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29. As detected by SDS–polyacrylamide gel electrophoresis of freshly prepared COMP and of dissolved crystals, the concentration of reduced disulfides varied from 10 to 20%, depending on the preparation, and increased with time in the crystal. As a result, fragments of the polypeptide chain adjacent to disulfides were initially poorly defined, especially in the structure of the M66A mutant. In the wild-type structure, disulfide bridges were better defined because of the higher degree of oxidation and the stabilizing effect of Met^{66} side chains, which decreased the local protein mobility through higher packing density in the crystal. The initial electron density was significantly improved by a simple skeletonization-like procedure. Residues Glu^{67} to Gly^{72} were excluded from the phase calculations, and the density calculated with coefficients $(2F_{obs} - F_{calc})$, α_{calc} for this part of the structure was filled with dummy atoms. After three iterative cycles, the quality of the map was sufficient to model all of the remaining residues, except Gly^{72}.
30. Supported in part by Swiss National Science Foundation grant 31-32251.91 to J.E. We thank J. N. Jansonius for support and for providing access to x-ray equipment, and also for valuable comments on the manuscript. Coordinates have been deposited in the Brookhaven Protein Bank (entry code, 1VDF).

20 June 1996; accepted 19 September 1996

Nested Retrotransposons in the Intergenic Regions of the Maize Genome

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The relative organization of genes and repetitive DNAs in complex eukaryotic genomes is not well understood. Diagnostic sequencing indicated that a 280-kilobase region containing the maize *Adh1*-F and u22 genes is composed primarily of retrotransposons inserted within each other. Ten retroelement families were discovered, with reiteration frequencies ranging from 10 to 30,000 copies per haploid genome. These retrotransposons accounted for more than 60 percent of the *Adh1*-F region and at least 50 percent of the nuclear DNA of maize. These elements were largely intact and are dispersed throughout the gene-containing regions of the maize genome.

Plant genomes, like those of other higher eukaryotes, consist of repetitive DNA sequences intermixed with genes (*1–4*), but neither the nature of these repetitive sequences nor the basis of their interspersion has been well defined. In general, larger eukaryotic genomes have higher percentages of repetitive DNAs. The maize nuclear genome, for instance, contains about 60 to 80% repetitive DNA (*1*).

In a contiguous 280-kb region flanking the maize *Adh1*-F gene, isolated on a yeast artificial chromosome (YAC) (*4*), 37 classes of repeats accounted for >60% of the DNA. Blocks containing different mixtures of these repeats make up most of the maize genome, range in size from 20 to 200 kb, and are hypermethylated in mature plant tissues (*3*). Fragment order, size, and composition in this region was further resolved (*4, 5*) (Fig. 1). Groups of repeats were observed more than once within the region. For instance, the L repeat was often found on nearby fragments (12 and 15, 41 and 45, 57 and 61, and 113 and 119; Fig. 1). Repeats M, N, O, and P were only found between two L repeats. Similarly, the Q, R, and S repeats were between pairs of I/J repeats, whereas repeat C was found between H repeats (Fig. 1). These patterns suggested that repeat pairs may be long terminal repeats (LTRs) and that the sequences between them may encode retroelement products (*6*).

We undertook diagnostic DNA sequencing of the presumptive LTRs of each of these proposed retrotransposons, and completely sequenced one element. In every case, the model was supported by the data. The complete element sequenced, which we named *Opie*-2, is 8987 base pairs (bp) with a 1271-bp 5′ LTR and a 1292-bp 3′ LTR. The LTRs correspond to the L repeat (Fig. 2).

Further sequencing uncovered three other families of LTR-retrotransposons: *Huck* (containing LTRs with the H repeat), *Kake* (containing the internal KK repeat), and *Fourf* (containing LTRs with the FF repeat) (Fig. 3). Three other novel retrotransposons were found by chance: *Milt*, *Reina*, and *Victim* (Fig. 3). We also identified three highly repetitive retroelements similar to those found in previous studies. *Ji* (with J/I LTRs) is related to retrotransposon PREM-2 (*7*); *Grande*-zm1 is homologous to *Grande* from *Zea diploperennis* (GenBank accession number X82087); and *Cinful* is homologous both to the defective retrotransposon *Zeon*-1 (*8*) and the solo

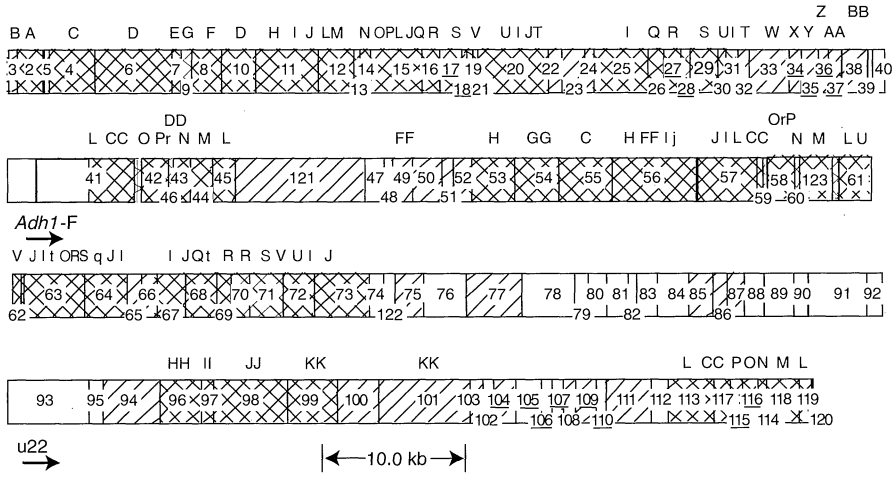

Fig. 1. Composition and arrangement of repetitive DNA flanking the maize *Adh1*-F locus. Bar patterns correspond to approximate copy numbers: cross-hatched bars, highly repetitive (thousands of copies); hatched bars, repetitive (hundreds of copies); and open bars, low copy number (<100 copies) (*4*). Letters above bars indicate the class of repetitive DNA, as defined by hybridization of each highly repetitive fragment to all fragments in the region (*4*). The arrows indicate two genes in this region, *Adh1*-F and u22 (*12*), and their direction of transcription. The order of adjacent underlined fragments is not known.

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LTR called Cin1 (9).

Twenty retrotransposons account for ~150 kb (62%) of the contiguous 240 kb analyzed, including almost all of the highly repetitive DNA. Solo LTRs *Ji*-2 and *Ji*-5 could have been generated by unequal recombination between LTR pairs during the cloning process. However, using a low–copy-number restriction fragment near *Ji*-2 as a probe in gel blot hybridization analysis, we observed that this solo LTR is present in two independent YAC clones carrying maize *Adh1*-F (*4*). A band of this size is also seen in maize genomic DNA (*10*). In 11 of the 12 cases investigated, retrotransposon insertions were flanked by identical 5-bp duplications of target DNA (Table 1). The one exception, *Victim*, is flanked by CTCAC and TTCAC. The presence of direct target repeats flanking an element marks pairs of LTRs as components of the same element.

Sequences internal to the LTRs identified two features necessary for retroelement replication: the primer binding site (PBS) downstream from the 5′ LTR and the polypurine tract (PPT) upstream of the 3′ LTR. In all 13 cases investigated, a PPT was present in the appropriate position (Table 1). In 10 of the 12 cases examined, a PBS with homology to the 3′ end of a tRNA was intact and appropriately positioned (Table 1). Of the two exceptions, *Kake*-1 appears to be a defective element. In *Fourf*, the presumptive PBS exhibits a 13/13-bp match to a sequence in its LTR, which suggests that *Fourf* self-primes its reverse transcription (*11*). Ten of the 20 elements were inserted within another element (Fig. 3), and five of these were inserted within LTRs. Fifteen of the 20 elements were in the same orientation, such that their transcription would be in the opposite direction from *Adh1*-F and u22 (*12*) (Fig. 3).

Previous studies of repetitive DNAs, cloned because of their location near genes (*3*) or at the ends of maize YACs (*13*), indicated that >85% of these random repeats were homologous to repeats near *Adh1*-F (*4*). These results suggest that the *Adh1*-F region is representative of the full maize genome. Further analysis of these data indicated that an *Opie* element is 5′ to the *Adh2*-N locus and that *Ji* elements are 3′ to the *Sh1* gene cloned in pZmSh1 and 5′ to the 19-kD zein gene cluster on lambda clone ZG19.31 (*3*, *10*).

All four of the highly repetitive DNAs previously used to fingerprint maize YACs (*13*) were found to be parts of retrotransposons by sequence comparisons (*10*). Repeat 1, present at ~50,000 copies per genome and on 45% of all maize YACs, is a segment of an *Opie* LTR. Repeat 2, present on 50% of YACs, is a fragment containing the junction of a *Huck* LTR and *Ji* internal sequences (indicating a *Huck* element inserted into a *Ji*). Repeat 3, present at ~50,000 copies and on 62% of YACs, is part of a *Ji* LTR and its PPT. Repeat 4, at ~30,000 copies and on 63% of all YACs, is an internal portion of *Opie* (*13*). Because of their interspersed nature, high copy number, and presence flanking most maize genes (*3*, *13*), this nested retroelement structure apparently represents the standard organization of intergenic regions in maize. Regions around telomeres, cen-

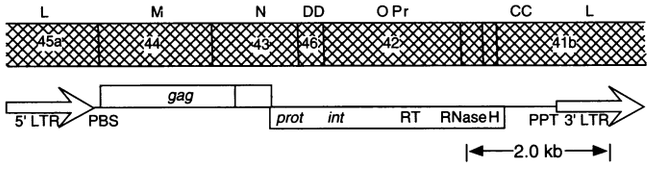

Fig. 2. Structure of the *Opie*-2 retrotransposon (GenBank accession number U68408). *Opie*-2 was subcloned, sequenced, and analyzed as described (*5*, *12*). Letters above the bar indicate repeat types, as in Fig. 1. Below the bar, arrows indicate the position, size, and orientation of the LTRs, and open bars below indicate two long ORFs with homology to key retrotransposon genes. Fragments are numbered within the bar as in Fig. 1; fragments 45a and 41b represent parts of fragments 45 and 41, respectively. RT, reverse transcriptase; RNase H, ribonuclease H.

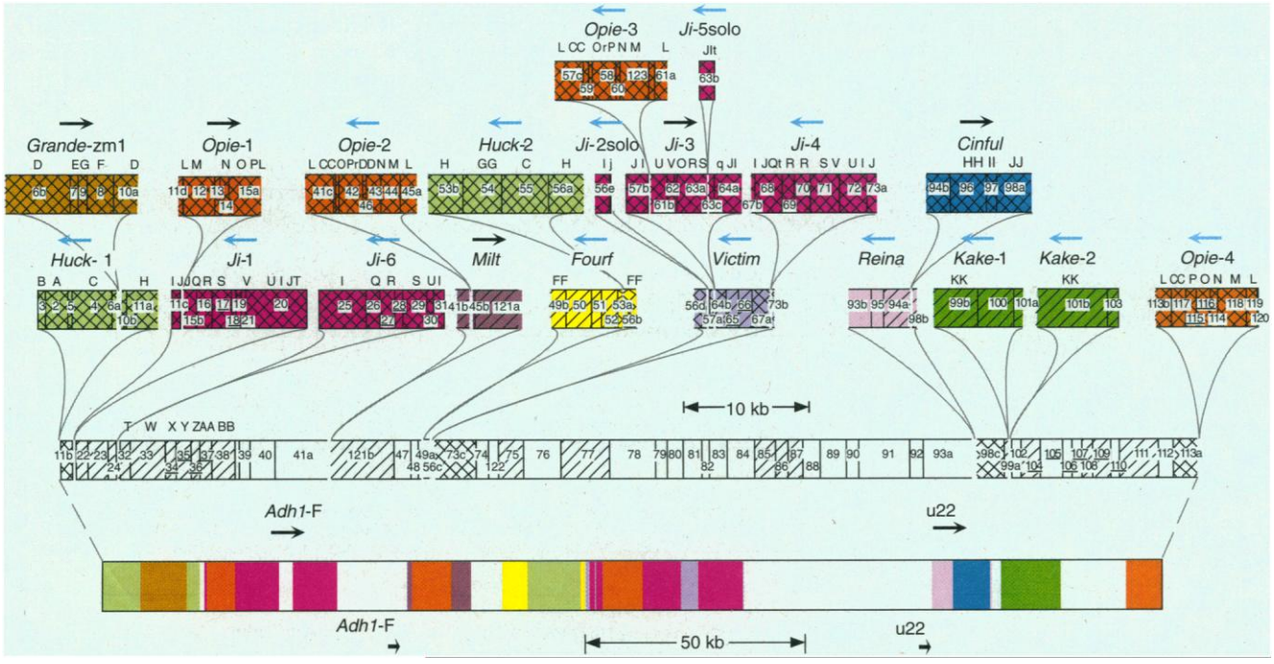

Fig. 3. Structure of the *Adh1*-F region of maize, showing identified retrotransposons. Labeling, bar patterns, and underlines are as in Fig. 1, but confirmed elements have been positioned above the DNA into which they have inserted. Curved lines below each element converge at the insertion site. The arrow above each element indicates its orientation. Fragments with lowercase a, b, or c designations are components of the fragment with the same number. GenBank accession numbers: *Fourf*, U68401; *Cinful*, U68402; *Grande*-Zm, U68403; *Huck*-2; U68404; *Ji*-3, U68405; *Kake*-1, U68406; *Milt*, U68407; *Reina*, U68409; and *Victim*, U68410.

tromeres, knobs, ribosomal DNA repeats, or other unusual structures would be likely exceptions.

Each highly repetitive retrotransposon family constitutes a substantial portion of the maize genome. The 30,000-copy *Opie* element, for instance, should constitute about 10 to 15% of the 2400-megabase maize genome (*14*). In total, *Opie*, *Cinful*, *Grande*, *Huck*, and *Ji* account for >25% of the maize genome. In addition, lower–copy-number retrotransposons *Fourf*, *Kake*, *Milt*, *Reina*, and *Victim* were discovered in this region largely as an accidental outcome of sequence analysis of the highly repetitive retrotransposons. Reverse Southern (DNA) blot analyses with *Fourf*, *Kake*, and *Victim* indicate copy numbers in the hundreds (*4*), whereas our Southern blot analysis with a *Reina* element probe indicated ~10 copies in maize (*10*). Because the five middle repetitive DNAs that we sequenced in this region all were found to be LTR-retrotransposons (*Fourf*, *Kake*-1, *Kake*-2, *Milt*, and *Victim*), we expect that the >50 kb of unexamined middle repetitive DNA surrounding *Adh1*-F contains additional LTR-retrotransposons, and that there are thousands of other families of these low–copy-number elements in maize. These observations indicate that the highly repetitive, middle-repetitive, and low–copy-number retroelements between genes combine to make up at least 50% of the maize genome.

Although retrotransposons are present in plants with smaller genomes, such as *Arabidopsis*, their variety and genomic copy number are usually very low (*6*). Larger genomes, like those of maize, barley, and lily, have both highly repetitive retrotransposons and many different families of retrotransposons in a full range of copy numbers (*6*). Hence, species-specific variation in LTR-retrotransposon amplification or maintenance might account for most genome size variation in plants (*14*).

Simple eukaryotic genomes, like those of *Arabidopsis*, nematodes, and yeast, primarily consist of tightly juxtaposed genes intermixed with rare mobile DNAs. The euchromatic regions of the *Drosophila* genome are similarly repeat-poor and gene-rich, but its β-heterochromatin consists of tandemly repeated sequences and variably fragmented mobile DNAs, including retrotransposons, interspersed with isolated genes (*15*). In contrast, interspersed repetitive DNAs in humans are usually non-LTR retroelements (for example, Alu and L1 elements) (*16*) that can be found in the large introns typical of many chordate genes. Plant genes usually have small introns that may only tolerate insertion by small elements (*17*). Mammalian genomes do contain ancient fragments of LTR-retrotransposons, often consisting of solo LTRs (*18*). Hence, complex animal and plant genomes (such as humans and maize) share retroelements as their major class of interspersed repetitive DNA. They differ, however, in the type of abundant retroelements and in their arrangement relative to genes.

The highly repetitive *Cinful*, *Grande*, *Huck*, *Ji*, and *Opie* elements have very few homologies with sequences in the maize sequence databases. In the cases of *Cinful*, *Grande*, and *Ji*, some of these homologies were to DNAs that were studied because of their repetitive nature, their expected mobility, or both (*7*–*9*). Of more than 200 genomic sequences of maize genes, database searches using LTR sequences yielded only three hits with *Cinful* (accession number U45859) (*19*), zero hits with *Grande*, two hits with *Huck* (GenBank accession number M23537) (*20*), four hits with *Ji* (GenBank accession numbers X91883 and L21007) (*21*), and two hits with *Opie* (*22*). Homologies were observed where sequencing continued for several kilobases either 5′ or 3′ to the analyzed locus (*19*–*22*).

The minor representation in the maize databases of sequences that make up >25% of the genome suggests that these elements specifically avoid genes. In contrast, some low–copy-number mobile DNAs like *Mu1* of maize preferentially insert into genes (*23*). Perhaps a highly repetitive retrotransposon can only exist if it targets regions between genes. Otherwise, the host cell would incur a lethal level of mutation.

Contrary to our expectations of a relatively unorganized mass of intermixed repetitive DNA between genes (*3*, *4*), we found that the retrotransposons that make up most of the maize genome are largely intact and simply organized. Future analyses will focus on understanding the significance, rate, and nature of changes in intergene structure and composition (*12*).

Table 1. Retrotransposons in the region flanking *Adh1*-F. The element family, either *copia* or *gypsy*, was determined by the order of internal sequence homologies to *pol* gene products (*6*, *10*). Element and LTR sizes were determined by sequence or by restriction mapping and are estimated to the nearest one-half or one-tenth kilobase, respectively. 5′DR, 5′ direct repeat (duplication of flanking target DNA); 3′DR, 3′ direct repeat; +, same potential direction of transcription as *Adh1*-F; –, the opposite direction; ND, not determined; NA, not applicable.

Name	Size	LTR size	5′DR	PBS	PPT	3′DR	Orientation
				Ty1/copia-*like elements*			
Opie-1	7.0	1.3	ND	ND	ND	ACAGG	+
Opie-2	9.0	1.3	GGACC	TGGTATCGGAGCCGT	AGGGGGAG	GGACC	–
Opie-3	9.0	1.3	ND	ND	AGGGGGAG	GGAAG	–
Opie-4	9.0	1.3	ND	ND	ND	ND	–
Ji-1	9.5	1.3	ND	ND	ND	ATAAT	–
Ji-2solo	1.3	1.3	GCAAG	NA	NA	GCAAG	–
Ji-3	8.5	1.3	CGAAG	TGGTATCAGAGCCCG	AGGGGGAG	CGAAG	+
Ji-4	10.0	1.3	GATTC	TGGTATCTGAGCCCG	AGGGGGAG	GATTC	–
Ji-5solo	1.3	1.3	GAAGC	NA	NA	GAAGC	–
Ji-6	9.0	ND	ND	ND	ND	ND	–
Fourf	7.0	1.1	TAATC	CCAAAAACCTAAT	TGGTGGGG	TAATC	–
Victim	5.5	0.1	CTCAC	TGGTACCAGAGCC	GCGGGGGG	TTCAC	–
				Ty3/gypsy-*like elements*			
Huck-1	11.5	1.5	GGGTG	TGGCGCGCCAGGTAGG	GGGGGCTA	ND	–
Huck-2	12.0	1.5	ATGTT	TGGCGCGCCAGGTAGG	GGGGGCTA	ATGTT	–
Grande-zm1	10.5	0.6	GCATC	TGGCGCGCCAGGTAGG	ATCGGGGG	GCATC	+
Cinful	8.5	0.6	GTGGC	TGGCGCCCACCCTCCG	AAGGGGCTA	GTGGC	+
Reina	5.5	0.3	ND	TGGTAATCGGAGCTGG	GGGAAGGGG	GGTTG	–
				Unknown			
Milt	4.5	0.7	ACAAG	TGGCGACTCCGCTGGG	AGGGGGGCA	ACAAG	+
Kake-1	7.0	0.2	TAGTT	CGTTATCAGCACTTT	AGGAGGAG	TAGTT	–
Kake-2	6.0	ND	ND	ND	ND	ND	–

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24. We thank S. Henikoff for helpful discussions and S. Frank for technical assistance. Supported by USDA grants to Z.A. (93-37300-8769) and to J.L.B. (94-37300-0299).

31 May 1996; accepted 23 August 1996

Requirement of Rigid-Body Motion of Transmembrane Helices for Light Activation of Rhodopsin

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Conformational changes are thought to underlie the activation of heterotrimeric GTP-binding protein (G protein)–coupled receptors. Such changes in rhodopsin were explored by construction of double cysteine mutants, each containing one cysteine at the cytoplasmic end of helix C and one cysteine at various positions in the cytoplasmic end of helix F. Magnetic dipolar interactions between spin labels attached to these residues revealed their proximity, and changes in their interaction upon rhodopsin light activation suggested a rigid body movement of helices relative to one another. Disulfide cross-linking of the helices prevented activation of transducin, which suggests the importance of this movement for activation of rhodopsin.

G protein–coupled receptors (GPCRs) form a superfamily that mediates the actions of extracellular signals as diverse as light, odorants, peptide hormones, and neurotransmitters (*1*). Activation of these receptors is assumed to require protein conformational changes. Understanding the nature of these structural changes is central to understanding the molecular mechanism of GPCR activation.

Rhodopsin is one of the best characterized GPCR systems. Secondary structure models have been proposed on the basis of biochemical, biophysical, and mutagenic data (Fig. 1) (*2*), and the location of the membrane-aqueous interfaces have been determined for some of the helices (*3*). A model for the packing of the transmembrane helices has been derived from cryoelectron microscopy (*4*), and schemes for mapping the rhodopsin sequence onto the resolved helices have been proposed (*3, 5*). Site-directed spin labeling (SDSL) studies reveal that isomerization of the 11-*cis*-retinal chromophore by light leads to reorganization of the tertiary contact surfaces of helices C and F, as well as to changes in the structure of cytoplasmic interhelical loops that are known to interact with transducin (*3*). These results were interpreted in terms of a possible rigid body motion of helices C and F upon light activation.

Rhodopsin mutants containing two reactive cysteines have been used together with disulfide–cross-linking and SDSL studies to probe the spatial proximity of Cys^{65}, which replaced His at that position, (helix A) and Cys^{316} (helix G) and their relative movement after photoactivation in rhodopsin (*6*). We used this approach to explore the proximity of the cytoplasmic ends of helices C and F in rhodopsin and light-induced alteration in their conformation. Six double cysteine mutants were constructed in a mutant of rhodopsin in which the native cysteines at positions 140, 316, 322, and 323 were replaced by serine (*7*). In each mutant, the position of one cysteine (Cys^{139}) was kept constant, whereas the location of the second cysteine was varied among amino acid positions 247 through 252 in helix F (Fig. 1). The mutant opsin proteins were expressed in COS-1 cells, regenerated with 11-*cis*-retinal, and purified in dodecyl maltoside (DM) detergent with an immunoaffinity procedure (*8, 9*). All mutants bound 11-*cis*-retinal to form pigments with properties similar to those of native rhodopsin [$\lambda_{max} \approx$ 498 nm and ratio of absorbances at 280 and 500 nm (A_{280}/A_{500}) between 1.6 and 1.8]. Upon illumination with light of wavelength (λ) greater than 496 nm, all showed an absorbance shift to 380 nm and subsequent retinal release characteristic of the native protein (*10*).

The sulfhydryl groups in the double cysteine mutants were derivatized with a methanethiosulfonate spin label (Fig. 2) (*11*). The double cysteine mutants so derivatized were designated as 139R1-248R1 through 139R1-252R1.

Static magnetic dipolar interactions between two nitroxide labels in an unoriented sample lead to spectral line broadening, and thus a decrease in signal intensity, for separations less than about 25 Å. Electron para-

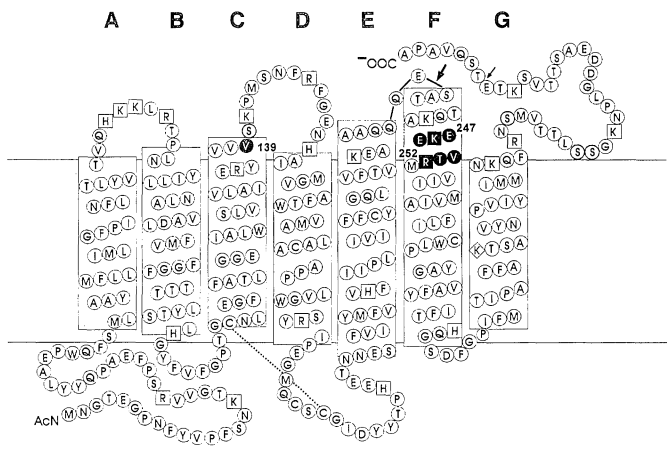

Fig. 1. A secondary structure model of rhodopsin showing the positions of cysteine substitutions (139 and 247 through 252; black residues). In all mutants, the native cysteines Cys^{140}, Cys^{316}, Cys^{322}, and Cys^{323} were substituted with serines (shaded residues). Single-letter amino acid abbreviations are used (*20*). The sites of V-8 proteolysis are indicated by arrows. Note that after V-8 digestion, rhodopsin was cleaved primarily into two large fragments, F1 (~27 kD) and F2 (~13 kD) (*21*). Ac indicates acetyl.

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