cm^2). In contrast, the generalists, with the exception of two *Pyrrharctia* larvae, consistently consumed only minimal areas (<0.25 cm^2) of the control leaf. For example, the *Spodoptera* larvae ingested 0.13 ± 0.07 cm^2 (n = 5) of the control leaf versus 1.97 ± 1.35 cm^2 (n = 20) of the experimental leaf. Evidently, milkweed tissue with intact veins is inherently unacceptable to these herbivores. The preference of generalists for the treated half of the experimental leaf can therefore be attributed to increased acceptability of this half, rather than to a possible decrease in acceptability of the untreated half.

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16. The sequence of presentation of control and experimental leaves had no detectable effect on the number of individuals feeding on either leaf.
17. Only some of the milkweed specialists fed on both the control and experimental leaves. Thus, in order to compare the total number of feeders that cut veins on these leaves, the paired experimental design must be ignored. We used Fisher's exact test for 2 by 2 tables in preliminary tests for independence between cutting veins on one leaf, and cutting and feeding on the other leaf. The lack of any statistically significant departure from independence was regarded as justification for ignoring the paired design.
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Ouabain Resistance Conferred by Expression of the cDNA for a Murine Na^+,K^+-ATPase α Subunit

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The molecular basis for the marked difference between primate and rodent cells in sensitivity to the cardiac glycoside ouabain has been established by genetic techniques. A complementary DNA encoding the entire α_1 subunit of the mouse Na^+- and K^+-dependent adenosine triphosphatase (ATPase) was inserted into the expression vector pSV2. This engineered DNA molecule confers resistance against 10^{-4} M ouabain to monkey CV-1 cells. Deletion of sequences encoding the carboxyl terminus of the α_1 subunit abolish the activity of the complementary DNA. The ability to assay the biological activity of this ATPase in a transfection protocol permits the application of molecular genetic techniques to the analysis of structure-function relationships for the enzyme that establishes the internal Na^+/K^+ environment of most animal cells. The full-length α_1 subunit complementary DNA will also be useful as a dominant selectable marker for somatic cell genetic studies utilizing ouabain-sensitive cells.

THE EXTREME DIFFERENCE IN OUABAIN sensitivity between rodent and primate cells has long been used as a selection strategy in somatic cell genetics (*1*). Although the Na^+- and K^+-dependent adenosine triphosphatase (Na^+,K^+-ATPase) is the apparent target of ouabain (*2*), the complex subunit structure of the enzyme (*3*) and the existence of multiple genes encoding alternative isoforms of the Na^+,K^+-ATPase (*4–6*) have left the genetic basis for differential ouabain resistance between rodent and primate cells uncertain. Furthermore, in most somatic cell genetic procedures involving the transfer of ouabain resistance from rodent to primate cells (*1*), a substantial amount of the rodent genome is transferred to recipient primate cells making it difficult to identify those rodent sequences which are directly responsible for ouabain resistance. Recently, complementary DNA (cDNA) clones encoding three rat α subunit isoforms (*4, 5*) and the rat β subunit (*7, 8*) have been identified. Hybridization of genomic DNA from monkey CV-1 cells made ouabain-resistant by transfer of metaphase mouse chromosomes to cDNAs specific for each α subunit isoform has led to the inference that the expression of the rodent α_1 subunit gene is likely to confer ouabain resistance to ouabain-sensitive primate cells (*9*). However, even in chromosome transferents, a substantial amount of rodent DNA is transferred to the recipient primate cells in addition to the gene encoding the Na^+,K^+-ATPase α_1 subunit. This consideration makes it impossible to definitively conclude that transfer of the rodent α_1 subunit gene is indeed responsible for species-specific differences in ouabain sensitivity. To resolve this issue, we have tested the capacity of a cDNA encoding the mouse α_1 subunit to directly confer ouabain resistance to monkey CV-1 cells.

To isolate full-length murine α_1 subunit cDNA, we screened a mouse pre-B cell cDNA library with a rat cDNA that encodes a portion of the rat α_1 subunit (*10*). Six clones, containing inserts of ≥3.6 kb in length, showed similar Eco RI and Bam HI restriction maps. The restriction map of one of these clones, mbα69, is shown in Fig. 1. Preliminary DNA sequence analysis indicated that clone mbα69 contained a complete coding sequence for the α_1 subunit polypeptide. A poly(A) addition consensus sequence was identified at one end of the mbα69 insert; at the opposite end we observed a continuous open reading frame containing the translation start site for the α_1 subunit polypeptide previously identified for human (*11*), sheep (*12*), pig (*13*), rat (*4, 5*), and Torpedo cDNA clones (*14*).

To test biological activity, the 3.6-kb Eco RI insert of mbα69 was introduced into the eukaryotic expression vector pSV2 (Fig. 1).

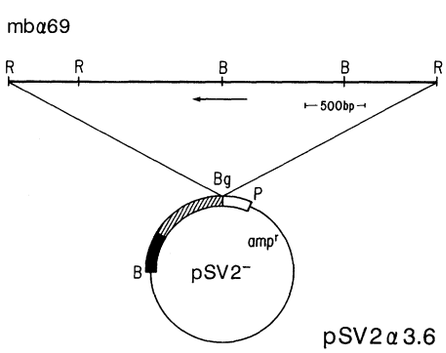

Fig. 1. Schematic representation of mouse Na^+,K^+-ATPase α_1 subunit cDNA clone mbα69 and insertion into the vector pSV2. The cDNA contains two internal Bam HI sites and one internal Eco RI site. The cDNA library containing this clone was constructed and screened with the rb5 probe (*10*) as described (*17*). Bcl I linkers were added to the mbα69 3.6-kb partial Eco RI digestion fragment for ligation to pSV2 DNA. The pSV2 vector was constructed from pSV2DHFR by excising the DHFR insert and adding Bgl II linkers (*18*). In the diagram of pSV2, the white area between Pvu II and Bgl II indicates the SV40 origin and promoter, hatched and black areas include the SV40 small T antigen splice site and poly(A) addition site, respectively. The arrow underneath mbα69 indicates direction of transcription. B, Bam HI; Bg, Bgl II; R, Eco RI; P, Pvu II.

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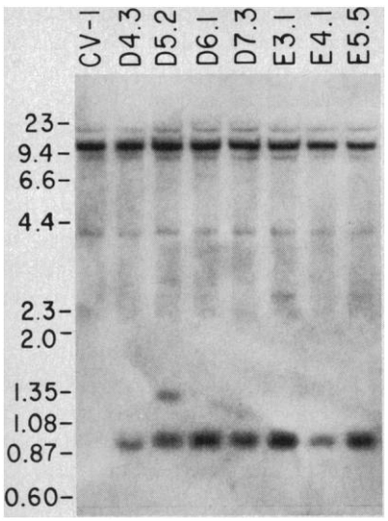

Fig. 2. Southern blot analysis of genomic DNA from cells transfected with pSV2α3.6. Genomic DNA (10 μg) from clones selected in $10^{-6}M$ ouabain and from CV-1 cells was digested with Eco RI. Digestion products were separated by electrophoresis in a 1% agarose gel containing $0.04M$ tris acetate (*p*H 8), $0.002M$ EDTA, and transferred to a hybridization membrane (Zetabind, AMF Cuno). The blot was hybridized with 10^7 count/min of ^{32}P-labeled 0.95-kb internal Bam HI fragment (specific activity 6×10^8 count/min per microgram of DNA) from the mbα69 clone (Fig. 1). Size markers, Hind III–digestion products of bacteriophage λ DNA and Hae III–digestion products of φX174 replicative form DNA.

Insertion in the sense orientation generated plasmid pSVα3.6. This plasmid was introduced into ouabain-sensitive CV-1 cells by the calcium phosphate coprecipitation procedure (*15*). Transfected cells were initially selected for the ability to proliferate in $10^{-6}M$ ouabain, a concentration of drug which is cytotoxic to these cells. In three separate experiments, introduction of pSV2α3.6 DNA into CV-1 cells gave between 200 and 650 ouabain-resistant colonies per 10^6 cells per microgram of DNA (Table 1). Colonies appeared within 2 weeks. Ouabain-resistant cells grew rapidly in the presence of ouabain at concentrations as high as $10^{-4}M$. Colonies were not observed in control CV-1 cells transfected with pSV2 DNA. No ouabain-resistant clones were observed in mock transfected cells. These results demonstrate that the introduced murine α_1 subunit cDNA confers ouabain resistance to the recipient cells and imply that the murine α_1 subunit is capable of forming an active Na^+,K^+-ATPase by association with the β subunit and other components contributed by the host cell. Ouabain-resistant colonies were not produced by transfection of clone pSVα3.0, which had a deletion of approximately 250 bp encoding the COOH-terminus of the α_1 subunit and 350 bp of the 3′ untranslated region. This result indicates that a cDNA encoding a complete polypeptide chain is required for biological activity in this assay.

Table 1. Efficiency of transfection of mouse Na^+, K^+-ATPase α_1 subunit cDNA clones into ouabain-sensitive monkey cells. DNA transfection of CV-1 African Green monkey cells was performed essentially as described (*15*). CV-1 cells were exposed to a calcium phosphate precipitate of pSV2α3.6, pSV2α3.0, or pSV2, or to calcium phosphate without DNA. Twenty-five hours later, cells were subcultured at dilutions of 1 to 4, 1 to 20, and 1 to 100, and ouabain was added to the cells 4 hours later to a final concentration of $10^{-6}M$. Two weeks after transfection, the colonies were fixed for 4 hours with 4% formaldehyde and stained with 0.1% methylene blue. ND, not determined.

DNA transfected	Colonies/μg DNA/10^6 cells		
	Expt. 1	Expt. 2	Expt. 3
pSV2α3.6	200	337	650
pSV2α3.0	ND	ND	0
pSV2	0	0	ND
None	0	0	ND

To confirm that the ouabain-resistant phenotype resulted from the introduction of the murine α_1 subunit cDNA construct, genomic DNA was isolated from ouabain-resistant transfectants, digested with the restriction enzyme Bam HI, and hybridized to a probe derived from a 0.95-kb internal Bam HI fragment of mbα69 (Fig. 2). The presence of the cDNA construct in all ouabain-resistant transfectants was indicated by hybridization to the probe of a 0.95-kb DNA fragment. The complete correlation between the presence of α_1 subunit cDNA in all transfectants and the high level of ouabain resistance of the transfected cells strongly supports the view that the α_1 cDNA indeed confers ouabain resistance to recipient CV-1 cells. Equal intensity of hybridization to an 11-kb Bam HI fragment, corresponding to the endogenous monkey α_1 subunit gene, was observed in all cell lines tested (including untransfected, ouabain-sensitive CV-1 cells). Therefore, amplification of the endogenous monkey α_1 subunit gene does not contribute to the ouabain resistance phenotype of the transfectants. To determine whether ouabain resistance could be related to increased expression levels of either introduced or endogenous α_1 subunit messenger RNA (mRNA), the total level of α_1 subunit mRNA was determined by Northern blotting and hybridization specific for α_1 subunit mRNA was quantitated by densitometry (Table 2). No significant differences in total α_1 subunit mRNA expression levels were detected between the ouabain-resistant transfectants and ouabain-sensitive controls, indicating that the ouabain resistance of the transfectants was not due to a major increase in expression level of the α_1 subunit gene.

Table 2. Expression of α_1 subunit mRNA in CV-1 control cells and cells transfected with pSV2α3.6 DNA. Total cytoplasmic RNA (10 μg) was separated by electrophoresis in a 1% agarose gel containing 7% formaldehyde, $0.02M$ MOPS (*p*H 7.0), $0.05M$ sodium acetate, and $0.001M$ EDTA. The RNA was transferred to a Gene Screen Plus membrane (Dupont) and the blot was probed with 10^7 count/min of a ^{32}P-labeled 0.95-kb internal Bam HI fragment from the mbα69 clone (specific activity 2×10^8 count/min per microgram of DNA). Blot hybridization and washing conditions were as described (*17*). Laser densitometry scanning (LKB Ultroscan) of an autoradiogram of the filter was used to quantitate the α_1 subunit mRNA level.

Cell line	Relative level of α_1 subunit mRNA
CV-1	1.0
D 5.2	1.4
D 6.1	1.3
D 7.3	1.2
E 4.1	1.4
E 5.5	1.6

The transfer of ouabain resistance by murine Na^+,K^+-ATPase α_1 subunit cDNA demonstrates that differential ouabain sensitivity between primate and rodent cells can be directly ascribed to primary sequence differences between the primate and rodent α_1 subunit genes. These results also suggest that the murine α_1 subunit is capable of association with the monkey β subunit to form a functional Na^+,K^+-ATPase.

The gene encoding the α_1 subunit of the Na^+,K^+-ATPase is a member of a multigene family. Three genes (*Atpa-1, Atpa-2,* and *Atpa-3*) encoding α subunit isoforms have been described in the mouse (*6*). In rat brain, Sweadner has demonstrated differential ouabain sensitivity of two Na^+,K^+-ATPase isoforms (*16*). However, the genetic basis for differential ouabain sensitivity among Na^+,K^+-ATPase isoforms has not been established. The differences in ouabain sensitivity of the Na^+,K^+-ATPase isoforms may reflect differences in the primary sequence of the α subunit polypeptides (*4*). It will clearly be of interest to determine how the DNA sequences which encode each isoform lead to differences in the ouabain sensitivity of the Na^+,K^+-ATPase. The results presented here provide a strategy for addressing this question. The construction of chimeric cDNA molecules between ouabain-resistant and ouabain-sensitive α subunit cDNAs should permit definitive identification of the sites within the α subunit that interact with ouabain or are responsible for differential ouabain sensitivity. The localization of sites within the α subunit that interact with ouabain would provide a valuable

tool in the study of Na^+,K^+-ATPase structure and mechanism. The understanding of cardiac glycoside/enzyme interaction also has practical significance because of the therapeutic use of this class of compounds in the treatment of congestive heart failure.

The ability to assess the biological activity of the Na^+,K^+-ATPase α_1 subunit gene via direct DNA transfer raises two additional points of interest. The construction of chimeric α subunit cDNAs, coupled with the application of site-directed mutagenesis, should allow the identification of other functional domains within the Na^+,K^+-ATPase. This approach should also prove useful for defining the functional differences between α subunit isoforms. Finally, our results demonstrate that the full-length mouse α_1 subunit cDNA can be used as a dominant selectable marker for somatic cell genetic studies utilizing primate and other ouabain-sensitive cells.

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Short Interval Time Measurement by a Parasitoid Wasp

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The number of eggs laid by the parasitoid wasp *Trichogramma* varies with host volume. The duration of the wasp's initial transit across the host surface during host examination is used to determine the number of eggs laid. A 2.5-second reduction in initial transit resulted in a 30% reduction in eggs oviposited, demonstrating that these wasps measure short time intervals. This measure is used for progeny allocation independent of host body size.

EACH YEAR A GREATER NUMBER OF *Trichogramma* (*1*) wasps are reared for biological control applications than any other animal used by man (*2*). These parasitoids have been used in massive release programs against a variety of pest insects, including the cotton bollworm (*Heliothis zea* Boddie) (*3*), the European corn borer (*Ostrinia nubilalis* Hb.) (*4*), and the sugarcane borer (*Diatraea saccharalis* Guilding) (*2, 4*). Although minute (adult length, 0.3 to 0.8 mm), *Trichogramma* display a range of complex behaviors. Since 1982 we have investigated the behavior and sensory physiology of *Trichogramma minutum* Riley to determine methods of improving their efficacy in biological control. We have also used *Trichogramma* as a vehicle for understanding the mechanisms that underlie the processing of mechanosensory information in the nervous system.

Trichogramma develop as parasitoids within the eggs of various other insects. The host eggs differ considerably in size, ranging from 0.3 mm to more than 2.5 mm in diameter. The size of the host determines the number of eggs (clutch size) laid by *Trichogramma* (*5–7*). Fewer eggs are allocated to smaller hosts, thereby minimizing larval competition and mortality resulting from limited nutrients and space (*8*). However, if too few eggs are laid into each host, the wasp may be unable to find sufficient hosts to dispose of its egg complement during its 2- to 6-day adult life span. In addition, sufficient larvae must develop within the host to consume most of the contents, as excess host residues can increase larval mortality (*9*). As a result, *Trichogramma* adjust the number of progeny to host volume, increasing clutch size proportionally with host size (*6, 7*).

Until recently, the mechanism by which *Trichogramma* measure host volume was unknown. In an earlier study (*7*), we found that the response of *Trichogramma* to host volume depends on mechanosensory rather than visual or chemical cues. The wasps allocate fewer progeny to *Manduca sexta* (L.) (*10*) hosts partially embedded in the substrate than to fully exposed hosts of the same species, both in daylight and in complete darkness. Since both embedded and fully exposed hosts have the same diameter, internal content, surface odor, and texture, the wasps cannot use these parameters to discriminate between hosts that differ only in exposed surface area (*7*).

To assess the use of external cues by the wasp, we performed a detailed analysis of its behavior on the host surface. Before oviposition, the wasp examines the host surface by walking over it, simultaneously drumming the surface with its antennae. When it encounters the juncture between the host and substrate, it makes an abrupt turn and continues its examination of the host surface (*5–7*). During its walk, the wasp may make many such contacts and turns while remaining on the host surface. Several of the parameters of this examination walk, including the number and frequency of substrate contacts and the intervals between them, depend on the exposed surface area of the host. Total time spent examining the host surface, however, is set by the wasp in response to host curvature, not to exposed surface area (*7*).

To determine which parameter was used by the wasps to set clutch size, we observed individual *Trichogramma* (*11*) during host examination on spherical *M. sexta* eggs (*12*) and recorded the frequency and intervals between substrate contacts and turns (*13*). These data were subsequently compared with the number of progeny allocated to the host (*14, 15*). Seven parameters were considered: total number of substrate contacts, mean interval between contacts, interval between last contact and oviposition, longest and shortest interval between contacts, total interval between first three contacts, and the interval between the first contact with the host and the first substrate contact (initial transit duration). Of these, only the initial transit duration showed a significant linear relation with progeny allocation [slope,

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