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## Review

# Inwardly rectifying potassium channels in *Drosophila*

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**Abstract:** Inwardly rectifying potassium channels (Kir) are a special subset of potassium selective ion channels which pass potassium more easily into rather than out of the cell. These channels mediate a variety of cellular functions, including control of membrane resting potential, maintenance of potassium homeostasis and regulation of cellular metabolism. Given the existence of fifteen Kir genes in mammals, current genetic studies using mutant animals that lack a single channel may have missed many important physiological functions of these channels due to gene redundancy. This issue can be circumvented by using a simple model organism like *Drosophila*, whose genome encodes only 3 Kir proteins. The sophisticated genetic approaches of *Drosophila* may also provide powerful tools to identify additional regulation mechanisms of Kir channels. Here we provide an overview of the progress made in elucidating the function of *Drosophila* Kir channels. The knowledge of *Drosophila* Kir channels may lead us to uncover novel functions and regulation mechanisms of human Kir channels and help on pathological studies of related diseases.

**Key words:** *Drosophila*; inwardly rectifying potassium channels

## 果蝇的内向整流型钾离子通道

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**摘要:** 内向整流型钾离子通道(Kir)是一类特殊的钾离子选择性通道, 具有内向整流性, 即钾离子内流较外流容易得多。这类离子通道介导许多细胞功能, 包括维持静息膜电位, 维持钾离子平衡稳态以及调节细胞代谢水平。由于哺乳动物中存在15个Kir基因, 目前在单个离子通道突变动物中进行的遗传学研究可能因为基因冗余而未能揭示这类通道的许多重要生理功能。这一问题可通过使用简单模型动物来解决, 比如整个基因组只含3个Kir基因的果蝇。在果蝇上开展的成熟的遗传学研究方法还可为发现更多的Kir通道调控机制提供有力的工具。因此在这里我们综述了果蝇Kir离子通道的研究进展。对果蝇Kir通道的了解将会引导我们发现人类Kir通道的新的功能及调控机制, 并有助于相关疾病的病理学研究。

**关键词:** 果蝇; 内向整流型钾离子通道

**中图分类号:** R329.2+5

Inwardly rectifying potassium channels (Kir) are a special subset of potassium selective ion channels which pass potassium more easily into rather than out of the cell [1]. The pore of Kir channels are formed by the assembly of four subunits. Each subunit contains two membrane-spanning alpha helices (M1 and M2) linked by an extracellular pore-forming domain (H5), and cy-

toplasmic NH<sub>2</sub>- and COOH-terminal domains [2-7]. Human genome encodes 15 Kir subunit genes, which are divided into seven subfamilies based on sequence homology. Since subunits within the same subfamily may form heterotetrameric channels, there may be many functionally distinct Kir channels.

Based on their functional properties, Kir channels

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can be classified into 4 groups: classical channels which are constitutively active, G protein gated channels ( $K_G$ ) which are regulated by G protein-coupled receptors, ATP sensitive channels ( $K_{ATP}$ ) which are related to cellular metabolism, and potassium transport channels [8]. Pharmacological and genetic studies have revealed a variety of physiological roles for these channels, such as control of membrane resting potential, maintenance of potassium homeostasis and regulation of cellular metabolism. However, given the potential redundancy in channel expression, current genetic studies with mutant animals lacking a single Kir subunit may have missed many important physiological functions of Kir channels.

Uncovering additional functions and regulatory mechanisms of Kir channels can benefit from using a simple model organism like *Drosophila*, whose genome encodes only 3 Kir proteins. The powerful genetic tools of *Drosophila* system have greatly improved the progress of neurobiology including characterizing novel ion channels and their functions. It is anticipated that studying Kir channels in *Drosophila* will help us to identify novel physiological functions and regulations of human Kir channels. In this review, we provide an overview of the still limited progress made in elucidating the function of *Drosophila* Kir channels.

The *Drosophila* genome encodes three Kir channel proteins<sup>[9]</sup>: Ir, Irk2 and Irk3 (Table 1). All three proteins contain the inward-rectifier-potassium-channel (IRK) domain that is conserved in mammalian Kir channel subunits. Here we discuss characteristics and potential functions of each fly Kir protein.

## 1 Ir

Ir, also known as Dir, Irk1, is encoded by the gene CG6747 located at 94E on chromosome 3. This Ir gene

encodes 4 transcripts: RB, RC, RD and RE (Table 1), which produce 4 proteins with slight differences at the  $NH_2$  terminal. Sequence comparison between Ir and the other two fly Kir protein (Irk2 and Irk3) reveals identities of 54% and 28% respectively [9].

Based on sequence homology, Ir is most closely related to the human Kir2 family that forms classic, constitutively active Kir channels. Heterologous expression of Ir in transfected S2 cell has evoked strong inwardly rectifying potassium currents [9], similar to those found in human Kir2 channels. However, this may not exclude potential assembly of Ir into other types of Kir channels. Ir is expressed in corpora cardiaca cells of the ring gland, together with the fly homolog of surphonylurea receptor (SUR) [10, 11] which serve as the ATP/NDP sensor of Kir6/ $K_{ATP}$  channels. Both Ir and the fly SUR appeared to be important for the resistance to cardiotoxic viral infections. Thus Ir may also function as a  $K_{ATP}$  channel.

Although the detailed gating mechanism of Ir remains to be characterized, it is suggested that residues in cytosolic tails participate in the control of channel activity [9], as in human Kir channels. Doring *et al.* [9] found that heterologous expression of a wild-type Ir protein in *Xenopus oocytes* failed to evoke any potassium current, whereas expression of chimeric channels with either  $NH_2$  or COOH tail produced typical inwardly rectifying currents. Similar potassium currents were recorded after the residue Val<sup>34</sup> in the wild-type protein was mutated to glutamine. It is likely that some cellular milieu factors, which are cell specific, may interact with cytosolic tails of Ir to control the channel activity.

The physiological function of a Kir channel depends on where it is expressed. According to FlyAtlas anatomical expression data [12], high levels of Ir expression are observed in larval and adult midgut, Malpighian tu-

Table 1. *Drosophila* genes for Kir channels<sup>[9]</sup>

	<i>Ir</i>	<i>Irk2</i>	<i>Irk3</i>
CG No.	CG6747	CG4370	CG10369
Conserve domain	Inward rectifier potassium channel	Inward rectifier potassium channel	Inward rectifier potassium channel
Transcripts	RB, RC, RD, RE	RA, RB, RC	RA, RB
Expression at high level	Larval/adult midgut, larval/adult Malpighian tubules, larval/adult salivary gland	Adult head, adult eye, adult CNS, adult crop, larval/adult hindgut, adult Malpighian tubules.	Larval/adult Malpighian tubules

CNS: central never system.

bules and salivary gland, while moderate levels are seen in adult head, adult crop and larval hindgut etc. (Table 2).

The *Drosophila* salivary gland mainly consists of secretory cells which synthesize and secrete proteins including digestive enzymes, and duct cells which form the salivary ducts [13]. The expression of Ir in salivary gland may suggest a role of this channel in promoting or inhibiting protein secretion from those secretory cells through modulating the membrane potential. Kim *et al.*[10] found that Ir is also expressed in corpora cardiaca, the endocrine cells in the ring gland. The potential role of Ir in control of cellular secretion is reminiscent of several mammalian Kir channels. First, enhancement of potassium current through a heteromeric Kir3.2/3.4 channel in the thyrotrophs of the pituitary gland lead to hyperpolarization of the thyrotroph membrane potential and thus inhibit thyrotrophin secretion by stopping Ca<sup>2+</sup> influx. Second, Kir3.1, Kir3.2, Kir3.3 and Kir3.4 form K<sub>G</sub> channels in pancreatic islet cells to inhibit insulin secretion. Third, K<sub>ATP</sub> channels expressed in endocrine cells may be involved in the control of hormone secretion [8].

Ir channels expressed in midgut and Malpighian tubules could regulate ion and water transportation and mediate osmoregulation in the fly. The epithelium of midgut is where the fly absorbs nutrients including ions from ingested food [14], while Malpighian tubules absorb solutes, water, and wastes from the surrounding hemolymph and produce primary urine[15]. *In situ* hybridization shows that Ir localizes in the principal cells of the tubule main segment and the ureter, suggesting roles both in secretion of and reabsorption from primary urine [16]. These potential functions of Ir are similar to those of mammalian potassium transporter channels Kir1, Kir4.X and Kir5.1, which are present in the stomach and kidney where they control salt homeostasis [8].

Although poorly expressed in the brain, Ir is enriched in ventral lateral neurons (LNv), a major group of circadian neurons [17, 18]. It is important to investigate whether Ir regulates fly circadian rhythms.

## 2 Irk2

The gene for Irk2, CG4370, is located at 95A on chromosome 3. It encodes 3 isoforms of Irk2 (Table 1) with

Table 2. FlyAtlas anatomical expression of *Drosophila* Kir channels<sup>[12]</sup>

Localizations	mRNA signals					
	<i>Ir</i>		<i>Irk2</i>		<i>Irk3</i>	
	Larval	Adult	Larval	Adult	Larval	Adult
Head	–	115	–	566	–	123
Eye	–	76	–	577	–	49
Brain	–	32	–	981	–	328
CNS	55	–	128	–	101	–
Thoracic-abdominal ganglion	–	78	–	1 676	–	178
Crop	–	281	–	1 722	–	6
Midgut	782	506	23	117	7	4
Hindgut	302	83	3 856	4564	4	48
Malpighian tubules	844	1 099	N/A	805	2 898	4 932
Fat body	87	41	N/A	25	N/A	N/A
Salivary gland	725	7 480	67	235	3	8
Heart	–	32	–	N/A	–	5
Trachea	166	–	N/A	–	4	–
Virgin female spermatheca	–	80	–	37	–	N/A
Inseminated female spermatheca	–	98	–	45	–	7
Ovary	–	202	–	10	–	1
Testis	–	6	–	19.4	–	1
Male accessory gland	–	122	–	41	–	N/A
Carcass	221	125	46	148	N/A	8

Note: Data was obtained from <http://flyatlas.org>. Values indicate intensities of RNA signal. N/A: no informative data. CNS: central never system.

sequence differences at either the NH<sub>2</sub> or the COOH terminal. The sequence of Irk2 is similar to that of Ir, and both are highly related to human Kir 2, 3, 5 and 6 proteins<sup>[9]</sup>. The NCBI database lists Irk2 as the fly homologue of Kir3.4. Kir3.4 forms a K<sub>G</sub> channel that can be activated by influx of sodium. The residue Asn<sup>223</sup> in Kir3.4 is reported to be the sodium sensor<sup>[8]</sup>. Interestingly, this Asn residue is conserved in all three fly Kir proteins including Irk2, suggesting functional relevance between Irk2 and Kir3.4. However, Irk2 channels in transfected S2 cells were constitutively active and showed strong inward rectification reminiscent of human Kir2 channels<sup>[9]</sup>. In addition, functional associations to SUR suggest that both Irk2 and Ir may form K<sub>ATP</sub> channels like human Kir6<sup>[11,19]</sup>. It is plausible that, depending on the cell type, Irk2 may have different mechanisms of gating or regulation.

Like Ir, Irk2 has high levels of expression in fly digestive and excretive tissues, including adult Malpighian tubules, crop, and larval/adult hindgut (Table 2)<sup>[12]</sup>. In Malpighian tubules Irk2 is expressed in the principal cells of main segment<sup>[16]</sup>. Malpighian tubules form the fly osmoregulatory system together with the hindgut<sup>[9]</sup>. The latter is important for re-absorption of salts and water. Thus, Irk2 may play similar roles as Ir in fly osmoregulation.

In addition to digestive and renal systems, Irk2 is highly expressed in the adult head, where it is concentrated in the brain and eye (Table 2)<sup>[12]</sup> and may regulate neuronal signaling in either neuron or surrounding glia. In mammals, both classic Kir2 and G protein-activated Kir3 channels function in neurons. These channels maintain resting membrane potentials and regulate neuronal excitability<sup>[8]</sup>. The glial cell astrocyte expresses potassium transport channels Kir4 and Kir5, which establish the high K<sup>+</sup> selectivity of the glial cell membrane and the strongly negative resting membrane potential. These features allow glia cells efficiently take up K<sup>+</sup> released from active neurons. This provides the basis for K<sup>+</sup> spatial buffering by which glia regulate neuronal activity<sup>[20]</sup>. Our first step to discovering the functional significance of Irk2 in the fly brain would be to investigate whether this channel functions in neuron, glia or both.

We have recently generated a deletion line of the Irk2 gene, and found that the mutant fly is homozygote lethal (Luan and Li, unpublished observations). By expressing a wild-type Irk2 protein in different tissues,

we will be able to find out whether the lethality is due to impaired brain functions or problems in osmoregulation. In combination with cell-specific RNAi, this approach may help to reveal functions of Irk2 in distinct cell types.

### 3 Irk3

The Irk3 (or *dKirIII*) gene CG10369 is located at 37A on chromosome 2, and encodes two isoforms of Irk3 protein (Table 1). The sequences of Irk3 are distantly related to those of Ir, Irk2 and human Kir proteins.

According to FlyAtlas anatomical expression data, high levels of Irk3 expression are observed in larval and adult Malpighian tubules while moderate levels are seen in adult head and CNS etc. (Table 2). Unlike the wide distributions of Ir and Irk2, Irk3 seems more specifically expressed in Malpighian tubules, where the signal is localized to the principal cells of main segment<sup>[16]</sup>. The level of Irk3 expression in Malpighian tubules is 80-fold enriched over the other part of *Drosophila*<sup>[21]</sup>, suggesting that Irk3 plays a critical function in the tubules. This distribution pattern of Irk3 is similar to that of human Kir1 (also known as renal outer-membranary potassium channel, ROMK), which is mainly expressed in renal epithelial cells for ion transport<sup>[8]</sup>.

Based on the distribution pattern, Chintapalli *et al.*<sup>[12]</sup> suggest that Irk3 is functionally analogous to Kir1. However, heterologous expression of Irk3 in S2 cells failed to produce a constitutive potassium current, which would be expected for a Kir1-like channel<sup>[9]</sup>. An explanation could be the lack of accessory molecules in S2 cells for the channel transport and/or membrane localization. Such an accessory molecule could be identified by screening for fly mutants that show the same phenotype as an Irk3 null fly.

Overall, studies on *Drosophila* Kir channels are still very limited. Their subunits, gating mechanisms, regulatory factors and physiological functions remain largely unknown. With powerful genetic tools, *Drosophila* is a great model organism for identification of regulatory factors and cell-specific functions of Kir channels. Information obtained from the fly system may lead us to revealing novel functions and regulations of human Kir channels, and provide clues to pathological studies of Kir-involved disorders such as Bartter syndrome (antenatal) type 2 (Kir1), Andersen's disease (Kir2.1), and hyperinsulinemic hypoglycemia of infant (Kir6.1)<sup>[22]</sup>.

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