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Detection and Characterization of Megasatellites in Orthologous and Nonorthologous Genes of 21 Fungal Genomes

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Megasatellites are large DNA tandem repeats, originally described in *Candida glabrata*, in protein-coding genes. Most of the genes in which megasatellites are found are of unknown function. In this work, we extended the search for megasatellites to 20 additional completely sequenced fungal genomes and extracted 216 megasatellites in 203 out of 142,121 genes, corresponding to the most exhaustive description of such genetic elements available today. We show that half of the megasatellites detected encode threonine-rich peptides predicted to be intrinsically disordered, suggesting that they may interact with several partners or serve as flexible linkers. Megasatellite motifs were clustered into several families. Their distribution in fungal genes shows that different motifs are found in orthologous genes and similar motifs are found in unrelated genes, suggesting that megasatellite formation or spreading does not necessarily track the evolution of their host genes. Altogether, these results suggest that megasatellites are created and lost during evolution of fungal genomes, probably sharing similar functions, although their primary sequences are not necessarily conserved.

Tandem repeats are a common component of all eukaryotic genomes sequenced so far (1). Besides the ubiquitous presence of microsatellites and the frequent occurrence of minisatellites, megasatellites represent a new class of larger tandem repeats that were initially identified in yeast genomes (2). Megasatellites were defined as tandem repeats whose base motif is longer than 100 nucleotides (whereas minisatellite motifs seldom reach this size [3]), tandemly repeated at least three times (to distinguish them from local duplications), and inserted within protein-coding genes. They are frequent in the pathogenic yeast *Candida glabrata*, in which two large families, called "SHITT" and "SFFIT," respectively (due to the conservation of these five amino acids within the motif), have been described in about 30 genes (2, 4). Another yeast genome, that of the well-studied baker's yeast *Saccharomyces cerevisiae*, contains eight tandem repeats that qualify as megasatellites: in the *FLO1* (*YAR050w*), *FLO5* (*YHR211w*), and *FLO9* (*YAL063c*) paralogous genes encoding cell wall proteins involved in yeast cell flocculation; in *FIT1* (*YDR534c*) and *HPF1* (*YOL155c*), two other cell wall genes; in *NUM1* (*YDR150w*), a cytoskeleton organization gene; and in *YIL169c*, a gene of unknown function sharing high similarity with *HPF1* (*YOL155c*). The *FLO1* megasatellite was experimentally shown to play a role in cell flocculation and adhesion, with longer repeats being associated with better adhesion and flocculation (5). *Kluyveromyces lactis* subtelomeric regions were shown to contain several genes encoding large tandem repeats (6), with four of them qualifying as megasatellites (*KLLA0A11935g*, *KLLA0B14916g*, *KLLA0C19316g*, and *KLLA0D00264g*) (see Table S1 in the supplemental material). There is no experimental evidence of their putative function in this yeast, but based on sequence similarity, they might be good candidates to be cell wall genes. The genome of *Candida albicans*, an opportunistic pathogenic yeast, contains eight ALS genes, each of them with 108-bp tandem motifs, corresponding to the megasatellite definition (7–9). The ALS genes are involved in adhesion of *C. albicans* to epithelial host cells by a mechanism involving binding to a large variety of ligands, including carbohydrates and peptides (8–10). Twenty-one different allele sizes have been found for the tandem array of the *ALS7* gene (*CAL0005421*) among different *C. albicans* strains (11), but it is not known whether some of them are associated with higher adhesion. However, tandem repeats of *ALS5* (*CAAL5736*) and *ALS3* (*CAAL1816*) were shown to be important for yeast cellular adhesion to epithelial cells or to fibronectin (12, 13). *Aspergillus fumigatus* was also shown to contain large tandem repeats, some of them included in genes proposed to encode cell wall components (14), but none of these megasatellites was shown to be directly involved in cellular adhesion. Among other tandemly repeated motifs detectable by our analysis, WD repeats are a family of tandem arrays frequently encountered in eukaryotic genes, having a structural function in proteins involved in functions as diverse as RNA processing, transcription, cytoskeleton assembly, vesicle trafficking, cell division, or sulfur metabolism in fungi (reviewed in: reference 15). WD motifs contain two highly variable regions, separating more conserved domains; therefore, all the motifs of a given tandem repeat do not necessarily share the same size, although their final structures are very similar.

Previous intraspecific comparisons between paralogous megasatellite-containing genes showed that megasatellite motifs are under purifying selection and that this selection is stronger in *C. glabrata* than in *S. cerevisiae* (16). It was proposed that megasatellites propagate by three different mechanisms: (i) duplication of a megasatellite-containing gene, (ii) gene conversion between homologous sequences, and (iii) "jumping" of one or several motifs from one megasatellite-containing gene to another gene (16).

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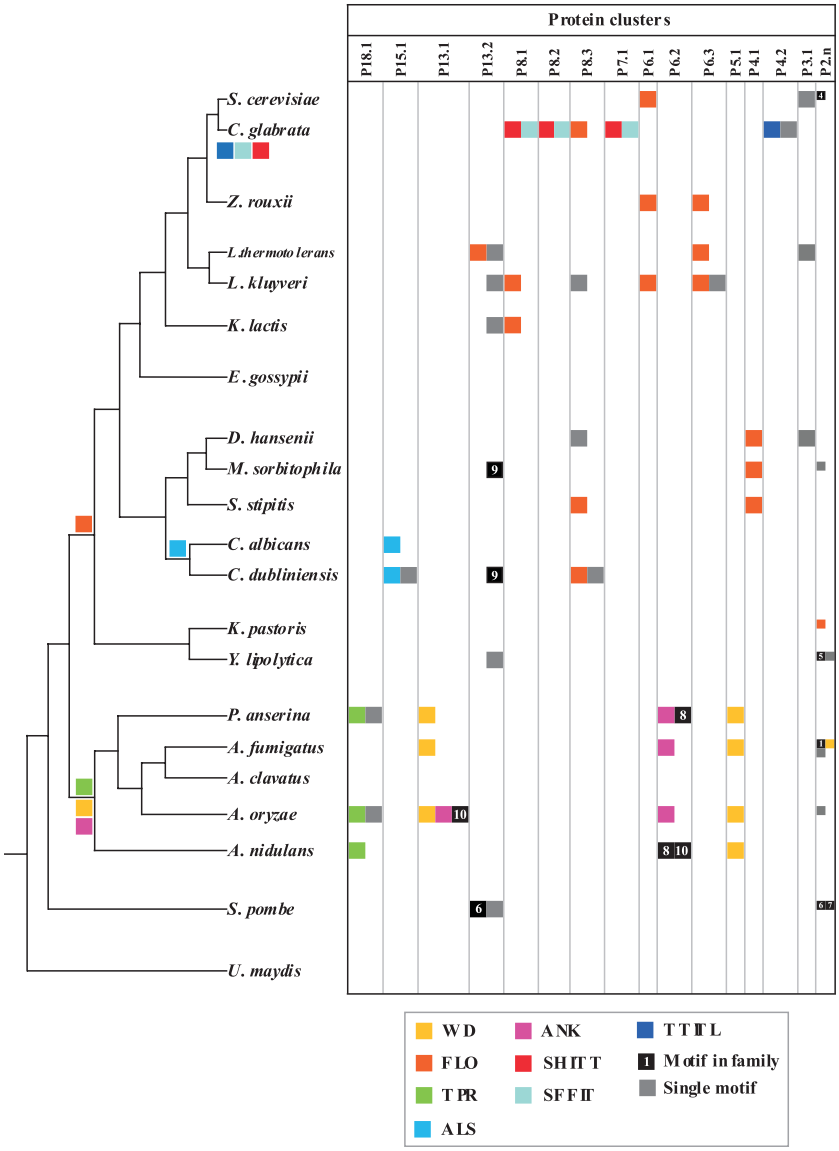


FIG 1 Distribution of megasatellites in the 21 genomes studied. Left, tree topology (68, 69). Branch lengths are arbitrary. Motif families are represented by a color code. Motifs drawn on the tree indicate their proposed time of appearance during evolution, under a parsimony hypothesis. Right, protein clusters containing two or more proteins are represented by vertical columns. Nonunique motifs are indicated by their number in a black box (see Table S1 in the supplemental material). Unique motifs are shown in gray. P2.n, all clusters containing only two proteins.

However, besides the intraspecific analyses carried out in *S. cerevisiae* and *C. glabrata*, very few studies in yeast or other fungal species are available to extensively characterize and compare megasatellite distribution.

In order to do so, we developed a methodology, based on the Tandem Repeat Finder (TRF) program (17, 18), to analyze 21 Dikarya genomes covering a large phylogenetic spectrum (mostly Ascomycota). Our analysis covered 15 ascomycetous yeasts (from *S. cerevisiae* to *Schizosaccharomyces pombe*), five filamentous ascomycetes (*Podospora anserina* and four *Aspergillus* species), and one basidiomycete (*Ustilago maydis*) (Fig. 1 and Table 1) (19–33). Megasatellites were classified according to their motif sequence similarity and characterized by their corresponding amino acid composition. About half of them encoded peptides particularly enriched in threonine residues. *In silico* structure prediction of the peptides encoded by these megasatellites indicates that most of them are not structured, suggesting that they do not form stable structures *in vivo*. Finally, we demonstrate that large tandem repeats are constantly created (and sometimes lost) during evolution, suggesting a rather fast molecular mechanism(s) that creates new functions in each fungal lineage.

MATERIALS AND METHODS

Megasatellite detection. A database containing 142,121 annotated genes from 21 fungal genomes was built (Table 1), and the Tandem Repeat Finder (TRF) program (17) was used to extract all motifs from this set of genes, using the following parameters: match weight = 2, mismatch penalty = 7, insertion/deletion penalty = 7, match probability = 80, insertion/deletion probability = 10, minimum alignment score to report = 50, and maximum period size to report = 2,000. For each genome, the fol-

TABLE 1 Fungal species analyzed

Species	Genome size, Mb (reference)	No. of:	
		Coding sequences	Megasatellites
Saccharomyces cerevisiae	12,068 (19)	5,862	7
Candida glabrata	12,280 (20)	5,202	28
Zygosaccharomyces rouxii	9,765 (21)	4,991	11
Lachancea thermotolerans	10,393 (21)	5,092	8
Lachancea kluyveri	11,346 (21)	5,321	9
Kluyveromyces lactis	10,631 (20)	5,076	7
Eremothecium gossypii	8,765 (22)	4,768	0
Debaryomyces hansenii	12,221 (20)	6,272	10
Millerozyma sorbitophila	21,460 (23)	11,252	6
Scheffersomyces stipitis	15,400 (24)	5,816	4
Candida albicans	14,855 (25)	6,112	8
Candida dubliniensis	Partial[a] (26)	5,860	15
Komagataella pastoris	9,430 (27)	5,040	5
Yarrowia lipolytica	20,503 (20)	6,448	15
Schizosaccharomyces pombe	12,463 (28)	4,993	12
Podospora anserina	Partial (29)	10,219	19
Aspergillus nidulans	30,069 (30)	10,701	17
Aspergillus oryzae	37,000 (31)	12,074	19
Aspergillus fumigatus	27,981 (30)	9,926	9
Aspergillus clavatus	29,400 (32)	4,574	1
Ustilago maydis	20,500 (33)	6,522	6
Total	>326,530	142,121	216

[a] Partial genome sequence; the genome size is therefore not precisely known.

lowing iterative approach was used to determine the minimal size of tandemly repeated motifs. Gene sequences were searched using TRF. Motifs with sizes equal to or greater than 90 bp were further analyzed for possible inclusion of repeated submotifs until no repeated motif was found. Submotifs of less than 90 bp were discarded. The presence of each megasatellite was confirmed by constructing self dot plots of the corresponding protein (34) and by comparing each corresponding gene sequence versus itself using bl2seq and blastn (35). At this step, only motifs repeated at least three times were retained as megasatellites.

Extraction of peptides encoded by megasatellites. Using the starting position of the megasatellite and its motif size (in nucleotides), each polypeptide was extracted from the translated gene sequence. Motif starting position and motif size were obtained by dividing by 3 the megasatellite starting position and motif size, as determined by TRF on the DNA sequence. Repeated polypeptides were further validated using bl2seq and blastp to compare the translated megasatellite to its corresponding polypeptide sequence. Manual inspection was often needed to find the precise border of each motif (computer programs are inefficient at finding the precise border of a tandem repeat, since any amino acid within the motif may be chosen as the beginning of the motif). In most cases, megasatellites correspond to tandemly directly repeated motifs, but some megasatellites are separated by amino acid segments varying from one to a few amino acids. Some megasatellites needed the insertion of one or two gaps to keep the periodicity of the tandem repeat, whereas in some cases, a few amino acids needed to be removed to keep the periodicity of the megasatellite. Each megasatellite was given a unique identification number, defined by its gene name (see Table S1 in the supplemental material). When a gene carries more than one megasatellite, each megasatellite within this gene bears an additional rank number (for example, *CADU0C86150-1* and *CADU0C86150-2* define the two megasatellites found in *CADU0C86150*).

Amino acid compositions and correspondence analysis. Amino acid compositions were computed for translation products of each megasatellite, for the set of proteins from which megasatellites were precisely removed, and for the 142,121 proteins of the 21 studied proteomes. Correspondence analysis is a multivariate method for exploration of large numerical data tables. It allows the projection of high-dimensional information onto low-dimensional spaces. Visual inspection of such projections onto a plane allows the detection of significant trends, which are often difficult to grasp in high-dimensional spaces. The method builds an orthogonal system called factorial axes (F1, F2, F3, etc.), with each axis representing a fraction (displayed in decreasing order) of all of the information contained in the analyzed data table. The statistical significance of this fraction determines the relative confidence attached to the displayed axes (megasatellites or amino acids). The orthogonality of the factorial axes allows the summation of their corresponding fractions. The first factorial plane corresponding to the first (F_1) and second (F_2) factorial axes includes the highest fraction of the total information, obtained by summing the fractions corresponding to the first (F_1) and to the second (F_2) factorial axes. Note that megasatellites and amino acids are displayed simultaneously on each factorial plane, in such a way that neighborhood between megasatellites and amino acids is indicative of significant relationships. Conversely, greater distance between megasatellites and amino acids is indicative of weaker relationships. The methodology and its applications in a similar case have been extensively described by Tekaia and Yeramian (36).

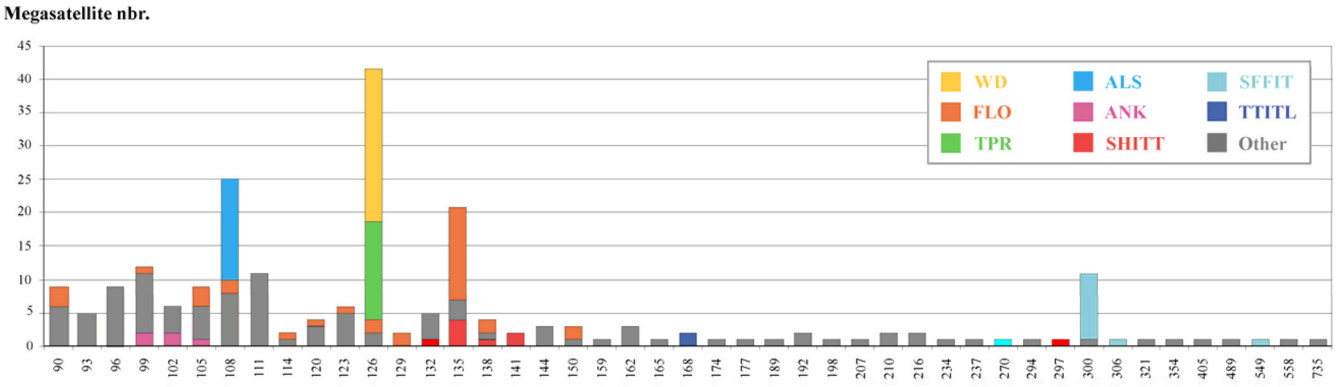
Comparison and clustering of all megasatellites against themselves. All peptidic motifs were compared to each other, using blastp (35). A blastp similarity score was considered significant when the corresponding E value was equal or lower than 10^{-2}. Nonunique peptides (i.e., those having significant similarity with at least one other peptide) were classified into clusters using mcl (37) with "−log(blastp(e-value))" and an inflation index *I* of 3.0. Each nonunique peptide was assigned to a cluster denoted Mp.q (for motif clusters), with p the number of peptides contained and q an arbitrary index number (38). Peptides included in each of the determined clusters were aligned using the ClustalW program (39), and conserved blocks were determined using the Gblock program (40).

Comparison and clustering of all proteins against themselves. All proteins, from which the tandemly repeated peptides were removed, were compared to each other, using blastp (35). A blastp similarity score was considered significant when the corresponding E value was equal to or lower than 10^{-9}, as previously described (41). Nonunique proteins were clustered using the mcl (37) program with the same options as indicated above for the megasatellites and assigned to a cluster denoted Pp.q (with p being the number of proteins contained and q an arbitrary index number).

Motif consensus and structure. When three or more megasatellites were found in a given family (ALS, FLO, SHITT, SFFIT, WD, etc.), motif consensus were determined by alignment of all the motifs using the Jalview program (42). All motifs sequences are given in Table S1 in the supplemental material. Subsequently, each of the motifs (or motif consensus) was analyzed using the metaserver MeDor (43) in order to determine whether any part of the motif was predicted to be disordered. The eight motifs that were predicted probably not to be disordered (see Results) were compared to the Protein Data Bank (PDB) (44) in order to determine if their structures were already known. In addition, megasatellite-containing proteins and peptidic motifs were also compared to the Conserved Domain Database (CDD) (45) version 3.02 (December 2011), including 40,815 domain sequences. Motif families (ALS, FLO, SHITT, SFFIT, WD, etc.) were also compared to several databases of known motifs (PROSITE, BLOCKS, ProDom, PRINTS, and Pfam) using the motif analysis tool found at http://www.genome.jp/tools/motif/.

RESULTS

Fungal genomes contain a large diversity of megasatellites. We have determined the complete set of tandem repeats detected in a total of 142,121 sequence-predicted protein-coding genes belonging to 21 fungal genomes (Dikarya) (Table 1). Out of more than 13,000 tandem repeats, we extracted 216 megasatellites (see Materials and Methods). The number of megasatellites detected



Species/Motif	WD	FLO	TPR	ALS	ANK	SHITT	SFFIT	TTITL	Other megasatellites in families	Single megasatellites	Total
Saccharomyces cerevisiae		3							2	2	**7**
Candida glabrata		1				9	13	2		3	**28**
Zygosaccharomyces rouxii		2								9	**11**
Lachancea thermotolerans		5								3	**8**
Lachancea kluyveri		5								4	**9**
Kluyveromyces lactis		5								2	**7**
Eremothecium gossypii											**0**
Debaryomyces hansenii		1							2	7	**10**
Millerozyma sorbitophila		2							1	3	**6**
Scheffersomyces stipitis		4									**4**
Candida albicans				8							**8**
Candida dubliniensis		2		7					1	5	**15**
Komagataella pastoris		5									**5**
Yarrowia lipolytica									4	11	**15**
Podospora anserina	9		6		1				1	2	**19**
Aspergillus fumigatus	4				1				2	2	**9**
Aspergillus clavatus										1	**1**
Aspergillus oryzae	6		1		3				1	8	**19**
Aspergillus nidulans	3		8						3	3	**17**
Schizosaccharomyces pombe									4	8	**12**
Ustilago maydis										6	**6**
Total	22	35	15	15	5	9	13	2	21	79	**216**

FIG 2 Distribution of megasatellites according to motif size. Upper panel, total number of megasatellites for each motif size. Lower panel, total number of megasatellites in each species, classified by families. The color code is the same in both panels.

ranges from 28 in *Candida glabrata* to none in *Eremothecium gossypii* and is correlated neither to genome size nor to gene content (Table 1). Motif sizes range from 90 bp (9 megasatellites in 5 species) to 735 bp (one megasatellite in *Yarrowia lipolytica*, *YALI0B09867g*). As expected for tandem repeats located within protein-coding genes, all motif sizes found are multiples of three nucleotides.

The most common motif found is the FLO motif, which was encountered in 35 megasatellites in 11 species, from *S. cerevisiae* to *Komagataella pastoris*, making it the most widespread of all megasatellite motifs. This motif encodes a Thr/Ser-rich sequence, often containing the Trp-Thr-Gly tripeptide (see Table S1 in the supplemental material). The FLO motif size is highly variable, ranging from 90 bp to 150 bp. By comparison, other frequent motifs, such as ALS, tetratricopeptide repeat (TPR), or WD, all share the same size (108 bp for ALS and 126 bp for TPR and WD). ALS motifs are only found in *C. albicans* and *Candida dubliniensis*, in eponymous genes and their homologues. TPR motifs occur in tandem arrays in more than 800 genes, from bacteria to humans. The motif corresponds to two antiparallel alpha helices separated by a turn (46). Megasatellites containing TPR motifs are particularly frequent in *Aspergillus nidulans* and *P. anserina*. WD repeats are generally encountered in proteins belonging to the whole eukaryotic world (15); however, in the present study, they were detected only in filamentous fungi (*P. anserina* and *Aspergillus* species).

Megasatellites found in *C. glabrata* and containing SHITT and SFFIT motifs were previously described and are widely spread in this genome (2, 4, 16). SHITT and SFFIT are sometimes encountered as motifs of slightly different sizes, suggesting that, like for FLO motifs, their containing proteins may accommodate some tandem repeat flexibility. Note that despite similar motif sizes, there is no detectable homology between SHITT and FLO motifs, suggesting that SHITT motifs either were *de novo* created in *C. glabrata* or rapidly evolved from an ancestral sequence.

Smaller families were also detected, such as the ankyrin (ANK) family, found within five megasatellites in *Aspergillus orizae*, *A. fumigatus*, and *P. anserina*, or the TTITL family, found in two megasatellites in *C. glabrata*. Ankyrin repeats consist of two alpha helices separated by loops and are involved in protein-protein interactions (47). Nothing is known about the structure or function of TTITL motifs. In addition to these, 21 other motifs belonging to small families (2 or 3 members) were found, and 79 other motifs did not share any detectable homology (Fig. 2).

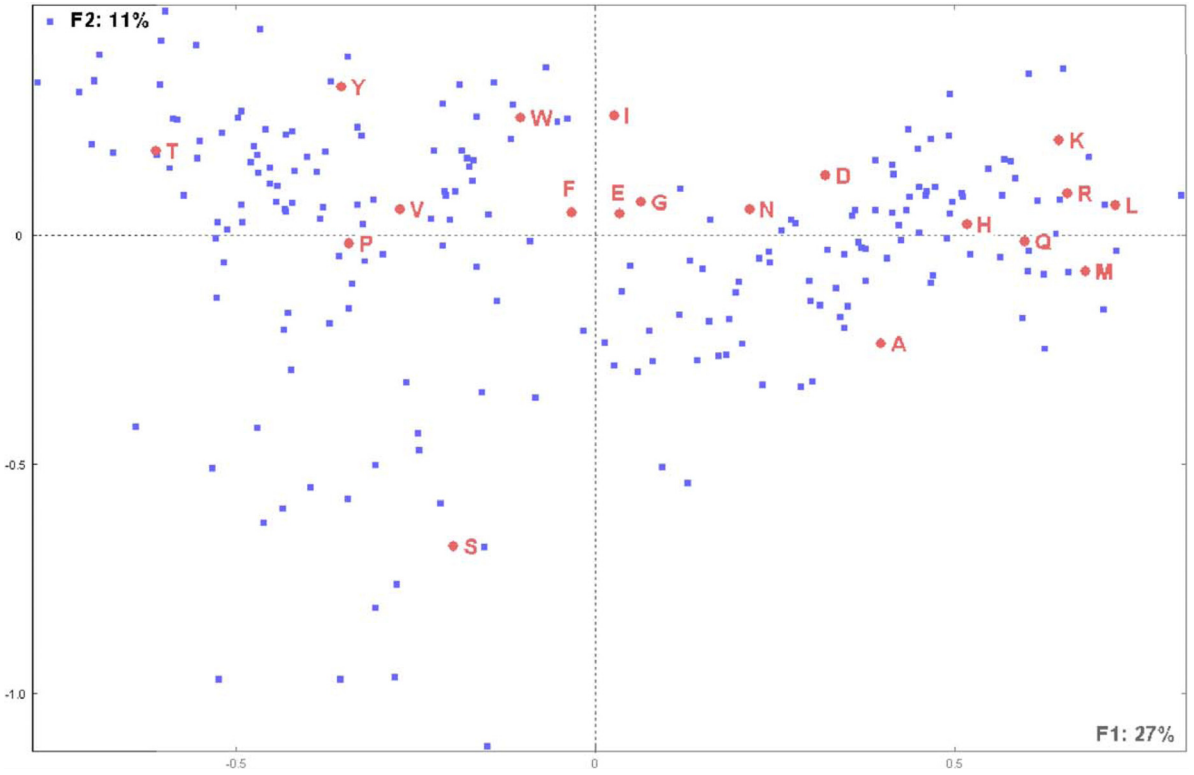


FIG 3 Correspondence analysis showing the distribution of megasatellites (blue dots) according to the 20 amino acids on the first factorial plane. F1 and F2 are the first and second factorial axes and represent, respectively, 27% and 11% of the total information included in the analyzed data table (observed megasatellites versus their amino acid composition).

Amino acid compositions of megasatellites. The amino acid compositions of all translated peptidic motifs were computed. Compared to the average amino acid composition of more than 60 million amino acids making altogether the 21 proteomes, Thr and Ser are often overrepresented, whereas Leu, Arg, Lys, Met, and Gln are often underrepresented. Correspondence analysis was used to determine possible amino acid composition biases of megasatellites (36). Megasatellites are displayed mostly in two groups along the first factorial axis (Fig. 3, horizontal axis F1, covering 27% of the total information in the analyzed data). One group (left) is characterized by high composition biases in Thr and by underrepresentation of Arg, Leu, Lys, and Gln. The second group (right) is characterized by high composition biases in Leu, Lys, Arg, and Gln and underrepresentation of Thr. Therefore, megasatellite composition according to the first axis is directly correlated with threonine content. It is interesting to note that few megasatellites are characterized by average compositions (few are plotted close to the axis origin), meaning that most of them exhibit biased amino acid composition. Note that FLO, ALS, SHITT, SFFIT, and TTITL are Thr rich, whereas ANK, WD, and TPR repeats are Thr poor.

Structure prediction for megasatellite peptidic motifs. Primary sequences of peptidic motifs encoded by megasatellites are generally not conserved, despite the existence of families as described above. This, however, does not exclude the possibility that common secondary structures exist. To address this question, several secondary structure and disorder predictors were used on each motif (see Materials and Methods). Out of 97 different peptidic motifs analyzed, 88 show an extensive level of disorder (50 to 100% of the motif) and no obvious secondary structure (data not shown). The eight remaining motifs, showing lower levels of disorder, were compared to the Protein Data Bank (PDB), and seven of them (all threonine-poor motifs) were found to correspond to known secondary structures. Motifs M8 (in PODANSg6698 and AN8019), M10 (in AN3543, AN8085 and AO090166000058), M21 (in PODANSg8665), and M56 (in AO090102000421) all correspond to ankyrin motifs, a common repeat in eukaryotic proteins, but also found in bacteria and archaea (PDB ID 2L6B). It is interesting to note that primary sequence similarity with the ANK motif described above in other megasatellites (Fig. 2) was not detected, but only the three-dimensional (3D) structure predicted that these four motifs should share the tertiary structure of ankyrin repeats. M69 (in ZYRO0G06028g) has a match in PDB with the structure of a carbohydrate epimerase from *Pseudomonas aeruginosa* (PDB ID 2IXI), hence linking this motif to carbohydrate metabolism (48). Finally, M49 (in AO090009000369) corresponds to a putative L-*allo*-threonine aldolase from *Listeria monocytogenes* (PDB ID 3PJ0).

In addition, each megasatellite peptidic motif was compared to the CDD (45). Only eight significant hits were detected, corresponding to ankyrin repeats, WD repeats (twice), TPR repeats, the cohesin-HEAT domain (associated with chromosome cohesion and condensation), DUF3659 (a 70-amino-acid domain of unknown function found in bacteria and eukaryotes), a putative 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase domain, and a deoxyhypusine synthase domain essential for translation initiation in eukaryotes. The overall conclusion is, therefore, that

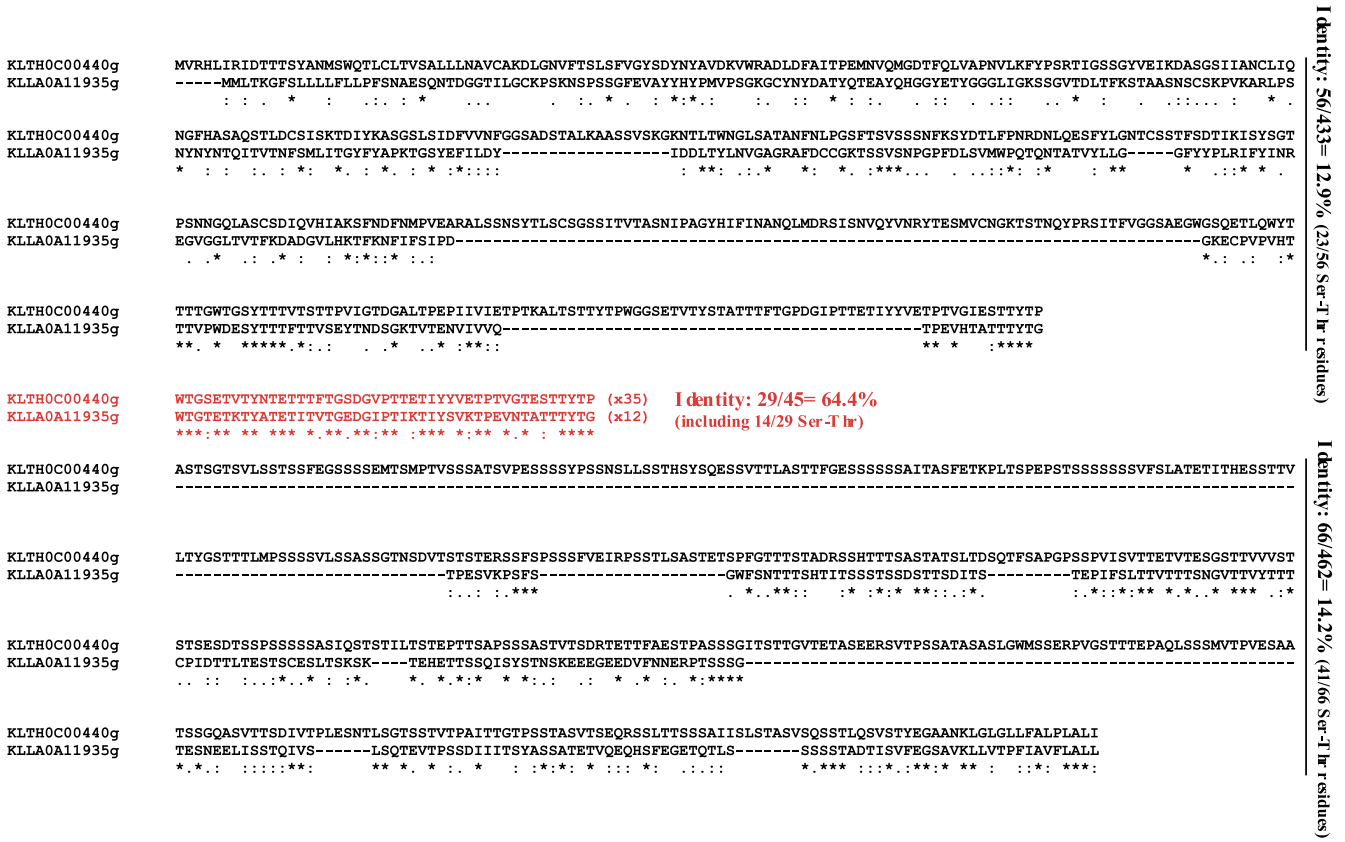


FIG 4 Example of similar megasatellites in two nonhomologous genes. Alignment of *KLTH0C00440g* and *KLLA0A11935g* translation products, two proteins belonging to two different clusters (P6.3 and P8.1, respectively) and containing the same peptidic motif (FLO, motif cluster M17.1 [see Table S1 in the supplemental material]), is shown. The peptidic motif is shown in red, along with the number of repeats in each protein. The N-terminal and C-terminal parts of both proteins exhibit little identity (12.9% and 14.2%, respectively), with most of the identical amino acids being serine and threonine residues, due to the compositional bias of both proteins. In comparison, both FLO motifs are very similar, despite a comparable compositional bias.

most peptidic motifs (88/97, ca. 91%) encoded by megasatellites are disordered and unstructured.

Formation and propagation of megasatellites during evolution. One of the main questions of the present work was to determine whether megasatellite motifs were species specific or lineage specific or whether they were distributed randomly, suggesting a possible propagation among fungi. Our results very clearly show that both happened during fungal evolution. SFFIT, SHITT, and TTITL motifs are restricted to *C. glabrata* (Fig. 1). The FLO motif is widespread in all hemiascomycetous yeasts, from *S. cerevisiae* to *Y. lipolytica*, although it is found in seven different clusters (see Table S1 in the supplemental material). Some FLO motifs are shorter, on average, than others (Fig. 2), but despite these size discrepancies, there is little doubt that both "short" and "long" FLO motifs, recognizable by their Trp-Thr-Gly tripeptide, come from a common ancestor. The ALS motif seems to be restricted to *C. albicans* and C. *dubliniensis*, and the WD, TPR, and ANK motifs are themselves restricted to the branch leading to Pezizomycotina (*P. anserina* to *Aspergillus* species). Thr-rich and -poor motifs are widespread among all fungi. No evidence for a case of horizontal gene transfer between two distant fungal species could be detected (49).

Subsequently, megasatellites were extracted from their containing genes and translated, and peptidic motif families were compared to protein families after megasatellite extraction (see Materials and Methods). Some large protein families were found, such as P18.1, found exclusively in *P. anserina* and *Aspergillus* species. Most of its members contain a TPR motif, but three of them contain a unique motif (M21, M22, and M23) (see Table S1 in the supplemental material). P6.1 and P4.1 contain genes that carry only the FLO motif, whereas P5.1 contains orthologous genes carrying only the WD motif (Fig. 1). All the other clusters contain at least two different kinds of motifs. Hence, similar megasatellites may be found in nonhomologous genes (Fig. 4), whereas orthologous genes often carry different megasatellites (Fig. 5).

DISCUSSION

In the present work, we present the first exhaustive comparative genomic analysis of megasatellite distribution in the genomes of 21 fungi. Peptidic motifs encoded by megasatellites were extracted from their containing proteins and compared to each other. Using the present approach, only megasatellites whose motifs have similar lengths can be detected. Therefore, none of the 55 WD repeats encoded by the *S. cerevisiae* genome was detected, since motif lengths are very different from each other.

Megasatellite-containing genes show hallmarks of plasma membrane or cell wall genes. We have identified 216 megasatellites spread in 18 different families and 79 unique megasatellites, with half of those encoding proteins including motifs enriched in

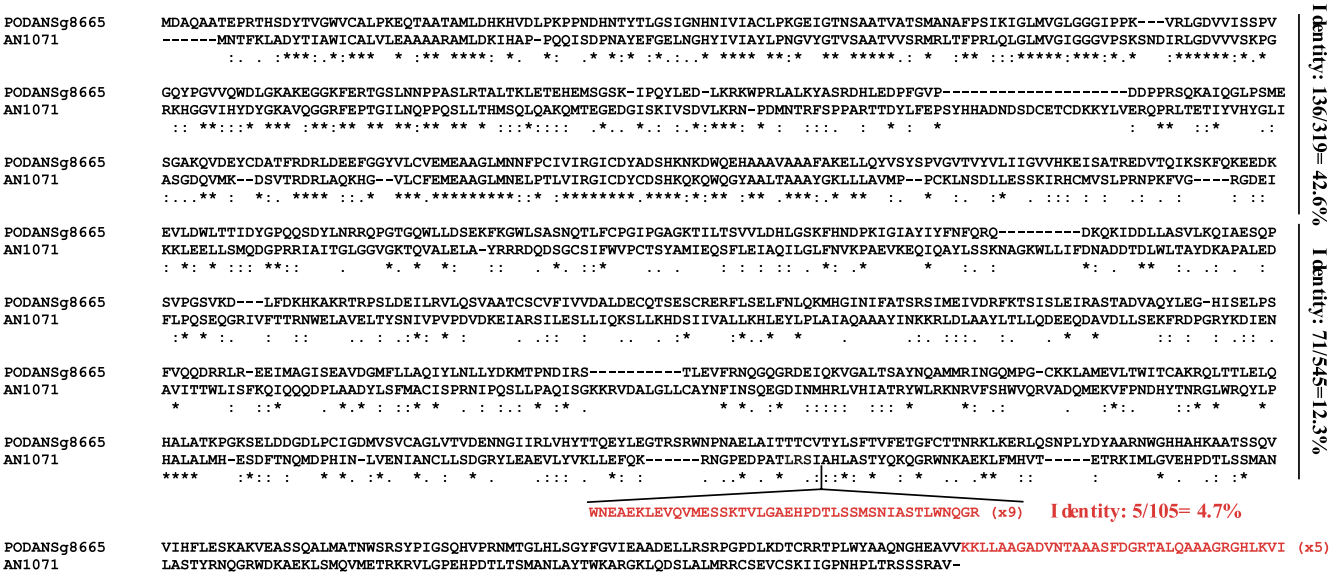


FIG 5 Example of different megasatellites in two homologous genes. Alignment of *PODANSg8665* and *AN1071* translation products, two homologous proteins (P18.1) containing different megasatellite motifs, is shown. Both proteins show very similar N-terminal parts (42.6% identity) followed by less conserved regions (12.3% identity) containing the repeated peptides. There is no homology between the peptidic motifs.

threonine residues. In *S. cerevisiae* cell wall proteins, such residues are sites of O mannosylations, which occur in the endoplasmic reticulum and are essential for localization of such proteins at the cell surface (50, 51). *FLO1* (*YAR050w*), *FLO5* (*YHR211w*), and *FLO9* (*YAL063c*) encode flocculins involved in cell-to-cell adhesion and yeast flocculation (52), *HPF1* (*YOL155c*) encodes a surface mannoprotein involved in protein aggregates in white wine fermentation, and *NUM1* (*YDR150w*) and *FIT1* (*YDR534c*) encode a cytoskeleton organization protein and a glycosylphosphatidylinositol (GPI) anchor-containing cell wall protein, respectively. Megasatellites are also found in adhesins in *C. albicans* (*ALS1* to *ALS7* and *ALS9* genes) (9) and in *C. glabrata* (*EPA1*, *EPA2*, and *EPA13* genes) (53, 54). In the well-studied model organism *Schizosaccharomyces pombe*, megasatellites are detected in the *MAP2* P-factor pheromone gene (*SPCC1795.06*), the *MAP4* gene (*SPBC21D10.06c*) (encoding an adhesin required for mating), and the *MAM3* gene (*SPAP11E10.02c*) (involved in cell-to-cell adhesion). Comparison of peptide motifs to databases of known motifs shows the presence of possible phosphorylation and glycosylation sites found in flocculins, as well as putative sites of myristoylation. Addition of myristate (a 14-carbon fatty acid) to proteins is a common posttranslational modification of proteins generally associated with the plasma membrane and/or involved in signal cascades. The myristoyl part of the protein is directly involved in the interaction with membrane lipids, in a reversible manner, helping to localize the protein at the plasma membrane (55). It is therefore tempting to propose that megasatellites encoding such Thr-rich motifs belong to genes encoding proteins localized at the plasma membrane and/or cell wall and involved, directly or indirectly, in cell adhesion. However, there is no information about the function of megasatellite-containing genes except for the handful described above.

Possible function(s) of megasatellites in fungal genes. The function of the megasatellite itself is puzzling. In *S. cerevisiae*, cell flocculation and adhesion to plasticware were correlated to the size of the *FLO1* megasatellite (5), but there is no experimental evidence that this is also the case for its two paralogues, *FLO5* and *FLO9*. In *C. albicans*, adhesion assays show reduced adhesiveness for strains with an *ALS3* allele containing only nine motifs, compared to 12 (12), suggesting a role in adhesion for the megasatellite. Given that the number of allelic lengths of a megasatellite may be quite large (for example, 21 different lengths of the *ALS7* megasatellite were found in patients infected with *C. albicans* [11]), megasatellite polymorphism offers the opportunity to modulate adhesion of such yeasts to their substrate. Finally, when *EPA1* is expressed in *C. glabrata* or *S. cerevisiae*, adhesion to epithelial cells is partly dependent on the presence of its megasatellite (56). However, it is not known if the same holds true for other megasatellite-containing *EPA* genes.

The molecular mechanism by which peptidic motifs encoded by megasatellites modulate adhesion is unclear, but it was suggested that they may serve as variable spacers between the N-terminal part (bearing the binding domain) and the C-terminal part (anchored to the cell wall) of the protein. This spacer needs to reach a given length in order to properly expose the N-terminal ligand-binding domain to the cell surface (9, 10, 56). It was also proposed (10, 14) that the high variability of megasatellites would help pathogens to escape the host immune system by modifying their surface antigens. A similar strategy, based on the activation/inactivation of cell wall genes by small tandem repeat size changes and called "phase variation," is extensively used by some human bacterial pathogens such as *Haemophilus influenzae* (57, 58) and *Neisseria meningitidis* (59–62). Similarly, the *Mycobacterium tuberculosis* genome contains two large families of proteins of unknown function called PE and PPE proteins; both have a disordered C-terminal domain made of tandemly repeated Pro-Glu or Pro-Pro-Glu motifs, which have been suggested to be a source of antigenic variation (63, 64).

It is commonly believed now that about 40% of human proteins contain long intrinsically disordered regions and that some

25% are probably disordered from beginning to end (65, 66). Predictions based on amino acid composition (43) suggest that 88 out of our 97 different megasatellite motifs (91%) are partially or fully disordered, a higher proportion than is commonly found for all eukaryotic proteins, suggesting that intrinsically disordered domains are a hallmark of megasatellites.

Formation and loss of megasatellites during evolution. The relative distribution of megasatellites in fungi varies among species. For example, TPR repeats were found only in the branch leading to filamentous fungi (Pezizomycotina), whereas the FLO motif was only detected in Saccharomycotina (Fig. 1). These motifs show very different amino acid compositions (FLO motifs contain 35.5% Thr residues, while TPR motifs contain 7.1% Thr residues), and no sequence homology could be detected between them. Therefore, the most parsimonious hypothesis is that FLO and TPR repeats do not share a ancestor. The same holds true for other motif families, suggesting that megasatellites belonging to different families are created and lost during evolution of fungal genomes.

In a comparative analysis of intragenic tandem repeats among 10 *Aspergillus* genomes, it was concluded that such repeat sequences were highly variable (only 21% of intragenic tandem repeats found in a given species were also detected in another one) and that repeat-containing proteins were less conserved than other proteins (67). In another study, comparisons of SHITT and SFFIT motifs in *C. glabrata* led the authors to propose a new mechanism, tentatively called "motif jump," to explain the presence of motifs belonging to a given family within a megasatellite-containing gene belonging to another family (16). Here, we can detect similar events occurring between nonorthologous gene families. For instance, FLO or WD repeats are found encoded by genes sharing no detectable homology, grouped in eight different protein clusters and two single proteins for FLO motifs and in three different protein clusters and four single proteins for WD motifs (see Table S1 in the supplemental material). To account for this observation, it may be proposed that the same megasatellite is recreated in different genes or that a megasatellite (or a discrete number of motifs) may "jump" from its original gene to another one, as proposed for SHITT and SFFIT motifs in *C. glabrata*. Alternatively, one may also propose that purifying selection operates more efficiently on megasatellites than on their containing genes, hence maintaining the same tandem repeat within genes that will eventually diverge to the point that any similarity between them will be erased. In support of the last hypothesis is the fact that megasatellite motifs in *C. glabrata* were found to be under a stronger purifying selection than their containing genes (16). Experiments aimed at determining how megasatellites appear and propagate within fungal genes are now needed to properly address this question.

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