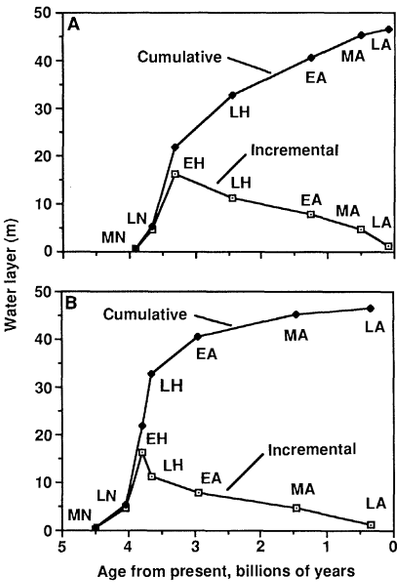

Fig. 1. Plot of water release on Mars inferred in association with volcanic processes (expressed as the thickness of a water layer that would cover the entire planet) for each geologic epoch Abbreviations: MN, Middle Noachian; LN, Late Noachian; EH, Early Hesperian; LH, Late Hesperian; EA, Early Amazonian; MA, Middle Amazonian; and LA, Late Amazonian. The "absolute" age scale is from Tanaka (*18*) and is derived from impact crater distributions based on work of Hartmann *et al.* (*9*) (model 1, **A**) and Neukum and Wise (*10*) (model 2, **B**).

suggested (*14*), they constitute less than 0.5% of the total volume of volcanic units. By using Earth as a first-order analog and by assuming that martian volcanism is dominated by mafic materials, a value of 1.0% by weight of water was used as an average for Mars.

Estimates of the amount of water on Mars released from the interior in association with volcanism are given in Table 1. Little can be said of the fate of the volatiles after their release to the surface, as a "martian water cycle" has not been formulated for either present or past conditions. Moreover, it is not known if water remained near its release area or migrated to other locations. By terrestrial analogy, some water probably migrated to shallow subsurface reservoirs in the regolith. Values given here pertain only to juvenile or "new" water released from the interior and not to recycled water. Because volcanism has spanned much of the "visible" history of Mars (the last ~4 billion years), water may have been released continuously from the interior, although not necessarily at a uniform rate. Figure 1 shows the incremental and cumulative release of water with time. Of particular interest is the very early history of Mars. Relatively little volcanic material is identified, yet most of the oldest terrain on Mars displays extensive valley networks and other evidence for the existence of water (*13, 15, 16*). This suggests that either there were other sources of water early in martian history (such as cometary contributions), or unidentified volcanic units of an early age, or both. Most of the ancient martian crust has been degraded or buried and thus its origin cannot be ascertained. However, by analogy with the moon, the martian crust probably developed by magmatic differentiation and extrusion of flood lavas. Thus, although few traces of earliest volcanism are visible, substantial water may have been released early in martian history and this water is not included in the estimates given here.

The greatest volumes of water appear to have been emplaced in Mars 3 to 4 billion years ago and coincided approximately with the development of the large outflow channels. Photogeologic evidence shows that the formation of outflow channels was episodic (*17*) and extended over a long interval of martian history (Late Hesperian through Middle Amazonian) and reflects on the availability of large volumes of water at or near the surface in some parts of the planet.

There are several uncertainties in the estimates presented here. Principal among these is lack of knowledge of volatile content for magmas; even terrestrial values, as used here, have large uncertainties and extrapolation to martian values is difficult. Uncertainties that stem from estimates of volcanic unit volumes can be reduced through more detailed mapping and determination of flow thicknesses. The values given here represent only amounts associated with volcanism. It is likely that significant additional volumes were released in association with plutonic intrusions. Thus the results substantiate the growing perception of Mars as a "wet" planet in the first third of its history.

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Interocean Differences in Size and Nutrition of Coral Reef Sponge Populations

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Sponges consume an order of magnitude more organic matter on Caribbean coral reefs than on the Great Barrier Reef. This rate of consumption is attributed to Caribbean sponge biomass being five to six times greater than that on the Great Barrier Reef, on average, and to the absence in the Caribbean of phototrophic sponges, which are a feature of clean water regions of the Great Barrier Reef. The long temporal and spatial separation of the Atlantic and Pacific oceans has resulted in the evolution of dissimilar sponge faunas, with Caribbean sponges being heterotrophic, whereas many Great Barrier Reef sponges rely on nutritional input from photosynthetic symbionts.

THE CORAL REEF FAUNA OF THE Caribbean and Great Barrier Reef (GBR) regions show remarkable differences. There are few species of corals, mollusks, echinoderms, and fishes in common (*1*). Coral reefs of the western Pacific are comparatively richer than the Caribbean in some animal groups; for example, there are 75% more genera and 85% more species

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of corals there (*2*). Interocean studies have stressed differences in species composition and community structure (*3*), in the evolution of predator patterns and subsequent countermeasures (*1*), and in reproductive characteristics (*4*). But no studies, to my knowledge, have compared the nutritional differences of the benthos or have examined sponges, which are frequently the second most important component of coral reef benthic fauna (*5, 6*). Marked interocean differences were suspected from comparisons of research on Caribbean (*5, 7*) and GBR sponges (*8, 9*). This study reports significant differences in species composition, biomass, mode of nutrition, and importance of photosynthetic symbioses in sponges in the two coral reef regions.

Sponge populations vary significantly on reefs along a 200-km transect across the continental shelf of the central GBR and out to the Coral Sea (*8*). Biomass of sponges is highest (mean of 484 g m^{-2}) (Table 1) on reefs adjacent to land, and biomass decreases away from the coast out to Coral Sea reefs, where it is lowest (mean of 64 g m^{-2}). Moreover, approximately half of the sponge biomass on "middle" and "outer" reefs remote from direct land influence (Table 1) consists of species that are phototrophic, that is, distinctly flattened sponges that derive at least 50% of their energy requirements from large populations of photosynthetic symbionts, usually cyanobacteria (blue-green algae) (*9*). The proportion of phototrophs is more than 90% on some outer reefs and 0% on reefs close to shore (*8*).

To determine whether similar patterns occur on Caribbean coral reefs, I compared sponge populations at 11 sites by using techniques similar to those used previously on or near the GBR and at two sites in Fiji (*10*). The Caribbean sites were chosen across wide latitudinal and longitudinal ranges to test the effects of the varying influences of land and ocean (Table 1). The results show that sponge populations in the two oceans are similar in species richness, number of individual sponges per unit area, and proportion of species with cyanobacterial symbionts; however, they are markedly different in species composition, biomass, average size of individual sponges, and mode of nutrition.

No sponge species are known to be common to coral reef communities in both oceans, although some questions of affinity between morphologically similar taxa are unresolved. Sponge biomass is five to six times greater at depths of 15 to 20 m on Caribbean reefs than on comparable reefs of the GBR (Table 1) (*11*). The largest difference is seen between Pacific oceanic reefs (Flinders, $\bar{x}$ = 64.4 g m^{-2}; Astrolabe, Fiji, $\bar{x}$ = 26.6 g m^{-2}) and similar "oceanic" reefs in the Caribbean where biomass is more than eight times higher (Glovers, Exuma, and Barbados East, $\bar{x}$ = 489.7 g m^{-2}). In both oceans there is a decrease in biomass from "inner" to "outer" reefs (Table 1) (*11*). Caribbean sponges are also larger on average than those on GBR reefs (*11*). This size difference is particularly evident on outer reefs where the weight difference is five to six times greater for Caribbean sponges, whereas on inner reefs, sponges are comparable in size in both regions. There are differences in the size of sponges across environmental gradients. On the GBR transect, mean sponge weight decreases from the inner to the outer Flinders reefs (142.3 to 25.3 g), whereas the reverse occurs in the Caribbean (inner, 180.4 g and outer, 569.3 g). The most striking difference between sponges in the two oceans is the virtual absence of phototrophic species on Caribbean reefs (Table 1). By contrast, sponge populations on middle and outer reefs of the GBR obtain approximately 50% or more of their nutrient energy from cyanobacterial symbionts (*12*). Linear regression analysis of the data in Table 1 showed no statistically significant linear relation with latitude within the range of 13° to 25°.

Despite these marked differences, neither species richness nor the population density

Table 1. Coral reef sponges in the western Pacific–Great Barrier Reef (GBR) and western Atlantic–Caribbean Sea. Reefs are subjectively listed in three environmental gradient groupings according to either distance from a major land mass or the balance of land and oceanic influences in the Caribbean. Biomass records sponge wet weight (after draining for 30 seconds) in a 120-m^2 area at a depth of 20 m or deepest available site (15 m: Pandora, Phillips, Corbett, and Wee Wee; 10 m: Clack). The phototroph column records the biomass percentage of sponges with predominantly phototrophic nutrition (gross production to respiration ratio is >1.5) (*9*). The symbiont column is the percentage proportion of species in 120 m^2 with at least some photosynthetic symbionts. Individual sponges ("colonies") are listed per square meter, and mean individual weight (not shown) is obtained by dividing this into biomass (grams per square meter).

Reef	Latitude	Land distance (km)	Biomass (g m^{-2})	Phototroph (%)	No. of species (per 120 m^2)	Symbiont (%)	No. of individuals (per m^2)
Inner reefs (inner shelf, land-influenced)							
Pandora*	18°49′S	17	569.9	0.0	45	13.3	3.37
Phillips*	18°58′S	19	398.6	0.0	45	11.1	4.59
Clack†	14°04′S	35	196.5	24.3	23	30.4	2.01
Corbett†	13°59′S	44	193.1	20.2	31	22.6	1.13
Wee Wee‡	16°46′N	13	1011.0	0.0	40	30.0	21.04
Key Largo‡	25°02′N	10	1259.3	0.0	38	31.6	5.39
Barbados West‡	13°11′N	1	2458.2	0.0	32	37.5	9.47
Middle reefs (middle shelf, mixed-influence)							
Rib*	18°29′S	77	48.1	27.7	14	50.0	1.45
John Brewer*	18°38′S	77	203.0	64.3	32	43.7	9.03
Lodestone*	18°42′S	76	98.3	37.1	27	44.4	1.80
Davies*	18°50′S	76	218.0	40.2	34	47.1	9.48
Southwater‡	16°49′N	18	498.7	0.0	34	47.1	1.85
Puerto Rico‡	17°56′N	7	791.6	0.0	39	28.2	3.51
St. Croix BI‡	17°47′N	5	654.2	0.1	37	29.7	7.60
St. Croix SRC‡	17°47′N	0.5	1353.8	0.0	30	40.0	7.37
Jamaica‡§	18°27′N	0.5	99.0	0.0	20	30.0	1.46
Outer reefs (outer shelf, ocean-influenced)							
Myrmidon*	18°16′S	132	157.6	5.1	18	55.5	0.51
Dip*	18°25′S	125	113.3	54.5	32	56.3	6.08
Bowl*	18°31′S	121	141.4	66.3	40	60.0	10.76
Flinders South*	17°53′S	201	57.2	97.2	18	44.4	6.53
Flinders Cay*	17°44′S	233	81.6	90.3	27	40.7	1.74
Flinders West*	17°43′S	231	54.4	11.5	31	25.8	2.71
Rodda†	13°56′S	55	63.9	69.2	29	58.6	2.42
Astrolabe Great	18°42′S	67	7.9	3.7	4	25.0	0.34
Astrolabe North	18°38′S	57	45.2	29.4	7	57.1	0.62
Barbados East‡	13°07′N	2	367.5	0.0	11	54.5	0.81
Exuma Cay‡	23°46′N	1	398.7	0.0	14	42.9	0.58
Glovers‡	16°45′N	47	702.9	0.0	28	42.9	1.24

*Platform reefs of the central GBR [as shown in maps in (*8*)]. †Reefs in the northern GBR near Princess Charlotte Bay; the Astrolabe reefs are in Kandavu group south of Viti Levu, Fiji. ‡Caribbean sites: Wee Wee, Southwater, and Glovers are small vegetated cays off Belize, near the Carrie Bow Cay Laboratory; Key Largo (Florida) at French Reef is near the Key Largo Marine Sanctuary Headquarters; Barbados West is an outer bank reef off the Bellairs Research Institute, and Barbados East is off Sam Lord's Castle; Puerto Rico is on the shelf edge off the University of Puerto Rico marine station, Isla Magueyes; St. Croix sites are north of Buck Island (BI), near West Indies Laboratory, and 1 km west of entrance to Salt River Canyon (SRC); Jamaica is 1 km west of Discovery Bay Marine Laboratory; Exuma Cay is east of Lee Stocking Island (Caribbean Marine Research Center). §The Jamaica site contained only small sponges growing on coral rubble left after Hurricane Allen in 1980.

of sponges varies significantly between the Caribbean and GBR (*11*). In both oceans there is a comparable decrease in the number of species present per 120 m^2 from inner to outer reefs (*11*). The lowest species diversity occurs on reefs with very strong ocean influence, for example, the east coast of Barbados and the Exuma Cay in the Atlantic and the Astrolabe reefs of Fiji (Table 1). Although phototrophic sponges are rare in the Caribbean, the incidence of sponge species containing at least some photosynthetic symbionts is comparable between the two oceans (*11*). In the Caribbean, approximately 40% of sponge species have a thin layer of tissue containing cyanobacteria that overlies the bulk of the sponge, whereas many GBR sponge species are thin with a greater proportion of cyanobacteria throughout the tissue (*9*). In both oceans the incidence of species with symbionts increased from inner reefs to outer reefs (*11*). Thus, the virtual absence of phototrophic sponge species in the Caribbean cannot be due to the nonexistence of photosynthetic symbionts.

On the GBR, sponge biomass is inversely proportional to distance from the Australian mainland along a gradient from higher nutrient, inshore waters to oligotrophic waters of the Coral Sea. This gradient is not apparent in the Caribbean where most reefs are directly associated with islands as fringing reefs. Sponge biomass at a depth of 20 m appears to be directly related to the amount of land-derived nutrients, as biomass decreases across the environmental gradients in both regions (Table 1). For example, on Barbados, the east coast, which is under strong ocean-influence, showed the lowest sponge biomass for the Caribbean, whereas sponge biomass was highest on the west coast, adjacent to large tourist developments where high levels of organic nutrients from raw or partially treated sewage have resulted in a decrease in coral growth (*13*). Key Largo provides a similar example where organic input from the Florida Keys has probably resulted in increased sponge growth compared to the nearby Exuma Cay site. Similarly, on St. Croix higher sponge biomass is evident close to shore, downstream of a large estuary (Salt River Canyon) and the town of Christiansted, whereas there is half as much biomass off Buck Island, upstream of island-derived nutrients. Other studies have shown that increased nutrient levels favor the growth of sponges at the expense of corals (*14*).

Caribbean sponge populations receive very little nutrition from photosynthetic symbionts. This fact refutes Wilkinson's suggestion (*9*) that translocation of photosynthates could make up for the shortfall in sponge nutrition of some Caribbean sponges that were not accounted for in measurements of heterotrophic filter feeding (*7*). Reiswig's original suggestion (*7*) that incorporation of dissolved organic matter is important in sponge nutrition remains to be tested, although sponges are able to incorporate dissolved amino acids (*15*).

The combination of higher biomass and virtual absence of phototrophic feeding means that Caribbean sponges consume an order of magnitude more organic matter than counterparts on middle and outer reefs of the GBR (*12*). No differences in the biology of sponges in the two oceans have been observed. Respiration rates (*12*), particle retention rates (*16*), and population densities (*11*) are comparable; there are no apparent differences in reproductive output. Therefore, the larger Caribbean biomass may be due to either a greater supply of food matter or markedly lower rates of predation. The latter explanation is unlikely as there are no discernible differences between sponge populations in areas where intense fishing activity would remove sponge-eating fishes (Barbados, Jamaica, St. Croix, and Puerto Rico) and other Caribbean sites where fishing is minimal or absent (Belize, Key Largo, and Grand Cayman) (*17*). Experiments with caged and uncaged sponges have shown that predation is not obvious on the GBR (*18*).

I hypothesize, therefore, that the higher sponge biomass in the Caribbean is caused by either a greater abundance of dissolved and particulate organic carbon from higher productivity in the benthos and the water column or a reduced consumption by other benthic animals or both. Higher Caribbean productivity was also suggested by Thresher (*4*), who related smaller egg volume for Caribbean fishes to higher productivity, and by Highsmith (*19*) who observed more bioerosion in waters with higher productivity, and also by participants at a workshop of the United Nations Educational, Scientific, and Cultural Organization (Unesco) (*20*). Phytoplankton productivity is reported to be higher in the western Atlantic than the western Pacific (*21*); however, confirmation of this productivity level for coral reef habitats is required to ascertain whether more organic matter is indeed available.

These observations stimulate speculation on the evolution of markedly different sponges in the two regions. Conditions of low productivity on the GBR favored the survival of evolving, flattened, phototrophic sponges, whereas such sponges either did not evolve in the higher productivity waters of the Caribbean or have subsequently died out during periods of severe climatic, tectonic, and sea level changes (*1*). The biogeographic patterns of these phototrophic sponges may mirror the patterns for hermatypic coral genera that are most numerous in the western Pacific and decrease away from this apparent focus of evolution (*2*).

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10. All sponges, except boring or cryptic species, that were along triplicate horizontal, 20 by 2 m transects were collected, sorted, and weighed. The presence of photosynthetic symbionts was determined by extraction of cleaned sponges in 90% acetone and spectrophotometric analysis at 664 nm. Microscopic examination confirmed the presence of cyanobacteria. At locations where collection was not possible (national parks or study areas), sponge biomass was estimated by measuring the length, breadth, and height underwater and by comparing calculated volumes with those from weighed specimens.
11. Data in Table 1 were compared by using two-way analysis of variance for unequal sample sizes within three environmental gradient groups (G) in two oceanic regions (R), western Pacific–Great Barrier Reef and western Atlantic–Caribbean Sea. The geographically remote Astrolabe reefs, Fiji, were not included. Sponge biomass (in grams per square meter) was significantly different between oceans ($P < 0.0001$) and across environmental gradients ($P < 0.0025$) with no significant $G \times R$ interaction ($P = 0.056$). The biomass proportion of phototrophic sponges was significantly different between oceans ($P < 0.0005$) but not between gradient groups ($P = 0.135$) with no $G \times R$ interaction ($P = 0.136$). The numbers of species were comparable between oceans ($P = 0.68$) but decreased across gradients in both oceans ($P = 0.017$; no interaction, $P = 0.173$). The proportion of sponge species with at least some photosynthetic symbionts was comparable between oceans ($P = 0.972$) but significantly different across gradients ($P = 0.0005$; with a $G \times R$ interaction, $P = 0.0394$). There was no significant difference for numbers of individuals (R, $P = 0.353$; G, $P = 0.089$; $G \times R$, $P = 0.013$), but there were significant differences in the mean size of sponges (R, $P < 0.0001$; G, $P < 0.0005$; $G \times R$, $P < 0.0005$).
12. The sponge population of 98.3 g (wet weight) at a depth of 20 m on Davies Reef, GBR, is estimated to require 0.084 g of carbon per square meter per 24 hours to sustain total respiration. Of this amount, 49.2% would be provided by photosynthetic symbionts. These data were extrapolated from respirometric measures of the ten most prominent sponges on the reef (*9*). Mean daily respiration of 19 prominent Caribbean sponges measured by similar techniques at Carrie Bow Cay, Belize (33.4 μmol g^{-1} day^{-1}, SD 11.74), was not significantly different from Davies Reef sponges (35.3 μmol g^{-1} day^{-1}, SD 15.41).
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Structural Evidence for the Authenticity of the Human Retinoblastoma Gene

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The retinoblastoma (Rb) gene is the prototype for a class of recessive human cancer genes in which loss of activity of both normal alleles is thought to be associated with tumorigenesis. Sixteen of 40 retinoblastomas examined with a complementary DNA probe shown to be the Rb gene had identifiable structural changes of the Rb gene including in some cases homozygous internal deletions with corresponding truncated transcripts. An osteosarcoma also had a homozygous internal deletion with a truncated transcript. In addition, possible hot spots for deletion were identified within the Rb genomic locus. Among those tumors with no identifiable structural changes there was either absence of an Rb transcript or abnormal expression of the Rb transcript. Comparison of the structural changes in the tumor cells and fibroblasts of certain patients provided support for Knudson's two-hit hypothesis for the development of retinoblastoma at the molecular level. The ability to detect germline structural deletions in fibroblasts from some patients with bilateral retinoblastoma also indicates that the isolated gene is useful for diagnostic purposes.

RETINOBLASTOMA (Rb) OCCURS IN both hereditary and nonhereditary forms and is the most common childhood cancer involving the eye (*1–4*). Sporadic bilateral cases are always hereditary while approximately 85% of sporadic unilateral cases are nonhereditary. On the basis of statistical analysis of clinical data, Knudson has proposed that the development of retinoblastoma requires two mutational events (*5*). In hereditary cases, where the germline mutation is present in all cells, Knudson postulated that a second random somatic mutation in a target retinoblast is required for tumorigenesis. The initial clue to the nature of the first mutational event came from cytogenetic studies of lymphocytes or fibroblasts from patients with retinoblastoma. A small subgroup of these patients were found to have a deletion including chromosomal region 13q14 (*6*). Initial tumor cytogenetic data also implicated chromosome 13 as the target for the second event. A loss or partial deletion of chromosome 13 including 13q14 was frequently found in retinoblastomas from patients with two apparently normal constitutional No. 13 chromosomes (*7*).

Additional evidence that the second event also involved the Rb susceptibility locus at 13q14 came from restriction fragment length polymorphism (RFLP) studies. By a comparison of the constitutional and tumor genotypes, as defined by RFLPs on chromosome 13, it was shown that the reduction to hemizygosity or homozygosity for all or part of an allele of chromosome 13 is a common event in the development of retinoblastoma (*2, 3*). Similarly, in osteosarcoma, a bone tumor which occurs frequently as an additional primary malignancy in patients with hereditary retinoblastoma (*8*), the development of homozygosity or hemizygosity for a chromosome 13 has been observed (*9*). All the available data suggest that the loss of activity of both normal alleles at the Rb locus is the key event in tumor development (*4*). However, it has not been possible until now to prove this hypothesis with certainty. In this paper, proof is provided by examining the genotypes of fibroblasts and tumor cells from the same individual with a probe of the putative Rb locus.

Our basic strategy of cloning involved chromosomal walking using probes in region 13q14.1, the locus of the Rb gene (*10*). Random probes free of highly repetitive sequences were isolated from the chromosome 13 specific library, LL 13NS01, obtained from the Lawrence Livermore Laboratory. We made use of two hamster-human hybrid cell lines; cell hybrid 3B6 was made with the cell strain 32T, which has a chromosomal deletion from 13q14.1 to 13q14.3 (*11*), whereas 2D12 contains a chromosome 13 with a deletion from 13q14.11 to 13q22.3 (*12*). Clones containing DNA inserts within chromosomal region 13q14.1 to 13q14.3 were identified by the absence of hybridization to DNA from 3B6 and 2D12. Two other probes, H2-42 and H3-8 (*13*), were obtained by similar strategy. In addition, since both the esterase D (EsD) and Rb loci are in 13q14.1 (*10*), we also isolated a full-length EsD complementary DNA (cDNA) clone to use as a probe.

H3-8 was found to be missing from several phage and cosmid libraries, presumably because it is in a region that is easily deleted. We therefore chose to focus on this troublesome region by cloning shorter restriction fragments detected with the H3-8 probe (Fig. 1A). The insert size of each clone obtained was verified by hybridization of the clone to genomic blots.

We were surprised to isolate a probe, PG4-4, which was located very near H3-8

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