been observed in three live individuals in Ueno Zoological Park in Tokyo, Japan, when they were grasping food plants.

We have shown that the hand of the giant panda has a much more refined grasping mechanism than has been suggested in previous morphological models[2,6–9].

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1. Lankester, E. R. & Lydekker, R. *Trans. Linn. Soc.* **8,** 163–171 (1901).
2. Davis, D. D. *Field. Zool. Mem.* **3,** 41–124, 146-198 (1964).
3. Gould, S. J. *Nat. Hist.* **87,** 20–30 (1978).
4. Pocock, R. I. *Nature* **143,** 206 (1939).
5. Endo, H. *et al. J. Anat.* **189,** 587–592 (1996).
6. Wood-Jones, F. *Nature* **143,** 157 (1939).
7. Wood-Jones, F. *Proc. Zool. Soc. Lond. B* **109,** 113–129 (1939).
8. Beijing Zoo *Morphology of the Giant Panda. Systematic Anatomy and Organ Histology* 148–152 (Science, Beijing, 1986).
9. Bourlière, F. *Traité de Zoologie XVII* 234–236 (Masson, Paris, 1955).

Yeast cell-type regulation of DNA repair

The mating-type locus (*MAT*) in the yeast *Saccharomyces cerevisiae* provides information about whether cells are of the **a** or α mating type, and genes at this locus encode transcriptional regulators that determine the phenotypes associated with the different cell types[1]. In **a**/α diploid cells, the **a**1/α2 repressor is formed, which inhibits haploid-specific gene expression and indirectly promotes meiosis. Mutations in *SIR* (silent information regulator) genes cause a loss of both heterochromatin and transcriptional silencing, resulting in the expression of cryptic **a** and α genes resident at the *HML* and *HMR* loci. As a result, *sir* mutant strains have the properties of **a**/α diploids.

Non-homologous end-joining (NHEJ) is required in mammals both for *V*(*D*)*J* recombination[2] and for repairing double-stranded DNA breaks. NHEJ also occurs in yeast[3,4], and it has been reported that Sir proteins are required for this process[5,6]. This observation was interpreted to mean that Sir proteins are involved directly in NHEJ, perhaps by forming a heterochromatin-like structure at double-stranded breaks. But we have found evidence for an alternative interpretation: that the **a**/α-state regulates NHEJ and that *sir* mutations affect NHEJ indirectly.

To distinguish between these two possibilities, we performed plasmid-rejoining assays. Plasmids that were linearized by restriction enzymes and contained a double-stranded break in vector sequences lacking homology to the yeast genome were transformed into yeast. The frequency of transformants was used as a measure of NHEJ[5,6]. Results obtained from SIR^+ and sir^- strains were consistent with previous findings[5,6]. NHEJ in sir^- strains was 20-fold less efficient than in wild-type strains (Table 1). However, assays performed in SIR^+ and sir^- strains in which all mating-type genes had been inactivated by a promoter deletion (*hml*αΔp *mat***a**Δp *hmr*αΔp, abbreviated here as $a^-a^-a^-$) revealed that the absence of mating-type heterozygosity suppressed the defect in NHEJ exhibited by the sir^- strains (Table 1).

We performed plasmid-rejoining assays on two SIR^+ diploid strains, an **a**/α diploid and a non-**a**/α diploid (*mat***a**Δp/*MAT*α, in which only α information is expressed). The non-**a**/α diploid strain accomplished NHEJ tenfold more efficiently than the **a**/α diploid (Table 1). NHEJ was therefore controlled by mating-type heterozygosity, and no cell-type-independent effect of *sir* mutations was detected.

The defect in NHEJ found in **a**/α cells indicates that a gene required for NHEJ was regulated by the **a**1/α2 repressor. RNA blot analysis of *HDF1*, *HDF2*, *DNL4*, *XRS2* and *MRE11*, the leading candidate genes[7–10] in wild-type, *sir3*, $a^-a^-a^-$ and $a^-a^-a^-$ *sir3* strains, revealed that all five genes were comparably expressed in *SIR3* and *sir3* strains (data not shown). These genes are therefore not relevant targets for the **a**1/α2 repression of NHEJ.

Our results provide evidence against a direct role for heterochromatin formation in NHEJ, indicating instead that the efficiency of NHEJ is controlled by cell type. But our data do not exclude the possibility that different strains might yield different results: indeed, the W303 strain we used contains a mild *rad5* mutation. However, the **a**/α regulation of NHEJ found here can explain problems associated with DNA repair in yeast. Diploid cells that suffer a double-stranded break have a homologous partner that can perform a homology-driven recombinational repair process. In cells that have more than one double-stranded break, NHEJ could lead to exchange-type aberrations[11], indicating that homology-driven repair should be the preferred pathway. But haploid cells in the G1 phase of the cell cycle lack homologues and so rely on NHEJ. With NHEJ under the control of the **a**1/α2 repressor, a yeast cell could adapt the repair process, using the NHEJ pathway primarily when homology-driven repair is not possible. This would require an **a**1/α2-repressed gene that is important for NHEJ. Alternatively, **a**1/α2 could inhibit NHEJ indirectly by upregulating the *RAD52* homologous repair pathway to outcompete the NHEJ pathway for the repair of double-stranded breaks.

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1. Herskowitz, I., Rine, J. & Strathern, J. *The Molecular Biology of the Yeast* Saccharomyces (eds Jones, E. W., Pringle, J. R. & Broach, J. R.) 583–656 (Cold Spring Harbor Laboratory Press, NY, 1992).
2. Lieber, M. R. *Curr. Biol.* **6,** 134–136 (1996).
3. Milne, G. T., Jin, S., Shannon, K. B. & Weaver, D. T. *Mol. Cell. Biol.* **16,** 4189–4198 (1996).
4. Boulton, S. J. & Jackson, S. P. *EMBO J.* **15,** 5093–5103 (1996).
5. Tsukamoto, Y., Kato, J. & Ikeda, H. *Nature* **388,** 900–903 (1997).
6. Boulton, S. J. & Jackson, S. P. *EMBO J.* **17,** 1819–1828 (1998).
7. Feldmann, H. & Winnacker, E. L. *J. Biol. Chem.* **268,** 12895–12900 (1993).
8. Feldmann, H. *et al. J. Biol. Chem.* **271,** 27765–27769 (1996).
9. Schiestl, R. H., Zhu, J. & Petes, T. D. *Mol. Cell. Biol.* **14,** 4493–4500 (1994).
10. Wilson, T. E., Grawunder, U. & Lieber, M. R. *Nature* **388,** 495–498 (1997).
11. Friedl, A. A., Kiechle, M., Fellerhoff, B. & Eckardt-Schupp, F. *Genetics* **148,** 975–988 (1998).

Table 1 **Efficiency of non-homologous end-joining in haploid and diploid strains**

Strain	Genotype	Relative efficiency of NHEJ
Haploid strains		
JRY2334	wild type	100
JRY4563	*sir2::TRP1*	7 ± 1
JRY3289	*sir3::TRP1*	4 ± 1
JRY4580	*sir4::TRP1*	6 ± 3
JRY3658	*hml***a**Δp *mat***a**Δp *hmr***a**Δp	66 ± 22
JRY6348	*hml***a**Δp *mat***a**Δp *hmr***a**Δp *sir2::TRP1*	114 ± 2
JRY3606	*hml***a**Δp *mat***a**Δp *hmr***a**Δp *sir3::TRP1*	64 ± 7
JRY6349	*hml***a**Δp *mat***a**Δp *HMR***a** *sir4::TRP1*	130 ± 4
Diploid strains		
JRY5384	*MAT***a**/*MAT*α	10 ± 2
JRY6328	*mat***a**Δp/*MAT*α	100

All strains were isogenic to W303-1a (*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad5-535*) unless indicated. Strains were transformed using *Hin*dIII-digested pRS316 or pRS416 by the lithium acetate method and plated onto supplemented minimal plates selecting for uracil prototrophy. Efficiency of non-homologous end-joining (NHEJ) was calculated by normalizing the number of transformants obtained to the number of transformants obtained in parallel transformations with supercoiled plasmid. The average absolute efficiency of NHEJ in strains JRY2334 and JRY6328 were 89% and 22%, respectively. Data are mean ± standard deviation of two or three independent experiments.