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22. Strain FY567 contains the *spt15-21* allele, which suppresses the His⁻ and Lys⁺ phenotypes caused by *his4-917δ* and *LYS2-173r2* insertion mutants [D. M. Eisenmann, C. Dollard, F. Winston, *Cell* **58**, 1193 (1989)]. Therefore, the strain is His⁺ and Lys⁻. Fragments that contained the double mutant TFIID genes, which do not suppress the insertions (*14*), were used to transform strain FY567. Cells in which the spt15-21 allele had been replaced were isolated by selecting for a His⁻ and Lys⁺ phenotype. Replacement by the double mutant TFIID allele was confirmed by testing for the temperature-sensitive phenotype.
23. We thank F. Winston, D. Eisenmann, K. Arndt, and other members of the Winston lab for their generosity with yeast strains, materials, and advice; S. Hahn for the generous gift of antiserum to TFIIA; J. Bittner for help in purification of proteins; and R. Buratowski, G. Fink, A. Koleske, D. Pellman, A. Sachs, and B. Tidor for critical reading of the manuscript. Supported by NIH grant R29-GM46498 and grants to the Whitehead Fellows program from the Lucille P. Markey Charitable Trust and the W. M. Keck Foundation.

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A Human Gene Responsible for Zellweger Syndrome That Affects Peroxisome Assembly

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The primary defect arising from Zellweger syndrome appears to be linked to impaired assembly of peroxisomes. A human complementary DNA has been cloned that complements the disease's symptoms (including defective peroxisome assembly) in fibroblasts from a patient with Zellweger syndrome. The cause of the syndrome in this patient was a point mutation that resulted in the premature termination of peroxisome assembly factor–1. The homozygous patient apparently inherited the mutation from her parents, each of whom was heterozygous for that mutation.

ZELLWEGER SYNDROME (ZS) [McKusick 214100 (*1*)] is a fatal autosomal recessive disease, with clinical evidence of severe neurologic abnormalities, dysmorphic features, hepatomegaly, and multiple renal cysts (*2*). This syndrome is characterized by the absence of catalase-containing particles (peroxisomes) (*3*), resulting in multiple metabolic disturbances such as defects in peroxisomal β-oxidation and plasmalogen biosynthesis (*4, 5*). Earlier studies suggested that ineffective assembly of peroxisomes was likely to be the syndrome's primary effect (*6, 7*). We previously showed that a cloned rat cDNA encoding the peroxisome assembly factor–1 (PAF-1) (*8*), a 35-kD peroxisomal integral membrane protein, restores the assembly of peroxisomes in one peroxisome-deficient Chinese hamster ovary (CHO) cell mutant (Z65) (*9*).

There are five to eight complementation groups that affect generalized peroxisomal disorders such as ZS, neonatal adrenoleukodystrophy, infantile Refsum's disease, and hyperpipecolic acidemia, implying that several genes are required for peroxisome assembly (*10–12*). To find clues to the etiology of generalized peroxisomal disorders, we searched for patients affected in the same complementation group as the CHO mutant Z65. We used fibroblasts from nine American and Japanese patients with generalized peroxisomal disorders from eight complementation groups. Fusion of the fibroblasts from all patients with Z65 resulted in the appearance of peroxisomes (Fig. 1A), thereby implying that all the patients' pathogenic genes differed from that of Z65. But one female infant, MM, diagnosed with ZS, could not be classified into any of the previously characterized complementation groups. Unlike the other hybrids, the cell hybrids of MM's skin fibroblasts with Z65 cells lacked peroxisomes (Fig. 1, B and C).

We transfected rat PAF-1 cDNA into

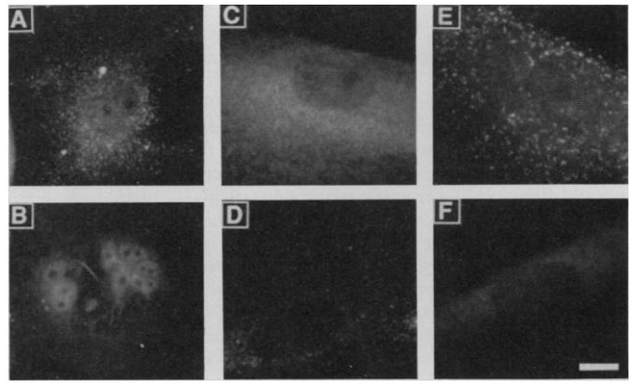

Fig. 1. Immunofluorescent staining of peroxisomes with the use of an antibody to catalase. (**A**) Hybrids (*14*) of Z65 cells and human fibroblasts GM228 (Human Genetic Mutant Cell Repository, Camden, New Jersey) from a patient with ZS [group E or group 1 (*12*)]. (**B**) Hybrids of Z65 with MM's fibroblasts (*15*). (**C**) MM's fibroblasts used for fusion in (B). (**D**) MM's fibroblasts transfected with the rat PAF-1 cDNA (*8, 16*). (**E**) MM's fibroblasts transfected with human PAF-1 cDNA. (**F**) MM's fibroblasts transfected with a PAF-1 cDNA isolated from MM's own fibroblasts. Antibodies to rat catalase [(A) and (B)] and human catalase [(C) through (F)] were used. Numerous catalase-positive particles, peroxisomes, were present in the cytoplasm of cells in (A), (D), and (E). Bar, 15 μm.

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Fig. 2. Deduced amino acid sequence (*17*) of human PAF-1. The nucleotide sequence of a 1630-bp cDNA encoding the human PAF-1 (first line) (*18*) was submitted to GenBank, accession no. M86852. The primary sequence of rat PAF-1 is indicated where amino acids differ from the human sequence (second line). Two putative membrane-spanning segments, similar to those noted in rat PAF-1 (*8*), are underlined. The amino acid changed by a nonsense mutation in patient MM is indicated by an arrowhead (Fig. 4).

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                                                                  60
MASRKENAKSANRVLRISQLDALELNKALEQLVWSQFTQCFHGFKPGLLARFEPEVKACL
  A E STQ                                                      F
                                                                 120
WVFLWRFTIYSKNATVGQSVLNIKYKNDFSPNLRYQPPSKNQKIWYAVCTIGGRWLEERC
 L                          Q    S   PV          LL
                                                                 180
YDLFRNHHLASFGKVKQCVNFVIGLLKLGGLINFLIFLQRGKFATLTERLLGIHSVFCKP
       R        A   M   V       E M       K
                                                                 240
QNIREVGFEYMNRELLWHGFAEFLIFLLPLINVQKLKAKLSSWCIPLTGAPNSDNTLATS
 SM                          V       I                STAG  S  GS
                                                                 300
GKECALCGEWPTMPHTIGCEHIFCYFCAKSSFLFDVYFTCPKCGTEVHSLQPLKSGIEMS
                        V   Y V       M                V

EVNAL
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MM's fibroblasts, using a mammalian expression vector pcD2 (*8*). Numerous peroxisomes were evident in the majority of the transfected cells (Fig. 1D). We screened a human liver cDNA library for human PAF-1, using the rat PAF-1 cDNA as a probe. Three positive colonies were isolated. One clone appeared to contain a full-length open reading frame (ORF) with polyadenylate [poly(A)]. This 1630-bp cDNA contained a putative 915-bp ORF, similar in length to that of the rat PAF-1 cDNA (*8*). The human and rat nucleotide sequences were 86% homologous; the deduced amino acid sequences were 88% homologous (Fig. 2). Both sequences encoded two highly conserved, putative membrane-spanning segments and seven cysteine residues in the COOH-terminal region. The two membrane-spanning regions may be responsible for localization of PAF-1 as an integral membrane protein in peroxisomes.

The function of human PAF-1 was assessed by transfection of its cDNA into MM's fibroblasts. Transfectants (17 out of 20 clones) contained numerous peroxisomes, thereby indicating that the human PAF-1 cDNA restored biogenesis of peroxisomes in MM's fibroblasts (Fig. 1E). Z65 cells transfected with human PAF-1 cDNA (*13*) [15 of 20 G418-resistant cells (Sigma)] or rat cDNA (*8*) also developed peroxisomes. Biogenesis of the peroxisomal enzymes appeared to be normal: a 75-kD precursor of acyl–coenzyme A (acyl-CoA) oxidase was appropriately cleaved into B (53 kD) and C (22 kD) components, and a 44-kD precursor of 3-ketoacyl–CoA thiolase was processed to the 41-kD mature enzyme (*13*). When fibroblasts from patients in the other complementation groups (A through E in Japan, 1 through 6 in the United States except for group 5, which is no longer available) were transfected with human PAF-1 cDNA (*8*), no peroxisomes were detected (*13*). This result suggests that the other complementation groups represent genes other than PAF-1 that are also essential for the biogenesis of peroxisomes.

To investigate the dysfunction in patient MM, we performed a Northern (RNA) blot analysis of RNA from MM's fibroblasts (Fig. 3). An RNA of ~1.9 kb, similar in size to the rat PAF-1 mRNA (*8*), was detected in MM's fibroblasts. An RNA of similar size and quantity was also detected in fibroblasts from an unaffected person and two patients with ZS of complementation groups A and E [the same as group 1 (*12*)], showing that the transcription of PAF-1 in patient MM was apparently normal. We determined the nucleotide sequence of PAF-1 cDNA from MM's fibroblasts by means of polymerase chain reaction (PCR) and subsequent cloning. In all six cDNA clones isolated, nucleotide C at position 355 (hereafter, starting from the first nucleotide of the initiator methionine codon, unless otherwise mentioned) was mutated to T, resulting in the creation of a termination codon TGA (Fig. 4A). When the PAF-1 cDNA from MM's fibroblasts was transfected back into her fibroblasts, almost all (95%) of the transfectants were peroxisome-negative (Fig. 1F),

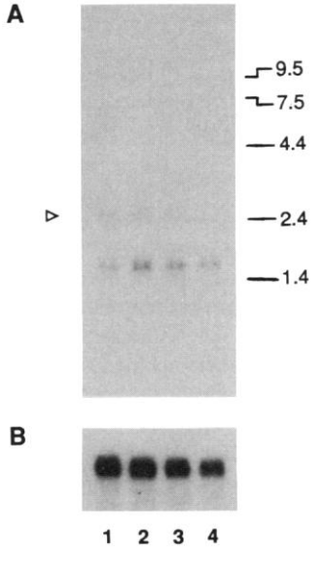

Fig. 3. Northern blot analysis of PAF-1 RNA. Poly(A)$^+$ RNA (*19*) on the Biodyne nylon membrane was hybridized with cDNA probes for human PAF-1 [(**A**) 3 μg of RNA per lane] and human β-actin [(**B**) 0.3 μg of RNA per lane], respectively (*20*). ^{32}P-labeled probes used were a 0.9-kb Pst I–Pst I cDNA fragment of human PAF-1 and a 0.44-kb Hinf I–Hinf I fragment of human β-actin DNA (Nippon Gene, Tokyo). Lane 1, SV40-transformed AG3022 fibroblasts from a female control; 2, fibroblasts from the patient MM; 3, SV40-transformed fibroblasts from a Japanese patient (TG) with ZS [complementation group A (*12*)]; 4, GM228 from another patient (Fig. 1). Open arrowhead indicates an unidentified RNA (~2.5 kb) that cross-hybridized with human PAF-1 cDNA. RNA size markers (in kilobases) are on the right. Exposure: (A), 10 days; (B), 12 hours.

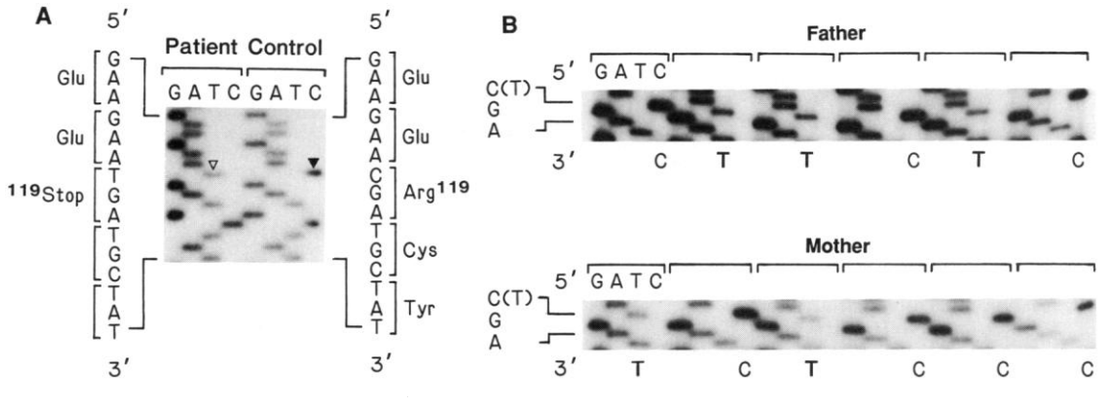

Fig. 4. Mutation in PAF-1 of the ZS patient MM and her parents. (**A**) Nucleotide sequence analysis of PAF-1 cDNA isolated from patient MM and a control (*21*); a point mutation changes codon 119 (solid arrowhead) to a stop codon, TGA (open arrowhead). (**B**) Nucleotide sequence of genomic DNA fragments from MM's parents. Only the region of the codon for Arg^{119} is shown in six clones each isolated from the father and mother of the patient (*22*).

whereas ~90% of the transfectants with the cDNA from the control fibroblasts (AG3022; Human Mutant Cell Repository) contained numerous peroxisomes, as seen in the control cells (*13*).

To determine if patient MM inherited the homozygous mutation from her parents, we investigated the nucleotide sequence in this region of their genomes. Genomic DNA fragments corresponding to a portion of the PAF-1 cDNA (nucleotides 340 through 371) were amplified by PCR and cloned. No intron was found in this region. In patient MM, T was substituted for C at position 355, as noted in the cDNA. In the genomic DNA clones isolated from the parents, T was detected in place of C in two and three of six clones, each from the mother and the father, respectively (Fig. 4B). This suggests that both father and mother were heterozygous for the same mutation in PAF-1 and that patient MM inherited the mutation from both parents.

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14. Cell fusion of a 6-thioguanine–resistant variant (*9*) of Z65, Z65TG^r, with human fibroblasts was done with polyethylene glycol as described [R. A. Zoeller *et al.*, *J. Biol. Chem.* **264**, 21872 (1989)], except that selection was done with 1 μM ouabain.
15. A Japanese girl (MM) who died at 8 months manifested typical findings and a clinical course of ZS, including accumulation of very long chain fatty acids in serum sphingomyelins, absence in liver homogenates of all three peroxisomal β-oxidation enzymes (*13*), and no peroxisomes in all skin fibroblasts immunochemically examined (Fig. 1C). Autopsy revealed macrogyria and polymicrogyria in the brain, hepatosplenomegaly, and many small cysts at the bilateral renal cortices. There was no consanguinity within the patient's family, although her parents were from the same village.
16. Transfected cells were fixed and incubated with rabbit antibody to human catalase or rat catalase, as described [Y. Suzuki, S. Yamaguchi, T. Orii, M. Tsuneoka, Y. Tashiro, *Cell Struct. Funct.* **15**, 301 (1990)]. The antigen-antibody complex was detected with fluorescein isothiocyanate–labeled goat antibody to rabbit immunoglobulin G (Bio-Rad).
17. Abbreviations for the amino acid residues are: 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.
18. Human PAF-1 cDNA was isolated by colony-hybridization screening of a human liver cDNA library constructed in vector pcD2 [C. Chen and H. Okayama, *Mol. Cell. Biol.* **7**, 2745 (1987)]. The probe used was the 0.9-kb Pvu I I–Eco RI fragment of rat PAF-1 cDNA that had been labeled with ^{32}P-labeled deoxycytidine 5′-triphosphate (Amersham) with a random primer labeling kit (Takara Shuzo, Kyoto, Japan). The colony membranes were washed twice sequentially with 2× saline sodium citrate (SSC) and 0.1% SDS for 15 min at room temperature, and with 1× SSC and 0.1% SDS for 15 min at 65°C. Inserts were excised from the vector with Bam HI and subcloned into Bluescript SK(−) (Stratagene). Overlapping deletions in both orientations and nucleotide sequencing were done as described (*8*).
19. Total RNA was isolated from cultured skin fibroblasts by the guanidinium thiocyanate method [J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979)]; polyadenylated [poly(A)$^+$] RNA was prepared with oligo(dT)-Latex (Nihon Roche, Tokyo) according to the manufacturer's procedure.
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21. The cDNAs for PAF-1 from control fibroblasts (AG3022) and MM's fibroblasts were separately prepared as follows: First, we synthesized single-strand cDNA from poly(A)$^+$ RNA (Fig. 3) with an antisense primer (AS-1; 29-bp oligonucleotide, 5′-TAAGAATTCTTTTCCTCAAAGGAAGCAAT-3′, an Eco RI linker plus nucleotide residues 946 through 927 in the human PAF sequence, starting the first nucleotide of the initiator methionic codon), using Moloney murine leukemia virus–reverse transcriptase (Bethesda Research Laboratories, Bethesda, MD). Second, we amplified the PAF-1 cDNA by PCR [R. K. Saiki *et al.*, *Science* **239**, 487 (1988)], using AS-1 and a sense primer (5′-GCAGGATCCGCAGGAACAGGAAAAAGAGA-3′, a Bam HI linker plus residues −34 to −15 of the PAF-1 sequence), of which the Eco RI–Bam HI fragment was subcloned into a multicloning site from the Bluescript vector constructed in pcD2 vector and sequenced.
22. Genomic DNA was prepared by standard procedure from cultured skin fibroblasts from the control, the patient MM, and the blood of the patient's parents [J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ed. 2, 1989)]. Primers used to amplify genomic DNA fragments near the mutation site were at nucleotides 321 through 339 and 372 through 391.

23. We thank H. Okayama, T. Kawai, H. Nakamura, S. Noguchi, Y. Kadokawa, M. Imada, T. Hashimoto, H. Moser, M. Ohara, and H. Tsunoo for advice, supplies, and patient samples. Supported in part by grants from the National Center of Neurology and Psychiatry (2-A-6) and Uehara Memorial Foundation, a grant for Pediatric Research (63-A) from the Ministry of Health and Welfare, Japan, and by a Grant-in-Aid for Scientific Research (03770558).

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Selective Transmission of Human Immunodeficiency Virus Type–1 Variants from Mothers to Infants

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Multiple human immunodeficiency virus type–1 sequences from the V3 and V4-V5 regions of the envelope gene were analyzed from three mother-infant pairs. The infants' viral sequences were less diverse than those of their mothers. In two pairs, a proviral form infrequently found in the mother predominated in her infant. A conserved N-linked glycosylation site within the V3 region, present in each mother's sequence set, was absent in all of the infants' sequence sets. These findings demonstrate that a minor subset of maternal virus is transmitted to the infant.

A HIGH DEGREE OF GENETIC VARIability in human immunodeficiency virus type–1 (HIV-1) can be found within infected individuals (*1*). Variants arise during retroviral replication by error-prone reverse transcription (*2*). The complex mixture of variants in an infected individual (*3*) may be the result of immunologic pressure for change (*4*), alterations in cell tropism (*5*), and replication efficiency (*6*) among the variants. We analyzed the distribution of distinguishable genotypes transmitted between mother and child to investigate the role of selection in perinatal HIV-1 transmission, a route of infection estimated to occur in 13 to 30% of infants born to HIV-1–infected mothers (*7*).

Sequence sets were examined from one sample of peripheral blood from three mothers and their 2- to 4-month-old infants (*8*). We extracted DNA directly from mononuclear cells and amplified it by polymerase chain reaction (PCR) using nested primer pairs (*9*). The primer sets flanked both the V3 region containing the immunodominant loop (*10*) and the V4-V5 regions that encompass a portion of the CD4-binding domain of the viral envelope gene (*11*). Product DNAs from each individual were cloned, and 5 to 27 clones from each sample were sequenced (*12*).

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