colysis is correlated with Na-K transport, knowledge of the regulation of aerobic glycolysis may be of major significance for understanding the vascular myopathies associated with hypertension, atherosclerosis, and aging, which are known to be correlated with changes in vascular Na-K transport (*16*) and carbohydrate metabolism (*13, 17*).

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10. The amount of glucose catabolized is directly proportional to the amount of glucose taken up from the bathing media since the intracellular concentration of glucose is zero. As calculated from the data in Table 1, glucose uptake therefore accounts for glucosyl units in the amount of 2.40 μmole/g during a 30-minute incubation with high K^+ (80 m*M*). Approximately 1.41 μmole/g is derived from glycogen during the same time period (Fig. 1), for total glucosyl units of 3.81 μmole/g. If complete mixing of glycolytic intermediates occurs then the predicted lactate specific activity ratio would be [(2.40/3.81) × glucose specific activity ratio (1.0)] + [(1.41/3.81) × glycogen specific activity ratio (0.107)] × 0.5, which is equal to 0.335. The time course of equilibration of the radioactive label (Fig. 2A), which has a time constant of 42 minutes, must also be taken into account. After 30 minutes, equilibration will be 44 percent complete, and a lactate specific activity ratio of 0.43 would be predicted. A lactate specific activity ratio less than 0.46 would be detected as statistically different from control (0.50), given the variance in the measured specific activity ratio in Fig. 2B. Similar derivation of a predicted lactate specific activity ratio for tissues treated with added K^+ after 60 minutes of incubation yields a value of 0.39.
11. Preferential degradation of labeled glycogen would lead to an initial flux of glucosyl units, amounting to 0.30 μmole/g (0.107 × 2.82), from glycogen with a specific activity equal to that of media glucose. Therefore [(2.40 + 0.30)/3.81] × 0.5 yields a lactate specific activity ratio of 0.350. Correction for equilibration of the radioactive label as in (*9*) gives a predicted specific activity ratio of 0.435, again a readily detectable alteration of the lactate specific activity ratio.
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Molecular Basis of Herbicide Resistance in *Amaranthus hybridus*

Abstract. *Resistance of different species of weeds to* s*-triazines, a commonly used class of herbicides, has been shown to involve a change in the binding affinity of the herbicide to a chloroplast polypeptide of 32,000 daltons. A single amino acid difference in this 32,000-dalton protein appears to be responsible for resistance to the herbicide in* Amaranthus hybridus.

Many commercially important herbicides inhibit photosynthesis. One such class of herbicides, the *s*-triazines, inhibit electron transfer on the reducing side of photosystem II (PS II) (*1, 2*). The mechanism of action of the triazines involves the initial binding of the herbicide to chloroplast thylakoid membranes and blocking of electron transport at the second stable electron acceptor of PS II (*3*). The high-affinity binding site for the herbicide is a thylakoid-membrane polypeptide of the PS II complex (*4*) and has been identified as the "rapidly turned over" 32,000-dalton chloroplast-encoded membrane polypeptide (*5*).

In agricultural areas where atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine) has been used extensively, atrazine-resistant biotypes of many species of weeds have appeared. The first evidence of intraspecific resistance to the *s*-triazines was reported for *Senecio vulgaris* in 1970 (*6*). Since this initial report, herbicide-resistant biotypes have been identified among 28 different species of weeds in North America and Europe (*7*). In all cases, the appearance of resistant weeds followed a continuous use of triazines (usually atrazine or simazine) over extended periods of time. In the examples of triazine resistance thus far examined, the biochemical basis of resistance has been identified as a change in the herbicide-target site in the chloroplast (*2, 8*). When chloroplasts were isolated from the resistant biotype of *Amaranthus hybridus*, it was demonstrated that a 32,000-dalton polypeptide of PS II is present in the thylakoids but does not bind the triazine (*8*).

Triazine resistance is maternally inherited (*9*), suggesting that this trait is coded for by the chloroplast genome. A chloroplast-encoded protein with a molecular size of 32,000 daltons has been characterized in many higher plants (*10*). This protein is tightly bound to thylakoid membranes and rapidly metabolized, and its synthesis is modulated by light (*11*). Translation of the messenger RNA (mRNA) for this protein occurs on chloroplast ribosomes (*12*) to produce a precursor polypeptide of 33,500 daltons in *Spirodela oligorhiza* and 34,500 daltons in maize and spinach (*13*). In order to investigate the genetic basis of triazine resistance, we have studied the gene which encodes this polypeptide, now designated psbA, in herbicide-resistant and herbicide-susceptible biotypes of the weed *Amaranthus hybridus*. Seeds used to propagate atrazine-resistant and atrazine-susceptible plants were from plant material originally collected in the state of Washington and previously characterized by chloroplast electron transport studies (*14*).

Chloroplast DNA was isolated (*15*) from both atrazine-susceptible and atrazine-resistant biotypes of *A. hybridus*. There is no apparent difference in the pattern of DNA fragments generated by Eco RI and Bam HI restriction endonucleases when chloroplast DNA's from the two biotypes are compared (*8*). Furthermore, with the use of the maize psbA gene cloned in the recombinant plasmid pZmc427 (*10*) as a probe, it was discovered that psbA in *A. hybridus* is present, in both susceptible and resistant plants, within a single Bam HI fragment [6 kilobase pairs (kb)] (*8*) and a single 3.68-kb Eco RI fragment (*16*).

We have cloned (*17*) the 3.68-kb Eco RI fragment of chloroplast DNA from herbicide-resistant and herbicide-susceptible plants in pBR322 with the recombinant plasmids designated pAH484 and pAH32S, respectively. The complete nucleotide sequence of the psbA gene from *A. hybridus* is presented in Fig. 1. There is only one open reading frame that is capable of coding for a precursor polypeptide of approximately 34,600 daltons. Translation of this sequence would produce a polypeptide of 317 amino acid residues. The nucleotide sequence of the gene psbA from *Spinacia oleracea* and *Nicotiana debneyi* has recently been determined (*18*), and an absolute homology in the amino acid sequence was found between these two species.

Zurawski *et al.* (*18*) deduced a polypeptide of 353 amino acids with a molecular size of 38,900 daltons by selecting the first available Met (ATG) (Met, methionine; A, adenine; T, thymine; G, guanine) codon on the open reading frame as the initiation site for translation. The same ATG sequence can be identified in the *A. hybridus* gene at nucleotide −108 (Fig. 1). However, if translation starts as the second ATG in the open reading frame (the nucleotide 1 in Fig. 1), the primary translation product is a polypeptide of 317 amino acid residues and a molecular size of 34,600 daltons. The size of this primary translation product is in agreement with the size of other species in which the unprocessed protein is about 34,000 daltons (*11, 13, 19*). In addition, a nucleotide sequence resembling a ribosome binding site (*20*) is located immediately prior to

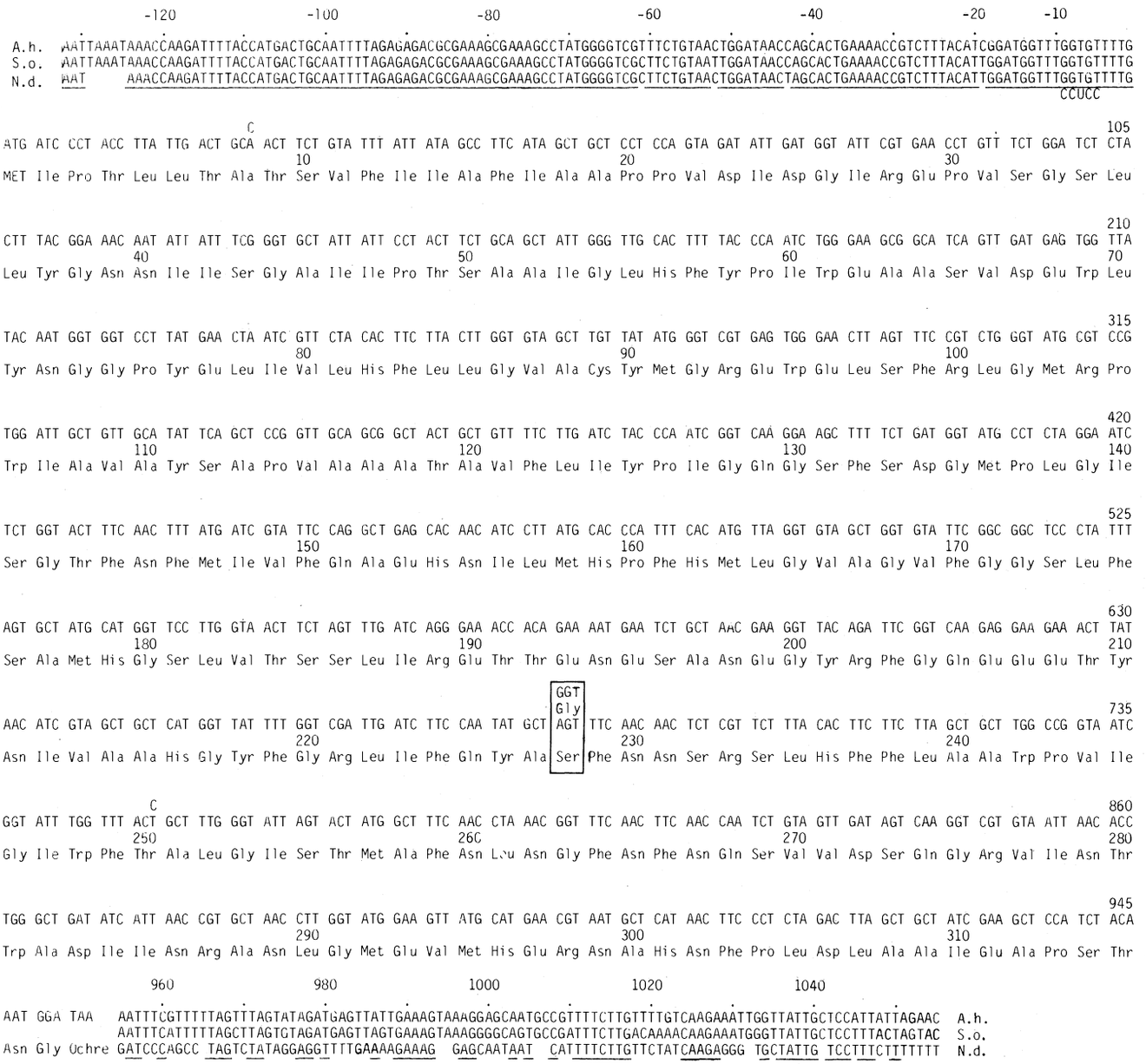

Fig. 1. The nucleotide sequence and the deduced amino acid sequence of the psbA from herbicide-susceptible *A. hybridus*. DNA sequence was determined by the method of Maxam and Gilbert (*25*). All regions were sequenced at least twice. The nucleotide sequence of the psbA from *Spinacia oleracea* (*S.o.*) and *Nicotiana debneyi* (*N.d.*) (*18*) is presented in the middle and lower lines, respectively, of the regions flanking the protein coding sequence. Regions of homology between these three species are underlined. Three nucleotide differences that were found in the psbA from herbicide-resistant plants are indicated above the sequence in nucleotides 24, 682, and 750. Only one of them leads to an amino acid change (boxed sequence). A putative ribosome binding site is indicated in nucleotide sequences −5 to −9.

the first ATG codon in the open reading frame (Fig. 1), an indication that this may be the initiation codon. Because of the size correlation described above and the high conservation in the amino acid sequence, we suggest that initiation of translation of the 32,000-dalton protein in *S. oleracea* and *N. debneyi* starts at the same codon as in *A. hybridus*, thus producing a polypeptide of a same size.

The second difference between the 32,000-dalton protein of *A. hybridus* and the protein of *S. oleracea* and *N. debneyi* involves one amino acid change. At position 245 (Fig. 1), it is isoleucine in *A. hybridus* and valine in the other two species. Fifty-eight silent base differences in the translated region of the psbA gene can be detected in the nucleotide sequence of *A. hybridus, N. debneyi*, and *S. oleracea* (*18*). The homology between *A. hybridus* and *S. oleracea* in the translated DNA is 97.2 percent, while between *S. oleracea* and *N. debneyi* it is 95.3 percent and between *A. hybridus* and *N. debneyi* it is 94.5 percent. Similarly, the homology between *A. hybridus* and *S. oleracea* along the 101 base pairs downstream from the termination codon is 79.2 percent, 42.5 percent between *N. debneyi* and *S. oleracea*, and 53.5 percent between *A. hybridus* and *N. debneyi*. These data probably reflect the phylogenetic classification in which Chenopodiaceae (*S. oleracea*) and Amaranthaceae (*A. hybridus*) both belong to the order Centrospermae while Solanaceae (*N. debneyi*) belongs to the order Tubiflorae.

Only three nucleotide differences, all of them in the protein-coding region, were found in comparing 1.5 kb of the psbA gene and its flanking regions from herbicide-resistant and herbicide-susceptible *A. hybridus*. Two differences in the codons of amino acids 8 and 250 (Fig. 1) are silent and one, in amino acid 228 (Figs. 1 and 2), results in a change from the amino acid serine in the susceptible ("wild type") to glycine in the resistant ("mutant") biotype.

The herbicide-binding protein is a thylakoid membrane polypeptide of about 32,000 daltons and the apparent atrazine-binding site is in the hydrophobic membrane phase (*5*). Previous data (*21*) predicted that, because atrazine resistance is a maternally inherited trait, we should expect to find a mutation in a chloroplast gene that confers this phenotype. The fact that the chloroplasts from atrazine-resistant *A. hybridus* plants used in our study had lost atrazine affinity (*14*) but contained the herbicide-binding polypeptide (*5*) suggests that a subtle change in the protein causes the decreased herbicide binding. The nucleotide sequence analysis presented here demonstrates that the 32,000-dalton protein in a triazine-resistant biotype of *A. hybridus* differs from the protein of a herbicide-susceptible biotype by only one amino acid. In view of the high conservation amino acid sequence, even among different species, this change is striking and is the most likely cause of the herbicide resistance (*22*).

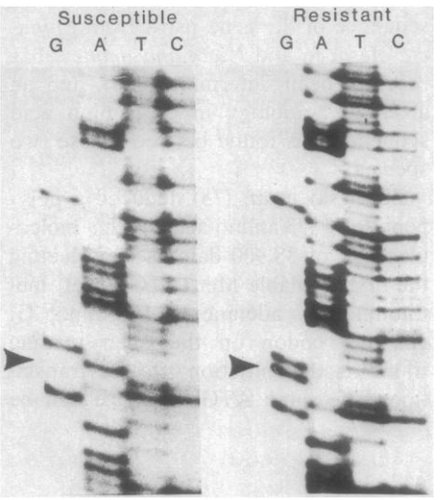

Fig. 2. Autoradiograms of DNA-sequencing gels of the region with a single nucleotide change that leads to an amino acid change in the herbicide-resistant plant (boxed sequence in Fig. 1). Arrowheads indicate the change of adenine in the psbA gene from a herbicide-susceptible plant to guanine in the gene from a herbicide-resistant plant.

The substitution of serine for glycine at position 228 in the polypeptide presumably changes the binding affinity of the triazine molecule. Since the actual triazine-binding site in the 32,000-dalton polypeptide is not known, the mechanism by which such a change in amino acid position alters herbicide binding is still unexplained. It is possible that the change from serine to glycine occurs within the binding site itself and thus alters the binding affinity. However, it is entirely possible that the mutation changes the tertiary structure of the polypeptide so that an atrazine-binding site, some distance from the altered amino acid, is modified.

Our analysis of the primary sequences of the herbicide binding protein in susceptible and resistant weed species could be a first step in the possible transfer of herbicide resistance to valuable crop plants by recombinant DNA techniques. To date, herbicide resistance has only arisen in weed species showing a high degree of phenotypic variation (*23*) and, indeed, it has been estimated that the possibility of finding such a variant in crop plants is unlikely (*24*). Since herbicide resistance allows for positive screening of possible transformants, this plant gene offers many advantages as a selection tool in the development of a transformation system for chloroplasts. In this case, the model would also have agronomic implications since the triazine herbicides are widely used in crop production, and transfer of resistance to now susceptible crops may have practical importance.

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Memory Retrieval: A Time-Locked Process in Infancy

Abstract. *Three-month-old infants learned to activate an overhead crib mobile by operant footkicking and received a visual reminder of the event (a "reactivation treatment") 2 weeks later, after forgetting had occurred. Subsequent manifestation of the association was a monotonic increasing function of time since the reactivation treatment, and performance of infants tested 8 hours after the reminder was related to the time spent sleeping in the interim* (r = *0.75). These data demonstrate that normal retrieval is time-dependent. Moreover, individual data suggest that retrieval may be continuous rather than discontinuous.*

Most of us have been unable to recall the name of an acquaintance even though we may recall some details of his appearance or the first letter of his name. Later, however, the name may intrude into our thoughts even though we had abandoned our attempt to recall it. This "tip-of-the-tongue" phenomenon is interesting because it demonstrates that a target memory attribute (for example, the name) which is inaccessible for immediate retrieval is nonetheless available in storage. In an early study of this phenomenon (*1*), subjects were given dictionary definitions of infrequently encountered words and asked to name the word. Most who could not said that they knew the word and recalled some of its letters, its number of syllables, the stressed syllable, and words similar to it in meaning or sound. These data are consistent with views (*2*) that memories are collections of independent attributes of an event and that they can be forgotten (or retrieved) at different rates. When cues similar or identical to these memory attributes are subsequently encountered, the memory attributes are presumably aroused, becoming accessible for retrieval. Because attributes that have been aroused will, in turn, activate others that constitute the same memory (*3*), the probability that the target attribute will be aroused and retrieved should improve over hours and perhaps even days. The tip-of-the-tongue phenomenon may be a protracted instance of the normal retrieval process, occurring when insufficient numbers of memory attributes are initially aroused.

Time dependence in normal retrieval has been difficult to demonstrate, probably because retrieval is usually rapid, facilitated by networks of associations constructed and organized by experience. We now report evidence from two studies of human infants that memory retrieval is a time-locked process. We considered 3-month-old infants as ideal subjects for the study of this problem because their facility in learning and remembering a unique association has been well characterized (*4*) and because they lack the verbal facility and extensive experience of older children and adults.

In both studies, infants received two 15-minute training sessions 24 hours apart and a procedurally identical retention test session 13 or 14 days later. During training, infants lay supine in their home cribs with an ankle ribbon connected to one of two overhead suspension hooks. In 9-minute reinforcement periods, the ribbon and a five-object mobile were attached to the same hook, and footkicks produced proportionally vigorous movements in the mobile. In the preceding and following 3-minute nonreinforcement periods, the ribbon and mobile were attached to different hooks so that the mobile, while in view, could not be moved by footkicking. The 3-minute phase at the beginning of session 1 provided an index of the pretraining kick rate; all other nonreinforcement phases were 3-minute cued-recall tests, with the nonmoving mobile components serving as cues for the anticipatory production of footkicking. This training procedure produces a rapid increase in kick rate that is solely attributable to the contingency (*5*). High post-training kick rates persist during cued-recall tests at the beginning of subsequent sessions, with responding gradually declining to the pretraining level over a 2-week period (*4*). This decline in conditioned responding operationally de-

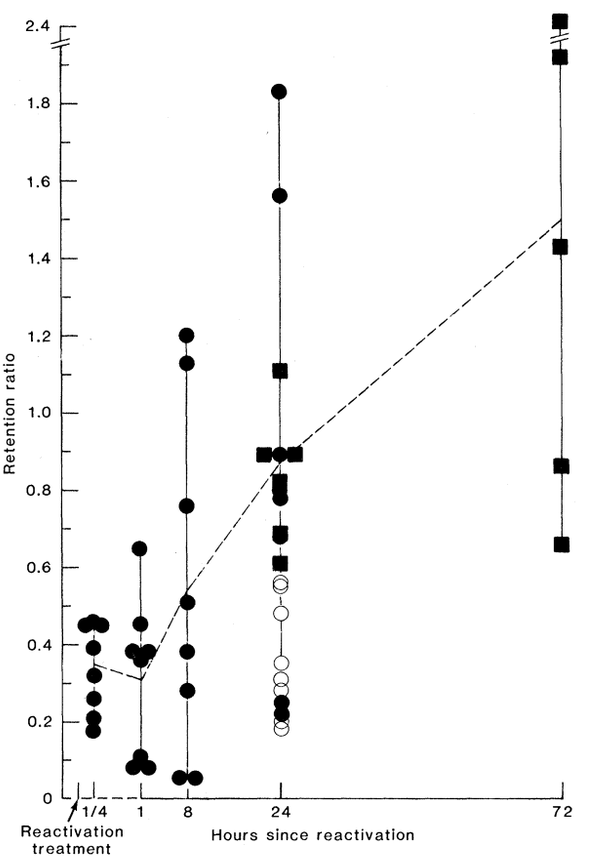

Fig. 1. Retention ratios of 32 infants at different times after a reactivation treatment (filled circles) administered 13 days after the last training session. A ratio of 1.0 indicates no change in performance over the retention interval. Ratios of 8 no-reactivation controls tested after a 14-day retention interval (open circles) and 11 infants, previously tested 24 or 72 hours after a reactivation treatment (filled squares) (*9*), are also shown. Vertical lines define the range of ratios at each delay, and the dashed line connects means for the infants that received the reactivation treatment (filled symbols). The abscissa is not drawn to scale between the reactivation treatment and 1 hour.