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13. 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.
14. The Coomassie blue–stained gel of Fig. 3 and the autoradiogram of a similar gel of phage labeled with [^{35}S]methionine were scanned in a densitometer. The peak areas were corrected for protein molecular size (stained gel) or sulfur content (autoradiogram) and converted to copies per virion by normalizing to proteins for which this value is known (gpH: 6 per virion; gpB: 12 per virion). The stained gel gave 8.3 copies per virion for gpStf and 12.5 copies per virion for gpTfa; the autoradiogram gave 11.4 copies per virion for gpStf and 9.7 copies per virion for gpTfa.
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Assignment of a Locus for Familial Melanoma, MLM, to Chromosome 9p13-p22

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Linkage analysis of ten Utah kindreds and one Texas kindred with multiple cases of cutaneous malignant melanoma (CMM) provided evidence that a locus for familial melanoma susceptibility is in the chromosomal region 9p13-p22. The genetic markers analyzed reside in a candidate region on chromosome 9p21, previously implicated by the presence of homozygous deletions in melanoma tumors and by the presence of a germline deletion in an individual with eight independent melanomas. Multipoint linkage analysis was performed between the familial melanoma susceptibility locus (MLM) and two short tandem repeat markers, D9S126 and the interferon-α (IFNA) gene, which reside in the region of somatic loss in melanoma tumors. An analysis incorporating a partially penetrant dominant melanoma susceptibility locus places MLM near IFNA and D9S126 with a maximum location score of 12.71. Therefore, the region frequently deleted in melanoma tumors on 9p21 presumably contains a locus that plays a critical role in predisposition to familial melanoma.

There are approximately 32,000 new cases of cutaneous malignant melanoma (CMM) diagnosed annually in the United States alone and 7,800 melanoma-related deaths. U.S. incidence rates for CMM have been increasing more rapidly than for any other cancer except that of the lung (*1*). Between 1973 and 1985 in Utah, the age-adjusted incidence rate for melanoma increased from 6.4 to 14.2 per 100,000 (*2*). Approximately 10% of melanoma cases arise in a familial setting (*3*); these cases are hypothesized to carry an inherited susceptibility to CMM. However, since melanoma may not be expressed in all individuals who inherit such a susceptibility, a proportion of apparently sporadic melanoma cases may also be due to genetic predisposition.

Several different studies have pinpointed a region on the short arm of chromosome 9 (9p) as one involved in the early stage development of melanoma tumors. In an attempt to determine whether the region contained a familial melanoma susceptibility locus (MLM), we examined genetic markers in the candidate region for genetic linkage with MLM using 11 kindreds with multiple cases of invasive CMM. Although the value of linkage studies in localizing homogeneous, fully penetrant dominant disorders has been established (*4*), the value of such studies in more complex disorders is unclear. The mode of inheritance of familial melanoma has not been established; investigators continue to debate the existence of a major gene, the localization of this putative gene, and the relationship between familial melanoma and an associated trait, the dysplastic nevus syndrome (DNS) (*5*). However, given chromosome 9p21 as a candidate region, we analyzed genetic markers from this region in these kindreds with a partially penetrant dominant genetic model and localized a susceptibility locus for familial melanoma.

In this study, 11 extended kindreds with 82 cases of melanoma diagnosed between the ages of 12 and 93 were analyzed. Each kindred is caucasian and of Northern European ancestry. Ten of the kindreds are from Utah (Fig. 1); one (K3346) is from Texas and has been studied for over 20 years (*6*). The kindreds were selected for the presence

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of melanomas in closely related individuals. The study was approved by the Institutional Review Board of the University of Utah Medical Center and informed consent was obtained from each participant. Histologic confirmation of invasive cutaneous malignant melanoma was obtained for all cases except for three that occurred in the early generations. Individuals in the kindreds with other types of proliferative melanocytic lesions, for example lentigo maligna melanoma (one case), melanoma in situ (two cases), ocular melanoma (one case), and congenital melanoma (one case), were classified as unaffected for the analysis. Table 1 presents the number of melanoma cases, median age at diagnosis, and number of individuals sampled for each kindred. The melanomas diagnosed in these individuals did not differ significantly in terms of age of diagnosis or sex from all Utah melanoma cases diagnosed between 1981 and 1990.

Cytogenetically detectable loss or rearrangement of chromosome 9p occurs in approximately 46% of all melanomas. The identification of 9p rearrangements in both melanoma precursor lesions (dysplastic nevi) and primary lesions implicated a 9p locus that participates in early melanoma development (*7*). Loss of heterozygosity (LOH) studies further indicate the existence of a tumor suppressor locus that is mutated in most, if not all, cutaneous melanomas (*8, 9*). In 86% of melanoma tumors and cell lines, the DNA markers in this region are deleted in a hemi- or homozygous fashion (*8*). A candidate region of 2 to 3 Mb on 9p21 was delineated on the distal side by the interferon-α (IFNA) genes and on the proximal side by the anonymous DNA marker D9S3 (*8*). A newly described marker, D9S126, lies between these loci, and homozygous deletions of or D9S3, or D9S126 and D9S3, have been detected in two unrelated melanoma cell lines (*8, 10*).

Support for a melanoma predisposition gene on chromosome 9p comes from the identification of a 34-year-old caucasian woman, with multiple atypical moles and eight primary cutaneous melanomas, who

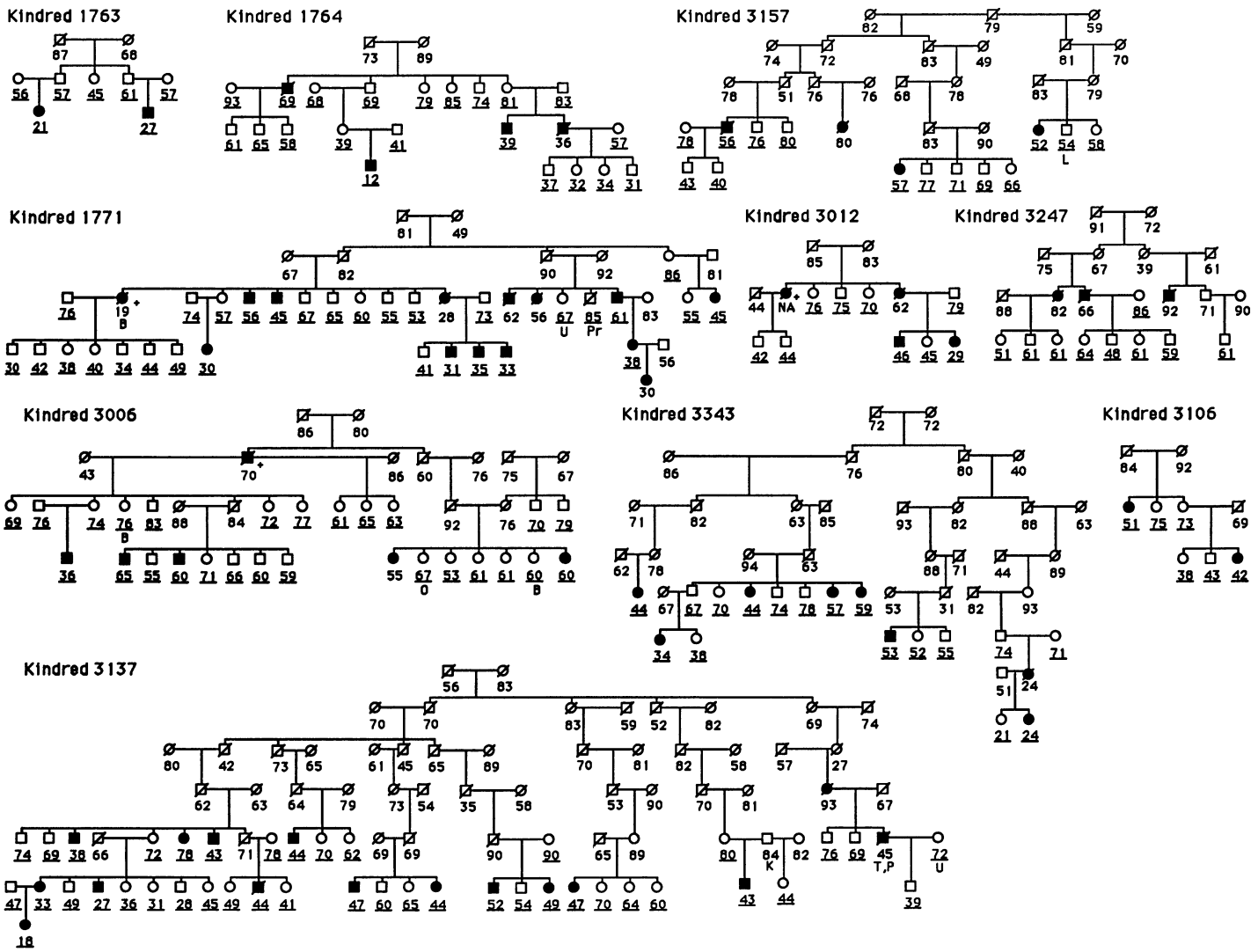

Fig. 1. Pedigree drawings of the ten Utah kindreds. Kindreds 1763, 1764, and 1771 were ascertained from local physicians in a previous study of melanoma and DNS for the presence of three or more closely related individuals with melanoma or DNS. Kindreds 3137, 3006, 3012, and 3106 were ascertained secondarily from a case control study of melanoma in which a set of sequentially diagnosed melanoma cases were selected for study from the Utah Cancer Registry. Probands of these kindreds reported at least one first-degree relative with melanoma. Kindreds 3157, 3247, and 3343 were ascertained from the Utah Population Database (*37*) for the presence of three or more related cases of melanoma. Affected individuals have solid symbols. A small plus sign to the right of a symbol indicates that no medical confirmation of cancer was available. Numerals beneath each symbol indicate the age of an affected individual at diagnosis or the age of an unaffected individual at exam or death. Underlined ages indicate those individuals from whom DNA samples are available. NA, the date of diagnosis was not available. Other cancers are listed by tissue site: B, breast; K, kidney; L, leukemia; P, pancreas; Pr, prostate; T, testicular; U, uterine.

Table 1. Description of Utah and Texas melanoma kindreds. One individual from K3012 and two from K3346 had unknown age of diagnosis for melanoma.

Kindred	Number of melanoma cases	Median age at diagnosis	Number of family members sampled
1763	2	24	7
1764	4	38	21
1771	14	37	30
3006	6	60	26
3012	4	46	9
3106	2	47	6
3137	16	44	41
3157	4	57	14
3247	3	82	9
3343	8	44	17
3346	22	60	53

Table 2. Lod scores between MLM and chromosome 9p markers for an age-specific penetrance model. The model was derived by assuming that the MLM locus accounted for 5% of all melanomas, a familial relative risk of 2.5 in first-degree relatives of cases compared to controls, and a population prevalence of 1.5%. The model was constrained to fit Utah age-specific incidence figures for melanoma for 1981–1990.

Kindred	Lod score at recombination fraction of:						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
			IFNA				
1763	−0.03	−0.03	−0.02	−0.02	−0.01	0.00	0.00
1764	0.89	0.87	0.78	0.66	0.42	0.20	0.05
1771	3.17	3.11	2.84	2.50	1.78	1.01	0.29
3006	−0.34	−0.17	0.11	0.20	0.18	0.09	0.03
3012	0.67	0.65	0.57	0.48	0.29	0.13	0.03
3106	0.09	0.09	0.07	0.05	0.02	0.01	0.00
3137	1.46	1.42	1.25	1.04	0.66	0.35	0.12
3157	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3346	4.34	4.99	5.10	4.72	3.62	2.36	1.10
3247	0.10	0.10	0.09	0.07	0.04	0.02	0.00
3343	−0.45	−0.46	−0.46	−0.42	−0.29	−0.17	−0.08
Total	9.90	10.57	10.33	9.28	6.71	4.00	1.54
			D9S126				
1763	−0.07	−0.07	−0.06	−0.05	−0.03	−0.01	0.00
1764	0.58	0.57	0.52	0.45	0.30	0.16	0.04
1771	1.89	1.85	1.68	1.46	1.01	0.57	0.18
3006	0.24	0.23	0.20	0.16	0.10	0.05	0.02
3012	0.38	0.36	0.32	0.26	0.16	0.07	0.02
3106	−0.13	−0.13	−0.10	−0.08	−0.04	−0.02	0.00
3137	0.88	0.99	1.11	1.05	0.75	0.43	0.17
3157	−0.01	−0.01	−0.01	−0.01	0.00	0.00	0.00
3346	2.82	2.75	2.45	2.08	1.37	0.74	0.28
3247	0.04	0.03	0.03	0.02	0.01	0.01	0.00
3343	−0.53	−0.45	−0.27	−0.18	−0.10	−0.06	−0.03
Total	6.09	6.12	5.87	5.16	3.53	1.94	0.68

has a de novo germline cytogenetic rearrangement that involves chromosomes 5p and 9p (*11*). Molecular analysis revealed loss of material from the 9p21 region. The IFNA and D9S126 loci from this individual were compared to those of her unaffected parents. Gene dosage studies showed hemizygosity at both loci, suggesting that the germline loss of this region predisposes to melanoma (*12*).

Additional evidence that the MLM locus functions as a tumor suppressor gene in melanomas and other types of cancer comes from somatic cell hybrid studies in which a gene that can suppress the tumorigenic phenotype of mouse melanoma was mapped to the central portion of mouse chromosome 4 (*13*), which is syntenic with human chromosome 9p (*14*). The interferon-α genes are also frequently deleted in chemically induced mouse lung tumors (*15*). Similarly, 9p is the site of frequent homozygous deletions, hemizygous deletions, or rearrangements in human acute lymphocytic leukemias, glioblastomas, and non–small cell lung cancers (*16*). The melanoma patient described above does not have any of these cancers, implying that the loss or mutation of a gene within this region may be rate-limiting in the development of melanoma, but secondary in the progression of these other human malignancies.

To test for an inherited familial melanoma susceptibility, we used two polymorphic short tandem repeat markers from the critical region of 9p, IFNA (*17*) and the D9S126 locus (*10*). Primer sequences for the IFNA marker were: TGCGCGTTAAGTTAATTGGTT and GTAAGGTGGAAACCCCCACT. Primer sequences for the marker D9S126 were: ATTGAAACTCTGCTGAATTTTCTG and CAACTCCTCTTGGGAACTGC. Allele frequencies were estimated from the observed or implied genotypes of the set of founder individuals marrying into each of the kindreds; 55 chromosomes were available for allele frequency estimates for each marker. Alleles not observed in founders were assigned an allele frequency of 0.01, with other frequencies adjusted slightly to allow gene frequencies to sum to 1.0. Six alleles were observed for IFNA with frequencies of 0.01, 0.16, 0.55, 0.07, 0.19, and 0.02 from the longest to shortest repeat. Five alleles were observed for D9S126 with frequencies of 0.07, 0.02, 0.45, 0.05, and 0.40 from the longest to shortest repeat. The frequencies for IFNA are comparable to those estimated from a set of 67 unrelated North Americans and Centre d'Étude du Polymorphisme Humain (CEPH) parents, in which only the five longest alleles were observed; the D9S126 frequencies are comparable to those estimated in an Australian study (*18*). The PCR reactions were done using a standard protocol (*19*).

The statistical analysis for the inheritance of susceptibility to melanoma used three approaches. Because no adequate models exist for the inheritance of melanoma, certain assumptions were made. The first approach, an age-specific model, assumed a rare autosomal dominant susceptibility locus, incorporated age-specific penetrances for melanoma, and allowed for sporadic cases of melanoma (cases not due to the hypothesized gene) in these kindreds. Five percent of all melanoma cases were assumed to be due to the hypothesized susceptibility; this estimate is similar to population-based estimates of familiality for breast cancer, another common cancer with recognized familial susceptibilities (*20*). A relative risk to first degree relatives of 2.5 was used, and was based on an estimate from a survey of published data (*21*). The age-specific model is a general model integrating age specificity and both predisposed and sporadic cases; however, because the parameters used are estimates only, other less specific models were also examined.

The second approach, an affected-only model, assumed (i) a rare dominant susceptibility with low penetrance, (ii) that all individuals were affected because of the inherited susceptibility, and (iii) that the penetrance of the susceptibility allele was very low, allowing unaffected individuals to contribute marker genotypic but not disease information. The affected-only model considers a dominant model with few parameters.

The third analytical approach was the affected pair method (APM), which is model-free and tests for excess sharing of alleles at the two marker loci among related affect-

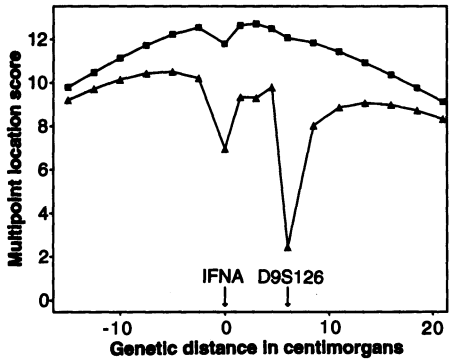

Fig. 2. Multipoint linkage analysis. The likelihood for various genetic map positions of the MLM locus was computed relative to a fixed map of the two marker loci with a recombination fraction between the two markers of 0.06, estimated directly from the data with a lod score of 16.30. These likelihoods were then transformed to location scores by subtracting the $\log_{10}$ likelihood assuming that the MLM locus was at a map position "x" on the fixed map from the $\log_{10}$ likelihood assuming that the MLM locus was unlinked to chromosome 9p. Age-specific model, solid squares; affected-only model, solid triangles.

ed individuals (*22*). Because the results of the two other genetic analyses are sensitive to inaccuracies in the assumed genetic model, this model was also considered.

Two-point lod scores (*23*) and multipoint location scores were calculated using the LINKAGE software package (*24*) under the first two models. Table 2 presents the two-point lod scores for linkage between each marker and the MLM locus when calculated with the age-specific model. These lod scores suggested a maximum likelihood location of MLM at a recombination fraction of 0.01 for IFNA with a lod score of 10.57, and a recombination fraction of 0.01 for D9S126 with a lod score of 6.12.

The affected-only model lod scores suggested a maximum likelihood location of MLM at a recombination fraction of 0.05 for IFNA with a lod score of 9.50, and a recombination fraction of 0.05 for D9S126 with a lod score of 4.00. For the kindreds that appear to be linked to 9p, the affected-only analysis gave essentially the same results as the age-specific analysis. A subset of the families with slightly negative lod scores in the age-specific analysis showed substantially stronger evidence against linkage in the affected-only analysis. A higher recombination fraction is estimated using the affected-only model, due to the assumption of no sporadic cases of melanoma. The lod scores were insensitive to increasing rare allele frequencies for the marker loci.

Figure 2 presents the multipoint linkage analysis location scores for both models. The multipoint location score maximized at 12.71 for MLM localization between IFNA and D9S126 for the age-specific model and 10.52 at a recombination fraction of 0.05 distal to IFNA for the affected-only model. The multilocus APM analysis used the $1/\sqrt{P}$ weighting function for allele frequencies (*22*) and indicated a significant excess of chromosome 9 haplotype sharing among affected individuals ($P < 0.001$). The agreement of all three approaches supported linkage to 9p.

The genetic heterogeneity of IFNA and D9S126 linkage to MLM was tested with the admixture test as implemented in the HOMOG program (*25*). No statistically significant evidence of heterogeneity was found for either marker for either genetic model. Two of the 11 kindreds showed strong linkage to 9p13-p22, with multipoint location scores greater than 3 at the most likely location in each kindred under both models (K1771 and K3346). Three other kindreds were suggestive of linkage to this region (K3137, K1764, and K3012), with location scores ranging from 0.64 to 1.90. The remaining kindreds were largely uninformative under the age-specific model. No significant heterogeneity was identified using multilocus location scores.

Melanoma susceptibility has been associated with DNS since its first description in 1978 (*26*). A number of families with multiple melanoma cases were analyzed for segregation (*27*). Members of the families were defined to have melanoma, DNS, or both, or were considered normal. Although segregation analysis did not reject a Mendelian dominant model for melanoma alone, the analysis of melanoma and DNS together did not conform to a Mendelian dominant model. Pathologists also disagree on the diagnosis of dysplasia in nevi. The NIH consensus development conference on melanoma (*28*) suggested that the diagnosis of dysplastic nevus is ambiguous and should not be used. Our studies of nevi in Utah kindreds suggest that a quantitative trait based on number and size of nevi is inherited as a common codominant major locus in some kindreds (*29*). CMM/DNS data studies consistently rejected rare dominant inheritance as a model for DNS (*5*, *30*). Because the number of nevi could be associated with melanoma risk (*31*), total number of nevi is also being examined as a melanoma-associated phenotype (*5*, *29*).

Linkage studies analyzing the combined trait of CMM/DNS have yielded conflicting results. A gene predisposing to CMM and DNS was linked to *Rh* on the short arm of chromosome 1 (*32*). A refined linkage analysis showed linkage of a CMM/DNS trait between the pronatiodilatin (*PND*) locus and an anonymous DNA segment, D1S47, on the chromosome band 1p36 (*33*). Most of the linkage evidence came from the DNS phenotype rather than the melanoma phenotype. However, five other analyses of linkage of melanoma to this region have refuted these findings (*34*). Analysis of linkage between CMM/DNS and the D9S3 locus in four melanoma-prone families excluded close linkage (*35*).

As family data from a large number of populations become available, the nature of the genetic predisposition to melanoma will begin to emerge. The existence of genetic heterogeneity may be suggested by kindreds exhibiting lack of 9p linkage; however, heterogeneity is confounded by the presence of sporadic cases and could be difficult to assess in small kindreds. Analysis is further complicated by tenfold differences in frequencies of CMM in caucasian populations of Northern European origin with different degrees of sun exposure (*36*). Genetic heterogeneity will only be resolved by the confirmation of linkage to other chromosomes. To date studies that exclude 9p linkage and support 1p linkage are based on a complex and controversial phenotype (CMM and DNS) and their analysis should be repeated using invasive melanoma as the phenotype (*32–35*). Independent studies are also needed to examine the relationship of the MLM locus to precursor nevus phenotypes and to assess the interaction of the MLM locus with known environmental risk factors. The ultimate identification of the germline mutations within the gene will allow estimation of the proportion of melanoma kindreds that are due to changes at the 9p locus, the frequency of the disease allele, and age- and sex-specific penetrances.

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A Functional Connection Between the Pores of Distantly Related Ion Channels as Revealed by Mutant K^+ Channels

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The overall sequence similarity between the voltage-activated K^+ channels and cyclic nucleotide–gated ion channels from retinal and olfactory neurons suggests that they arose from a common ancestor. On the basis of sequence comparisons, mutations were introduced into the pore of a voltage-activated K^+ channel. These mutations confer the essential features of ion conduction in the cyclic nucleotide–gated ion channels; the mutant K^+ channels display little selectivity among monovalent cations and are blocked by divalent cations. The property of K^+ selectivity is related to the presence of two amino acids that are absent from the pore-forming region of the cyclic nucleotide–gated channels. These data demonstrate that very small differences in the primary structure of an ion channel can account for extreme functional diversity, and they suggest a possible connection between the pore-forming regions of K^+, Ca^{2+}, and cyclic nucleotide–gated ion channels.

Ion channels are integral membrane proteins that form ion conduction pathways across cell membranes. The channel-mediated movement of ions into and out of cells underlies a variety of cellular functions such as muscle contraction, cell volume regulation, and the production of electrical signals in the nervous system. In order to fill their many different roles the ion channels are a diverse class of proteins. Some ion channels open in response to a change in membrane electrical potential, and others open in response to the binding of specific ligands. After having opened, some ion channels are not very selective and allow many different ions to pass, but others are highly selective and allow the conduction of only one kind of ion present in physiological solutions.

The voltage-activated Na^+, Ca^{2+}, and K^+ channels are members of the same gene superfamily; they open and close in response to membrane electrical potential and they are similar in their primary structures (*1*). The most characteristic structural feature shared by members of this superfamily is an amino acid segment called S4, which is an unusual stretch of hydrophobic and basic amino acids that is thought to serve as a transmembrane voltage sensor for channel gating (*2*).

The cyclic nucleotide–gated ion channels (CNG channels) from olfactory and retinal neurons are unexpected members of the S4-containing superfamily of ion channels (*3*, *4*). Unlike the voltage-activated channels, the CNG channels open and close in response to the binding of an intracellular ligand. Nevertheless, their amino acid sequences reveal a distant ancestral connection to the voltage-activated ion channels. In addition to having a residual S4 sequence (*5*), the CNG channels also contain a pore-forming region (P region) that bears a striking resemblance to the equivalent region of the K^+ channels (*6*, *7*).

The sequence similarity between voltage-activated K^+ channels and CNG channels is intriguing because they are functionally so dissimilar. The P regions are conserved (Fig. 1A), yet the ion conduction properties of these channels are not at all alike. The CNG channels do not discriminate between the alkali metal cations Na^+ and K^+; both are highly permeant (*8*, *9*). Furthermore, the CNG channels are blocked by the divalent cations Ca^{2+} and Mg^{2+} (*9*, *10*). The K^+ channels, in contrast, are highly selective for K^+ over Na^+, and they are not efficiently blocked by Ca^{2+} or Mg^{2+}.

We have identified the essential feature that distinguishes the pore regions of the voltage-activated K^+ channels from the CNG ion channels. The different ion conduction properties can be explained in terms of two amino acids that are present in K^+-selective channels and absent from CNG ion channels. These results demonstrate a close relationship between ion channels that are functionally different, and they show how the interconversion of one kind of ion conduction pore to another might have occurred in nature.

An alignment of the P region of a Shaker K^+ channel with the corresponding region of the bovine retinal CNG channel shows that the sequences between amino acid residues 434 and 444 are highly similar (Fig. 1A) (*11*). To the right of Gly^{444} we introduced a gap and aligned the Glu (E) of the CNG channel with the Asp (D) of the Shaker K^+ channel (*12*). This choice of sequence alignment implies that the CNG channel is missing two amino acids that are present in the K^+ channel pore.

Two mutant K^+ channels were designed such that their pore sequences would mimic that of the CNG channel. The first (Chimera, Fig. 1A) replaces YGDMTPV (resi-

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