The amount of UV-B light used in our experiments did not exceed twice the average minimal erythema dose, an amount of UV-B radiation that causes barely detectable skin reddening and is equivalent to 2 to 3 min of solar irradiation on a summer day (*27*). Prolonged sunlight exposure results in fever and a local inflammation. This response may result from activation of JNK and related MAPKs, leading to stimulation of AP-1 activity and cytokine production (*23*). Prolonged exposure to UV-B light causes skin wrinkling, another effect that was attributed to AP-1 activation (*27*). Osmotic stress also induces inflammatory responses in humans similar to the ones induced by IL-1 (*28*). Our findings provide a mechanistic explanation to these observations as well.

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Mapping of a Gene for Parkinson's Disease to Chromosome 4q21–q23

Mihael H. Polymeropoulos,* Joseph J. Higgins, Lawrence I. Golbe, William G. Johnson, Susan E. Ide, Giuseppe Di Iorio, Giuseppe Sanges, Edward S. Stenroos, Lana T. Pho, Alejandro A. Schaffer, Alice M. Lazzarini, Robert L. Nussbaum, Roger C. Duvoisin

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, affecting approximately 1 percent of the population over age 50. Recent studies have confirmed significant familial aggregation of PD and a large number of large multicase families have been documented. Genetic markers on chromosome 4q21–q23 were found to be linked to the PD phenotype in a large kindred with autosomal dominant PD, with a $Z_{max} = 6.00$ for marker *D4S2380*. This finding will facilitate identification of the gene and research on the pathogenesis of PD.

The first clear description of the common neurodegenerative disorder designated as Parkinson's disease (PD) was provided by James Parkinson in 1817 (*1*). Clinical manifestations include resting tremor, muscular rigidity, bradykinesia, and postural instability. Additional features are characteristic postural abnormalities, dysautonomia, dystonic cramps, and dementia. The specific pattern of neuronal degeneration is accompanied by eosinophilic intracytoplasmic inclusions known as Lewy bodies in surviving neurons in the substantia nigra, locus ceruleus, nucleus basalis, cranial nerve motor nuclei, central and peripheral divisions of the autonomic nervous system, hypothalamus, and cerebral cortex (*2*).

Parkinson's successors speculated on the relative roles of environment and heredity. Gowers in 1880 noted that 15% of his PD patients had affected relatives (*3*). Modern studies of familial aggregation in PD along with the observation of an autosomal dominant pattern of inheritance seen in multicase families have indicated that genetic factors play a substantial etiologic role (*4*). The availability of well-documented families provides an opportunity to carry out DNA linkage studies (*5*).

The discovery of the parkinsonogenic effects of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) led many investigators to search for an environmental factor in the etiology of PD (*6*). The highly selective destruction of the dopaminergic neurons of the substantia nigra caused by MPP^+, the toxic metabolite of MPTP, through inhibition of complex I of the mitochondrial respiratory chain provided a model that explained how an exogenous agent could induce PD (*7*). However, MPTP-induced parkinsonism did not replicate either the clinical course or the pathology of PD. Moreover, subsequent studies did not establish a direct link between PD and mutations in mitochondrial DNA (*8*) or

M. H. Polymeropoulos, S. E. Ide, A. A. Schaffer, R. L. Nussbaum, Laboratory of Genetic Disease Research, National Center for Human Genome Research, National Institutes of Health, Bethesda, MD 20892–1430, USA.
J. J. Higgins and L. T. Pho, Clinical Neurogenetics Unit, Medical Neurology Branch, National Institutes of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892–1430, USA.
L. I. Golbe, W. G. Johnson, E. S. Stenroos, A. M. Lazzarini, R. C. Duvoisin, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA.
G. Di Iorio and G. Sanges, Istituto di Scienze Neurologiche, Facoltá di Medicina, Seconda Universitá degli Studi di Napoli, Naples, Italy.

*To whom correspondence should be addressed.

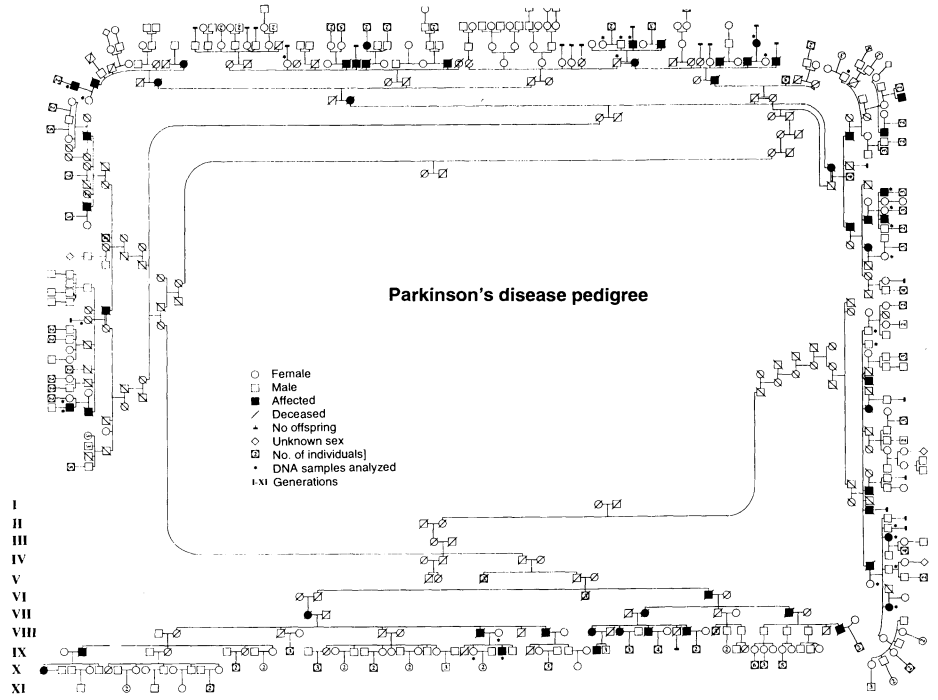

Fig. 1. A large family with PD. The clinical and pathological features of some members of this kindred were previously reported (*11*).

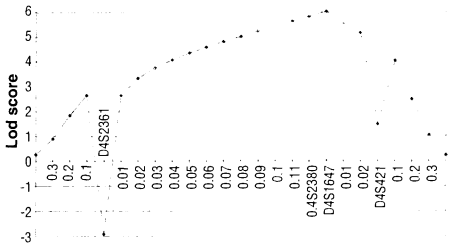

Fig. 2. Multipoint lod score analysis between chromosome 4q markers and the PD locus.

Table 1. Two-point LOD scores between chromosome 4q markers and the PD locus.

Locus	Two-point lod scores at recombination fractions of:							Z_{max}	θ_{max}
	0.00	0.01	0.05	0.10	0.20	0.30	0.40		
D4S2361	−5.60	−0.83	0.30	0.54	0.43	0.21	0.06	0.55	0.12
D4S2380	6.00	5.90	5.30	4.60	3.00	1.50	0.50	6.00	0.00
D4S1647	5.22	5.07	4.47	3.71	2.26	1.05	0.30	5.22	0.00
D4S421	−2.42	0.45	0.77	0.65	0.38	0.22	0.09	0.77	0.05

polymorphisms in nuclear genes involved in the metabolism of potentially neurotoxic substances (9).

To identify a genetic locus responsible for the PD phenotype we performed a genome scan (*10*) in a large kindred of Italian descent with pathologically confirmed PD (Fig. 1) (*11*). The average age of onset for the illness in this pedigree is 46 ± 13 years. A total of 140 genetic markers were typed in this pedigree at an average spacing of ~20 cM. Genetic markers at the cytogenetic location 4q21–q23 were the only ones to show linkage to the disease phenotype with a Z_{max} [maximum logarithm of the likelihood ratio for linkage (lod) score] = 6.00 at recombination fraction $\theta = 0.00$ for marker *D4S2380* (Table 1). Recombinations between the disease phenotype and genetic markers were observed in the proximal region for marker *D4S2361* and in the distal region for marker *D4S421*. Genetic markers *D4S2380* and *D4S1647* showed no obligate recombination events in the affected individuals. Multipoint lod score analysis between markers *D4S2361*-13cM-*D4S1647*-3cM-*D4S421* and the disease locus places the PD gene between markers *D4S2361* and *D4S421* at a recombination distance of 0.00 cM from marker *D4S1647* with a Z_{max} = 6.04 (Fig. 2). This location is favored from the alternative genetic intervals by a difference in the lod score of greater than 3 lod units.

Although expansions of unstable trinucleotide repeats are found in a number of human neurodegenerative conditions, there is no evidence for an association of a CAG trinucleotide repeat expansion in families with PD (*12*). In addition, genetic linkage studies in other families with PD-like illnesses do not support the involvement of several candidate genes (glutathione peroxidase, tyrosine hydroxylase, brain-derived neurotrophic factor, catalase, amyloid precursor protein, CuZn superoxide dismutase, and debrisoquine 4-hydroxylase) in the etiology of the disorder (*13*). Genes previously mapped in the general region of linkage include the loci for alcohol dehydrogenase, formaldehyde dehydrogenase, synuclein, UDP-N-acetylglycosamine phosphotransferase, and others. Other distinct clinicopathological entities associated with parkinsonian features are probably linked to other genetic loci. For example, Wilhelmsen-Lynch disease (disinhibition-dementia-parkinsonian-amyotrophy complex) is linked to the 17q21–q22 chromosomal region (*14*). If the pathogenesis of diseases affecting the nigrostriatal pathway includes environmental influences, then a range of mutations affecting vulnerable sites in the electron transport chain or enzyme polymorphisms influencing neurotoxin metabolism may vary the penetrance of PD by altering an individual's resistance to exogenous or endogenous agents. However, our finding of a highly penetrant genetic locus linked to PD suggests that abnormalities of a single gene may be sufficient to cause the PD phenotype.

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nated in the town of Contursi in the Salerno province of southern Italy (*11*); some members emigrated to the United States, Germany, and other countries. The extended family pedigree consists of 592 members with 60 individuals affected by PD (Fig. 1). High molecular weight genomic DNA was isolated from whole-blood lysate as described [G. I. Bell, J. Karam, W. Rutter, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5979 (1981)]. Genotyping was performed as described [J. M. Gyapay *et al.*, *Nature Genet* **7**, 262 (1994)]. Pairwise linkage analysis was performed by means of the MLINK program of the FASTLINK package. [G. M. Lathrop, J. M. Lalouel, C. Julier, J. Ott, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3442 (1984); R. W. Cottingham, R. M. Idury, A. A. Schaffer, *Am. J. Hum. Genet.* **54**, 252 (1984); S. K. Gupta, A. A. Schaffer, A. L. Cox, S. Dwarkadas, W. Zwaenepoel, *Comp. Biomed. Res.* **28**, 116 (1995).] Allele frequencies were used as reported in the Genome Data Base (http://gdbwww.gdb.org) and the Cooperative Human Linkage Consortium (CHLC) database (http://www.chlc.org). Multipoint analysis was performed with the LINKMAP program of the FASTLINK package. For the multipoint analysis allele frequencies were set to 1/*n* where *n* equals the number of alleles observed. In the two-point analysis, lod scores were calculated for both the reported and the 1/*n* allele frequencies with minimal effect on the maximum lod score observed. Simulations of multipoint analysis in a subset of the pedigree with different allele frequencies similarly indicated no significant effect on the scores calculated. Maximum lod scores as shown were observed for the heterozygote and homozygote disease allele penetrance set to 0.99 which is similar to the PD allele penetrance previously reported ranging from 0.88 to 0.94 (*11*). All unaffected individuals used in the study were of age above the mean for onset of illness. Disease allele frequency was set to 0.001. Distances and order of genetic markers were set as reported in the CHLC database. Overlapping three point analysis was performed for markers *D4S2361*, *D4S1647*, *D4S421*, and the PD locus. The 12-allele *D4S2380* locus was not included because of prohibitive run time. Multipoint analysis was performed on an IBM SP2 parallel computer and the SGI Challenge machine. Changes have been made in the pedigree shown to protect patient confidentiality.

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Identification of BIME as a Subunit of the Anaphase-Promoting Complex

Jan-Michael Peters,*† Randall W. King,* Christer Höög, Marc W. Kirschner‡

The initiation of anaphase and exit from mitosis require the activation of a proteolytic system that ubiquitinates and degrades cyclin B. The regulated component of this system is a large ubiquitin ligase complex, termed the anaphase-promoting complex (APC) or cyclosome. Purified *Xenopus laevis* APC was found to be composed of eight major subunits, at least four of which became phosphorylated in mitosis. In addition to CDC27, CDC16, and CDC23, APC contained a homolog of *Aspergillus nidulans* BIME, a protein essential for anaphase. Because mutation of *bimE* can bypass the interphase arrest induced by either *nimA* mutation or unreplicated DNA, it appears that ubiquitination catalyzed by APC may also negatively regulate entry into mitosis.

Mitotic cyclins bind to the protein kinase $p34^{cdc2}$ to promote entry into mitosis in all eukaryotic cells (*1*). During anaphase, mitotic cyclins are rapidly degraded by ubiquitin-dependent proteolysis, leading to the inactivation of Cdc2 and allowing exit from mitosis (*2*). This proteolytic system is also thought to initiate anaphase by ubiquitinating a hypothetical inhibitor of chromosome segregation (*3*, *4*). Cyclin B ubiquitination can be reconstituted in reactions containing the ubiquitin activating enzyme E1, either one of the ubiquitin conjugating enzymes UBC4 or UBCx (*5*, *6*), and APC (*5*, *7*). *Xenopus* APC contains homologs of CDC27 and CDC16 (*5*), proteins that are essential for the metaphase-anaphase transition in yeast and mammalian cells (*4*, *8*). However, the precise subunit composition of APC and the mechanisms of its activity and cell-cycle regulation remain unknown.

We immunopurified APC from interphase and mitotic *Xenopus* egg extracts and analyzed its subunit composition and activity (*9*). Both forms contained eight major polypeptides, that we refer to as APC1 through APC8, in approximately stoichiometric amounts (Fig. 1A and Table 1). The electrophoretic mobilities of APC1, APC3, APC6, and APC8 were reduced in APC purified from mitotic extracts. These mobility differences were apparently caused by phosphorylation because treatment of purified mitotic APC with λ protein phosphatase (λ-PPase) (*10*) produced a subunit pattern resembling the interphase form (Fig. 1D). Mitotic APC supported cyclin B ubiquitination in the presence of either an interphase 100,000*g* supernatant fraction (S100) or a mixture of purified E1 with UBC4 or UBCx (Fig. 1C) (*9*). Interphase APC had approximately 20% of the activity of mitotic APC. Treatment of mitotic APC with λ-PPase (*10*) resulted in a similar reduction of cyclin ubiquitination activity (Fig. 1E), suggesting that phosphorylation of APC subunits is essential for mitotic levels of activity. Similarly, the ubiquitination activity of partially purified cyclosome from clam is inhibited by addition of a phosphatase-containing fraction (*11*).

Protein microsequencing of individual APC subunits revealed that APC1, APC3, APC6, and APC7 are similar to previously described cell-cycle proteins (Table 1) (*12*). Peptide sequences obtained from APC3 and APC6 were 50 to 100% identical to sequences of human CDC27 and CDC16 (*8*), respectively, confirming our previous conclusion (*5*) that these proteins are subunits of APC. A peptide derived from APC7 was identical to a sequence encoded by the human expressed sequence tag H59410, which showed highest similarity to *Saccharomyces cerevisiae* Cdc23p (*13*). This suggests that APC7 is a *Xenopus* homolog of Cdc23p, which has previously only been found in budding yeast, where it interacts with Cdc27p and Cdc16p (*8*) and is required for cyclin degradation (*4*).

None of the peptide sequences revealed any similarity to Cse1p or Cdc26p—proteins required for cyclin degradation in budding yeast (*4*, *14*). We have found that a *Xenopus* homolog of Cse1p does not cofractionate with APC during column chromatography or density gradient centrifugation (*15*). It remains possible that a homolog of Cdc26p, which appears to be a component of yeast APC (*14*), is only loosely associated with the *Xenopus* complex, and is therefore lost during affinity purification. However, in *Xenopus*, neither Cse1p nor Cdc26p is essential for the reconstitution of cyclin B ubiquitination in vitro.

We obtained four peptide sequences from APC1 (*12*), all of which showed 60 to 83% identity to mouse Tsg24, a 216-kD protein that is 30% identical to *Aspergillus nidulans* BIME (*16*). In immunoblot experiments, antibodies to Tsg24 recognized a 210-kD protein in crude *Xenopus* interphase extracts and a

J.-M. Peters, R. W. King, M. W. Kirschner, Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115, USA.
C. Höög, Department of Cell and Molecular Biology, Medical Nobel Institute, Karolinska Institutet, S-17177 Stockholm, Sweden.

*These authors contributed equally to this work.
†Present address: Research Institute of Molecular Pathology, Dr. Bohr-Gasse 7, A-1030 Vienna, Austria.
‡To whom correspondence should be addressed.