Spatial organisation and behaviour of the parental chromosome sets in the nuclei of *Saccharomyces cerevisiae* × *S. paradoxus* hybrids

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Summary

We demonstrate that the genomes of *Saccharomyces cerevisiae* and *S. paradoxus* are sufficiently divergent to allow their differential labeling by genomic in situ hybridisation (GISH). The cytological discrimination of the genomes allowed us to study the merging of the two genomes during hybrid mating. GISH revealed that in hybrid nuclei the two genomes are intermixed. In hybrid meiosis, extensive intraspecific nonhomologous pairing takes place. GISH on chromosome addition and substitution strains (with chromosomes of *S. paradoxus* added to or replacing the homoeologous chromosome of an otherwise *S. cerevisiae* background) was used to delineate individual chromosomes at interphase and to examine various aspects of chromosome structure and arrangement.

Key words: Yeast, Hybrid, Zygote, Nuclear architecture, FISH, Interphase, Meiosis, Chromosome

Introduction

Hybrid organisms often show increased vigour over their parental species but they usually trade in fertility for the high degree of heterozygosity that confers heterosis. Chromosome behaviour has been studied in several hybrid organisms because it is suspected that the selective silencing of genes derived from one parental species might be correlated with a nonrandom distribution of the chromosome sets within the nucleus. Moreover, the reduced fertility of hybrids is often reflected by defects in the meiotic pairing of the chromosomes of different origin.

When studying chromosome behaviour in hybrids, it is of great advantage if chromosomes derived from different species can be discriminated. Total genomic DNA from one species was used to detect introgressed chromosomes from this species in the nuclei of another species by in situ hybridisation (Manuelidis, 1985; Schardin et al., 1985), this technique was later also applied for the differential labelling of the two genomes in hybrids (Schwarzacher et al., 1989; Le et al., 1989) and was termed genomic in situ hybridisation (GISH) (Schwarzacher et al., 1989). These studies showed the nonrandom positioning of the two parental chromosome sets with respect to each other in interphase nuclei and on the mitotic spindle and stated that in hybrids of several plants, parental genomes are separated in the nuclei of differentiated tissues. Genome separation was also found in somatic cell hybrids in animals (Rechsteiner and Parsons, 1976; Zelesco and Marshall-Graves, 1988) and plants (Gleba et al., 1987), in at least some differentiated cell types of mouse (*Mus musculus* × *M. spretus*) hybrids (Mayer et al., 2000a), and in nuclei formed upon fusion of human sperm with golden hamster eggs (Brandriff et al., 1991). Separation of the paternal and maternal chromosome sets is not limited to the cells of hybrid organisms or cultured hybrid cells, but was also observed to be maintained for several cell cycles following fertilisation in mouse embryos (Odartchenko and Keneklis, 1973; Mayer et al., 2000b).

S. cerevisiae and *S. paradoxus* (syn. *S. douglasii*) (Naumov and Naumova, 1990) are closely related yeast species with an estimated genome divergence of about 8-20% determined by DNA sequence comparison of certain coding and noncoding sequences (Herbert et al., 1988; Adjiri et al., 1994; Chambers et al., 1996). Natural and artificial hybrids are viable, but practically sterile (Hawthorne and Philippsen, 1994; Hunter et al., 1996). Nevertheless, rare progeny must occur since natural introgression was observed (Naumov et al., 1997). Here we show that nuclear DNA sequences of the sibling species of the genus *Saccharomyces* are sufficiently diverse to elicit differential labelling by fluorescence in situ hybridisation (FISH) with genomic DNA of *S. cerevisiae* and *S. paradoxus.*

Yeasts of the genus *Saccharomyces* have a primarily diplontic life cycle, but undergo meiosis and form haploid spores upon starvation. Spores of complementary mating types (**a** and α) can conjugate whereby cell fusion is directly followed by karyogamy (Byers, 1981). The diploid nucleus of the zygote replicates its DNA and enters a mitosis, the result of which is a bud, the first cell of the diploid generation (reviewed by Marsh and Rose, 1997).

Here we used GISH to study the behaviour and redistribution of the parental genomes in zygotes and subsequent cells of *Saccharomyces cerevisiae* × *S. paradoxus* hybrids to ask whether the phenomenon of genome separation exists in yeasts. We also investigated the pairing of the differentially

Table 1. Strains used in this study

Strain no.	Species	Relevant genotype	Source/reference
SK1	*S. cerevisiae*	*MAT***a**/*MAT*α, *HO*/*HO*	Kane and Roth, 1974
Y55	*S. cerevisiae*	*MAT***a**/*MAT*α, *HO*/*HO*	McCusker and Haber, 1988
N17	*S. paradoxus*	*MAT***a**/*MAT*α, *HO*/*HO*	Naumov and Naumova, 1990
NKY857	*S. cerevisiae*	*MAT***a**, *ho*::*LYS2, lys2, leu2*::*hisG, his4X, ura3*	N. Kleckner*
N17-41	*S. paradoxus*	*MAT*α, *ho*Δ, *lys2, ura3, can1, cyh2-1*	S. Chambers and R. Borts*
NHD47	Hybrid	*MAT***a** *his4-RI leu2*Δ*/Mat*α *HIS4 LEU2*; *ho*Δ*Pst trp1-bsu36 ade8-1/ho*Δ*Pst TRP1 ADE8; met13-4 CYH2/MET13 cyh2-1; can1 ura3-nco/CAN1 ura3-1*	Hunter et al., 1996
SLY2006	Hybrid	*MAT***a**/*MAT*α, *ho*::*LYS2*/*ho*Δ, *lys2*/*lys2, leu2*::*hisG*/*LEU2, his4X*/*HIS4, ura3*/*ura3, CAN1*/*can1*, *CYH2*/cyh2-1	NKY857 × N17-41, this paper
SKC5	*S.cerevisiae*, addition strain	*MAT***a**/*MAT*α Trisomic; one chromosome *IV* from *S. paradoxus*	S. Chambers and R. Borts*
SCD22	*S.cerevisiae*, substitution strain	*MAT***a** *HML*::*ADE1 his4-r leu2-r thr4-a*/*MAT*α *HML HIS4 LEU2 THR4, KAR1*/*kar1-Δ13, ade1-1*/*ade1-1, can1-1 ura3-n*/*CAN1 ura3-n, met13-4 CYH2*/*met13-4 cyh2-1, lys2-d*/*LYS2*; *S. paradoxus* chromosome *III*	Chambers et al., 1996
SLY2007	*S.cerevisiae*, substitution strain	*MAT***a**/*MAT*α, *ade1-1*/*ade1-1, ura3-n*/*ura3-n, met13-4*/*met13[*YCp50*(HO, URA3)]* Both chromosomes *III* from *S. paradoxus*	This paper

*For location, see Materials and Methods.

labelled genomes in meiosis. Moreover, a trisomic chromosome addition strain (with one chromosome of *S. paradoxus* added to a diploid set of *S. cerevisiae*) and a substitution strain (with two chromosomes *III* of *S. paradoxus* replacing both their homoeologous chromosomes in an otherwise pure *S. cerevisiae* background) were used to trace individual chromosomes in interphase by GISH.

Materials and Methods

Yeast strains

The yeast strains used in this study are listed in Table 1. Strains Y55, N17, N17-41, NHD47, SKC5 and SCD22 were gifts from Rhona Borts (University of Leicester, UK). NKY857 was kindly provided by Nancy Kleckner (Harvard University, USA).

Cell culture and preparation

Vegetative cells were obtained by culturing the strains in liquid YPD at 30°C. For meiotic cell preparations, cultures were grown to a density of 2×10^7 cells/ml in YPA. The cells were collected by centrifugation and resuspended in 2% (w/v) potassium acetate at a density of 4×10^7 cells/ml. The resulting cell suspension was incubated shaking at 30°C to induce meiosis. For obtaining zygotes, dense suspensions of cells of opposite mating types were thoroughly mixed by vortexing and ultrasonication, put on YPD plates and incubated for 3 hours. Progress of mating was monitored via phase-contrast microscopy every 30 minutes until zygotes reached the desired developmental stage (normally after 3-5 hours).

Cells were collected from liquid cultures or plates and spheroplasted with Zymolyase 100T (140 μg/ml; Seikagaku, Tokyo) or with Zymolyase 100T plus Novozym 234 (70 μg/ml; Sigma, St Louis, MO) in 0.8 M sorbitol supplemented with 10 mM DTT. Spheroplasting of hybrid strains worked better if cells had been killed by washing in 1 mM NaN_3, 50 mM NaF, 10 mM EDTA in 0.9% NaCl-solution. Spheroplasting was terminated by adding 10 volumes of ice-cold 1 M sorbitol. Cells were pelleted and resuspended at a concentration of 4×10^8 cells/ml. This suspension was then mixed with detergent and fixative on a slide for spreading the cells [procedure B (Jin et al., 2000)]. For some mitotic nuclei and zygotes we applied the detergent after fixation [semi-spreading procedure C (Jin et al., 2000)]. For detailed protocols on the preparation of yeast nuclei see Loidl et al., 1998 (Loidl et al., 1998).

For the preparation of morphologically well-preserved zygotes amenable to in situ hybridisation, cells were spheroplasted, fixed with formaldehyde, put on slides, dried and postfixed with methanol and acetone as described previously (Gotta et al., 1996).

Genomic in situ hybridisation (GISH)

For GISH, genomic DNA was isolated from *S. cerevisiae* SK1 and from the *S. paradoxus* N17 strain. A plasmid containing the *S. cerevisiae* Ty1 retrotransposon (Boeke et al., 1985) and a pool of 36 PCR products of mostly single copy sequences from the left arm of chromosome *IV* of *S. cerevisiae* (J.F., A.L. and J.L., unpublished) were also used as probes. Finally, probes for the nucleolar organiser regions were generated. For this, total rDNA repeats from both species were amplified using oligos 5′-GTGCATGGCCGTTCTTAGTTGG-3′ and 5′-GCGCTTACTAGGAATTCCTCG-3′ as primers by long-range PCR (Expand Long Template PCR System, Roche Diagnostics, Mannheim, Germany). Probes were labelled by nick translation with Biotin-21-dUTP (Clontech Laboratories, Palo Alto, CA), Biotin-14-dATP (Invitrogen, Carlsbad, CA), Digoxigenin-11-dUTP, FITC-12-dUTP (Roche Diagnostics) or Cy3-dUTP (Amersham Pharmacia, Little Chalfont, UK) as described (Loidl et al., 1998).

Labelled probes were dissolved in hybridisation solution (50% formamide, 10% dextran sulfate, 2× SSC) to a final concentration of ~10 ng/μl for single copy and Ty FISH probes, ~30 ng/μl for genomic probes in hybrids, and ~50 ng/μl for genomic probes in substitution and addition lines. After 5 minutes of denaturation at 95°C the probes were dropped onto the slides, denatured for 10 minutes at 80°C and hybridised for at least 36 hours at 37°C. For FISH with total genomic DNA, the disproportionally strong hybridisation to rRNA gene tracts was blocked by adding unlabelled rDNA in ~10-fold excess. Post hybridisation washes were carried out in 50% formamide in 2× SSC (37°C), 2× SSC (37°C) and 1× SSC (room temperature) for 5 minutes each. Subsequently, biotinylated probes were detected using FITC-conjugated avidin (Sigma) and Digoxigenin-11-dUTP labelled probes were detected by anti-Digoxigenin-Rhodamine (Roche Diagnostics). Finally, slides were mounted under a coverslip in Vectashield mounting medium for fluorescence (Vector Laboratories, Burlingame, CA) supplemented with 1 μg/ml DAPI (4′ 6-diamidino-2-phenylindole) as a DNA-specific counter-stain.

Microscopy and evaluation

After FISH and detection preparations were evaluated using a Zeiss

Axioskop epifluorescence microscope equipped with single-band-pass filters for the excitation of red, green and blue. Images of high magnification were obtained using a cooled black and white CCD camera controlled by IPLab Spectrum software (Scanalytics, Fairfax, VA) or the ISIS imaging system (MetaSystems, Altlussheim, GER).

Results

Differential FISH labelling of the *S. cerevisiae* and *S. paradoxus* genomes

To test the feasibility of GISH in yeast, we hybridised differently labelled *S. cerevisiae* and *S. paradoxus* genomic DNA probes to preparations that contained a ~1:1 mixture of cells of the two species. We found a distinct labelling of individual nuclei with either the one or the other probe (Fig. 1A), which demonstrates that the two genomes can be discriminated by in situ hybridisation. We next performed GISH on hybrid nuclei and found that *S. cerevisiae* and *S. paradoxus* probes produce a mosaic pattern (Fig. 1B) (see below).

To investigate to what degree diverged single copy or repetitive Ty1 sequences contribute to the genome-specific hybridisation signals obtained, separate hybridisations were performed with these two genome fractions. First, a *S. cerevisiae* Ty1 probe was hybridised to preparations containing equal proportions of *S. cerevisiae* and *S. paradoxus* cells. Half of the nuclei exhibited a speckled FISH pattern whereas the others were virtually devoid of Ty1 signals (Fig. 1C), which indicates preferential hybridisation of the Ty1. Those nuclei that were devoid of the Ty1 signal were labelled by FISH with *S. paradoxus* genomic probe (Fig. 1C). This confirmed that the Ty1 probe indeed recognises *S. cerevisiae* nuclei. In order to test whether nonrepetitive sequences are able to elicit differential FISH signals, we used a pool of 36 PCR products of mostly single copy sequences from the left arm of *S. cerevisiae* chromosome *IV*. This compound probe produced a strong and a weak linear signal in hybrid nuclei (Fig. 1D), which demonstrates that differences in single copy sequences contribute significantly to the discrimination of the two genomes by GISH. It also shows that in the hybrid (which had been kept in culture for at least 50 generations) this 450 kb region had maintained its integrity.

Hybridisation with genomic DNAs was particularly strong at rDNA regions where the signal outshone the remainder of

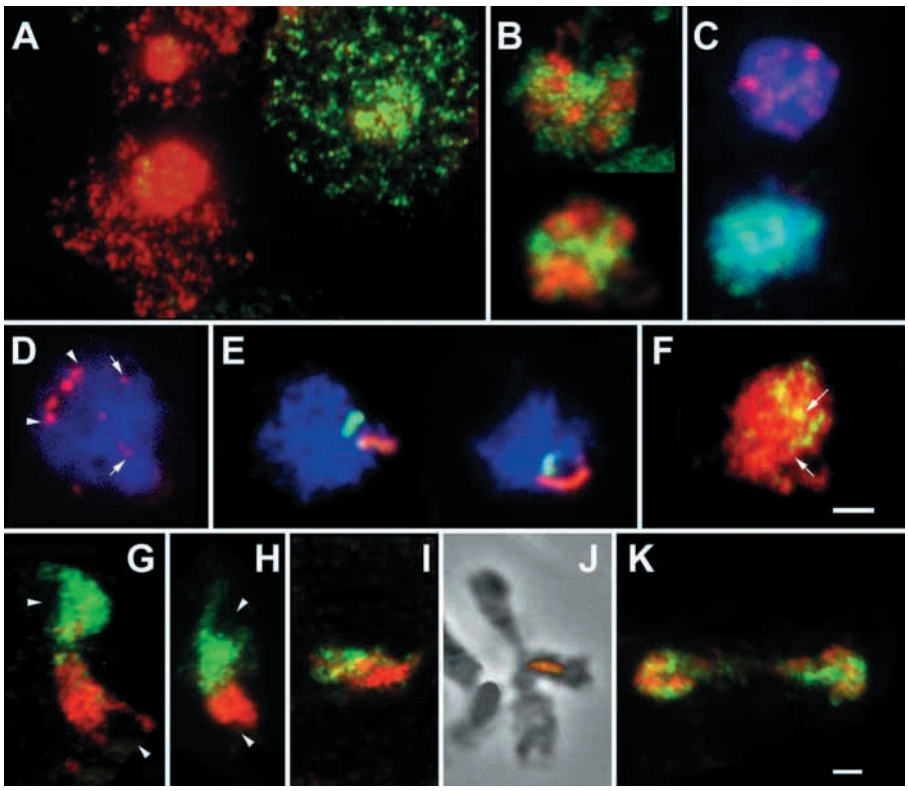

Fig. 1. Differential staining of *S. cerevisiae* and *S. paradoxus* genomes by FISH with DNA probes from the two species. Red, *S. cerevisiae* DNA; green, *S. paradoxus* DNA; blue, DAPI-counterstaining of unlabelled DNA regions. (A) Cells from a mixed *S. cerevisiae*-*S. paradoxus* culture. The nuclei are differently marked by simultaneous hybridisation with total genomic DNA probes from the respective species. The halos around the nuclei stem from the differential staining of mitochondria by hybridisation with species-specific mitochondrial DNA sequences that were contained in the probes. (B) *S. cerevisiae* × *S. paradoxus* nuclei show a mosaic hybridisation pattern after simultaneous FISH with total genomic DNA from the two parental species, indicating the intermixing of the chromosome complements. (C) Differential labelling of the nuclei of a mixed culture with a Ty1 probe from *S. cerevisiae* (red) and genomic DNA from *S. paradoxus* (green). Only *S. cerevisiae* nuclei are labelled with the Ty1 probe. (D) FISH of a composite single sequence probe (covering most part of the left arm of chromosome *IV*) from *S. cerevisiae* to hybrid nuclei. Each nucleus contains a strong (arrowheads) and a weak signal (arrows), corresponding to *S. cerevisiae* and *S. paradoxus* chromosomes *IV*, respectively. This demonstrates that species-specific single sequence probes cross-hybridise only weakly. (E) Hybrid nuclei simultaneously hybridised with rDNA probes from the two parental species. The two rDNA tracts are differentially stained, which indicates the high species-specificity of the rDNA sequences. (F) Trisomic addition strain of *S. cerevisiae* with an additional chromosome *IV* from *S. paradoxus*. The *S. paradoxus* chromosome occupies a distinct oblong territory (arrows) that is delineated by the *S. paradoxus* probe. The *S. cerevisiae* probe highlights the remainder of the nucleus. (G-K) Karyogamy and mixing of the parental genomes in hybrid *S. cerevisiae* × *S. paradoxus* zygotes as seen by GISH. The unstained sectors of nuclei (arrowheads) mark the sites of nucleoli where hybridisation of labelled probes was blocked by excess unlabelled rDNA. (G) Haploid parental nuclei in a zygote. (H) Zygote containing a diploid hybrid nucleus after karyogamy. The two genomes are still spatially separated. (I) Incipient intermixing of genomes in a nucleus whose shape suggests that mitosis has started. (J) Overview of a budding zygote at lower magnification with the nucleus in mitosis (similar stage as in I). Phase contrast picture of the entire zygote was merged with the FISH image of the nucleus. (K) Anaphase of a zygote nucleus with the genomes completely mixed. Bar, 2 μm (in F, for A-F); 2 μm (in K, for G-I,K).

the nucleus. To reveal the signals at the nuclear DNA, rDNA hybridisation was suppressed by addition of an excess of unlabelled rDNA to the hybridisation probe. Species-specific rDNA probes produced differential staining of the two NORs in diploid hybrid nuclei (Fig. 1E). Thus, rDNA repeats seem to have undergone a similar degree of divergence between the two species, as did the genome as a whole.

The genomic DNA extracted from the two species also contained mitochondrial DNA. In semi-spread preparations of cells, nuclei were surrounded by DAPI-bright mitochondria that upon GISH showed the same species-specific labelling as the corresponding nuclei (Fig. 1A).

The relative positioning of the two genomes in hybrid nuclei

GISH on *S. cerevisiae* × *S. paradoxus* hybrids showed that the parental chromosome complements are intermingled, with hybrid nuclei containing a mosaic of red and green patches (Fig. 1B). In the 30 well preserved nuclei that were scored, none of the parental chromatin sets formed a single contiguity. This contrasts with reports on the separation of parental genomes in the nuclei of hybrid plants and somatic cell hybrids. Since zygotic genome separation was reported from mammalian embryos, we wanted to see whether a newly formed hybrid nucleus would maintain parental genome separation for some time. We mated *S. cerevisiae* and *S. paradoxus* cells of opposite mating types on plates and prepared zygotes at different stages of karyogamy for GISH. In two-nucleate zygotes the parental nuclei could be well differentiated (Fig. 1G). Immediately after karyogamy the two genomes were separate and the nucleoli were situated at the distal ends of the elongated nucleus (Fig. 1H). The position of the nucleoli suggests that the two chromosome sets are facing each other with their centromeres since the nucleolus and centromeres occupy opposite poles of nuclei (Jin et al., 2000). Moreover, during karyogamy nuclei fuse at the regions of the spindle pole bodies (SPBs) (Marsh and Rose, 1997) to which the centromeres are attached (Jin et al., 2000). Fused elongated zygote nuclei that had their longitudinal axis oriented transversal to the long axis of the zygote showed the beginnings of intermixing of the parental chromosome sets (Fig. 1I). FISH on zygotes with well-preserved cell walls confirmed that these oblong nuclei represent early mitotic nuclei that just enter or pass through the neck of the zygotic bud (Fig. 1J). This suggests that intermixing starts with the onset of zygotic mitosis. By the end of the zygotic mitosis, the mingling of chromosomes was virtually complete (Fig. 1K).

Delineation of individual chromosomes in addition and substitution strains

GISH on nuclei of addition strains or substitution strains was applied to delineate entire chromosomes. To this end we used or constructed *S. cerevisiae* strains in which an additional chromosome *IV* from *S. paradoxus* was present (strain SKC5) or where both authentic chromosomes *III* were replaced by their *S. paradoxus* homoeologs (strain SLY2007). Hybridisation of these strains with *S. paradoxus* genomic DNA clearly delineated the *S. paradoxus* chromosomes in many of the interphase nuclei (Fig. 1F). This indicates a territorial organisation of yeast chromosomes in at least some stages of interphase.

Observations in meiosis

It was reported that meiotic recombination between an *S. cerevisiae* and *S. paradoxus* homeologous chromosome *III* pair is decreased by 25-fold compared with homologs in *S. cerevisiae* (Chambers et al., 1996) and that spore viability in the complete hybrid is only 1% (Hunter et al., 1996). We therefore wanted to study whether and how the homoeologous genomes pair in hybrid meiosis. Since the strain in which the genetical studies had been performed turned out to be unfavourable for meiotic cytology, we carried out our investigation in the hybrid strain SLY2006 with SK1 as the *S. cerevisiae* parent. In this strain spore viability was 7% (10 of 144). We prepared whole mount spreads of synaptonemal complexes (SCs) and investigated them by immunostaining of the SC component Zip1 (Sym et al., 1993), by electron microscopy and by GISH. Zip1 is a part of the transversal filaments and it is present between (homologously and nonhomologously) synapsed regions of chromosomes at zygotene and pachytene of meiosis (Sym et al., 1993). Immunostaining of hybrid nuclei showed several long individual threads of Zip1 indicating extensive synapsis (Fig. 2A). Electron microscopy of silver-stained synaptonemal complexes produced a more complex image, since it reveals not only the synapsed chromosome regions but also unpaired axial elements at pairing partner switches. It was found that, unlike in non-hybrid pachytenes, the axes of many chromosomes engaged in synapsis with changing partners (Fig. 2B). This promiscuous behaviour was also observed in pachytene SCs of the hybrid strain NHD47 that had been studied by Hunter et al. (Hunter et al., 1996). There are two possible explanations for the switching of synaptic partners. First, chromosomes of the two species might not be co-linear (i.e. regions homologous to a single chromosome in one species are dispersed over several chromosomes in the other), so that chromosomes have to switch partners in order to achieve homologous synapsis. This possibility is considered unlikely (see Discussion). Alternatively, synapsis could occur between nonhomologous regions. GISH on pachytene nuclei showed that not only green and red genome portions were associated (Fig. 2C), but occasionally there were two chromosome regions of the same color paired (Fig. 2D). This indicates that nonhomologous pairing of chromosomes or chromosome regions within the same species does occur. It further suggests that the pairing of *S. cerevisiae* and *S. paradoxus* chromosomes may also be at least partially heterologous.

In pachytene nuclei of chromosome addition and substitution strains, chromosomes derived from *S. paradoxus* could be delineated. In contrast to the hybrid, meiotic pairing was undisturbed and the synapsis of *S. paradoxus* chromosomes *III* in the *S. cerevisiae* background was normal (Fig. 2E).

Discussion

The genomes of *S. cerevisiae* and *S. paradoxus* can be discriminated by GISH

In GISH experiments performed in plants and animals, the differentiation of the parental genomes is mainly caused

by species-specific dispersed repetitive DNA sequences (Anamthawat-Jónsson et al., 1990). Here we show that unique sequences of *S. cerevisiae* and *S. paradoxus* are sufficiently divergent to discriminate the two genomes by GISH. In the two yeast species analysed, DNA reassociation kinetics had suggested the nuclear DNA heterology to be as high as ~50% (Vaughan Martini, 1989). On the basis of DNA hybridisations the sequence divergence between *S. cerevisiae* and *S. douglasii* (which is regarded as a variety of *S. paradoxus*) was estimated to be 30-40% (Hawthorne and Philippsen, 1994). By contrast, the *ARG4* and the *YSD83* coding regions of *S. paradoxus* differ from their *S. cerevisiae* homologs only by 8.1% and 12.5%, respectively. The noncoding regions are less conserved, with small AT-rich insertions/deletions and 20% base substitutions (Adjiri et al., 1994). Our data demonstrate that this diversity of the two genomes is sufficient for the unambiguous discrimination of the two genomes within hybrid nuclei.

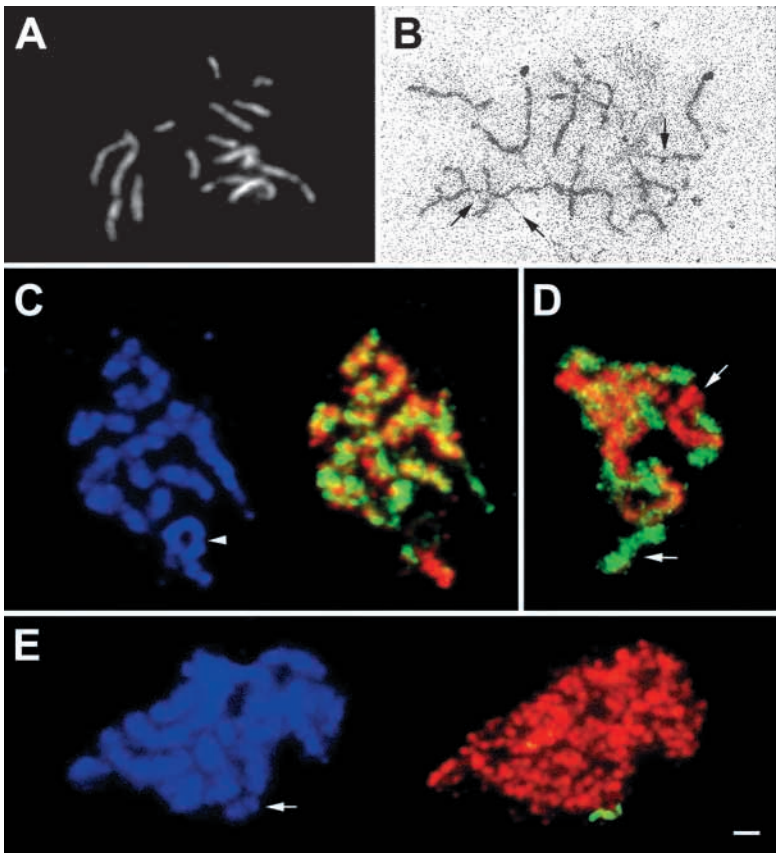

Fig. 2. (A-D) Meiotic pairing in pachytene nuclei of the hybrid SLY2006. (A) Extensive stretches of SC delineated by anti-Zip1 immunostaining. (B) Electron microscopy of silver-stained nuclei shows that the synapsed chromosome portions are connected by axial elements (arrows) and that most chromosomes are engaged in multiple pairing partner switches. (C,D) GISH on spread pachytene nuclei. (C) Pairing between *S. cerevisiae* and *S. paradoxus* chromosome regions prevails as most red and green threads are running side-by-side; (left) corresponding DAPI image. The rDNA tract, whose labelling is blocked in GISH is clearly visible (arrow). (D) In this nucleus uniformly red or green staining structures (arrows) indicate synapsis of chromosomes from one and the same genome. (E) Pachytene of the diploid chromosome *III* substitution strain SLY2007. The chromosome *III* bivalent is painted green with *S. paradoxus* genomic DNA; (left) corresponding DAPI image. Arrow denotes the bivalent. Bar, 2 μm.

Pulsed field-gel electrophoresis showed that chromosome number and sizes are largely conserved between the two species (Naumov et al., 1992; Hunter et al., 1996). Moreover, all of 15 genes investigated located to the corresponding chromosomes in the two species (Naumov et al., 1992; Hunter et al., 1996). Likewise, Southern hybridisation on electrophoretic karyotypes with probes from close to the centromere and one from near each end of each of the 16 chromosomes demonstrated that the *S. paradoxus* karyotype is colinear (i.e. they show no detectable translocations) with that of *S. cerevisiae* (Fischer et al., 2000). The fact that *S. cerevisiae* strains in which the authentic chromosome *III* is replaced by the corresponding *S. paradoxus* chromosome grow vigorously under laboratory conditions [(Chambers et al., 1996) and this study], demonstrates that the genes on this chromosome can substitute for the roughly 200 genes on *S. cerevisiae* chromosome *III*, and confirms the colinearity (synteny) of the chromosomes between the two species. However, recombination between the homoeologous genomes is low, as was shown by the high frequencies of aneuploidy and low frequencies of genetic exchange of the rare offspring of hybrids (Hunter et al., 1996).

Yeast hybrid nuclei do not show separation of parental genomes

We co-cultivated haploid *S. cerevisiae* and *S. paradoxus* strains of opposite mating types to obtain hybrid zygotes. In preparations from these cultures, we found that even the earliest diploid nuclei (except those in the zygotes themselves) had the two genomes arranged at random. This indicates that their intermixing starts during or soon after karyogamy; either in the zygote or during the first mitosis. Hence also the nuclei of *S. cerevisiae* × *S. paradoxus* hybrids that had been in culture for extended periods contained entirely intermixed parental chromosome sets. The rapid randomisation of chromosome distribution is in contrast to the separation of parental genomes that has been observed in a wide range of hybrid cell types and organisms. Genome separation was described in several hybrid plants and in cultured hybrid cells (see Introduction).

Since the different genomes in yeast hybrids appear to mix during or immediately after karyogamy, it is reasonable to assume that the chromosomes in normal (non-hybrid) matings will also intermingle. This is different from fertilisation in animals, where it was shown that the parental genomes remain spatially separated for several cell generations following the zygote (Odartchenko and Keneklis, 1973; Mayer et al., 2000c). Sperm chromatin undergoes extensive remodelling and modification that causes its transition from the densely packed and transcriptionally inactive state in the sperm to an open chromatin configuration in the male pronucleus (e.g. Brandriff et al., 1991). This remodelling is accompanied by rapid DNA demethylation (Mayer et al., 2000b) and probably other epigenetic modifications, which render the parental genomes in the zygote structurally and transcriptionally different (Vielle-Calzada et al.,

2000). Spatial separation may help to maintain these differences. Continued inactivation of one parental genome could be of functional significance since silencing of one genome will extend a functionally haploid state in diplontic or diplohaplontic organisms, in which the haplophase is the only period when a defective recessive allele can be efficiently selected against (Vielle-Calzada et al., 2000). However, this requirement does not apply to yeast, as this organism normally forms several haploid cell generations after sporulation.

Yeast chromosomes occupy distinct territories in interphase

In metazoans and plants individual chromosomes occupy well-separated regions of the interphase nucleus. This territorial organisation of chromosomes has been proposed to be important for the functional compartmentalisation of the cell nucleus (Cremer et al., 1993). In yeast, the occurrence of ectopic mitotic recombination events between loci within or between chromosomes at similar frequencies has led to the interpretation that this organism lacks chromosome territories (Haber and Leung, 1996). The observation that ectopic recombination is efficient suggests that chromatin fibers are loosely packaged and intermix with chromatin of other chromosomes. However, by using GISH, we observed dense, mutually exclusive stained areas in hybrid nuclei (Fig. 1B) and distinct domains for individual chromosomes in addition and substitution strains (Fig. 1F). This provides evidence that in interphase nuclei of budding yeast there exist chromosome territories similar to those in higher eukaryotes.

Meiotic pairing in the hybrid is partially random

In meiosis of the hybrid, chromosomes do not pair as bivalents but they are engaged in pairing partner switching that produces multivalents (Fig. 2B). Since the karyotypes of the two species do not seem to differ by multiple translocations (see above) the synaptic switches do not reflect pairing of homologous regions dispersed over different chromosomes. These switches are rather due to nonhomologous synapsis, as is, for instance, also found in the meiosis of haploid yeast, where chromosomes lack homologous partners (Loidl et al., 1991). The occurrence of nonhomologous synapsis is also supported by the observation of occasional pairing between chromosomes of one and the same parental set (Fig. 2D).

In the budding yeast, SC formation depends on the initiation of recombination (Alani et al., 1990; Padmore et al., 1991) and seems to initiate at sites where recombination has occurred (Agarwal and Roeder, 2000). In the absence of homologous chromosomes recombination tends to occur between minor ectopic homologies and promotes the formation of nonhomologous SC (Loidl and Nairz, 1997). Since in the *S. cerevisiae* × *S. paradoxus* hybrids there is considerable pairing of nonhomologous chromosomes, it appears that sequence homology between the parental genomes is not sufficient to support exclusive homoeologous recombination and synapsis. Thus one could speculate that homoeologous recombination is rarely initiated and/or it does not progress to a stage that promotes homoeologous synapsis. While the NHD47 hybrid produces only 1.2% viable spores in which genetic exchange is reduced and aneuploidy is high, hybrids lacking the mismatch repair genes *PMS1* or *MSH2* are improved with respect to spore viability and recombination and segregation (Hunter et al., 1996). Chambers et al. (Chambers et al., 1996) and Hunter et al. (Hunter et al., 1996) proposed that recombination that initiates between regions of inadequate homology (e.g. the homoeologous chromosomes of the hybrid) is abolished by the mismatch repair system. It will be interesting to test whether the increase in homoeologous crossing over after disruption of *PMS1* or *MSH2* is accompanied by more extensive homoeologous synapsis.

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