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Review

The genetics of calcium signaling in *Drosophila melanogaster*☆

Tetyana Chorna, Gaiti Hasan *

Laboratory of intracellular calcium signaling, National Center for Biological Sciences, Tata Institute of Fundamental Research, Bangalore 560065, India

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ABSTRACT

Background: Genetic screens for behavioral and physiological defects in *Drosophila melanogaster*, helped identify several components of calcium signaling of which some, like the Trps, were novel. For genes initially identified in vertebrates, reverse genetic methods have allowed functional studies at the cellular and systemic levels.
Scope of review: The aim of this review is to explain how various genetic methods available in *Drosophila* have been used to place different arms of Ca^{2+} signaling in the context of organismal development, physiology and behavior.
Major conclusion: Mutants generated in genes encoding a range of Ca^{2+} transport systems, binding proteins and enzymes affect multiple aspects of neuronal and muscle physiology. Some also affect the maintenance of ionic balance and excretion from malpighian tubules and innate immune responses in macrophages. Aspects of neuronal physiology affected include synaptic growth and plasticity, sensory transduction, flight circuit development and function. Genetic interaction screens have shown that mechanisms of maintaining Ca^{2+} homeostasis in *Drosophila* are cell specific and require a synergistic interplay between different intracellular and plasma membrane Ca^{2+} signaling molecules.
General significance: Insights gained through genetic studies of conserved Ca^{2+} signaling pathways have helped understand multiple aspects of fly physiology. The similarities between mutant phenotypes of Ca^{2+} signaling genes in *Drosophila* with certain human disease conditions, especially where homologous genes are causative factors, are likely to aid in the discovery of underlying disease mechanisms and help develop novel therapeutic strategies. This article is part of a Special Issue entitled Biochemical, biophysical and genetic approaches to intracellular calcium signalling.

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1. Introduction

Genetic studies in the fruit fly, *Drosophila melanogaster*, have helped understand the complexity of metazoan biology at all levels, including development, behavior and more recently, metabolism [1]. *Drosophila* offers several advantages for the investigation of a complex signaling system such as Ca^{2+} signaling, with its likely impact on multiple aspects of cellular and systemic physiology [2]. The advantages arise both from practical considerations based on the existence of a range of sophisticated molecular and genetic techniques and the extent of developmental, physiological and behavioral studies that are possible in this system. The first draft of the *D. melanogaster* genome was completed over ten years ago [3]. This enabled the identification of genes encoding Ca^{2+} signaling molecules represented by homologs within the vertebrate genome, and to the discovery of some novel genes. Importantly, a number of Ca^{2+} signaling molecules in *Drosophila* are encoded by single genes. Thus *Drosophila* provides a genetic background where mutant analysis of physiological functions dependant on Ca^{2+} signaling is considerably simplified.

Mutants in Ca^{2+} signaling genes of *Drosophila* have been generated using traditional methods of mutagenesis like X-irradiation, chemical mutagens such as ethyl methane sulfonate [4] and insertional mutagenesis techniques with P-elements. More recently, methods for knocking-out or altering gene function using targeted recombination of the gene of interest have been developed and standardized [5]. Further, the ability to carry out large scale genetic interaction screens for enhancers and suppressors of mutant phenotypes has helped identify novel components of signaling pathways in several instances. Genetic methods for spatio-temporal expression of mutant and wild-type transgenes were pioneered with use of the yeast transcription factor GAL4 and the Upstream Activation Sequence [6] by Brand and Perrimon [7]. This system and its subsequent modifications have allowed for over-expression of genes of interest in specific cell and tissue sub-types at particular stages of development or in adulthood [8]. Given the importance of calcium signaling in development, mutations in genes affecting calcium channels and other calcium signaling proteins are frequently homozygous lethal at an early developmental stage, precluding functional studies of the gene in adults. The ability

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* Corresponding author. Tel.: +91 80 23666140; fax: +91 80 23666462.
E-mail address: gaiti@ncbs.res.in (G. Hasan).

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to reduce or inhibit gene expression in a tissue-specific manner after development has helped understand how loss of particular arms of calcium signaling affect adult physiological and behavioral phenotypes. Traditionally these questions were addressed with the help of viable alleles or temperature-sensitive alleles of the gene of interest. Genetic mosaic techniques have also been used where homozygous mutant patches of cells or tissue are obtained by mitotic recombination in a heterozygous mutant background. In more recent times variations of these techniques have been developed where genetic mosaics are created at a higher frequency with the FLP-FRT system adapted from yeast. In this system the resulting homozygous mutant cell clones can be identified by either the absence or presence of a visible marker like green fluorescent protein [9] (GFP) [10–12].

Tissue and stage specific knock-down of calcium signaling genes in *Drosophila* has been further eased by the public availability of gene-silencing strains [http://stockcenter.vdrc.at and 13]. Knockdown is initiated by short double stranded RNA fragments generated by genetically encoded constructs for individual genes, which are under the control of the GAL4-UAS system. Cellular dsRNA screens have also been standardized in *Drosophila* S2 cells [14]. These approaches have helped identify and investigate the consequences of knockdown of a range of *Drosophila* Ca^{2+} signaling molecules. Basal cytosolic Ca^{2+} levels are controlled by an efficient interplay of different Ca^{2+} transport systems localized on the surface plasma membrane and on the membranes of intracellular compartments, some of which function as internal Ca^{2+} stores. Free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) also depends on the presence of a number of Ca^{2+} binding proteins, some of which integrate changes in $[Ca^{2+}]_c$ with downstream effectors such as Ca^{2+} activated kinases, phosphatases and transcription factors. A critical aspect of calcium signaling lies in the dynamics and spatial localization of cellular calcium changes that occur after activation of a pathway. The development of genetically encoded Ca^{2+} indicators has allowed for these measurements *in vivo* in multi-cellular animals [9]. In *Drosophila* genetically encoded calcium indicators have been targeted to specific cells and sub-cellular compartments in combination with the GAL4-UAS system. These studies have helped identify and measure the response of regions of the brain to various sensory stimuli including olfaction, taste, and thermosensation [15–18]. Here we have reviewed the current status of how *Drosophila* genetics has helped understand several novel aspects of calcium signaling in the context of development, physiology and behavior. Where ever appropriate data exist, their relevance to vertebrate function has also been discussed.

2. Plasma membrane Ca^{2+} transport systems

2.1. Voltage-gated Ca^{2+} channels

Voltage-gated Ca^{2+} channels allow the influx of extracellular Ca^{2+} into the cytosol in response to plasma membrane depolarization and regulate a variety of physiological processes. They have been classified based on their gating kinetics, single channel conductance and pharmacological properties [19]. Voltage-gated Ca^{2+} channels form hetero-oligomeric assemblies that are typically comprised of α_1 (pore-forming), α_2, δ, β and γ subunits [19]. In vertebrates, the α_1 subunit genes are grouped into three families, Ca_v1, Ca_v2, and Ca_v3.

The *Drosophila* genome encodes three α_1 (Dmca1D, Dmca1A, and Ca-α_{1T}) subunits which can be classified as Ca_v1-, Ca_v2-, and Ca_v3-type channels, respectively. Channels formed by Dmca1D are dihydropridine (DHP) sensitive, similar to L-type vertebrate channels, and are expressed in the embryonic nervous system and adult muscles [20–22]. The Dmca1A channel is insensitive to DHP and is widely expressed in the embryonic nervous system [23]. It is encoded by the *cacophony* (*cac*) gene also referred to as *Dmca1A*. Null alleles of each channel cause embryonic lethality [21,23]. Several viable *cac* alleles exhibit reduced synaptic transmission, altered motor terminal growth at neuromuscular junctions, increased convulsions, altered vision and courtship song [24–31]. Electrophysiological recordings that measured Ca^{2+} currents in *cac* mutant neurons from embryos [32], larvae [33], pupae and adults have shown that the channel is modulated differently through development [24]. Recordings from *cac* null embryonic neurons show that it is essential for fast synaptic transmission. In cultured neurons from larval brains of *cac* null and hypomorphic alleles both slow and fast inactivating synaptic Ca^{2+} currents were reduced or lost [33]. However, when Ca^{2+} currents were measured from pupal neurons of two hypomorhic *cac* alleles a significant change was observed for the slow-inactivating component of Ca^{2+} currents only [24]. Thus while Dmca1A encoded by *cac* appears to be a major constituent of neuronal voltage gated Ca^{2+} channels in *Drosophila*, a role for channels encoded by other genes needs to be assessed, particularly in the adult nervous system. Both Dmca1A and D subunits are also found in Malpighian tubules and were shown to be involved in CAP_{2b}-mediated Ca^{2+} entry and epithelial fluid transport [34]. Mutations in a α_2-δ subunit gene (*straightjacket; CG12295*) share many of the phenotypes known of the α_1 subunit mutation encoded by *cac* (*Dmca1A*), such as embryonic lethality, defects in synaptic transmission at the neuromuscular junction and aberrant electro-retinograms. Most of these phenotypes can be rescued by expression in the nervous system [35,36] and appear to be caused by reduced level of *cac* surface expression in synaptic zones [35]. More recently *straightjacket* (*stj*) has been identified in a genetic screen for reduced sensitivity to heat. Adults and larvae with pan-neuronal knockdown of *stj* become insensitive to the tested temperature of 46 °C, which is normally avoided by wild-type animals [37].

2.2. Ligand-gated ion channels

2.2.1. Ionotropic glutamate receptors

Ionotropic glutamate receptors (iGluRs) represent a family of ligand-gated ion channels that are activated upon binding of their agonist glutamate and conduct Ca^{2+} *in vivo*. They are widely expressed in both vertebrates and invertebrates. The postsynaptic iGluRs expressed at the larval neuromuscular junction (NMJ) are well characterized in *Drosophila* [38–41]. There are five subunits that selectively form tetramer complexes of functional GluRs consisting of either GluRIIA or GluRIIB subunits with the other three subunits, GluRIIC (also known as GluRIII), GluRIID and GluRIIE [42–44]. The GluRIIA and GluRIIB subunits are most similar in sequence to mammalian kainate receptors [45] and the presence of either subunit imparts distinct synaptic properties as measured by electrophysiology and regulation by second messengers [38,39]. The other three subunits are essential for receptor formation and function [46]. The unique feature of the GluRIID subunit is that it is expressed both at the NMJ and at central synapses [43]. The GluR channel lacking GluRIIA subunit has smaller synaptic currents [47,48], suggesting either selective or higher Ca^{2+} ion flow through GluRIIA than GluRIIB [49]. Both hypomorphic and null alleles for various GluR subunits have been studied, primarily in the context of the larval neuromuscular junction. Apart from their functional role in excitability of the larval NMJ, GluRs also play an important role in synapse maturation [46]. Moreover, the precise GluR composition of postsynaptic densities during synapse maturation is shaped by presynaptic glutamate release [50]. Null alleles of *GluRIIA* (*GluRIIA^{3P16}* and *GluRIIA^{AD9}*) are viable and do not exhibit obvious behavioral abnormalities, but they do have physiological deficits, such as a significant decrease in the postsynaptic response to spontaneous transmitter release [40]. *GluRIIB* null mutants are also viable, and both mutant lines with no *GluRIIA* and a 50% reduction in *GluRIIB*, showed a 75% significant decrease in amplitude of miniature excitatory junctional potentials [40]. Simultaneous deletion of both *GluRIIA* and *GluRIIB* results in embryonic lethality [40,48] and a presumed complete loss of functional glutamate receptors [43].

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