

DNA structural alterations can occur in the human genome and that these alterations can demarcate the precise boundaries of sites of recurrent chromosomal breakage. We have also provided evidence that the RAG complex is responsible for the t(14;18) translocation *in vivo* and *in vitro* and that its role here is different from ones invoked by previously described mechanisms. □

Methods

Plasmid construction and V(D)J recombination assay

The plasmid constructs were made by modifying the SV40-based plasmid, pGG51 (ref. 8). See Supplementary Methods.

Transfection of the 293T cells with pXW5 or pSCR45 along with full-length RAG1/2 (Fig. 1), mutant RAG1/full-length RAG2 (Fig. 1 and Table 1) or core RAG1/2 (Table 1) was done using the calcium-phosphate method as described earlier⁹. The coordinates of the core RAGs are the same as those used for protein production below. Plasmid DNA was alkaline harvested and analysed as described in the Supplementary Methods.

Bisulphite modification assay

The bisulphite modification assay was used as described previously¹¹ (see Supplementary Methods).

Ligation-mediated PCR

For details of the ligation-mediated PCR see Supplementary Methods.

In vitro RAG nicking assay

Core murine glutathione S-transferase (GST)–RAG1 (amino acids 330–1040) and GST–RAG2 (amino acids 1–383), or MBP murine core RAG1 and RAG2, or core RAG1 and full-length MBP RAG2 proteins were overexpressed in the human 293T cells and purified as previously described^{10,18}. See Supplementary Methods.

Received 18 October 2003; accepted 19 January 2004; doi:10.1038/nature02355.

1. Lieber, M. R. in *The Causes and Consequences of Chromosomal Aberrations* (ed. Kirsch, I.) 239–275 (CRC Press, Boca Raton, 1993).
2. Lewis, S. M. The mechanism of V(D)J joining: lessons from molecular, immunological and comparative analyses. *Adv. Immunol.* **56**, 27–150 (1994).
3. Dalla-Favera, R. in *The Causes and Consequences of Chromosomal Aberrations* (ed. Kirsch, I. R.) 313–332 (CRC Press, Boca Raton, 1993).
4. Korsmeyer, S. J. Chromosomal translocations in lymphoid malignancies reveal novel proto-oncogenes. *Annu. Rev. Immunol.* **10**, 785–807 (1992).
5. Tycko, B. & Sklar, J. Chromosomal translocations in lymphoid neoplasia: A reappraisal of the recombinase model. *Cancer Cells* **2**, 1–8 (1990).
6. Raghavan, S. C., Kirsch, I. R. & Lieber, M. R. Analysis of the V(D)J recombination efficiency at lymphoid chromosomal translocation breakpoints. *J. Biol. Chem.* **276**, 29126–29133 (2001).
7. Jager, U. *et al.* Follicular lymphomas BCL-2/IgH junctions contain templated nucleotide insertions: novel insights into the mechanism of t(14;18) translocation. *Blood* **95**, 3520–3529 (2000).
8. Gauss, G. H. & Lieber, M. R. Mechanistic constraints on diversity in human V(D)J recombination. *Mol. Cell. Biol.* **16**, 258–269 (1996).
9. Schwarz, K. *et al.* RAG mutations in human B cell-negative SCID. *Science* **274**, 97–99 (1996).
10. Swanson, P. C. The DDE motif in RAG-1 is contributed in trans to a single active site that catalyzes the nicking and transesterification steps of V(D)J recombination. *Mol. Cell. Biol.* **21**, 449–458 (2001).
11. Yu, K., Chedin, F., Hsieh, C.-L., Wilson, T. E. & Lieber, M. R. R-loops at immunoglobulin class switch regions in the chromosomes of stimulated B cells. *Nature Immunol.* **4**, 442–451 (2003).
12. Gough, G. W., Sullivan, K. M. & Lilley, D. M. The structure of cruciforms in supercoiled DNA: probing the single-stranded character of nucleotide bases with bisulphite. *EMBO J.* **5**, 191–196 (1986).
13. Tevelev, A. & Schatz, D. G. Intermolecular V(D)J recombination. *J. Biol. Chem.* **275**, 8341–8348 (2000).
14. Santagata, S. *et al.* The RAG1/RAG2 complex constitutes a 3' flap endonuclease: implications for junctional diversity in V(D)J and transpositional recombination. *Mol. Cell* **4**, 935–947 (1999).
15. Ramsden, D. A., McBlane, J. F., van Gent, D. C. & Gellert, M. Distinct DNA sequence and structure requirements for the two steps of V(D)J recombination signal cleavage. *EMBO J.* **15**, 3197–3206 (1996).
16. Besmer, E. *et al.* Hairpin coding end opening is mediated by the recombination activating genes RAG1 and RAG2. *Mol. Cell* **2**, 817–828 (1998).
17. Ma, Y., Pannicke, U., Schwarz, K. & Lieber, M. R. Hairpin opening and overhang processing by an Artemis:DNA-PKcs complex in V(D)J recombination and in nonhomologous end joining. *Cell* **108**, 781–794 (2002).
18. Yu, K. & Lieber, M. R. The nicking step of V(D)J recombination is independent of synapsis: implications for the immune repertoire. *Mol. Cell. Biol.* **20**, 7914–7921 (2000).

Supplementary Information accompanies the paper on www.nature.com/nature.

Acknowledgements We thank S. J. Korsmeyer for guidance during the early phases of this work. We also thank I. Haworth, J. S. Lee, P. Chastian and D. Shibata for discussions of the work, and NIH for grants to M.R.L.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to M.R.L. (lieber@usc.edu).

Tension between two kinetochores suffices for their bi-orientation on the mitotic spindle

Hilary Dewar¹, Koza Tanaka¹, Kim Nasmyth² & Tomoyuki U. Tanaka¹

¹School of Life Sciences, University of Dundee, Wellcome Trust Biocentre, Dundee DD1 5EH, UK

²Research Institute of Molecular Pathology, Dr Bohr-Gasse 7, A-1030 Vienna, Austria

The movement of sister chromatids to opposite spindle poles during anaphase depends on the prior capture of sister kinetochores by microtubules with opposing orientations (amphitelic attachment or bi-orientation)¹. In addition to proteins necessary for the kinetochore–microtubule attachment, bi-orientation requires the Ipl1 (Aurora B in animal cells) protein kinase^{2–7} and tethering of sister chromatids by cohesin^{8,9}. Syntelic attachments, in which sister kinetochores attach to microtubules with the same orientation, must be either ‘avoided’ or ‘corrected’. Avoidance might be facilitated by the juxtaposition of sister kinetochores such that they face in opposite directions; kinetochore geometry is therefore deemed important. Error correction, by contrast, is thought to stem from the stabilization of kinetochore–spindle pole connections by tension in microtubules, kinetochores, or the surrounding chromatin arising from amphitelic but not syntelic attachment^{10,11}. The tension model predicts that any type of connection between two kinetochores suffices for efficient bi-orientation. Here we show that the two kinetochores of engineered, unreplicated dicentric chromosomes in *Saccharomyces cerevisiae* bi-orient efficiently, implying that sister kinetochore geometry is dispensable for bi-orientation. We also show that Ipl1 facilitates bi-orientation by promoting the turnover of kinetochore–spindle pole connections in a tension-dependent manner.

Evidence for an error correction mechanism facilitating bi-orientation has been hitherto confined to meiotic cells, where homologous kinetochores are connected by chiasmata, whose remoteness from the sites of microtubule attachment precludes a key role for kinetochore geometry. Thus, in grasshopper spermatocytes whose kinetochores and spindle poles are repeatedly connected and disconnected by microtubules until they bi-orient, the kinetochore-to-pole connections of a ‘maternal’ chromosome can be stabilized by using a glass needle to pull on the ‘paternal’ chromosome attached to it^{10,11}. If bi-orientation were established in mitotic cells by a similar tension-sensitive error correction mechanism, then any kind of connection between two kinetochores would suffice for their bi-orientation, including two kinetochores situated on the same chromatid. We therefore set out to analyse the behaviour of dicentric minichromosomes that are unable to replicate during S phase.

We addressed this issue in the budding yeast *S. cerevisiae*, whose kinetochores possess only a single microtubule-binding site¹² and whose centromeres (the DNA underlying the kinetochore) have been pinpointed to sequences no longer than 120 base pairs (bp)¹³. Their activity can therefore be turned off by transcription from an adjacent promoter¹⁴. The behaviour of yeast centromeres can be followed by marking them with bacterial operators bound by repressor proteins fused to green fluorescent protein (GFP)^{8,15,16}. Traction caused by ‘amphitelic’ attachment overwhelms sister chromatid cohesion at centromeres, but not in the flanking sequences, which leads to their precocious separation before onset of anaphase. The tension so created causes centromeric chromatin to unravel, forming 10-nm (beaded nucleosomes) or even thinner fibres.

To address the behaviour of unreplicated dicentric chromosomes, we created a circular minichromosome carrying two centromeres, one constitutive and the other conditionally inactivated owing to its juxtaposition to the *GAL1-10* promoter (ref. 14 and Fig. 1a). The chromosome also possessed a single replication origin flanked by recombination sites for the *Zygosaccharomyces rouxii* R recombinase¹⁷ and contained a tandem array of *tet* operators that bind Tet repressor–GFP fusion proteins¹⁸. The host cell expressed the recombinase gene from the *MET3* promoter, which is transcribed only in the absence of methionine. The minichromosome was stably propagated as long as cells were grown in the presence of galactose, which inactivates one of the centromeres, and in the presence of methionine, which prevents expression of the recombinase.

Removal of methionine from the culture medium caused efficient recombination between recombination sites and the accumulation of minichromosomes lacking any replication origin (Fig. 1b, 2 × RS), leading to a rapid increase in cells born either without any minichromosome or with an unreplicated minichromosome (see below). The origin was not lost from minichromosomes containing only a single recombination site (Fig. 1b, 1 × RS). After most minichromosomes had lost their origins, galactose was replaced by glucose in the medium, which rapidly activated the second centromere of the minichromosome (Supplementary Note 1 and Fig. S1).

The behaviour of unreplicated dicentric (2 × CEN) and mono-

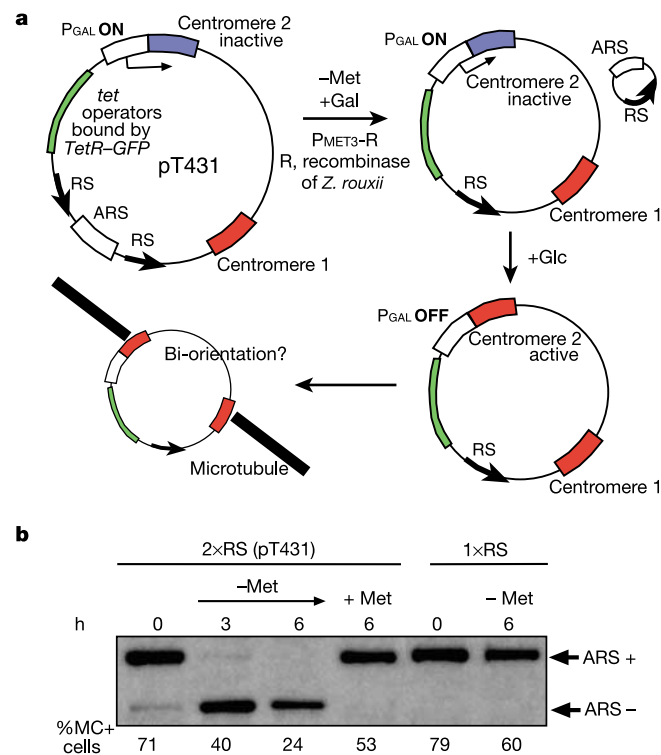


Figure 1 Making unreplicated dicentric minichromosomes. **a**, Scheme for making an unreplicated dicentric minichromosome (see text). After removing the DNA replication origin, the intervals between centromeres are 7.0 kb on the side containing *tet* operators and 4.2 kb on the other side. ARS, DNA replication origin; RS, recombination site; *P_{MET3}*, *MET3* promoter; *P_{GAL}*, *GAL1-10* promoter; Met, methionine; Gal, galactose; Glc, glucose. **b**, Southern blot analysis and percentage of cells containing minichromosomes. *TetR-GFP P_{MET3}-R* cells carrying pT431 or pT432 were incubated in medium with galactose either with (+Met) or without (-Met) methionine. The percentage of cells containing minichromosomes out of all cells observed (% MC+ cells) is shown for each time point. 2 × RS (pT431), DNA replication origin flanked by two recombination sites (see **a**); 1 × RS (pT432), DNA replication origin flanked by only one recombination site.

centric (1 × CEN) minichromosomes was observed by time-lapse microscopy. Both types of minichromosome were marked by the Tet repressor fused to GFP, and spindle pole bodies (SPBs) were marked by Spc42 fused to yellow fluorescent protein (YFP). During metaphase, the GFP signals that were associated with all 38 unreplicated monocentric minichromosomes remained in the close vicinity of one SPB (Fig. 2a, top). Although they changed their SPB partner on rare occasions (see Supplementary Note 5), they never spent any appreciable time in the space between the SPBs.

The GFP signals of all 34 unreplicated dicentric minichromosomes, by contrast, spent most of this period halfway between the two SPBs (Fig. 2a, bottom). They frequently moved vigorously back and forth along the axis connecting the SPBs, and most (75%) were stretched along this axis. The compaction¹⁹ of stretched operators was reduced between 6- and 30-fold, and was reduced even further in *yku80* deletion mutant cells (data not shown), as

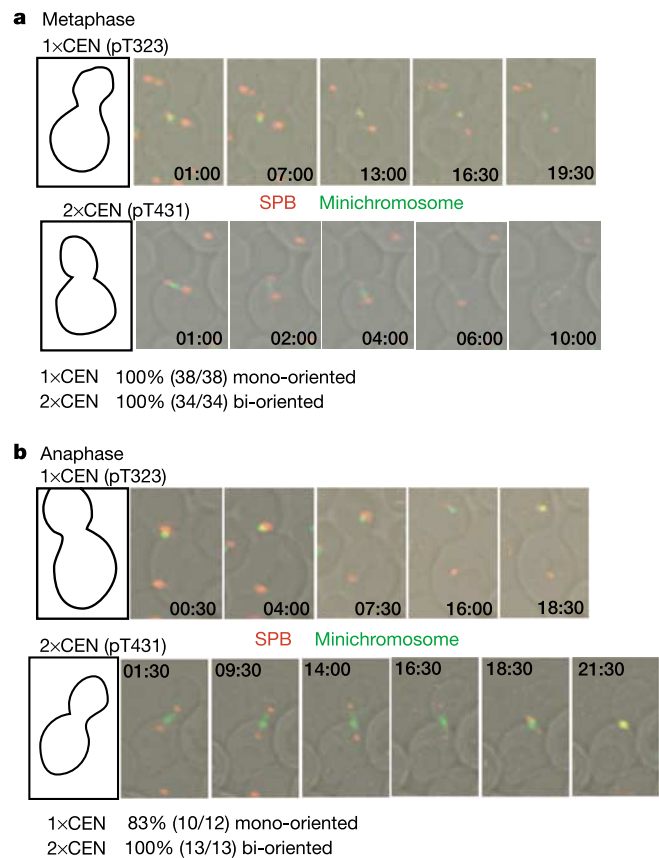


Figure 2 Behaviour of unreplicated monocentric and dicentric minichromosomes. **a**, Behaviour in metaphase. *TetR-GFP SPC42-YFP P_{MET3}-R* cells carrying pT323 (T2755, top) or pT431 (T3000, bottom) were incubated in the absence of methionine, first with galactose for 4 h and then with glucose. Time-lapse images were collected for 20 min at 30-s intervals, commencing 20 min after transfer to glucose medium. Shown are representative time-lapse images of cells with unreplicated minichromosomes. The time in minutes from the start of image acquisition is indicated. When both separated SPBs were in the mother cell, the cell was considered to be in metaphase. The behaviour of minichromosomes (mono-oriented or bi-oriented) was scored as described in Methods. When minichromosome behaviours did not belong to either category (less than 3% of total counts) or when the bipolar spindle was short (less than 1 μm) on a projected image, they were not scored. See also Supplementary Movie 2a. **b**, Behaviour in anaphase. T2755 and T3000 cells were treated as in **a**. When spindles were at least twice as long as those in metaphase at least at one time point, with one SPB in the bud and the other in the mother cell, the cell was considered to have initiated anaphase. The behaviour of unreplicated minichromosomes was scored as in **a**. See also Supplementary Movie 2b.

previously reported for replicated dicentric chromosomes²⁰. These behaviours of GFP signals emanating from unreplicated dicentric minichromosomes imply that their two kinetochores are simultaneously attached to opposite poles and pulled in opposite directions; in other words, they bi-orient on the spindle.

During anaphase, 10 out of 12 monocentric unreplicated minichromosomes remained in the vicinity of one of the two SPBs. These monocentric minichromosomes followed the SPB as it moved towards the cell cortex (Fig. 2b, top). For unknown reasons,

2 out of 12 monocentric minichromosomes did not segregate with an SPB. By contrast, all 13 dicentric unreplicated minichromosomes remained in the space between the SPBs on initiation of anaphase (Fig. 2b, bottom). Their GFP signals usually remained stretched and sometimes moved vigorously between SPBs. In most cells containing these dicentric minichromosomes, the SPBs started to segregate from each other but their movement was halted before they reached the cell cortex (Supplementary Note 2).

Do unreplicated dicentric minichromosomes establish bi-orientation as efficiently as replicated monocentric ones in cell cycles? To answer this question, we compared the kinetics of their bi-orientation establishment in synchronized cells. We arrested cells containing unreplicated monocentric or dicentric minichromosomes or replicated monocentric minichromosomes with α -factor and released them into fresh medium. The rate of bi-oriented minichromosomes was scored during the bud emergence and establishment of a bipolar spindle. As expected, unreplicated monocentric minichromosomes did not show any appreciable bi-orientation (Fig. 3a). Both unreplicated dicentric and replicated monocentric minichromosomes established bi-orientation soon after bipolar spindle formation (SPB separation) with similar kinetics (Fig. 3b, c). We also found that many cells underwent SPB separation during time-lapse image acquisition. Most if not all unreplicated dicentric (24 out of 25 observed; Supplementary Fig. S2) and replicated monocentric (14 out of 15 observed) minichromosomes showed evidence of bi-orientation (see Methods) within 2 min of SPB

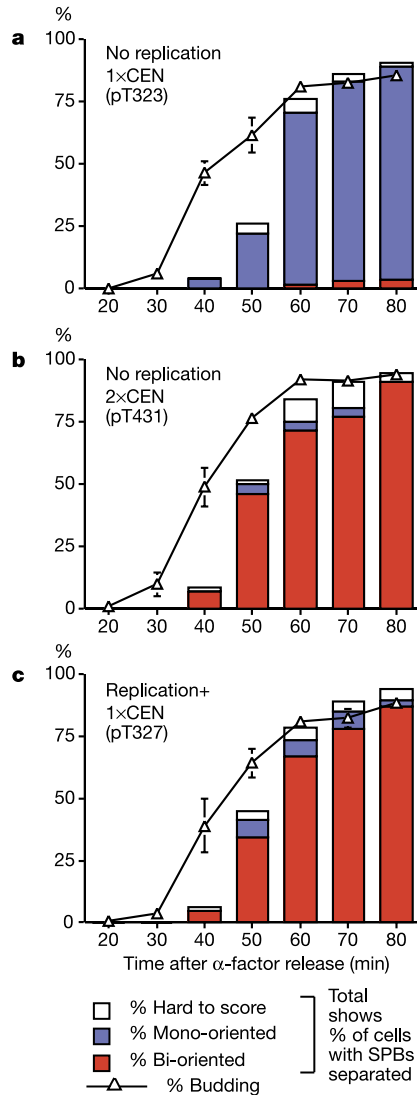


Figure 3 Comparing the kinetics of bi-orientation establishment during a synchronous cell cycle. *TetR-GFP SPC42-YFP P_{MET3}-R* cells carrying pT323 (T2755; **a**), pT431 (T3000; **b**) or pT327 (T2951; **c**) were incubated in the absence of methionine with galactose for 2 h and subsequently treated with α -factor for 2.5 h. They were then released into fresh YPA medium containing glucose. After the release, samples were collected for bud counts and time-lapse image acquisition every 10 min. Commencing immediately after sample collection, live cell images were acquired at 30-s intervals for 6 min. The behaviour of the three minichromosomes was scored as described in Methods. In addition, pre-anaphase separation of sister minichromosomes³¹ was counted as bi-orientation (only for pT327). From three trials of the release from α -factor arrest of each strain, 50–80 minichromosomes were scored at each time point, including ones with unseparated SPBs. Scoring was difficult if the distance between SPBs was too short in the projected images ('hard to score'). If SPBs separated within 2 min of the start of image acquisition, the cells were considered to have separated SPBs. The mean \pm s.d. percentage of cells with buds from three trials of α -factor release is plotted at each time point.

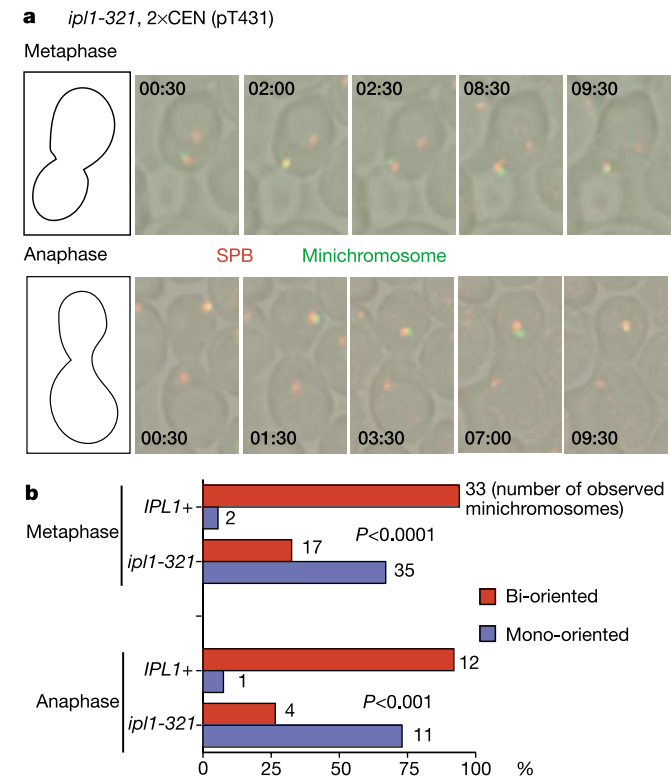


Figure 4 Behaviour of unreplicated dicentric minichromosomes in *ip11* mutant cells. *IP11+* (T3000) and *ip11-321* (T3001) cells (*TetR-GFP SPC42-YFP P_{MET3}-R* pT431) were incubated in medium without methionine but with galactose at 25 °C for 3 h and then at 35 °C (the restrictive temperature for *ip11-321*) for 1 h, and subsequently with glucose at 35 °C. The images were collected at 35 °C, commencing 20 min after transfer to glucose medium. The behaviour of minichromosomes was scored as in Fig. 2. **a**, Representative time-lapse images. **b**, Scoring. When synchronous cultures (release from α -factor arrest) of the two strains were observed in glucose medium at 35 °C, similar results were obtained in metaphase and anaphase during the first cell cycle (data not shown).

separation. These data suggest that unreplicated dicentric minichromosomes establish bi-orientation as efficiently as replicated monocentric minichromosomes.

It could be argued that proximity of the two centromeres on the unreplicated dicentric minichromosomes precludes their syntelic attachment and that this ensures efficient bi-orientation. The shortest distance between them is 4.2 kilobases (kb), which might be expected to span 12–18 nm (ref. 19). Because this is smaller than the diameter of microtubules (25 nm), there might be insufficient space for both centromeres to attach to microtubules with the same orientation. To address this issue, we enlarged the distance between centromeres to 10 kb (28–42 nm) on both sides of the circular minichromosome, but this had no effect on the efficiency of bi-orientation (27 out of 27 bi-oriented). It is therefore unlikely that efficient bi-orientation is caused by centromere proximity. Instead, our data imply that the connection of two kinetochores alone is sufficient for their efficient bi-orientation. The simplest explanation for this phenomenon is that any connection between kinetochores can provide the tension needed to stabilize kinetochore-to-pole connections.

This tension is normally made possible by connections between sister chromatids that are mediated by cohesin, whose absence causes frequent mono-orientation of replicated kinetochores^{8,9}. If the primary role of cohesin in promoting bi-orientation is to supply tension, then it should be possible to restore bi-orientation to cohesin-depleted cells by providing an alternative means of connection between sister DNAs. A possible way to achieve this would be by inactivating topoisomerase II, which is required to decatenate sister chromatids after DNA replication^{21–23}. We therefore compared the efficiency of sister kinetochore bi-orientation in *TOP2+* and *top2-4* (ref. 22) cells after depleting the Scc1 subunit of cohesin (Supplementary Note 3 and Fig. S3). The centromeres on chromosome V (*CEN5s*) were bi-oriented in 55% of *TOP2+* cells and in 77% of *top2-4* cells. Thus bi-orientation was partially restored when topoisomerase II function was impaired in cohesin-depleted cells. A similar result was obtained when Scc1-depleted DT40 chicken cells were treated with an inhibitor of topoisomerase II (ref. 24). These data suggest that any kind of connection between sister kinetochores may be sufficient to induce their bi-orientation.

To address whether the bi-orientation of unreplicated dicentric minichromosomes is, like that of authentic replicated sister kinetochores^{2,3,6}, dependent on the Ipl1 kinase, we compared the behaviour of unreplicated dicentric minichromosomes in *IPL1+* and *ipl1-321* mutant² cells. Bi-orientation of unreplicated dicentric minichromosomes took place in 94% of *IPL1+* cells (45 out of 48 in metaphase and anaphase; Fig. 4b). This occurred in only 31% of *ipl1-321* cells (21 out of 67). In the remaining 69%, minichromosome GFP signals remained in the vicinity of one SPB (Fig. 4a, b). They made connections to a single pole; that is, they mono-oriented (Supplementary Note 4). Inactivation of Ipl1 therefore greatly reduces the incidence with which unreplicated dicentric minichromosomes bi-orient. Ipl1 seems to be just as important for the bi-orientation of unreplicated dicentric minichromosomes as it is for replicated monocentric minichromosomes. We obtained similar results with a mutant of Sli15 (data not shown), which is an orthologue of Inner centromere protein (INCENP) and forms a complex with Ipl1 (refs 25–27). The Ipl1–Sli15 complex must be therefore capable of promoting bi-orientation through mechanisms that do not involve sister kinetochore geometry.

It has been suggested that Ipl1 promotes bi-orientation by facilitating the turnover of kinetochore–spindle pole connections when tension has not been generated⁶, as occurs during syntelic attachment. However, previous work has never properly documented the role of Ipl1 in detaching a kinetochore from one SPB and attaching it to the opposite pole after SPB separation. This process should occur with unreplicated monocentric minichromosomes on

which tension cannot be exerted. We developed an assay by which we could directly visualize the process of re-orientation that unreplicated monocentric minichromosomes undergo after SPB separation (Supplementary Note 5 and Fig. S4). We found that *ipl1-321* mutants showed less frequent re-orientation than *IPL1+* cells. These data suggest that Ipl1 does indeed have an important role in promoting the turnover of kinetochore–spindle pole connections when they cannot come under tension normally generated by amphitelic attachment.

Three decades ago it was shown that the connection of a kinetochore to a spindle pole by microtubules during meiosis I of grasshopper spermatocytes is stabilized by tension¹⁰. This implied that error correction has a key role in bi-orienting maternal and paternal chromosomes during meiosis I. It has long been disputed whether a similar principle operates during mitosis¹. Because sister kinetochores lie back to back, it has been thought that kinetochore geometry and not error correction might be the driving force behind the bi-orientation of sister kinetochores. Studies have implicated the Ipl1 (Aurora B) kinase and cohesin in promoting mitotic bi-orientation^{2–9}. Do they act by creating a back-to-back kinetochore geometry conducive to bi-orientation, or instead as part of an error correction mechanism? The finding that Ipl1 promotes the realignment of kinetochore–spindle pole connections in budding yeast, from old to new spindle poles, is consistent with an error correction mechanism⁶. Our results provide further evidence that erroneous connections are indeed corrected in a tension-dependent manner.

Our data suggest that the main, if not only, role of cohesin during mitosis in *S. cerevisiae* is to provide the physical connection between sister kinetochores necessary to generate tension when they bi-orient, and that an important function of Ipl1 is to eliminate kinetochore–spindle pole connections that do not generate such tension. This notion is also supported by the report that syntelic attachments are removed by Aurora-B-dependent mechanisms in mammalian cells⁷. How tension is actually sensed and how this affects whether or not Ipl1 promotes re-orientation remain to be addressed. Phosphorylation of the Dam1 kinetochore protein could be one of the key events in this process²⁸. Our data do not of course exclude the possibility that sister kinetochore geometry is also conducive to bi-orientation. For instance, Ipl1 (Aurora B) and/or cohesin might have additional roles in promoting kinetochore geometry²⁹. In particular, fission yeast and animal cells must have a single kinetochore attached to more than one microtubule. Chromosome bi-orientation fails if a single kinetochore is captured by microtubules from the opposite spindle poles (merotelic attachment)³⁰. In these organisms, sister kinetochore geometry may have more important roles to ensure bi-orientation than in *S. cerevisiae* cells (see Supplementary Note 6). □

Methods

Yeast genetics and molecular biology

The background of yeast strains (W303), yeast media, Southern blotting, α -factor arrest/release, *TetR-GFP/tet* operator system, and the construction of pT323, pT327, *CEN5-tetOs* and *SPC42-YFP* have been described^{6,8,16,18}. To construct *P_{MET3}-R*, pT343 was made by cloning the *R* recombinase gene (from pHM153; ref. 17) under control of the *MET3* promoter into YIplac204 (National Center for Biotechnology Information, X75461) and integrated at the *trp1* locus. pT431 and pT432 were constructed by cloning *CEN4* (730-bp DNA containing *CDEI-II-III*) under control of the *GAL1-10* promoter into pT323 and pT327, respectively. pT323 and pT327 are the same, except that the former has two recombination sites that flank a replication origin, whereas the latter has only one⁶. See Supplementary Note 7 for further methodological details.

Microscopy

The general procedures for time-lapse microscopy have been described⁸. We collected time-lapse images every 20–30 s, either for 20–30 min at 23 °C (ambient temperature) or for 10–15 min at 35 °C or 37 °C unless otherwise stated. One bright field image and 7–9 z-stacks (each 0.5–0.7- μ m apart) of GFP or YFP images were acquired with a J3 filter set (Chroma) at each time point. The z-stacks were deconvoluted and projected to two-dimensional images⁸ with the Openlab (Improvision) or SoftWoRx (Applied Precision) software.

Minichromosome scoring

The behaviour of minichromosomes (mono-oriented or bi-oriented) was scored as follows unless otherwise stated. When minichromosomes were not more than one-fifth of the spindle length away from an SPB at any two consecutive time points, they were scored as 'mono-oriented'. When minichromosomes were more than one-fifth of the spindle length away from both SPBs at most time points or they moved vigorously between two SPBs, they were scored as 'bi-oriented'. To score the behaviour of pT323 and pT431, rare replicated minichromosomes were distinguished (and ignored) owing to the greater intensity of their GFP signals. For pT327, a few cells with excessive copies of minichromosomes were not included in scoring.

Received 14 October 2003; accepted 12 January 2004; doi:10.1038/nature02328.
Published online 11 February 2004.

1. Tanaka, T. U. Bi-orienting chromosomes on the mitotic spindle. *Curr. Opin. Cell Biol.* **14**, 365–371 (2002).
2. Biggins, S. *et al.* The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. *Genes Dev.* **13**, 532–544 (1999).
3. He, X., Rines, D. R., Espelin, C. W. & Sorger, P. K. Molecular analysis of kinetochore–microtubule attachment in budding yeast. *Cell* **106**, 195–206 (2001).
4. Adams, R. R., Maiato, H., Earnshaw, W. C. & Carmena, M. Essential roles of *Drosophila* inner centromere protein (INCENP) and aurora B in histone H3 phosphorylation, metaphase chromosome alignment, kinetochore disjunction, and chromosome segregation. *J. Cell Biol.* **153**, 865–880 (2001).
5. Kaitna, S., Pasierbek, P., Jantsch, M., Loidl, J. & Glotzer, M. The aurora B kinase AIR-2 regulates kinetochores during mitosis and is required for separation of homologous chromosomes during meiosis. *Curr. Biol.* **12**, 798–812 (2002).
6. Tanaka, T. U. *et al.* Evidence that the Ipl1–Slh15 (Aurora kinase–INCENP) complex promotes chromosome bi-orientation by altering kinetochore–spindle pole connections. *Cell* **108**, 317–329 (2002).
7. Hauf, S. *et al.* The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore–microtubule attachment and in maintaining the spindle assembly checkpoint. *J. Cell Biol.* **161**, 281–294 (2003).
8. Tanaka, T., Fuchs, J., Loidl, J. & Nasmyth, K. Cohesin ensures bipolar attachment of microtubules to sister centromeres and resists their precocious separation. *Nature Cell Biol.* **2**, 492–499 (2000).
9. Sonoda, E. *et al.* Scc1/Rad21/Mcd1 is required for sister chromatid cohesion and kinetochore function in vertebrate cells. *Dev. Cell* **1**, 759–770 (2001).
10. Nicklas, R. B. & Koch, C. A. Chromosome micromanipulation. III. Spindle fiber tension and the reorientation of mal-oriented chromosomes. *J. Cell Biol.* **43**, 40–50 (1969).
11. Nicklas, R. B. How cells get the right chromosomes. *Science* **275**, 632–637 (1997).
12. Winey, M. *et al.* Three-dimensional ultrastructural analysis of the *Saccharomyces cerevisiae* mitotic spindle. *J. Cell Biol.* **129**, 1601–1615 (1995).
13. Hegemann, J. H. & Fleig, U. N. The centromere of budding yeast. *BioEssays* **15**, 451–460 (1993).
14. Hill, A. & Bloom, K. Genetic manipulation of centromere function. *Mol. Cell Biol.* **7**, 2397–2405 (1987).
15. Goshima, G. & Yanagida, M. Establishing biorientation occurs with precocious separation of the sister kinetochores, but not the arms, in the early spindle of budding yeast. *Cell* **100**, 619–633 (2000).
16. He, X., Asthana, S. & Sorger, P. K. Transient sister chromatid separation and elastic deformation of chromosomes during mitosis in budding yeast. *Cell* **101**, 763–775 (2000).
17. Matsuzaki, H., Nakajima, R., Nishiyama, J., Araki, H. & Oshima, Y. Chromosome engineering in *Saccharomyces cerevisiae* by using a site-specific recombination system of a yeast plasmid. *J. Bacteriol.* **172**, 610–618 (1990).
18. Michaelis, C., Ciosk, R. & Nasmyth, K. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell* **91**, 35–45 (1997).
19. Guacci, V., Hogan, E. & Koshland, D. Chromosome condensation and sister chromatid pairing in budding yeast. *J. Cell Biol.* **125**, 517–530 (1994).
20. Thrower, D. A. & Bloom, K. Divalent chromosome stretching during anaphase reveals roles of Sir2/Ku in chromatin compaction in budding yeast. *Mol. Biol. Cell* **12**, 2800–2812 (2001).
21. DiNardo, S., Voelkel, K. & Sternglanz, R. DNA topoisomerase II mutant of *Saccharomyces cerevisiae*: topoisomerase II is required for segregation of daughter molecules at the termination of DNA replication. *Proc. Natl Acad. Sci. USA* **81**, 2616–2620 (1984).
22. Holm, C., Goto, T., Wang, J. C. & Botstein, D. DNA topoisomerase II is required at the time of mitosis in yeast. *Cell* **41**, 553–563 (1985).
23. Uemura, T. *et al.* DNA topoisomerase II is required for condensation and separation of mitotic chromosomes in *S. pombe*. *Cell* **50**, 917–925 (1987).
24. Vagnarelli, P. *et al.* Analysis of Scc1-deficient cells defines a key metaphase role of vertebrate cohesin in linking sister kinetochores. *EMBO Rep.* **5**, 167–171 (2004).
25. Kim, J. H., Kang, J. S. & Chan, C. S. Slh15 associates with the Ipl1 protein kinase to promote proper chromosome segregation in *Saccharomyces cerevisiae*. *J. Cell Biol.* **145**, 1381–1394 (1999).
26. Kaitna, S., Mendoza, M., Jantsch-Plunger, V. & Glotzer, M. INCENP and an Aurora-like kinase form a complex essential for chromosome segregation and efficient completion of cytokinesis. *Curr. Biol.* **10**, 1172–1181 (2000).
27. Adams, R. R. *et al.* INCENP binds the Aurora-related kinase AIRK2 and is required to target it to chromosomes, the central spindle and cleavage furrow. *Curr. Biol.* **10**, 1075–1078 (2000).
28. Cheeseman, I. M. *et al.* Phospho-regulation of kinetochore–microtubule attachments by the Aurora kinase Ipl1p. *Cell* **111**, 163–172 (2002).
29. Losada, A., Hirano, M. & Hirano, T. Cohesin release is required for sister chromatid resolution, but not for condensin-mediated compaction, at the onset of mitosis. *Genes Dev.* **16**, 3004–3016 (2002).
30. Cimini, D. *et al.* Merotelic kinetochore orientation is a major mechanism of aneuploidy in mitotic mammalian tissue cells. *J. Cell Biol.* **153**, 517–527 (2001).
31. Tanaka, T., Cosma, M. P., Wirth, K. & Nasmyth, K. Identification of cohesin association sites at centromeres and along chromosome arms. *Cell* **98**, 847–858 (1999).

Supplementary Information accompanies the paper on www.nature.com/nature.

Acknowledgements We thank A. Toth for discussions leading to the use of unreplicated dicentric chromosomes; W. Earnshaw for discussing inhibition of Top2 in Scc1-depleted cells and for sharing unpublished data; M. J. R. Stark and J. Swedlow for discussion and critically reading the manuscript; N. Mukae and C. Newlon for discussion; P. Andrews and S. Swift for help with time-lapse microscopy; and R. Ciosk, S. Biggins, E. Uhlmann, X. He, P. Sorger, R. Tsien, W. Fangman, H. Araki, C. Holm, R. Sternglanz, C. Chan and EUROSCARF for reagents. This work was supported by The Wellcome Trust, Cancer Research UK, EMBO Young Investigator Program, and a fellowship (to K.T.) from Japan Society for the Promotion of Science.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to T.U.T. (t.tanaka@dundee.ac.uk).

.....
Preferential *cis-syn* thymine dimer bypass by DNA polymerase η occurs with biased fidelity

Scott D. McCulloch¹, Robert J. Kokoska¹, Chikahide Masutani², Shigenori Iwai³, Fumio Hanaoka^{2,4} & Thomas A. Kunkel¹

¹Laboratory of Molecular Genetics and Laboratory of Structural Biology, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, North Carolina 27709, USA

²Graduate School of Frontier Biosciences, Osaka University and CREST, Japan Science and Technology Corporation, 1-3 Yamada-oka, Suita, Osaka 565-0871, Japan

³Division of Chemistry, Graduate School of Engineering Science, Osaka University, 1-3 Machikaneyama, Toyonaka, Osaka 560-8531, Japan

⁴Discovery Research Institute, RIKEN, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan

.....
Human DNA polymerase η (Pol η) modulates susceptibility to skin cancer by promoting DNA synthesis past sunlight-induced cyclobutane pyrimidine dimers that escape nucleotide excision repair (NER)^{1,2}. Here we have determined the efficiency and fidelity of dimer bypass. We show that Pol η copies thymine dimers and the flanking bases with higher processivity than it copies undamaged DNA, and then switches to less processive synthesis. This ability of Pol η to sense the dimer location as synthesis proceeds may facilitate polymerase switching before and after lesion bypass. Pol η bypasses a dimer with low fidelity and with higher error rates at the 3' thymine than at the 5' thymine. A similar bias is seen with *Sulfolobus solfataricus* DNA polymerase 4, which forms a Watson–Crick base pair at the 3' thymine of a dimer but a Hoogsteen base pair at the 5' thymine (ref. 3). Ultraviolet-induced mutagenesis is also higher at the 3' base of dipyrimidine sequences^{4–6}. Thus, in normal people and particularly in individuals with NER-defective xeroderma pigmentosum who accumulate dimers, errors made by Pol η during dimer bypass could contribute to mutagenesis and skin cancer.

To compare the efficiency with which human Pol η bypasses a *cis-syn* thymine–thymine (TT) dimer or undamaged thymines in the same sequence context, we used a large excess of primer template and incubation times short enough to ensure that the DNA products result from a single cycle of synthesis⁷. Thus, the dimer is encountered while the polymerase is processively synthesizing DNA using all four dNTPs. This provides bypass efficiency parameters that differ from those in previous studies that measured the rate of single nucleotide insertion.

Human Pol η has low processivity with undamaged DNA (ref. 8 and Fig. 1a, left, and 1b–d, open bars). With damaged DNA, processivity is unexpectedly higher at the 3' and 5' T of dimers