QTL Dissection of Lag Phase in Wine Fermentation Reveals a New Translocation Responsible for Saccharomyces cerevisiae Adaptation to Sulfite

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Abstract

Quantitative genetics and QTL mapping are efficient strategies for deciphering the genetic polymorphisms that explain the phenotypic differences of individuals within the same species. Since a decade, this approach has been applied to eukaryotic microbes such as Saccharomyces cerevisiae in order to find natural genetic variations conferring adaptation of individuals to their environment. In this work, a QTL responsible for lag phase duration in the alcoholic fermentation of grape juice was dissected by reciprocal hemizygosity analysis. After invalidating the effect of some candidate genes, a chromosomal translocation affecting the lag phase was brought to light using de novo assembly of parental genomes. This newly described translocation (XV-t-XVI) involves the promoter region of ADH1 and the gene SSU1 and confers an increased expression of the sulfite pump during the first hours of alcoholic fermentation. This translocation constitutes another adaptation route of wine yeast to sulfites in addition to the translocation VIII-t-XVI previously described. A population survey of both translocation forms in a panel of domesticated yeast strains suggests that the translocation XV-t-XVI has been empirically selected by human activity.

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Introduction

Adaptation by natural selection occurs through the emergence of mutations that improve the fitness of an organism and its reproductive success in its environment. Identifying the genetic bases of adaptation is a great challenge in microbiology that may be carried out by different approaches including experimental evolution [1–3] and linkage analysis [3–8]. Diverse mutation types may impact trait variability and adaptation to an environment. In many cases, point mutations affecting transporters, transcription factors, and enzymatic activities produce variation in quantitative traits such as fitness, metabolite production, and gene expression level, as shown by many examples in yeast [3,9–11] and bacteria [12]. Small insertions and deletions (INDEL) that generate frame shifts may affect also the integrity of proteins, causing drastic trait changes [3,11,13,14]. Larger genome reorganizations may also drive the emergence of more adapted individuals. Yeasts, especially those belonging to the Saccharomyces genus, have been widely investigated for their genome plasticity and several examples of chromosomal rearrangements conferring a phenotypic advantage to individuals have been found [15]. Segmental duplications have been selected under laboratory [16,17] or environmental conditions [18,19]. Moreover, horizontal transfer of genetic material from eukaryotic [20,21] or prokaryotic [22] origins has been brought to light. Recently, the physiological impact of some of them has been demonstrated for wine yeast [23]. Extra-copies of genes located in subtelomeric regions [24] as well as aneuploidies [25] are other examples of the role of yeast genomic plasticity in environmental adaptation. Another possible mechanism driving adaptation and evolution in yeast is chromosomal translocation [26]. This chromosomal rearrangement is initiated by a DNA double-strand break (DSB) that may generate both reciprocal and non-reciprocal translocations [27,28] and that may be induced by physiological situations or by exogenous DNA damaging agents [26,29,30].

In yeast, translocation events are thought to play a role in adaptation and species evolution. For example, in the Saccharomyces clade reciprocal translocations may contribute with other mechanisms to hybrid sterility [31,32]. Translocations may occur between small homologous sequences such as Ty elements, tRNAs, or microsatellites [2,33,34] and contribute to the karyotypic polymorphism found in yeast [31,35,36]. Such chromosomal rearrangements may have physiological consequences, affecting gene expression [19,37–39], fitness [2,19] and cell morphology [40]. Translocation events also play a role in environmental adaptation as described in wine yeast. Indeed a
translocation between chromosome VIII and XVI increases sulfite resistance due to the creation of a new genetic environment regulating differentially the sulfate membrane pump, Ssu1p [37,39,41,42]. The large number of chromosomal rearrangements found among yeast strains and their striking physiological consequences suggest that other unidentified translocation events may impact on the adaptation of wild yeast to their ecological niche. With the advent of next generation sequencing (NGS) chromosomal rearrangements can be more easily detected using specific approaches [43] or de novo assembling [26]. However, linking these events with a specific trait change remains a difficult task. In this work we describe the molecular dissection of a QTL controlling lag phase, the time necessary to start the alcoholic fermentation of grape must. In a previous study, this QTL was localized on the left arm of chromosome XV and was used to drive breeding strategies using molecular markers [44]. By analyzing the genomic sequence at this locus for both parental strains we bring to light a new reciprocal translocation between chromosome XV and chromosome XVI that confers an adaptive advantage to yeast strains in an ecologocal context.

Materials and methods

Genomic sequence analysis of the QTL region

Spore dissection and haploid strain mating

Sporulation was induced on ACK medium (1% potassium acetate, 2% agar) after three days at 24°C. After incubation (1 h, 30°C) in a 2 mg/ml solution of cytochelase (Sigma, Lisle d’Abeau Chesnes, France); spores were dissected by a micromanipulator Singer MSM Manual (Singer Instrument, Watchet, Somerset, UK) on YPD-agar. Hybrids were obtained by mixing haploid cells on YPD-agar for 6–18 h at 30°C; mating types were determined by using mating testers of both sex type.

Genomic sequence analysis of the QTL region

Whole genome data sequences of strains SB and GN were obtained by using an Illumina pair end strategy. Briefly, genomic DNA was extracted from a saturated culture of 100 ml under anaerobic condition (YPD) using the genomic tip-100 kit (Qiagen,Courtaboeuf, FRANCE). Paired-end Illumina sequencing libraries were prepared from sonicated genomic DNA according to manufacturer protocols (Genomic DNA Sample Preparation) and were carried out by the Genomic and Transcriptomic facility (Euroscarf, Franckfurt, Germany) or RG13 (kindly given by Professor Richard Gardner, Auckland, New Zeeland). The primers p25 and p26 allowed the amplification of a disruption cassette containing ~500 bp of the flankling region of the HO locus. The deletions of HAL9, ATG34 and SSU7 were carried out following a similar strategy, using as template the genomic DNA of the strains Y01865, Y01774, Y02160, respectively. Primer couples p494/p495, p500/501, and p988/989 were used to amplify the deletion cassette. All the constructions were verified by both insertion and deletion PCR test. The insertion test consists in positively amplifying by PCR a fragment containing the 5’ part of the KanMx4 cassette and ~600 bp of the 5’-flanking region of the target gene. The deletion test consists in observing the absence of amplification of a central portion of the target gene. All the primers used for these tests are listed in Table S2. The parental strains SB and GN were transformed using the lithium acetate protocol described by Gietz and Schiestl [49]. Hemizygous hybrids were then constructed by classical genetics using tetrad microdissection and haploid mating techniques.

Gene deletion and hemizygous hybrids construction

The haploid derivative ho-SB and ho-GN were obtained by deletion of the HO locus using the Kan-Mx4 and Nat-Mx cassettes, respectively [48]. Briefly, a deletion cassette was obtained by PCR using as template the genomic DNA of the strains Y03925 (Euroscarf, Franckfurt, Germany) or RG13 (kindly given by Professor Richard Gardner, Auckland, New Zeeland). The primers p25 and p26 allowed the amplification of a disruption cassette containing ~500 bp of the flankling region of the HO locus. The deletions of HAL9, ATG34 and SSU7 were carried out following a similar strategy, using as template the genomic DNA of the strains Y01865, Y01774, Y02160, respectively. Primer couples p494/p495, p500/501, and p988/989 were used to amplify the deletion cassette. All the constructions were verified by both insertion and deletion PCR test. The insertion test consists in positively amplifying by PCR a fragment containing the 5’ part of the KanMx4 cassette and ~600 bp of the 5’-flanking region of the target gene. The deletion test consists in observing the absence of amplification of a central portion of the target gene. All the primers used for these tests are listed in Table S2. The parental strains SB and GN were transformed using the lithium acetate protocol described by Gietz and Schiestl [49]. Hemizygous hybrids were then constructed by classical genetics using tetrad microdissection and haploid mating techniques.

PCR screening of translocations

To rapidly detect translocations involving the gene SSU7, PCRs were developed by designing appropriate primers (MWG Biotech, Germany). The translocations VIII-t-XVI and its reciprocal (XVI-t-VIII) were detected using the primers p764–p765 and p762–p763 respectively. The newly described translocation XV-t-XVI was detected using the primers P758–p765, while its reciprocal from (XVI-t-XV) was detected using the primers p760–761. The wild type copies of chromosomes VIII, XV and XVI were detected using the primers p786–p787, p758–p761, and p788–p789, respectively. Finally, an additional couple of primers was specifically designed for detecting wt-XVI chromosome (p788–p1032) and XV-t-XVI (p1031–p1032) in a ΔSSU7 background. All primers amplified small genomic regions (<1000 bp) around the chromosomal break point. All the primer sequences are given on Table S2.
transcripts were amplified by using the following primers time PCR using iCycler iQ (Bio-Rad, Hercules, CA). ORF sample was analyzed two times independently by quantitative real- using RNA as a template in real-time PCR assays. Each cDNA of contaminant genomic DNA in RNA preparations was verified was quantified using the ND-1000 UV-visible light spectropho-

tethered to Bio-Rad using iScriptTM cDNA Synthesis Kit (Bio-Rad, Hercules, CA). The extracted RNA was quantified using the ND-1000 UV-visible light spectrophotometer (NanoDrop Technologies, Wilmington, DE). The absence of contaminant genomic DNA in RNA preparations was verified using RNA as a template in real-time PCR assays. Each cDNA sample was analyzed two times independently by quantitative real-
time PCR using iCycler iQ (Bio-Rad, Hercules, CA). ORF transcripts were amplified by using the following primers SSU1: p766/p767 (151 bp), ALG9: p605/p606 (163 bp), ACT1: p323/p904 (123 bp). The ALG9 genes were used as a second reference gene as proposed previously [50]. Real-time quantitative qPCRs (qPCRs) were carried out using the iQ SYBR Green Super Mix (Bio-Rad). Primers were added at a concentration of 0.3 mM each. The PCR program was as follows: 3 min at 95°C for initial denaturation, then 40 cycles of 10 s at 95°C, 30 s at TM and 30 s at 72°C. A final melt curve was carried out for control specific amplification by 36 cycles of 10 s starting at 65°C, with increasing steps of 0.5°C at each cycle. The PCR efficiencies were 90.2%, 89.7%, 97.7% for SSU1, ACT1 and ALG9, respectively. A standard curve was determined for each gene, where x is the threshold cycle and y is the log value of the starting quantity (ng): SSU1 (γ = -3.90x+21.44, adjusted R² = 0.995); ACT1 (γ = -3.62x+22.44, adjusted R² = 0.983); ALG9 (γ = -3.65+23.81, adjusted R² = 0.994). Standard curves were obtained from eight points in triplicate and linearity was observed from 0.0366 ng to 183 ng of DNA. A threshold value for the fluorescence of all samples was set manually, to maintain the same value in each experiment. The relative amounts of SSU1 with respect to each reference gene were calculated from the standard curve. As similar results were obtained from both reference gene only SSU1/ACT1 are graphically presented.

Measure of lag phase time during alcoholic fermentation

Fermentations were carried out using the model synthetic medium (KP-medium) as previously described [51]. Pre-cultures were run for 24 h at 24°C under orbital agitation (150 rpm) in the fermentation media filtered through a 0.45 μm nitrate-cellulose membrane (Millipore, Molsheim, France) and diluted 1:1 with milli-Q water. The inoculum concentration was 10⁴ viable cells per ml. Fermentations were run in closed 150 mL glass-reactors, locked to maintain anaerobiosis, with permanent stirring (300 rpm) at 24°C. The SO₂ amount was adjusted in the fermentation media but not in the preculture at a concentration ranging from 0 to 40 mg/L as specified in the text. The CO₂ released was monitored by measurement of glass-reactor weight loss regularly and expressed in g L⁻¹. The raw fermentation kinetics data were smoothed by a Loess function allowing the estimation of the lag phase time (h) that was the time between

Table 1. Yeast strains used.

| Strain      | Genetic background | Genotype                  | Origin                  |
|-------------|--------------------|---------------------------|-------------------------|
| Y02160      | S288c              | BY4741; Mat a; his3Δ1; leu2Δ; met15 Δ; ura3Δ; YPL092::kanMX4 | Euroscarf               |
| Y01774      | S288c              | BY4741; Mat a; his3Δ1; leu2Δ; met15 Δ; ura3Δ; YOL083::kanMX4 | Euroscarf               |
| Y01865      | S288c              | BY4741; Mat a; his3 Δ 1; leu2 Δ; met15Δ; ura3Δ; YOL089::kanMX4 | Euroscarf               |
| Y03925      | S288c              | BY4741; Mat a; his3 Δ 1; leu2 Δ; met15Δ; ura3Δ; HO::kanMX4 | Euroscarf               |
| GN          | monosporic clone of VL1 | HO/ HO; chr: VIII; chr: XV-XVI; | Marullo et al. 2007b    |
| SB          | monosporic clone of BO213 | HO/ HO; chr:VIII; chr XV; chr: XVI | Marullo et al. 2007b    |
| hoGN        | GN                 | haploid derivate of GN; ho::NATMX4, mat a | Albertin et al. 2013    |
| GΔ083       | GN                 | HO/ HO; YOL089ΔΔ::kanMX4/YOL083ΔΔ::kanMX4 | this study               |
| GΔ089       | GN                 | HO/ HO; YOL092ΔΔ::kanMX4/YOL089ΔΔ::kanMX4 | this study               |
| GΔ092       | GN                 | HO/ HO; YPL092ΔΔ::kanMX4/YPL092ΔΔ::kanMX4 | this study               |
| hoSB        | SB                 | haploid derivate of SB; ho::kanMX4, mat a | Albertin et al. 2013    |
| RG13        | SB                 | haploid derivate of SB; ho::NATMX4, mat a | Richard Gardner (Auckland university) |
| SΔ083       | SB                 | HO/ HO; YOL083ΔΔ::kanMX4/YOL083ΔΔ::kanMX4 | this study               |
| SΔ089       | SB                 | HO/ HO; YOL089ΔΔ::kanMX4/YOL089ΔΔ::kanMX4 | this study               |
| SΔ092       | SB                 | HO/ HO; YPL092ΔΔ::kanMX4/YPL092ΔΔ::kanMX4 | this study               |
| BN          | F1 hybrid          | SB x GN hybrid            | Marullo et al. 2007b    |
| G5S-083     | BN                 | hemizygote hybrid YOL083ΔΔ::kanMX4/YOL083ΔΔ::kanMX4 | this study               |
| SΔG083      | BN                 | hemizygote hybrid YOL083ΔΔ::kanMX4/YOL083ΔΔ::kanMX4 | this study               |
| GΔ089       | BN                 | hemizygote hybrid YOL089ΔΔ::kanMX4/YOL089ΔΔ::kanMX4 | this study               |
| SΔG092      | BN                 | hemizygote hybrid YOL092ΔΔ::kanMX4/YPL092ΔΔ::kanMX4 | this study               |
| SΔG092      | BN                 | hemizygote hybrid YOL092ΔΔ::kanMX4/YPL092ΔΔ::kanMX4 | this study               |
| F10-4A      | monosporic clone of F10 | YOL083ΔΔ | Marullo et al. 2009    |

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inoculation and the release of 3 g/L of CO₂. All the fermentations were done in triplicate.

Cell concentration and viability monitoring

During the lag phase period, bioreactors were sampled and yeast populations were analyzed using a flow cytometer (Quanta SC MPL, Beckman Coulter, Fullerton, California), equipped with a 488 nm laser (22 mW) and a 670 nm long-pass filter. Samples were diluted in McIlvaine buffer pH = 4.0 (0.1 M citric acid, 0.2 M sodium phosphate dibasic) and propidium iodide (0.3% v/v) was added in order to stain dead cells (FL3 channel). Viability and cell concentration were expressed in %, and cells per ml respectively. In order to measure the physiological change of yeast during the lag phase the LP viability was calculated as follow: \( \frac{V_{\text{min}}-V_{p}}{V_{p}} \). Were \( V_{p} \) and \( V_{\text{min}} \) are the viability of the preculture and minimal viability value observed during the lag phase. Indeed, negative values of LP viability indicate that yeast cells underwent a drop of viability during the lag phase.

Analytical methods

The total SO₂ and free SO₂ (mg/L) were measured by Pararosaniline titration [52] by the analytical laboratory SARCO (Bordeaux, France).

Statistical and graphical analyses

All the statistical and graphical analyses were carried out using the R program. The variation of each trait analyzed was estimated by an analysis of variance (ANOVA) according to the model described in the result section. For each variable, the homogeneity of the variance was assessed using a Levene test by means of R’s car package version 2.15.1 [53], as well as the normality of residual distribution using a Shapiro test [53]. Duncan’s multiple comparison was used to determine which means differ significantly (Duncan’s multiple comparison, p<0.05) using the agricolae package. When required, pairwise comparisons were carried out using the Wilcoxon test with at least 5 independent repetitions.

Results

QTL dissection assisted by whole genome sequencing reveals a translocation event between the chromosomes XV and XVI

In a previous work [44] we identified by QTL mapping a major locus controlling the time necessary to initiate the alcoholic fermentation in winemaking conditions (lag phase). This QTL named QTL-XV was narrowed between the gene HAL9 (YOL089c) and ATG19 (YOL082w) in a region of nearly 20 kb containing 8 genes. The aim of this work is to dissect this QTL and find the genetic polymorphisms explaining the phenotypic difference between the parental strains SB and GN. By sequencing the whole genome of parental strains using an Illumina pair end strategy, between the parental strains, 33 Single Nucleotide Polymorphisms were detected in this region generation fifteen non synonymous mutations affecting five genes (Table S3). Putative deleterious mutations were found for two of them (HAL9 and ATG34) and we tested their impact on lag phase by reciprocal hemizygosity assay (RHA) according to Steinmetz et al. [6] [Figure S1 and S2]. This first tentative failed to identify the cause of lag phase difference suggesting the role of other genetic modifications such as gross chromosomal rearrangements. To identify such modifications, the genomes of parental strains SB and GN were de novo assembled generating between 360 to 400 contigs per strains (Table S4). We used as query sequence, the 20 kb region of QTL-XV of the strain S288c and we carried out a blastn analysis against the parental set of de novo contigs. For the strain SB the entire sequence match with one contig showing the same synteny than S288c strain. In contrast for the parental strain GN, we found evidence for a reciprocal translocation between the chromosome XV and the chromosome XVI covered by the contigs c545 and c23. The translocation event was localized between the coding sequences of ADH1 and PHM7 (chromosome XV) and between the coding sequence of SSU1 and NOG1 (chromosome XVI). The exact chromosomal break point occurs at the position 161342 and 373561 for the chromosome XV and XVI, respectively, as confirmed by PCR sequencing. This break point occurs in a low complexity AT rich region without evident homology between chromosomes. As shown in Figure 1, this chromosomal rearrangement generates one XV-t-XVI chromosome, remodeling the promoter environment of the SSU1 gene. This gene encodes for a plasma membrane protein playing a major role in sulfite detoxification. The expression regulation of SSU1 has been widely investigated in the past and depends on the transcription factor Fzf1p, a protein subjected to positive selection within Saccharomyces species [54] and among wine S. cerevisiae strains [42]. In the translocation XV-t-XVI described in this work, the start codon of SSU1 is then located at 501 bp of an Fzf1p binding site (CTATCA) and at 607 bp of an Adr1p binding site (GGGGG) known to activate the transcription of alcohol dehydrogenase during fermentation conditions [55]. The presence of these promoter regions suggest that the expression of this gene might be enhanced. The role of the SSU1 gene in lag phase was further investigated by analyzing the time course of cell growth and CO₂ production during the first hours of alcoholic fermentation by RHA. As shown in Figure 2, the SSU1GN allele allowed initiating the fermentation 28.3 h sooner than its SSU1SB counterpart (Wilcoxon test, p<0.01). Interestingly, the reduction of the SSU1 copy number in the BN hybrid has an effect, and both hemizygous hybrids showed a longer lag phase than control. This means that both SSU1 alleles (GN and SB) play an active role in starting alcoholic fermentation, but that the SSU1GN allele is more efficient than SSU1SB. Physiologically, the lag phase difference observed was in part explained by yeast viability, which is significantly higher when the GN allele was present (74% vs 59%). However the relation between lag phase and viability is not simple, the parent SB initiated the fermentation much more quickly than the GN. However, demonstrating that the SSU1GN allele is the main cause of lag phase discrepancy between strains GN and SB and validated the QTL dissection at a gene level.

Impact of two chromosomal translocations involving SSU1 on lag phase

The Ssu1p sequence of SB and GN were compared with those of 30 Saccharomyces cerevisiae yeasts. Between GN and SB, five single amino-acid polymorphisms were found (F16S, V19M, A122S, I151V, R291K). The substitutions F16S and I151V were found only for the SB strains while other SAPs have allelic frequencies ranging from 0.1 to 0.6 among the 30 sequences investigated. However, no deleterious effect of these SAPs were found using the SIFT algorithm (\( \sigma = 0.05 \)) [56] by aligning these SAP with 39 eukaryote sulfite pump sequences (data not shown). In addition, the comparison of S092 with SB and GN with G092 strains demonstrated that in both parents Ssu1p is functional and able to reduce lag phase in the presence of SO₂. This evidence suggests that the reorganization of the SSU1 promoter region due to the translocation XV-t-XVI, rather the function of the Ssu1 protein, might be the cause of the impact of the SSU1GN allele.
Interestingly, a translocation event implying the SSU1 gene with the promoter region of EMC34 has been described for wine yeast [41]. This translocation concerns chromosomes VIII and XVI [VIII-t-XVI] [41], allowing the formation of the so-called SSU1-R allele [57] and increasing sulfite tolerance by enhancing SSU1 expression level [37,39,58]. In order to compare the effect of the new XV-t-XVI translocation described here with the VIII-t-XVI and the wild type forms [nt-XVI], lag phase duration was investigated in a synthetic must containing different amounts of total SO2 (0, 20 and 40 mg/L) using the strains GN (XV-t-XVI), F10 (VIII-t-XVI) and SB (nt-XVI). 40 mg/L SO2 correspond to the standard concentration found in wine making conditions at the beginning of alcoholic fermentation [59]. The lag phase was monitored by weight loss each 2 hours and culture samples were regularly taken at different times since the alcoholic fermentation started. Cell growth and viability were monitored by flow cytometry and a transcriptional analysis of SSU1 was carried out during the lag phase period. The Table 2 summarizes the analysis of variance carried out; the Figure 3 shows the lag phase, lag viability, and SSU1 expression level for all the strain-media conditions. As expected and previously reported in winemaking conditions, lag phase time is impacted by SO2 concentration in the medium [60,61]. However strains harboring translocations XV-t-XVI and VIII-t-XVI are much less affected than the strain SB and initiated the fermentation after 18 hours even in presence of 40 mg of total SO2. At this concentration, the strain SB showed a much longer lag phase (46 hours) (panel A). The behavior of SB can be explained by cellular viability during the lag phase. As shown on panel B the lag viability of SB is drastically affected when SO2 concentration increases, while other strains harboring a translocation form are not significantly affected. In presence of 40 mg/L of SO2 the viability of strain SB decreases up to 80% of its initial value, explaining the long lag phase observed for this strain in this condition. These results suggest a toxic effect of SO2 on the strain SB, explaining the lag phase discrepancy observed in the BN progeny. Regarding the expression levels of SSU1, no induction by SO2 can be detected (Table 2) as previously reported for other wine yeast [37]. In contrast, as shown on panel C, a clear translocation effect was detected by variance analysis whatever the SO2 concentration in the medium (ANOVA, p<0.001). The strain GN (XV-t-XVI) showed a 3 fold increased expression of SSU1 compared to other strains during the lag phase while the strains SB (nt-XVI) and F10 (VIII-t-XVI) cannot be statistically discriminated from each other (Duncan test, α = 0.01). This result suggested that SSU1 expression level during the lag phase is the cause of trait discrepancy between SB and GN. Despite no global strain effect being detected between F10 and SB, a significant difference (3.7 fold) was found between F10 (VIII-t-XVI) and SB (nt-XVI) when the SO2 concentrations was increased up to 40 mg/L (Wilcoxon, p<0.05) indicating that in high SO2 concentration the strain F10 may export more efficiently the SO2 than the strain SB, subsequently reducing the lag phase.

**Figure 1. Description of the XV-t-XVI translocation.** The diagram represents the breakpoint of translocation XV-t-XVI for the strain GN. The genomic region of contig c343 includes the S' portion of gene ADH1 and the coding sequence of SSU1 (red arrows) and their respective regulating regions Adr1p and Fz11 binding regions (yellow diamond). The small arrows represent the primers p758 and p761 allowing identifying by PCR the XV-t-XVI translocation.

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**Landcape of XV-t-XVI and VIII-t-XVI translocations among industrial yeast strains**

In order to identify rapidly the various forms of chromosome XVI described in this work, a set of PCR based tests were set up as described in methods. The occurrence of the newly described form of translocation XV-t-XVI within a panel of 48 yeast strains from different origin was then investigated (Table S1). To test if this chromosomal rearrangement is a rare event or a natural selection outcome, a panel of 24 commercial starters was compared with 12 natural grape isolates, 3 distillery strains, 5 brewery strains, 3 bakery strains and one oak isolate. The distribution of the three forms of chromosome XVI is shown in Figure 4. Yeast strains isolated form other industries (bakery, brewery and distillery) as well as the natural isolate OS104 did not possess translocated form of chromosome XVI. By contrast, almost wine yeasts (32/36) showed at least one translocation involving the SSU1 gene (XVI-t-XVI or VIII-t-XVI). In a few cases, at least three copies of chromosomes XVI were present in the yeast genome, confirming the aneuploidy nature of many wine yeasts [35]. Strikingly, only the industrial starters (16/24) have at least one copy of XV-t-XVI in their genome, suggesting that this specific translocation has been selected by humans within the best performing individuals. The already described VIII-t-XVI form was found in 26 wine strains including 18 starters and constitutes the major allelic form of chromosome XVI in the wine group. The fact that many commercial strains, selected for their fermentation performances, carry the XV-t-XVI form suggests that this chromosomal rearrangement must be considered as the consequence of anthropic selection to improve the fermentation capacities of yeast. To test the impact of the different chromosomes of type XVI on SSU1 expression, an additional experiment was carried out at
40% of CO₂ released in a synthetic medium. Of the eight strains used, four have wild-type copies of chromosome XVI (nt-XVI) (302, A24, XMC30 and OS104), two carry the VIII-t-XVI translocation (F10, Fx10), one has the XV-t-XVI translocation (NL3), and the last possesses all the possible forms (F33). As shown in Figure 5, the strains carrying translocations have the highest

Figure 2. The SSU1GN allele impact the lag phase in BN background. On the left panel, the time course of viable cell concentration (open circle) and the CO₂ production (black square) during the 60 first hours after yeast inoculation are shown. Data presented are the mean of three independent repetitions with standard error for parental strains (SB and GN), F1 hybrid (BN) as well as hemizygous hybrids (GO92S, S092G) for the gene SSU1 (YPL092w). The lag phase time computed is indicated by a red arrow. The bar plots on the right panel represent mean values and standard error measured for 3 independent repetitions for the lag phase (h) and the LP viability. These traits were measured for parental strains (SB and GN), F1 hybrid (BN), the hemizygous hybrids (GO92S, S092G) for the gene SSU1 (YPL092w) and the SSU1 double deletion mutant of GN and SB (GO92 and S092). Isogenic strains are represented with the same color, GN = light green, BN = green, SB = blue. The statistic difference between isogenic strains was tested using a Wilcoxon test. The significant level is coded **, for p-values < 0.01.

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expression values for $SSU1$; however, from a statistical point of view, only the strains VL3 and F10 expressed significantly more $SSU1$ than the nt-XVI group. The strain F33, despite the presence of the two types of translocation forms, has a $SSU1$ expression level identical to the wild type form. Altogether, these results suggest that other genetic determinisms may have an epistatic control on the $SSU1$ expression during the alcoholic fermentation in some genetic backgrounds.

Discussion

QTL dissection: searching for SNPs, finding a translocation

Over the ten past years, *Saccharomyces cerevisiae* has become an excellent model of quantitative genetics in order to unravel the complex determinism of natural trait variation [4]. Indeed, QTL mapping approaches match perfectly with the intrinsic characteristics of budding yeast: huge genetic and phenotypic variation, short life cycle, genome compactness, high recombination rate, and molecular genetics facilities. The advent of this new yeast genetics discipline is due to decisive technological breakthroughs that provide numerous markers in single experiments, such DNA microarrays [3,9,10,62–65] and NGS approaches [5,8,66–68]. In these numerous studies, markers were obtained by physically mapping genetic polymorphisms detected within strains onto a reference strain genome (S288c). In many cases this approach was justified by the fact that one of the parental strains was the laboratory strain itself. This avoids the reconstitution of the true genetic map, assuming that the parental strains genomes are collinear. However, QTL programs can be carried out with two [9,69] or more natural strains [66,68] that might have a different chromosomal architecture than the reference genome. In our study we first mapped the SNP found between the parental strains GN and SB, onto the chromosomal organization of S288c and we invalidated our candidate genes by an RHA. In the present case, this strategy was elusive since the genetic modification involved in lag phase was a translocation affecting the expression level of the $SSU1$ gene. The existence of a reciprocal translocation at this locus was previously suggested by analyzing the germination rate of backcrossed lines [44]. However, on the basis of the microarray data that were collected, the linkage between chromosome XV and chromosome XVI was undetectable (data not shown). Using de novo assembly of pair-end reads, we identified in the parental strain GN a new translocation between chromosomes XV and XVI that explains most of phenotypic variation in the BN hybrid progeny. To our knowledge, this is the first example of QTL

**Figure 3.** Effect of translocations XV-t-XVI and VIII-t-XVI on lag phase, LP viability and $SSU1$ expression. The lag phase, LP viability, and expression level of $SSU1$ gene are shown in panel A, B and C, respectively. Data were obtained from alcoholic fermentation using the strains F10, GN and SB carrying the chromosome forms VIII-t-XVI, XV-t-XVI and nt-XVI, respectively. For each strain, fermentations were carried out in synthetic media containing increasing concentrations of total SO$_2$ (0, 20 and 40 mg), graphically represented by orange scale 0 mg = light orange, 20 mg = orange, 40 mg = dark orange. For panel A and B, bar plots represent the mean with standard error of three independent repetitions. For panel C the expression level of $SSU1$ was normalized by $ACT1$. Data represent the mean and standard error of 6 to 9 samples collected during the lag phase.

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| Translocation | part of variance | p value | Translocation | part of variance | p value | Translocation | part of variance | p value |
|---------------|----------------|---------|---------------|----------------|---------|---------------|----------------|---------|
| Lag phase (h)$^a$ | 36.4 | $<0.001$ | 36.2 | $<0.001$ | 28.4 | $<0.001$ |
| SO$_2$ | 22.2 | $<0.001$ | 8.0 | $<0.05$ | 0.5 | ns |
| Translocation:SO$_2$ | 34.9 | $<0.001$ | 26.4 | $<0.01$ | 2.3 | ns |
| qPCR block | nd | nd | 8.0 | $<0.05$ |
| Residual | 6.4 | 29.4 | 60.8 |

$^a$the model used was $Z1 = m +$translocation$_{ijk}$*SO$_2$$_{ijk}$*e, which allows the estimation of the part of the variance explained by the chromosome XVI forms (n = 3) and the SO$_2$ concentration (n = 3). For each condition, 3 repetitions were analyzed. nd = not determined.

The model used was $Z2 = m +$translocation$_{ijk}$*SO$_2$$_{ijk}$*qPCR$_{ijk}$*e, which allows the estimation of the part of the variance explained by the chromosome XVI forms (n = 3), the SO$_2$ concentration (n = 3), and the qPCR blocks. For this model 6 to 9 repetitions were analyzed for each conditions depending on the lag phase time.

ns = not significant.

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dissection that demonstrates the impact of a gross chromosomal rearrangement on phenotypic trait variation. This work also highlights the importance of using de novo assembly rather than mapping assembly for dissecting QTLs with natural yeast. This point is crucial when the parental strains are derived from industrial backgrounds, which are known to carry many chromosomal rearrangements and aneuploidies [15].

Convergent evolutionary routes affect the SSU1 expression level

Sulfite resistance has been widely documented in yeast and in particular for the winemaking context as recently reviewed by Divol et al [70]. After diffusing in the cell by passive transport [71] under its molecular form (SO2⋅H2O), sulfite interacts with yeast metabolism mainly under its bisulfite ion form (HSO3−). Sulfites inhibit key glycolytic enzymes like Tdh and Adh proteins, bind carbonyl [72] compounds such pyruvate and acetaldehyde, affect transporter activity by binding membrane proteins [70], and down-regulate the expression of many central metabolism genes [73]. These multiple targets explain why sulfite resistance is a quantitative trait [74] involving different detoxification routes such as sulfite export [42], acetaldehyde accumulation [75] and the sulfur reduction pathway [76]. The most efficient detoxification mechanism is sulfite efflux by the Ssu1p pump. The molecular mechanisms modifying the activity and expression of this transporter have been widely investigated: punctual mutations [77], differential gene expression [42,57], as well as possible post-transcriptional regulations of SSU1 [37], are the main molecular mechanisms identified.

Here we have described a new allelic variation widely spread among wine yeasts that increases the SSU1 expression level by modifying drastically the upstream region of SSU1. The relative distance of the consensus binding sites of Adr1 (~607 pb) and Fz1p (~501 bp) from the SSU1 start position closely matches with the optimal position of these regulatory sites [78]. To date, all the natural allelic variations explaining the adaptation of wine yeast to sulfite modulate directly or indirectly the expression level of SSU1 gene. Interestingly, other wine microbes such as the bacterium Oenococcus oeni seem to have a gain in fitness when carrying a sulfite pump (tauE) similar to SSU1 [79]. This convergence indicates that sulfite pumping over is the most efficient way for a microorganism to adapt to sulfite by natural selection. These mutations can be classified into two groups. First, a trans-acting group, that affects both the activity [42,54], and expression level [54] of Fz1p and by consequence could impact the expression level of SSU1. Interestingly, comparative genomics within the Saccharomyces clade demonstrated that this gene is under strong positive selection [54]. Second, a cis-acting group characterized by two independent translocation events VIII-t-XVI [41] and XV-t-XVI (this study) is highly prevalent in wine yeast population.

Adaptative role of translocations in yeast

The role of translocations in yeast adaptation have been widely described for the Saccharomyces clade but also for other yeast genera [80] and pathogenic fungi [81]. Within Saccharomyces species, translocations contribute with other mechanisms to reduce the fertility at a post zygotic level [31,32]. At the intraspecific level, translocations are thought to be a rapid way to gain an adaptive mutation by creating a new chromosomal environment [15]. Moreover translocations are more stable than collinear segmental duplications and may confer a more stable gain of fitness [82]. In a laboratory environment, many examples demonstrate the adaptive benefit of translocation under nutrient limitation [2,83] or inhibitory conditions [84]. However, reports of translocation
events occurring in a natural population and conferring a selective advantage to environmental conditions are rare. For a long time, the translocation event VIII-t-XVI reported by [41] was the unique example of a translocation in natural yeast population. Recently another example of translocation between the chromosomes VII and VIII associated with segmental duplications has been reported for a Saccharomyces cerevisiae population adapted to high copper concentrations. The new example of translocation described here confirms that chromosomal translocations are an important adaptive route in harsh environmental conditions. Moreover we demonstrated here that both translocations VIII-t-XVI and XV-t-XVI conferred a selective advantage by shortening by many hours the growth lag phase in a medium containing SO2. These conditions match better to the natural conditions than the classical toxin resistance test carried out by plating yeast on YPD. A competition assay was performed with strains carrying XV-t-XVI or nt-XVI inoculated at the same rate. The strains carrying XV-t-XVI translocation clearly outcompeted their non-translocated counterparts, as more than 95% of colonies carried the XV-t-XVI chromosome form at the end of the fermentation, demonstrating the translocation advantage in only five generations (data not shown). The translocation benefit was also confirmed by their prevalence in wine yeast (both grape isolate and industrial starters). Interestingly, the commercial starter group is the unique one carrying the translocation XV-t-XVI. Further experiments could test the relative advantage between both translocation forms in isogenic conditions.

**Concluding remarks**

In this study, we elucidated the molecular basis of a QTL controlling a fermentation trait that explains the adaptation of yeast strains to human activity (i.e., winemaking in presence of sulfite). For the first time the nature of genetic variation elucidated was not here a SNP or a small INDEL but a newly described chromosomal rearrangement creating a “gain of function” allele. Indeed most of the QTL dissected here brought to light deleterious alleles in one of the parental strain affecting the function or the expression of a protein. Finally, we demonstrated that the translocation XV-XVI form is present in many yeast strains used in industry that are likely selected for their ability to rapidly colonize grape must.

**Supporting Information**

Figure S1 The time course of viable cell concentration (open circle) and the CO2 production (black square) during the 60 first hours after yeast inoculation are shown. Data presented are the mean of three independent repetitions with standard error for parental strains (SB and GN), F1 hybrid (BN) as well as hemizygous hybrids (G083S, S083G, G089S and S089G) for the gene ATG34 (YOL083w) and HAL9.
Reciprocal heterozygosity analysis of ATG34 and HAL9 for lag phase. The lag phase (h) as well as the LP viability was measured for parental strains (SB and GN), F1 hybrid (BN) as well as hemizygous hybrids (G083S, S083G, G089S, S089G) for genes ATG34 (YOL083w) and HAL9 (YOL089c). The bar plots represent mean values and standard errors measured for 3 independent repetitions. The statistic difference between isogenic strains was tested using a Wilcoxon test. Isogenic strains are represented with the same color, difference between isogenic strains was tested using a Wilcoxon test. The bar plots represent mean values and standard errors measured for 3 independent repetitions. The significant levels was coded *, ** for p-values<0.05 and <0.01 respectively.

Table S1

Table S2

Table S3

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