Functional divergence of gene duplicates through ectopic recombination

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INTRODUCTION

How organisms evolve and adapt to new environments remains a central question in biology. Gene duplication events have a crucial role in evolutionary processes, especially in the rapid development of new functions [1,2]. Duplication might yield adaptive benefits through increased dosage of the parental gene. In addition, gene duplication can also stimulate evolutionary innovation as mutations in one or both gene duplicates can lead to subfunctionalization or neofunctionalization [3–8].

Gene duplications are not spread evenly over the genome, occurring much more frequently in the subtelomeres, regions directly adjacent to the telomeres [9–11]. As a result, subtelomeric gene families are often large, with some families carrying as many as several hundred paralogs [9,11]. In Trypanosoma and Plasmodium species, variable expression of subtelomeric variants of a cell-surface antigen allows these pathogens to elude the host immune system. Subtelomeric gene families in the more compact genome of Saccharomyces cerevisiae are generally smaller and are enriched for cell-surface genes, as well as genes involved in nutrient transport and metabolism [9,12–18].

Recombination between paralogs could generate more sequence diversity in duplicated genes [19]. However, apart from anecdotal examples in specific gene families such as the VAR and VSG cell-surface genes of Plasmodia and Trypanosomes and the major histocompatibility complex (MHC) class genes in vertebrates, the occurrence and biological relevance of ectopic recombination between gene duplicates have not been systematically investigated [16,17,20].

Here, we present the results of a comprehensive in silico analysis of ectopic recombination in all paralog gene families in the model eukaryote S. cerevisiae. Our results show that intergenic recombinations occur predominantly in gene families that are located at the subtelomeres and/or encode cell-surface genes. To verify whether these intergenic recombination events could lead to altered phenotypes, we mimicked the intergenic recombination events that shaped the FLO (flocculation) adhesin gene family. Phenotypic analyses of these artificial FLO chimaera...
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RESULTS AND DISCUSSION

Ectopic recombination in gene families

To investigate the occurrence of intergenic recombination events in *S. cerevisiae*, we first identified paralogous genes by BLASTing the reference strain S288c’s proteome against itself. Next, we used the MCL clustering algorithm and manual curation to define a list of 210 gene families, each comprising at least two paralogs (see methods and supplementary information online for details) [21]. For all genes in these families we collected the known GO categories as well as chromosomal locations. We attributed these characteristics to gene families if at least two genes in a family shared the annotation (the rationale being that it takes at least two genes to create a chimaeric allele).

Next, we BLAST-searched each family against a database containing 24 recently published high-quality *S. cerevisiae* genomes (supplementary Table S1 online). For each family, we performed several in silico tests to check for evidence of intergenic recombination in the family. First, we used the SplitsTree4 programme [22] to produce reticulate phylogenetic trees of each family. In the absence of recombination, this procedure results in a classic, unrooted phylogenetic tree. Alternatively, when a recombination event took place, this is represented in the reticulate tree as a closed rectangle. To further analyse these families and provide statistically significant proof for the occurrence of intergenic recombination, we used the Pairwise Homoplasy Index (PHI) test [23]. The null hypothesis for this test is that the observed sequence differences are due to convergent mutations, which implies that all PHI values are similar for all pairs (i.e., the PHI values do not vary with the physical distance between residues). Alternatively, in the presence of recombination, distant sites show low PHI values. These calculations then result in a single PHI value for the paralog gene family. For further details about these procedures, please refer the supplementary information online.

The distribution of PHI values indicates the presence of certain highly recombinogenic families (Fig 1). Interestingly, almost all chimaeric sequences are part of families that contain either subtelomeric or cell-surface genes, or both (Table 1). Statistical analyses of these distributions with a Komolgorov–Smirnov test revealed a statistically significant enrichment for subtelomeric gene families (P-value $3.48 \times 10^{-6}$) and for cell-surface gene families (P-value $5.5 \times 10^{-3}$). The distribution of a combination of all subtelomeric and cell-surface gene families was also significantly enriched for chimaeric alleles. Moreover, when all cell-surface gene families are removed from the analysis, we still find a statistically higher occurrence of chimaeric subtelomeric genes and vice versa. We were unable to find other subgroups that are enriched or depleted for ectopic recombination (e.g., the P-value for the comparison between families containing intragenic tandem repeats, and those without is 0.38; supplementary Fig S1 online).

Several recombinations shaped the adhesin gene family

To investigate the functional implications of ectopic recombination events, we focused on the subtelomeric FLO gene family, which encodes lectin-like cell-surface adhesins that confer adhesion to abiotic surfaces and/or other yeast cells [24–29]. These two phenotypes are biologically important for yeast cells and are relatively easy to measure and quantify [24,29–33]. The amino terminus of these rod-shaped proteins is a lectin-like globular domain that contains a pentapeptide involved in adhesion to specific carbohydrate residues present at the surface of other yeast cells or host tissues [34,35]. The central adhesin domain is formed by a repetitive pattern of a heavily glycosylated serine/threonine-rich peptide, which is thought to act as a variable rod-like spacer that helps to display the N-terminal domain to the environment [36].

To perform an in-depth analysis of recombination events between FLO paralogs, we gathered 58 more FLO sequences (including a few partial sequences or pseudogenes) from both NCBI and ENA (supplementary Table S2 online). Phylogenetic analysis revealed three subclades that cluster with the FLO1, FLO10 and FLO11 genes found in the reference S288c strain. In line with previous findings, we found extensive variation in tandem repeat length, even among adhesins from the same class [36,37].

We first verified whether the full set of FLO genes shows signs of intergenic recombination across the repeat region. Unrooted phylogenetic trees of the N- (Fig 2A) and carboxy-terminal (Fig 2B) domains revealed that several alleles might originate from recombination across the repeat region (Figs 2A–C). The PHI test further confirmed the occurrence of several intergenic recombination events in the complete FLO open reading frames (P-value $<10^{-16}$). Whereas this shows that ectopic recombination between intragenic tandem repeats does occur, analyses using all *S. cerevisiae* families do not show a significant enrichment of chimaera among genes that contain internal tandem repeats (see above).

**Fig 1** Analyses of paralog gene families indicate intergenic recombination. Distribution of the PHI values for all paralog gene families in the *S. cerevisiae* genome. Most gene families cluster towards the left hand side of the graph, with high PHI values that are indicative of the absence of intergenic recombination. However, several gene families on the far right hand side (Table 1) show inter-paralog recombination. Note that this group exists almost exclusively of subtelomeric and/or cell-surface gene families. For more information on the calculation of PHI values, see Bruen et al [23] and (supplementary information online) methods. PHI, pairwise homoplasy index.

revealed that they were functional and conferred phenotypes that differed from their parental adhesins.
Table 1 | S. cerevisiae gene families showing ectopic recombination

| Family description | Sequences in S288c | Total number of sequences retrieved | Subtelomeric | Cell-surface |
|--------------------|---------------------|------------------------------------|--------------|------------|
| Genes coding for (putative) Helicase-like proteins | 31 | 323 | Yes | No |
| HXT family | 16 | 213 | Yes | Yes |
| COS family | 9 | 116 | Yes | Yes |
| Genes coding for (Iso)maltases | 7 | 86 | Yes | No |
| AAD gene family | 7 | 74 | Yes | No |
| FLO gene family | 6 | 19 | Yes | Yes |
| Type I transmembrane sorting receptor for vacuolar hydrolases and similar sequences | 6 | 69 | Yes | No |
| PHO gene family | 5 | 79 | Yes | Yes |
| Pheromone-regulated protein with a motif involved in COPII binding/ putative integral membrane protein | 4 | 27 | No | Yes |
| Genes coding for MAL activators | 4 | 75 | Yes | No |
| ENA gene family | 3 | 19 | Yes | No |
| TDH gene family | 3 | 52 | No | No |
| FRE gene family | 3 | 55 | No | Yes |
| Transporters of thiamine/nicotinamide riboside | 3 | 44 | No | Yes |
| TPO gene family | 2 | 24 | No | Yes |

FLO, flocculation. Examples of some representative S. cerevisiae gene families that undergo ectopic recombination (for a full list, see supplementary Dataset S1 online). The table contains 15 gene families that contain at least one chimaeric sequence (PHI value < 10⁻¹⁶). First column lists function of characterized members of the identified gene family, ‘sequences in S288c’ lists the number of members of each gene family present in the S288c reference genome, whereas ‘sequences retrieved’ lists represents the total number of sequences found in 24 genomes (supplementary Table S1 online). Gene families are classified as subtelomeric or cell-surface if at least two sequences in the family meet the characteristic.

As repeat regions can cause artifacts in alignments, we repeated the analyses to specifically search for recombination events in the N-terminal domains of the FLO genes. These domains contain several sites important for the recognition and binding of specific carbohydrates [34,35], which is important for pathogenicity in Candida strains [38,39], and for FLO characteristics in brewing strains [28,40]. The altered carbohydrate-binding properties due to intergenic recombination could therefore have significant phenotypic consequences. These analyses revealed extensive recombination between the N-terminal domains (P-value < 10⁻¹⁶), especially in the FLO1-like N-terminal domains. The recombination occurred across the central repeat domain. These recombination events lead to variation in the length and sequence of the repeats, which in turn can also result in new combinations of functional FLO domains.

Chimaeric adhesins also occur in pathogens

Previous studies in yeasts such as Candida albicans and Candida glabrata have speculated that the rapid phenotypic variation produced by chimaeric adhesins could contribute to pathogenicity in these species by avoiding the host immune response [39,41]. To investigate this, we performed a similar in silico analysis on adhesins of pathogenic yeasts by collecting sequences both for the EPA (epithelial adhesins) genes of C. glabrata (11 sequences) and the ALS (agglutinin-like sequences; 14 sequences) genes of C. albicans. Both cases revealed significant evidence for intergenic recombination (P-value < 10⁻¹⁶), suggesting that recombination between adhesins is a common occurrence across yeast strains.

Engineered chimaeric adhesins confer distinct phenotypes

To assess the functional implications of recombination events between paralogs, we mimicked recombination by constructing several chimaeric FLO genes, each combining the 5’ end of one domain (supplementary Fig S3 online). Detailed analysis of these genes revealed that many recombination events in the N-terminal domain occurred in-between regions important for substrate binding [35]. Such events could subtly alter the strength and preference of substrate binding in the N-terminal domain and therefore influence FLO. In the second group, recombination occurred across the central repeat domain. These recombination events lead to variation in the length and sequence of the repeats, which in turn can also result in new combinations of functional FLO domains.
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**Fig 2** Phylogenetic analyses of FLO genes reveal extensive intergenic recombination. A–C show the principle of reticulate analysis. Shown in blue are sequences used as representative haplotypes in supplementary Fig S2 online. (A) Phylogenetic tree of nucleotide sequences coding for N-terminal domains of all complete FLO1-like sequences. (B) Phylogenetic tree of nucleotide sequences coding for C-terminal domains of the same subset of FLO1-like sequences. (C) Trees of (A) and (B) are combined in an unrooted reticulate network (i.e., the overlay of trees shown in A and B). Such a phylogenetic reticulate allows to represent recombination events between either current or ancestral sequences and provides a more precise visualization of the evolutionary history of the FLO genes. Sequence (27) appears in different places in (A) and (B), thus placing it at an extension of the corner of a closed square in the reticulate tree. Such closed squares are generated by a predicted recombination event. (D) Reticulate displaying recombination events in the N-terminal domain of all FLO1-like sequences. Note that all these analyses were performed using all available FLO sequences but for the sake of clarity, only a subset of sequences was used to generate these figures. FLO, flocculation.

FLO gene with the 3’ end of another (supplementary Table S3 online). We used three genes (FLO1, FLO10 and FLO11) from S288c that represent FLO gene classes with distinct FLO characteristics as parents for the chimaeric genes. The protein products of the engineered chimaera retain the traditional three-domain structure of fungal adhesins while differing in N-terminal domains, total length, number and positions of tandem repeats and glycosylation sites.

To assess the functionality of these engineered chimaera, we expressed each construct separately in the non-flocculent and non-adherent S288c strain. Expression levels (determined by quantitative PCR) were comparable for each of the constructs, with an average expression level around 75% of the ACT1 transcription level, which is comparable to the natural FLO gene expression in flocculent strains derived from the feral yeast EM93 [33].

We then determined flocculation strength, adhesion to diverse surfaces and cell wall hydrophobicity conferred by each of the chimaeric Flo proteins. Our results demonstrate that different domains contribute to different phenotypes and that the chimaeric adhesins confer phenotypes that differ from their parental adhesins (Fig 3; Table 2; supplementary Table S4 online for details). This demonstrates that recombination events between paralogs can generate new alleles that can display new combinations of phenotypes and/or variation in the degree of adhesion.

**CONCLUSION**

Our results reveal an elegant mechanism by which gene duplicates might be used as a molecular toolbox for the generation of a large array of different new alleles and phenotypes. These findings also demonstrate that paralogs do not necessarily evolve completely independently through mutation. Instead, ectopic recombination events also contribute to the generation of sequence divergence in paralogs, which in turn propels evolutionary innovation [1–8].

Intergenic recombination events are observed predominantly in subtelomeric gene families, which are known to harbour
lifestyle-specific genes, or in cell-surface gene families, which are involved in interactions with the cells environment. Hence, this mechanism could provide yeasts with an ever-changing reservoir of genes to quickly adapt and tune the way they interact with specific conditions and opportunities. Our results also confirm speculation that C. albicans and C. glabrata might have chimaeric adhesins [39,42], suggesting that this allows pathogens to adapt to the host. This mechanism is also similar to those of some pathogenic protozoans, such as Plasmodium and Trypanosoma spp. where modular cell coat proteins continuously recombine to form new variants and avoid host immune system recognition [16,17,43]. Hence, recombination of cell-surface and subtelomeric genes seems to be a common theme in (eukaryotic) microorganisms, yielding a substantial source of phenotypic variability with only a few genes. Moreover, as recombinations are clearly not limited to yeasts but have also been observed in the MHC class genes of vertebrates, it seems likely that similar mechanisms also exist in higher eukaryotes, including humans [20].

Our results show that in yeast, two distinct categories of gene families show enrichment for chimaeric alleles: families containing genes located near subtelomeres, and gene families encoding cell-surface proteins. Importantly, this enrichment remains significant for both categories, even if all families belonging to the other category are removed from the analysis, indicating that the observed enrichment is independent. One main unresolved question is whether intergenic recombination occurs more frequently between subtelomeric and cell-surface genes, or whether all paralog families show similar rates of recombination but show differences in the adaptive advantage of such recombination events. It is, for example, possible that ectopic recombination between non-subtelomeric and non-cell-surface genes often leads to dysfunctional chimaera. Although it seems likely that both mechanisms might contribute to the enrichment of chimaeric alleles of cell-surface and subtelomeric genes, further research is needed to investigate the underlying mechanism(s).

METHODS
Paralog gene families were defined on the basis of relative pairwise blast scores between all known S288c proteins using the MCL algorithm (supplementary Dataset S1 online) [21]. For each family, the S288c sequences it comprises were blasted against a database containing 24 high-quality Saccharomyces genomes (supplementary Table S1 online). Resulting sequences were
Table 2 | Overview of adhesin phenotypes

| FLO<sub>x</sub> | FLO<sub>y</sub> | Length | Floc. | Hydrop. | Agar A. | Polys A. |
|---------------|-------------|--------|-------|---------|---------|----------|
| WT            | —           | 4,614  | 9 ± 0 | 5 ± 2   | +       | 1.0 ± 0.2|
| FLO1          | —           | 4,614  | 98 ± 1| 72 ± 6  | +       | 3.0 ± 0.3|
| FLO10         | —           | 3,510  | 55 ± 2| 50 ± 10 | + + +   | 2.1 ± 0.3|
| FLO11         | —           | 4,404  | 12 ± 5| 80 ± 8  | + ++    | 1.5 ± 0.1|
| FLO1<sub>1–807</sub> | FLO10<sub>847–3510</sub> | 3,471  | 89 ± 6| 61 ± 29 | + +     | 4.0 ± 0.3|
| FLO1<sub>1–807</sub> | FLO10<sub>847–3510</sub> | 3,441  | 59 ± 7| 63 ± 9  | + +     | 4.3 ± 0.3|
| FLO1<sub>1–819</sub> | FLO10<sub>877–3510</sub> | 3,453  | 59 ± 6| 80 ± 11 | + +     | 3.9 ± 0.2|
| FLO1<sub>1–831</sub> | FLO10<sub>987–3510</sub> | 3,465  | 78 ± 3| 87 ± 2  | + +     | 4.0 ± 0.3|
| FLO1<sub>1–807</sub> | FLO1<sub>1–574–4104</sub> | 4,338  | 84 ± 4| 69 ± 2  | + + + + | 4.5 ± 0.2|
| FLO1<sub>1–831</sub> | FLO1<sub>1–574–4104</sub> | 4,362  | 87 ± 7| 80 ± 3  | + + + + | 4.0 ± 0.4|
| FLO10<sub>1–864</sub> | FLO1<sub>1–799–4614</sub> | 4,680  | 75 ± 5| 64 ± 2  | +       | 3.7 ± 0.2|
| FLO10<sub>1–864</sub> | FLO1<sub>1–799–4614</sub> | 4,395  | 50 ± 4| 65 ± 13 | + + + + | 1.2 ± 0.1|
| FLO1<sub>1–579</sub> | FLO1<sub>1–799–4614</sub> | 4,395  | 14 ± 6| 34 ± 6  | +       | 0.8 ± 0.1|
| FLO11<sub>1–633</sub> | FLO1<sub>1–799–4614</sub> | 4,449  | 18 ± 1| 72 ± 8  | +       | 0.7 ± 0.1|
| FLO11<sub>1–455</sub> | FLO1<sub>1–877–3510</sub> | 3,069  | 17 ± 2| 54 ± 13 | + +     | 0.7 ± 0.1|
| FLO11<sub>1–633</sub> | FLO1<sub>1–877–3510</sub> | 3,297  | 12 ± 1| 83 ± 6  | + +     | 0.9 ± 0.2|

FLO, flocculation; WT, wild type. FLO<sub>x</sub> and FLO<sub>y</sub> represent the origin of the N-terminal and C-terminal part of the adhesin gene, respectively (with the numbers denoting the nucleotide positions in the open reading frame). Length is the length of the final adhesin. Floc. is the flocculation (%) of the strains expressing the adhesin. Hydrop. is the hydrophobicity level of a strain expressing the respective adhesin (%). Agar A. represents after the observed cell-surface adhesion as estimated by a plate-washing assay (+, weak adhesive growth; +++, strong adhesive growth). Polys A. represents the adherence of the strains to polystyrene relative to WT.

Ectopic recombination generates chimaeric adhesins was measured by an adaptation of the method of Rosenberg [49].

Supplementary information is available at EMBO reports online (http://www.embreports.org).

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

REFERENCES
1. Conant GC, Wolfe KH (2008) Turning a hobby into a job: how duplicated genes find new functions. Nat Rev Genet 9: 938–950
2. Ohno S (1970) Evolution by Gene Duplication (Springer-Verlag, Berlin).
3. Hahn MW (2009) Distinguishing among evolutionary models for the maintenance of gene duplicates. J Hered 100: 605–617
4. Lynch M, Conery JS (2000) The evolutionary fate and consequences of duplicate genes. Science 290: 1151–1155
5. Kondrashov FA, Rogozin IB, Wolf YI, Koonin EV (2002) Selection in the evolution of gene duplications. Genome Biol 3: RESEARCH0008.
6. Hughes AL (2004) Birth-and-death evolution of protein-coding regions and concerted evolution of non-coding regions in the multi-component genomes of nanoviruses. Mol Phylogenet Evol 30: 287–294
7. Talirico S, Whitefield SF, Fero J, Haas R, Salama NR (2012) Regulation of Helicobacter pylori adherence by gene conversion. Mol Microbiol 84: 1050–1061
8. Voorderckers K, Brown CA, Vanneste K, van der Zande E, Voet A, Maere S, Verstrepen KJ (2012) Reconstruction of ancestral maltese enzymes reveals molecular mechanisms underlying evolutionary innovation through gene duplication. (in the press)
9. Brown CA, Murray AW, Verstrepen KJ (2010) Rapid expansion and functional divergence of subtelomeric gene families in yeasts. Curr Biol 20: 895–903
10. Fairhead C, Dujon B (2006) Structure of Kluyveromyces lactis genomes of nanoviruses. FEMS Yeast Res 6: 428–441
11. Linardopoulou E, Mefford HC, Nguyen O, Friedman C, van den Engh G, Farwell DG, Coltrea M, Trask BJ (2001) Transcriptional activity of multiple copies of a subtelomERICally located olfactory receptor gene that is polymorphic in number and location. Hum Mol Genet 10: 2373–2383
12. Louis EJ, Naumova ES, Lee A, Naumov G, Haber JE (1994) The chromosome end in yeast: its mosaical nature and influence on recombinalional dynamics. Genetics 136: 789–802
13. Verstrepen KJ, Fink GR (2009) Genetic and epigenetic mechanisms underlying cell-surface variability in protozoa and fungi. Annu Rev Genet 43: 1–24
14. Barry JD, Ginger ML, Burton P, McCulloch R (2003) Why are parasite contingency genes often associated with telomeres? Int J Parasitol 33: 29–45
15. Berriman M et al (2005) The genome of the African trypanosome Trypanosoma brucei. Science 309: 416–422
16. Taylor JE, Rudenko G (2006) Switching trypanosome coats: what’s in the wardrobe? Trends Genet 22: 614–620
17. Freitas-Junior LH, Bottius E, Piritt LA, Deitsch KW, Scheidig C, Guinet F, Nebrass U, Wellemes TE, Scherf A (2000) Frequent ectopic recombination of virulence factor genes in telomeric chromosome clusters of P. falciparum. Nature 407: 1018–1022
18. Smith JD, Chitnis CE, Craig AG, Roberts DJ, Hudson-Taylor DE, Peterson DS, Pinches R, Newbold CI, Miller LH (1995) Switches in expression of Plasmodium falciparum var genes correlate with changes in antigenic and cytoadherent phenotypes of infected erythrocytes. Cell 82: 101–110
19. Cramer A, Raillard SA, Bermudez E, Stammer WP (1998) DNA shuffling of a family of genes from diverse species accelerates directed evolution. Nature 391: 288–291
20. Jeffreys AJ, Kauppi L, Neumann R (2001) Intensely punctate meiotic recombination in the class II region of the major histocompatibility complex. Nat Genet 29: 217–222
21. Enright AJ, Van Dongen S, Ozouzouis CA (2002) An efficient algorithm for large-scale detection of protein families. Nucleic Acids Res 30: 1575–1584
22. Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Mol Biol Evol 23: 254–267
23. Bruen TC, Philippe H, Bryant D (2006) A simple and robust statistical test for detecting the presence of recombination. Genetics 172: 2665–2681
24. Verstrepen KJ, Klis FM (2006) Flocculation, adhesion and biofilm formation in yeasts. Mol Microbiol 60: 5–15
25. Dranginis AM, Rauceo JM, Coronado JE, Lipke PN (2007) A biochemical guide to yeast adhesins: glycoproteins for social and antisocial occasions. Microbiol Mol Biol Rev 71: 282–294
26. Hoyer LL (2001) The ALS gene family of Candida albicans. Trends Microbiol 9: 176–180
27. Verstrepen KJ, Reynolds TB, Fink GR (2004) Origins of variation in the fungal cell surface. Nat Rev Microbiol 2: 533–540
28. Govender P, Domingo JL, Bester MC, Pretorius IS, Bauer FF (2008) Controlled expression of the dominant flocculation genes FLO1, FLO5, and FLO11 in Saccharomyces cerevisiae. Appl Environ Microbiol 74: 6041–6052
29. Reynolds TB, Fink GR (2001) Bakers’ yeast, a model for fungal biofilm formation. Science 291: 878–881
30. Stövicek V, Vachova L, Kuthan M, Palkova Z (2010) General factors important for the formation of structured biofilm-like yeast colonies. Fungal Genet Biol 47: 1012–1022
31. Van Mulders SE, Christianen E, Saerens SM, Daenen L, Verstrepen KJ, Delvaux FR (2009) Phenotypic diversity of a Flo protein family-mediated adhesion in Saccharomyces cerevisiae. FEMS Yeast Res 9: 178–190
32. Stratford M (1992) Yeast flocculation: a new perspective. Adv Micro Physiol 33: 2–71
33. Smukalla S et al (2008) FLO1 is a variable green beard gene that drives biofilm-like cooperation in budding yeast. Cell 135: 726–737
34. Zupancic ML, Frieman M, Smith D, Alvarez RA, Cummings RD, Cormack BP (2008) Glycan microarray analysis of Candida glabrata adhesin ligand specificity. Mol Microbiol 68: 547–559
35. Veelders M, Bruckner S, Ott D, Unverzagt C, Mosch HU, Essen LO (2010) Structural basis of flocculin-mediated social behavior in yeast. Proc Natl Acad Sci USA 107: 22511–22516
36. Verstrepen KJ, Jansen A, Lewitter F, Fink GR (2005) Intragenic tandem repeats generate functional variability. Nat Genet 37: 986–990
37. Fidalgo M, Barrales RR, Jimenez J (2008) Coding repeat instability in the FLO11 gene of Saccharomyces yeasts. Yeast 25: 879–889
38. Cormack BP, Ghorri N, Fallow S (1999) An adhesin of the yeast pathogen Candida glabrata mediating adhesion to human epithelial cells. Science 285: 578–582
39. Butler G et al (2009) Evolution of pathogenicity and sexual reproduction in eight Candida genomes. Nature 459: 657–662
40. Kobayashi O, Hayashi N, Kuroki R, Sone H (1998) Region of FLO1 proteins responsible for sugar recognition. J Bacterial 180: 6503–6510
41. Zhao X, Oh SH, Jaji K, Diekema DJ, Pfaffer MA, Pujol C, Soll DR, Hoyer LL (2007) Analysis of ALS5 and ALS6 allelic variability in a geographically diverse collection of Candida albicans isolates. Fungal Genet Biol 44: 1298–1309
42. De Las Penas A, Pan SJ, Castano I, Alder J, Cregg R, Cormack BP (2003) Virulence-related surface glycoproteins in the yeast pathogen Candida albicans are encoded in subtelomeric clusters and subject to RAP1- and SIR-dependent transcriptional silencing. Genes Dev 17: 2245–2258
43. Roth C, Brignaud F, Lyden RE, Baltz T, Eisen H (1989) Active late-appearing variable surface antigen genes in Trypanosoma equiperdum are constructed entirely from pseudogenes. Proc Natl Acad Sci USA 86: 9375–9379
44. Thompson JD, Higgins DG, Gibson TJ (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
45. Saitou N, Nei M (1987) The neighbor-joining method: a new method for constructing phylogenetic trees. Mol Biol Evol 4: 406–425
46. Bryant D, Moulton V (2004) Neighbor-net: an agglomerative method for the construction of phylogenetic networks. Mol Biol Evol 21: 255–265
47. D’Hautcourt O, Smart K (1999) Measurement of brewing yeast flocculation. J Am Soc Brew Chem 57: 123–128
48. Roberts RL, Fink GR (1994) Elements of a single MAP kinase cascade in Saccharomyces cerevisiae mediate two developmental programs in the same cell type: mating and invasive growth. Genes Dev 8: 2974–2985
49. Rosenberg M (1984) Bacterial adherence to hydrocarbons: a useful technique for studying cell surface hydrophobicity. FEMS Microbiol Lett 22: 289–295

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