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Stability of Large Segmental Duplications in the Yeast Genome

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ABSTRACT

The high level of gene redundancy that characterizes eukaryotic genomes results in part from segmental duplications. Spontaneous duplications of large chromosomal segments have been experimentally demonstrated in yeast. However, the dynamics of inheritance of such structures and their eventual fixation in populations remain largely unsolved. We analyzed the stability of a vast panel of large segmental duplications in *Saccharomyces cerevisiae* (from 41 kb for the smallest to 268 kb for the largest). We monitored the stability of three different types of interchromosomal duplications as well as that of three intrachromosomal direct tandem duplications. In the absence of any selective advantage associated with the presence of the duplication, we show that a duplicated segment internally translocated within a natural chromosome is stably inherited both mitotically and meiotically. By contrast, large duplications carried by a supernumerary chromosome are highly unstable. Duplications translocated into subtelomeric regions are lost at variable rates depending on the location of the insertion sites. Direct tandem duplications are lost by unequal crossing over, both mitotically and meiotically, at a frequency proportional to their sizes. These results show that most of the duplicated structures present an intrinsic level of instability. However, translocation within another chromosome significantly stabilizes a duplicated segment, increasing its chance to get fixed in a population even in the absence of any immediate selective advantage conferred by the duplicated genes.

RECIPROCAL translocations and large duplications are major driving forces in genome evolution. The role played by these chromosomal rearrangements in shaping the genomic architectures of eukaryotic species has been notably illustrated by comparative analyses between related species, including yeasts (Fischer *et al.* 2000; Kellis *et al.* 2003; Dujon *et al.* 2004), plants (Schmidt 2002; Yogeeswaran *et al.* 2005; Yu *et al.* 2005), animals, and humans (Dutrillaux 1979; Stanyon *et al.* 1999; Bailey *et al.* 2002, 2004; Stankiewicz and Lupski 2002; Jaillon *et al.* 2004; Bourque *et al.* 2005). Several experimental studies have shown that the fixation of translocations and duplications in populations of yeast maintained in continuous culture was obtained in response to growth-limited conditions and/or in competition experiments (Hansche *et al.* 1978; Adams *et al.* 1992; Brown *et al.* 1998; Dunham *et al.* 2002; Infante *et al.* 2003; Colson *et al.* 2004). Adaptive evolution of wine yeast strains also results from translocations within their genome (Perez-Ortin *et al.* 2002).

Various mechanisms leading to the duplication of whole or large portions of genomes have been described in fungi. Whole-genome duplication, aneuploidization, extrachromosomal amplification, unequal crossing over, cycle of break-fusion-bridge, nonreciprocal translocation, and retrotransposition events all result in the duplication of large DNA sequences (Bainbridge and Roper 1966; Sexton and Roper 1984; Whittaker *et al.* 1988; Dorsey *et al.* 1993; Wolfe and Shields 1997; Moore *et al.* 2000; Dunham *et al.* 2002; Dietrich *et al.* 2004; Kellis *et al.* 2004; Schacherer *et al.* 2004). Duplications of large DNA segments are also commonly found in laboratory strains as suppressors of deletion mutants (Hughes *et al.* 2000). In a previous work, we developed a gene dosage selection assay to recover spontaneous duplications from the right arm of chromosome XV in a haploid yeast strain (Koszul *et al.* 2004). We showed that a large variety of inter- and intrachromosomal segmental duplications appears spontaneously, at an estimated frequency of 10^{-9}/cell/division (revised from our initial estimate of 10^{-10}) and possibly through a new mechanism related to the replication process. All the intrachromosomal events corresponded to the direct tandem duplication (DTD) of large DNA segments ranging in size from 41 to 288 kb. We also found three different types of interchromosomal duplications where the right arm of chromosome XV was translocated onto other chromosomes, within a

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subtelomere (subtelomerically translocated duplication, STD), or in an internal region (internally translocated duplication, ITD), or onto a supernumerary chromosome corresponding to the fusion between the duplicated parts of two different chromosomes, one of which is chromosome XV (supernumerary chromosome duplication, SCD).

The study of the intrinsic stability of different duplication structures has been tackled so far in different ways. Pioneer works on genetic recombination have revealed that tandem arrays of small repetitive units are subject to amplification and deletion through unequal recombination events (Szostak and Wu 1980; Fogel and Welch 1982; Jackson and Fink 1985). It is also well established that subtelomeres are dynamic chromosomal regions that undergo frequent recombinational exchanges (Louis and Haber 1990a; Pryde *et al.* 1997; Liti *et al.* 2005). Finally, disomic chromosomes are known to be spontaneously lost mitotically at a rather high frequency (Campbell *et al.* 1975). However, by specifically engineering strains carrying large duplicated segments that can be spontaneously lost without any detrimental effect, our work aims at giving a comprehensive picture of the relative stability of different types of duplicated segments. The large panel of duplicated structures recovered from our previous study gives a unique opportunity to precisely measure and compare the mitotic and the meiotic stability of various large inter- and intrachromosomal duplicated segments in a given genetic background. Our results show that the combination between translocations and large segmental duplications results in stabilizing newly duplicated regions within the yeast genome.

MATERIALS AND METHODS

Yeast strains: All strains used for the mitotic assays are haploid derivatives from *Saccharomyces cerevisiae* BY4743 (*MAT***a**/*MAT*α, *his3*Δ*1/his3*Δ*1, leu2*Δ*0/leu2*Δ*0, ura3*Δ*0/ura3*Δ*0, LYS2/lys2*Δ*0, MET15/met15*Δ*0*) (Winzeler *et al.* 1999). The haploid strains carrying large segmental duplications from chromosome XV [encompassing the *RPL20B* (*YOR312c*) gene] used in this work are derived from the original strains YKF1036, -1038, -1057, -1050, and -1246; from YKF1114 described in Koszul *et al.* (2004); and from a newly isolated strain YKF2048 carrying a 230-kb duplicated segment from chromosome XV (also encompassing *RPL20B*) at the extremity of chromosome I (YKF210 in Figure 1B). A wild-type copy of the originally deleted *RPL20A* (*YMR242c*) gene was reintroduced in these strains by crossing and tetrad micromanipulation, resulting in 7 different recombinant haploid strains carrying both *RPL20A* and a duplicated segment encompassing *RPL20B* (YKF166, -210, -211, -179, -181, -164, and -161, see Figure 1 and results). In each of these strains, one of the two copies of *RPL20B* was replaced by transformation with a *URA3* marker. The position of the *URA3* marker was characterized precisely by karyotype hybridization and by restriction mapping, resulting in 14 different strains, each carrying *URA3* located in either of the duplicated blocks (Figure 1, positions 1–14 indicated in the schematics).

For meiotic assays, the haploid strains carrying various duplicated blocks (YKF1050, -1038, and -1057 and YKF1246 from Koszul *et al.* 2004; the backward translocated strain type II* in Figure 2C) and control strains (BY4741 and BY4742) with no duplication were crossed with either Y2159 (*MAT***a**, ade1-1, trp1-b) or Y2160 (*MAT*α, ade1-1, trp1-b), two derivatives of the Y55 strain. The five resulting diploids, heterozygous for a large duplicated segment from chromosome XV, were named YKF155, -145, -129, -127, and -187; sporulated; and 158, 249, 300, 98, and 149 tetrads were dissected, respectively. One hundred tetrads were also dissected for the two control strains. The presence/absence of the duplicated blocks in the meiotic products was sought by PCR amplification of the breakpoint junctions as previously described (Koszul *et al.* 2004) or by pulsed-field gel electrophoresis (PFGE) karyotyping.

Estimation of the rate of 5-fluoroorotic acid resistance: The rate of appearance of 5-fluoroorotic acid (5-FOA)-resistant colonies was determined by a fluctuation test analysis. They were calculated from the average of two independent experiments, each corresponding to 4–12 independent cultures. Cells were diluted from an overnight preculture ($1.5–2.5 \times 10^8$ cells/ml) to ~25–50 cells/ml and grown in YPD to $2.5–3 \times 10^8$ cells/ml. Cells were diluted in sterile water to the appropriate concentration and plated on 5-FOA (DUCHEFA F0176, 1 mg/ml) -containing medium to select for resistant cells (two plates per culture). After 3 days of incubation at 30°, resistant colonies were counted and the median value for each set of cultures of an experiment was estimated. For each strain the spontaneous mutation rate was then calculated using the median method (Lea and Coulson 1948).

PFGE and Southern hybridizations: Plugs of intact chromosomal DNA, PFGE, and transfer onto Hybond-N+ membranes (Amersham Biosciences, Arlington Heights, IL) as well as probe labeling and hybridizations were carried out as described previously (Fischer *et al.* 2001).

RESULTS

Construction of the strains used for mitotic stability assays: Seven large segmental duplications from the right arm of chromosome XV (six of which were previously obtained in Koszul *et al.* 2004 and a newly isolated strain) that all encompass the *RPL20B* (*YOR312c*) gene were studied: an ITD of a 115-kb segment within chromosome III (Figures 1A and 2); two STDs of 230 and 256 kb at the extremities of chromosome I and V, respectively (Figures 1B and 3); a SCD of 268 kb (Figures 1C and 4); and three DTDs internal to chromosome XV of 41, 115, and 213 kb, respectively (Figures 1D and 5). These large segmental duplications resulted from the selection of a duplication of the *RPL20B* gene in strains that were deleted for *RPL20A*. The deletion of *RPL20A* produced a severe growth defect that was compensated by the duplication of *RPL20B* (Koszul *et al.* 2004). The *RPL20A* gene was reintroduced in the genomes of the seven parental strains used in this study. The resulting strains carried one copy of *RPL20A* and two copies of *RPL20B*. One of the two copies of *RPL20B* was subsequently replaced by a *URA3* cassette (see materials and methods) without detrimental effect on the growth rate. This resulted in the construction of 14 strains, each carrying a *rpl20b::URA3* marker in

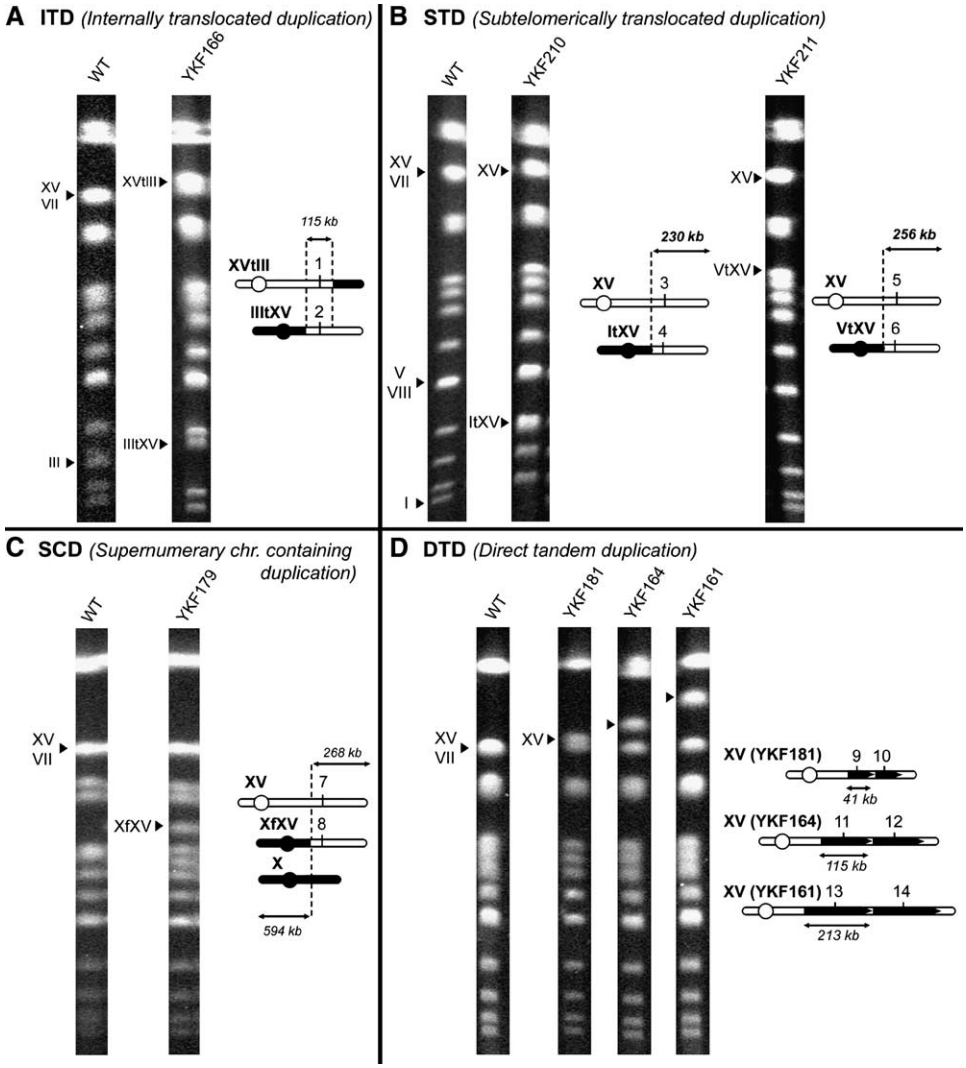

FIGURE 1.—Different types of large segmental duplications. PFGE karyotypes of WT and mutant strains carrying segmental duplications. Chromosomes XV and VII comigrate in the WT strain, resulting in a band with a double intensity on the gel. Relevant chromosome numbers are indicated, and solid arrowheads show the positions of the modified or additional chromosomes in the karyotypes. For each duplication type a schematic of the chromosomes involved in the duplication events is shown, and the positions where the *rpl20B::URA3* marker was inserted are indicated (1–14). The extent of the duplicated sequences is indicated by black double-headed arrows.

one or the other duplicated block (Figure 1, positions 1–14).

The *URA3* gene confers prototrophy to uracil but also sensitivity to 5-FOA. Thus, the loss of the duplicated block containing the *rpl20B::URA3* marker confers resistance to 5-FOA. The mitotic stability of the duplicated segments was therefore measured from the rate of 5-FOA-resistant colonies formation by a fluctuation test (Table 1). 5-FOA-resistant cells appear in the control strain (BY4743) at a frequency of $\sim 10^{-7}$/cell/division as a result of inactivation of the *URA3* gene probably through point mutations. In the duplicated strains, the events responsible for 5-FOA resistance were classified in three categories: loss of the whole duplicated block carrying *URA3* (type I), loss of the *rpl20b::URA3* locus by gene conversion with the *RPL20B* gene as donor located on the other duplicated segment (type II), and inactivation of *URA3* through point mutations (type III). Among these three categories, only type I events correspond to the loss of the duplicated segment.

Stability of an ITD: The *rpl20B::URA3* marker was inserted in the ITD carried either by chromosome XVtIII (Figures 1A and 2B, position 1; Table 1, strain YKF173) or by chromosome IIItXV (position 2, strain YKF256). The rate of 5-FOA^R mutants formation in both strains presents only a threefold increase compared to wild type (WT) (Table 1). Thirty-two and 27 independent 5-FOA-resistant colonies were isolated from the progenies of YKF173 and YKF256 and analyzed by PFGE. The corresponding karyotypes showed a complete stability of the ITD, whose loss was never observed among the 59 strains analyzed (no type I event). For positions 1 and 2, 27 and 23 cells presented an unmodified karyotype compared to the parental strain YKF166, respectively (Figure 2B and Table 1). Among those, hybridization with *URA3* as a probe showed that 20 and 9 strains presented an absence of the *rpl20B::URA3* marker for positions 1 and 2, respectively. These cases correspond to type II events, reflecting a gene conversion event between the *RPL20B* and *rpl20B::URA3* loci carried by the two duplicated blocks. The remaining

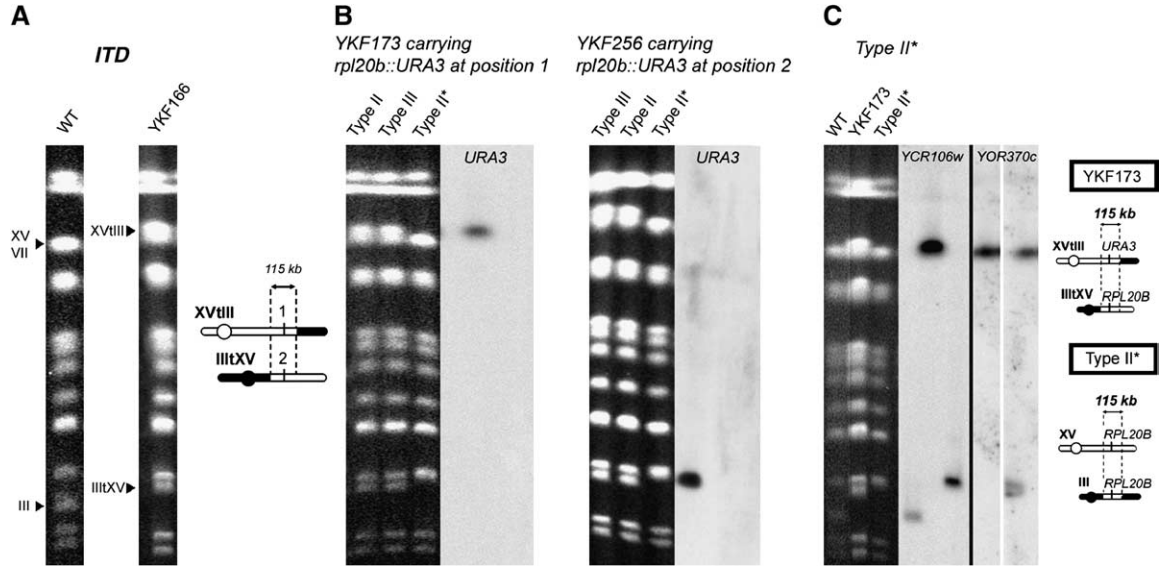

FIGURE 2.—Mitotic stability of an internally translocated duplication (ITD). (A) PFGE karyotypes of WT and YKF166 strains. The solid arrowheads show the positions of the chromosomes of interest mentioned in the text. A schematic of the duplicative translocation in YKF166 including positions 1 and 2 where the *rpl20B::URA3* marker was inserted is also presented. (B) Karyotypes of representative 5-FOA^R mutant strains recovered for each position and hybridization of the corresponding Southern blot with the *URA3* gene as probe. Type II corresponds to the loss of the *URA3* marker by a gene conversion event between *rpl20B::URA3* and *RPL20B* located in the other duplicated block. Type II* corresponds to the same gene conversion event but associated with a mitotic crossing over. Type III corresponds the inactivation of *URA3* by a point mutation. (C) PFGE karyotypes of WT, YKF173, and type II* 5-FOA^R mutant strains and hybridizations of the corresponding Southern blot with subtelomeric probes from chromosomes III (*YCR106w*) and XV (*YOR370c*). The right side shows schematics of both the original ITD in strain YKF173 (XVtIII and IIItXV) and the backward translocation corresponding to type II*.

strains showing unmodified karyotypes (7 strains for position 1 and 14 for position 2) still possess a *URA3* gene (type III, Figure 2B and Table 1) probably inactivated by point mutations, as supported by the sequencing of this gene in five independent 5-FOA-resistant clones (three derived from YKF173 and two from YKF256). These five clones presented point mutations resulting in an amino acid substitution (A184P); or in premature stop codons at positions 56, 83, and 150; or in a frameshift (position 247 due to a single-base deletion, ΔG_{741}). In addition, the proportions of these events (7/32 and 14/27, *e.g.*, frequencies of $\sim 0.7 \times 10^{-7}$ and 1.5×10^{-7} event/cell/division for positions 1 and 2, respectively) are similar to the frequency of point mutation in *URA3* in the WT ($\sim 10^{-7}$/cell/division).

Five and four 5-FOA^R strains, for positions 1 and 2, respectively, presented a karyotype different from the karyotypes of both the parental strain YKF166 and the WT. Hybridization with *URA3* as a probe revealed that these strains have lost the reporter gene (type II* events, Figure 2B and Table 1). In these strains, chromosome XV comigrates with chromosome VII as in the WT strain, and chromosome III now comigrates with chromosome IX. Hybridizations with subtelomeric probes from either chromosome III (*YCR106w*) or XV (*YOR370c*) revealed a backward translocation between the translocated arms of chromosomes III and XV that preserved the duplicated block within chromosome III (Figure 2C). These strains, therefore, carry a block from chromosome XV internally duplicated within chromosome III, resulting in a size increase of 115 kb of this chromosome [consistent with the observed PFGE pattern, chromosome (chr)IX = 440 kb; chrIII + 115 kb = 430 kb]. Chromosome XV is intact in these strains, consistent with its comigration with chromosome VII. A crossing over associated with a gene conversion event between *RPL20B* and *rpl20B::URA3* is likely to be responsible for the formation of these backward translocations.

The meiotic transmission of this ITD was analyzed by crossing the corresponding strain with a strain devoid of both the duplicated region and the translocation. As expected, the fertility of the diploid was affected strongly by the reciprocal translocation, resulting in an overall spore viability of ~47%. This is in good agreement with the theoretical fertility of 50% expected from a cross between two strains heterozygous for a reciprocal translocation. The backward-translocated strains obtained during the mitotic assay (type II*, see schematic in Figure 2C) were also crossed with the strain devoid of the ITD. In the resulting diploid, the only structural difference between the two sets of homologous chromosomes consists of a 115-kb segment from chromosome XV duplicated internally to one chromosome III. In this case, the backward translocation increases the meiotic fertility of the diploid up to 79% (compared to 89% for the control strain with no translocation or

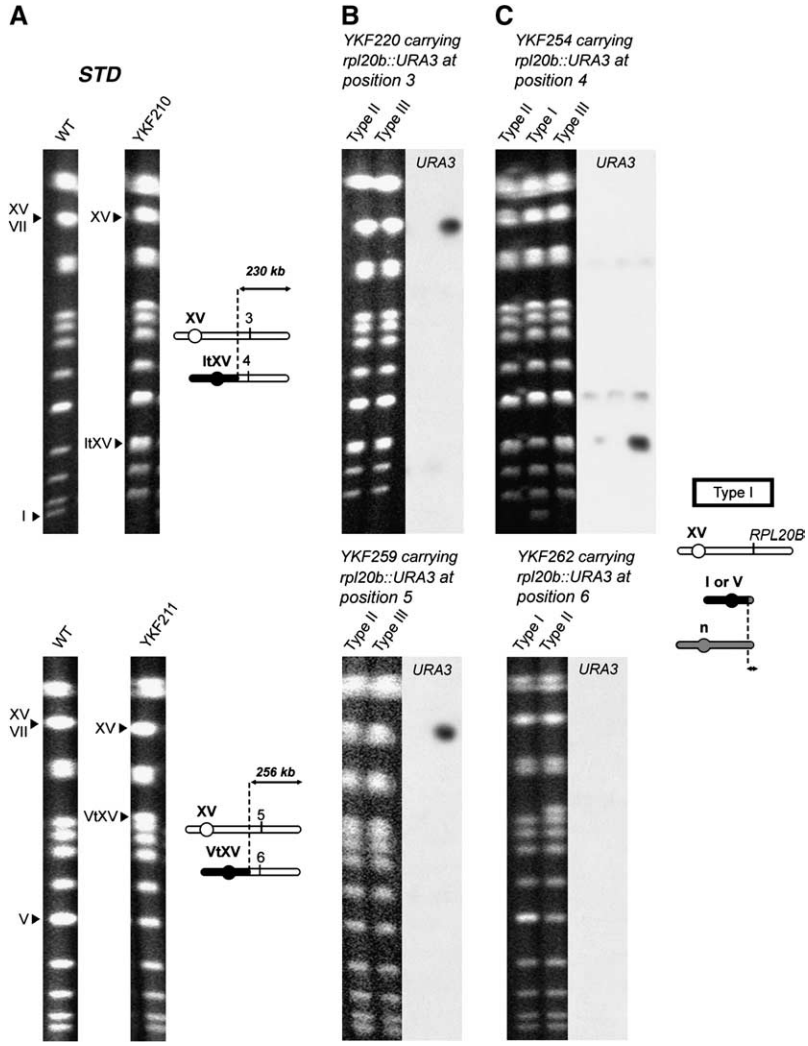

FIGURE 3.—Mitotic stability of a subtelomerically translocated duplication (STD). (A) PFGE karyotypes of WT, YKF210 (top), and YKF211 (bottom) strains. The solid arrowheads show the positions of the chromosomes of interest mentioned in the text. The schematics of the STD event in both YKF210 (top) and YKF211 (bottom), including positions 3, 4, 5, and 6 where the *URA3* gene was inserted, are presented. (B and C) Karyotypes of representative 5-FOA^R mutants recovered for each position and hybridization of the corresponding Southern blot with *URA3* as a probe. Type II and type III correspond to inactivation of *URA3* by gene conversion with *RPL20B* and by point mutation, respectively. Type I corresponds to the loss of the *URA3*-containing duplicated segment. For position 4, faint additional signal onto chromosome V corresponds to hybridization of the probe with the *ura3Δ851* allele, which corresponds only to a partial deletion of the *URA3* gene. The right side shows a schematic of the chromosomal structure of type I mutants recovered in the progeny of strains carrying *URA3* at either position 4 or 6.

duplication). Therefore, backward translocation partially restores the fertility defect conferred by the initial translocation. Moreover, the duplicated block does not appear to be subject to excision during meiosis as all 20 tetrads tested presented a 2:2 segregation of the duplicated block.

In summary, among 59 5-FOA-resistant strains analyzed, none has lost the ITD (loss frequency is therefore $<10^{-8}$ event/cell/division). The duplicated translocated strains carry a duplicated block of 115 kb encompassing 53 genes that is stably inherited during both mitosis and meiosis despite the absence of any selective advantage conferred by the presence of the duplicated region (*i.e.*, the WT and the duplicated strains show identical growth rates in rich medium).

Mitotic stability of two STDs: In strains YKF210 and YKF211, carrying a STD of 230 kb from the right arm of chromosome XV in the right subtelomere of chromosome I (chromosome ItXV, Figure 1B) and a STD of 256 kb from the right arm of chromosome XV in a subtelomere of chromosome V (chromosome VtXV, Figure 1B), respectively, the *rpl20B::URA3* marker has been inserted either onto chromosome XV (strain YKF220, position 3 and strain YKF259, position 5, respectively) or onto the STD (strain YKF254, position 4 and strain YKF262, position 6, respectively, Table 1 and Figure 3).

Both strains carrying the *rpl20B::URA3* marker onto chromosome XV present a slight increase in the 5-FOA^R clone formation compared to that in WT (×6 and ×12 for strains YKF220 and YKF259, respectively; Table 1). The 32 5-FOA^R mutants isolated in the progeny of YKF220 presented karyotypes identical to that of their progenitor. For strain YKF259, 4 of the 32 mutants had lost the STD of 256 kb on chromosome V, illustrating the high intrinsic instability of this structure (see below). The majority of mutants (26/32 and 28/32 for strains YKF220 and YKF259, respectively; Table 1) have lost the *URA3* marker through type II events (Figure 3B), probably as a result of a gene conversion event between *RPL20B* from the STD and *rpl20B::URA3* on chromosome XV or a break-induced replication (BIR) event between the translocated segment and the right arm of chromosome XV. The six and four remaining strains

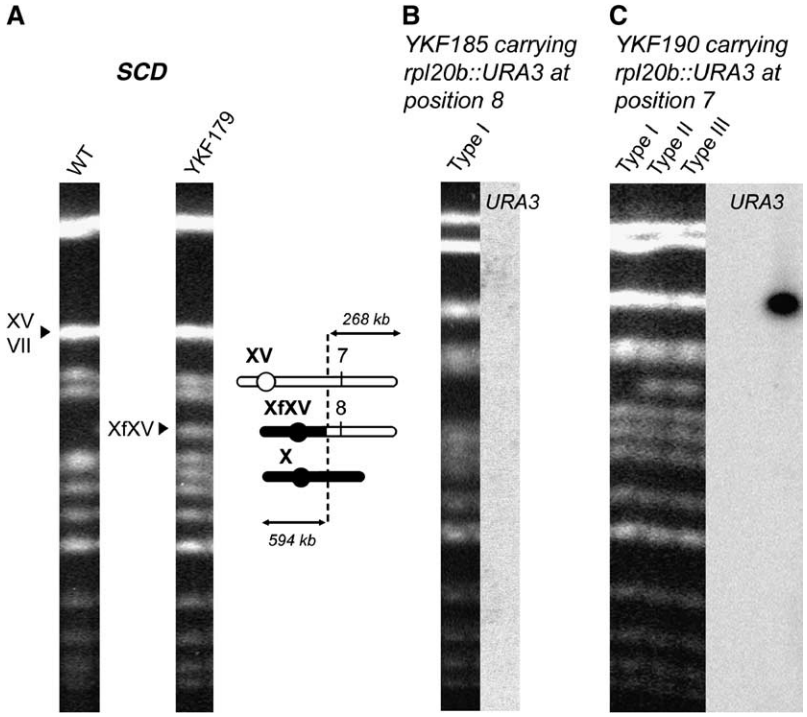

FIGURE 4.—Mitotic stability of a supernumerary chromosome duplication (SCD). (A) PFGE karyotypes of WT and YKF179 strains. The solid arrowheads show the positions of the chromosomes of interest mentioned in the text. Schematic of the structure of the supernumerary chromosome in YKF179, including positions 7 and 8 where the *URA3* gene was inserted, is also presented. (B and C) Karyotypes of representative 5-FOA^R mutants recovered for each position and hybridization of the corresponding Southern blot with *URA3* as a probe. Type I corresponds to the loss of the supernumerary chromosome. Type II and type III correspond to inactivation of *rpl20b::URA3* by gene conversion with *RPL20B* and by point mutation, respectively.

correspond to type III events, as illustrated by the sequence of the *ura3* gene characterized in one of the six 5-FOA^R clones deriving from YKF220 showing a frameshift at position 146 due to a single-base deletion (ΔG_{436}).

The strains YKF254 (position 4) and YKF262 (position 6), carrying *URA3* onto the STD located on chromosomes I and V, respectively, present very different 5-FOA^R clone formation rates. Whereas the rate of 5-FOA^R mutant formation in YKF254 presents only a fourfold increase compared to that in WT, the rate in YKF262 is 2812-fold higher than that in WT (Table 1).

For YKF254, the karyotypes of 30 resistant mutants (among 32 analyzed) were found to be similar to the parental karyotype of strain YKF210 (Figure 3C and Table 1). Hybridization with *URA3* as a probe revealed that 25 of them correspond to type II events while the 5 remaining strains still possess a copy of *URA3* and correspond to type III events [point mutations leading to amino acid substitutions (T119K and V233F) were characterized in the *ura3* sequences from two clones]. The karyotypes of the remaining two resistant strains are consistent with the loss of the STD, with chromosome I migrating in the pulse-field gel at a position corresponding to a size slightly smaller than the WT chromosome I (type I, Figure 3C). As expected from this karyotype, the new chromosome I does not carry the *URA3* marker anymore. The terminal structure of the new chromosome I has not been precisely studied; however, the STD loss is likely to result from an ectopic recombination event between the *PAU7* (*YAR020c*) located centromere proximal to the site of insertion of the original STD and another member of this large subtelomeric gene family. The loss of this 230-kb STD located on chromosome I occurs at a frequency of 2.6×10^{-8} event/cell/division.

Thirty-two independent 5-FOA-resistant colonies were isolated from the progenies of YKF262, the strain carrying the *rpl20B::URA3* marker on the STD of 256 kb on chromosome VtXV, and analyzed through PFGE. All karyotypes but one were consistent with the loss of the STD. This suggests that this block was duplicated in a highly unstable subtelomeric region. The loss of this 256-kb duplication occurs at a frequency of $\sim 2.7 \times 10^{-4}$ event/cell/division. This STD was mapped at the far extremity of one subtelomere of chromosome V (KOSZUL *et al.* 2004). To pinpoint further the site of translocation, the junction of the rearrangement was PCR amplified using a chromosome XV-specific primer (CAACTAATGAACTCTGGATA) and a primer within a Y′ element (TTCGAGCAGAGAAGTTGGAG) and subsequently sequenced. The junction occurred precisely between an internal TG_{1-3} tract distal to the Y′ element (TGTGTGGGTGTGGTGTGT) and an interstitial telomere-related sequence of 18 bp from chromosome XV (TGTGTGTGTATGTGTGTG) located in the intergenic region between YOR273c and YOR274w (coordinates 836471–836488). Therefore, the massive instability of this STD probably results from frequent ectopic recombination events between Y′ elements possibly as a result of the fragility of the TG_{1-3} tract (see DISCUSSION).

Mitotic stability of a SCD: The strain YKF179 carries a SCD (XfXV) corresponding to the fusion between 594 kb duplicated from chromosome X (containing the centromere) and the terminal 268 kb duplicated from

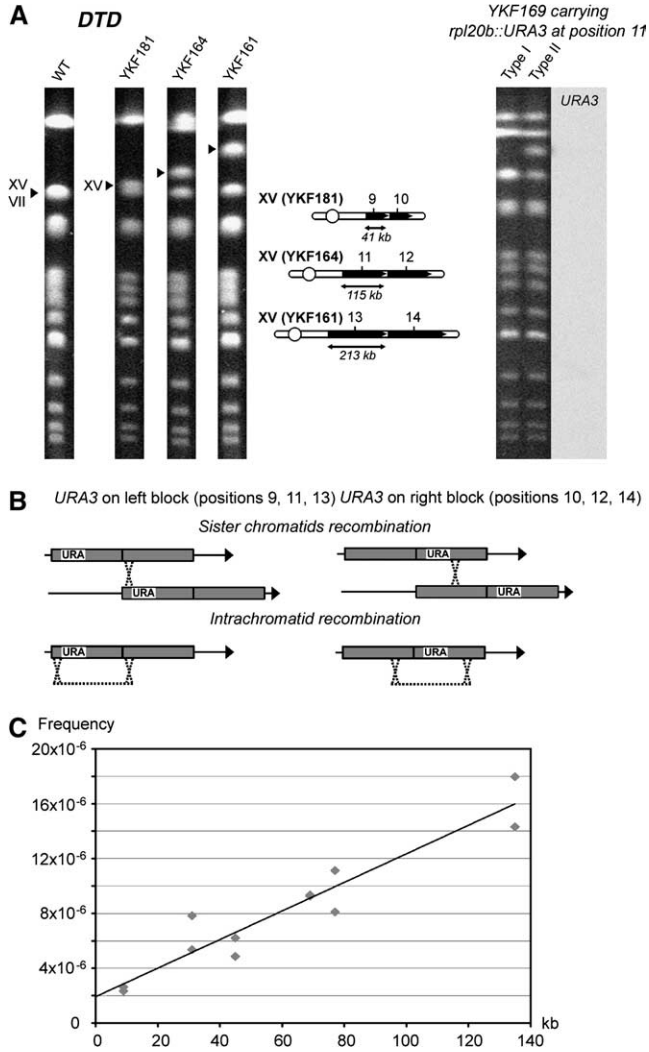

FIGURE 5.—Mitotic stability of direct tandem duplication (DTD). (A) Left, PFGE karyotypes of the strains carrying direct tandem duplications on chromosome XV. The solid arrowheads show the positions of the comigrating chromosomes VII and XV in the WT strain as well as the positions of the 41-, 115-, or 213-kb duplication-containing chromosomes XV in strains YKF181, YKF164, and YKF161, respectively. Middle, schematic of the six positions where *URA3* was inserted (also see Table 1). Right, karyotypes of representative 5-FOA^R mutants recovered in the progeny of YKF169 (*URA3* at position 11), and hybridization of the corresponding Southern blot with the *URA3* gene as probe. Type I corresponds to the loss of the tandemly duplicated block, and type II corresponds to the loss of *URA3* by gene conversion with *RPL20B* present in the other duplicated segment. (B) Schematic of unequal crossing over leading to the loss of the *URA3*-containing block. (C) Frequency of loss of the duplicated block plotted as a function of the size of the sequence available for unequal recombination (indicated in Table 1) ($R^2 = 0.90$). For each size two independent experiments were performed.

the right arm of chromosome XV (Figure 1C). The *rpl20B::URA3* marker has been inserted either onto chromosome XV (strain YKF190, position 7) or onto the supernumerary chromosome XfXV (strain YKF185, position 8, Figure 4). In the latter case, resistance to 5-FOA appears at a rate of 3.4×10^{-5} mutational event/cell/division (corresponding to a 359-fold increase compared to WT, Table 1). Among the 32 5-FOA-resistant mutants karyotyped, all presented a karyotype similar to the WT (type I, Figure 4B) in which the supernumerary chromosome that carried the *rpl20B::URA3* marker was lost. Therefore, supernumerary chromosomes are highly unstable during mitosis.

Strain YKF190 presents a frequency of resistance to 5-FOA of $\sim 1.2 \times 10^{-6}$ ($\times 12$ compared to that in WT). Among 32 mutants analyzed, 29 have conserved the supernumerary chromosome but lost the *URA3* gene on chromosome XV (type II, Figure 4C). Two have kept both the supernumerary chromosome and the *URA3* locus and have probably acquired resistance by point mutations, in agreement with their frequency of appearance comparable to WT (type III, Figure 4C). The last strain analyzed has lost both the chimeric chromosome and the *URA3* gene (type I, Figure 4C). Type I and type II strains probably result from gene conversion between *RPL20B* on chromosome XfXV and *rpl20B::URA3* on chromosome XV or from BIR events between these two chromosomes, initiated in a region centromere proximal to *RPL20B*.

Stability of intrachromosomal DTDs: The most frequent duplication event at the *RPL20B* locus recovered in our former analysis consists of DTD (KOSZUL *et al.* 2004). Twenty-six to 32 independent 5-FOA-resistant colonies were isolated from the progenies of YKF189, YKF169, and YKF170, corresponding to *rpl20B::URA3* at positions 9, 11, and 13, respectively (Table 1, Figures 1 and 5A). Two types of 5-FOA-resistant mutants were found. In type I mutants (the majority of cases, Table 1), the PFGE karyotype is similar to WT, consistent with the loss of the duplicated block (Figure 5A, type I). Hybridization of these karyotypes with *URA3* as a probe confirms the loss of this gene concomitantly with the loss of the duplicated segment. Type II resistant mutants present an unmodified karyotype compared to that of their parental strains but an absence of *rpl20B::URA3* marker, as revealed by hybridization (Figure 5A, type II). Type I strains result from mitotic unequal crossing over (UCO) between the tandemly duplicated segments, by either intra- or interchromatid recombination (Figure 5B). For each parental strain, we plotted the frequency of type I mutants as a function of the size of the sequence flanking the *URA3* gene and into which the UCO events must have occurred to result in a strain that has lost the *URA3*-containing duplicated segment (Table 1 and Figure 5C). A linear relationship is observed ($R^2 = 0.9$). From the regression curve, the mitotic instability (frequency of loss per cell per generation) of tandemly duplicated segments in the yeast genome follows the relationship $I = C + (10^{-7} \times S)$, where S is the size of the duplicated sequence (in kilobases) and C is a constant value of 2×10^{-6}. According to this equation, theoretical

TABLE 1

Rate of appearance of 5-FOA-resistant mutants in cultures

Position[a]	Strain name	Parental strain and type of duplication	5-FOA^R frequency	Fold increase	Type of mutants I	Type of mutants II (II*)	Type of mutants III	Frequency of loss of the *URA3*-containing block	Size of the flanking sequence (kb)[c]
WT	BY4741	—	9.6×10^{-8}	×1					
1	YKF173	YKF166 (ITD)	3.3×10^{-7}	×3	0	25 (5)	7	$<10^{-8}$[b]	
2	YKF256	YKF166 (ITD)	2.9×10^{-7}	×3	0	13 (4)	14	$<10^{-8}$[b]	
3	YKF220	YKF210 (STD)	6.1×10^{-7}	×6	0	26	6	$<10^{-8}$[b]	
4	YKF254	YKF210 (STD)	4.2×10^{-7}	×4	2	25	5	2.6×10^{-8}	
5	YKF259	YKF211 (STD)	1.2×10^{-6}	×12				$<10^{-8}$[b]	
6	YKF262	YKF211 (STD)	2.7×10^{-4}	×2812	32	0	0	2.7×10^{-4}	
7	YKF190	YKF179 (SCD)	1.2×10^{-6}	×13	1	29	2	3.7×10^{-8}	
8	YKF185	YKF179 (SCD)	3.5×10^{-5}	×350	32	0	0	3.5×10^{-5}	
9	YKF189	YKF181 (DTD)	2.6×10^{-6}	×27	31	1	0	2.5×10^{-6}	9
10	YKF183	YKF181 (DTD)	6.8×10^{-6}	×71				6.6×10^{-6}	31
11	YKF169	YKF164 (DTD)	6.5×10^{-6}	×68	27	5	0	5.5×10^{-6}	45
12	YKF168	YKF164 (DTD)	1.1×10^{-5}	×115				9.3×10^{-6}	69
13	YKF170	YKF161 (DTD)	1.0×10^{-6}	×104	25	1	0	9.6×10^{-8}	77
14	YKF192	YKF161 (DTD)	1.7×10^{-5}	×174				1.6×10^{-5}	135

[a] Positions refer to the location of the *URA3* gene (see Figures 1–5).
[b] Loss of the duplication segment was never observed.
[c] Size of the sequence flanking the *URA3* gene and into which the UCO events must have occurred (see Figure 5B).

values for the blocks of 41, 115, and 213 kb are 6.1×10^{-6}, 1.3×10^{-5}, and 2.3×10^{-5}/cell/generation, respectively.

To determine the meiotic stability of large direct tandem duplications, the three strains YKF1050, -1038, and -1057 carrying intrachromosomally duplicated blocks of 41, 115, and 213 kb, respectively, were crossed with a strain devoid of segmental duplications and tetrad analysis was performed on the resulting diploids. As shown in Figure 6A, the global viability of the meiotic products decreases when the size of the block increases. This is illustrated by a significant increase in the number of tetrads with only three viable spores, the number of tetrads with four viable spores decreasing concomitantly. This result suggests that large tandem duplications alter meiotic segregation of chromosomes, which may lead to abnormal and unviable meiotic products. For each diploid strain, tetrads with four viable spores were further analyzed to follow the segregation of the duplicated segment (Table 2). The proportion of 2:2 segregation of the duplicated block decreases as the size of the block increases. On the contrary, the proportion of 3:1 segregation corresponding to the absence of the duplication in three of the four meiotic products increases concomitantly with the size of the block (Table 2). Therefore, tandemly duplicated segments of this region are frequently lost during meiosis. The meiotic DNA double-strand break (DSB) frequencies in the genome of a *S. cerevisiae* strain related to BY4743 have been monitored previously (Gerton *et al.* 2000). In the region covered by the three tandem duplications, four loci presenting high DSB frequencies (meiotic hotspots) have been characterized (Figure 6B). The instability of the block during meiosis is higher if one (or several) of this (these) meiotic hotspot(s) is (are) encompassed in the duplicated sequence. Following the generation of a meiotic DNA DSB, crossing over occurs preferentially between homologs rather than sisters (Schwacha and Kleckner 1997). Recombination between homologs heterozygous for the tandem duplication would end in a 2:2 segregation of the duplicated block. However, we showed that the proportion of 3:1 tetrads increases when the size of the duplication increases. In the case of structural heterozygosity interhomologs DNA exchanges can be rejected and the DSB ends redirected into intersister pathways (Hunter and Kleckner 2001). However, UCO between sister chromatids must lead to a tandem triplication in one of the four spores. We sought for the presence of such structures in the meiotic products of the 3:1 tetrads from YKF129 by PFGE karyotyping and subsequent hybridization with *RPL20B* as a probe. We found only one triplication of 18 tetrads (not shown). Thus the higher level of spore lethality in the progeny of the diploids heterozygous for the large segmental duplications would not be due to the triplications being inviable. In addition, predominance of the 3:1 tetrads with one spore containing a tandem duplication over the 3:1 tetrads with a tandem triplication suggests that intrachromatid recombination leading to the loop out of one copy of the duplication would be more frequent than sister-chromatid UCO that would lead to the creation of a tandem triplication. Our results suggest that, in cases of large tandem duplications, DSB ends would be preferentially redirected into intrachromatid rather than intersister recombination.

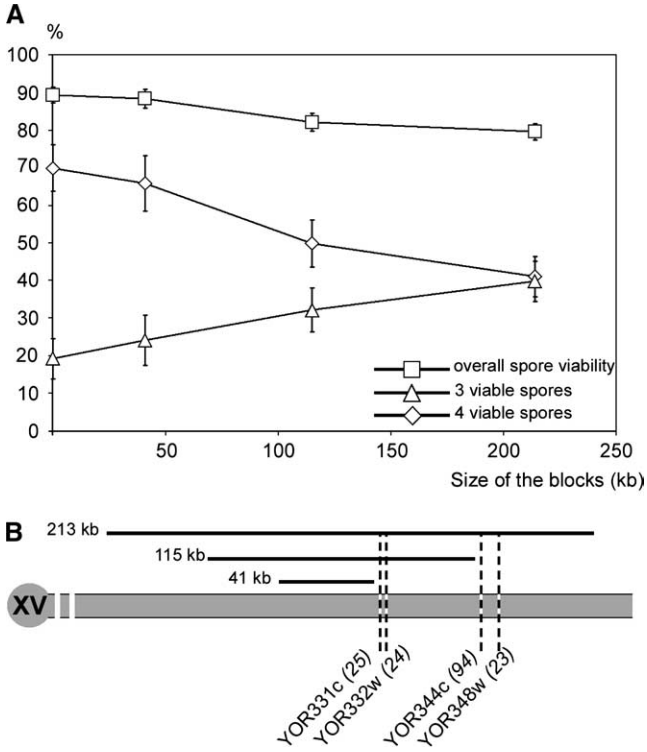

FIGURE 6.—Meiotic instability of DTDs. (A) Influence of the size of the tandemly duplicated block on the viability of the meiotic products after sporulation of a diploid strain heterozygous for the duplication. (B) The three duplicated blocks tested (41, 115, and 213 kb) and the meiotic hotspot ORFs they encompassed are mapped on chromosome XV. The number in parentheses corresponds to the rank of each hotspot in the yeast genome, according to GERTON *et al.* (2000).

DISCUSSION

Segmental duplications present highly variable levels of stability: In this work, we monitored and quantified the spontaneous intrinsic instability of different types of large segmental duplications in the yeast genome. We showed that most of the structures formed by segmental duplications present some levels of instability when no selective advantage is conferred by the presence of the duplication (*i.e.*, the duplication can be lost without detrimental effect on the growth rate of the strain). Our study is in good agreement with former results obtained on the stability of duplicated DNA sequences structurally related to ours. Thus, a SCD, resulting from the fusion between sequences from two different chromosomes, is lost mitotically at a high frequency (3.4×10^{-5}), similar to the frequency of loss of an aneuploid chromosome [a disomic chromosome III is lost at a frequency of 10^{-4} during mitosis (CAMPBELL *et al.* 1975)].

Furthermore, it is known that subtelomeres are highly recombinogenic (PRYDE *et al.* 1997), which could explain the relative instability of the STDs analyzed here. These losses result probably from BIR events involving the repeated sequences interspersed throughout the subtelomeric regions of the chromosomes. However, we found highly variable rates of loss between the two STDs studied. Discrepancies in loss rates might be related to the number and the types of repeated sequences present within subtelomeres and available for recombination. The less unstable STD (loss rate of 2.6×10^{-8} event/cell/division) presents a copy of the *PAU7* gene flanking the site of insertion of the 230 kb from the right arm of chromosome XV in the right subtelomere of chromosome I. This gene belongs to a family of 23 members, with proteins showing 80–85% identity, most of them showing a subtelomeric localization (PRYDE *et al.* 1997). The highly unstable STD of 256 kb from the right arm of chromosome XV (loss rate of 2.7×10^{-4} event/cell/division) has inserted in an interstitial telomere-related sequence directly flanking a Y′ element in the left subtelomere of chromosome V. The Y′ repeated sequence family is composed of long and short elements that reside at 17 of the 32 chromosome ends (LOUIS and HABER 1990b). Despite several insertions/deletions between and within Y′-longs and Y′-shorts, these elements share more extensive sequence identity than the members of the *PAU* family, which could explain in part the higher rate of loss of the STD on chromosome V than on chromosome I. However, the rate of recombinational interactions involving a particular Y′ was previously estimated to 2×10^{-6} (LOUIS and HABER 1990a), 100 times lower than the rate of STD loss observed in this study. Intrachromosomal telomeric sequences have been described as fragile sites in higher eukaryotes (BOUFFLER 1998; MUSIO and MARIANI 1999; RUIZ-HERRERA *et al.* 2005). In addition, replication forks pause as they pass through internal tracts of $TG_{(1–3)}$ (IVESSA *et al.* 2002). The particularly high level of instability of the STD on chromosome V could result from an intrinsic fragility of the $TG_{(1–3)}$ telomere-related sequence flanking the Y′ element at the rearrangement breakpoint: the resulting double-strand breaks would be repaired using an ectopic Y′ and subsequently lead to loss of the STD.

We also showed that large DTDs, from 41 to 213 kb in size, are lost during mitosis at frequencies proportional to their size. Unequal crossovers between sister chromatids are likely to be the cause of these losses, as

TABLE 2

Meiotic stability of the tandemly duplicated blocks in four viable spored asci

		Segregation of the duplicated block[a]			
Strain	Block size (kb)	Total[b]	2+:2−	1+:3−	4−
YKF155	41	23	22	1	0
YKF145	115	24	19	5	0
YKF129	213	35	15	16	4

[a] +, block present; −, block absent.
[b] Total number of tetrads analyzed.

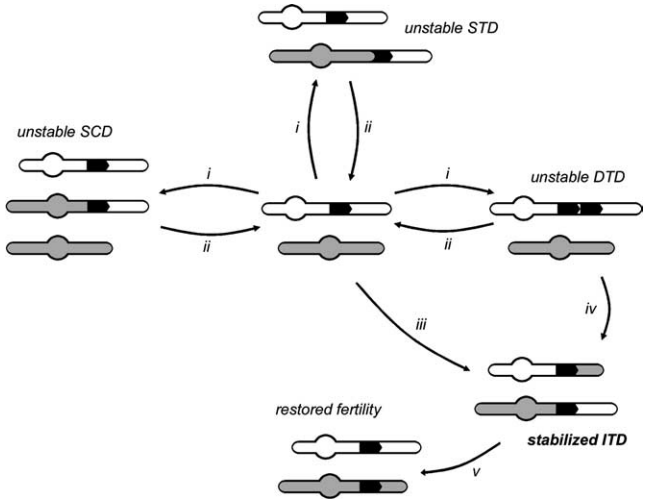

FIGURE 7.—Dynamics of segmental duplications in the yeast genome. Schematic of two arbitrary chromosomes (open and shaded areas) is shown. The orientated black boxes represent the chromosomal segments subject to duplication and loss. (i) Duplication of a chromosomal segment that generates an unstable structure (STD, SCD, and DTD). (ii) Loss of the duplications through UCO or BIR events. (iii and iv) Duplication associated with a reciprocal translocation between nonhomologous chromosomes (ITD). (v) Backward translocation restoring the original collinearity between chromosomes (see text).

previously described for much smaller tandem repeats (SZOSTAK and WU 1980; FOGEL and WELCH 1982; WELCH *et al.* 1990; PAQUIN *et al.* 1992; LAMBERT *et al.* 1999). Moreover, we demonstrated that a duplication internally translocated onto another chromosome (ITD) is permanently stabilized both in asexual and in meiotic cells.

The intrinsic instability of tandem and subtelomeric duplications favors a translocation-based model to reach fixation: Previous results on the spontaneous formation of segmental duplications suggest that, at each generation, a large chromosomal segment can be spontaneously duplicated (KOSZUL *et al.* 2004). This event is rare but its frequency is such that numerous duplications can occur in a population. The sole *RPL20B* gene is duplicated at a frequency of 10^{-9} event/cell/division in a DNA segment whose median size is 115 kb (KOSZUL *et al.* 2004). In most cases, the duplicated block lies in direct tandem next to the original block but interchromosomal duplications have also been characterized (Figure 7, i). Despite the intrinsic instability of most of these structures, if the duplication confers a selective advantage, the duplication may become fixed in the population. For instance, a DTD of 11 kb has been found in the genome of *Kluyveromyces lactis* (DUJON *et al.* 2004). If no selective advantage is associated with the duplication, the present analysis reveals that such duplicated blocks tend to be mitotically lost (Figure 7, ii). The meiotic viability is slightly decreased if one parent carries a tandem duplicated segment (Figure 6A). Except if the selective advantage conferred by the duplicated block exceeds this fitness reduction, the duplication will tend to be lost also during meiosis (Table 2). It was previously shown that loss of a tandemly duplicated block encompassing the yeast *HIS4* hotspot occurs frequently during meiosis (JACKSON and FINK 1985). Here, tandem block excision frequencies seem also to depend on the positions of recombination hotspots in the genome (Figure 6B).

Translocation of the duplicated segment internally onto another chromosome suppresses its intrinsic instability (Figure 7, iii and iv), allowing its fixation in the population even in the absence of any associated selective advantage. A twofold reduction in meiotic fertility is expected for such a strain carrying a reciprocal translocation between two chromosomes. But a mitotic backward translocation both preserving the segmental duplication and restoring collinearity between the chromosomes of the two parental strains has been independently isolated several times in the course of this study (Figure 7, v). This backward translocation can partially restore the fertility defect of the diploid heterozygous for the duplicated region (79% of spore viability *vs.* 89% for the control strain without any duplication). Thus, the chance of fixation of a segmental duplication both in mating and in asexual populations is favored by reciprocal translocation events.

Conciliation of our model with preexisting observations and models from higher eukaryotes: Translocation events often occur by ectopic recombination between the LTR sequences in laboratory and industrial strains (RACHIDI *et al.* 1999; DUNHAM *et al.* 2002) as well as in wild isolates (FISCHER *et al.* 2000). LTRs are also commonly found at the breakpoints of direct tandem duplications (KOSZUL *et al.* 2004). Other sequences such as microhomologous sequences may also be used as recombination breakpoints leading to both segmental duplications and translocations (PEREZ-ORTIN *et al.* 2002; KOSZUL *et al.* 2004). Studies have demonstrated the association of segmental duplications with synteny breakpoints between the human and mouse genomes (ARMENGOL *et al.* 2003; BAILEY *et al.* 2004) and between the human and *Gorilla gorilla* genomes (STANKIEWICZ *et al.* 2001). Our study provides experimental evidence that segmental duplications can be associated directly with translocations in a eukaryotic genome, to concomitantly duplicate and stabilize dozens of genes. The breakpoint junctions of the segmental duplications we analyzed mapped within low-complexity DNA sequences, replication fork convergence regions, and LTR sequences associated with tRNA genes (KOSZUL *et al.* 2004). These particular regions are scattered throughout the genome and provide numerous possibilities for genomic rearrangements. Our study fits with a model of genome evolution intermediate to the random breakage model of NADEAU and TAYLOR (1984) and to the "fragile site" model of PEVZNER and TESLER (2003): rearrangements may arise at numerous locations

nonuniformly distributed but depending on the chromosomal context (Trinh *et al.* 2004). In addition, our experimentally based model presents strong similarities to the translocated-based model of segmental duplication polymorphism for human subtelomeres proposed recently by Linardopoulou *et al.* (2005).

The results presented here might also apply to describe the behavior of large-scale duplications and translocations in the development of some cancer and tumorigenesis progression (Lengauer *et al.* 1998; Pihan and Doxsey 2003).

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