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Gene Expression and Distribution of Swi6 in Partial Aneuploids of the Fission Yeast *Schizosaccharomyces pombe*

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ABSTRACT. **Imbalances of gene expression in aneuploids, which contain an abnormal number of chromosomes, cause a variety of growth and developmental defects. Aneuploid cells of the fission yeast *Schizosaccharomyces pombe* are inviable, or very unstable, during mitotic growth. However, *S. pombe* haploid cells bearing minichromosomes derived from the chromosome 3 can grow stably as a partial aneuploid. To address biological consequences of aneuploidy, we examined the gene expression profiles of partial aneuploid strains using DNA microarray analysis. The expression of genes in disomic or trisomic cells was found to increase approximately in proportion to their copy number. We also found that some genes in the monosomic regions of partial aneuploid strains increased their expression level despite there being no change in copy number. This change in gene expression can be attributed to increased expression of the genes in the disomic or trisomic regions. However, even in an aneuploid strain that bears a minichromosome containing no protein coding genes, genes located within about 50 kb of the telomere showed similar increases in expression, indicating that these changes are not a secondary effect of the increased gene dosage. Examining the distribution of the heterochromoatin protein Swi6 using DNA microarray analysis, we found that binding of Swi6 within ~50 kb from the telomere occurred less in partial aneuploid strains compared to euploid strains. These results suggest that additional chromosomes in aneuploids could lead to imbalances in gene expression through changes in distribution of heterochromatin as well as in gene dosage.**

Key words: *Schizosaccharomyces pombe*/aneuploid/DNA microarray

Introduction

Imbalance of gene expression in aneuploidy causes a variety of biological disorders. When the copy number of a certain gene is increased due to aneuploidy, expression of the gene is thought to increase. The primary increase in expression of one gene could cause secondary changes in the expression of other genes, leading to an imbalance of gene expression throughout the genome. Consequently, a variety of disorders can occur in an aneuploid cell, sometimes resulting in lethality. Autosomal trisomy in humans is known of the 13th, 18th, and 21st chromosomes. Individuals with trisomy of these chromosomes can be born, although they will experience developmental disorders, whereas trisomy of the other chromosomes causes fetal miscarriage or death (Therman and Susman, 1993). This may be because the number of genes on these additional chromosomes from viable trisomes is smaller than that of the other chromosomes, and thus gene expression in these trisomy individuals is not so severely disturbed as to prevent birth. Trisomy of chromosomes that contain a larger number of genes probably causes a more severe imbalance in gene expression, resulting in lethality.

Growth defects as a result of aneuploidy are also known in the fission yeast *Schizosaccharomyces pombe*. *S. pombe* cells vegetatively grow usually as haploid (n=3). In *S.*

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Abbreviations: CGH, comparative genome hybridization; PFG electrophoresis, pulsed field gel electrophoresis; ChIP, chromatin immunoprecipitation; GEO, gene expression omnibus.

pombe, aneuploid cells can be generated from triploid cells through meiosis. Triploid cells can be selected with appropriate genetic markers, and meiosis in triploid cells generates spores of various kinds of ploidy. However, the ploidy of viable spores is limited to three kinds, that is, haploid, diploid, and disomic haploid in which there is an extra copy of the shortest chromosome 3 (Niwa and Yanagida, 1985). This disomic haploid strain is the only viable aneuploid ever generated in *S. pombe*, and yet is difficult to proliferate stably with the extra chromosome. Derived from this disomic haploid strain by gamma ray irradiation, a stable disomic strain was isolated, which contains a smaller portion of the chromosome 3 as the extra copy. This minichromosome, named Ch16, is a linear chromosome of about 530 kb of sequence spanning the centromeric region, and lacks about 85% of the chromosome 3 (Niwa *et al*., 1986). Telomere repeat sequences were found at both ends of Ch16 (Matsumoto *et al*., 1987). Thus, the haploid cell containing Ch16 is a partial aneuploid, in which about 3% of the genome is duplicated (relative to 14 M bp in the *S. pombe* genome). This level of partial aneuploidy is likely to be tolerated in *S. pombe*. To determine how aneuploidy causes imbalances in gene expression from the whole genome, we used DNA microarrays to analyze the gene expression of aneuploid strains bearing Ch16 and its derivatives S28 and Ch10.

Results and Discussion

Determination of the breakpoints of minichromosomes by an array CGH method

Ch16 and S28 are linear minichromosomes that include the centromere of the chromosome 3 (Fig. 1A). Their lengths, estimated by pulse field gel electrophoresis, are about 530 kb and 750 kb, respectively (Fig. 1B). It is presumed that the right arm of S28 contains additional sequence of about 200 kb distal to that of Ch16, and that their left arms are the same (Niwa *et al*., 1989). Their truncated ends are healed by telomere repeat sequences (Matsumoto *et al*., 1987). Although it is expected that these minichromosomes contain several genes around the centromere, the precise positions of their breakpoints have yet to be determined. For analysis of gene expression in the partial aneuploid strains bearing the minichromosomes Ch16 and S28, it is necessary to know which genes are contained in these minichromosomes. Thus, we first determined the positions of the breakpoints of these minichromosomes by using a comparative genome hybridization (CGH) method on a DNA microarray.

Panels C and D of Fig. 1 show the results of the array CGH experiments. The vertical bars in the graph indicate the genomic DNA content of the chromosome 3 in the partial aneuploids relative to that in the normal haploid. The measured value in the pericentromeric region of the chromosome 3 was approximately two in the partial disomic haploid containing Ch16 (Fig. 1C) and approximately three in the partial trisomic haploid containing both Ch16 and S28 (Fig. 1D), indicating the copy number of the genes in these regions. Analysis of the results indicate that the end of the left arm of Ch16 corresponds to a point located between the ORFs SPCPB16A4.06c and SPCC1742.01 (Fig. 1E), and that the right end of Ch16 corresponds to a point between SPCC11E10.02c and SPCC11E10.03 (Fig. 1F). As the telomere sequence was expected to occur at the end of Ch16, we amplified the DNA fragment including the ends of the minichromosome by PCR. An internal sequence adjacent to the putative breakpoint and the telomeric repeat sequence were used as primers, and genomic DNA from the partial aneuploid containing Ch16 was used as the template. The PCR product from the left end contained the coding region of SPCC1742.01 which is transcribed from the centromere towards the telomere. The telomeric repeat sequence occurred 760 nucleotides from the beginning of this ORF. The PCR product amplified from the right end contains the latter half of the coding region of SPCC11E10.02c which is transcribed from the telomere toward the centromere. The telomeric repeat sequence was found 832 nucleotides from the beginning of this ORF. Restriction fragments from the regions including the break points determined here were detected by Southern blotting with genomic DNA from the partial aneuploid containing Ch16 (data not shown). The total length from SPCC1742.01 to SPCC11E10.02, excluding the centromere, is about 430 kb. As the length of the centromere of the chromosome 3 is about 100 kb, this value is consistent with the length of Ch16 (530 kb) estimated by pulse field gel electrophoresis. The sequence of the right end of S28, which was determined in the same manner, corresponded to the region between SPCC663.17 and SPCC663.03. Based on the *S. pombe* genome database, these results support the conclusion that Ch16 contains 165 ORFs from SPCC1742.01 to SPCC11E10.02 (163 complete ORFs and truncated ORFs at both ends), and S28 contains 233 ORFs from SPCC1742.01 to SPCC663.17 (231 complete ORFs and truncated ORFs at both ends). The number of ORFs included in the minichromosomes corresponds to 3.3% or 4.7% of all of the ORFs of *S. pombe*. Therefore, the trisomic haploid containing both Ch16 and S28 has an excess of ORFs of 8%. Because these partial aneuploids containing minichromosomes grow normally, it may be concluded that they are able to tolerate this level of genomic imbalance.

Gene expression in the partial aneuploid strain containing Ch16

We examined gene expression of the minichromosome-containing partial aneuploids using a DNA microarray analysis. The partial aneuploid strain bearing Ch16 contains 2 copies of the 163 complete ORFs. The partial aneuploid strain bearing both Ch16 and S28 contains 3 copies of the

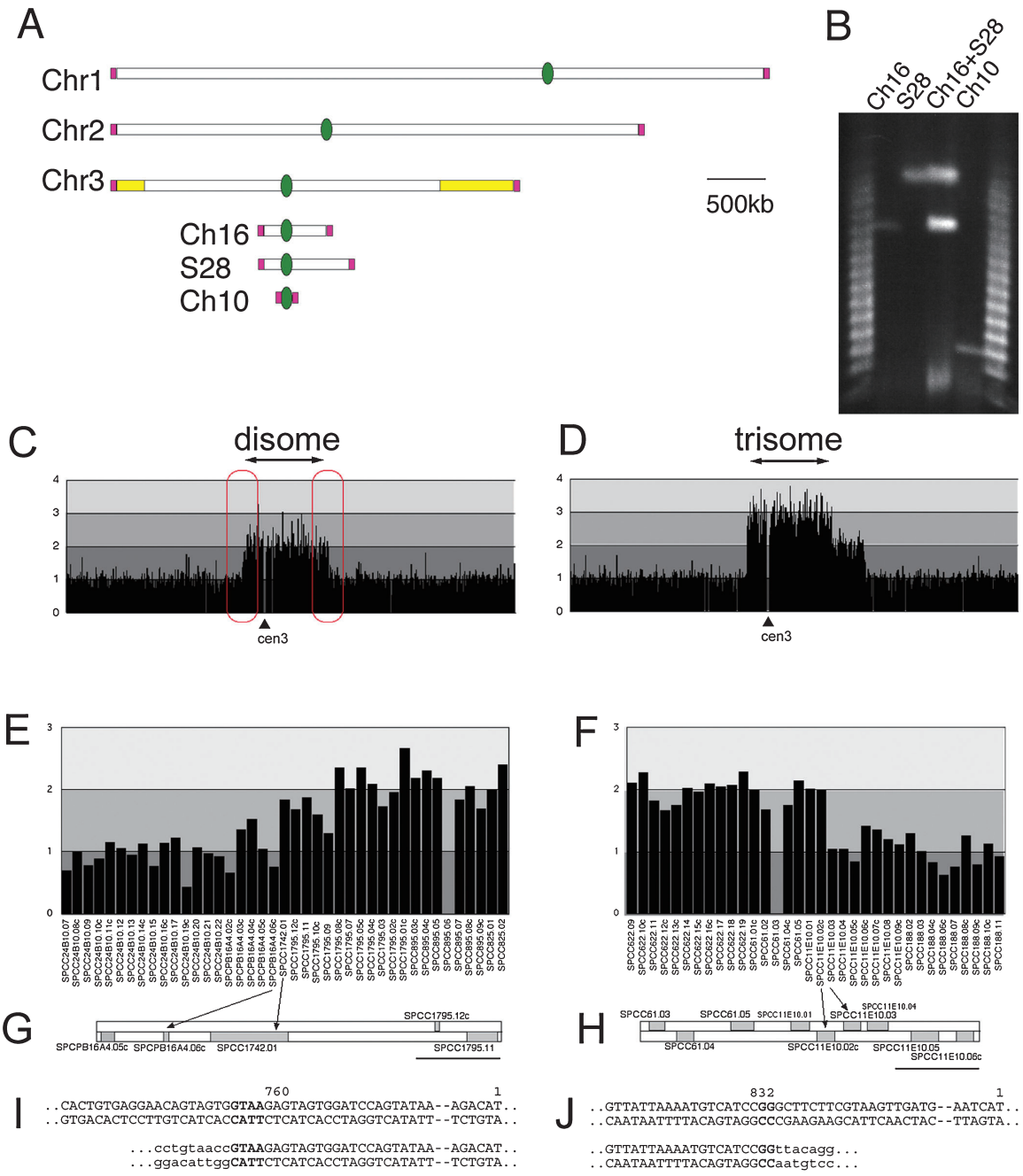

Fig. 1. Breakpoints of the minichromosomes. A: The three chromosomes and minichromosomes derived from the chromosome 3 (Ch16, S28, and Ch10) that were used in this study. Centromeres, telomeres and rRNA genes are shown in green, red, and yellow, respectively. B: Minichromosomes separated by pulsed field gel electrophoresis. Lambda DNA ladder was applied in lanes on both sides of the gel. The monomer of the lambda standard is 48.5 kb. C, D: Results of array based CGH of the partial disomic haploid containing Ch16 (C) and the partial trisomic haploid containing both Ch16 and S28 (D). The vertical axis indicates the ratio of copy number of genomic DNA for the chromosome 3 in the partial aneuploids relative to normal haploid. The horizontal axis shows ORFs in the order that they occur on the chromosome 3, excluding the rRNA gene regions. E, F: Magnification of the red enclosed regions in C. G, H: Map of ORFs around the breakpoint. Bar is 5 kb. I, J: DNA sequence around the breakpoint of the chromosome 3 (upper line) and of Ch16 (lower line).

163 genes shared by Ch16 and S28, and 2 copies of the 68 ORFs that are located only on S28. We measured expression ratios of the 163 genes in these partial aneuploid strains in the vegetative growth phase. Among these 163 genes, expression levels of 123 genes in the disomic strain and 132 genes in the trisomic strain were sufficient to allow calcu-

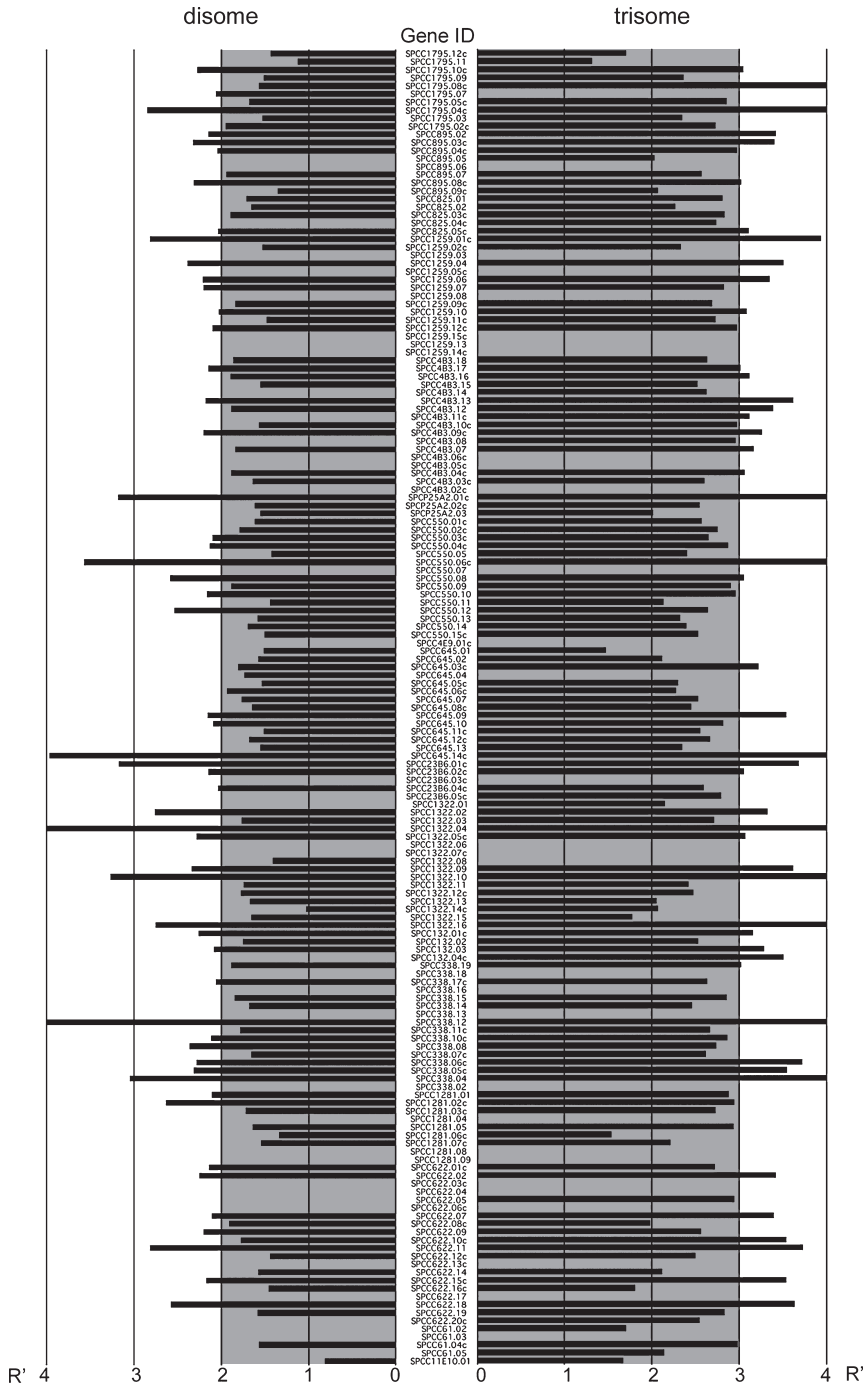

Fig. 2. Expression of genes included in the minichromosome Ch16. The expression ratios of the 163 genes included in Ch16 are shown as R' values (see Materials and Methods). The horizontal axis shows R' values plotted on a linear scale obtained from two independent experiments. (Left) Partial disomic haploid containing Ch16. (Right) Partial trisomic haploid containing both Ch16 and S28. No bars are indicated when expression level of the respective genes were below the detection limit.

lation of the expression ratio reproducibly in two independent experiments (see Materials and Methods). The average of the expression ratio was 1.917 in the disomic cells, and 2.813 in the trisomic cells (Fig. 2), indicating that the level of expression increases in proportion to the copy number of the gene.

It is noteworthy that the expression ratio of some genes in the monosomic region increased remarkably in the partial

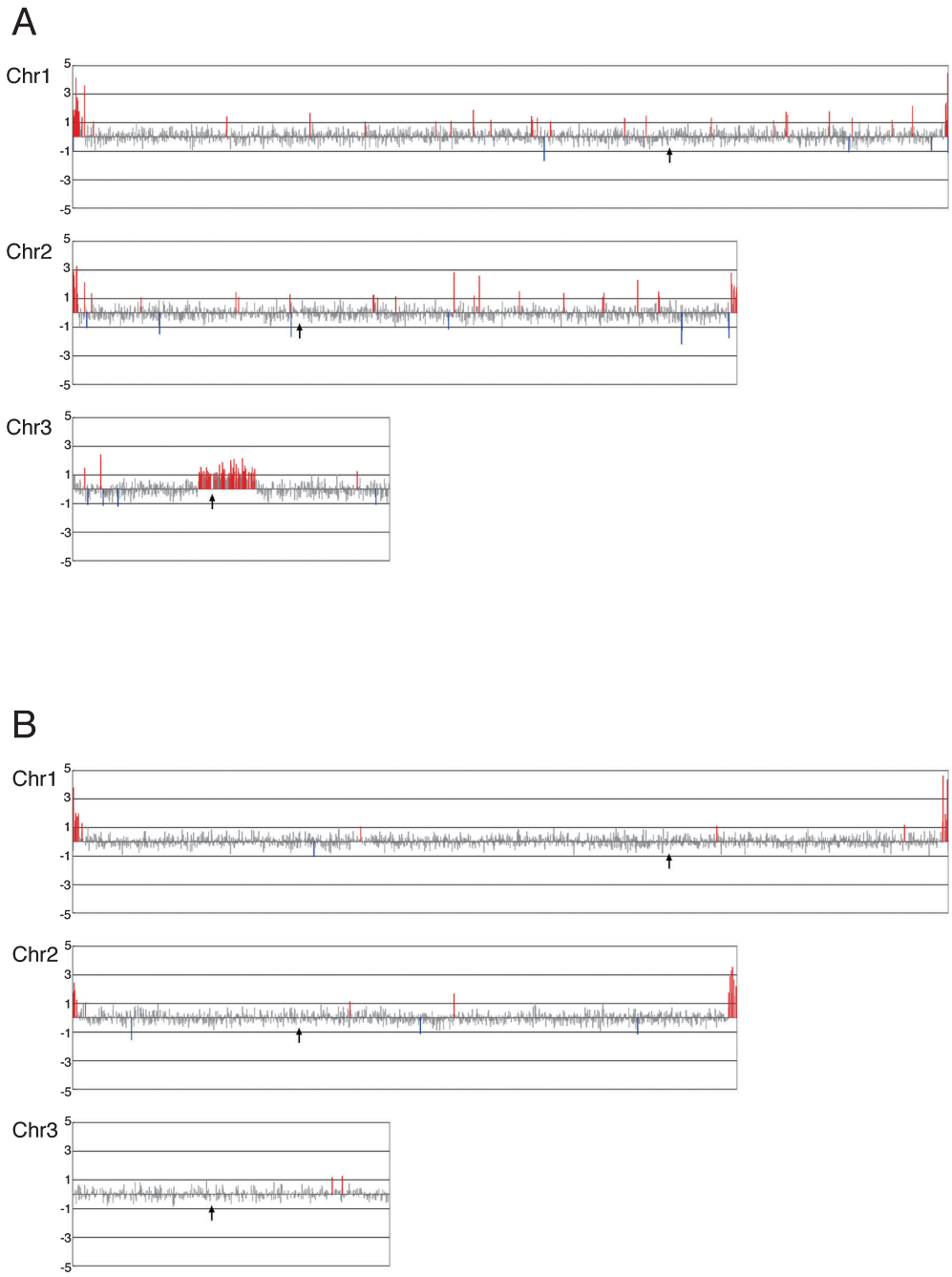

Fig. 3. Gene expression from the whole genomes of the partial aneuploids. The expression ratios at the whole genome level are shown by the r' value (see Materials and Methods). The vertical axis is the averaged expression ratio (r') of two independent experiments in a logarithmic scale to the base of 2. Genes with a greater than two-fold increase in expression are shown in red (see Table I), those with a greater than two-fold decrease are in blue (see Table II). The arrow indicates the position of the centromere. (A) The aneuploid containing Ch16. (B) The aneuploid containing Ch10.

aneuploid containing Ch16 (Fig. 3A). While such genes were distributed widely along all of the chromosomes, they were particularly concentrated in the vicinity of the telomeres of chromosomes 1 and 2. 79 genes in the monosomic region of the partial aneuploid containing Ch16 showed a greater than 2-fold increase in expression compared with that in the normal haploid (Table I). Out of these 79 genes, 28 genes were located in the vicinity of the telomeres of chromosomes 1 and 2. The remaining 51 genes seem to be distributed randomly on the chromosomes; interestingly 27

Table I. 79 GENES LOCATED IN THE MONOSOMIC REGION OF THE ANEUPLOID CONTAINING CH16, SHOWED A GREATER THAN TWO-FOLD INCREASE IN EXPRESSION RELATIVE TO THE NORMAL HAPLOID

Systematic Name	r'(Ch16) [a]		r'(Ch10) [b]	Systematic Name	r'(Ch16) [a]		r'(Ch10) [b]
SPAC750.07c	4.437	Swi6-bound [c]	4.329	SPBC725.03	1.416	Up-stress	0.182
SPAC212.02	4.095	Swi6-bound	1.978	SPBC11C11.06c	1.337	Up-stress	–0.150
SPBC1348.03	2.876	Swi6-bound	n.d.	SPBC660.05	1.328	Up-stress	n.d.
SPBPB2B2.06c	2.751	Swi6-bound	3.252	SPAC19G12.09	1.310	Up-stress	–0.011
SPAC977.02	2.732	Swi6-bound	n.d.	SPAC328.03	1.291	Up-stress	0.797
SPBC1348.05	2.588	Swi6-bound	2.377	SPAC513.02	1.285	Up-stress	–0.123
SPAC977.04	2.524	Swi6-bound	1.758	SPBC23G7.10c	1.234	Up-stress	0.286
SPAC750.04c	2.358	Swi6-bound	n.d.	SPCC830.07c	1.201	Up-stress	0.353
SPAC750.02c	2.229	Swi6-bound	1.913	SPBC32F12.03c	1.171	Up-stress	–0.136
SPBPB2B2.07c	2.004	Swi6-bound	1.685	SPACUNK4.16c	1.158	Up-stress	0.185
SPAC212.04c	1.883	Swi6-bound	1.450	SPAC26F1.07	1.156	Up-stress	–0.108
SPBC1348.01	1.862	Swi6-bound	1.796	SPAC7D4.07c	1.148	Up-stress	0.346
SPBPB2B2.14c	1.852	Swi6-bound	1.520	SPBC21H7.06c	1.107	Up-stress	n.d.
SPAC212.08c	1.851	Swi6-bound	3.754	SPBC725.10	1.081	Up-stress	–0.028
SPAC212.01c	1.791	Swi6-bound	1.675	SPAC22H10.13	1.071	Up-stress	n.d.
SPAC977.06	1.768	Swi6-bound	1.952	SPAC1F8.03c	3.570	Others	–0.023
SPBCPT2R1.01c	1.758	Swi6-bound	n.d.	SPBC1348.11	2.864	Others	n.d.
SPBC1348.07	1.739	Swi6-bound	1.885	SPBC19C7.04c	2.560	Others	n.d.
SPBPB2B2.13	1.733	Swi6-bound	2.623	SPCC794.04c	2.357	Others	n.d.
SPBPB2B2.12c	1.554	Swi6-bound	n.d.	SPBC1683.09c	2.079	Others	n.d.
SPAC212.05c	1.527	Swi6-bound	n.d.	SPAC977.07c	1.752	Others	n.d.
SPBPB2B2.19c	1.406	Swi6-bound	2.168	SPAC17G6.13	1.452	Others	0.767
SPAC212.06c	1.383	Swi6-bound	0.966	SPBC32C12.02	1.435	Others	–0.108
SPBC1348.02	1.140	Swi6-bound	1.670	SPAC821.10c	1.409	Others	0.753
SPAC750.05c	1.139	Swi6-bound	1.476	SPAC977.15	1.360	Others	1.256
SPAC750.06c	1.106	Swi6-bound	1.563	SPBC16D10.11c	1.355	Others	0.547
SPBPB2B2.18	1.082	Swi6-bound	n.d.	SPBC1348.14c	1.299	Others	n.d.
SPAC977.01	1.011	Swi6-bound	1.717	SPAC1B3.03c	1.277	Others	0.516
SPBC1348.12	3.228	Up-stress [d]	1.204	SPBC83.17	1.249	Others	0.594
SPBC3E7.02c	2.778	Up-stress	1.639	SPBC23G7.09	1.184	Others	0.419
SPBC21C3.19	2.247	Up-stress	–1.174	SPAC9E9.09c	1.115	Others	0.408
SPAP8A3.04c	2.129	Up-stress	–0.219	SPBC3B9.01	1.114	Others	0.532
SPAC23H3.15c	1.835	Up-stress	–0.041	SPBC16D10.08c	1.073	Others	0.995
SPAC22F8.05	1.758	Up-stress	–0.750	SPBC649.04	1.058	Others	0.702
SPAC27D7.09c	1.721	Up-stress	0.472	SPAC13G7.02c	1.050	Others	0.170
SPAC167.06c	1.633	Up-stress	0.549	SPAC5H10.01	1.046	Others	0.059
SPAC637.03	1.524	Up-stress	0.189	SPAC31G5.09c	1.040	Others	0.470
SPBC30D10.14	1.470	Up-stress	–0.360	SPAPB1A10.05	1.024	Others	n.d.
SPCC330.06c	1.435	Up-stress	0.587	SPBC1711.08	1.023	Others	0.389
SPACUNK4.17	1.432	Up-stress	n.d.				

a): the expression ratio for the aneuploid containing Ch16.
b): the expression ratio for the aneuploid containing Ch10. The expression ratio of genes which showed a greater than two-fold increase is written in red.
c): genes were located in the vicinity of the telomeres of chromosomes 1 and 2, and bound Swi6 in the normal haploid.
d): stress response genes (Chen *et al*., 2003).
n.d.: not detected.

of these were stress response genes.

Two possible explanations for the observed increased expression in the monosomic regions are apparent. First, if transcription factor genes or regulators of such genes occur in the disomic region, expression of the monosomic genes could be changed in the partial aneuploid. The 163 genes on Ch16 might include genes that increase expression of telomere adjacent genes or stress response genes. A second

Table II. 18 GENES SHOWED A GREATER THAN TWO-FOLD DECREASE IN EXPRESSION RELATIVE TO THE NORMAL HAPLOID

Systematic Name	r'(Ch16) [a)]
SPAC212.10	–1.152
SPAC212.09c	–1.001
SPAPB24D3.07c	–1.672
SPAPB8E5.03	–1.072
SPAC1039.02	–1.004
SPAC750.08c	–1.131
SPBC1198.02	–1.029
SPBC947.04	–1.493
SPBC29B5.02c	–1.678
SPBC25B2.08	–1.157
SPBC1861.01c	–2.187
SPBC1861.02	–1.233
SPBC8E4.01c	–1.158
SPBP4G3.02	–1.764
SPCC320.14	–1.088
SPCC794.12c	–1.157
SPCC1682.09c	–1.210
SPCC1494.07	–1.085

a): the expression ratio for the aneuploid containing Ch16.

possibility is that the change in expression of the monosomic genes may be a direct consequence of bearing an extra chromosome. Because both heterochromatin and euchromatin are increased by the presence of an additional chromosome in aneuploidy, some chromatin formation factors might be insufficient to trigger expression changes in the monosomic region. To distinguish these possibilities, we examined expression in a different aneuploid that contains the minichromosome Ch10 which lacks any protein coding sequences.

Gene expression in the aneuploid strain containing the Ch10 minichromosome

Ch10 was made by deleting the majority of the arm region of Ch16 to eliminate all protein coding genes (Niwa *et al*., 1989). It consists of the centromere, the telomeres and the sup3-5 gene as a selectable marker which encodes the opal suppressor of Ser-tRNA (Rafalski *et al*., 1979). If the expression changes were transcriptionally regulated by increased expression of the disomic 163 genes in the partial aneuploid containing Ch16, this effect should disappear in the partial aneuploid containing Ch10. Fig. 3B shows the expression ratio of each gene in the partial aneuploid containing Ch10. Of the 27 stress response genes that exhibited increased expression in the partial aneuploid containing Ch16, 25 genes did not show a significant increase in expression in the partial aneuploid containing Ch10. This suggests that the increased expression of these genes in the partial aneuploid containing Ch16 is probably induced by some of the 163 genes in Ch16. On the other hand, of the 28 telomere-adjacent genes showing increased expression in the partial aneuploid containing Ch16, 20 genes also exhibited increased levels of expression in the partial aneuploid containing Ch10 (Table I). This indicates that the observed increase in expression of the telomere-adjacent genes is a direct consequence of bearing the additional chromosome, instead of transcriptional regulation by specific disomic genes.

Expression of telomere adjacent genes and distribution of Swi6 in aneuploidy

The observed increase in expression of the telomere-adjacent genes that are common to the partial aneuploids bearing either Ch16 or Ch10, suggests that chromatin structures near the telomere might be altered in these partial aneuploids. We measured the distribution of the heterochromatin protein Swi6 in a wild type strain and in the aneuploid containing Ch10 by chromatin immunoprecipitation (ChIP) and DNA microarray (Fig. 4). The relative amounts of DNA in the anti Swi6 antibody immunoprecipitates were compared with the whole genomic DNA by DNA microarray. The vertical bars in the upper part of Fig. 4 show the distribution of Swi6 in subtelomeric regions of the chromosomes 1 and 2 in the normal haploid and the aneuploid containing Ch10. In the normal haploid, Swi6 proteins bind to sequence from ~50 kb from the left end to ~70 kb from the right end of the chromosome 1 and from ~50 kb from the left end to ~90 kb from the right end of the chromosome 2. On the other hand, the binding of Swi6 in the aneuploid containing Ch10 was reduced at the region indicated by red squares in Fig. 4. While the red squared regions contain 51 genes, 28 of these 51 genes gave expression levels below detectable limits both in the anueploid and the normal haploid (Table III). Out of the remaining 23 genes with detectable levels of gene expression, 22 genes showed a greater than two-fold increase in expression in the aneuploid containing Ch10 compared with that in the normal haploid (Fig. 3B, Tables III and IV). These results suggest that the higher expression of the telomere adjacent genes in the aneuploid is caused by a decrease in Swi6 binding to this region.

The DNA microarray used in these experiments consists of ORF sequences only and does not contain centromeric sequences. To examine the distribution of Swi6 at the centromere, we constructed a high-density DNA microarray which mainly consists of the centromeric region. The whole sequence from the $lys1^+$ gene located 30 kb from the cen1 (centromere of the chromosome 1) to the central sequence of cen1 (cntI), and sequences unique to cen2 and cen3 were arrayed in the high-density centromere DNA array (see Materials and Methods). ChIP analysis with Swi6 antibody

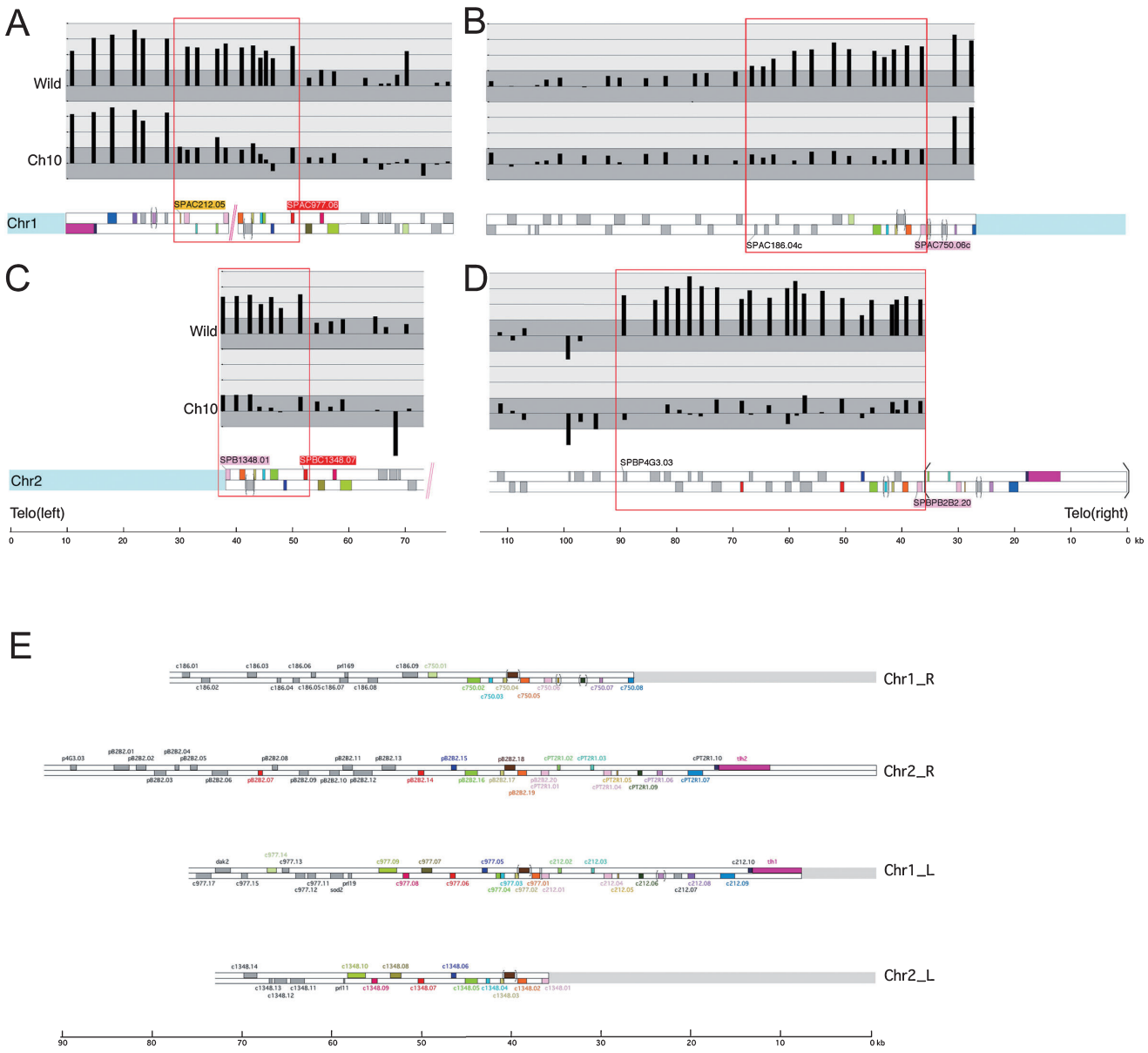

Fig. 4. Distribution of Swi6 at subtelomeric regions. Panels A and B show the left and right subtelomeric regions of the chromosome 1. Panels C and D show the left and right subtelomeric regions of the chromosome 2. The vertical bars show the relative amount of Swi6 bound at each locus in the normal haploid (indicated by Wild) and in the aneuploid containing Ch10 (indicated by Ch10). Positions of ORF probes are indicated at bottom of each panel. Regions in a bracket were not included as probes on the microarray. The light blue regions extending from the map show telomeric gap regions where DNA sequences have not been determined. In the regions outlined by red squares, the binding of Swi6 was found to be reduced in the aneuploid containing Ch10 relative to the normal haploid. Panel E shows a map of the ORFs in the subtelomeric regions of the chromosomes 1 and 2 according to the *S. pombe* GENE DB (http://www.genedb.org/genedb/pombe/index.jsp). ORFs are shown in colors along the double strands. ORFs on the upper strand are transcribed from the centromere towards the telomere, and ORFs in the lower strands are transcribed from the telomere towards the centromere. ORFs shown in the same color are homologous with each other. The ORFs in brackets are not annotated in the *S. pombe* GENE DB. However, sequences flanking these regions are homologous with the respective regions of other chromosomes, thus we speculate that homologous sequences continue throughout these regions.

on the high-density centromere DNA array showed that Swi6 binds to the sequences from probe C1-67 to probe C1-131 (Fig. 5). This result is consistent with the measurements that were previously reported (Cam *et al*., 2005; Partridge *et al*., 2000).

As shown in Fig. 5, a significant difference in binding of Swi6 to centromeres was not observed between the wild type and the aneuploid containing Ch10 strains. Binding of

Table III. ANALYSIS OF THE 51 GENES LOCATED IN THE REGION INDICATED BY RED SQUARES IN FIGURE 4

Systematic Name	r'(Ch10) [a)]	chromosomal location [b)]
SPAC212.05c	n.d.	1-L
SPAC212.04c	1.450	1-L
SPAC212.03	n.d.	1-L
SPAC212.02	1.978	1-L
SPAC212.01c	1.675	1-L
SPAC977.01	1.717	1-L
SPAC977.02	n.d.	1-L
SPAC977.03	n.d.	1-L
SPAC977.04	1.758	1-L
SPAC977.05c	n.d.	1-L
SPAC977.06	1.952	1-L
SPAC186.04c	1.366	1-R
SPAC186.05c	4.592	1-R
SPAC186.06	n.d.	1-R
SPAC186.07c	n.d.	1-R
SPAC186.08c	n.d.	1-R
SPAC186.09	n.d.	1-R
SPAC750.01	0.765	1-R
SPAC750.02c	1.913	1-R
SPAC750.03c	n.d.	1-R
SPAC750.04c	n.d.	1-R
SPAC750.05c	1.476	1-R
SPAC750.06c	1.563	1-R
SPBC1348.01	1.796	2-L
SPBC1348.02	1.670	2-L
SPBC1348.03	n.d.	2-L
SPBC1348.04	n.d.	2-L
SPBC1348.05	2.377	2-L
SPBC1348.06c	n.d.	2-L
SPBC1348.07	1.885	2-L
SPBP4G3.03	n.d.	2-R
SPBPB2B2.01	n.d.	2-R
SPBPB2B2.02	2.887	2-R
SPBPB2B2.03c	n.d.	2-R
SPBPB2B2.04	n.d.	2-R
SPBPB2B2.05	n.d.	2-R
SPBPB2B2.06c	3.252	2-R
SPBPB2B2.07c	1.685	2-R
SPBCPT2R1.02	n.d.	2-R
SPBPB2B2.09c	3.504	2-R
SPBPB2B2.10c	n.d.	2-R
SPBPB2B2.11	n.d.	2-R
SPBPB2B2.12c	n.d.	2-R
SPBPB2B2.13	2.623	2-R
SPBPB2B2.14c	1.520	2-R
SPBPB2B2.15	n.d.	2-R
SPBPB2B2.16c	n.d.	2-R
SPBPB2B2.17c	n.d.	2-R
SPBPB2B2.18	n.d.	2-R
SPBPB2B2.19c	2.168	2-R
SPBCPT2R1.01c	n.d.	2-R

a): the expression ratio for the aneuploid containing Ch10. The expression ratio of genes which showed a greater than two-fold increase is written in red.
b): 1-L is the left end of chromosome 1, 2-R is the right end of chromosome 2.
n.d.: not detected.

Table IV. 35 GENES SHOWED A GREATER THAN TWO-FOLD INCREASE IN EXPRESSION IN THE ANEUPLOID CONTAINING CH10 RELATIVE TO THE NORMAL HAPLOID

Systematic Name	r'(Ch10) [a)]		
SPAC186.05c	4.592	Swi6-bound [b)]	Swi6-decreased [d)]
SPBPB2B2.09c	3.504	Swi6-bound	Swi6-decreased
SPBPB2B2.06c	3.252	Swi6-bound	Swi6-decreased
SPBPB2B2.02	2.887	Swi6-bound	Swi6-decreased
SPBPB2B2.13	2.623	Swi6-bound	Swi6-decreased
SPBC1348.05	2.377	Swi6-bound	Swi6-decreased
SPBPB2B2.19c	2.168	Swi6-bound	Swi6-decreased
SPAC212.02	1.978	Swi6-bound	Swi6-decreased
SPAC977.06	1.952	Swi6-bound	Swi6-decreased
SPAC750.02c	1.913	Swi6-bound	Swi6-decreased
SPBC1348.07	1.885	Swi6-bound	Swi6-decreased
SPBC1348.01	1.796	Swi6-bound	Swi6-decreased
SPAC977.04	1.758	Swi6-bound	Swi6-decreased
SPAC977.01	1.717	Swi6-bound	Swi6-decreased
SPBPB2B2.07c	1.685	Swi6-bound	Swi6-decreased
SPAC212.01c	1.675	Swi6-bound	Swi6-decreased
SPBC1348.02	1.670	Swi6-bound	Swi6-decreased
SPAC750.06c	1.563	Swi6-bound	Swi6-decreased
SPBPB2B2.14c	1.520	Swi6-bound	Swi6-decreased
SPAC750.05c	1.476	Swi6-bound	Swi6-decreased
SPAC212.04c	1.450	Swi6-bound	Swi6-decreased
SPAC186.04c	1.366	Swi6-bound	Swi6-decreased
SPAC750.07c	4.329	Swi6-bound	
SPAC212.08c	3.754	Swi6-bound	
SPBC3E7.02c	1.639	Up-stress [c)]	
SPBC1348.12	1.204	Up-stress	
SPBP4G3.02	1.720	others	
SPAC977.15	1.256	others	
SPCC576.09	1.251	others	
SPCC1906.04	1.151	others	
SPAPJ760.03c	1.150	others	
SPBC1A4.06c	1.103	others	
SPAC23A1.08c	1.073	others	
SPBC1683.12	1.017	others	
SPAC1002.19	1.012	others	

a): the expression ratio for the aneuploid containing Ch10.
b): genes were located in the vicinity of the telomeres of chromosomes 1 and 2, and bound Swi6 in the normal haploid.
c): stress response genes (Chen *et al*., 2003).
d): the binding of Swi6 to the genes in the aneuploid containing Ch10 is reduced compared to the normal haploid strain.

Swi6 to the silence mating-type locus, examined by ChIP and PCR, identified no obvious differences between the aneuploid containing Ch10 and the normal haploid strains (data not shown). Therefore, the difference in distribution of Swi6 in the aneuploid containing Ch10 strain from the normal haploid strain is limited to the 20–50 kb subtelomeric region of the chromosomes 1 and 2. It has been reported that

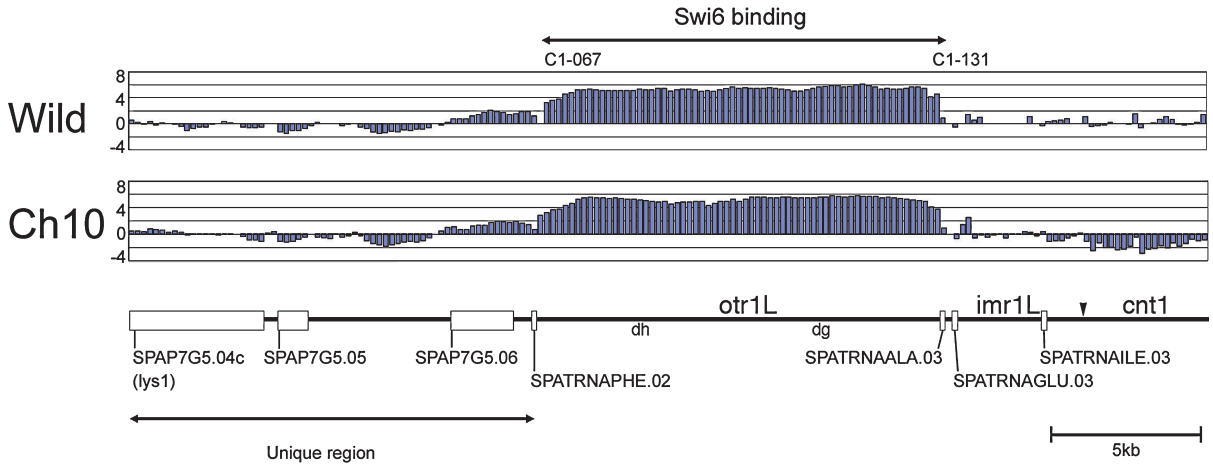

Fig. 5. Distribution of Swi6 in the cen1 region. The vertical bars show the relative amount of Swi6 bound for each probed sequence. A physical map of cen1 region is drawn under the bar graph as a reference for positions of probes. Genes around the cen1 region are shown by open squares with gene ID. The arrowhead on the map indicates the boundary between cnt1 and imr1L.

the binding region of Swi6 to the left subtelomeric region of the chromosome 1 can be divided into a major region of 20 kb from the chromosome end and a minor region of ~17 kb proximal to the major region (Cam *et al*., 2005). The region which exhibits lower levels of Swi6 binding in the aneuploid strain, corresponds well to this minor binding region. These results suggest that heterochromatin in this region is less stable than in other regions and can be easily transformed by the presence of an extra chromosome.

Conclusion

In this report, we constructed a DNA microarray that consists of all ORFs from the genome of *S. pombe*, and utilized it for three types of experiments: (1) measurement of genomic DNA content by the array CGH method, (2) measurement of mRNA for expression analysis of genes, and (3) analysis of protein-bound DNA sequences by chromatin immunoprecipitation (ChIP on Chip experiment). Using these three microarray-based experiments, we analyzed chromosome structures and gene expression in *S. pombe* partial aneuploids. From the expression analysis we found that in the aneuploids the level of expression relative to the normal haploids increased in the vicinity of the telomeres. The distribution analysis of heterochromatin protein Swi6 suggests that the change in Swi6 distribution causes changes in the expression of genes from the telomeric regions of aneuploids. The extra chromosomes present in the aneuploids probably cause the imbalance in gene expression not only by increasing gene dosage but also by altering chromatin structure.

Materials and Methods

Media and strains

YES (yeast extract, glucose, supplements) liquid media were used for culture (Moreno *et al*., 1991). Exponentially growing *S. pombe* cells were used. The genotypes of the strain used here are as follows:

L972: h^-, HM381: h^- leu1-32 ade6-216 S28(LEU2), AY238-8B: h^- ade6-210 Ch16(ade6-216), CRLe33: h^- ade6-210 Ch16(ade6-216) ura4-D18 taz1Δ::ura4$^+$, CRL600: h^+ his2-245 leu1-32 ade6-M26 S28(ade6-210 LEU2) ura4-D18 taz1Δ::ura4$^+$, CRLb35: h^- leu1-32 ade6-375 or ade6-210 Ch16(ade6-216) S28(ade6-210 LEU2), CRLe70: h^- leu1-32 ade6-704 Ch10(sup3-5), CRLf90: h^- leu1-32 lys1$^+$::sup3-5 ade6-704. For efficient PCR amplification of the DNA fragment including the ends of minichromosomes, the genomic DNA of taz1Δ strains bearing minichromosomes were used for the PCR template as telomere sequences are elongated in taz1Δ cells.

Pulsed field gel electrophoresis (PFG)

The minichromosomes were separated by PFG electrophoresis using a CHEF-DRII system (BIO-RAD). Specimen agarose blocks were prepared as described in Niwa *et al*. (1986). A Lambda DNA Ladder, containing monomeric lambda DNA of 48.5 kb in length (TAKARA), was used as a size marker.

Chromatin immunoprecipitation (ChIP)

Exponentially growing *S. pombe* cells (5×10^8 cells) were fixed with 3% formaldehyde in YES for 30 min at 18°C. After fixation, glycine was added to a final concentration of 0.125 M and the cells were washed twice with ice-cold PBS buffer. Cells were trans-

ferred to a 2 ml tube and resuspended in 100 μl lysis buffer (50 mM Hepes-KOH, pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% DOC (deoxycholic acid), 1 mM PMSF) with ice-cold glass beads. Cells were broken with a bead beater (Multi-beads shocker, Yasui-Kikai, 2700 rpm, 20 min in total). The base of the 2 ml tube was punctured with a needle, and the tube placed in a 5 ml tube. The cell lysate was collected in the 5ml tube by centrifugation at 1500 rpm for 2 min. After adding 500 μl lysis buffer to the 2 ml tube, the remaining lysate was collected again by centrifugation at 1500 rpm for 2 min. The collected lysate was sonicated, on ice, to shear the chromatin to 500 bp-1 kb. After sonication, the lysate was transferred to a 1.5 ml tube and centrifuged at 15000 rpm for 5 min. The supernatant was collected and incubated with anti-Swi6 antibody (Nakayama *et al*., 2000) for 4 h at 4°C, and then incubated with Protein A agarose beads for 1h at 4°C. The Protein A agarose beads were precipitated by centrifugation at 5000 rpm for 1 min to collect the Swi6 bound fraction.

Construction of the ORF DNA microarray

DNA probes used for the ORF microarray were generated by PCR from the coding region of the genes that are annotated in published *S. pombe* genome sequences (Wood *et al*., 2002). The continuous 300 bp±10 bp exon sequence nearest to the 3'-end of the gene was selected as a probe. For genes that had no exons longer than 290 bp, the longest exon was used as a probe. Primers used for PCR were designed using the primer design support tool, Primer3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The length of each primer was 30±3 bp with Tm of 60–70°C. Forward primers were modified by the addition of an amino group at their 5' end to allow covalent binding of only the sense strand of probes to the coated glass slide. Genomic DNA from the wildtype L972 strain was used as a template for the PCR in which DNA probes were generated for the first time. For the later generations of DNA probes, the first PCR products were used as templates. A GeneAmp PCR System 9700 (Applied Biosystems) and z-Taq polymerase (TAKARA) were used for PCR. PCR conditions were as follows: 40 cycles of denaturation at 95°C, extension at 72°C, and annealing, depending on the Tm values of the primers, at 54–63°C. The PCR products were purified by using Multiscreen96 (Millipore) or NucleoFast96 (MACHEREY-NAGEL) kits and their concentrations were measured using a SpectraMax190 (Molecular Devices). A small portion of each PCR product was analyzed by agarose gel electrophoresis to confirm its purity and concentration. Biomek2000 (Beckman Coulter) was used for the above-mentioned purification, concentration measurement and dilution.

For each probe, 4 μg of probe DNA was dissolved in 20 μl spotting buffer (150 mM Na-phosphate buffer, pH 8.5). Probe DNA was stamped onto a coated glass slide (Surmodics 3D-Link Slide) by a GTMASS STAMPER (Nippon Laser Electronics) using a 16-pin stamper: stamping 324 times in 16 blocks to form 5184 spots (18×18 spots in each block labeled from A to P). The diameter of each spot was approximately 150 μm and the pitch 190 μm.

To covalently bind probe DNA to the glass slide, a blocking procedure was carried out as follows: the stamped slides were incubated in a humidity chamber (~75% humidity) for 2 days, and then were soaked in a pre-warmed blocking buffer (50 mM ethanolamine, 0.1 M Tris-Cl, pH 9, 0.1% SDS) at 50°C for 15–30 min. The slides were rinsed twice with distilled water (DW), soaked in a wash buffer (4×SSC, 0.1% SDS) at 50°C for 15–60 min, and again rinsed twice with DW. To remove the unmodified antisense strand of the probe DNA, the slides were boiled in DW for 2 min. After rinsing twice in DW, the slides were dried.

The 5184 spots included: 4929 fission yeast genes (cdc2, nuc1, and nda3 excluded) each spotted once on the slide, and the cdc2, nuc1, nda3, and GFP genes spotted once per block (16 blocks in total). Representative values of r' for these 4 genes were calculated as the average of their 16 spots. ID numbers from 5185 to 5188 were assigned to these representative values. Eleven negative control spots were included in each block (A to P except for G block, 175 in total). In G block, one of the 11 negative controls spots was replaced with LEU2 (*S. cerevisiae*). The reaction mixture without primers was used for the negative control spots. Eight buffer only spots were included as blanks. The 4932 probe sequences were blasted against the *S. pombe* genome, the resulting 4726 out of the 4932 giving a blast score <200. The remainder of the probes possibly cross-hybridize with their homologous sequence. The sequences of the probes were deposited with GEO (http://www.ncbi.nlm.nih.gov/geo/index.cgi; accession number GSE8782).

Construction of a high-density tiling DNA microarray for centromeres

Of the 255 probe sequences from centromere regions generated by PCR, 173 of them occurred between lys1 to the right edge of cntI, 56 sequences were from cen2, and the remaining 26 sequences from cen3. Probes for cen2 and cen3 were selected only from specific regions unique to each centromere. The probe sequences are available through GEO (accession number GPL5747). C1-50 probes were not included in the array because they were not amplified by PCR. To make specific probes for each strand of the sequence, two primer sets were prepared for each sequence. In one set, the 5' end of the forward primer was modified with an amino group. In the other set, the 5' end of the reverse primer was modified with an amino group. The PCR and subsequent purification of the PCR products were done as described above. These probes were spotted on a Surmodics 3D-Link Slide by a PixSys4500 (Genomic Solutions) to produce the centromere array. Spotting was carried out by the Nippn Techno Cluster e-Array system, with 534 spots were applied to each cen array. The diameter of each spot was 380 μm and the pitch 920 μm. The 534 spots included: 514 spots which corresponded to the forward or the reverse strands of 257 sequences (ura4, GFP and 255 sequences from cen regions), and 20 negative control spots. The stamped slides were treated in the same manner as those for the ORF microarray.

Preparation of targets for microarray

· For polyA-RNA targets

A single colony of *S. pombe* cells on a YES plate was inoculated

into YES liquid medium. Cells were incubated at 30°C and collected when they reached a density of 5×10^6 cell/ml. Total RNA was isolated by acid phenol methods described at http://www.sanger.ac.uk/PostGenomics/S_pombe/, and the polyA-RNA was then purified using an Oligotex-dT30 (super) mRNA purification kit (TAKARA). The polyA-RNA(3–6 μg) was labeled using a CyScribe Post-Labeling Kit (GE Healthcare) with Cy3 or Cy5 and an Oligo dT primer. For the measurements of polyA-RNA, polyA-RNA from the normal haploid and from aneuploids was labeled with Cy3 and Cy5, respectively. The labeled targets were purified using GFX columns (GE Healthcare). It was confirmed that the difference between the incorporation ratio (IR) of Cy3 and Cy5 is within ±10%. Equal amounts of the labeled targets, estimated by measuring the Cy3 and Cy5 fluorescence signals, were added to the hybridization solution. The Incorporation ratio was calculated as follows:

IR=(pmol of CyDye in a sample)/(μg of nucleic acid in sample)

pmol of CyDye in a sample=(A/E)*(1/W)*Z*df*10^6

A=absorbance of Cy3 at 550 nm or Cy5 at 650 nm

E (Extinction coefficient)=150000 l/mol.cm at 550 nm for Cy3, 250000 l/mol.cm at 650 nm for Cy5.

Z=original volume (μl)

W=optical path of cuvette (cm)

df=dilution factor

· *For genomic DNA targets*

Genomic DNA was isolated from *S. pombe* using the methods described in Matsumoto *et al*. (1987). The isolated genomic DNA was divided into 3 aliquots. The DNA aliquots were incubated with AluI, RsaI, or Sau3AI and then were mixed. Short DNA fragments (16 bp or less) were removed using CENTRI-SEP Spin columns (Applied Biosystems) and the remaining DNA fragments were labeled with Cy3-dUTP for the normal haploid and Cy5-dUTP for the aneuploid using an Oligotailing kit (Roche Diagnostics). Following purification on CENTRI-SEP Spin columns, 1–1.5 μg of the labeled targets were added to the hybridization solution.

· *For ChIP targets*

Genomic DNA, isolated from nuclei or immunoprecipitates, was amplified according to previously described methods (Katou *et al*., 2003). In the first round of amplification, primer A (GGAATTCCAGCTGACCACCNNNNNNNNN) and the template DNA were incubated in reaction mixture containing Sequenase Ver2.0 T7 DNA polymerase (USB). In the second round of amplification, primer B (GGAATTCCAGCTGACCACC) and the product of the first round were incubated in reaction mixture with Ex Taq polymerase (TAKARA). The amplified DNA, from the whole nuclei or from the immunoprecipitates, was labeled with Cy3-dUTP or Cy5-dUTP, respectively, using an Oligotailing kit (Roche Diagnostics).

Hybridization

The labeled targets (~100 ng) were added to the hybridization solution to hybridize to the DNA probes on the microarray, using Genomic solutions GeneTac Hybridization Station, for four hours at 40°C in Genomic solutions GeneTac Hyb buffer (120 μl; including 42% formamide). After hybridization, the slides were washed in the following sequence: (1) 2×SSC 0.1% SDS at 40°C for 5 min, (2) 0.2×SSC at 25°C for 1 min, and (3) 0.1×SSC at 25°C for 1 min.

Data acquiring and processing

Microarrays were scanned using an ArrayWoRx array scanner, and the acquired data was analyzed by ArrayWoRx software (Applied Precision, Inc.). The fluorescence intensity of each spot circle (150 μm diameter) was measured using SoftWorx tracker.

The measured fluorescent intensity, designated by I, was corrected as follows to give a corrected intensity designated by C:

C=I–M in case of I≥M+2s

C=I*2s/(M+2s) in case of I<M+2s

M and s are the average and standard deviation of I of negative control spots for each wavelength, respectively. When I=M+2s, that is C=2s, it was set as the detection limit. When C for either Cy3 or Cy5, or both, was greater than 2s, the values were considered to be effective data. The value r' of the each effective detection spot obtained was calculated as follows:

r'=r–m, r=logR(the base of 2), R=(Ccy5/Ccy3), m is the average of r for all effective detection spots. For the cells including minichromosome Ch16 or S28, m was calculated by excluding the spots that originated in the minichromosomal region from the effective detection spots. In the cen array experiments, m was calculated only for the unique region (from C1-01 to C1-65) shown in Fig. 5, which is on the outside of cen1. In the gene expression analysis, r' values of two independent experiments were averaged. The average r' was used as expression ratio in Fig. 3 and Tables I–IV, and R' in Fig. 2 were calculated by $R'=2^{(\text{average } r')}$. The R' ($=2^{r'}$) value was also used in Fig. 1C, D as a DNA copy number relative to the normal haploid. In the chromatin IP experiments shown in Fig. 4 and Fig. 5, the z value ($z=r'/\sigma$: σ is the standard deviation of r') was used instead of r'. In the cen array experiments, r' values for forward and reverse probes were averaged. The averaged r' was used for calculation of σ and z values. The σ was calculated only for the unique region shown in Fig. 5, which is on the outside of cen1. Original data from the microarray experiments have been submitted to GEO (http://www.ncbi.nlm.nih.gov/geo/index.cgi; accession number GSE8782).

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