cell–specific regulatory factors. Indeed, a highly conserved octanucleotide sequence has been identified approximately 70 bp upstream from the transcriptional initiation start site of all sequenced Ig κ genes (*9, 11*). The region containing the octanucleotide sequence is essential for correct initiation of transcription from the Ig κ promoter function in lymphoid cells (*10*). Results analogous to our data have been reported, and RNA mapping studies verified that correct initiation from the Ig κ promoter occurs only in Ig-producing cells (*14*).

In L cells, the Ig κ gene promoter containing 225 bp of 5′ flanking sequence was activated by the HaMuSV enhancer in tandem, whereas the promoter with 625 bp of flanking sequence was not activated. Initially these results seem to suggest the presence of an inhibitory sequence in the upstream region, but the short promoter was found to be inactive with the enhancer in the other orientation or at another position in the vector. Thus we believe that the different results obtained with the short and long promoter fragment relate to the distance between the enhancer and promoter elements. The requirement for a tissue-specific environment for function of the Ig κ gene promoter can apparently be overcome by placing it close to a strong enhancer in L cells. These cells may contain permissive nonspecific transcriptional factors, since a number of cloned, developmentally regulated genes function when transferred into these cells.

Tissue-specific expression of genes under the control of *cis*-acting transcriptional elements is thought to reflect the interaction of such elements with regulatory factors present in certain cells and not in others. Cells of lymphoid origin would seem therefore to contain at least two specific transcriptional factors, one that interacts with the enhancer element (*15*), and a second that interacts with sequences within the promoter. Both the Ig enhancer and promoter can be shown to function independently in lymphoid cells with a nontissue-specific promoter or enhancer, respectively, but neither the Ig promoter or enhancer will function consistently in nonlymphoid cells.

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Mutation to Herbicide Resistance Maps Within the *psbA* Gene of *Anacystis nidulans* R2

Abstract. *A* psbA *gene encoding the target of photosystem II herbicide inhibition, the 32,000-dalton thylakoid membrane protein, has been cloned from a mutant of* Anacystis nidulans *R2, which is resistant to 3-(3,4-dichlorophenyl)-1,1-dimethylurea-(diuron). A cloned DNA fragment from within the coding region of this gene transforms wild-type cells to herbicide resistance, proving that mutation within* psbA *is responsible for that phenotype. The mutation consists of a single nucleotide change that replaces serine at position 264 of the wild-type protein with alanine in that of the diuron-resistant mutant.*

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Mutation of a chloroplast gene, *psbA*, has been correlated with resistance to urea- and triazine-class herbicides in *Amaranthus hybridus* and *Solanum nigrum* (*1, 2*) and the green alga *Chlamydomonas reinhardii* (*3*). The product of this gene is an integral protein of the photosynthetic apparatus which, with a bound quinone, serves as the second stable electron acceptor of photosystem II (PS II) (*4*), termed Q_B (*2*). The Q_B protein is the site of herbicide binding (*5*). We have isolated a *psbA* gene from a diuron-resistant mutant of the cyanobacterium *Anacystis nidulans* R2 (*6*) and report that a cloned internal fragment of the mutant gene can transform wild-type *A. nidulans* cells to diuron resistance. Like the mutant from which the diuron-resistance gene was obtained (*6*), cells transformed with the internal fragment are also resistant to the herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamine-*s*-triazine) and the quinone analog 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO), indicating that the same mutation in the *psbA* gene affects interaction with each of these inhibitors of photosynthetic electron transport.

When chromosomal DNA from *A. nidulans* is digested with the restriction enzyme Eco RI and probed with cloned *psbA* genes from spinach chloroplast DNA (*7*) or from *Anabeana* 7120 (*8*), three bands of approximately 11, 9, and 7 kilobase pairs (kb) are detected with equal intensity of hybridization (data not shown). At least two of these bands (11 and 9 kb) carry complete structural genes; the extent of *psbA* coding sequence on the 7-kb fragment has not yet been determined. The 11-kb Eco RI fragment was isolated from a bacteriophage λ gt7-ara6 (*9*) library of DNA fragments (*10*) from the *A. nidulans* mutant R2D2-X1 (*6*). This cloned DNA was able to transform wild-type *A. nidulans* cells to diuron resistance, presumably by homologous recombination (*11*). No diuron-resistant transformants were obtained when recombinant λ DNA carrying the 9-kb fragment from resistant-cell DNA was incubated with wild-type cells. Analysis of fragments of the diuron resistance-conferring λ clone by hybridization with the spinach *psbA* probe, and assays of the ability to transform cells to diuron resistance indicated tight linkage of the herbicide-resistance locus and *psbA* gene homology.

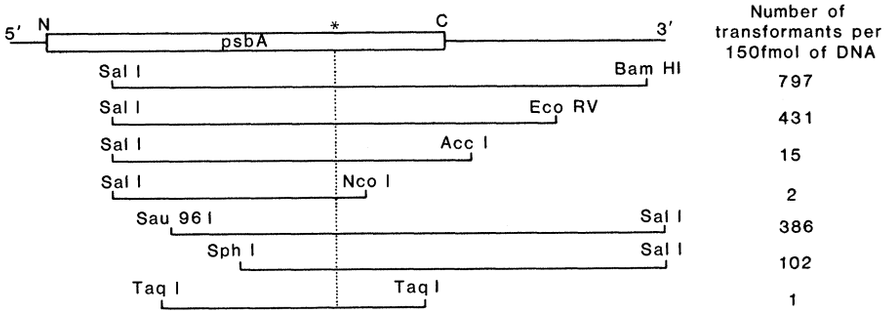

Fig. 1 (right). Transformation to diuron resistance through the use of restriction fragments of R2D2-X1 DNA containing portions of *psbA*. A 1.5-kb fragment generated by the restriction enzyme Sal I and extending from amino acid 59 of the coding region of *psbA* to 600 base pairs beyond the 3′ end of the gene was purified from the cloned 11-kb Eco RI fragment from diuron-resistant R2D2-X1. This fragment was further cleaved with other enzymes, and the products of these digests were used in transformation assays. A portion of each digest was subjected to electrophoresis on a 1 percent agarose gel to determine that the restriction digests were complete. Wild-type *A. nidulans* cells were grown (*17*), washed with 10 m*M* NaCl, and concentrated to 5×10^8 cells per milliliter in BG-11 growth medium. A portion of each restriction digest was incubated with 300-μl portions of cells overnight in foil-wrapped tubes to exclude light (*11*). The transformation mixtures were plated on BG-11 agar plates in duplicate at 150 μl per plate; after plates were incubated for 4 hours under normal growth conditions, diuron was added under the agar at a final concentration of 10^{-6}*M*. Plates were incubated an additional 6 days and colonies were counted. Numbers in the diagram next to the fragments indicate the total number of diuron-resistant transformants obtained for that digest. Each assay contained approximately 150 fmol of each fragment; this DNA concentration is approximately saturating for transformation of *A. nidulans* R2 (*11*). The *psbA* gene is shown at the top of the figure with the amino (N) and carboxyl (C) terminals of the coding region marked for orientation. The position of the single nucleotide mutated in *psbA* from R2D2-X1 is indicated by an asterisk. Fig. 2 (below). Nucleotide sequence and derived amino acid sequence of an *A. nidulans psbA* gene. The sequence of the *psbA* gene from the 11-kb Eco RI fragment obtained from wild-type *A. nidulans* and the mutant R2D2-X1 was analyzed by the method of Maxam and Gilbert (*18*), except for the piperidine cleavage step, which followed Smith and Calvo (*19*). The sequence of the R2D2-X1 gene was determined on both DNA strands at all nucleotide positions and compared with one strand of the wild-type sequence. Approximately 65 percent of the wild-type sequence was also determined for both strands, including the region containing the mutation. *Anacystis nidulans* amino acid positions that are the same in the *psbA* sequences of both *Anabaena* and spinach are shown in bold type. Plain type represents amino acid positions encoded uniquely in the *A. nidulans* gene. Residues identical to spinach alone are underlined, and those identical to *Anabaena* alone are in bold type and underlined. Amino acids 352 to 356 are not present in the plant sequence. The nucleotide mutated in the gene from R2D2-X1 is the first position of codon 264. The Taq I sites bordering the insert of pTaq700 are underlined in the nucleotide sequence.

ATG	ACC	AGC	ATT	CTT	CGC	GAG	CAA	CGC	CGC	GAT	AAC	GTT	TGG	GAT	CGG	TTT	TGT	GAG	TGG
met	**thr**	ser	ile	**leu**	arg	glu	gln	arg	arg	asp	asn	val	trp	asp	**arg**	**phe**	**cys**	glu	**trp** 20
GTA	ACC	AGC	ACC	GAC	AAC	CGC	ATC	TAC	GTG	GGT	TGG	TTC	GGC	GTG	CTG	ATG	ATC	CCC	ACT
val	thr	**ser**	thr	asp	**asn**	arg	ile	tyr	val	**gly**	**trp**	**phe**	**gly**	val	leu	**met**	**ile**	**pro**	**thr** 40
CTG	CTG	ACC	GCC	ACC	ATC	TGC	TTC	ATC	GTT	GCG	TTC	ATT	GCA	GCC	CCT	CCC	GTC	GAC	ATC
leu	**leu**	thr	**ala**	**thr**	ile	cys	**phe**	**ile**	val	**ala**	**phe**	ile	**ala**	**ala**	**pro**	**pro**	**val**	**asp**	**ile** 60
GAC	GGC	ATC	CGT	GAG	CCC	GTT	GCC	GGC	TCT	CTC	ATG	TAT	GGC	AAC	AAC	ATC	ATT	TCC	GGC
asp	**gly**	**ile**	**arg**	**glu**	**pro**	**val**	ala	**gly**	**ser**	**leu**	met	**tyr**	**gly**	**asn**	**asn**	**ile**	**ile**	**ser**	**gly** 80
GCT	GTT	GTT	CCT	TCC	AGC	AAC	GCC	ATC	GGC	CTG	CAT	TTC	TAT	CCG	ATT	TGG	GAA	GCC	GCT
ala	val	val	**pro**	ser	**ser**	asn	**ala**	**ile**	**gly**	**leu**	**his**	**phe**	**tyr**	**pro**	**ile**	**trp**	**glu**	**ala**	**ala** 100
AGC	CTC	GAC	GAG	TGG	CTG	TAC	AAC	GGT	GGT	CCT	TAC	CAA	TTA	GTG	GTC	TTC	CAC	TTC	TTG
ser	leu	asp	**glu**	**trp**	**leu**	**tyr**	**asn**	**gly**	**gly**	**pro**	**tyr**	gln	**leu**	val	val	phe	**his**	**phe**	**leu** 120
CTG	GGT	ATC	AGC	TGC	TAC	ATG	GGT	CGT	CAA	TGG	GAG	CTG	TCG	TAC	CGC	CTC	GGT	ATG	CGC
leu	**gly**	ile	ser	**cys**	**tyr**	met	**gly**	**arg**	gln	**trp**	**glu**	**leu**	**ser**	tyr	**arg**	**leu**	**gly**	**met**	**arg** 140
CCT	TGG	ATC	TGT	GTT	GCA	TAC	AGT	GCT	CCA	CTC	TCG	GCT	GCT	TTT	GCA	GTG	TTT	CTG	ATC
pro	**trp**	**ile**	cys	**val**	**ala**	**tyr**	**ser**	**ala**	**pro**	leu	**ser**	ala	**ala**	**phe**	**ala**	**val**	**phe**	**leu**	**ile** 160
TAC	CCG	ATC	GGC	CAA	GGT	TCG	TTC	TCG	GAC	GGC	ATG	CCC	CTG	GGT	ATC	AGC	GGC	ACC	TTC
tyr	**pro**	**ile**	**gly**	**gln**	**gly**	**ser**	**phe**	**ser**	**asp**	**gly**	**met**	**pro**	**leu**	**gly**	**ile**	**ser**	**gly**	**thr**	**phe** 180
AAC	TTC	ATG	TTC	GTG	TTC	CAA	GCA	GAG	CAC	AAC	ATT	TTG	ATG	CAC	CCC	TTC	CAC	ATG	CTG
asn	**phe**	**met**	phe	**val**	**phe**	**gln**	**ala**	**glu**	**his**	**asn**	**ile**	**leu**	**met**	**his**	**pro**	**phe**	**his**	**met**	**leu** 200
GGT	GTG	GCT	GGT	GTG	TTC	GGT	GGT	TCG	CTG	TTC	TCG	GCA	ATG	CAC	GGT	TCG	TTG	GTG	ACC
gly	**val**	**ala**	**gly**	**val**	**phe**	**gly**	**gly**	**ser**	**leu**	**phe**	**ser**	**ala**	**met**	**his**	**gly**	**ser**	**leu**	**val**	**thr** 220
AGC	TCG	CTG	GTG	CGT	GAG	ACG	ACC	GAG	ACC	GAG	AGC	CAA	AAC	TAC	GGC	TAC	AAA	TTT	GGT
ser	**ser**	**leu**	val	**arg**	**glu**	**thr**	**thr**	**glu**	thr	**glu**	**ser**	gln	**asn**	tyr	**gly**	**tyr**	lys	**phe**	**gly** 240
CAA	GAG	GAA	GAG	ACC	TAC	AAC	ATC	GTG	GCA	GCC	CAC	GGT	TAC	TTC	GGT	CGC	TTG	ATC	TTC
gln	**glu**	**glu**	**glu**	**thr**	**tyr**	**asn**	**ile**	**val**	**ala**	**ala**	**his**	**gly**	**tyr**	**phe**	**gly**	**arg**	**leu**	**ile**	**phe** 260
			G																
CAA	TAC	GCA	TCG	TTC	AAC	AAC	AGC	CGT	TCG	CTG	CAC	TTC	TTC	CTG	GGT	GCA	TGG	CCG	GTC
gln	**tyr**	**ala**	ser (ala)	**phe**	**asn**	**asn**	**ser**	**arg**	ser	**leu**	**his**	**phe**	**phe**	**leu**	**gly**	**ala**	**trp**	**pro**	**val** 280
GTG	GGC	ATC	TGG	TTT	ACC	TCC	ATG	GGC	ATC	AGC	ACC	ATG	GCG	TTC	AAC	CTG	AAT	GGT	TTC
val	**gly**	**ile**	**trp**	**phe**	**thr**	**ser**	**met**	**gly**	ile	**ser**	**thr**	**met**	**ala**	**phe**	**asn**	**leu**	**asn**	**gly**	**phe** 300
AAC	TTC	AAC	CAG	TCG	GTT	TTG	GAT	AGC	CAA	GGC	AAA	GTG	ATC	AAC	ACT	TGG	GCA	GAT	GTG
asn	**phe**	**asn**	**gln**	**ser**	val	**leu**	**asp**	**ser**	**gln**	**gly**	lys	**val**	**ile**	**asn**	**thr**	**trp**	**ala**	**asp**	**val** 320
TTG	AAC	CGT	GCC	AAC	TTG	GGC	ATG	GAA	GTG	ATG	CAC	GAG	CGT	AAT	GCT	CAC	AAC	TTC	CCG
leu	**asn**	**arg**	**ala**	**asn**	**leu**	**gly**	**met**	**glu**	**val**	**met**	**his**	**glu**	**arg**	**asn**	**ala**	**his**	**asn**	**phe**	**pro** 340
CTC	GAC	TTG	GCA	GCA	GGC	GAA	GCG	ACC	CCG	GTC	GCT	TTG	ACT	GCG	CCT	TCA	ATT	CAC	GGT TAA
leu	**asp**	**leu**	**ala**	**ala**	gly	**glu**	**ala**	**thr**	pro	val	ala	**leu**	thr	**ala**	**pro**	ser	ile	**his**	**gly** **end**

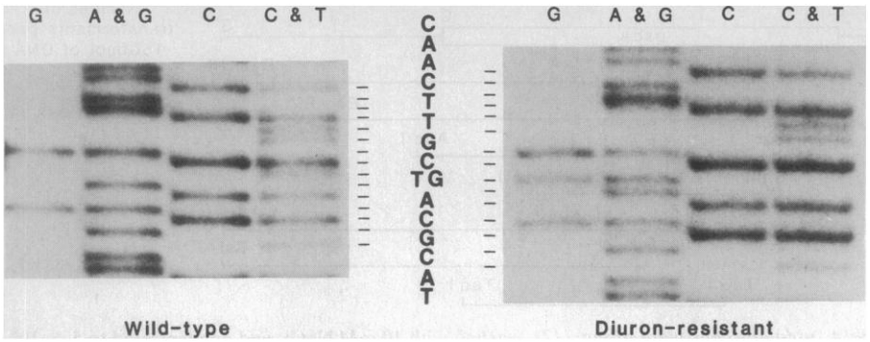

Fig. 3. Sequence comparison of wild-type and diuron-resistance *psbA* genes. The polyacrylamide sequencing gels used to determine the nucleotide sequence encoding amino acids 262 to 266 are shown. The T → G transversion results in substitution of alanine for serine at amino acid 264.

A more detailed analysis of this linkage was made with a 3.5-kb Xho I fragment containing all of *psbA* as starting material for transformation assays. Restriction enzymes were used to generate subfragments, which were separated by electrophoresis on 1 percent agarose gels. Each resulting band was eluted and purified by binding to glass in the presence of sodium iodide (*12*). A portion of each fragment was subjected to electrophoresis to determine DNA concentration and homology with a *psbA* gene probe; another portion was assayed for the ability to transform *A. nidulans* to diuron resistance (*11*). Transformants were obtained with fragments at least 1 kb in size containing the 3′ two-thirds of the *psbA* gene. No diuron-resistant transformants were obtained in the absence of added DNA or when fragments lacking homology to *psbA* were added. Restriction fragments used in a quantitative transformation assay are aligned in Fig. 1 with respect to the coding region of the *psbA* gene and the position, marked by an asterisk, of a single nucleotide difference between the mutant and wild-type sequences. The efficiency of transformation depended on the distance from the site of the mutation to the end of the DNA fragment. However, this dependence was not symmetrical; the Sph I site on the left is closer to the mutation than the Acc I site on the right, yet the fragment containing the former was more efficient in transformation.

Mapping the diuron-resistance locus to an internal portion of the *psbA* gene was limited by a tenfold or greater drop in transformation frequencies when fragments of less than 1 kb were used (Fig. 1). To ensure that rare transformants obtained with internal fragments of the mutant *psbA* gene were not generated by contaminating larger fragments, the internal Taq I fragment from the *psbA* gene of R2D2-X1 was cloned into the Cla I site of the *Escherichia coli* plasmid pBR328 (*13*), which is incapable of replication in *A. nidulans*. Circular DNA of the resulting plasmid, pTaq700, transformed wild-type *A. nidulans* to diuron resistance at a frequency of 5500 transformants per each 150 fmol of plasmid DNA. Genomic DNA was prepared from diuron-resistant clones resulting from transformation by pTaq700, digested with Eco RI, and analyzed by Southern blots (*10*) with *psbA* and pBR328 probes. The *psbA* probe hybridized to the expected bands at 11, 9, and 7 kb, while pBR328 sequences were absent (data not shown). Thus, transformation was due either to reciprocal exchange between pTaq700 and the chromosome or to a gene conversion event.

Having established that the mutation to diuron resistance maps within the *psbA* gene coding region, we determined the complete nucleotide sequence of that region from R2D2-X1 DNA and the corresponding wild-type *A. nidulans* gene (Fig. 2); the single nucleotide difference between mutant and wild-type genes is shown in a portion of a sequencing gel in Fig. 3. This difference is a transversion from T to G at the first codon position of amino acid 264, which results in the replacement of serine with alanine. This is the same replacement noted earlier in herbicide-resistant *C. reinhardii* (*3*). In *A. hybridus*, the serine residue at position 264 in the wild type is coded by AGT, which cannot mutate to an alanine residue by a single base change; atrazine-resistant *A. hybridus* and *S. nigrum* encode glycine at this position (*1, 2*).

The amino acid sequence shown in Fig. 2 is 84 percent homologous with the corresponding sequences of plants (*7*) and 88 percent with that of the corresponding gene of *Anabaena*, including a six-amino-acid insertion near the carboxyl terminus relative to the plant sequences (*8*). Thirty amino acid positions differ from both the plant and *Anabaena* sequences. As in *Anabaena*, the *psbA* gene of *A. nidulans* includes lysine residues not found in the plant sequences.

Mutants of *A. nidulans* selected for resistance to diuron are also resistant to other inhibitors of photosynthetic electron transport (*6*). The availability of diuron-resistant cells resulting from transformation with pTaq700 made it possible to determine whether a single mutation is responsible for all of the resistances observed. The PS II electron transport rates of wild-type *A. nidulans*, the diuron-resistant mutant R2D2-X1, and R2Taql, a diuron-resistant clone obtained by transformation with pTaq700, were assayed in the presence of various concentrations of the inhibitors diuron, atrazine, and HQNO. We used a Clark-type oxygen electrode to assay whole cells at 20°C under saturating white light with reaction mixtures previously described by Guikema and Sherman (*14*). The inhibitor concentration causing 50 percent reduction in oxygen evolution was determined for each strain (*6*). The transformant R2Taql exhibited resistances that were, relative to that of wild-type, 100 times that to diuron, 10 times that to atrazine, and 6 times that to HQNO, as has been reported for the parent strain R2D2-X1 (*6*). These data indicate that the mutation replacing amino acid 264 affects the interaction of Q_B with all three inhibitors. This result is especially interesting for HQNO, which is thought to act at the cytochrome b_6/f complex (*15*).

We have exploited the transformability of the cyanobacterium *A. nidulans* R2 to analyze the phenotypic effects of alteration of a component of the photosynthetic apparatus. The ability to introduce a mutated gene into the genome of wild-type cells provides a means for genetic dissection of the photosynthetic apparatus not yet possible for chloroplast genes in plants. Expansion of this method to include introduction of genes that have been mutagenized in vitro will allow controlled manipulation of photosystem components. This approach can be used to identify portions of the Q_B protein that are important for the binding of different classes of herbicides acting at overlapping but nonidentical sites (*16*).

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Responsiveness and Receptive Field Size of Carp Horizontal Cells Are Reduced by Prolonged Darkness and Dopamine

Abstract. *In the fish retina the interplexiform cells contain dopamine and provide a centrifugal pathway from the inner plexiform layer to horizontal cells of the outer plexiform layer. Dopamine application reduced the responsiveness and receptive field size of cone horizontal cells, as did a prolonged period of complete darkness. Other results suggest that the interplexiform cells may release dopamine after a prolonged period in the dark. The interplexiform-horizontal cell system may modify the strength of the antagonistic surrounds of retinal neurons as a function of time in the dark.*

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In 1957 Barlow *et al.* (*1*) reported that the strength of the antagonistic surround of cat retinal ganglion cell receptive fields is severely reduced or eliminated after a prolonged period in the dark. This change in receptive field organization did not relate to a switch from cone to rod vision (*1*) and the mechanisms that underlie the phenomenon have remained unknown. Evidence presented here suggests that interplexiform cells, neurons whose perikarya lie among amacrine cells and whose processes extend into both plexiform layers of the retina (*2*), may mediate such a phenomenon in fish.

In the fish retina, interplexiform cells contain dopamine and their processes make numerous synaptic contacts onto horizontal cells in the outer plexiform layer (*2*). Application of dopamine to the retina results in an accumulation of adenosine 3′,5′-monophosphate (cyclic AMP) in horizontal cells (*3*) and a reduction in the response of these cells to full-field illumination (*4*). Furthermore, dopamine, cyclic AMP analogs, and forskolin, an adenylate cyclase activator, reduce the receptive field size of horizontal cells by decreasing the electrical coupling among these cells (*5–7*). Horizontal cells provide receptor, bipolar, and many ganglion cells with their receptive field surrounds (*8, 9*); thus a reduction in horizontal cell responses or receptive field size would be expected to affect the surround antagonism observed in receptor, bipolar, and ganglion cells. In support of this, application of dopamine to the goldfish retina decreases the antagonistic surround responses of bipolar and receptor cells (*4*).

We report that, following a prolonged period in the dark (100 to 110 minutes), L-type (H1) cone horizontal cells in the carp showed alterations in responsiveness and receptive field size very similar to those induced by dopamine. Furthermore, exogenous dopamine no longer had effects on the cells. These observations suggest that interplexiform cells in fish release dopamine after prolonged darkness and thus modulate inhibitory surround effects mediated by horizontal cells as a function of time in the dark.

Experiments were performed on superfused retinas from carp *(Cyprinus carpio)* maintained on a 12:12-hour light: dark cycle. During the light phase whole retinas were dissected in dim red light from fish kept in the dark for 20 minutes (control) or 90 minutes and were mounted receptor side up in a superfusion chamber. On average it took 10 to 20 minutes after the initial dark period for the preparation to be set up and for a satisfactory cell to be impaled. Thus, total time in the dark or dim red light for control preparations was 30 to 40 minutes, compared to 100 to 110 minutes for preparations kept in prolonged darkness. The superfusion medium consisted of Ringer's solution containing 110 m*M* NaCl, 2.5 m*M* KCl, 20 m*M* $NaHCO_3$, 20 m*M* glucose, 0.1 m*M* $CaCl_2$, and 0.1 m*M* $MgSO_4$, maintained at ~19°C and *p*H 7.6 and aerated with a mixture of 97 percent O_2 and 3 percent CO_2 (*10*). The medium flowed by gravity at a rate of 1.5 ml/min into a superfusion chamber (volume, 0.5 ml). Test drugs were added to the medium.

Membrane potentials and light-evoked responses of cone-driven H1 horizontal cells were recorded intracellularly. The cells were identified by their responses to monochromatic spectral stimuli, by response waveform, and by the depth of the penetrated unit (*4, 10*). The resting membrane potential after both 30 to 40

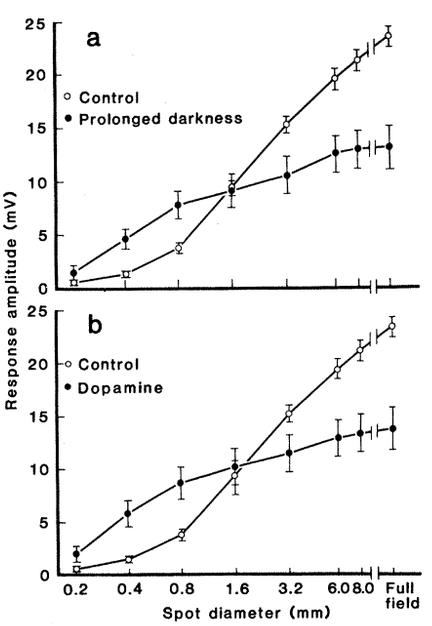

Fig. 1. Average H1 horizontal cell response amplitudes as a function of stimulus spot diameter. (a) Comparison of average response amplitudes of cells to spot stimuli after 30 to 40 minutes in the dark (control, $n = 16$) and after 100 to 110 minutes in the dark ($n = 8$). (b) Comparison of average response amplitudes of cells to spot stimuli after application of 25 μ*M* dopamine ($n = 8$) and after 30 to 40 minutes in the dark (control, $n = 16$). Dopamine application and 100 to 110 minutes in the dark caused average response amplitudes to small spot stimuli to be significantly larger and average response amplitudes to large spot stimuli to be significantly smaller. Each data point represents the mean ± standard error.