, S. & Skaletsky, H. J. (2000). Primer3 on the WWW for general users and for biologist programmers. In *Bioinformatics Methods and Protocols: Methods in Molecular Biology*, pp. 365–386. Edited by S. Krawetz & S. Misener. Totowa, NJ: Humana Press.
- Schlake, T. & Gutz, H. (1993). Mating configurations in *Schizosaccharomyces pombe* strains of different geographical origins. *Curr Genet* **23**, 108–114.

- Singh, G. & Klar, A. J. (2003).** DNA sequence of the *mat2,3* region of *Schizosaccharomyces kambucha* shares high homology with the corresponding sequence from *Sz. pombe*. *Yeast* **20**, 1273–1278.
- Sipiczki, M. (2000).** Where does fission yeast sit on the tree of life? *Genome Biol* **1**, reviews 1011.1–1011.4.
- Steiner, W. W. & Smith, G. R. (2005).** Natural meiotic recombination hot spots in the *Schizosaccharomyces pombe* genome successfully predicted from the simple sequence motif M26. *Mol Cell Biol* **25**, 9054–9062.
- Stiller, J. W. & Hall, B. D. (2002).** Evolution of the RNA polymerase II C-terminal domain. *Proc Natl Acad Sci U S A* **99**, 6091–6096.
- Strand, M., Prolla, T. A., Liskay, R. M. & Petes, T. D. (1993).** Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* **365**, 274–276.
- Sunnerhagen, P. (2002).** Prospects for functional genomics in *Schizosaccharomyces pombe*. *Curr Genet* **42**, 73–84.
- Sutherland, G. R. & Richards, R. I. (1995).** Simple tandem DNA repeats and human genetic disease. *Proc Natl Acad Sci U S A* **92**, 3636–3641.
- Takahashi, K., Murakami, S., Chikashige, Y., Funabiki, H., Niwa, O. & Yanagida, M. (1992).** A low copy number central sequence with strict symmetry and unusual chromatin structure in fission yeast centromere. *Mol Biol Cell* **3**, 819–835.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994).** CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Tompa, P. (2003).** Intrinsically unstructured proteins evolve by repeat expansion. *Bioessays* **25**, 847–855.
- Tóth, G., Gaspari, Z. & Jurka, J. (2000).** Microsatellites in different eukaryotic genomes: survey and analysis. *Genome Res* **10**, 967–981.
- Vaughan Martini, A. (1991).** Evaluation of phylogenetic relationships among fission yeast by nDNA/nDNA reassociation and conventional taxonomic criteria. *Yeast* **7**, 73–78.
- Vergnaud, G. & Denoeud, F. (2000).** Minisatellites: mutability and genome architecture. *Genome Res* **10**, 899–907.
- Verstrepen, K. J., Jansen, A., Lewitter, F. & Fink, G. R. (2005).** Intragenic tandem repeats generate functional variability. *Nat Genet* **37**, 986–990.
- Viguera, E., Canceill, D. & Ehrlich, S. D. (2001).** Replication slippage involves DNA polymerase pausing and dissociation. *EMBO J* **20**, 2587–2595.
- Ward, J. J., Sodhi, J. S., McGuffin, L. J., Buxton, B. F. & Jones, D. T. (2004).** Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. *J Mol Biol* **337**, 635–645.
- Wood, V., Gwilliam, R., Rajandream, M. A., Lyne, M., Lyne, R., Stewart, A., Sgouros, J., Peat, N., Hayles, J. & other authors (2002).** The genome sequence of *Schizosaccharomyces pombe*. *Nature* **415**, 871–880.
- Zimmer, M., Welser, F., Orlaer, G. & Wolf, K. (1987).** Distribution of mitochondrial introns in the species *Schizosaccharomyces pombe* and the origin of the group II intron in the gene encoding apocytochrome *b*. *Curr Genet* **12**, 329–336.

Edited by: H. A. B. Wösten