Table 2. Rates of uptake (nanomoles per minute per centimeter of intestine) and total transport capacities (nanomoles per minute per gram$^{0.75}$) for three species each of mammals and reptiles. Rates of uptake were measured in the proximal part of the small intestine and are given as the range of values, with the mean value in parentheses. Transport capacities are relative to the metabolic live mass ($W^{0.75}$) of the animals and are given as the mean ± standard error of the mean.

Class	Rate of uptake (mean)		Transport capacity	
	D-Glucose*	L-Proline†	D-Glucose*	L-Proline†
Reptiles	294 to 831 (485)	249 to 377 (329)	109 ± 32	119 ± 32
Mammals	401 to 615 (487)	329 to 676 (511)	734 ± 328	800 ± 301

*Carrier-mediated uptake. †Total uptake.

tance from mouth to anus is 4.1 ± 0.5 for the eight species of small mammals studied but only 1.6 ± 0.3 for the five species of reptiles. Second, the actual surface area of the intestine is 4 to 13 times greater than that of the corresponding smoothbore cylinder (compare columns 7 and 8 of Table 1) because of elaborations of the intestinal mucosa at the microscopic level (finger-like villi in mammals and long ridges in lizards) and is increased another order of magnitude by microvilli. The factor for increase in surface area at the microscopic level may be higher for mammals (7.4 ± 1.6 for five species studied) than for lizards [4.0 for one species; see (*16*)]. When rates of uptake were integrated over the whole length of the small intestine to yield the total transport capacity [see (*6, 7*) and Table 1], these capacities, whether related to weight or to metabolic live mass, were an average of 6.8 times greater for mammals than for reptiles, for both glucose and proline (Table 2).

Thus, in the evolution of mammals from reptiles the key adaptation of the digestive system to the need for higher nutrient uptake is analogous to the key adaptation of the respiratory system to the need for higher oxygen uptake (*17*), that is, a great increase in absorptive surface area. Through lengthening of the gut (and possibly by microscopic elaborations), the intestine is enabled to process food in a shorter time without any sacrifice in extraction efficiency. This pattern of intestinal adaptation during the evolution of endothermy resembles the adaptations, within an individual animal's lifetime, to many other conditions associated with increased requirements for nutrient uptake, such as pregnancy, lactation, diabetes, intestinal resection, and perhaps reduced environmental temperatures (*5*). Under these conditions, the intestine adapts by a proliferation of mucosal surface per unit length of intestine. Such mucosal proliferation has the same result as lengthening of the gut: namely, increased rates of uptake for all nutrients. These anatomical responses contrast with the other main adaptive pattern of the gut, that is, induction or repression of specific transport mechanisms that enable animals to adapt phenotypically or evolutionarily to changes in diet (*5–7*).

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9. The four species listed in Table 1, plus box turtles (*Terrapene carolina*) and hamsters, were given a diet of alfalfa pellets and free access to water. Lizards were fed hydrated pellets by gavage to maintain constant body weight and were able to bask under heat lamps for 10 hours per day. Thus, their body temperatures were 37° to 40°C during the day and room temperature (24°C) at night. We made selected measurements on six species of herbivorous or omnivorous mammals [kangaroo rat (*Dipodomys merriami*), Belding's ground squirrel (*Spermophilus beldingi*), laboratory rat, green monkey (*Cercopithecus aethiops*), and fruit bats (*Artibeus jamaicensis* and *Carollia perspicillata*)] and on two species of carnivorous lizards [desert spiny lizard (*Sceloporous magister*) and leopard lizard (*Gambelia wislizenii*)]. These species were fed on other diets.
10. Limits for mean retention times (t_r) in Table 1 were calculated from the mass of digesta in the gut (M), the rate at which food enters the gut (R_i) and the rate at which feces leave the gut (R_o) as $(M/R_o) > t_r > (M/R_i)$ [R. M. Sibley, in *Physiological Ecology*, C. R. Townsend and P. Calow, Eds. (Sinauer, Sunderland, Mass., 1981), p. 109]. These estimated t_r values were confirmed for lizards by direct measurements with food dyed with Sudan III [desert iguanas, 95.5 ± 50.4 hours (n = 5); chuckwallas, 74.4 ± 2.4 hours (n = 4); mean ± standard error of the mean].
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12. Apparent extraction efficiency was calculated as $[(m_{food} - m_{feces})/m_{food}] \times 100$, where m is the flux of total dried weight or of a specific diet component per day. This calculation underestimates actual efficiencies if feces contain significant amounts of organic matter from intestinal secretions or microbes.
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15. Similar conclusions were obtained whether the rates of transport were normalized to the length, dry or wet weight, mucosal weight, nominal or microscopic surface area, or protein content of the tissue segment (*6*).
16. Microscopic surface area was measured as described (*7*). The ratio of microscopic to nominal area (see Table 1) is 4.0 for iguana, 5.0 for mouse, and 12.8 for woodrat; corresponding figures for three other small mammals are 7.4 (kangaroo rat), 7.1 (*Carollia perspicillata*), and 4.5 (*Artibeus jamaicensis*). Morphometric measurements and allometric analysis of gut dimensions in more species are needed to test whether mammals really do exceed reptiles in the ratio of microscopic to nominal area and to compare the contributions of an increase in this ratio and of the increased gut length to the increased gut area of mammals.
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Herbicide Resistance and Cross-Resistance: Changes at Three Distinct Sites in the Herbicide-Binding Protein

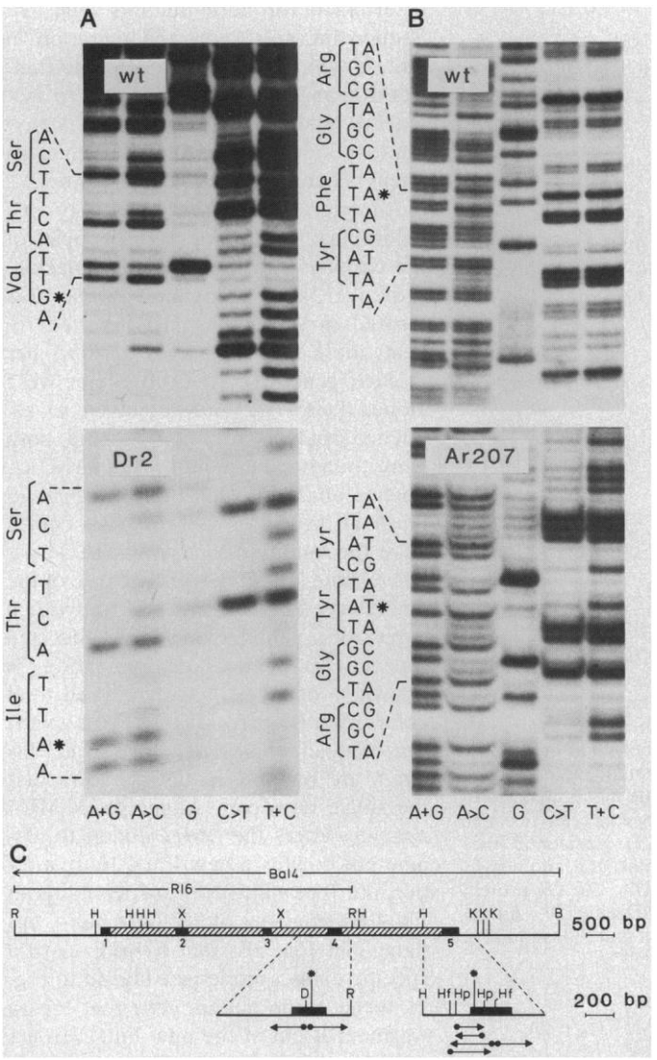
Abstract. *Plants and algae resistant to the commonly used* s-*triazine herbicides display a wide spectrum of cross-resistance to other herbicides that act in a similar manner. Analysis of uniparental mutants of the green alga* Chlamydomonas reinhardi *showed that three different amino acid residues in the 32-kilodalton thylakoid membrane protein can be independently altered to produce three different patterns of resistance to* s-*triazine and urea-type herbicides. These results clarify the molecular basis for herbicide resistance and cross-resistance. Two of the mutations do not alter normal electron transport and thus may have applications of agronomic interest.*

The light reactions of photosynthesis take place in the chloroplast thylakoid membrane, mediated in part by the membrane-bound complexes of photosystem I (PS I) and photosystem II (PS II). Many commonly used herbicides, such as atrazine [2-chloro-4-ethylamino-6-(isopropylamino)-*s*-triazine] and diuron, or DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea], inhibit photosynthesis by preventing electron transfer at the reducing side of PS II (*1*). Recent studies (*2–4*)

indicate that the molecular basis for such resistance is a change in the DNA coding for one of the proteins found in the PS II complex. This protein, originally called the rapidly labeled 32-kD protein and since identified as a herbicide-binding protein (*5*), is coded for by the chloroplast *psb*A gene (*6*).

We report here the results of sequence analysis of *psb*A from two *Chlamydomonas reinhardi* uniparental mutants with normal electron transport but with different levels of resistance to diuron and atrazine (*7*). Each mutant has a unique base pair change, with respect to the wild-type gene, that produces a unique amino acid substitution in the 32-kD protein. Both these substitutions are at residues other than the serine 264 previously identified as altered in an atrazine- and diuron-resistant *C. reinhardi* mutant (*4*) and in atrazine-resistant higher plant species (*2, 3*). These results, which show that a single change at any one of three residues in the same protein can be correlated with a different pattern of herbicide resistance, help to clarify the molecular basis for herbicide resistance and cross-resistance.

It has been proposed that, as certain



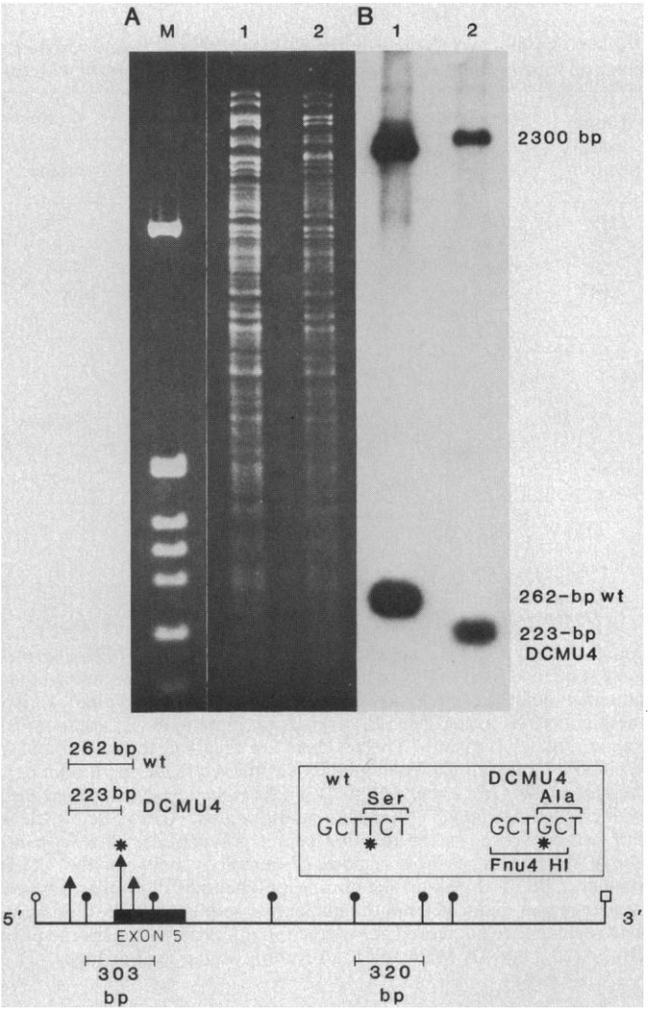


Fig. 1 (left). (A and B) Nucleotide sequencing gels for portions of *psb*A exon 4 (A) and exon 5 (B) from *C. reinhardi cw*15, referred to as wild type (wt) with respect to herbicide resistance, and from mutant strains Dr2 and Ar207. (C) Map of chloroplast restriction fragments R16 and part of Ba14 (*13*), showing the positions of the five *psb*A exons (solid bars), the introns (crosshatched bars), and (below) the DNA sequencing strategy for exons 4 and 5 from the herbicide-resistant mutants. Restriction enzyme sites are indicated for Eco RI (R), Hind III (H), Xba I (X), Kpn I (K), Bam HI (B), Dde I (D), Hinf I (Hf), and Hpa II (Hp). The fragments were labeled with ^{32}P at the 5′ or 3′ ends and the strands were separated before sequences were determined. The chemical cleavage reactions performed (*28*) are indicated below each lane on the autoradiograms of 8 percent (Dr2, Ar207, exon 5 wt) or 20 percent (exon 4 wt) urea polyacrylamide gels. Given at the side of each gel are nucleotide sequences (gel shows noncoding strand in exon 4, coding strand in exon 5), the corresponding amino acid sequence, and the single base that differs between the wt strain and each mutant (*). The noncoding sequences are listed as 5′ to 3′ from bottom to top (Dr2 and both wt) or top to bottom (Ar207). Abbreviations: Ser, serine; Thr, threonine; Val, valine; Ile, isoleucine; Arg, arginine; T, thymine; A, adenine; G, guanine; and C, cytosine. Fig. 2 (right). (A and B) Southern blot transfer showing that all copies of *psb*A from DCMU4 are mutant. *Chlamydomonas reinhardi* chloroplast (ct) DNA from strains *cw*15 and DCMU4 was purified (*13*), digested with Fnu4 HI, and electrophoresed on a 2 percent agarose gel. (A) Ultraviolet (UV) visualization of ethidium bromide–stained gel with 2.5 μg of *cw*15 ct DNA (lane 1), 1 μg of DCMU4 ct DNA (lane 2), and 0.8 μg of Hinf I–digested pBR322 DNA as a size marker (M). (B) Autoradiogram of lanes 1 and 2 from (A) after Southern blot transfer to GeneScreen (New England Nuclear) and hybridization. The filter was exposed to UV light from a transilluminator for 3 minutes before baking. Sizes of hybridizing bands are given. The map at the bottom shows the Hind III (○) and Bam HI (□) fragment of 2.5 kilobase pairs containing *psb*A exon 5. This fragment was digested with Hinf I (●). The 303- and 320-bp fragments comigrated on a preparative acrylamide gel and were eluted, ^{32}P nick-translated, and hybridized with the filter (*13*). Also indicated are restriction sites for Fnu4 HI (▲), the new Fnu4 HI site created by the DCMU4 mutation affecting *psb*A serine codon 264 (*), and the size of Fnu4 HI fragments predicted for *cw*15 (wt) and DCMU4. The band of 2300 bp seen in the autoradiogram for both strains probably results from hybridization to the 320-bp Hinf I fragment and part of the 303-bp Hinf I fragment.

herbicides compete with quinone for binding to thylakoid membranes (*8–10*), herbicide resistance occurs when an alteration in the membrane reduces herbicide binding, thus allowing quinone to bind even in the presence of herbicide (*10, 11*). It is not clear whether binding of these compounds to thylakoid membranes is determined solely by the primary structure of the 32-kD protein or by a structure consisting of several proteins in the PS II complex. However, the mutations that alter the serine 264 residue also significantly reduce the affinity of azido-[^{14}C]atrazine binding to a membrane protein of 32 kD (*4, 5, 12*). In the *Amaranthus hybridus* (*2*) and *Solanum nigrum* (*3*) biotypes and in the *C. reinhardi* mutant DCMU4 (*4*), which differ in their herbicide resistances (Table 1), the same serine residue 264 is changed to glycine (higher plants) or to alanine (DCMU4). Uniparental mutants of *C. reinhardi* (*7*) resistant to diuron (Dr2) or to atrazine (AR207) provide an opportunity to further correlate herbicide-resistant genotypes and phenotypes.

Using the strategy previously designed to clone and determine the nucleotide sequence of the wild-type algal *psb*A gene (*13*), which contains four introns, we analyzed *psb*A from Dr2 and Ar207. Nucleotide sequences for the coding regions contained in exons 4 and 5 were determined for both mutants (Fig. 1). Sequencing gels show the mutation in Dr2, in which a guanine to adenine transition changes valine codon 219 to isoleucine, and in Ar207, in which a thymine to adenine transversion changes phenylalanine codon 255 to tyrosine.

Although *C. reinhardi* has only one chloroplast, it contains 50 to 80 copies of the circular chloroplast DNA molecule. Moreover, since *psb*A in *C. reinhardi* is located in the chloroplast inverted repeat, there are two copies of *psb*A per circular genome (*13*). Both copies were cloned from Dr2 and subjected to sequence analysis for exon 4, and both were found to be mutant. Similarly, sequence analysis of *psb*A from *C. reinhardi* DCMU4 shows that both copies have the mutation that alters the serine 264 residue (*4*). However, because of the ambiguity in defining the individual copies of a gene located in the inverted repeat (*13*), where recombination is known to occur (*14*), we analyzed total chloroplast DNA from DCMU4 and verified that all copies of *psb*A from this strain are mutant. Figure 2 shows that the single base pair change in DCMU4 not only alters the serine codon to alanine but creates a new Fnu4 HI restriction site. The wild-type *psb*A contains an Fnu4 HI fragment of 262 base pairs (bp) (lane 1 in Fig. 2B) that hybridizes to a specific exon 5 probe (see legend to Fig. 2), while in the mutant gene this 262-bp fragment is cut at the new Fnu4 HI site to produce fragments of 223 bp (lane 2 in Fig. 2B) and 39 bp (not seen on this gel). In the ethidium bromide–stained gel (Fig. 2A), the restriction patterns for the wild-type and DCMU4 chloroplast DNA appear to be otherwise identical, suggesting no major changes in the chloroplast genome of the mutant. The mutations in Dr2 and Ar207 do not alter any known restriction endonuclease sites. However, on the basis of sequence information for Dr2 and DCMU4 and the data in Fig. 2, we suggest that all copies of *psb*A in a given mutant are altered. It may be that both copies in one circular molecule are mutated independently, but it is more likely that under selective

Table 1. Amino acid changes in the 32-kD protein, atrazine resistance, DCMU resistance, and electron transport in *C. reinhardi, A. hybridus*, and *S. nigrum* with respect to wild-type strains.

Amino acid position	Codons		Resistant strain	Resistance		Electron transport
	Wild type	Mutant		Atrazine	DCMU	
219	Val	Ile	Dr2 (*7*)	2×	15 ×	Normal
255	Phe	Tyr	Ar207 (*7*)	15×	0.5×	Normal
264	Ser	Ala	DCMU4 (*4*)	100×	10 ×	Altered
264	Ser	Gly	*A. hybridus* and *S. nigrum* (*3*)	1000×	1 ×	Altered

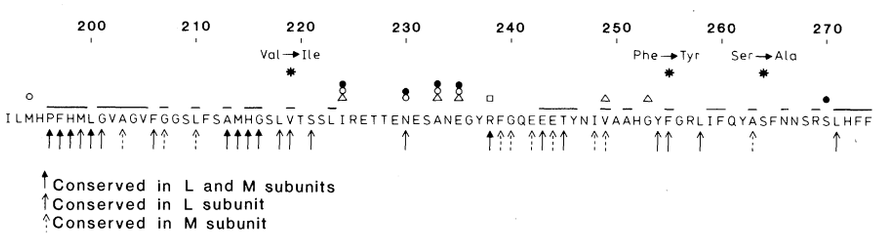


Fig. 3. Amino acid residues 192 to 274 of the *C. reinhardi* 32-kD protein deduced from the *psb*A gene sequence (*13*) and given in the one-letter notation for amino acid sequences (A, alanine; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; and Y, tyrosine). The residues are identical to those found in higher plant protein, as typified by spinach (*6*). Symbols above the sequence mark amino acid residues that differ in *Anabaena* (●) (*16*), *Fremyella* (○) (*17*), *Euglena* (△) (*18, 19*), and the residue that is changed to lysine (□) in *Anabaena, Fremyella*, and *Euglena*. Arrows below the sequence indicate residues that are conserved in the reaction center polypeptides of *R. capsulata* (*22*). Horizontal lines above the sequence show regions of homology between the 32-kD protein and another *C. reinhardi* PS II thylakoid membrane polypeptide of similar size called D2. The D2 protein sequence was deduced from the nucleotide sequence of the chloroplast *psb*D gene (*20*). Amino acid residues are numbered across the top (*6*). Asterisks mark the position of mutations found in Dr2, Ar207, and DCMU4 and the resulting amino acid changes.

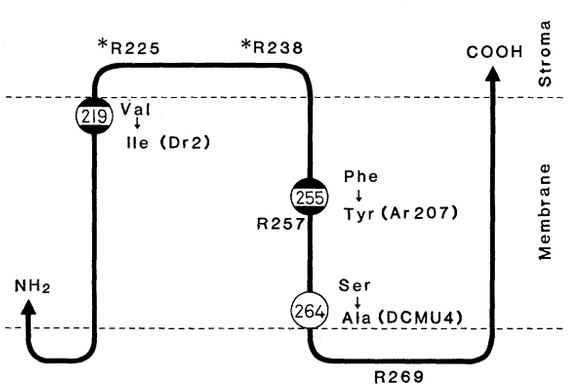


Fig. 4. Membrane-folding model for part of the 32-kD protein (*24*), showing the three membrane-spanning regions toward the carboxyl terminus, which is in the stroma (*25*). The circles indicate the location of three amino acid residues that are changed in *C. reinhardi* mutants. The filled circles denote places where the changes do not affect electron transport. Tryptic digestion of membranes cleaves arginine residues marked *R but not those marked R (*25*). The boxed-in region has homology with reaction center polypeptides L and M of photosynthetic bacteria (see legend to Fig. 3).

conditions a single mutation spreads by intermolecular recombination (*15*) or by gene conversion.

The three amino acid residues altered by point mutations in the herbicide-resistant mutants are found in a region of the protein (Fig. 3) that is totally conserved between *C. reinhardi* (*13*) and spinach (*6*) and highly conserved in cyanobacteria (*16, 17*) and *Euglena* (*18, 19*). In addition, this area has considerable homology with another PS II thylakoid membrane protein, D2 (*20*), and with the reaction center polypeptide subunits L and M of photosynthetic bacteria (*21, 22*). It was proposed that portions of this region may affect quinone binding (*23*). All three altered residues are conserved in every deduced "32-kD" protein whose gene sequence has been determined to date. The valine 219 and phenylalanine 255 residues are also conserved in the D2 protein and in the L subunit. Hydropathic analysis of the 32-kD protein, in which hydrophobic and hydrophilic regions are identified and potential membrane–spanning alpha helices designated (*24*), places all three amino acid residues that affect herbicide resistance in the membrane (Fig. 4). Although such a model is speculative given the absence of rigorous physical and chemical analysis of this protein, the model in Fig. 4 is consistent with data on trypsin cleavage of arginine residues and carboxyl-terminal processing of the protein (*25*). It is also consistent with the results of competitive binding studies, which suggest that the lipid-soluble compounds atrazine, diuron, and quinone have separate but overlapping binding domains (*8–11*). Wolber and Steinback (*26*) have shown that azido-[^{14}C]atrazine binds to a tryptic digest fragment of the 32-kD protein that contains residue 219 and the proposed quinone-binding region (boxed region in Fig. 4).

Our results reveal that mutations affecting three different amino acid residues of the 32-kD protein are correlated with different types and levels of herbicide resistance. This may explain why several weed biotypes with various levels of resistance to *s*-triazines display strikingly different cross-resistances to other PS II inhibitors (*11, 27*). Interestingly, the mutations affecting valine 219 and phenylalanine 255 do not alter electron transport as measured by fluorescence induction (*7*), while the mutation affecting serine 264 does (*4, 11*). Thus, changes such as those in residues 219 and 255 may be of use in genetic engineering of herbicide resistance.

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