by TNF-α. However, in the same cells, TNF-α activated JNK 10-fold. Similar results were obtained when endogenous Raf-1 and B-Raf were immunoprecipitated from PC12 cells and catalytically inactive MEK1 was used as a substrate; although EGF activated Raf-1 and B-Raf, TNF-α did not.

23. Purified recombinant GST-MEK1 and GST-MEK2 were provided by K. L. Guan (*35*). Recombinant histidine-tagged ERK2 protein was expressed as described [T. G. Boulton *et al.*, *Cell* **65**, 664 (1991)] and purified by a Nickel-NTA-agarose column (Qiagen).
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26. A. Minden and C. Lange-Carter, unpublished results. MEKKΔ is a truncated form of MEKK that lacks amino acids 1 to 351 and in which translation is initiated at an internal Nco I site. Complementary DNA encoding the COOH-terminal portion (1.7 kb) of MEKK was subcloned into pBluescript KS⁻ (Stratagene) between the Eco RI and Xho I sites. A Bam HI–Kpn I fragment encoding the entire COOH-terminal portion of MEKK was then subcloned between the Bam HI and Kpn I sites of pJ5Ω [J. P. Morgenstern and H. Land, *Nucleic Acids Res.* **18**, 1068 (1990)], in which expression is under the control of the MMTV long terminal repeat. MEKKΔ(K432M) was constructed like MEKKΔ, but it also contains a $Lys^{432} \rightarrow Met$ substitution. To compare expression of MEKK and MEKKΔ, we transfected COS-1 cells with equal amounts of MEKK and MEKKΔ CMV5 expression vectors (*15*) by the calcium phosphate coprecipitation technique [S. Osawa *et al.*, *Cell* **63**, 697 (1990)]. Cell extracts were prepared and analyzed after 48 hours by immunoblotting (*37*), and the amounts of MEKK and MEKKΔ were quantitated by densitometry. The extent of MEKKΔ expression was five- to sixfold that of MEKK.
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31. Transfections were done with lipofectamine (Gibco/BRL). Cells on 35-mm plates were transfected with 1 μg of each plasmid and collected 48 hours later (*9*). Cell extract (100 μg of protein) was analyzed by immune complex kinase assays, after precipitation with antibodies to HA (Boehringer Mannheim), in the presence of kinase buffer containing 20 μM ATP, [γ-^{32}P]ATP, and either 2 μg of GST–c-Jun(1–79) or MBP as substrate (*9*).
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37. For immunoblot analysis, whole-cell extract (25 μg of protein) was resolved by SDS-PAGE and transferred to Immobilon P membranes (Millipore). After blocking of nonspecific sites, the filters were incubated with antibodies to a COOH-terminal peptide from MEKK (C-22; Santa Cruz Biotechnology), and antibody-antigen complexes were visualized by the chemiluminescence detection system (Amersham), as described (*7–9*).
38. We thank M. Cobb, K. L. Guan, and M. Weber for the supply of essential reagents. A.M. and A.L. were supported by postdoctoral fellowships from the Tobacco Related Disease Research Program. The M.K., G.L.J., and R.J.D. laboratories were supported by grants from the National Institutes of Health, American Cancer Society, and Council for Tobacco Research.

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Suppression of Hyphal Formation in *Candida albicans* by Mutation of a *STE12* Homolog

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A *Candida albicans* gene (*CPH1*) was cloned that encodes a protein homologous to *Saccharomyces cerevisiae* Ste12p, a transcription factor that is the target of the pheromone response mitogen-activated protein kinase cascade. *CPH1* complements both the mating defect of *ste12* haploids and the filamentous growth defect of *ste12/ste12* diploids. *Candida albicans* strains without a functional *CPH1* gene (*cph1/cph1*) show suppressed hyphal formation on solid medium. However, *cph1/cph1* strains can still form hyphae in liquid culture and in response to serum. Thus, filamentous growth may be activated in *C. albicans* by the same signaling kinase cascade that activates Ste12p in *S. cerevisiae*; however, alternative pathways may exist in *C. albicans*.

Candida albicans is the most frequently isolated fungal pathogen in humans. The ability to switch between the yeast and filamentous form has been postulated to contribute to the virulence of this organism (*1*). Difficulties in the genetic manipulation of *C. albicans* have hindered the identification of factors that contribute to the dimorphic switch. *Candida albicans* has no known sexual cycle and is at least diploid. Thus, it is difficult to isolate and identify mutations that affect pathogenicity (*1*).

The observation that *S. cerevisiae* is also dimorphic has permitted the analysis of the switch from budding cells to filaments in a genetically more tractable fungus. Under conditions of nitrogen starvation on solid medium, *S. cerevisiae* diploids switch their growth motif from round cells to pseudohyphae, which are chains of elongated cells that remain attached to each other (*2*). Elements of the yeast mating signal-transduction pathway [the mitogen-activated protein (MAP) kinase cascade] are required for pseudohyphal growth (*3*). The kinases Ste20p, Ste11p(MEKK), and Ste7p(MEK) are required in haploids for the phosphorylation of the transcription factor Ste12p, which stimulates the expression of mating-specific genes (*4–6*). Pseudohyphal formation is greatly suppressed in diploids homozygous for mutations in *STE20*, *STE11*, *STE7*, or *STE12* (*3*).

The existence of a filamentous phase in *S. cerevisiae* provides a suitable background in which to clone *Candida* genes that enhance pseudohyphal growth in *S. cerevisiae*. A diploid *S. cerevisiae* strain, CGx69 (**a**/α *ura3-52/ura3-52*) (*7*), was transformed by electroporation with a *C. albicans* genomic library constructed in a high–copy number *S. cerevisiae* vector, and Ura^+ transformants were selected on a medium that suppresses pseudohyphal formation (*8*). Nine transformants that formed pseudohyphae on this medium were isolated by their agar invasion phenotype and elongated cell morphology (*9*). Restriction analysis of the plasmids isolated from these nine clones indicated that they represented two *C. albicans* genes, *CPH1* and *CPH2* (*Candida* pseudohyphal regulator). *CPH1* markedly enhanced the pseudohyphal growth of *Saccharomyces* on nitrogen starvation medium (Fig. 1, A and B).

The DNA sequence from the open reading frame of the *CPH1* clones showed that the predicted amino acids 20 to 187 of Cph1p are 74% identical to residues 32 to 200 of *S. cerevisiae* Ste12p and 83% identical to residues 27 to 194 of *Kluyveromyces lactis* Ste12p (Fig. 2) (*10–12*). This region contains the DNA-binding domain of *Saccharomyces* Ste12p (*13*). Another stretch of eight residues (Cph1p residues 291 to 298) that are identical between Cph1p and Ste12p of *S. cerevisiae* or *K. lactis* is located in the middle of the region required for pheromone-inducible transcriptional activation (*4*, *14*). *CPH1* complements both the defect in filament formation and the mating defect of *S. cerevisiae ste12* mutants. The *ste12/ste12* diploid strains that carry the *CPH1* gene on a plasmid show restored filament formation (Fig. 1, C and D). The suppression of the *Saccharomyces* mating defect by *Candida* *CPH1* is striking because no sexual cycle is known in *Candida*. The existence of a functional *Candida* homolog of the *S. cerevisiae* mating pathway component Ste12p suggests that *C. albicans* may have an undetected sexual cycle. In a manner similar to *K. lactis* *STE12*, *Candida* *CPH1* complements the *ste12* mating defect better in *MAT***a** strains than in *MAT*α strains (Fig. 3), which presumably reflects the additional requirement for α1 protein to interact with Ste12p in *MAT*α strains for successful mating (*12*).

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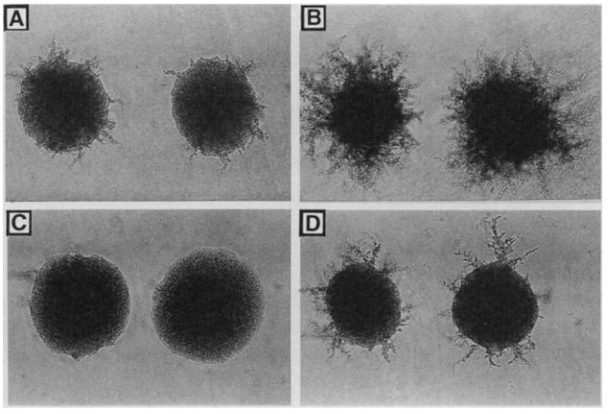

Fig. 1. Enhancement of pseudohyphal growth in *S. cerevisiae* by *CPH1*. Diploids of *S. cerevisiae* were grown on SLAD medium for 3 days at 30°C (*7*). (**A**) L5366 (**a**/α *ura3-52/ura3-52*) carrying a *URA3* vector. (**B**) L5366 carrying a *CPH1 URA3* plasmid (pHL160). (**C**) HLY352 (**a**/α *ste12::LEU2/ste12::LEU2 ura3-52/ura3-53 leu2::hisG/leu2::hisG*) carrying a *URA3* vector. (**D**) HLY352 carrying a *CPH1 URA3* plasmid (pHL160).

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Ca Cph1p   MKESLRLIEDLKFFLATAPANWQENQVIRRYYLNHDEGFVSCVYWNNLYFIT  71
Sc Ste12p  VEESLRLIGDLKFFLATAPVNWQENQIIRRYYLNSGQGFVSCVFWNNLYYIT  84
Kl Ste12p  VEESLRLIEDLKFFLATRPANWQENQVIRRYYLSNDEGFVSCVFWNNLYYIT  78

Ca Cph1p   GTDIVRQIVYKFEHFGRKIIDRKKFEEGIFSDLRNLKCQADAILEPFRSEFL 123
Sc Ste12p  GTDIVKCQLYRMQKFGREVVQKKKFEEGIFSDLRNLKCGIDATLEQPKSEFL 136
Kl Ste12p  GTDIVRCQAYRMQKFGRETVERKKFEEGIFSDLRNLKCGIDATLEKPKSDLL 130

Ca Cph1p   EFLFKNSCLRTQKKQKVFFWFNVPHDKLMADALERDLKKEKMGQRPTTMGHR 175
Sc Ste12p  SFLFRNMCLKTQKKQKVFFWFSVPHDKLFADALERDLKREGLNQPSTTKPVN 188
Kl Ste12p  AFLYKNMCLKTQKKQKVFFWFSVPHDRLFADALERDLKRSSTGSQPTTRAVS 182

Ca Cph1p   EPALSFHYDESS--SLYTQLGKFMETQKRINDAATSS----------TSNTA 215
Sc Ste12p  EPALSFSYDSSSDKPLYDQLLQHLDSRRPSSTTKSDNSPPKLESENFKDNEL 240
Kl Ste12p  EPALSFRWEANSDVSLYDQITNHVDSQR------TDSRPSTVGAQDVTQKQN 228

Ca Cph1p   TTLTDTGVSSGLNNTTSGGQ--SDSATSTHNNNEASTKPSNGSEKSSPEYTT 265
Sc Ste12p  VTVTNQPL---------LGVGLMDDDAPE----------SPSQINDFIPQKLI 274
Kl Ste12p  NRVTDTPVSASKDVEPFESNVVEDEVQIVDNNKMCYGLPHSESSNNYVPQQLI 280

Ca Cph1p   TARGR-------DEHGFLNEA-TPSQYKANSDYEDDFPLDYINQTTQNS   306
Sc Ste12p  IEPNTLELNGLT---EETPHDLPKNT--AKGRDEEDFPLDYFPVSVEYP   318
Kl Ste12p  VPQSDLERNELTNEFDELNADLKPSDILTSNQEDDDFPLDYFPVEIEYS   329
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Fig. 2. Sequence comparison of *C. albicans* Cph1p with *S. cerevisiae* Ste12p and *K. lactis* Ste12p. The GenBank accession number for *CPH1* is U15152 (*27*). Cph1p was aligned with Ste12p of *S. cerevisiae* and *K. lactis* with the MEGALIGN program (DNAstar, Madison, Wisconsin) according to the Clustal method (*25*). The region of greatest homology (Cph1p residues 20 to 306) is shown. Identical residues are boxed; dashes represent gaps introduced to optimize alignment.

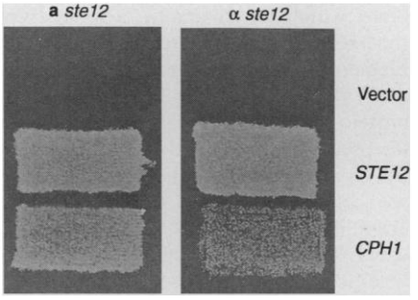

Fig. 3. Complementation of the *ste12* mating defect by *CPH1*. Haploid *ste12* mutants, HLY633 (**a** *ste12::LEU2 ura3-52 leu2::hisG*) and HLY635 (α *ste12::LEU2 ura3-52 leu2::hisG*), were transformed with a vector, *STE12* (B2552), or *CPH1* [pHL160 (*24*)]. For a mating test, rectangular patches of the transformants grown on SC-ura medium were replica-plated to a fertile (Ste$^+$) lawn of the opposite mating type on YPD. After 6 hours at 30°C, the mixture was replica-plated to YNB medium, on which only diploids that result from a mating can grow. The white patches of diploids indicate that mating ability was restored to the *ste12/ste12* transformant by the plasmid.

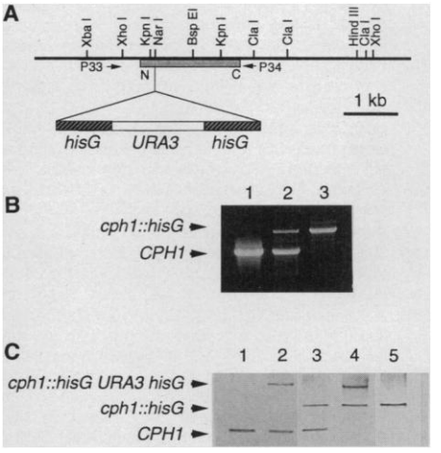

Fig. 4. Disruption of the *CPH1* gene in *C. albicans*. (**A**) The disruption construct contains a *hisG URA3 hisG* insertion at the Nar I site in the DNA-binding domain in *CPH1* coding sequence [pHL156 (*24*)]. (**B**) Identification of *Candida cph1* mutants by PCR analysis. Lane 1, CAI4, *CPH1/CPH1*; Lane 2, JKC16, *CPH1/cph1::hisG*; Lane 3, JKC18, *cph1::hisG/cph1::hisG*. The PCR primers (P33, 5′-CGACCTTCCCCCCACACTCGTTCCC; and P34, 5′-TGGAATCATGCCAATCATAGCACC) amplify from a Xho I site and from the 3′ end of *CPH1* (A). The primers are outside of the *cph1::hisGURA3 hisG* transforming DNA region. (**C**) Southern analysis with a *CPH1* fragment located between P33 and P34 (a Xho I–Sac I fragment of the *CPH1* insert from pHL153) as the probe. The DNA digested with Kpn I was from the following strains: Lane 1, CAI4, *CPH1/CPH1*; lane 2, JKC17, *CPH1/cph1::hisG URA3hisG*; lane 3, JKC16, *CPH1/cph1::hisG*; lane 4, JKC19, *cph1::hisG/cph1::hisGURA3hisG*; Lane 5, JKC18, *cph1::hisG/cph1::hisG*. For more details on the strain constructions, see (*26*).

A *CPH1* disruption construct (Fig. 4A) was used to transform a Ura3$^-$ *C. albicans* strain in order to replace the chromosomal *CPH1* gene with the disrupted sequence by homologous recombination. The disruption of both *CPH1* genes requires a multistep procedure (*15*–*17*). The structures of the *CPH1/cph1* and *cph1/cph1* disruptions were confirmed by both the polymerase chain reaction (PCR) and Southern (DNA) analysis (Fig. 4, B and C). The *cph1/cph1* strains showed suppressed hyphal formation when compared with the *CPH1/CPH1* and *CPH1/cph1* strains (Fig. 5) grown on two different solid media (*18*). The defect in hyphal formation is unlikely to result from a growth defect, because all the strains grew with approximately equivalent doubling times. The only other reported gene whose function is required for hyphal growth in *Candida* is *PHR1* (*16*).

Several experiments indicated that defective hyphal formation is a direct consequence of loss of *CPH1* function: (i) Two independently constructed *cph1/cph1* strains showed the same defect in hyphal formation. (ii) Reintroduction of a wild-type *CPH1* gene by integrative transformation restored the ability of *cph1/cph1* strains to form hyphae (Fig. 5D). Five integrative transformants were obtained in one transformation experiment, all of which contained a functional copy of *CPH1* and formed hyphae (*19*).

Although *cph1/cph1* strains are defective in hyphal formation on solid medium, no difference was observed between these strains and *CPH1/CPH1* strains for either hyphal formation upon growth in liquid Lee's medium or for serum-induced germ tube formation (*20*, *21*). Thus, there must exist *CPH1*-independent pathways capable of signaling the shift from the yeast to the hyphal form that are induced under these diverse conditions. These data suggest the existence of multiple pathways for the induction of hyphal growth in *Candida*.

Comparisons between *S. cerevisiae* and *C. albicans* must take into account the differences between the potential developmental alternatives available to each organism. Both grow as similarly shaped yeast forms; however, the filamentous forms differ markedly. *Saccharomyces cerevisiae* forms only pseudohyphae, which are filaments comprised of chains of distinct cells, whereas *C. albicans* forms both pseudohyphae and true hyphae, which are

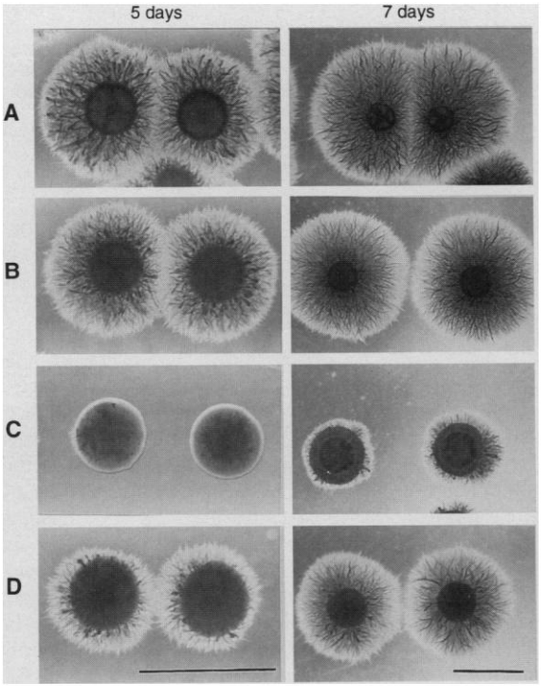

Fig. 5. Suppression of hyphal growth on solid medium by mutation of the *CPH1* gene. Strains were grown on solid Spider medium at a density of ~100 colonies per plate and photographed after growth for 5 or 7 days at 37°C (*18*). (**A**) *CPH1/CPH1* (SC5314, Ura$^+$ ancestor of CAI4) (*15*). (**B**) *CPH1/cph1* (JKC17). (**C**) *cph1/cph1* (JKC19). (**D**) *cph1/cph1::CPH1* (JKC28). All the strains shown are Ura3$^+$, because Ura3$^-$ strains show defects in hyphal formation. Scale bars in (D) represent 0.5 cm. The *CPH1/CPH1* strain (A) developed observable hyphae after 3 days of growth and extensive hyphae after 5 days. The *cph1/cph1* deletion strain (C) showed no observable hyphae after 3 days and markedly reduced hyphal formation after 5 days. Although many of the *cph1/cph1* colonies showed some hyphae after 7 days, these were shorter and much less abundant than those of strains containing a functional *CPH1* gene. The heterozygous *CPH1/cph1* strain (B) showed a slight reduction in the extent of hyphal formation compared with *CPH1/CPH1* (A). The *cph1/cph1* strains transformed with *CPH1* regained the ability to form hyphae (D). These transformants were unstable and segregated colonies with suppressed hyphal growth similar to those in (C) (*19*).

long multicellular tubes with no constrictions at septa between the cells. The organisms also differ with respect to the external signals that induce the yeast cells to form filaments: *Saccharomyces* forms pseudohyphae in response to nitrogen starvation, whereas *Candida* forms only a few small pseudohyphae when starved for nitrogen but forms florid hyphae on many different rich media. Other factors that induce the dimorphic switch in *Candida* include pH, temperature, and carbon source (*21*, *22*). Moreover, serum, which has no effect on *Saccharomyces*, induces *Candida* yeast cells to develop a germ tube and, ultimately, hyphae. The differences in the developmental repertoires available to these two organisms may be a consequence of adaptations to life in different habitats.

In spite of these differences between *Candida* and *Saccharomyces*, the observation that the Ste12p/Cph1p transcription factor mediates filament formation in both organisms suggests that the same kinase cascade may signal filamentous growth in both organisms. This kinase cascade may be a general conduit for signals that induce the yeast-to-filament transition in fungi. Filament formation is blocked in the corn smut, *Ustilago maydis*, by mutations in the Ste7p/MEK homolog Fuz7p (*23*). Our results with the *cph1/cph1* mutant suggest that there may be more than one signal that induces filament formation in *Candida* and more than one pathway that can transmit this signal.

Note added in proof: After submission of this manuscript, Malathi *et al.* (*27*) reported the cloning of a *C. albicans* gene *APRP1* whose protein sequence is identical to that of Cph1p.

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9. Ura$^+$ transformants of *Saccharomyces* were selected on SC-ura+1M sorbitol, a medium that suppresses pseudohyphal formation and invasion. Plates containing transformants were washed with water to remove noninvasive colonies. Approximately 200 of the 10^5 transformants remained, and nine of these formed the elongated cell morphology typical of pseudohyphal growth.
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19. JKC18 (*ura3/ura3 cph1/cph1*) was transformed to Ura$^+$ with pHL221 (*URA3 CPH1*) linearized at the unique Bsp EI site in *CPH1*. All five transformants examined by Southern analysis had reconstituted a wild-type *CPH1* gene integrated at the resident *CPH1* locus: Two had integrations at the COOH-terminal side and one at the NH_2-terminal side of the *hisG* disruption; the other two contained two tandem integrations at the *CPH1* locus. All transformants showed restored hyphal formation, but they were extremely unstable and sectored nonhyphal varients at a high frequency. Presumably, this instability is a consequence of the duplications resulting from plasmid integration.
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24. Plasmids pHL13, pHL14, and pHL16 were isolated once and pHL15 twice from our *Candida* genomic library (*8*). pHL15 was used to sequence *CPH1*. Two of the *CPH1* clones, pHL13 and pHL16, did not complement the *ste12* mating defect in *Saccharomyces*. DNA sequence comparison between pHL13 and pHL15 reveals several differences within the *CPH1* coding region. The shortest *CPH1* subclone, pHL160, contains the Xho I–Cla I fragment from pHL14 in the same *URA3*/2μ plasmid, pRS202. The *cph1::hisGURA3hisG* plasmid, pHL156, was constructed by inserting the *hisGURA3hisG* Bgl II–Bam HI fragment from PCUB-6 (*17*), via a Bgl II linker, into the unique Nar I site in pHL153, which was constructed by cloning the *CPH1* Bgl II–Xho I fragment from pHL15 into the Bam HI–Xho I site of a pBluescript SK plasmid (Stratagene). A *Candida* integration plasmid, pHL159, was constructed by cloning the *URA3* Xba I–Bam HI fragment from pMB-7 (*15*) into the Xba I–Bam HI site of a pBluescript SK plasmid. The *CPH1/URA3* integration plasmid, pHL221, was generated by cloning the whole *CPH1* insert from pHL15 into the Sac I site of pHL159.
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and were then screened by PCR for acquisition of a higher molecular mass product, *cph1::hisG*. This process of transformation and 5-fluoroorotic acid treatment was repeated to disrupt the second copy of *CPH1*. *cph1/cph1* isolates were screened by PCR for complete loss of the wild-type *CPH1* PCR fragment.
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TECHNICAL COMMENTS

The Entropic Cost of Binding Water to Proteins

In a recent Perspective (*1*) about the entropic cost of binding water molecules to proteins and other macromolecules, Jack D. Dunitz uses thermodynamic data on water, anhydrous salts and their hydrates to set limits on the entropy decrease for transferring a water molecule from liquid water to the macromolecule. The limits set were 0 to 7 cal mol^{-1} K^{-1} with larger entropy decreases corresponding to more tightly held waters. Dunitz also states that thermodynamic data from which these entropy changes can be directly calculated are "nonexistent." Such data do exist and calculations of these entropy changes have been reported in the literature.

Data for calculating dS/dn, the entropy change occuring when a mole of water is transferred from liquid water to solid protein, as a function of n, the moles of water bound per mole of protein, can be obtained from measurements of sorption isotherms of water vapor on solid proteins at several temperatures (*2*). Isotherms of water vapor on proteins generally exhibit hysteresis, but it has been shown that correct entropy calculations can be made even in the absence of isotherm reversibility (*3*). For example, calculations of dS/dn values for water bound to native ovalbumin (*2*) have been made on data from the literature (*4*). The variation of such entropies with n has also been discussed (*5*). Values of $-dS/dn$ for ovalbumin varied from approximately 0 to 12 cal mol^{-1} K^{-1} for absorption isotherms and from approximately 0 to 20 cal mol^{-1} K^{-1} for desorption isotherms. Larger entropy decreases were generally seen at lower values of n. The fact that some of these entropy decreases are greater than the estimated limit of 7 cal mol^{-1} K^{-1} suggests that the binding of more tightly held waters to a protein can cause a decrease in protein entropy as well as a decrease in water entropy. Thus, uptake of water could lead to fewer, or more ordered protein conformations, or both.

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Dating Hominid Sites in Indonesia

C. C. Swisher *et al.* (*1*) recently published two new dates for hominid sites in Java based on $^{40}Ar/^{39}Ar$ laser-incremental heating analyses. They propose mean-weighted ages of 1.81 ± 0.04 million years ago (Ma) for the Mojokerto and 1.66 ± 0.04 Ma for the Sangiran site. On the basis of these dates they draw far-reaching conclusions about the early migration of the ancestor of *Homo erectus* out of Africa as well as an explanation for the absence of the Acheulean stone tool culture in Asia. These new $^{40}Ar/^{39}Ar$ ages are based on hornblende separated from pumice recovered at Sangiran and Mojokerto. However, the geological context of these hornblende samples is not clear, and the new ages are contradicted by a wide range of established data.

A discrepancy of about 0.9 Ma between the $^{40}Ar/^{39}Ar$ ages (1.81 and 1.66 Ma) given by Swisher *et al.* and the existing magnetostratigraphy [which is based on detailed sections of Sangiran (0.97 to 0.73 Ma) and Mojokerto (0.97 Ma) reported by Hyodo *et al.* (*2*) in 1993] is not adequately explained by Swisher *et al.* The Hyodo *et al.* (*2*) magnetostratigraphy, based on a solid lithostratigraphy (*3*), corroborates perfectly with a series of fission track ages (*4*) indicating dates all less than 1.0 Ma. In this light, the geological context of samples which yielded the older dates must be critically reviewed. At the Mojokerto site the pumice was taken from a conglomeratic volcanic sandstone, which invites the interpretation that the pumice was likely reworked and redeposited. Swisher *et al.* state, about the Sangiran sample, that the pumice was handpicked from a volcanic pumice-rich layer. There is inadequate information about the lithostratigraphy and exact stratigraphic position of this sample in the Sangiran section and about the relationship of the volcanic pumice-rich layer to the high number of well-described and recognizable tuff layers in the Sangiran area of which some have fission track data (*3*).

There is agreement between the normal polarity found at the Mojokerto site by Swisher *et al.* (*1*) and that reported by Hyodo *et al.* (*2*) but, on the basis of the $^{40}Ar/^{39}Ar$ age of 1.8 ± 0.04, Swisher *et al.* place this site in the Olduvai event. On the basis of the paleomagnetic properties of the section in Sangiran as well as in Mojokerto, Hyodo *et al.* (*2*) demonstrate that the normal polarity of these sites represent the Jaramillo event, which suggests an age of approximately 0.97 Ma. We see no reason to doubt this paleomagnetic sequence, which is also corroborated by fission track ages (*4*). In addition to the discrepancy of the new $^{40}Ar/^{39}Ar$ ages compared with the paleomagnetic and fission track data, the biostratigraphy of Sangiran and Mojokerto (*5*) contradict the newly proposed ages for these sites. Trinil, which contains the type specimen of *Homo erectus* discovered by Dubois (*6*) and is characterized by Stegodon, is widely considered to have an age of about 1 Ma. The Kedung Brubus fauna, characterized by new arrivals of the Asiatic mainland, like *Elephas*, to which the Mojokerto fauna belongs, is younger (*5*) than the Trinil fauna based on all key biostratigraphic markers (*5*).

The $^{40}Ar/^{39}Ar$ dates of Swisher *et al.* may themselves be "technically correct," but until their geological context is established, it is premature to attach such far reaching conclusions to these new age estimates for the hominid of Java.

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