A *Drosophila* behavioral mutant, *down and out* (*dao*), is defective in an essential regulator of Erg potassium channels

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To signal properly, excitable cells must establish and maintain the correct balance of various types of ion channels that increase or decrease membrane excitability. The mechanisms by which this balance is regulated remain largely unknown. Here, we describe a regulatory mechanism uncovered by a *Drosophila* behavioral mutant, *down and out* (*dao*). At elevated temperatures, *dao* loss-of-function mutants exhibit seizures associated with spontaneous bursts of neural activity. This phenotype closely resembles that of *seizure* mutations, which impair activity of ether-a-go-go-related gene (Erg)-type potassium channels. Conversely, neural overexpression of wild-type Dao confers dominant temperature-sensitive paralysis with kinetics reminiscent of *paralytic* sodium-channel mutants. The over-expression phenotype of *dao* is suppressed in a *seizure* mutant background, suggesting that Dao acts by an effect on Erg channels. In support of this hypothesis, functional expression of Erg channels in a heterologous system is dependent on the presence of Dao. These results indicate that Dao has an important role in establishing the proper level of neuronal membrane excitability by regulating functional expression of Erg channels.

hERG potassium channels | membrane excitability | neurogenetics | seizure

Proper electrical signaling requires an appropriate balance of inward and outward currents, which, in turn, are dependent on the relative densities of different types of ion channels. However, the precise mechanisms that establish and maintain this balance remain largely unknown. Screening for behavioral mutations in *Drosophila* that perturb neural signaling has been an effective means of identifying key proteins and cellular pathways that regulate excitability. Particularly useful for such studies have been temperature-sensitive (ts) paralytic mutants including *paralytic* (*para*) and *slowpoke* (*slo*), which encode voltage-gated sodium channels and calcium-activated potassium channels, respectively, and *seizure* (*sei*), which encodes the *Drosophila* ortholog of the voltage-gated human ether-a-go-go-related gene (hERG) potassium channel (1–4).

Genetic analysis of other ts paralytics enabled identification of previously unknown regulators of sodium-channel activity. Characterization of *no action potential* (*nap*) and its genetic interaction with *para* led to the discovery that the RNA helicase encoded by *nap* plays an important role in RNA editing of the *para* transcript (5, 6). The resultant missplicing of the *para* transcript in a *nap* background leads to the generation of defective *para* mRNAs with a consequent deficit in sodium-channel levels. *Temperature-induced paralysis* (*tipE*) mutants are also similar in phenotype to *nap* and *para*. TipE is required for functional expression of *para* channels in *Xenopus* oocytes and likely acts as a regulatory or structural component of these channels (7, 8).

Here, we use a similar genetic approach to identify a regulator of Erg-type potassium channels. In *Drosophila*, mutations of *sei* cause neuronal hyperexcitability and seizures on exposure to elevated temperatures (9–11). In humans, mutations of *hERG*, which encodes channels required for cardiac repolarization (12, 13), cause a familial form of cardiac arrhythmia associated with ventricular fibrillation and sudden death (14). Thus, understanding mechanisms that regulate expression of Erg channels is of broad biological and medical significance. We identified *down and out* (*dao*) as a ts paralytic mutant that confers electrophysiological and behavioral phenotypes similar to *sei*. However, *dao* is unique among ts paralytic mutants, because overexpression of the wild-type gene causes ts paralysis reminiscent of mutations affecting sodium channels. This overexpression phenotype is suppressed in a *sei* mutant background, suggesting that Dao acts through an effect on Erg channels. In support of this hypothesis, functional expression of Erg channels in a heterologous system is absolutely dependent on the presence of Dao. These results indicate that Dao has a critical role in regulating functional expression of Erg channels, thereby controlling levels of neuronal membrane excitability.

Results

Isolation and Characterization of *dao*. Among our existing collection of ts paralytic mutants, we identified two independently generated recessive alleles (*b4* and *g1*) of a gene located on the second chromosome that we named *dao*. Although dao^{b4} and dao^{g1} seem essentially normal at room temperature (20–22 °C), mutant homozygotes and the heteroallelic combination undergo seizure-like behavior with bouts of uncontrolled flight motor activity followed by paralysis when suddenly shifted to 37 °C (Fig. 1*A*). This behavior is paralleled by bursts of spontaneous activity in intracellular recordings from the dorsal longitudinal muscles (DLMs) at elevated temperatures (Fig. 1*B*). These behavioral and electrophysiological phenotypes of *dao* are very similar to *sei* mutants, which disrupt Erg-type K^+ channels (3, 4, 9–11). However, complementation tests revealed that *dao* and *sei* affect different genes.

Mapping and Molecular Identification of *dao*. The ts paralytic phenotype of *dao* is uncovered by *Df(2L)do1* and *Df(2L)rd9*, delimiting *dao* to the cytological interval 35C3-5 that encompasses three genes (Fig. 2*A*). We identified *CG15267* as the *dao* locus on the basis of the following evidence. *SH901*, a transposon inserted between two of the candidate genes (*CG15267* and *CG3994*) (15) 8 bp upstream of the transcriptional start site of *CG15267*, complements *dao*. However, a small deletion generated by imprecise excision of this element that extends distally from the insertion site fails to complement *dao* (Fig. 2*B*). This excision allele, dao^{C7}, is viable as a homozygote, and it exhibits a ts paralytic phenotype identical with that of other *dao* alleles. Subsequently, we found that dao^{b4} and dao^{g1} also had alterations in the *CG15267* nucleotide sequence (Fig. 2*B*). dao^{b4} contains a small upstream deletion in the same

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The authors declare no conflict of interest.

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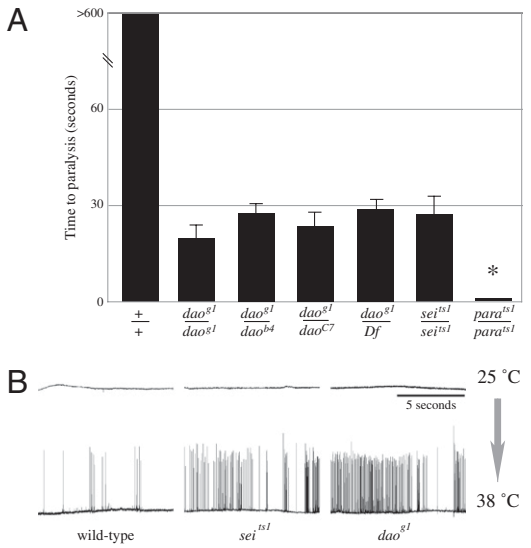

Fig. 1. *dao* exhibits ts seizures and paralysis similar to *sei* mutants. (*A*) After exposure to 39 °C, *dao* homozygotes and heteroallelic combinations undergo initial seizures followed by paralysis with kinetics similar to that of *sei* mutants. *Df* is *Df(2)rd9*. In contrast, *para* mutants, which affect sodium channels, undergo much more rapid paralysis (asterisk indicates $P < 0.05$). Values represent mean time until loss of movement. (*B*) Electrophysiological recordings from the dorsal longitudinal muscles of control, and mutant animals are shown at permissive and nonpermissive temperatures. Bursting activity is observed in *dao* and *sei* mutants when the temperature is increased to greater than 37 °C, whereas control animals exhibit little to no prolonged bursting activity.

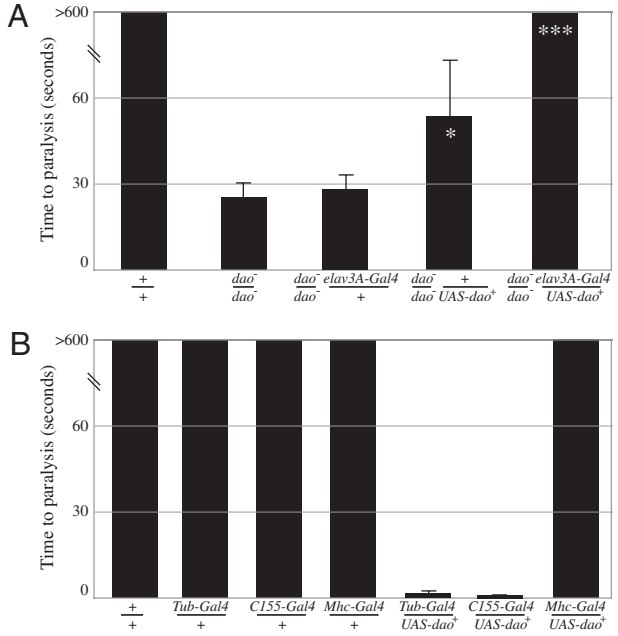

Fig. 3. Strong neuronal overexpression of dao^+ causes rapid ts paralysis. (*A*) Expression of *UAS-dao* under the control of the weak neuronal driver, *elav3A-Gal4*, rescues the ts paralytic phenotype of *dao* mutants. (*B*) In contrast, high-level expression of *UAS-dao* under the control of *Tub-Gal4* or the strong neuronal driver, *C155-Gal4*, results in a gain-of-function, ts paralytic phenotype with rapid kinetics reminiscent of *para* mutants (Fig. 1). This phenotype is not observed when *UAS-dao* is expressed under control of the strong muscle driver *Mhc-Gal4*.

region as dao^{C7}, and dao^{g1} has an 8-bp deletion in the fourth exon that results in a frameshift and the introduction of a premature stop codon. Finally, the *CG15267* transcript is undetectable by RT-PCR in dao^{g1}, most likely owing to nonsense-mediated mRNA decay (16), whereas *CG3994* transcripts were present at normal levels.

To confirm identification of CG15267 as the *dao* locus, we performed transgenic rescue experiments using the upstream activation sequence (UAS-GAL4) system, whereby controlled expression of the yeast transcription factor GAL4 sequentially drives expression of transgenes linked to the UAS promoter region. We found that a *UAS-CG15267* cDNA construct was able to partially rescue the behavioral phenotype of *dao*, even in the absence of any GAL4 driver (Fig. 3*A*). This rescue is dependent on the particular site of insertion of the transgenes and the number of transgenes present. This rescue likely results from weak or "leaky" expression of the transgene and suggests that a very low level of expression of the gene is sufficient to confer a wild-type phenotype. More complete rescue was achieved by driving expression of *UAS-CG15267* with the weak neuronal-specific *elav3A-GAL4* driver (Fig. 3*A*). Rescue was specific for *CG15267*, because we were unable to rescue *dao* mutants with transgenic constructs for the neighboring gene, *CG3994*. We conclude that *CG15267* is the *dao* locus.

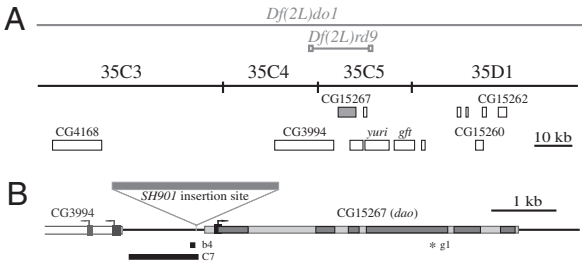

Fig. 2. Mapping and molecular identification of *dao*. (*A*) *Df(2L)do1* and *Df(2L)rd9* both uncover the ts paralytic phenotype of *dao*. PCR analysis of genomic DNA from homozygous *Df(2L)rd9* animals established the location of the indicated breakpoints. (*B*) The P element insertion line *SH(l)901*, containing an insert between CG3994 and CG15267, complemented *dao*. However, small deletions generated through imprecise excision of this element, including *C7*, fail to complement *dao*. Sequence analysis identified a smaller overlapping deletion in dao^{b4} and an 8-bp deletion (denoted by the asterisk) in the coding region in dao^{g1} that generates a premature stop codon.

dao encodes an 882 amino acid protein containing two recognizable domains. The first is a myeloid, Nervy, and deformed epidermal autoregulatory factor-1 (DEAF-1) (MYND) domain that consists of a cluster of cysteine and histidine residues that form a zinc-finger motif (17, 18). The second is a tetratricopeptide repeat (TPR) motif, which is a degenerate, 34 amino acid repeat with eight consensus residues (19). TPRs are helical domains that mediate protein–protein interactions in a variety of proteins. Whereas the *dao* sequence is strongly conserved within the *Drosophila* genus (e.g., $E = e^{-27}$ for *D. grimshawi* in a BLAST search), it shows a remarkable lack of sequence similarity with any genes in species outside this group (e.g., $E = e^{-06}$ for *Anopheles gambiae* and $E = 0.05$ for *Apis mellifera*). We were unable to identify any vertebrate homologs of Dao in database searches.

Overexpression of Dao Causes Rapid ts Paralysis. Unexpectedly, we found that high-level overexpression of *UAS-dao*$^+$ under control of the strong *C155-GAL4* pan-neural driver, either in a *dao* mutant or wild-type background, causes a ts paralytic phenotype with rapid kinetics of paralysis and recovery that are reminiscent of *para* and other mutants with impaired sodium-channel activity (1) (Fig. 3*B*). In contrast, overexpression of Dao in muscles has no detectable phenotype (Fig. 3*B*). Thus, loss-of-function mutations of *dao* cause a ts paralytic phenotype associated with hyperexcitability, whereas neuronal overexpression of Dao causes a ts

paralytic phenotype resembling that of mutants with impaired axonal conduction or hypoexcitability.

Because the phenotype caused by neuronal overexpression of Dao resembles *para*, we tested whether or not it could also suppress ether-sensitive leg-shaking behavior. We found that Dao overexpression did not suppress leg shaking of *ether-a-go-go Shaker* (*eag Sh*) flies; this suggests that, despite phenotypic similarities with *para* mutants, elevated Dao does not reduce sodium-channel expression or function.

Dao Acts Through *sei*-Encoded Erg-Type Potassium Channels. The behavioral and electrophysiological phenotypes of *dao* and *sei* mutants are strikingly similar. Moreover, *dao sei* double mutants showed no additive effects, suggesting that both mutations disrupt a common pathway (Fig. 4 *Left*). One possibility is that expression or activity of Erg-type potassium channels encoded by *sei* is impaired in a *dao* mutant background. If so, neuronal overexpression of UAS-sei^{+} might be able to suppress *dao* mutant phenotypes. Although UAS-sei^{+} transgenes can rescue *sei* mutants, we found that they did not suppress *dao* (time to paralysis = 25.5 ± 3.8 seconds) (Fig. 4). However, if Dao is required for efficient expression or function of Erg channels, it might not be possible to achieve sufficiently high levels of Erg expression in a *dao* mutant background to alleviate ts paralysis of *dao*.

We tested the effect of *dao* on Erg-channel expression in a different manner by asking whether the rapid ts paralysis associated with overexpression of Dao was dependent on the presence of functional Erg channels. Strikingly, when Dao was overexpressed in a *sei* mutant background, the flies exhibited only the *sei* mutant phenotype with seizures followed by paralysis after 27.3 ± 4.3 seconds at 37 °C. The Dao-dependent paralysis that occurs in <1 second in all other backgrounds was completely eliminated (Fig. 4 *Right*). This result shows that rapid ts paralysis associated with Dao overexpression is dependent on expression of Erg channels; in the absence of these channels, Dao overexpression has no observable phenotypic effects. These results suggest that Dao is a regulator of Erg-channel expression or function and that *dao* loss-of-function phenotypes result from a deficit in the levels of functionally active Erg channels.

Dao Enables Heterologous Expression of Erg Channels. Although potassium channels encoded by *hERG*, the human ortholog of *sei*, and the closely related *Drosophila eag* have been successfully expressed in heterologous systems, it has not been possible to express the *sei*-encoded Erg channels in *Xenopus* oocytes (Fig. 5*A*). These results suggest that endogenous expression of *Drosophila* Erg channels in vivo may require an additional cofactor

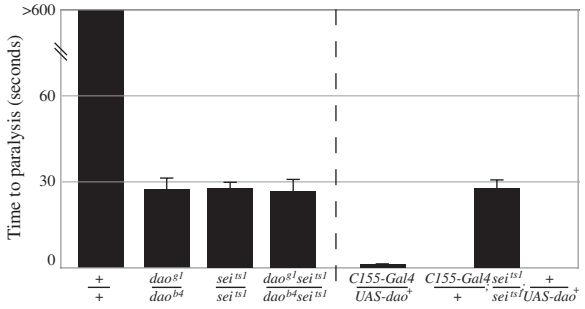

Fig. 4. The combination of *dao* and *sei* mutations does not enhance temperature sensitivity, seizure behavior, or kinetics of paralysis (*Left*). Rapid ts paralysis caused by overexpression of dao^{+} is dependent on *sei* (*Right*). In a *sei* mutant background, strong neuronal expression of dao^{+} does not cause rapid ts paralysis, and only the *sei* mutant phenotype is observed, indicating that rapid paralysis depends on *sei*-encoded Erg potassium channels.

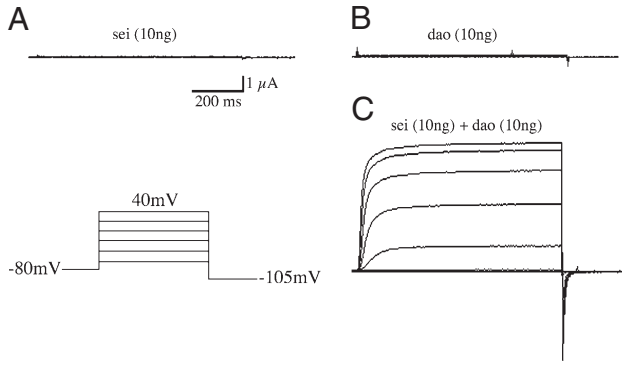

Fig. 5. Functional expression Erg-type potassium channels in a heterologous system requires Dao. *Xenopus* oocytes injected with the indicated RNA species were examined for ionic currents when depolarizing voltage increments (*A Lower*) were applied. No detectable currents are observed in oocytes injected only with *sei* (*A*) or *dao* (*B*) RNA. In contrast, oocytes injected with both RNA species (*C*) exhibit robust voltage-dependent potassium currents, showing that functional expression of Erg channels requires Dao.

that is lacking in heterologous systems. Our results identify Dao as a candidate for this role.

To test directly whether Dao can mediate expression of Erg channels, we performed coinjection experiments in *Xenopus* oocytes. Whereas injection of *sei* or *dao* mRNA alone did not produce detectable currents (Figs. 5 *A* and *B*), coinjection produced robust voltage-dependent potassium currents (Fig. 5*C*), which are similar to those resulting from expression of Eag channels. These results provide compelling evidence that Dao mediates functional expression of Erg channels.

Although these studies show that Dao is required for production of Erg currents, they do not reveal the point at which Dao acts. In principle, Dao could be required for processing, trafficking, membrane distribution, or biophysical function of Erg channels. To explore these possibilities, we examined the effect of Dao on the localization and distribution of the Erg protein in vivo. For these experiments, we expressed a GFP-tagged Erg construct in larval muscles using a myosin heavy chain GAL4 (*Mhc-GAL4*). The large size and distinct subcellular compartments of these cells facilitated precise localization of the tagged protein. In the absence of Dao, only faint GFP expression was detected, and it seemed to be confined to the endoplasmic reticulum (ER) (Fig. 6*A*). In contrast, expression of GFP-tagged Erg in combination with Dao results in strongly enhanced fluorescence in the muscle, which is particularly apparent in the subsynaptic reticulum (SSR), the highly convoluted plasma membrane enveloping synaptic boutons (Fig. 6 *B–D*). When GFP-tagged Dao is expressed by itself in larval muscles, it is distributed widely throughout the plasma membrane with no enrichment at the SSR (Fig. 6*E*), and this distribution is not strongly affected by coexpression of Erg. Taken together, these data suggest that Dao is most likely required for processing and/or trafficking of Erg channels to the plasma membrane.

Discussion

Dao Regulates Neuronal Excitability. Proper electrical signaling in excitable cells requires the correct balance of various ion channels. Beyond the transcriptional activity of genes encoding ion-channel subunits, a variety of posttranscriptional regulatory mechanisms will contribute to the relative densities and distribution of ion channels, including splicing and editing of channel transcripts, controlling translation, associating with auxiliary subunits, and processing, transport, and membrane stability of channel proteins. Forward genetic screens in *Drosophila* have uncovered many genes encoding ion channels as well as genes important in different regulatory mechanisms. Here, we have identified a gene that regulates func-

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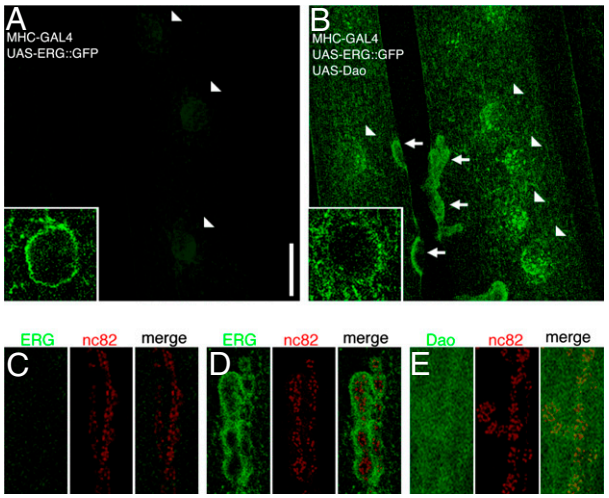

Fig. 6. Expression and localization of Erg channels in vivo is affected by Dao. The *Mhc-GAL4* driver was used to express a cDNA construct encoding a GFP-tagged Erg in larval body-wall muscles alone (*A*) or together with Dao (*B*). The GFP signal in *A* is faint and restricted to the endoplasmic reticulum (arrowheads). Coexpression of Dao along with GFP-Erg in these cells results in a robust GFP signal in the endoplasmic reticulum (arrowheads) and subsynaptic reticulum (arrows). (*C* and *D*) Higher magnification photomicrographs showing localization GFP-Erg channels in the absence (*C*) or presence (*D*) of coexpressed Dao. When Dao is coexpressed, Erg channels accumulate in the subsynaptic reticulum, which is the complex postsynaptic membranous network that envelopes presynaptic boutons. Active zones are marked with anti-nc82 antibody (red). (*E*). GFP-tagged Dao expressed in muscle is distributed at high levels throughout the plasma membrane. (Scale bar, 20 μm.)

tional expression of Erg channels. *dao* was discovered as a ts paralytic mutant whose convulsive behavior at elevated temperature closely resembles that of *sei* mutants, which disrupt Erg potassium channels. This phenotypic similarity suggested that *dao* might also affect these channels at some level. Consistent with this interpretation, *dao* mutants exhibit flight motor-pathway hyperexcitability at elevated temperatures similar to that of *sei*. In contrast with the loss-of-function phenotype, neuronal overexpression of dao^+ causes rapid and complete ts paralysis that is reminiscent of *para* sodium-channel mutants. Moreover, this overexpression phenotype is also strictly dependent on Erg potassium channels, because it is entirely ablated in a *sei* mutant background. The simplest interpretation of these phenotypes is that Dao exerts a dose-dependent effect on the production of functional Erg channels: loss of Dao results in a deficit of Erg channels and overexpression of Dao results in excessive levels of Erg channels. These phenotypes are analogous to those observed in the case of *para*, where increasing the gene dosage by one copy (e.g., 3 vs. 2) resulted in hyperexcitability similar to that of *Sh* mutants; however, reducing the gene dosage by one copy (e.g., 1 vs. 2) resulted in a reduction in membrane excitability (20). Thus, simply by increasing or decreasing the levels of wild-type sodium channels relative to potassium channels, it is possible to produce hyperexcitable and hypoexcitable behavioral and electrophysiological phenotypes, respectively. Similarly, our results suggest that decreasing or increasing the levels of Erg potassium channels relative to sodium channels is responsible for the observed *dao* loss-of-function and overexpression phenotypes. Interestingly, overexpression of a *UAS-sei*$^+$ cDNA itself does not produce the same rapid ts paralytic phenotype as overexpression of *dao*. One possibility is that expression of Erg channels cannot be driven to sufficiently high levels by this method to achieve the excess needed to produce the imbalance that leads to ts paralysis. Alternatively, the level of Dao protein present may set limits on the number of functional Erg channels incorporated into the plasma membrane. Thus, to create flies with high levels of functional Erg channels in neurons, it may be necessary to overexpress Dao. The fact that functional expression of Erg channels in *Xenopus* oocytes is absolutely dependent on the coexpression of Dao provides direct evidence that Dao plays an essential role in the production of functional Erg channels, as we inferred from the analysis of mutant phenotypes.

It is perhaps somewhat surprising that Dao shows no evolutionary conservation outside of the *Drosophila* genus. In other species, it is possible that other proteins of unrelated sequence provide the same biological function as Dao. An analogous situation is found in the case of TipE, a protein required for function expression of *Drosophila* sodium channels in vivo and in *Xenopus* oocytes (7, 8, 21–23). Like Dao, TipE shows no evolutionary conservation outside of *Drosophila*. It is unclear why these proteins should have evolved so rapidly.

Role of Dao in Erg-Channel Expression. Although expression of Erg channels in a heterologous system is dependent on Dao, the exact function of Dao is still unknown. Dao is a globular protein containing two domains that may provide functional insights. The first is a MYND zinc-finger domain that has been suggested to mediate protein–protein interactions rather than DNA binding (18). The second domain is a TPR motif. TPR motifs are thought to mediate protein–protein interactions, and other TPR-containing proteins have functions in cell-cycle control and protein transport and are used as co-chaperones (19, 24). Recent studies show that FKBP38, which contains a TPR motif, associates with hERG channels in a cardiac-derived cell line and in HEK-293 cells (25). hERG trafficking is hampered when FKBP38 levels are reduced by small interfering RNA, and the trafficking of an hERG mutant protein associated with long QT (LQT) syndrome is partially rescued when FKBP38 is overexpressed. Like FKBP38, Dao may facilitate maturation or membrane localization of Erg channels.

When a tagged Erg construct is coexpressed with Dao in larval muscles, Erg channels are present at substantial levels and accumulate in the SSR in synaptic regions. In contrast, in the absence of Dao, only low levels of Erg protein are detectable, and the protein seems to be largely confined to the ER. Dao itself is widely expressed throughout the plasma membrane with no preferential localization in synaptic regions either in the presence or absence of Erg channels. Taken together, these data suggest that Dao is not a subunit of Erg channels and does not colocalize with Erg, but rather, it is important in facilitating the production of mature Erg channels and/or ensuring their proper subcellular distribution.

Identification and characterization of proteins mediating expression of Erg-type potassium channels is of particular interest, because they mediate cardiac repolarization and serve as targets of inherited or acquired LQT syndrome, a potentially lethal form of cardiac arrhythmia (12–14). Some familial forms of LQT syndrome are caused by the production of mutant hERG proteins that are inappropriately retained in the ER (26–28). Remarkably, this deficit can sometimes be overcome by treatment with certain hERG-binding drugs that somehow promote the subsequent processing and transport of the mutant channels to the plasma membrane, where they function normally (26, 29, 30). Dao may be an endogenous factor that functions in an essentially similar manner to promote the appearance of Erg channels at the plasma membrane. Identification of human proteins playing equivalent roles in expression of hERG channels would be of substantial interest as therapeutic targets.

Materials and Methods

Fly Strains. Fly stocks were cultured on cornmeal-molasses medium at 22–25 °C. Strains used in this study include $para^{ts1}$, $para^{LK5}$, eag^1, Sh^{133}, sei^{ts1}, *Df(2L)do1*, *Df(2L)rd9*, *pCASPER-sei*$^+$ genomic lines 1–3 and 4–4, *UAS-sei*$^+$ cDNA line 110, and *l(2)SH901* (15). Transgenic lines were generated by P element-mediated germline transformation as described (31). Briefly, dechorionated embryos expressing transposase from the stable genomic Δ2–3 element were injected

with plasmid constructs at 0.8 mg/mL in 5 mM KCl and 0.1 mM PO4 (pH 7.8) with 3% Durkee green food coloring. The Gal4-UAS system (32) was used for tissue-specific expression of cDNA constructs cloned into the pUAST vector. Gal4 driver lines used in this study were *C155-Gal4* (high-level neuronal expression), *elav3A-Gal4* (low-level neuronal expression), tubulin (*Tub-Gal4*), and myosin heavy chain (*Mhc-Gal4*). Unless otherwise stated, wild type and control refer to Canton S.

Behavioral Tests. Adults were collected under CO_2 at 0–3 days after eclosion and kept for 1–2 days before behavioral analysis. Animals were aspirated from vials at room temperature into vials immersed in a 39 °C water bath. Time to seizure, paralysis, and recovery after return to room temperature were recorded for each genotype.

***Drosophila* Electrophysiology.** Recordings from the giant-fiber (GF) escape circuit were performed as previously described (33–35). Briefly, the animals were etherized, mounted in clay with the head and thorax exposed, and then placed in a humidified chamber for at least 20 minutes to allow recovery. Glass microelectrodes were used to record potentials from the DLMs, and the reference electrode was placed in the eye. A sampling rate of 650 microseconds was used with an Axopatch1D amplifier in current clamp = 0 configuration to record endogenous activity. Using a Cambion Bipolar temperature-controller plate, the temperature was quickly increased from 21 °C to 39 °C, and activity was monitored.

Expression of *sei* and *dao* in *Xenopus* Oocytes. The *sei* and *dao* cRNA was synthesized in vitro from linearized templates using an mMESSAGE mMachine kit (Ambion). RNA was diluted in sterile water to working concentrations (~10 ng). Stage V oocytes from adult female *Xenopus laevis* were defolliculated with collagenase treatment. Defolliculated oocytes were injected with 10 ng of each type of RNA. Oocytes were cultured at 18 °C in storage solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM Hepes supplemented with 10 μg/mL gentamicin and 1 mg/mL BSA at pH 7.4). After 3 days, currents were recorded using the two-electrode voltage-clamp technique. PClamp software was used for data acquisition. The resistance of the electrodes was ~1 M Ohm in 2 M KCl. The bath solution contained 94 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 0.3 mM $CaCl_2$, and 5 mM Hepes (pH 7.4). Holding potential was −80 mV, and all recordings were performed at room temperature, roughly 22–24 °C. No leak subtraction was used.

Immunohistochemistry. Anti-GFP was used at 1:500 (A-6455; Molecular Probes/Invitrogen), and anti-nc82 was used at 1:100 (A. Hofbauer). Secondary antibodies Alexa-488 anti-RABBIT (A-6455; Molecular Probes/Invitrogen) and Alexa-568 anti-MOUSE (A-11031) were both used at 1:500. Fluorescent images were acquired on a Zeiss LSM 510 Meta confocal microscope.

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1. Loughney K, Kreber R, Ganetzky B (1989) Molecular analysis of the *para* locus, a sodium channel gene in *Drosophila*. *Cell* 58:1143–1154.
2. Atkinson NS, Robertson GA, Ganetzky B (1991) A component of calcium-activated potassium channels encoded by the Drosophila slo locus. *Science* 253:551–555.
3. Titus SA, Warmke JW, Ganetzky B (1997) The *Drosophila* erg K^+ channel polypeptide is encoded by the *seizure* locus. *J Neurosci* 17:875–881.
4. Wang XJ, Reynolds ER, Déak P, Hall LM (1997) The *seizure* locus encodes the *Drosophila* homolog of the HERG potassium channel. *J Neurosci* 17:882–890.
5. Hanrahan CJ, Palladino MJ, Ganetzky B, Reenan RA (2000) RNA editing of the *Drosophila* para $Na^{(+)}$ channel transcript. Evolutionary conservation and developmental regulation. *Genetics* 155:1149–1160.
6. Seeburg PH (2000) RNA helicase participates in the editing game. *Neuron* 25:261–263.
7. Feng G, Deák P, Chopra M, Hall LM (1995) Cloning and functional analysis of TipE, a novel membrane protein that enhances *Drosophila* para sodium channel function. *Cell* 82:1001–1011.
8. Warmke JW, et al. (1997) Functional expression of *Drosophila para* sodium channels. Modulation by the membrane protein TipE and toxin pharmacology. *J Gen Physiol* 110:119–133.
9. Jackson FR, Wilson SD, Strichartz GR, Hall LM (1984) Two types of mutants affecting voltage-sensitive sodium channels in *Drosophila melanogaster*. *Nature* 308:189–191.
10. Jackson FR, Gitschier J, Strichartz GR, Hall LM (1985) Genetic modifications of voltage-sensitive sodium channels in *Drosophila*: Gene dosage studies of the *seizure* locus. *J Neurosci* 5:1144–1151.
11. Elkins T, Ganetzky B (1990) Conduction in the giant nerve fiber pathway in temperature-sensitive paralytic mutants of *Drosophila*. *J Neurogenet* 6:207–219.
12. Sanguinetti MC, Jiang C, Curran ME, Keating MT (1995) A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. *Cell* 81:299–307.
13. Trudeau MC, Warmke JW, Ganetzky B, Robertson GA (1995) HERG, a human inward rectifier in the voltage-gated potassium channel family. *Science* 269:92–95.
14. Curran ME, et al. (1995) A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell* 80:795–803.
15. Oh SW, et al. (2003) A P-element insertion screen identified mutations in 455 novel essential genes in *Drosophila*. *Genetics* 163:195–201.
16. Chang YF, Imam JS, Wilkinson MF (2007) The nonsense-mediated decay RNA surveillance pathway. *Annu Rev Biochem* 76:51–74.
17. Feinstein PG, Kornfeld K, Hogness DS, Mann RS (1995) Identification of homeotic target genes in *Drosophila melanogaster* including *nervy*, a proto-oncogene homologue. *Genetics* 140:573–586.
18. Gross CT, McGinnis W (1996) DEAF-1, a novel protein that binds an essential region in a Deformed response element. *EMBO J* 15:1961–1970.
19. Lamb JR, Tugendreich S, Hieter P (1995) Tetratrico peptide repeat interactions: To TPR or not to TPR? *Trends Biochem Sci* 20:257–259.
20. Stern M, Kreber R, Ganetzky B (1990) Effects of *para* gene dosage on behavior and axonal excitability in *Drosophila*. *Genetics* 124:133–143.
21. Jackson FR, Wilson SD, Hall LM (1986) The *tip-E* mutation of *Drosophila* decreases saxitoxin binding and interacts with other mutations affecting nerve membrane excitability. *J Neurogenet* 3:1–17.
22. Kulkarni S, Padhye A (1982) Temperature sensitive paralytic mutations on the 2nd and 3rd chromosomes of *Drosophila melanogaster*. *Genet Res* 40:191–200.
23. Ganetzky B (1986) Neurogenetic analysis of *Drosophila* mutations affecting sodium channels: Synergistic effects on viability and nerve conduction in double mutants involving *tip-E*. *J Neurogenet* 3:19–31.
24. Blatch GL, Lässle M (1999) The tetratricopeptide repeat: A structural motif mediating protein-protein interactions. *Bioessays* 21:932–939.
25. Walker VE, Atanasiu R, Lam H, Shrier A (2007) Co-chaperone FKBP38 promotes HERG trafficking. *J Biol Chem* 282:23509–23516.
26. Ficker E, et al. (2000) Novel characteristics of a misprocessed mutant HERG channel linked to hereditary long QT syndrome. *Am J Physiol Heart Circ Physiol* 279:H1748–H1756.
27. Furutani M, et al. (1999) Novel mechanism associated with an inherited cardiac arrhythmia: Defective protein trafficking by the mutant HERG (G601S) potassium channel. *Circulation* 99:2290–2294.
28. Anderson CL, et al. (2006) Most LQT2 mutations reduce Kv11.1 (hERG) current by a class 2 (trafficking-deficient) mechanism. *Circulation* 113:365–373.
29. Rajamani S, Anderson CL, Anson BD, January CT (2002) Pharmacological rescue of human $K^{(+)}$ channel long-QT2 mutations: Human ether-a-go-go-related gene rescue without block. *Circulation* 105:2830–2835.
30. Zhou Z, Gong Q, January CT (1999) Correction of defective protein trafficking of a mutant HERG potassium channel in human long QT syndrome. Pharmacological and temperature effects. *J Biol Chem* 274:31123–31126.
31. Robertson HM, et al. (1988) A stable genomic source of P element transposase in Drosophila melanogaster. *Genetics* 118:461–470.
32. Brand AH, Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118:401–415.
33. Kuebler D, Tanouye MA (2000) Modifications of seizure susceptibility in *Drosophila*. *J Neurophysiol* 83:998–1009.
34. Lee J, Wu CF (2002) Electroconvulsive seizure behavior in *Drosophila*: Analysis of the physiological repertoire underlying a stereotyped action pattern in bang-sensitive mutants. *J Neurosci* 22:11065–11079.
35. Fergestad T, Bostwick B, Ganetzky B (2006) Metabolic disruption in *Drosophila* bang-sensitive seizure mutants. *Genetics* 173:1357–1364.