inserts were melted at 72°C, diluted 500× in TE buffer [1 mM EDTA and 10 mM tris (pH 8.0)], and mounted for optical mapping. More than 10,000 irradiated DNA molecules were studied for the presence of circles or other aberrant structures.
31. M. J. Daly and K. W. Minton, *J. Bacteriol.* **178**, 4461 (1996).
32. J. Lin, data not shown.
33. B. B. Bederson *et al.*, in *Human Factors in Web Development*, L. Ratner, E. Grose, C. Forsythe, Eds. (Erlbaum, Mahwah, NJ, 1998), p. 255.
34. J. Lin, data not shown.
35. _____, data not shown.
36. We thank T. Burland for Geneplot software, S. Paxia for Pad software (*33*), and F. Blattner for prepublication *E. coli* sequence. Partially funded by the U.S. Department of Energy and NIH, by Department of Energy Office of Biological and Environmental Research grants DE-FG02-98ER62583, DE-FG02-97ER62492, DE-FG07-97ER20293, and DE-FG02-98ER62554, and by U.S. Public Health Service grants GM39933 and HG0025-08.

11 February 1999; accepted 2 August 1999

Historical Genetics: The Parentage of Chardonnay, Gamay, and Other Wine Grapes of Northeastern France

John Bowers,[1]* Jean-Michel Boursiquot,[2] Patrice This,[3] Kieu Chu,[1] Henrik Johansson,[1] Carole Meredith[1]†

The origins of the classic European wine grapes (*Vitis vinifera*) have been the subject of much speculation. In a search for parental relationships, microsatellite loci were analyzed in more than 300 grape cultivars. Sixteen wine grapes that have long been grown in northeastern France, including 'Chardonnay', 'Gamay noir', 'Aligoté', and 'Melon', have microsatellite genotypes consistent with their being the progeny of a single pair of parents, 'Pinot' and 'Gouais blanc', both of which were widespread in this region in the Middle Ages. Parentage analysis at 32 microsatellite loci provides statistical support for these relationships.

The wines of northeastern France, notably those of the Burgundy and Champagne regions, have been highly regarded for centuries. Like most of the world's finest wines, they are made entirely from old cultivars of *Vitis vinifera* L. The cultivars most strongly associated with this part of France are 'Pinot noir' and 'Chardonnay', which are used both for Champagne (*1*) and also for the best red and white wines, respectively, of the Côte d'Or in the heart of Burgundy. These two grapes are now grown in many of the world's wine regions. In the southern part of Burgundy, the red wines of Beaujolais are made primarily from 'Gamay noir'. Several other cultivars, including 'Aligoté', 'Melon', and 'Sacy', are used in wines carrying regional Burgundy appellations.

Grapevines are propagated vegetatively, so that the individual vines of a cultivar are genetically identical to each other (except for somatic mutations) and to the single original seedling from which the cultivar originated. While some cultivars may have originated in the regions with which they are now associated, others are thought to have been introduced by traders or conquerors, most notably the Romans. Although a few varieties have been produced by controlled crosses since the mid-1800s, most *V. vinifera* cultivars in existence today are centuries old and are thought to have arisen by several mechanisms—domestication of wild vines, spontaneous crosses between wild vines and cultivars, and spontaneous crosses between cultivars (*2*). Such a spontaneous cross between two cultivated varieties gave rise to 'Cabernet Sauvignon', the most important cultivar of Bordeaux and arguably the most highly regarded red wine grape in the world today (*3*).

Any wild vines that were parents of today's important wine grapes cannot be identified, because they no longer exist. Parents that are themselves cultivars, however, may still exist in collections if not in cultivation. In a search for the parents of some important French wine grapes, we analyzed 322 cultivars of *V. vinifera* (*4*), including most extant old French cultivars.

Samples of 51 cultivars were obtained from the vineyards at the University of California at Davis and the rest were taken from the variety collection maintained by Institut National de la Recherche Agronomique at Domaine de Vassal, near Montpellier, France. After an initial screening of all cultivars at 17 microsatellite loci (*5*), we compared microsatellite alleles within all possible sets of three to identify pairs of cultivars that could have contributed the alleles of the third cultivar (*6*). A subset of cultivars was then further analyzed at 15 additional loci (*7*).

On the basis of 32 loci, 16 cultivars had microsatellite genotypes consistent with their being the progeny of a single pair of parents—'Pinot' and 'Gouais blanc' (Fig. 1) (*8–10*). For each of the 16 putative 'Pinot' × 'Gouais blanc' progeny, we calculated parentage indices to compare the probability of the observed progeny alleles if it had the putative parents to the probability of those alleles, if it had two random parents, or if the parents were close relatives of the putative parents. We show the detailed parentage indices for 'Chardonnay' in Table 1 and summaries for all 16 progeny cultivars in Table 2. The likelihood ratios show that the putative parents are 10^{12} to 10^{15} times

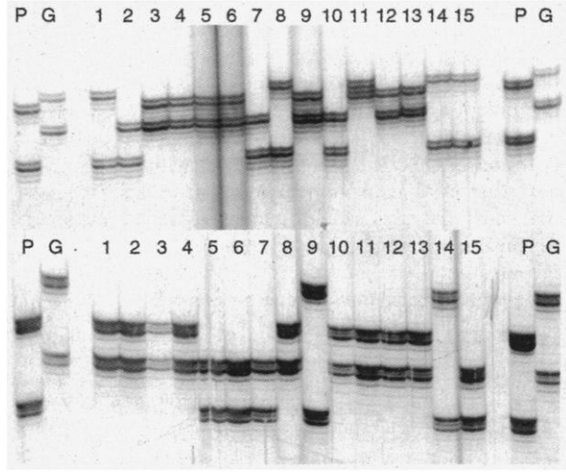

Fig. 1. Inheritance of parental microsatellite alleles by progeny cultivars for locus VVMD5 (top) and VVMD28 (bottom). Microsatellites were amplified from genomic DNA, electrophoresed in polyacrylamide gels, and visualized by silver staining. Lanes represent (from left to right) (P) Pinot noir, (G) Gouais blanc, (1) Aligoté, (2) Aubin vert, (3) Auxerrois, (4) Bachet noir, (5) Beaunoir, (6) Chardonnay, (7) Franc noir de la Haute Saône, (8) Gamay blanc Gloriod, (9) Gamay noir, (10) Knipperlé, (11) Melon, (12) Peurion, (13) Romorantin, (14) Roublot, (15) Sacy, (P) Pinot noir, and (G) Gouais blanc.

[1]Department of Viticulture and Enology, University of California, Davis, CA 95616, USA. [2]Unité de Formation et de Recherche de Viticulture, Ecole Nationale Supérieure Agronomique, 34060 Montpellier, France. [3]Unité de Recherches de Génétique et d'Amélioration des Plantes-Viticulture, Institut National de la Recherche Agronomique, 34060 Montpellier, France.

*Present address: Plant Genome Mapping Laboratory, University of Georgia, Athens, GA 30602, USA.
†To whom correspondence should be addressed. E-mail: cpmeredith@ucdavis.edu

more probable than two random parents and are 447 to 28,000 times more probable than either 'Pinot' or 'Gouais blanc' relatives.

The origins of these varieties have been the subject of much speculation. While most are thought to be French, foreign origins have been suggested for some. 'Chardonnay', for example, has been proposed as coming from the Middle East, 'Gamay noir' from Dalmatia and 'Sacy' from Italy (*11*).

Because some of these varieties are morphologically similar, some authors have suggested that they are close relatives. The varieties are indeed related, but none of the specific relationships they proposed are the ones revealed by our data. In the 17th century, for example, 'Aligoté' was suspected to be a seedling of 'Gouais blanc' and 'Chardonnay'. 'Gamay blanc Gloriod' was thought to perhaps be a seedling of 'Gamay noir'. 'Chardonnay' was considered to be a white form of 'Pinot noir' (*11*). Based on their observations that wine cultivars historically associated with a region often share morphological similarities, several authors have proposed ecogeographic groupings for French wine grapes (*12*), some of which include varieties discussed here.

It is no surprise that these 16 varieties are the progeny of 'Pinot'. No variety is thought to have been in Burgundy for a longer time, and it also has a long history in other parts of northeastern France. References to a variety that was probably 'Pinot' go back as far as the Roman agricultural writer Columella in the first century A.D. 'Pinot' may already have been present in the Burgundy region at the time of the Roman conquest (*11*).

The surprise is that the second parent is 'Gouais blanc', a variety considered so mediocre that it was banned (unsuccessfully) at various times in at least two regions and is no longer planted in France. The name "Gouais" derives from the old French adjective "gou," a term of derision. In the Middle Ages, this variety was very widespread in northeastern France and probably occupied the majority of the vineyards, but only the mediocre sites, the better sites being reserved for more noble varieties such as 'Pinot' (*11*).

We believe that 'Gouais blanc' is not indigenous to northeastern France but was introduced from elsewhere because it is the same variety as 'Heunisch weiss', once widely grown in Eastern Europe (*13*). The third century Roman emperor Probus, a Dalmatian, encouraged viticulture in the provinces and is said to have given the Gauls a grape from his homeland. While it has been suggested that this grape was 'Gamay noir' (*11*), there is no evidence that this variety was grown outside of France until relatively recently, so perhaps Probus' gift to the Gauls was 'Gouais blanc'.

We do not know how many separate crossing events produced these progeny but it is likely that each was produced at a different

Table 1. Detailed parentage analysis for 'Chardonnay' (C) and its presumptive parents 'Pinot' (P) and 'Gouais blanc' (G). The likelihood of the progeny alleles for the putative parents versus alternative possibilities was calculated as in (*3*) and (*21*). For each locus, the likelihood ratio is the quotient of the probability of the progeny genotype if it had the presumptive parents and the probability of that genotype if it had the alternative parents. Observed allele frequencies are based on 322 genotypes. The calculations were also performed with upper 95% confidence limits of allele frequencies to compensate for sampling error.

Locus	Genotype			Frequency of progeny alleles		Likelihood ratios of P × G* versus alternative parents (observed allele frequencies)				Likelihood ratios of P × G versus alternative parents (upper confidence limits of allele frequencies)				
	P	C	G	Observed	Upper 95% confidence limit	X × Y†‡	G × X§	P × X§	G × P‖ relative	P × G‖ relative	X × Y	G × X	P × X	G × P relative	P × G relative
VVMD28	221	221	231	0.06	0.07	18.81	8.67	4.34	1.79	1.63	12.81	6.93	3.70	1.75	1.57
	239	231	249	0.12	0.14										
VVS2	137	137	133	0.04	0.05	18.07	12.39	2.92	1.85	1.49	12.18	9.50	2.56	1.81	1.44
	151	143	143	0.17	0.20										
VVMD31	216	214	212	0.09	0.10	13.47	4.66	5.78	1.65	1.71	10.00	4.16	4.81	1.61	1.57
	–	216	214	0.21	0.24										
VrZAG79	239	243	237	0.09	0.11	12.14	4.60	5.28	1.64	1.68	8.63	3.90	4.42	1.59	1.63
	245	245	243	0.11	0.13										
Cumulative product for these 4 loci						55,588	2,303	386	8.98	6.94	13,466	1,068	202	8.12	6.12
Cumulative product for all 32 loci						4.36×10^{17}	5.42×10^{13}	3.22×10^{9}	4.00×10^{4}	2813	5.9×10^{13}	4.17×10^{11}	4.79×10^{7}	1.01×10^{4}	691

*No direction is implied by the order of the parents in each cross. †Where X and Y are random unrelated cultivars. ‡The identity of both parents is unknown. §The identity of one parent is assumed but that of the other is unknown. ‖The identity of one parent is assumed but the possibility is considered that the other parent is a close relative of the cultivar proposed as the second parent. A close relative is a parent, progeny or full sibling.

time and place. Historical references to the individual progeny cultivars point to beginnings in different locales ranging from the easternmost Loire Valley to Champagne to Alsace. Both parents were widespread in northeastern France and could have been in close proximity in all of these places. 'Pinot' was also grown in other parts of Europe in the late Middle Ages, so that crosses between the two parents could have occurred in places other than France. However, the progeny discovered in this study are all historically associated with northeastern France and not with other places, suggesting that the crosses occurred here. Other 'Pinot' × 'Gouais blanc' progeny may well exist in other parts of Europe.

Several of the progeny can be traced back to the early Middle Ages or before ('Chardonnay', 'Gamay noir', 'Melon'). The others are mentioned in literature from 100 to 400 years ago. Several progeny varieties that were first described only in the late 19th century (such as 'Gamay blanc Gloriod' and 'Peurion') may have existed before this time. Although 'Gouais blanc' would have been a more probable parent earlier, when it was more widespread, even a relatively rare crossing event in the 19th century could have produced successful progeny cultivars. 'Pinot' and 'Gouais blanc' are clearly a good parental combination, whereas any other varieties growing in the vicinity would likely be 'Pinot' or 'Gouais blanc' relatives and would be less fit as a consequence of inbreeding depression.

Nine of the progeny cultivars have light-colored fruit (white-yellow to amber), four are blue-black or violet-black and two have intermediate pink berries. 'Gouais blanc' has yellow-gold berries. The parent of the dark-berried cultivars ('Bachet noir', 'Beaunoir', 'Franc noir', 'Gamay noir') must have been 'Pinot noir'. But because 'Pinot noir' is heterozygous for berry color, we cannot say whether 'Pinot noir', 'Pinot gris' or 'Pinot blanc' was the parent in the other cases. However, 'Pinot noir' has always been the most common form so that it is the more probable parent.

Although 'Gouais blanc' has never been highly regarded and is maintained in only a few grape variety collections today, its genetic potential as a parent has been clearly demonstrated here, particularly in the case of 'Chardonnay', considered one the world's greatest wine varieties. Knowledge of parental relationships such as those reported here can facilitate rational decisions regarding the size of grape germplasm core collections, which are constantly threatened by economic constraints. A core collection containing 'Pinot noir' and 'Gouais blanc' will contain the same allelic resources as a larger collection that also includes cultivars that are the progeny of these two.

Although most grape cultivars are hermaphroditic and largely self-pollinating, grape is intolerant of inbreeding (*14*). In searching for parental relationships among more than 300 cultivated grape varieties, we did not find any that are the selfed progeny of another cultivar (*15*). Furthermore, despite their geographic proximity over a long period of time, none of the 'Pinot' × 'Gouais blanc' progeny cultivars seem to have produced any successful progeny. Our results suggest that heterosis has played a significant role in the emergence of successful wine grapes. 'Gouais blanc' is genetically relatively dissimilar to 'Pinot', sharing only 20 out of 64 alleles at the 32 loci studied, consistent with an eastern European origin for 'Gouais blanc'. The large number of successful progeny these two parents have produced may be a consequence of their genetic distance (*16*). Modern grape breeding programs might benefit from the use of comparably distant parents.

Table 2. Summary of parentage analyses for 16 putative progeny cultivars of 'Pinot' (P) and 'Gouais blanc' (G). X and Y denote random unrelated cultivars. The likelihood ratio shown for each possible parentage for each progeny cultivar is the cumulative product of the ratio for each of 32 loci. Ratios are calculated from 95% upper confidence limits of allele frequencies.

Progeny	Likelihood ratios of Pinot × Gouais blanc versus alternative parents				
	X × Y	G × X	P × X	G × P relative	P × G relative
Aligoté	1.09×10^{15}	1.23×10^{12}	9.14×10^{8}	2.04×10^{4}	2.25×10^{3}
Aubin vert	1.86×10^{13}	1.41×10^{11}	1.05×10^{8}	7.92×10^{3}	9.14×10^{2}
Auxerrois	7.92×10^{12}	2.77×10^{11}	1.82×10^{7}	1.34×10^{4}	4.47×10^{2}
Bachet noir	2.51×10^{14}	6.02×10^{11}	5.20×10^{8}	9.83×10^{3}	1.26×10^{3}
Beaunoir	3.86×10^{13}	7.33×10^{11}	1.81×10^{8}	2.19×10^{4}	8.34×10^{2}
Chardonnay	5.91×10^{13}	4.17×10^{11}	4.79×10^{7}	1.01×10^{4}	6.91×10^{2}
Dameron	3.70×10^{14}	2.60×10^{12}	1.21×10^{8}	2.80×10^{4}	8.35×10^{2}
Franc noir de la Haute Saône	7.53×10^{14}	2.32×10^{12}	2.53×10^{8}	1.95×10^{4}	1.05×10^{3}
Gamay blanc Gloriod	4.69×10^{14}	8.17×10^{10}	3.03×10^{9}	7.77×10^{3}	3.11×10^{3}
Gamay noir	6.99×10^{13}	1.66×10^{11}	5.27×10^{8}	9.25×10^{3}	1.87×10^{3}
Knipperlé	8.92×10^{13}	4.73×10^{11}	8.96×10^{8}	1.18×10^{4}	1.31×10^{3}
Melon	5.79×10^{14}	2.61×10^{10}	1.37×10^{10}	2.29×10^{3}	6.22×10^{3}
Peurion	2.05×10^{15}	2.68×10^{11}	5.05×10^{9}	5.75×10^{3}	4.05×10^{3}
Romorantin	3.76×10^{14}	1.73×10^{13}	9.44×10^{8}	2.00×10^{4}	1.92×10^{3}
Roublot	1.92×10^{14}	1.31×10^{11}	1.90×10^{10}	4.11×10^{3}	6.26×10^{3}
Sacy	2.04×10^{14}	2.35×10^{12}	2.49×10^{8}	1.26×10^{4}	1.48×10^{3}

References and Notes

1. The third variety used in Champagne, 'Meunier', is considered a separate variety by winemakers but is actually a chimeric mutant of 'Pinot'. 'Pinot noir', 'Pinot gris', and 'Pinot blanc' are all treated as separate varieties by winemakers, by virtue of their different fruit colors, but they are simply color mutants of the same variety and all have the same microsatellite genotype, indicating that they have originated from a single individual seedling. 'Pinot' is the name used to encompass all of these forms. 'Pinot fin teinturier', a form of 'Pinot' with colored juice, also has the same microsatellite genotype except for one allele at locus VVS2.
2. L. Levadoux, *Ann. Amelior. Plant.* **6**, 59 (1956).
3. J. E. Bowers and C. P. Meredith, *Nature Genet.* **16**, 84 (1997).
4. We began with 351 cultivars but eliminated 29 that have duplicate microsatellite genotypes because they are either mutants (for example, the color forms of 'Pinot' and other varieties) or synonymous cultivars. The 322 cultivars analyzed are listed in a supplementary table available at www.sciencemag.org/feature/data/1042157.shl.
5. Young leaves and shoot tips were collected from actively growing vines at the University of California at Davis and the Institut National de la Recherche Agronomique collection at Domaine de Vassal near Montpellier, France. Genomic DNA was isolated by a modified cetyltrimethyl ammonium bromide method [J. E. Bowers, E. B. Bandman, C. P. Meredith, *Am. J. Enol. Vitic.* **44**, 266 (1993)]. Microsatellite loci were amplified as described (*17*), separated on denaturing 6% polyacrylamide sequencing gels, and visualized by silver staining with a commercial kit (Promega). Allele sizes were initially determined by visual comparison to a sequencing reaction and in subsequent analyses by visual comparison to reference cultivars included in the same gel.
6. Microsatellite genotypes were compared in all possible groups of three by means of a BASIC program written for this purpose.
7. The 17 microsatellite loci analyzed for all cultivars were VVMD5, VVMD6, VVMD7 (*6*); VVMD21, VVMD24, VVMD25, VVMD26, VVMD27, VVMD28, VVMD31, VVMD32, VVMD34, VVMD36 (*18*); VVS2 [M. R. Thomas and N. S. Scott, *Theor. Appl. Genet.* **86**, 985 (1993)]; VVS29 (*19*); VrZAG83 and VrZAG 93 (*20*). The 15 additional loci analyzed on a subset of cultivars were VVMD14, VVMD17 (*18*); VVS4, VVS19 (*19*); VrZAG62, VrZAG64, VrZAG79 (*20*); VMC2C3, VMC2A5, VMC2H4 (N. Goto, personal communication); VMC5A1, VMC5G1.1, VMC5G6, VMC5H2, VMC5H5 (S. Riaz and C. Meredith, unpublished results)
8. We do not refer to 'Gouais blanc' as 'Gouais', to avoid confusion with 'Gouais noir', which is a synonym for a different variety, 'Enfariné'.
9. One of the putative progeny, 'Romorantin', does not share an allele with 'Pinot' at locus VVS2, exhibiting a 129–base pair (bp) allele instead. We have observed an allele of this size in 'Pinot fin teinturier', a red-juiced variant of 'Pinot', but not in any other cultivars. Another putative progeny, 'Dameron', does not share an allele with 'Gouais blanc' at locus VVMD36, requiring either a mutation of one of the 'Gouais blanc' alleles to a 254-bp allele or to a null allele.
10. A supplementary data table containing the genotypes of the putative parents and progeny is available at www.sciencemag.org/feature/data/1042157.shl
11. P. Viala and V. Vermorel, *Ampélographie, Volumes I–VII* (Masson, Paris, 1901–1910).
12. A. M. Negrul [*C. R. Acad. Sci. URSS* **18**, 585 (1938)] placed the ancient wine grapes of France, with their small leaves and small fruit clusters, into his group *prole occidentalis* within which he identified a 'Pinot' sortotype (subgroup) including 'Pinot noir', 'Chardonnay', 'Meunier', and 'Aligoté'. Levadoux (*2*) categorized a number of similar varieties in northeastern France as the 'Noiriens', including 'Pinot', 'Gamay', 'Chardonnay', 'Tressot', and 'Savagnin'. More recently J. Bisson [*J. Int.*

Sci. Vigne Vin **29**, 63 (1995)] defined 12 "ecogeographs." He included in the Noirien group 'Pinot noir', 'Chardonnay', 'Gamay', 'Meunier', 'Melon', 'Troyen', 'Auxerrois', and 'Gouget'.
13. We compared 'Gouais blanc' and 'Heunisch weiss' at 11 microsatellite loci and found them to be identical (J.-M. Boursiquot, C. Roux, P. This, unpublished results). H. Goethe [*Handbuch der Ampelographie* (P. Parey, Berlin, 1887)] indicates that 'Heunisch weiss' is of Croatian origin.
14. Grape cultivars are highly heterozygous. Although modern cultivars are hermaphroditic and naturally self-pollinate, the viability of selfed progeny declines beyond a few generations. Wild grapes and some primitive cultivars are dioecious and therefore obligate outcrossers.
15. We found 'Malaga II' to be the selfed progeny of 'Muscat of Alexandria'. It was selected as a seedling in the 1950s and is not cultivated.
16. The parents of 'Cabernet Sauvignon', 'Cabernet franc', and 'Sauvignon blanc' are also genetically dissimilar, sharing only 12 of 56 alleles at 28 loci.
17. J. E. Bowers, G. S. Dangl, R. Vignani, C. P. Meredith, *Genome* **39**, 628 (1996).
18. J. E. Bowers, G. S. Dangl, C. P. Meredith, *Am. J. Enol. Vitic.* **50**, 243 (1999).
19. M. Thomas, personal communication.
20. K. Sefc, F. Regner, E. Turetschek, J. Glössl, H. Steinkellner, *Genome* **42**, 367 (1999).
21. E. Hagelberg, I. C. Gray, A. J. Jeffreys, *Nature* **352**, 427 (1991); B. S. Weir, *Genetic Data Analysis II* (Sinauer, Sunderland, MA, 1996), pp. 209–215.
22. Partially supported by the American Vineyard Foundation and the California Department of Agriculture Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board.

27 May 1999; accepted 28 July 1999

Differential Stimulation of PKC Phosphorylation of Potassium Channels by ZIP1 and ZIP2

Jianping Gong, Jia Xu,* Magdalena Bezanilla,† Rika van Huizen, Rachel Derin, Min Li‡

Targeting of protein modification enzymes is a key biochemical step to achieve specific and effective posttranslational modifications. Two alternatively spliced ZIP1 and ZIP2 proteins are described, which bind to both Kvβ2 subunits of potassium channel and protein kinase C (PKC) ζ, thereby acting as a physical link in the assembly of PKCζ-ZIP-potassium channel complexes. ZIP1 and ZIP2 differentially stimulate phosphorylation of Kvβ2 by PKCζ. They also interact to form heteromultimers, which allows for a hybrid stimulatory activity to PKCζ. Finally, ZIP1 and ZIP2 coexist in the same cell type and are elevated differentially by neurotrophic factors. These results provide a mechanism for specificity and regulation of PKCζ-targeted phosphorylation.

Protein phosphorylation is important for the regulation of neuronal excitability and many other non-neuronal processes. Phosphorylation of ion channels, which could change channel expression as well as kinetic properties, is thought to be one of the key regulatory mechanisms of membrane excitability (*1*). The existence of signaling complexes containing both ion channels and closely associated protein kinases and phosphatases has been inferred from biochemical and electrophysiological studies (*2*). Such ion channel–kinase/phosphatase complexes are thought to provide the necessary macromolecular organization for signaling specificity and regulation (*3, 4*). Many ion channels are phosphorylated by serine-threonine kinases such as PKC and PKA (*5*). The mechanisms for targeting the specificity and regulating the activity of PKC or PKA to ion channel proteins are not well understood.

Potassium channels are important for controlling neuronal excitability (*6*). Shaker-type potassium channels have been implicated in associative memory in model systems (*7*). The auxiliary Kvβ subunits specifically interact with a subset of Kvα subunits, which, in some cases, results in modulatory effects (*8–10*). The mammalian Kvβ2 is an abundant subunit found in both excitable and nonexcitable cells, and the native neuronal Shaker-type potassium channel has an estimated stoichiometry of $(Kv\alpha)_4(Kv\beta)_4$ (*11*).

To identify proteins that functionally interact with potassium channels, a yeast two-hybrid library made from rat hippocampal mRNA was screened against full-length Kvβ2. The interacting clones include multiple partial cDNA fragments of Kvβ2 (*12*). In addition, we found two cDNA fragments, B20 and B24, encoding two previously unknown Kvβ2-binding proteins. Sequence comparison of full-length cDNAs showed that B20 and B24 are identical except for a stretch of 27 residues missing in B24. Although B24 has never been reported, B20 was identical to a previously reported gene known as ZIP (PKC-zeta interacting protein) that is isolated as a protein interacting with an atypical PKCζ isozyme (*13*). Human homologs for this protein, known as A170 or p62, have also been identified (*14*). Thus, B20 and B24 were termed ZIP1 and ZIP2.

We generated primers flanking the insertion site, which allows polymerase chain reaction (PCR) to amplify DNA covering the putative splicing site, thereby producing DNA fragments of distinct sizes (*15*). Reverse transcription (RT)–PCR using rat cerebellum mRNA amplified two DNA fragments (Fig. 1C, lane 5). They possessed the same mobility as the PCR products obtained using cloned ZIP1 and ZIP2 as templates and corresponded individually to the coding sequences of ZIP1 and ZIP2 (Fig. 1C, lanes 2 through 4). These data provide evidence that ZIP1 and ZIP2 resulted from alternative splicing, and both transcripts are present in the brain. The two forms contain a number of protein domains suggestive of their biochemical features (Fig. 1, A and B). The NH_2-terminal acidic motif is a novel domain homologous to a segment of CDC24 in yeast. They were found in diverse proteins of evolutionarily distant species ranging from bacteria to mammal. All share a characteristic sequence pattern of (Y/W)XDXXGD(L/F) (V/I). The zinc finger domain contains three CXXC motifs and a DYDL signature (*16, 17*). Both PEST and ubiquitin associated (UBA) domains are involved in regulation of protein turnover by either coding protein stability or directing proteins to degradation pathways. In addition, the PEST sequence is implicated in interaction with calmodulin (*18*).

ZIP was identified from rat brain cDNA library, but its expression was not restricted to the nervous system. A 69-kD polypeptide was detected in all tissues tested (Fig. 1D). Different from most tissues, regions in the central nervous system gave rise to two bands, 69 and 66 kD, both of which could be completely blocked with purified GST-ZIP1 fusion protein (*19*). The sizes of the two polypeptides are consistent with those of ZIP1 and ZIP2, although we cannot rule out that the two species result from differential posttranslational modifications. RT-PCR was employed to detect the expression and relative ratio of ZIP1 and ZIP2 transcripts. In nonexcitable tissues, ZIP1 was present in significantly higher amounts than ZIP2, whereas in regions of the central nervous system, the ZIP1/ZIP2 ratio was closer to 1 (Fig. 1D, lower panel). The expression of the ZIP proteins was also temporally regulated. In the cerebellum, an area with a high expression level of ZIP (Fig. 2C), the expression of the ZIP proteins peaked at postnatal day 13 (Fig. 1E). The two ZIP polypeptides differed both in expression level

Department of Physiology and Department of Neuroscience, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA.

*Present address: Aurora Biosciences Corporation, San Diego, CA 92121, USA.
†Present address: The Salk Institute for Biological Studies, San Diego, CA 92186, USA.
‡To whom correspondence should be addressed. E-mail: minli@jhmi.edu