rally sink. The discharge of saline fluids may be concentrated at the base of the escarpment because the dense pore fluids collect within the limestones behind the impermeable abyssal sediments until they spill out onto the seafloor at the very base of the escarpment.

We found healthy communities or evidence of former communities on four of six dives made to the base of the Florida Escarpment. This suggests that saline seeps may occur frequently along the base of this and possibly other carbonate escarpments. The duration of seepage in these areas may be long in comparison with the duration at ridge crest hydrothermal vents (*14*). If similar seeps are common and persistent, then the corrosion associated with them may exert an effect on the morphologies of carbonate continental margins.

Isolation of chemosynthetic bacterial strains from Pacific ridge crest vent areas has confirmed that primary production occurs based on energy released in the oxidation of H_2S (*7*). Indeed abyssal chemosynthesis remains the only plausible explanation for the existence of these highly productive deep-sea communities. The requirements are a source of reduced inorganic compounds and the presence of molecular oxygen. The waters emanating at the Florida Escarpment seeps appear to be at nearly ambient temperatures when they arrive at the sea floor. Therefore, hydrothermal vents are but one vehicle to bring dissolved reduced inorganic compounds into contact with oxygenated seawater so that chemosynthesis can occur.

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9. The mussels differ from those found at the Pacific vent sites and probably belong to an undescribed species. The clam is a new species of *Calyptogena* (R. Turner, personal communication). The vestimentiferans are congeneric with an undescribed species collected from a hydrothermal spring associated with a strike-slip fault off San Diego [P. Lonsdale, *Nature (London)* **281**, 531 (1979); M. Jones, personal communication]. The limpet is closely related to a species found near Pacific vents (J. Mclean, personal communication).
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15. Profile (collected by the University of Texas, Institute of Geophysics) crosses the escarpment about 90 km north of the seeps in a morphologically similar area.
16. We wish to thank Captain Baker and the crew of the *Atlantis II* Ralph Hollis and the Alvin Group for their efforts and enthusiasm which ensured the success of the cruise. J. Edmond personally gave up one of his scheduled dives on a later cruise so that we could further explore these sites. The *Alvin* dives were funded through NSF grant OCE 82-08177 and by the Naval Ocean Research and Development Activity.

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Salivary Proline-Rich Protein Genes on Chromosome 8 of Mouse

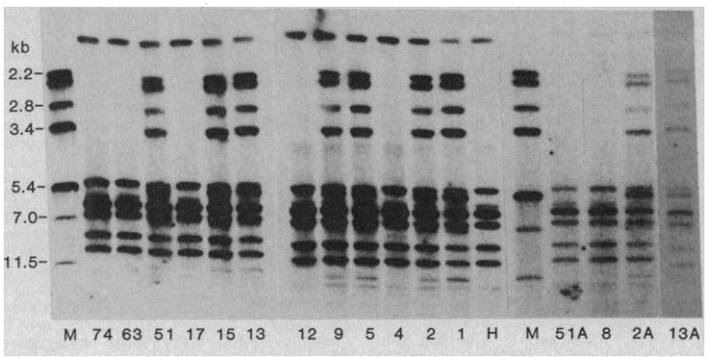
Abstract. *Endonuclease restriction (Hind III) fragments of DNA from Chinese hamster × mouse somatic cell hybrids hybridized with proline-rich protein complementary DNA clones only when the DNA was isolated from cells containing mouse chromosome 8, or a fragment of chromosome 8. The evidence suggests that proline-rich protein genes are located at the proximal portion of chromosome 8 toward the centromere.*

The proline-rich protein (PRP) gene family is large and complex and is important in tooth- and respiratory-related functions (*1, 2*). The PRP genes constitute a single autosomal linkage group in the human as determined by family studies and somatic cell hybridization data (*3, 4*). The rat PRP's are probably also encoded by a multigene family for the following reasons: there are at least ten PRP's for which there is no evidence of interconversion (*5, 6*); all PRP's label with a single amino-terminal [^{35}S]methionine during cell-free translations (*7*); and PRP complementary DNA's (cDNA's) hybridize selectively with at least two families of messenger RNA's (mRNA's) (*8*). Some human and rat PRP's show similarities in amino acid composition (*1*) and peptide sequences (*9*).

Treatment of rats with isoproterenol, a β-agonist, causes rapid glandular secretion and dramatic changes in the parotid and submandibular glands (*5, 6*) such as hypertrophy and the induction of synthesis of the PRP's, which are very high in proline (25 to 45 percent), glutamate plus glutamine (19 to 25 percent), and glycine (17 to 19 percent) (*6*). In the parotid glands of isoproterenol-treated rats, a group of ten or more PRP's comprise about 60 to 70 percent of the total soluble protein. A similar induction of PRP's in the parotid glands of rats (*10*) and mice (*11, 12*) is observed after feeding a diet containing tannins.

To determine the chromosomal location of mouse PRP genes, two nick-translated rat PRP cDNA probes (pRP8 and pRP33) were used for hybridization experiments. DNA's were isolated from Chinese hamster × mouse somatic cell hybrids that were differentially lacking mouse chromosomes while retaining all the hamster chromosomes. The preparation and analysis of these hybrids for chromosomal content (*13*) and the preparation (*14*) and analysis (*8, 9*; including sequences) of the rat cDNA clones have been described. The two cDNA's code for different rat PRP's as determined by hybridization selection studies, sequence analyses, Northern blots, and the lack of cross-hybridization under stringent conditions (*8, 9*).

Digestion of DNA's by Hind III results in prominent and characteristic multiple fragments for the mouse and hamster genes. Seven of the mouse fragments hybridize with PRP33 (Fig. 1). The hybrids, called EBS 1, 2, 5, 9, 13,



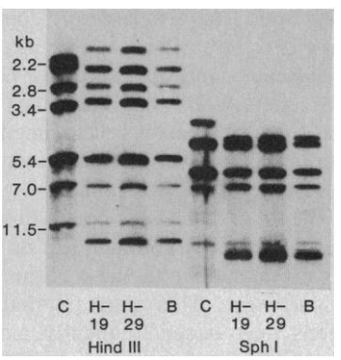


Fig. 1 (left). Hybridization patterns of mouse, Chinese hamster, and somatic cell hybrid DNA fragments to the rat PRP cDNA probe (pRP33). Each DNA sample (10 μg) was digested to completion with Hind III, subjected to electrophoresis on an agarose (0.8 percent) slab gel, and transferred by blotting to nitrocellulose filters. The probe was labeled with ^{32}P by nick-translation and hybridized to the filters (*27*). DNA fragments are from mouse (M), Chinese hamster (H), and the somatic cell hybrids (numbered). Fig. 2 (right). Hybridization of ^{32}P-labeled rat PRP cDNA to Hind III fragments of DNA from congenic mice and progenitor strains. Lanes are indexed according to mouse strain: lane B, C57BL/6; lane C, BALB/c; lane H-19, congenic strain B6.C-*H-19*^{c}/By; lane H-29, congenic strain B6.C-*H-29*^{c}/By.

15, 51, 2A, and 13A possess all of the mouse bands, while EBS 4, 12, 17, 63, 74, 51A, and PBH8 do not. The same results were obtained with a 0.2-kb fragment of pRP8 as a probe. The presence or absence of mouse bands hybridizing to PRP cDNA probes was then correlated with the mouse chromosome content of the cell hybrid (Table 1). Only mouse chromosome 8 occurs concordantly with hybridization to PRP cDNA's. Since mouse DNA fragments segregate coordinately, the PRP genes are probably all on the same chromosome, although it is possible that the probes may not have detected all of the genes. We confirmed the chromosome 8 localization by hybridizing a ^{32}P-labeled mouse metallothionein Mt-1 gene fragment (*15*) to 14 of the Eco RI–digested hybrid DNA's. There is complete concordance between PRP and Mt-1 probe results ($P < 0.0001$).

To locate PRP genes at a particular region of chromosome 8, the DNA's extracted from the livers of two congenic strains of mice (B6.C-*H-19*^{c}/By and B6.C-*H-29*^{c}/By) were studied (*16*). These congenic strains (*17*) have different fragments of chromosome 8 of the BALB/cBy donor strain inserted into the chromosomal background of the C57BL/6By strain. A total of about 40 centimorgans, which represents approximately half of chromosome 8 from the BALB/cBy mouse, is present in the congenic strains (*18*) although there may be some overlap between the two fragments. Fragments prepared by the digestion of the DNA's of congenic and progenitor BALB/cBy and C57BL/6By strains with Hind III and Sph 1 were transferred to nitrocellulose and hybridized to the ^{32}P-labeled pRP33 probe. The fragments of the congenic strain DNA's that hybridized to the labeled probe are those of the background C57BL/6By strain rather than those of the BALB/cBy donor strain (Fig. 2). Therefore, the PRP loci are not located in the central region of chromosome 8 in the vicinity of the *H-19* and *H-29* selected markers (*19*). Since the *Es-1* marker is included in the B6.C-*H-29*^{c}/By congenic strain, the PRP loci are not present in the donor chromosome 8 segment between the *Es-1* and *H-29* loci (*18*).

Positive evidence for sublocalization of mouse PRP loci was obtained by studies of the EBS hybrid subclone 2A. Its hybridization pattern with the pRP33 probe is shown in Fig. 1. The EBS hybrid subclone 2A is morphologically lacking either a recognizable intact mouse chromosome 8 or its fragment; however, the presence of a chromosomal 8 fragment is inferred by the presence of the mouse chromosome 8 marker, glutathione reductase (*Gr-1*). Another mouse chromosome 8 marker, adenine phosphoribosyl transferase (*Aprt*), is not present in the hybrid. The location of *Aprt* within mouse chromosome 8 has not been determined (*19*). Since the *Gr-1*

Table 1. Mouse chromosome content and results of hybridization with rat PRP cDNA's to DNA fragments of Chinese hamster × mouse somatic cell hybrids. The two columns on the right show results of hybridizing rat PRP cDNA's (pRP33 and pRP8) to Hind III fragments. The other columns show the mouse chromosome content in the hybrids. The clones designated EBS 2A, 51A, and 13A are subclones of the primary EBS clones of the same number. Open triangles indicate that only a fragment of the complete chromosome is present. Individual hybrid clones were scored as positive for a given chromosome if more than 15 percent of the metaphases examined contained it; the clone was scored as negative if less than 5 percent of the metaphases contained it. If 5 to 15 percent of the metaphases contained it, then the hybrid was not used in the analysis. D/C represents the ratio of discordance to concordance for each mouse chromosome. Concordance means that the specific chromosome (or fragment) and the hybridization to the rat PRP probe are either both present or both absent. Discordance means that opposite results were obtained for hybridization and chromosome content.

Hybrids	Mouse Chromosomes																				Hybridization of PRP probe	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	33	8
EBS 1	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+
4	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	+	-	-
5	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+
9	-	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+
10	-	+	+	-	-	-	+	-	-	+	-	+	+	+	+	+	-	+	-	+	-	
11	+	-	-	-	-	-	+	-	-	+	-	+	-	-	+	+	-	-	-	+	-	
12	-	-	+	-	-	-	-	-	-	+	-	-	-	-	Δ	Δ	-	+	+	+	-	-
13	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
15	-	+	+	+	-	+	+	+	-	+	-	+	+	-	+	+	+	-	+	+	+	+
17	+	+	-	-	+	-	+	-	+	-	-	-	+	-	+	-	+	-	-	+	-	-
51	-	+	-	-	+	-	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+
63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Δ	-	-
71	+	+	+	-	+	-	+	-	+	-	-	+	-	+	+	+	+	+	-	+	-	
74	-	+	+	-	-	+	-	-	-	-	-	+	-	+	+	+	-	-	-	+	-	-
2A	-	-	+	-	-	+	-	Δ	+	-	-	+	+	+	+	+	+	-	-	+	+	
51A	-	+	-	-	-	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	-	
13A	-	+	+	+	-	+	+	+	-	+	-	+	+	-	+	-	+	+	-	+	+	
PBH 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	
D/C	8/11	6/13	5/14	3/16	7/12	2/17	6/13	0/19	5/14	5/14	9/10	4/15	4/15	7/12	9/10	8/11	4/15	7/12	3/16	10/9		

marker is known to be near the centromere (*19*), the association of PRP loci and the *Gr-1* locus on a chromosome fragment in conjunction with the congenic mice experiment described above, suggests that the PRP loci may be in the proximal (toward the centromere), and not in the central, region of chromosome 8.

Some mouse chromosomal 8 genes, such as, *Gr-1*, *Got-2*, *Prt-2*, and *Aprt* have counterparts on human chromosomes 8 and 16 (*20*). Human PRP genes have now been localized to chromosome 12 rather than 8 or 16 (*4*). The reasons for maintenance or disruption of linkage groups during evolution are unknown, although closely linked genes tend to be conserved (*20–22*).

In previous studies, the structural or regulatory genes for salivary proteins in the mouse have not been found on chromosome 8, but occur on at least four different chromosomes (*23–26*) including chromosome 1 (renin), chromosome 3 (amylase and nerve growth factor), chromosome 2 (parotid secretory protein), and chromosome 7 (tamase and other salivary proteins).

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Conformational Variability of NAD^+ in the Free and Bound States: A Nicotinamide Sandwich in NAD^+ Crystals

Abstract. *X-ray analysis of the free-acid crystal form of the coenzyme nicotinamide adenine dinucleotide (NAD^+) revealed a conformational difference between the free NAD^+ molecule and one bound in enzymes or complexed to Li^+ ions. The pyrophosphate group showed asymmetry in the phosphate-oxygen bonds of the phosphate-oxygen-phosphate link; this bond at the nicotinamide side of the link is longer than that at the adenosine side by 0.04 angstrom. The crystal structure showed a novel intermolecular stacking of adenine and water molecules on opposite sides of nicotinamide that gives rise to a nicotinamide sandwich.*

The coenzyme nicotinamide adenine dinucleotide (NAD^+), formerly called DPN^+, coenzyme I, cohydrase I, or cozymase, plays a dominant role in the transfer of hydride (a proton and two electrons) in biological reduction-oxidation (redox) processes. Along with the other coenzyme flavin adenine dinucleotide, NAD^+ is responsible for most enzyme-catalyzed dehydrogenation reactions. It consists of adenylic acid and nicotinamide-5′-ribonucleotide linked through a 5′-5′ pyrophosphate linkage (Fig. 1); the nicotinamide ring system is redox active, while the adenylic acid seems to serve mainly as a handle for binding the coenzyme. In about 250 known enzymatic redox reactions involving NAD^+ the reactive site is at C-4, and 1,4-dihydronicotinamide is the exclusive isomer formed biologically. Ever since the elucidation of its chemical structure in 1936, numerous studies have been carried out to determine the three-dimensional conformation of NAD^+ in the free and bound states (*1–4*). It was inferred to have a folded conformation in aqueous solutions (*5*), and x-ray crystallographic studies of NAD^+ bound to several dehydrogenases at low resolution showed an extended form, with the nucleotides exhibiting nonstandard, higher energy conformations (Fig. 1 and Table 1) (*4*). X-ray analysis of Li^+-NAD^+ complex (*3*, *6*) at medium resolution showed an extended conformation for the NAD^+ molecule. We now describe our high-resolution x-ray study of the free-acid form of NAD^+, in which the NAD^+ molecule adopts a conformation strikingly different from that found for NAD^+ when it is bound to enzymes or complexed to Li^+ ions.

The crystal structure of NAD^+ shows interesting chemical, conformational, and stacking features. In this structure N-1 of adenine is protonated, so both the purine and pyrimidine bases are positively charged; both phosphates are ionized, and each one carries a negative charge. If the differences in the heterocycles are neglected, NAD^+ has a pseudo diad axis passing through the pyrophosphate oxygen, OPP. However, the two phosphates are not chemically equivalent. The PN–OPP bond of 1.617(3) Å is significantly longer than the PA–OPP bond of 1.578(2) Å. The P–O–P angle is 133.3(2)°, a value similar to that found in other pyrophosphates (*3*). The longer P–O bonds, together with a wider P–O–P angle, allow considerable flexibility in the pyrophosphate links. Another interesting chemical feature in this structure is the pronounced bond-shortening anomeric effect in the C–O bonds of the ribose rings (*7*, *8*); the C-1′–O-4′ and C-4′–O-4′ bond lengths are 1.380(5) and 1.458(5) Å, respectively, for the ribose moiety attached to nicotinamide and 1.404(5) and