one to five to reduce the probability of the formation of homomeric GluR1 receptors. Voltage-clamp recordings with two electrodes were made 2 to 15 days after injection. For routine recording, oocytes were bathed in a solution containing 90 mM NaCl, 1 mM KCl, 1.7 to 1.8 mM $MgCl_2$, 0.1 mM $CaCl_2$, and 15 mM Hepes, pH 7.6. The low Ca^{2+} concentration minimized the secondary activation of the Ca^{2+}-dependent Cl^- currents (*17*). Kainate was applied by bath perfusion. In most experiments, we used 300 μM kainate to activate glutamate receptors, but lower concentrations were used on a few oocytes that produced large responses to 300 μM kainate. We obtained *I-V* relations by applying 2-s voltage ramps (from −100 to +50 or +100 mV) in the presence and absence of kainate and then by subtracting the resting *I-V* curve from that observed in the presence of kainate.

12. We calculated slope conductance by measuring the current 5 mV positive and negative to the indicated potentials and dividing the difference by 10 mV.
13. Our substitution of Ba^{2+} for Ca^{2+} was not sufficient to abolish the secondary activation of the Ca^{2+}-dependent Cl^- current (*18*). In the experiments illustrated, we suppressed this current by including 0.4M EGTA as well as 3 M KCl in the recording and current pipettes and by keeping the application of kainate brief. We also achieved suppression of the outward Cl^- current by replacing all the extracellular Cl^- with methanesulfonate (*19*) and including EGTA in the recording pipettes. For all combinations of subunits, the reversal potentials in the Cl^--containing and methanesulfonate-containing solutions were similar. In contrast to the records shown in Fig. 2, long applications of kainate (>15 s) often produced substantial outward currents in oocytes injected with any of the combinations of subunits that had inwardly rectifying *I-V* relations in low Ca^{2+}, because of the secondary activation of the Cl^- current.
14. The reversal potential for Cl^- was estimated from tail currents following a depolarizing step that activated the Ca^{2+}-dependent Cl^- currents. In these experiments, the recording pipettes did not contain EGTA. The Cl^- reversal potential was −25 ± 1.8 mV (n = 10) in the high Ca^{2+} solution and −32 ± 2.8 mV (n = 9) in the high Ba^{2+} solution.
15. Permeability ratios were calculated from the Goldman equation modified to include external divalent cations. In these calculations, it was assumed that anion permeability was negligible, that all monovalent cations were equally permeable, that intracellular monovalent cation concentration was 90 mM, and that the ion activities were 0.8 for monovalent cations and 0.5 for divalent cations. Calculations were made with and without compensation for the negative surface potential (*20*). The magnitude of this effect depends critically on the number and distribution of charges around the extracellular mouth of the channel, information not yet available. A surface charge equivalent to −20 mV at the channel would reduce the estimated permeability ratio of GluR1 plus GluR2(R607Q) receptors from 2.6 to 1.9.
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HRR25, a Putative Protein Kinase from Budding Yeast: Association with Repair of Damaged DNA

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In simple eukaryotes, protein kinases regulate mitotic and meiotic cell cycles, the response to polypeptide pheromones, and the initiation of nuclear DNA synthesis. The protein HRR25 from the budding yeast *Saccharomyces cerevisiae* was defined by the mutation *hrr25-1*. This mutation resulted in sensitivity to continuous expression of the HO double-strand endonuclease, to methyl methanesulfonate, and to x-irradiation. Homozygotes of *hrr25-1* were unable to sporulate and disruption and deletion of *HRR25* interfered with mitotic and meiotic cell division. Sequence analysis revealed two distinctive regions in the protein. The NH_2-terminus of HRR25 contains the hallmark features of protein kinases, whereas the COOH-terminus is rich in proline and glutamine. Mutations in *HRR25* at conserved residues found in all protein kinases inactivated the gene, and these mutants exhibited the *hrr25* null phenotypes. Taken together, the *hrr25* mutant phenotypes and the features of the gene product indicate that *HRR25* is a distinctive member of the protein kinase superfamily.

THE REPAIR OF DNA DAMAGE requires the coordination of a large number of gene products (*1*). For example, in responding to ultraviolet (UV) irradiation, cells can use photoreactivation or excision repair functions to correct genetic lesions. The repair of strand breaks, such as those created by x-rays, can proceed through recombinational mechanisms. Many forms of DNA damage cause cells to arrest in G2 (*2*). During this G2 arrest, DNA lesions are repaired to ensure chromosomal integrity before mitotic segregation. In eukaryotes such as *S. cerevisiae*, genetic studies have defined repair-deficient mutants and have identified more than 50 radiation-sensitive (*RAD*) mutants with defects in genes that function in repair of damaged DNA (*3*).

To understand the functions involved in recognizing and repairing a broken chromosome, we have isolated mutants sensitive to continuous expression of the *HO* gene, which codes for a 65-kD site-specific endonuclease that cuts double-stranded DNA and initiates mating-type interconversion (*4*). The products of at least three DNA repair genes (*RAD51, RAD52*, and *RAD54*) are required for the repair of the HO-created double-strand break (*3, 5*). However, the characteristics of the proteins encoded by these genes are not known (*6*). In a yeast strain containing a galactose-inducible *HO*, we identified mutants that were unable to grow on galactose. Of the mutants that were subsequently complemented by various galactose metabolism (*gal*) mutants, several showed varying sensitivity to the radiomimetic alkylating agent methyl methanesulfonate (MMS). Five alleles of known *rad* mutations were identified in this screen (7), and one mutation, *hrr25-1* (*HO* and radiation repair), displayed severe defects in DNA repair.

The *hrr25-1* mutation conferred a recessive DNA repair defect that included sensitivity to MMS (Fig. 1). The *hrr25-1* strains also showed sensitivity at 5 to 20 krad of x-irradiation (*8*), similar sensitivity to that observed with mutations in the radiation repair genes *RAD50* and *RAD52* (*3, 6*). These *hrr25-1* strains are no more sensitive to UV irradiation than are wild-type strains and are not temperature-sensitive for growth at 37°C. Although some *rad* mutants have several of the *hrr25-1* DNA repair phenotypes, *hrr25-1* strains differ in that they undergo nearly normal mitotic recombination (*3, 9*). Frequencies of spontaneous mitotic gene conversion and crossing-over were similar for homozygous *hrr25-1* and wild-type strains (Table 1). However, *HRR25* is required for the correct completion of meiosis. Homozygotes of *hrr25-1* showed fewer than 0.5% spores under conditions in which an isogenic wild-type strain sporulated to 75 to 85%. The *hrr25-1* mutation could be complemented by a number

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of radiation-sensitive mutations (*rad6, rad50, rad52, rad54*, and *rad57*) that present some of the *hrr25* phenotypes (Fig. 1), suggesting that *hrr25-1* is a new, *rad*-like mutation and not one of these previously described genes. These results also indicate that *HRR25* functions in DNA repair and meiosis but is not specifically required for the repair of spontaneous mitotic lesions by recombination.

We isolated the *HRR25* gene by screening a genomic DNA library for genes that complement MMS sensitivity (*10*). A 12-kb genomic fragment was identified and complementing activity was localized to a 3.1-kb fragment (Fig. 1) by transposon mutagenesis (*11*) and subcloning. This region complemented DNA repair defects and meiotic deficiencies. Transposon insertion mutations within the 3.1-kb fragment (Fig. 1) that were targeted to integrate into yeast chromosomal DNA did not complement *hrr25-1* for MMS sensitivity, whereas adjacent chromosomal insertions outside the complementing region segregated with *hrr25-1* in genetic crosses.

The DNA sequence of this 3.1-kb fragment (GenBank accession number M68605) revealed a 1482-nucleotide, centrally located open reading frame. A transposon insertion mutation in this open reading frame inactivated *HRR25* comple-mentation (Fig. 1), whereas insertions elsewhere in the 12-kb clone did not affect *HRR25* complementation. Transposon-mediated disruption of *HRR25* (Fig. 1) produced several phenotypes not seen with *hrr25-1* and indicated that mutations in *HRR25* can affect a variety of processes. Insertion of a Tn*10*-based transposon (*11*) into the middle of a plasmid-borne *HRR25* coding region (*hrr25::LUK*) inactivated complementation for MMS sensitivity. Integration of this insertion into the genomic *HRR25* gene resulted in a severe growth defect (Fig. 1), MMS sensitivity, and meiotic inviability (Table 1). This severe growth defect was not observed with *hrr25-1* strains.

To determine whether the mutant phenotypes revealed by the *hrr25::LUK* disruption allele represent a null phenotype, we deleted the entire *HRR25* coding sequence (*12*). Yeast carrying the deletion-disruption allele (*hrr25Δ*) showed phenotypes identical to those with the *hrr25::LUK* allele for all properties examined, including MMS sensitivity, slow growth, and the sporulation defect, indicating that wild-type *HRR25* protein is associated with these processes and that the *hrr25::LUK* allele does not indirectly interfere with DNA repair, growth, or sporulation. In direct parallel comparisons, the *hrr25::LUK* and *hrr25Δ* alleles behaved identically.

Microscopic examination of germinating and actively growing mid-log phase cells lacking *HRR25* revealed aberrant cellular morphologies (Fig. 2). Deletion and disruption of *HRR25* resulted in abnormally large cells, and 25 to 40% of the cells were filamentous or extended. Nuclear staining (*13*) and flow cytometric analysis (*14*) of mid-log populations showed that orderly cell cycle progression in *hrr25* deletion and disruption mutants was lost (Fig. 2). Many cells lacked detectable nuclei, and, by single-cell manipulations, these were shown to be inviable. Consistent with this nuclear segregation defect, the plating efficiency of *hrr25* deletion and disruption haploids was reduced (Table 1). However, this reduction in plating efficiency was insufficient to account for the severe growth rate reduction. Flow cytometric analysis (*14*) showed that a large number of the cells in a haploid *hrr25Δ* deletion population were delayed in the cell cycle and exhibited G2 DNA content, but the population was not arrested uniformly in the cell cycle (Fig. 2).

The predicted translation product of *HRR25* contains the 11 conserved subdomains characteristic of the catalytic domain of serine-threonine protein kinase superfamily members (*15*). For comparison, the *HRR25* translation was aligned with the catalytic domains for two subgroups of yeast protein kinases, the *CDC28, cdc2* group and the *KSS1, FUS3* group (Fig. 3A). Located between amino acids 15 and 30 is a region that contains the conserved GXGXXG region (where X is any amino acid). Just COOH-terminal to this region are conserved lysine and glutamic acid residues present in most kinases. These regions are thought to function in the nucleotide binding and phosphotransfer steps of the kinase reaction (*15*). Between amino acid residues 120 and 150 are regions containing the HRD and DFG motifs; these motifs are found in most protein kinase family mem-

Table 1. Phenotypes associated with mutations in *HRR25*.

Genotype	MMS resistance	Doubling time (min)	Mitotic recombination proficiency*	Spore formation (%)†	Anucleate cells (%)‡	Plating efficiency§
HRR25	+	90–110	1.0	75–85	2.4	1.0
hrr25-1	−	150–220	1.4	<0.5	1.9	0.9
hrr25Δ	−	600–720	0.8	<0.5	15.7	0.6

*Recombination frequencies were normalized to *HRR25* wild-type strains as described by Montelone *et al.* in (*26*). †Sporulation was assessed microscopically as described by Malone and Hoekstra in (*26*), and at least 200 cells were counted. For *hrr25-1* and *hrr25Δ*, no spores were observed. ‡Distribution of anucleate cells was measured as described by Farnet *et al.* in (*13*), and at least 200 to 400 cells per strain were examined. §Plating efficiency was measured from mid-log phase cells by comparing the efficiency of colony formation on rich medium relative to the total number of cells determined by hemocytometer count. Values are normalized to wild type.

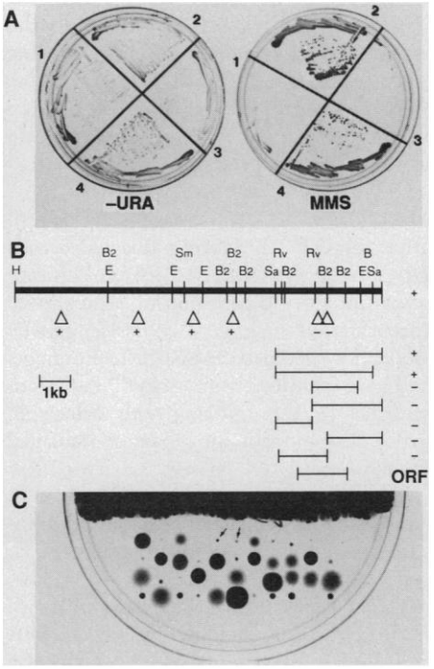

Fig. 1. Isolation, genomic organization, and disruption of *HRR25*. (**A**) Yeast strain MHML3-36d (*ura3 hrr25-1*) was transformed with a YCp50-based library (*10*) and screened for MMS resistance. Quadrant 1 shows MHML3-36d with cloning vector YCp50 (*27*), quadrant 2 shows the 12-kb *HRR25* clone, quadrant 3 shows a Tn*10*LUK (*12*) insertion into the *HRR25* coding region, and quadrant 4 shows a subclone containing a 3.1-kb Sal I–Bam HI fragment that complements *hrr25-1*. (**B**) Restriction map of the *HRR25* complementing region. Mini-Tn*10*LUK transposons (△) (*11*) delineated the location of *HRR25*. The insertion farthest to the right is shown in (A), quadrant 3. The activities of various subclones for complementing *hrr25-1* MMS sensitivity are indicated by + and −, and the *HRR25* open reading frame (ORF) is shown. B is Bam HI, B2 is Bgl II, E is Eco RI, H is Hind III, RV is Eco RV, Sa is Sal I, and Sm is Sma I. (**C**) Yeast strain MFH14 (*MATa/MATα ura3/ura3*) was transformed with Bg1 II–linearized YCp50-HRR25::LUK (quadrant 3), and a heterozygous insertion mutant was dissected. Cells were allowed to germinate at 30°C for 7 days before photography. After a normal germination period of 2 days, the severe growth defect of *hrr25::LUK* (highlighted with arrows) suggested that the deletion of *HRR25* was lethal. The shading variation seen in this photo is due to mutations in adenine biosynthesis. MFH14 is *ade5/ADE5 ade2/ade2*. An *ade5 ade2* strain is gray in this photo, whereas an *ADE5 ade2* strain is black.

bers. In addition, sequence examination of all known serine-threonine protein kinases indicates that *HRR25* shares some additional similarities with the *Raf,PKS,mos* subgroup (*15*). The strongest similarities are in areas around the GXGXXG, DFG, and DXXSXG conserved regions in protein kinase catalytic domains (Fig. 3A).

To investigate the functional relevance of the observed sequence similarity between *HRR25* and protein kinases, we altered specific residues within the *HRR25* kinase domain and examined the phenotypic consequences of these changes. We mutated a lysine at position 38 (Lys^{38}) to an arginine (*16*). Lys^{38} in *HRR25* corresponds to the lysine found in all known protein kinases, and this subdomain is involved in adenosine triphosphate binding. Mutations at the conserved lysine in protein kinases such as v-Src, v-Mos, and *DBF2* inactivate these proteins (*17, 18*). The mutant *hrr25-Lys*^{38} allele was incapable of complementing *hrr25-1*, *hrr25::LUK*, and *hrr25Δ* alleles (*19*) for all properties examined, an indication that the *HRR25* kinase domain is required for in vivo function of *HRR25*. Mutations at other conserved residues also inactivated *HRR25* (*19*).

The predicted *HRR25* translation product has other notable features. For example, the last 100 amino acids are rich in proline and glutamine, containing 50 of these residues (Fig. 3B). Other proteins with regions rich in these two amino acids include the transcription factors Sp1, JUN, and HAP2, steroid hormone receptors, the ran1 protein kinase from *Schizosaccharomyces pombe*, and Mak, the male germ cell–associated kinase from rat (*20*). In the case of Sp1 and JUN, the proline and glutamine regions are involved in transcription transactivation, whereas the proline and glutamine region in the human mineralocorticoid receptor is thought to serve as an intramolecular bridge. How the proline and glutamine region in *HRR25* might function is unclear, but it could act as a structural feature for substrate interaction or for subcellular localization. Also, the glutamine richness of this region is similar to the *opa* or M-repeat seen in the *Drosophila* notch and *Xenopus* xotch proteins (*21*). The function of the *opa* repeat is not certain, but this repeat is found in several *Drosophila* genes. Lastly, the sequence TKKQKY at the COOH-terminal end of the protein kinase region is similar to the nuclear localizing signal of SV40 large T antigen and yeast histone H2B (*22*).

The *HRR25* kinase joins an expanding group of protein kinases involved in growth control and cell division. In budding and fission yeasts, G1/start and G2/M transitions are under the control of protein kinases such as CDC28 or cdc2, wee1, and nim1 (*23*). In *S. cerevisiae* specifically, the progression through S phase requires at least two protein kinases encoded by *CDC7* and *DBF2* (*18, 24*). The CDC7 protein kinase, which acts downstream of CDC28 "start" function in the cell cycle, may participate in error-prone DNA repair and is required for the commitment to meiosis (*24*). In higher organisms, the c-Mos protein kinase is a positive factor in meiotic development (*25*), and the ran1 protein kinase in *S. pombe* induces cells to enter meiotic differentiation (*20*). The HRR25 protein kinase is important for normal cellular

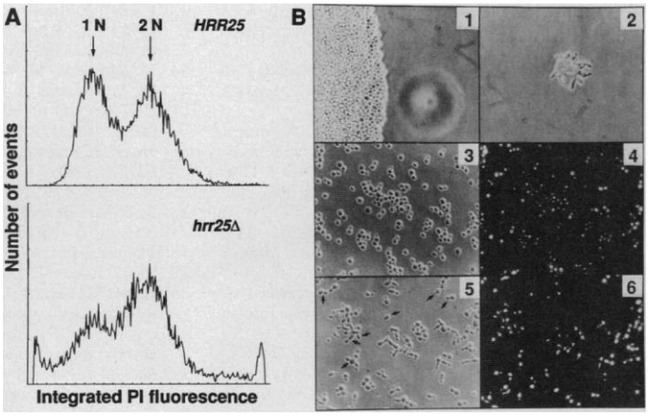

Fig. 2. Flow cytometric and microscopic analysis of germinating and proliferating *hrr25* cells. (**A**) Flow cytometry of *HRR25* and *hrr25Δ* by standard procedures (*14*). DNA content (1N or 2N) of the cells is indicated. (**B**) Photomicrographs of *HRR25* (1) and *hrr25::LUK* (2) colonies after germination on rich medium (*28*). Phase contrast (3 and 5) and 4′,6′-diamino-2-phenylindole (DAPI) staining (4 and 6) of proliferating *HRR25* (3 and 4) and *hrr25::LUK* (5 and 6) cells are shown. DAPI staining was by standard procedures (*13*). The arrows highlight cells in *hrr25::LUK* that lack DAPI-stainable nuclei.

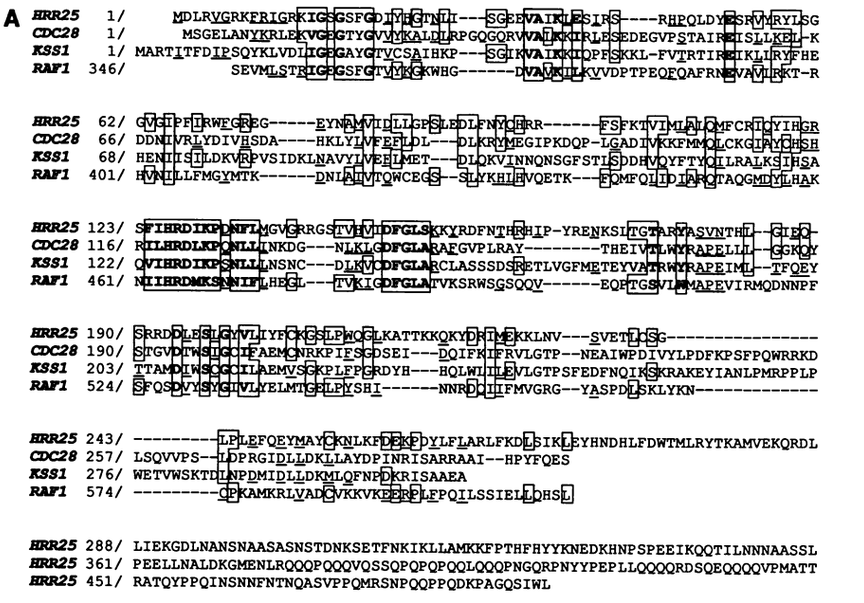

Fig. 3. Sequence comparison of *HRR25* with *CDC28, KSS1*, and *RAF1*. (**A**) The predicted translation product of *HRR25* is compared with the catalytic domains of several members of the serine-threonine protein kinase superfamily (*15*). Bold letters represent conserved features of protein kinases (*15*), boxes indicate amino acids that are identical to those in HRR25, and underlining indicates amino acid similarity to HRR25. Structurally similar groupings are nonpolar side chains (M, L, I, V, and C); aromatic or ring-containing side chains (F, Y, W, H); small side chains with near neutral polarity (A, G, S, T, and P); acidic and uncharged polar side chains (D, E, N, and Q); and basic polar side chains (K, R, and H). HRR25 shows 21% identity and 41% similarity to *CDC28* and 19% identity and 43% similarity to *KSS1*. HRR25 shows highest similarity to members of the *Raf1,PKS,mos* family (*19*) of protein kinases. Through the catalytic domain, *HRR25* shows 30% identity and 49% similarity to *Raf1*. (**B**) Schematic representation of the structure of HRR25. The protein kinase homology is represented by a black box, and the proline and glutamine region is indicated by an open box. Location of the mTn*10*LUK insertion used in Figs. 1 and 2 is shown (▲). This insertion disrupts residue 239 within the protein kinase homology, and the insertion is an out-of-frame mutation (*11*). Abbreviations for the amino acids are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

growth, nuclear segregation, DNA repair, and meiosis, and deletion of *HRR25* results in cell cycle defects. These phenotypes, coupled with the similarity of the *HRR25* sequence to the sequence of the Raf,c-Mos protein kinase subgroup (Fig. 3A), suggest that *HRR25* might play a similar role in *S. cerevisiae* growth and development. The defects in DNA double-strand break repair and aberrant growth properties revealed by mutations in the *HRR25* kinase extend the possible functions of protein kinases in cell growth and place *HRR25* with *CDC7* in a functional category of yeast kinase associated with DNA metabolism.

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9. Intragenic mitotic recombination was measured by the formation of prototrophs at heteroalleles (*26*), whereas intergenic recombination was measured by drug resistance at heterozygous loci (*26*).
10. The *HRR25* gene was isolated by complementing for MMS sensitivity with a genomic library constructed in the plasmid YCp50 (*27*). An *hrr25-1* strain was transformed by standard methods (*27*), and transformants were replicated to media containing 0.01% MMS. Among 1200 transformants, a single MMS-resistant isolate was identified.
11. Transposon mutagenesis with mTn*10*LUK was by the methods described by O. Hoisman *et al.* [*Genetics* **116**, 191 (1987)]. Double-stranded DNA sequencing primers used to locate the end points of the mTn*10* insertion in Figs. 1 and 3 were 5′-CTGCCCGGATTACAGCA-3′ and 5′-GACGTTGTAAAACGACGG-3′.
12. Deletion of the *HRR25* coding sequence used a *hisG::URA3::hisG* cassette [E. Alani *et al.*, *Genetics* **116**, 541 (1987)]. The 3.1-kb *HRR25* Sal I fragment (Fig. 1) was first cloned into pBluescript (Stratagene). This plasmid was digested with Bgl II, and the two Bgl II fragments that span the entire *HRR25* gene and its flanking sequences were deleted (Fig. 1). Into this deletion was introduced the 3.8-kb Bam HI–Bgl II *hisG::URA3::hisG* fragment from pNKY51 to create the *hrr25Δ* allele. Sal I digestion yielded a linearized fragment that deleted the entire *HRR25* locus.
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28. An MFH14 *hrr25::LUK* heterozygous transformant was dissected onto a thin film of YPD-rich medium on a sterilized microscope slide, and segregants were allowed to germinate under a cover slip by incubation of the slide in a moist 30°C chamber. Photographs of colonies were taken after 2 days of growth.

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A Difference in Hypothalamic Structure Between Heterosexual and Homosexual Men

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The anterior hypothalamus of the brain participates in the regulation of male-typical sexual behavior. The volumes of four cell groups in this region [interstitial nuclei of the anterior hypothalamus (INAH) 1, 2, 3, and 4] were measured in postmortem tissue from three subject groups: women, men who were presumed to be heterosexual, and homosexual men. No differences were found between the groups in the volumes of INAH 1, 2, or 4. As has been reported previously, INAH 3 was more than twice as large in the heterosexual men as in the women. It was also, however, more than twice as large in the heterosexual men as in the homosexual men. This finding indicates that INAH is dimorphic with sexual orientation, at least in men, and suggests that sexual orientation has a biological substrate.

SEXUAL ORIENTATION—SPECIFICALLY, the direction of sexual feelings or behavior toward members of one's own or the opposite sex—has traditionally been studied at the level of psychology, anthropology, or ethics (*1*). Although efforts have been made to establish the biological basis of sexual orientation, for example, by the application of cytogenetic, endocrinological, or neuroanatomical methods, these efforts have largely failed to establish any consistent differences between homosexual and heterosexual individuals (*2, 3*).

A likely biological substrate for sexual orientation is the brain region involved in the regulation of sexual behavior. In nonhuman primates, the medial zone of the anterior hypothalamus has been implicated in the generation of male-typical sexual behavior (*4*). Lesions in this region in male monkeys impair heterosexual behavior without eliminating sexual drive (*5*). In a morphometric study of the comparable region of the

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