The Genetic Architecture of Degenerin/Epithelial Sodium Channels in *Drosophila*

Kathleen M. Zelle, Beika Lu, 1,2 Sarah C. Pyfrom, and Yehuda Ben-Shahar³ Department of Biology, Washington University in St. Louis, St. Louis, Missouri 63130

ABSTRACT Degenerin/epithelial sodium channels (DEG/ENaC) represent a large family of animal-specific membrane proteins. Although the physiological functions of most family members are not known, some have been shown to act as nonvoltage gated, amiloride-sensitive sodium channels. The DEG/ENaC family is exceptionally large in genomes of Drosophila species relative to vertebrates and other insects. To elucidate the evolutionary history of the DEG/ENaC family in Drosophila, we took advantage of the genomic and genetic information available for 12 Drosophila species that represent all the major species groups in the Drosophila clade. We have identified 31 family members (termed pickpocket genes) in Drosophila melanogaster, which can be divided into six subfamilies, which are represented in all 12 species. Structure prediction analyses suggested that some subunits evolved unique structural features in the large extracellular domain, possibly supporting mechanosensory functions. This finding is further supported by experimental data that show that both ppk1 and ppk26 are expressed in multidendritic neurons, which can sense mechanical nociceptive stimuli in larvae. We also identified representative genes from five of the six DEG/ ENaC subfamilies in a mosquito genome, suggesting that the core DEG/ENaC subfamilies were already present early in the dipteran radiation. Spatial and temporal analyses of expression patterns of the various pickpocket genes indicated that paralogous genes often show very different expression patterns, possibly indicating that gene duplication events have led to new physiological or cellular functions rather than redundancy. In summary, our analyses support a rapid early diversification of the DEG/ENaC family in Diptera followed by physiological and/or cellular specialization. Some members of the family may have diversified to support the physiological functions of a yet unknown class of ligands.

KEYWORDS

Degenerin/ epithelial sodium channel chemosensation mechanosensation phylogeny fruit fly

All cells use a complex array of ion channels to maintain the appropriate ionic gradients across membrane barriers, including the plasma membrane and intracellular compartments and organelles. One enigmatic group of ion channels is the Degenerin/epithelial Na⁺ channel (DEG/ENaC) family. Although the physiological functions of most family members are not well understood, at least some members seem

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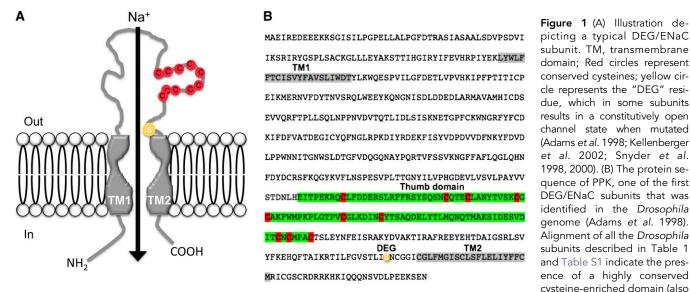
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¹These authors contributed equally to this work.

to act as nonvoltage-gated, amiloride-sensitive sodium channels (Bianchi and Driscoll 2002; Garty and Palmer 1997). Various natural ligands and mechanical stimuli can activate or modulate channel functions. These include the neuropeptides FMRFamide (Askwith et al. 2000; Durrnagel et al. 2010; Golubovic et al. 2007; Green et al. 1994; Kellenberger and Schild 2002; Lingueglia et al. 1995; Xie et al. 2003), FFamide, SFamide (Deval et al. 2003; Sherwood and Askwith, 2008, 2009), and dynorphin-related opioid peptides (Sherwood and Askwith 2009). In addition, some mammalian family members are gated by extracellular protons (Benson et al. 2002; Price et al. 2001; Waldmann et al. 1997; Xie et al. 2003; Xiong et al. 2004). Recently, several sulfhydryl compounds (Cho and Askwith 2007) and small polyamines such as agmatine (Yu et al. 2010) also were shown to modulate the channel functions of specific mammalian family members. Finally, data also support a role for specific DEG/ENaC subunits in pheromone-dependent behaviors as well as in chemosensory functions underlying male courtship behaviors in Drosophila (Ben-Shahar 2011; Ben-Shahar et al. 2007; 2010; Lin et al. 2005; Lu et al. 2012; Starostina et al. 2012; Thistle et al. 2012; Toda et al. 2012).

²Present address: School of Life Sciences and Technology, Tongji University, Shanghai 200092, China.

³Corresponding author: Department of Biology, Washington University in St. Louis, One Brookings Dr., St. Louis, MO 63130. E-mail: benshahary@wustl.edu



cysteine-enriched domain (also see Figure 7A, thumb domain), highlighted in green. Conserved cysteines are highlighted in red; DEG, a predicted "deg" residue, is highlighted in yellow. TM1 and TM2 represent the predicted transmembrane domains 1 and 2, respectively.

DEG/ENaC family members also have been implicated in mechanosensation in *Caenorhabditis elegans*, mammals, and *Drosophila* (Arnadottir *et al.* 2011; Bazopoulou *et al.* 2007; Geffeney *et al.* 2011; Lu *et al.* 2009; O'Hagan *et al.* 2005; Price *et al.* 2001; Simon *et al.* 2010; Tsubouchi *et al.* 2012; Zhang *et al.* 2004; Zhong *et al.*

2010). Together, these data indicate that DEG/ENaC channels have evolved to serve many different physiological functions, acting as ionotropic receptors to diverse extracellular stimuli.

Functional and structural studies of DEG/ENaC channels demonstrated that channels are likely hetero or homotrimeric (Benson *et al.*

■ Table 1 ppk genes identified in the Drosophila melanogaster genome

Name	Symbol	Alternative Name	CG No.	FB ID	Location
pickpocket 1	ppk	ppk1	CG3478	FBgn0020258	2L: 35B1-35B1
ripped pocket	rpk	ppk2	CG1058	FBgn0022981	3R: 82C5-82C5
pickpocket 3	ppk3		CG30181	FBgn0050181	2R: 59E3-59E3
Nach	Nach	ppk4	CG8178	FBgn0024319	2R: 53C14-53C14
pickpocket 5	ppk5		CG33289	FBgn0053289	3L: 78D5-78D5
pickpocket 6	ppk6		CG11209	FBgn0034489	2R: 56F11-56F11
pickpocket 7	ppk7		CG9499	FBgn0031802	2L: 26C3-26C3
pickpocket 8	ppk8		CG32792	FBgn0052792	X: 3D6-3D6
pickpocket 9	ppk9		CG34369	FBgn0085398	2R: 58A4-58A4
pickpocket 10	ppk10		CG34042	FBgn0065110	2L: 31E3-31E4
pickpocket 11	ppk11		CG34058	FBgn0065109	2L: 30C8-30C9
pickpocket 12	ppk12		CG10972	FBgn0034730	2R: 58E1-58E1
pickpocket 13	ppk13		CG33508	FBgn0053508	2L: 39A1-39A1
pickpocket 14	ppk14		CG9501	FBgn0031803	2L: 26C3-26C3
pickpocket 15	ppk15		CG14239	FBgn0039424	3R: 97B1-97B1
pickpocket 16	ppk16		CG34059	FBgn0065108	2L: 30C8-30C8
pickpocket 17	ppk17		CG13278	FBgn0032602	2L: 36A14-36A14
pickpocket 18	ppk18		CG13120	FBgn0032142	2L: 30C7-30C8
pickpocket 19	ppk19		CG18287	FBgn0039679	3R: 99B7-99B7
pickpocket 20	ppk20		CG7577	FBgn0039676	3R: 99B7-99B7
pickpocket 21	ppk21		CG12048	FBgn0039675	3R: 99B6-99B6
pickpocket 22	ppk22		CG31105	FBgn0051105	3R: 96B1-96B1
pickpocket 23	ppk23		CG8527	FBgn0030844	X: 16B4-16B4
pickpocket 24	ppk24		CG15555	FBgn0039839	3R: 100B9-100B9
pickpocket 25	ppk25	lounge lizard (llz)	CG33349	FBgn0053349	2R: 42E1-42E1
pickpocket 26	ppk26		CG8546	FBgn0035785	3L: 66A1-66A1
pickpocket 27	ppk27		CG10858	FBgn0035458	3L: 63E9-63E9
pickpocket 28	ppk28		CG4805	FBgn0030795	X: 15A9-15A10
pickpocket 29	ppk29		CG13568	FBgn0034965	2R: 60B6-60B6
pickpocket 30	ppk30		CG18110	FBgn0039677	3R: 99B7-99B7
pickpocket 31	ppk31		CG31065	FBgn0051065	3R: 97E5-97E6

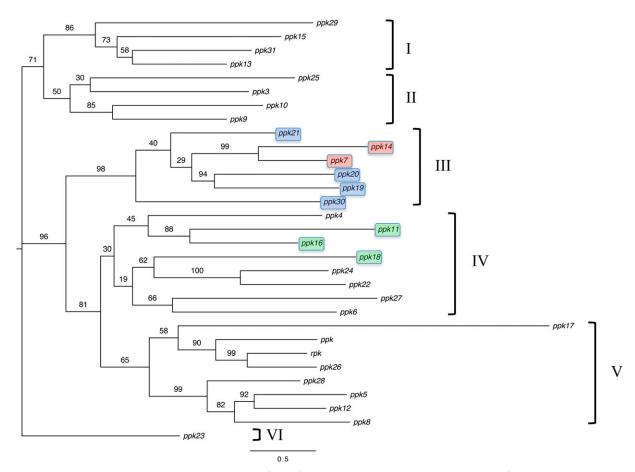


Figure 2 Maximum-likelihood unrooted phylogenetic tree inferred from multiply aligned amino acid sequences for *D. melanogaster* DEG/ENaC *ppk* genes. A total of 31 DEG/ENaC amino acid sequences are divided into six clusters and labeled as groups I-VI. Bootstrap values are given on branches and amino acid substitution rate is given at the bottom of the figure. Colors represent chromosomally clustered subunits (see Figure 5 for details).

2002; Canessa *et al.* 1994; Eskandari *et al.* 1999; Jasti *et al.* 2007; Kellenberger and Schild 2002; Zha *et al.* 2009b). Electrophysiological studies indicated that subunit composition has a significant effect on the pharmacological and kinetic properties of assembled channels, suggesting that channel subunit composition plays a critical regulatory mechanism (Askwith *et al.* 2004; Benson *et al.* 2002; Chu *et al.* 2004; Xie *et al.* 2003; Zha *et al.* 2009a; Zhang *et al.* 2008). Hence, channel subunit diversity in a single animal is likely to represent diversity in activating stimuli and/or complex channel regulation.

Although the DEG/ENaC family is highly diverse across animalia, all family members share several highly conserved structural and topological features (Bianchi 2007; Bianchi and Driscoll 2002; Corey and Garcia-Anoveros 1996; Tavernarakis and Driscoll, 2000, 2001). Conserved topologies include two transmembrane helixes, two short intracellular domains, and a large cysteine-rich extracellular loop (Figure 1) (Ben-Shahar 2011).

Surprisingly, mammalian genomes encode only eight to nine independent DEG/ENaC subunits, whereas the genomes of the worm *C. elegans* and various *Drosophila* species harbor a significantly larger number of DEG/ENaC-like genes [31 in *Drosophila melanogaster* and 30 in *C. elegans* (Bazopoulou *et al.* 2007; Ben-Shahar 2011; Liu *et al.* 2003a; Liu *et al.* 2003b; Studer *et al.* 2011)]. Consequently, DEG/ENaC genes represent one of the largest ion channel families in the *Drosophila* genome. The high diversification of DEG/ENaC protein sequences across distant animal species makes it difficult to evaluate whether the

family expanded in some invertebrate species or whether it contracted in vertebrates. Nevertheless, the remarkable diversity of ppk genes in Drosophila suggests two alternative hypotheses. The first would suggest DEG/ENaC ion channels serve a wider range of physiological functions relative to their roles in mammals. An alternative hypothesis would be that DEG/ENaC channels in Drosophila evolved to serve highly specialized functions, predicting that each specific DEG/ENaC channel type in flies is responsible for a narrow slice of the physiological functions performed by a mammalian family member. However, identifying physiological and functional homology between family members across distant species is often impossible due to the poor overall protein sequence conservation of the extracellular loop domains. Thus, protein alignment analyses alone are typically not sufficient to draw physiological homology conclusions. Consequently, newly identified family members typically require physiological analyses de novo.

The increasing interest in DEG/ENaC-dependent signaling, their emerging importance in diverse physiological functions, and their high variability across different animal genomes suggests these ion channels may have played an important role in animal evolution. Here we reason that the dramatic diversity of the DEG/ENaC family in the *Drosophila* lineage represents an excellent opportunity to use evolutionary and molecular studies to gain new insights into the possible unique role of these channels in diverse physiological systems in general and insect biology in particular.

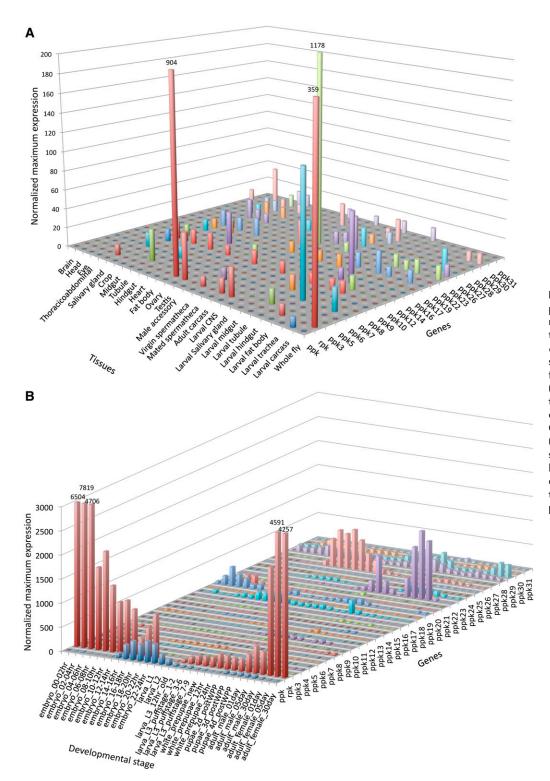


Figure 3 (A) Spatial expression patterns of ppk genes. Microarray expression data were extracted from FlyAtlas (Chintapalli et al. 2007). Expression represents the average signal from four independent microarrays. (B) Temporal expression patterns of ppk genes. Data were extracted from the modEN-CODE RNA-seq database (Celniker et al. 2009). Expression levels are represented as log₂ values of the original coverage. Numbers at the tops of truncated bars show actual expression values.

MATERIALS AND METHODS

Phylogenetic analyses

Drosophila melanogaster ppk family member protein sequences were mined in FlyBase and multiply aligned using Clustal Omega (Sievers *et al.* 2011). To determine the best model of protein evolution for our data, we entered the alignment into ProtTest v 2.4. The appropriate substitution matrix was selected from the Akaike informa-

tion criterion and Bayesian information criterion scores (Abascal *et al.* 2005; Darriba *et al.* 2011; Drummond and Strimmer 2001; Guindon and Gascuel 2003). Phylogenetic analysis was then completed using a maximum likelihood approach and rapid bootstrapping algorithm within RAxML v 7.2.8 Black Box (Stamatakis 2006; Stamatakis *et al.* 2008), on the Cipres web portal (Miller *et al.* 2010). Visualizations of the bipartition files were made using FigTree v 1.3.1 (http://tree.bio.ed. ac.uk/software/figtree/).

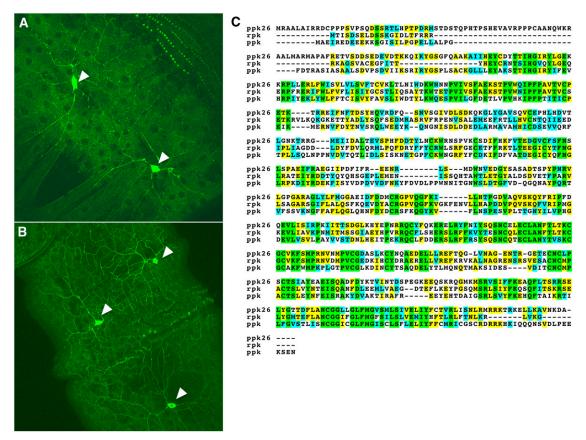


Figure 4 ppk and ppk26 expression in larval multidendritic neurons. (A) ppk-GAL4 x UAS-mCD8::GFP. (B) ppk26-GAL4 x UAS-mCD8::GFP. White arrows indicate cell body. (C) Alignment of ppk, rpk, and ppk26 amino acid sequence. Green, residues are conserved across all proteins examined; yellow, residues are conserved in some species; blue, conserved substitutions.

Expression of ppk genes

Expression patterns of each member of the ppk gene family across different fly tissues were mined from FlyAtlas (Chintapalli et al. 2007). Microarray expression data from four independent microarrays were normalized and then graphed according to the expression level in different tissues. Temporal expression patterns of the ppk gene family were extracted from the modENCODE RNA-sequencing database (Celniker et al. 2009; Graveley et al. 2011). Normalized maximum expression was represented at different developmental stages, from the embryo to the adult fly in both males and females. To observe the spatial expression patterns of ppk and ppk26 at a single cell resolution, we used the UAS-GAL4 binary expression system (Brand and Perrimon 1993) to express a membrane tethered version of EGFP

(UAS-mCD8::GFP) using a previously published ppk-GAL4 line and a new ppk26-GAI4 line we have generated. ppk-GAI4 line was obtained from the Bloomington Drosophila Stock Center (stock no. 32078). The ppk26-GAL4 line was produced by amplifying a 2.2-kb fragment that included the first intron as well as sequences upstream of ppk26 transcriptional start site (coordinates were 3L: 7447230-7449432 in release 5.47 of the *Drosophila* genome)

PPK protein structure modeling

There are currently seven different accession numbers for structural models of DEG/ENaC channels in the PDB database, all which are based on the chicken acid-sensing ion channel (ASIC)1a protein. We chose to base our structural analyses of the *Drosophila ppk* gene family

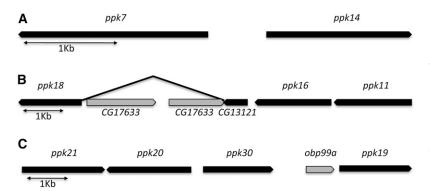


Figure 5 Chromosomal clusters of ppk genes. (A) Cluster of ppk7 and ppk14 located at 2L: 26C3-26C3. (B) Cluster of ppk18, ppk16, and ppk11 located at 2L: 30C8-30C9. Note that although CG13121 is currently annotated as a separate gene, molecular analyses of mRNA clones indicate that it is part of the ppk18 locus (not shown). (C) Cluster of ppk21, ppk20, ppk30, and ppk19 located at 3R: 99B6-99B7. Black boxes, ppk genes; gray boxes, none-ppk genes.

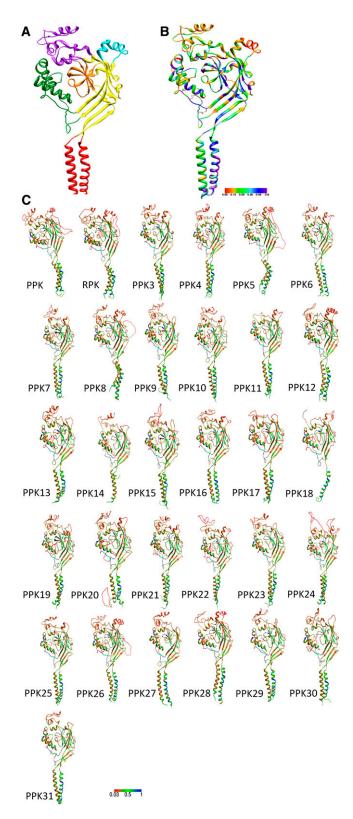


Figure 6 Structural modeling of the *ppk* family in *Drosophila*. (A) Domain organization of the chicken ASIC1a subunit (Jasti *et al.* 2007) Red: TM1 (left helix), TM2 (right helix); yellow: Palm; cyan: Knuckle; orange: beta-ball; purple: Finger; green: Thumb. (B) ASIC1a subunit rendered by conservation information from its alignment with the *ppk* family. The regions colored in purple are highly conserved residues, whereas those colored in red are most variable in the alignment. (C) Predicted

on the original 2QTS model (Jasti et al. 2007) because of the following reasons: (1) The 2QTS model has the best resolution (1.9 Å), which serves better as a template of homology modeling; and (2) 2QTS is a ligand-free model, which we predicted would work better as a modeling template since ASIC1a is a proton receptor, which is not necessarily a general property of DEG/ENaC channels. To generate structural predictions in silico, all PPK reference sequences and the template sequence (PDB ID: 2QTS) were aligned onto Hidden Markov model of amiloride-sensitive sodium channel family from PFAM [PFAM ID: PF00858(Punta et al. 2012)] by the program hmmalign in HMMER3 (Finn et al. 2011) and visualized by CLC Sequence Viewer. From the pair-wise sequence alignment of each PPK protein and the template, multiple structural models were generated by MODELER with default homology modeling protocol (Sali and Blundell 1993). The model with the best score was selected for further analysis. The molecular graphics software UCSF Chimera was used for structural visualization and analysis (Pettersen et al. 2004).

RESULTS AND DISCUSSION

The ppk family in Drosophila melanogaster

The authors of previous studies have identified several DEG/ENaC family members, which were termed *pickpocket* (*ppk*) genes (Darboux *et al.* 1998; Liu *et al.* 2003a,b). However, a comprehensive scan of the fly genome for all family members has not been performed to date. We used a combination of current genome annotations as well as various homology search engines to identify 31 independent genes encoding for family members, which we named *ppk-ppk31* in complete agreement with prior annotations (Table 1).

Alignment of all identified PPK sequences revealed a highly conserved cysteine-enriched domain, which contains five disulfide bonds by 10 highly conserved cysteines in the thumb domain (Figure 1, A and B). Unrooted protein phylogenetic analysis of all identified ppk genes in the D. melanogaster genome indicated that this protein family is composed of at least six distinct subfamilies (labeled as I-VI; Figure 2). Overall, the relationship between ppk genes in subfamilies III, IV, and V are well resolved and supported by high bootstrap values. However, few genes such as ppk17 and ppk23 are not well resolved in our phylogeny, despite multiple (N = 4) runs of the alignment and phylogenetic tree programs, which produced the same results for each run. The inability to resolve certain ppk relationships is likely due to the high amount of divergence in amino acid sequence between ppk family members (Supporting Information, Table S1).

ppk genes are highly conserved in the Drosophila lineage

We subsequently extended our gene search analyses to the sequenced genomes of additional 11 *Drosophila* species as well as to the genome of *Anopheles gambiae* (African malaria mosquito), which served as a dipteran outgroup (Table S2) (Holt *et al.* 2002). These analyses revealed that the majority of the *D. melanogaster ppk* radiation is preserved in all 12 sequenced *Drosophila* genomes (Bhutkar *et al.* 2008; Singh *et al.* 2009), indicating *ppk* diversification occurred early in the evolution of the *Drosophila* lineage.

structure for all *Drosophila* PPK subunits. The rainbow scale represents the residue conservation scores. The regions colored in red are most variable whereas regions in blue are highly conserved.

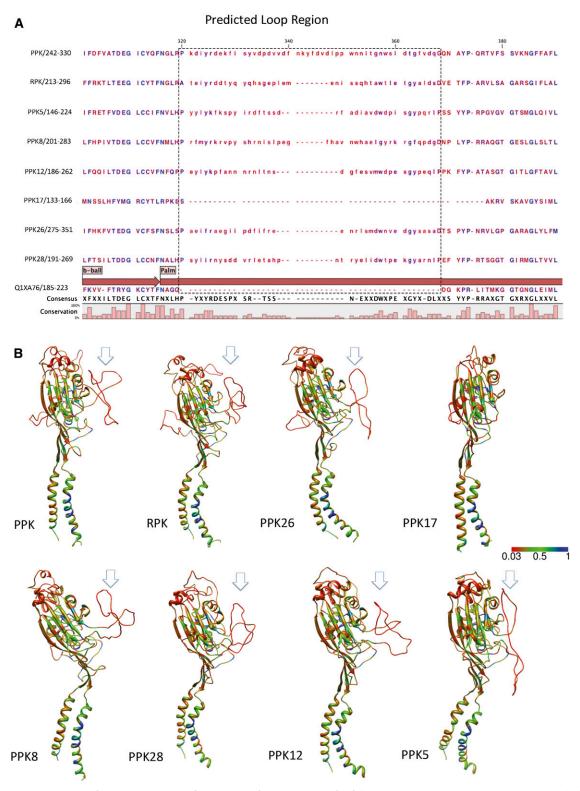


Figure 7 (A) The alignment of individual subunits from ppk subfamily Group V (for full Group V alignment, see Figure S1). The dashed frame marks the unstructured loop region. Note that PPK17 does not have the unstructured loop region. Q1XA76 is the chicken ASIC Uniprot Accession ID. Consensus sequence was built from the majority of the aligned residues. The bars in the bottom represent conservation percentage after alignment. (B) Unstructured loop region in the subfamily Group V. Predicted structures for all D. melanogaster PPK subunits are shown in Figure 6. The rainbow scale represents residue conservation as in Figure 6.

Expression patterns, structural variations, and predictions of function

Analyses of mRNA expression levels across various D. melanogaster tissues (Figure 3A) and developmental stages (Figure 3B) indicated that individual ppk family members show different expression profiles in both mRNA expression level and temporal and spatial expression patterns. These data suggest that this family has evolved to serve a wide variety of physiological functions. Although a handful of subunits have been implicated in mechanosensation and chemosensory perception, the contribution of sequence variation to physiological function remains unclear. Of particular interest is subfamily V, which includes the ppk, rpk, and ppk26 cluster (Figures 2 and 4). Both rpk and ppk have been implicated in mechanosensation in larvae, although in different types of multidendritic neurons, and are likely to have similar but independent functions in neurons (Adams et al. 1998; Kim et al. 2012; Tsubouchi et al. 2012; Zhong et al. 2010). The spatial expression pattern of ppk26, which is a close paralogue of the ppk and rpk subunits is very similar to ppk suggesting the two subunits might be co-expressed (Figure 3A). To further explore this, we generated a transgenic Drosophila line that can report the expression patterns of the gene using the UAS-GAL4 system (Brand and Perrimon 1993). As predicted by the mRNA expression data, the expression of the ppk26 gene is enriched in class IV multidendritic sensory neurons, which also express ppk (Figure 4). These data suggest that ppk26 and ppk are either redundant or are corequired for some aspect of mechanosensation in these nociceptive neurons. In sum, though the functions of all DEG/ENaC subunits are not yet known, we hypothesize that ppk, rpk, and ppk26, which show sequence and structural similarities and are expressed in multidendritic neurons, may have similar functions in nociceptive mechanosensation.

Subfamily III is not present in mosquitoes

As expected, *ppk* family gene conservation between the *D. melanogaster* and the mosquito genomes was lower than across the *Drosophila* lineage (Table S2). We identified only 18 family members in the genome of *A. gambiae*, of which 17 had homologs in the *Drosophila* genome and one that seemed to be a mosquito-specific subunit (AGAP006704; Table S2). These data suggest that the extreme diversity we observed in the *Drosophila* lineage is not shared by all dipteran species.

Closer examination of the conservation of *Drosophila ppk* subfamilies in *A. gambiae* revealed that none of the genes represented in subfamily III was present in the mosquito genome, suggesting this subfamily is not common in all dipteran species. (Figure 2 and Table S2). In contrast, we have indentified at least one homologous gene from each of the remaining ppk subfamilies in the mosquito genome (Table S2). These data may suggest that each ppk subfamily (with the exception subfamily III) represents a core DEG/ENaC physiological function in Diptera.

Diversity, duplications, gene syntenies, and sequence homologies

Examination of overall gene conservation across all sequenced *Drosophila* species indicated that protein phylogeny followed closely the predicted species phylogeny (Clark *et al.* 2007). We examined in more detail several subfamilies of conserved *ppk* genes across the 12 sequenced *Drosophila* genomes as well as the malaria mosquito *A. gambiae*. We first examined the highly conserved subgroup that included *ppk*, *rpk*, and *ppk26*. All three genes are highly conserved across all 12 genomes (Table S2).

Although each *Drosophila* genome includes one subunit that corresponds most closely to *ppk*, *rpk*, or *ppk26*, the mosquito genome encodes four related subunits, all of which are clustered with the *Drosophila ppk26* (Table S2). These data suggest that *ppk26* represents an early dipteran subunit, which may have independently diversified in the *Drosophila* and mosquito lineages.

Nine of the 31 ppk genes we have identified in the D. melanogaster genome are chromosomally clustered (Figure 5). Protein phylogeny indicated that the majority of genomic clusters were likely the result of gene duplications since the clustered genes showed high sequence similarities and belonged to the same ppk subfamilies (Boxed genes names in Figure 2). An exception is ppk18, which is clustered with ppk11 and ppk16 (Figure 5B), two less related subunits (Figure 2). These data suggest that the clustering of these three subunits might have been the result of selection underlying shared physiological and/ or cellular functions. ppk11 has been implicated in salt taste (Liu et al. 2003b). We speculate that these three subunits might contribute to salt taste in Drosophila by forming the sodium sensitive ion channel. (Adams et al. 1997; Chandrashekar et al. 2006; Chandrashekar et al. 2010; McDonald et al. 1995; Snyder et al. 1995). We found that all identified D. melanogaster ppk genomic clusters are conserved across all 12 Drosophila species genomes (not shown), indicating that the molecular events that led to clusters formation happened early in the species radiation of the Drosophila genus.

In addition to linear protein sequence analyses, we also built structural models of all PPK proteins by using the published crystal structure of the chicken ASIC (Jasti *et al.* 2007) as a guide. According to the protein conservation information from multiple alignment of the *ppk* family, we rendered a general *Drosophila* PPK model (Figure 6A). Furthermore, we used the resolved ASIC structure to predict structural models for all individual *Drosophila ppk* subunits (Figure 6B). Close inspection of the structure and the overall protein alignment revealed 10 highly conserved cysteines (>90% conservation), which are likely to form up to five disulfide bonds.

We also found that most family members from group V (Figure 2) have a long unstructured loop without a matched structural template in the resolved vertebrate model (Figure 7, with the exception of PPK17). Whether this unstructured loop plays a functional role is unknown. However, ppk is expressed in type IV multidendritic neurons, which play a role in thermal and mechanical nociception in fly larvae (Adams et al. 1998; Ainsley et al. 2003; Hwang et al. 2007; Kim et al. 2012; Zhong et al. 2010). The recent publication, which implicates rpk in mechanosensotive functions in Class III multidendritic neurons, and our finding that ppk26 is expressed in Class IV multidendritic neurons in a similar pattern to ppk suggest that other members of this cluster might be playing similar roles in mechanotransduction pathways. Further, our data raise the intriguing hypothesis that the large unstructured side loop that is a signature of cluster V may be playing a role in mechanosensory functions, possibly by interacting with extracellular matrix proteins (Arnadottir and Chalfie 2010; Arnadottir et al. 2011; Brown et al. 2008; Chalfie 2009; Geffeney et al. 2011; Huber et al. 2006; Zhang et al. 2004).

Here we show a comprehensive analysis of an emerging and important family of ion channels in the genetically tractable fruit fly model. As the importance of the DEG/ENaC family continues to increase, studies in *Drosophila* could reveal novel insights into the physiological functions of this enigmatic group of ion channels. Taking advantage of the wealth of genetic and evolutionary data in the *Drosophila* group as well as other insect species, we intend to generate novel testable structure-function hypotheses that would likely shed additional light on the physiological functions of these proteins in species ranging from the worm to humans.

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