the same length as the inverted repeat and corresponds to the highly variable section of the Sau repeat units.

A third argument is based on the observation that sequences similar to the INS segment occur elsewhere in the bovine genome. This was shown by hybridization of a cloned fragment comprising the INS segment to total calf thymus DNA and various fractions obtained by isopycnic centrifugation. A major region of homology was found in a 1600-bp Eco RI fragment from the 1.711b satellite DNA. Additional bands seen in the autoradiograms were partly a result of larger fragments from the 1.711b satellite that were resistant to Eco RI cleavage because of sequence divergence, and partly a result of minor repetitive components that have not been identified.

The third segment in the 1.711a repeat unit is remarkably similar to Pvu segments B and D, respectively, of the 1.706 satellite DNA (*5*). The 23-bp prototype sequence of this segment differs only by 2 bp from the prototype sequence of Pvu segment D (Fig. 1c) and is closely related to the prototype sequence of the 1.720 satellite (*9*). When the sequences of pairs of individual Pvu segments are compared, the following numbers of base changes are found: between 1.711a and 1.706-B, 52; between 1.711a and 1.706-D, 35; and between 1.706-B and 1.706-D, 20. This suggests that the evolutionary pathways leading to the 1.706 and 1.711a satellite DNA's separated before segment B was generated. Therefore, the 611-bp INS segment should have been incorporated into an ancestor of the 1.706 satellite that probably had only one Pvu and one Sau segment. Such an intermediate structure has been postulated (*5*).

The same sequence of events can be reconstructed from the deletions present in the Pvu segment. There is a deletion of 1 bp in all three segments, an additional deletion of 1 bp in segments B and D, and a further deletion of 4 bp only in segment B. This indicates again that the lines of descent leading to the 1.706 and 1.711a satellite DNA's separated before segment B in the 1.706 satellite was generated by duplication.

From the DNA sequences of cloned preproinsulin and globin genes, Perler *et al.* (*10*) have estimated a minimum value of 7×10^{-9} substitutions per nucleotide site per year for the mutation rate at silent positions and within introns. If this figure holds also for satellite DNA's, then the duplication of the Pvu segment in the 1.706 satellite should have occurred about 5 million years ago, and the separation of the 1.706 and 1.711a satellites, 10 million years ago. With additional sequence data of Pvu-like segments in satellite DNA's from species related to the cow it would be possible to decide whether the rate of mutation in satellite DNA is the same as in introns and silent positions of coding regions.

The structures of the 1.711 satellite DNA's suggest a novel mechanism for the generation of satellite DNA's: the insertion of DNA into repetitive sequences already present in the genome and the subsequent amplification of a new repeat unit containing the inserted sequence and a part of the repetitive sequences. This way of generating satellite DNA's may have been widespread. Related structures have been found for the highly repetitive sequences in the telomeric heterochromation of rye (*11*). The complex satellite DNA's of the human genome (*12*) may be similarly composed. The size variation of the repeating unit found in a satellite DNA from *Drosophila* has been explained by a related mechanism (*1*).

Now five of the eight major satellite DNA's, which together form almost one-fifth of the bovine genome, are known. The nucleotide sequences of the various related satellites have suggested mechanisms for their evolution from common ancestors. Moreover, sequence analyses have shown that complex satellite DNA's may contain sequences believed to be essential for the transcription of eukaryotic genes [see also (*5*)]. This encourages experiments to investigate whether heterochromatin is really genetically as inert as it is presently considered to be.

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Size of the Chloroplast Genome in *Codium fragile*

Abstract. *Chloroplasts isolated from the siphonous green alga* Codium fragile *yield circular DNA molecules averaging 27.3 micrometers in length and* 56×10^6 *daltons in molecular size. This chloroplast genome is 25 to 30 percent smaller than any reported. The small size of the* Codium *chloroplast genome may represent a primitive evolutionary condition in green plants.*

In 1962 it was demonstrated that chloroplasts contain DNA (*1*). Over the past decade chloroplast DNA (ctDNA), extracted from a wide variety of plant groups, has exhibited marked uniformity in its physical and chemical properties, existing as covalently closed circular molecules of 37 to 62 μm in length and molecular size of 80×10^6 to 134×10^6 daltons (*2*). A single exception has been reported in *Acetabularia*, where the estimated genome size is 1500×10^6 daltons, as judged from kinetic complexity measurements (*3*).

We now report that the ctDNA from the green alga *Codium fragile* can be isolated as covalently closed circular molecules with an average contour length of 27.3 μm (measured by electron microscopy) (*4*) and a molecular size of 56×10^6 daltons, as determined by gel electrophoresis of restriction endonuclease fragments. This size is smaller than any yet described for ctDNA.

Until recently it was thought that ctDNA was entirely prokaryotic in character (*5, 6*). With the discovery of introns (intervening sequences) in *Chlamydomonas* ctDNA (*7*), a characteristic of eukaryotic DNA, the picture has become more complicated. Two hypotheses have been advanced to account for the evolu-

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tion of the chloroplast genome (*5, 8*). According to the endosymbiont theory the chloroplast began as a free-living prokaryote that became associated symbiotically with a host cell and in time evolved into an organelle. By contrast, the cluster-clone theory proposes that small segments of DNA were compartmentalized as organelles within the prokaryote ancestor and that the primitive character of the DNA was conserved in the modern chloroplast.

With the unresolved questions of chloroplast evolution in mind, we chose to investigate the ctDNA of *Codium fragile*, a siphonous green alga belonging to the order Caulerpales. Our selection of *Codium* was dictated, in part, by reports of an unusually large chloroplast genome in *Acetabularia*, a related genus in the order Dasycladales (*3, 9*). Chloroplasts of *Codium* and *Acetabularia* are discoid and unusually hardy after isolation, often retaining the capacity for CO_2 fixation in the laboratory for 5 days (*10*). Harvested by saccoglossan mollusks, *Codium* chloroplasts continue to photosynthesize within the animal for up to 3 months after ingestion (*11*). *Codium* occupies a comparatively isolated taxonomic position and appears to be a relic genus of great antiquity. Fossil ancestors of both *Codium* and *Acetabularia* dating from the Ordovician period are among the oldest known fossil macroalgae (*12*).

Codium chloroplasts were isolated by filtration, differential centrifugation, and subsequent flotation through a discontinuous sucrose gradient. The isolated chloroplasts were lysed and treated with proteinase K. Chloroplast DNA was purified by two cycles of density equilibrium centrifugation in cesium chloride (*13*). We found that it was essential to maintain a high molarity of ethylenediaminetetraacetic acid (EDTA), tetrasodium salt (up to 0.5*M*), throughout the extraction procedure to obtain intact circular molecules of ctDNA (*14*).

Samples of *Codium* ctDNA were prepared for electron microscopy with PM2 phage DNA as a marker, with the use of Kleinschmidt's cytochrome c surface-spreading technique (*15*). In some preparations the molecules were still partly protein-bound, partly supercoiled, and partly unraveled. Circular molecules ranged from 23 to 31 μm, averaging 27.3 μm (Fig. 1, a and b).

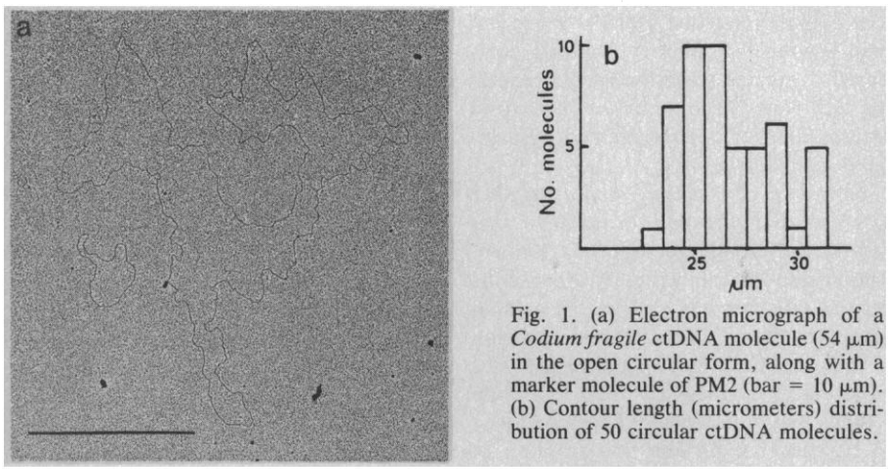

Fig. 1. (a) Electron micrograph of a *Codium fragile* ctDNA molecule (54 μm) in the open circular form, along with a marker molecule of PM2 (bar = 10 μm). (b) Contour length (micrometers) distribution of 50 circular ctDNA molecules.

Purified ctDNA was digested with three restriction endonucleases: Hind III, Xho I, and Hpa II. The resulting fragments were end-labeled with [α-^{32}P]-deoxyguanosine 5′-triphosphate (dGTP) with the use of T_4 polymerase, and separated on linear agarose gels simultaneously with a size marker, lambda DNA, digested with Hind III (Fig. 2). Undigested ctDNA (Fig. 2d) was essentially intact. Digestion of ctDNA with Xho I yielded 11 fragments totaling 57×10^6 daltons, Hind III gave 26 fragments totaling 53×10^6 daltons, and Hpa II gave 26 fragments totaling 58×10^6 daltons.

Purified *Codium* ctDNA sheared by sonication to approximately 0.5×10^6 daltons melted over a narrow temperature range with a T_m of 84.5°C corresponding to G + C content of 37 percent. Lambda DNA run simultaneously had a T_m of 89.0°C, as expected (*16*).

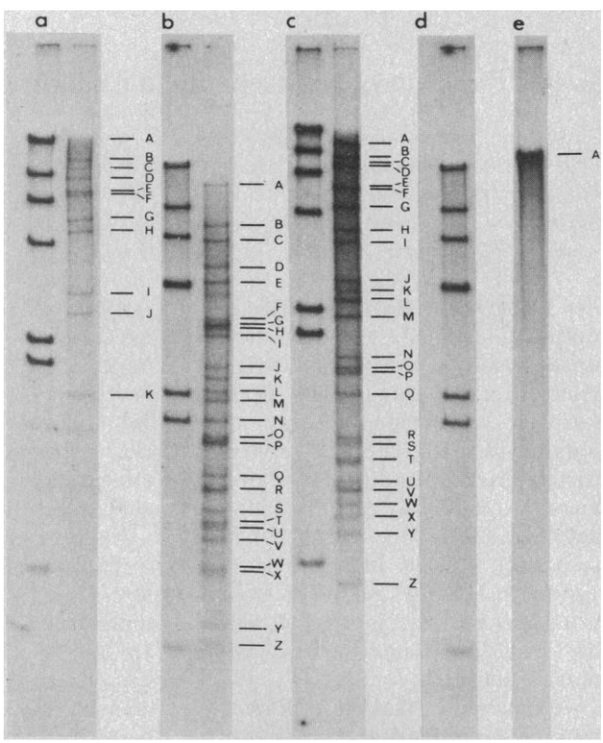

Fig. 2. Autoradiogram of *Codium fragile* ctDNA gel fragments, digested with restriction endonucleases and end-labeled with [α-^{32}P]dGTP. (channel a) Chloroplast DNA digested with Xho I; (channel b) ctDNA digested with Hind III; (channel c) ctDNA digested with Hpa II; and (channel d) ctDNA before endonuclease treatment. In each case the co-run size marker lambda phage DNA digested with Hind III appears on the left of the ctDNA channel and the lettered band positions are on the right. The ctDNA in channels a, b, c, and d were run in a 1.0 percent agarose vertical slab gel 24 cm long for 9 hours at 82 V. The ctDNA in channel c was run similarly in 1.2 percent agarose for 12 hours at 75 V.

The small size of the *Codium* chloroplast genome may represent an advanced condition in which DNA has been lost or transferred to the nuclear genome, or a primitive condition in which additional DNA sequences have not been inserted into the ctDNA. The problematical relation between size and degree of evolutionary advancement is illustrated in the case of mitochondrial DNA (mtDNA): mammalian mtDNA averages around 5 μm in length, whereas yeast mtDNA averages 25 μm in length, and higher plant mtDNA is even larger. In the case of yeast, mtDNA contains split genes and spacer DNA whereas the mammalian mtDNA does not (*17*).

With our findings, the question of the conservative or nonconservative character of chloroplast DNA grows in perplexity. Both the endosymbiont and cluster-clone theories suggest that the chloroplasts of photosynthetic eukaryotes may have originated in Cyanobacteria or oth-

er similar prokaryotes, such as the genus *Prochlorion*, which contains chlorophyll b (*8*). This interpretation is confirmed by studies of nucleotide sequences in 16*S* RNA's (*18*). A large chloroplast genome of the order of 1000×10^6 daltons that approaches the bacterial genome in size is what one might expect to find in the truly primitive chloroplast according to the endosymbiont theory. Indeed, the large molecular size of 1500×10^6 daltons reported for ctDNA in *Acetabularia* has been interpreted as evidence for the primitive nature of this genome (*3*). Our discovery of an exceptionally small chloroplast genome (56×10^6 daltons) in *Codium* raises the alternative possibility that small genome size may be primitive in extant species of green plants, and underscores the need for an extended survey of the properties of ctDNA in algae.

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Viral Epitopes and Monoclonal Antibodies: Isolation of Blocking Antibodies That Inhibit Virus Neutralization

Abstract. *The inability of pathogenic animal viruses to be completely neutralized by antibodies can lead to chronic viral infections in which infectious virus persists even in the presence of excess neutralizing antibody. A mechanism that results in this nonneutralized fraction of virus was defined by the topographical relationships of viral epitopes identified with monoclonal antibodies wherein monoclonal antibodies bind to virus and sterically block the binding of neutralizing antibodies.*

Interactions of a wide variety of viruses with neutralizing antibodies usually result in a small fraction of the viral population escaping neutralization even in the presence of excess antibody (*1*). The biological importance of this phenomenon derives both from observations that infectious viruses can persist in animals in the presence of neutralizing antibody (*2*) and from its implications for persistent viral infections in a wide array of systems including those of significance to man (*3*). Although the mechanism for the generation of the nonneutralized fraction is not completely understood, one of several mechanisms suggested by Dulbecco *et al.* (*1*) was that antiserum contained uncharacterized molecules, presumably antibodies, which combined with viruses so as to inhibit the attachment of neutralizing antibody without inhibiting virus infectivity. It was subsequently shown (*4*) that the nonneutralized fraction of virus could be neutralized by the addition of a secondary antibody specific for the primary antibody. This result implied that the surviving virus had reacted with primary antibody but had remained infectious. To understand how one antibody can bind virus and not neutralize it but can interfere with the binding of a second antibody that does neutralize, requires knowledge both of the topography of the combining sites for each antibody and the mechanism of antibody-mediated virus neutralization.

Because of their homogeneity and spe-

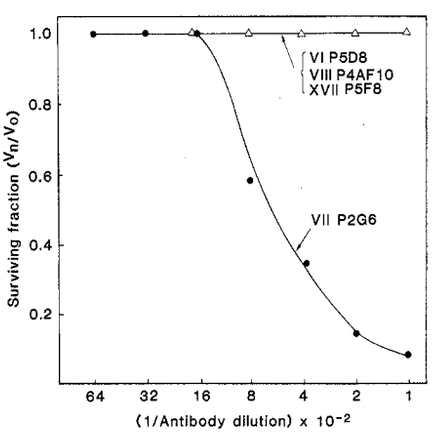

Fig. 1. Neutralization of KiSV (C3H MMTV) pseudotype with monoclonal antibodies. Because there is no direct quantitative infectivity assay for MMTV in vitro, neutralization was measured by the ability of the various monoclones to neutralize the focus-forming capacity of Kirsten sarcoma virus (KiSV) C3H MMTV pseudotype. This pseudotype contained the envelope glycoprotein gp52 of C3H MMTV and it has been previously shown that gp52 is a target for neutralization (*6*). Hybridomas producing monoclonal antibodies to C3H MMTV were isolated as described (*7*) from cell clones derived from a fusion between lymphocytes from an immunized mouse and the NS-1 myeloma cell line (*8*). All the monoclones were judged to be reactive with gp52 on the basis of radioimmunoprecipitation assays (data not shown). The antigenic specificities of these antibodies are: VII P2G6 (IgG_{2a}) type-specific, reactive only with the C3H strain of MMTV; VI P5D8 (IgM) and VIII P4AF10 (IgG_3), class-specific, reactive with C3H and GR strains of MMTV's; XVII P5F8 (IgG_3), group-specific, reactive with C3H, GR, RIII, and C3Hf strains of MMTV's. Ascites fluids containing each of the monoclones at a 1:100 dilution were heat-inactivated at 56°C for 30 minutes and filtered. Serial twofold dilutions were mixed with 200 focus-forming units of virus and incubated at 37°C for 30 minutes. Virus-antiserum mixtures were added to duplicate 60-mm dishes containing Fisher rat embryo cells as described (*5*). Foci appeared between 10 and 14 days. The number of foci induced in the presence of antibodies (V_n) and the number of foci induced in the absence of antibodies (V_o) were used to calculate the surviving fraction.

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