associated with action. Note also that the activation is mostly lateralized to the right hemisphere and that lesions to the right inferior parietal lobule are typically associated with body schema disorders (*18*). Enhancing brain activity of this area during action production can be a computationally simple way to preserve body identity ("it is my body that is moving") during imitation (*19*).

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8. Two recent behavioral studies demonstrated that movement observation strongly affects movement execution in a paradigm similar to the one adopted here (M. Brass, H. Bekkering, A. Wohlschlager, W. Prinz, *Brain Cogn.*, in press; M. Brass, H. Bekkering, W. Prinz, *Acta Psychol.*, in press). In both of these studies, as in this imaging study, a "mirror" configuration (right hand imitation of left hand action) was selected because it has been repeatedly shown that there is a natural tendency to imitate in the mirror configuration [H. Head, *Brain*, **43**, 87 (1920); N. C. Kephart, *The Slow Learner in the Classroom* (Charles Merrill, Columbus, OH, 1971); W. N. Schofield, *Q. J. Exp. Psychol.* **28**, 571 (1976)].
9. A total of 16 participants were studied, following the UCLA Human Subject Protection Committee guidelines. Four participants belonged to a pilot study, which was performed to evaluate the feasibility of the experiment in a fMRI setting. The four participants in the pilot study were three males and one female, and their mean age was 37.75 years (±15.84). The 12 participants in the experiment were nine males and three females, and their mean age was 25.42 years (±5.8). All participants were right-handed, as assessed with a questionnaire that was modified from the Edinburgh Handedness Inventory [R.C. Oldfield, *Neuropsychologia* **9**, 97 (1971)], and had no neurological abnormalities identified at the neurological examination that was performed just before the scanning procedure.
10. We used an echo planar T_2*-weighted gradient echo sequence [repetition time (TR) = 4000 ms; echo time (TE) = 70 ms; flip angle = 90°; 64 by 64 volume element (voxel) matrix; 26 axial slices; 3.125-mm in-plane resolution; 4-mm thickness; interslice gap (skip) = 1 mm)]. We also acquired a coplanar high-resolution echo planar imaging (EPI) volume (TR = 4000 ms; TE = 54 ms; flip angle = 90°; 128 by 128 voxel matrix; 26 axial slices; 3.125-mm in-plane resolution; 4-mm thickness; skip = 1 mm), to obtain anatomical data on our participants. The software program MacProbe [E. Zaidel and M. Iacoboni, *Brain* **119**, 2155 (1996)] was used for stimulus presentation. Four fMRI scans of 5 min and 20 s each were performed on every participant. The six tasks were alternated with seven rest periods. Each task or rest period lasted 24 s, except for the last rest period, which lasted 36 s. Each trial lasted 3 s. Thus, each task period comprised eight trials. The task order was counterbalanced across participants. In-plane Gaussian filtering was applied to produce a final image resolution of 8.7 mm by 8.7 mm by 8.6 mm. Image registration for each participant was performed by aligning the functional volumes to the coplanar high-resolution EPI volume with a rigid-body linear registration algorithm [R. P. Woods, S. T. Grafton, C. J. Holmes, S. R. Cherry, J. C. Mazziotta, *J. Comput. Assisted Tomogr.* **22**, 139 (1998)]. Image registration for the group of participants was performed with fifth-order polynomial nonlinear warping [R. P. Woods, S. T. Grafton, J. D. G. Watson, N. L. Sicotte, J. C. Mazziotta, *J. Comput. Assisted Tomogr.* **22**, 153 (1998)] of each participant's images into a Talairach-compatible brain magnetic resonance (MR) atlas [R. P. Woods, M. Dapretto, N. L. Sicotte, A. W. Toga, J. C. Mazziotta, *Hum. Brain Mapp.* **8**, 73 (1999)]. Statistical analysis was performed with analysis of variance (ANOVA) [R. P. Woods, M. Iacoboni, S. T. Grafton, J. C. Mazziotta, in *Quantification of Brain Function Using PET*, R. Myers, V. Cunningham, D. Bailey, T. Jones, Eds. (Academic Press, San Diego, CA, 1996), pp. 353–358]. Participants (n = 12), fMRI scans (n = 4), task (n = 2: observation and observation-execution), and stimuli (n = 3: animated hand, static hand, and geometric figure) were included in the ANOVA, whereas rest periods were excluded. Because of the "blurred" hemodynamic response, the six brain volumes acquired per task period cannot be considered independent observations. Thus, we used the sum of the signal intensity at each voxel throughout each task period as the dependent variable. To account for the delayed hemodynamic response [R. S. Menon and S.-G. Kim, *Trends Cogn. Sci.* **3**, 207 (1999)], we excluded the first brain volume of each task period and included the first brain volume of the following rest period. The statistical threshold, estimating variance at each voxel, was corrected for spatial multiple comparisons [K. J. Worsley *et al.*, *Hum. Brain Mapp.* **4**, 58 (1996)]. Although cluster size is reported here, cluster size was not used in computing statistical significance; that is, even a single activated voxel would be statistically valid. The alpha level was also Bonferroni corrected for multiple comparisons. Given that we were interested in the cortical correlates of imitation, only cortical regions with motor properties were considered. Thus, our search region of interest was limited to the cerebral cortex of the frontal and parietal lobes.
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Heterozygous Germ Line *hCHK2* Mutations in Li-Fraumeni Syndrome

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The *hCHK2* gene encodes the human homolog of the yeast Cds1 and Rad53 G_2 checkpoint kinases, whose activation in response to DNA damage prevents cellular entry into mitosis. Here, it is shown that heterozygous germ line mutations in *hCHK2* occur in Li-Fraumeni syndrome, a highly penetrant familial cancer phenotype usually associated with inherited mutations in the *TP53* gene. These observations suggest that *hCHK2* is a tumor suppressor gene conferring predisposition to sarcoma, breast cancer, and brain tumors, and they also provide a link between the central role of p53 inactivation in human cancer and the well-defined G_2 checkpoint in yeast.

Li-Fraumeni syndrome (LFS) is a rare familial multicancer syndrome characterized by the occurrence of sarcomas, breast cancer, brain tumors, leukemia, and adrenal cortical tumors in multiple relatives (*1*). In most cases, LFS results from inheritance of a mutant *TP53* allele, followed by somatic loss of the remaining wild-type allele, which thus constitutes the primary initiating event leading to cancer (*2, 3*). The central role of *TP53* mu-

tation during cancer progression is also demonstrated by its inactivation in ~50% of all sporadic human cancers (*4*). p53 is thought to mediate a cell cycle checkpoint, whose activation following ionizing radiation (IR) is dependent on the ataxia telangiectasia (ATM) gene product and leads to either G_1 arrest or apoptosis (*5*).

In addition to this G_1 checkpoint, which prevents the replication of damaged DNA, chromosomal integrity before mitosis is monitored by the G_2 checkpoint, a pathway that has been best studied in yeast (*6*). In *Schizosaccharomyces pombe*, IR triggers activation of the Chk1 and Cds1 kinases, which is dependent on the ATM homolog Rad3 (*7*). Mutation of *CHK1* is sufficient to prevent activation of this checkpoint in response to DNA damage, but disruption of the *CHK1* and *CDS1* genes is required to abrogate the response to hydroxyurea (HU), a ribonucleotide reductase inhibitor that triggers a Rad3-independent DNA replication checkpoint (*8*). Activation of Chk1 and Cds1 leads to the inhibitory phosphorylation of the phosphatase Cdc25, excluding it from the nucleus and preventing it from activating Cdc2, hence blocking entry of the cell into mitosis (*9*).

Mammalian homologs of the yeast G_2 checkpoint genes show sequence conservation and, in some cases, partial functional rescue of a null phenotype in yeast. In human cells, hCHK1 phosphorylates Cdc25C at Ser^{216}, leading to its binding and cytoplasmic sequestration by 14-3-3 proteins (*10*). The p53 target gene product 14-3-3σ also sequesters Cdc2–cyclin B1, suggesting a role for p53 in the G_2 checkpoint (*11*). *hCHK2* encodes a kinase with ~30% amino acid identity to both the *S. pombe* Cds1 and its *Saccharomyces cerevisiae* homolog, Rad53 (*12*). Like hCHK1, hCHK2 is phosphorylated in an ATM-dependent manner in response to DNA damage and can phosphorylate Cdc25C on Ser^{216}. Activation of the DNA replication checkpoint by treatment of cells with HU leads to the ATM-independent phosphorylation of hCHK2 (*12*).

To determine whether disruption of the G_2 checkpoint is associated with human cancer, we first screened for heterozygous germ line mutations in *hCHK1* or *hCHK2* in four cases of classical LFS [see (*13*) for clinical criteria] that lacked mutations in *TP53*. The respective cDNAs, derived from either Epstein-Barr virus (EBV)–immortalized lymphoblasts or primary cultured fibroblasts, were amplified in overlapping fragments by reverse transcriptase–polymerase chain reaction (RT-PCR), and uncloned products were screened for mismatches by denaturing high-performance liquid chromatography (DHPLC) (*14*), followed by nucleotide sequence analysis (*15*). Mutations were confirmed by analysis of genomic DNA. A heterozygous germ line mutation in *hCHK2* was detected in kindred MA81, cosegregating with the cancer predisposition phenotype (Fig. 1). In this family, remarkable for the incidence of rhabdomyosarcoma, brain tumors, and multiple cases of early-onset and bilateral breast cancer, a single nucleotide deletion was present within the kinase domain [deletion of C at nucleotide 1100 (1100delC)], resulting in premature termination. The mutation was present in three affected family members but it was absent from an unaffected sibling. The 1100delC mutation was not detected in 50 control individuals (100 alleles).

We extended our germ line mutational analysis to individuals with LFS-variant. These probands belong to families with multiple cancers that do not meet the clinical criteria for classical LFS (*13*). Although ~70% of classical LFS kindreds are due to germ line *TP53* mutations, these are detected in only ~20% of LFS-variant (*3*), suggesting that these more moderate phenotypes are linked to other genes. Germ line specimens were available from 18 cases of LFS-variant in which *TP53* mutations were excluded. A single nucleotide deletion within the kinase domain of *hCHK2* (deletion of T at nucleotide 1422) leading to a frameshift was detected in LFS-variant DF593 (Table 1). The proband had multiple colonic polyps, colorectal cancer, and bilateral ocular melanomas and had a family history of sarcomas and breast, colorectal, gastric, and lung cancers. A second mutation, an Ile → Thr missense mutation at codon 157 of *hCHK2* was present in LFS-variant MGH005, in which the proband had developed three primary tumors: early-onset breast cancer, melanoma, and lung cancer (Fig. 2). This nonconservative substitution is within the forkhead homology-associated (FHA) domain of hCHK2, a highly conserved protein-interaction domain of 60 amino acids that is essential for activation of the yeast homolog Rad53 in response to DNA damage (*16*). The mutation was not detected in 100 alleles from 50 control individuals of comparable ethnic background, indicating that it is not a polymorphic variant that is prevalent in the population. The germ line $Ile^{157} \rightarrow Thr^{157}$ mutation in *hCHK2* is therefore likely to be responsible for this case of LFS-variant.

To identify common sequence variations in *hCHK2* within the population, we analyzed the entire coding sequence in 28 EBV-immortalized lymphoblasts from healthy controls. A silent polymorphism, $Ala^{252} \rightarrow Gly^{252}$, was detected in 4 of 28 individuals, but no amino acid substitutions in the *hCHK2* coding sequence were observed in control specimens (Table 1). Given the similar function of hCHK1 and hCHK2, we also analyzed

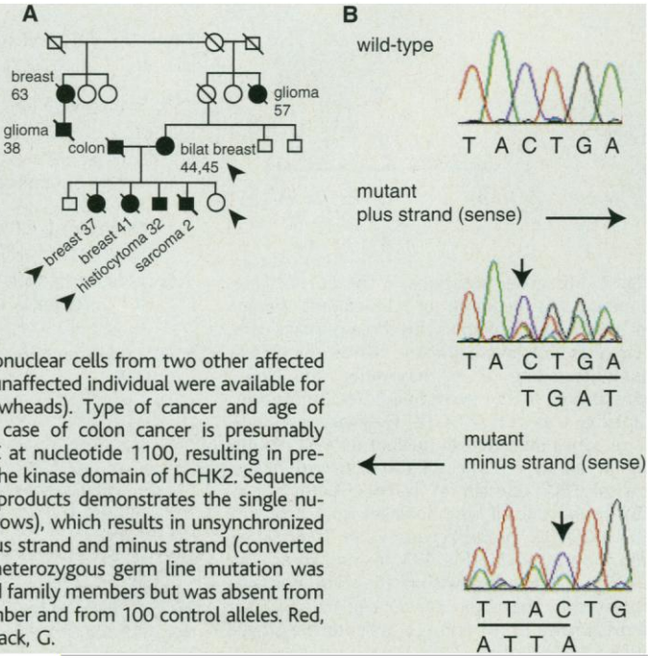

Fig. 1. Germ line truncating mutation in *hCHK2* in a classical LFS family with wild-type p53. (**A**) Pedigree of kindred MA81 with classical LFS. Solid circles (females) or squares (males) denote affected individuals. Slashed symbols indicate deceased individuals. EBV-immortalized lymphoblasts from one affected family member, peripheral blood mononuclear cells from two other affected family members, and one unaffected individual were available for analysis (denoted by arrowheads). Type of cancer and age of diagnosis are noted. The case of colon cancer is presumably sporadic. (**B**) Deletion of C at nucleotide 1100, resulting in premature truncation within the kinase domain of hCHK2. Sequence analysis of uncloned PCR products demonstrates the single nucleotide deletion (short arrows), which results in unsynchronized sequence, shown in the plus strand and minus strand (converted to sense sequence). This heterozygous germ line mutation was present in all three affected family members but was absent from the unaffected family member and from 100 control alleles. Red, T; green, A; blue, C; and black, G.

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our cohort of LFS and LFS-variants for mutations in *hCHK1*. No germ line mutations in *hCHK1* were detected in individuals with these familial cancer syndromes. Two silent polymorphisms in *hCHK1* were identified, each present in 1 of 23 individuals (Table 1).

To screen for somatic inactivation of *hCHK2*, we analyzed 49 cancer cell lines, derived from a wide variety of sporadic human cancers (Table 1). We detected a mutation in only one: The colon cancer cell line HCT15 contained a heterozygous Arg → Trp missense mutation at codon 145, within the FHA domain (Fig. 2). This mutation may contribute to tumorigenesis either as a result of reduced gene dosage or through a dominant negative effect, as has recently been proposed for heterozygous mutations in the spindle-assembly checkpoint kinase gene *hBUB1* (*17*). The observation that *hCHK2* mutations are rare in cell lines derived from sporadic tumors is unexpected given the striking phenotype associated with its inactivation in the germ line. Conceivably, the high frequency of G_1 checkpoint disruption in sporadic tumors, either by mutation of *TP53* itself or by destabilization of p53 protein (*18*), may preclude the need for simultaneous disruption of the G_2 checkpoint though *hCHK2* mutation. In this context, it is of interest that the HCT15 colon cancer cell line contains one wild-type allele of *TP53* and has homozygous inactivation of *MSH6*, resulting in a defect in the repair of single point mutations (*19*). Analysis of the *hCHK1* transcript in our panel of cancer cell lines did not reveal any sequence variations, other than the silent polymorphisms noted above. Alterations in a polyadenosine stretch within the *hCHK1* coding region have been reported in a few colon cancers with mismatch repair defects (*20*), but we did not detect such mutations in sporadic cancer cell lines.

Our observation that LFS and LFS-variant kindreds with wild-type p53 harbor mutations in the G_2 checkpoint kinase *hCHK2* suggests that germ line mutations in these two genes have similar consequences. Sarcomas and breast and brain tumors, the most common cancers seen in LFS associated with mutant p53 (*1*), were also prevalent in carriers of germ line *hCHK2* mutations. However, LFS is a rare syndrome, and only a few cases with wild-type p53 were available for analysis. Gene-specific differences in tumor spectrum may emerge from the study of additional families. Similarly, more extensive studies will be required to determine whether the FHA domain of *hCHK2* is a mutational hotspot and whether truncating and missense mutations in this gene are associated with distinct phenotypes.

The comparable clinical features of *TP53* and *hCHK2* germ line inactivation have implications for our understanding of the critical functional pathways for these two gene products. It is possible that disruption of either the G_1 or G_2 checkpoints may be distinct but functionally equivalent in triggering genomic instability. Alternatively, the shared phenotype of *TP53* and *hCHK2* inactivation may underscore the contribution of p53 itself to maintenance of the G_2 checkpoint (*11, 21*). Finally, there may be a direct functional interaction between hCHK2 and p53. Activation of p53 and hCHK2 after DNA damage is ATM-dependent, raising the possibility that hCHK2 may be an intermediate kinase in the phosphorylation of p53 [reviewed in (*22*)].

The identification of *hCHK2* as a human tumor suppressor gene is noteworthy in that the gene was isolated and its presumed function was determined by virtue of its homology to yeast genes known to function in a critical DNA damage response pathway (*12*). A comparable degree of evolutionary conservation has been observed for the mismatch

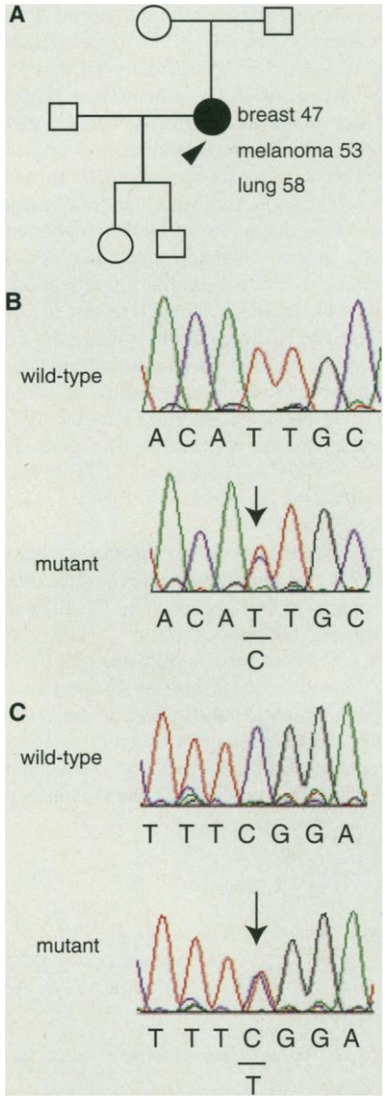

Fig. 2. Missense mutations in the *hCHK2* FHA domain. (**A**) Pedigree of LFS-variant family MGH005. The proband, with three primary cancers, was a cigarette smoker; hence, the development of lung cancer may reflect this exposure as well as the germ line *hCHK2* mutation. Labeling is as in Fig. 1A. (**B**) Heterozygous T → C missense mutation at nucleotide 470, resulting in an Ile → Thr substitution within the critical FHA domain of hCHK2, detected in EBV-immortalized lymphoblasts from proband MGH005. (**C**) Heterozygous C → T missense mutation at nucleotide 433, leading to an Arg → Trp substitution within the FHA domain, detected in the colon cancer cell line HCT15. Short arrows in (B) and (C) indicate the single nucleotide deletion.

Table 1. *hCHK1* and *hCHK2* sequence variations in LFS kindreds and in cancer cell lines; nt, nucleotide.

Sequence variation	Codon	Nucleotide change and position*	Effect on protein
hCHK2 mutations			
Germ line of familial cases†			
LFS MA81	366	Deletion of C at nt 1100	Frameshift (stop at codon 381)
LFS-variant DF593	475	Deletion of T at nt 1422	Frameshift (stop at codon 481)
LFS-variant MGH005	157	T to C at nt 470	Missense (Ile → Thr in FHA domain)
Sporadic cancer cell lines‡			
HCT15 (colon)	145	C to T at nt 433	Missense (Arg → Trp in FHA domain)
hCHK2 polymorphisms§			
4/28	84	A to G at nt 252	None
hCHK1 polymorphisms			
1/23	396	T to C at nt 1188	None
1/23	400	G to A at nt 1200	None

*All detected mutations were heterozygous. †Familial cancer: classical LFS (n = 4) and LFS-variant (n = 18). For definitions, see (*13*). ‡Cancer cell lines (n = 49) studied were breast cancer (MCF7/Adr, MDA-MB-435, T-47D, HS578T, BT-549, BT-483, MDA-MB-468, MDA-MB-415, MDA-MB-175-VII, MDA-MB-231, MDA-MB-436, MDA-MB-453, MDA-MB-157, UACC-893, HS467T, HS496T, and HS275), ovarian cancer (OVCAR-2, OVCAR-5, OVCAR-8, IGROV-1, and SKOV-3), glioblastoma (SNB-19, U251, and SF-295), leukemia (K562, MOLT-4, RPMI 8226, SR, and CCRF-CEM), prostate cancer (DUL-145 and PC-3), colon cancer (HT-29, HCT-116, SW-620, COLO-205, and HCT-15), renal cell cancer (UO-31, SN-12C, CAKI-1, 786-0, and ACHN), melanoma (SK-MEL-2, UACC-62, M14, and LOX-IMVI), and lung cancer (NCI-H460, NCI-H522, and HOP-92). §Frequency of polymorphisms was determined by analysis of EBV-immortalized lymphoblasts derived from healthy controls. The two *hCHK1* polymorphisms were detected in separate individuals.

repair genes, inactivated in hereditary nonpolyposis colon cancer (*23*). The presence of *hCHK2* mutations in a human cancer syndrome highlights the importance of cell cycle checkpoints in tumorigenesis. However, inactivation of the G_2 checkpoint may also have therapeutic implications (*6*), because it may render cancer cells particularly sensitive to DNA damaging agents, triggering a mitotic catastrophe (*24*) as they attempt segregation of damaged chromosomes.

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13. Clinical criteria for diagnosing a family as having LFS are the combination of (i) proband with sarcoma diagnosed under age 45, (ii) first-degree relative with an LFS component tumor (sarcoma, breast cancer, brain tumor, leukemia, or adrenal cancer) diagnosed under age 45, and (iii) first- or second-degree relative with any cancer diagnosed under age 45 or with sarcoma diagnosed at any age. Clinical criteria for LFS-variant are an individual with three separate primary cancers, with the first cancer diagnosed under age 45, or the combination of (i) proband with childhood cancer or LFS component tumor diagnosed under age 45, (ii) first- or second-degree relative with LFS component tumor diagnosed at any age, and (iii) first- or second-degree relative with any cancer diagnosed under age 60 [J. M. Birch *et al.*, *Cancer Res.* **54**, 1298 (1994); C. Eng *et al.*, *Cancer Epidemiol. Biomark. Prev.* **6**, 379 (1997)].
14. RNA was isolated from pelleted peripheral blood mononuclear cells, cultured fibroblasts, or EBV-immortalized lymphoblasts using RNA–Stat 60 (Teltest, Friendswood, TX) and reverse transcribed with random hexanucleotides. Overlapping 250– to 350–base pair RT-PCR fragments of the *hCHK1* and *hCHK2* transcripts were designed to generate homogeneous melting profiles with the Stanford prediction program (available at http://insertion.stanford.edu/melt.html) and were amplified in nested reactions (primers and conditions are available upon request). Uncloned RT-PCR products were analyzed by DHPLC (Transgenomics, Omaha, NE) to detect mismatched sequences resulting from heterozygous mutations [W. Liu, D. Smith, K. J. Rechtzigel, S. N. Thibodeau, C. D. James, *Nucleic Acids Res.* **26**, 1396 (1998)]. Synthetic mutations were tested for all fragments to ensure optimal analytic conditions. To search for homozygous mutations in cancer cell lines, we mixed equal amounts of PCR products derived from different cell lines to generate detectable mismatches. To search for polymorphic variants in the population, we analyzed EBV-immortalized lymphoblasts from 50 healthy individuals for alterations in the *hCHK1* and *hCHK2* transcripts. All samples with abnormal chromatography profiles were analyzed by nucleotide sequencing.
15. For automated nucleotide sequencing analysis, uncloned RT-PCR products were sequenced in both directions, with Energy Transfer Dye Primer Chemistry (Amersham Pharmacia Biotech), and analyzed for the presence of heterozygous mutations. Point mutations and small deletions detected in the *hCHK2* transcript were confirmed by amplification of genomic DNA and nucleotide sequencing. Nucleotides are numbered from the initiator methionine (GenBank accession number AF086904).
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Photic Induction of *mPer1* and *mPer2* in *Cry*-Deficient Mice Lacking a Biological Clock

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Mice lacking *mCry1* and *mCry2* are behaviorally arrhythmic. As shown here, cyclic expression of the clock genes *mPer1* and *mPer2* (mammalian *Period* genes 1 and 2) in the suprachiasmatic nucleus and peripheral tissues is abolished and *mPer1* and *mPer2* mRNA levels are constitutively high. These findings indicate that the biological clock is eliminated in the absence of both mCRY1 and mCRY2 (mammalian cryptochromes 1 and 2) and support the idea that mammalian CRY proteins act in the negative limb of the circadian feedback loop. The *mCry* double-mutant mice retain the ability to have *mPer1* and *mPer2* expression induced by a brief light stimulus known to phase-shift the biological clock in wild-type animals. Thus, mCRY1 and mCRY2 are dispensable for light-induced phase shifting of the biological clock.

Many physiological and behavioral systems are controlled by an internal self-sustaining molecular oscillating mechanism with a periodicity of approximately 24 hours, known as the biological clock. The core oscillator consists of an autoregulatory transcription-(post) translation–based feedback loop involving a set of clock genes (*1*). In mammals, as in *Drosophila*, three recently identified *mPer* genes (*mPer1*, *mPer2*, and *mPer3*) are thought to be oscillator genes (*2–4*). Transcription of *mPer1* is driven by the CLOCK/BMAL transcriptional activator complex and in turn is repressed by its own gene product (*5*). To maintain synchrony with the solar day/night cycle, the master clock in the suprachiasmatic nucleus (SCN) of the brain needs to be reset by daily light through receptors in the eye (*6*). In mammals, mCRY1 and mCRY2, members of the light-harvesting cryptochrome/photolyase protein family (*7*), have been proposed as candidate photoreceptors required for light-entrainment of the biological clock (*8*). Mouse mutants lacking *mCry1* show an acceleration of the free-run-

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