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硕 士 学 位 论 文

两个 TRP 离子通道受体在黑腹果蝇免疫反应中的初步研究

Preliminary study of two TRP ion channel receptors in *Drosophila melanogaster* immune response.

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Abstract

Innate immunity is the first line to defense host against all kinds of pathogens. In last two decades, *Drosophila melanogaster* has served as a particularly useful model organism for studying and genetically dissecting the aspect of innate immunity. The immense knowledge derived from such studies has led to the important discovery of the receptors like the toll like receptors and their signalling pathways in mammalian systems. Innate immunity has been shown to be evolutionarily conserved from insect to mammals. My research work is focused on two transient receptor potential (TRP) channel receptors in *Drosophila melanogaster*: CG31284 and CG34123. In my thesis, mutant flies of the two TRP genes have been generated by P-element mediated imprecise excision. One gain of function mutation for CG31284 and one loss of function mutation for CG34123 were produced. We infected these mutant flies with a number of pathogens and the two alleles showed different effects to infection. The flies with increased expression of CG31284 were more resistant to infection, while those with decreased expression of CG34123 were more susceptible to infection. These results indicate that TRP receptors have a role in *Drosophila* immune responses.

Key words: *Drosophila*, TRP receptors, innate immunity

摘要

先天性免疫是机体抵抗一切病原体的第一道防线。 在过去的二十年中，黑腹果蝇（*Drosophila melanogaster*）作为研究先天性免疫的一种特别有用的模型生物，从遗传学的角度详细剖析了先天性免疫的各个层面。由这些研究而来的诸多认识直接导致了哺乳动物体系的一些受体如 Toll-like 受体，及其信号通路的重大发现。先天性免疫在从昆虫到哺乳动物进化上的保守已经被证实。我的研究工作主要围绕黑腹果蝇的两种瞬时受体电位通道受体（TRP channel receptor）：CG31284 和 CG34123。在论文里，我通过 P 因子介导的不精确剪切获得这两个 TRP 基因各自的果蝇突变体，其中，一个是 CG31284 基因的功能增益突变，另一个则为 CG34123 的功能缺失突变。我们用一些病原体感染这些果蝇突变体，发现这两个突变体有不同的免疫效应：CG31284 表达量增多的果蝇对这类感染更有抵抗力，而 CG34123 表达量减少的果蝇对这类感染表现的比较敏感。以上结果表明：TRP 通道受体在果蝇的免疫反应中扮演着一定的角色。

关键词：果蝇，TRP 通道受体，先天性免疫

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1- TRANSIENT RECEPTOR POTENTIAL (TRP) ION CHANNEL RECEPTORS.

By 1990 the founding members of most of the major functionally identified channel families had been molecularly identified. However, there was still a diverse range of non-voltage-gated cation channels, such as second-messenger operated channels (SMOCs), store-operated channels (SOCs or I_{crac}), calcium-activated non-selective cation channels (CAN channels) and a variety of sensory transduction channels, including those responsible for taste, hearing and temperature sensation, for which there were no molecular candidates. Although between them, their properties would not necessarily have suggested that they might belong to the same family, it turns out that at least one further major ion channel family had yet to be discovered, which now seems likely to account for most of these. This is the transient receptor potential (TRP) ion channel family, first discovered in *Drosophila*, and which is notable for its diversity of function and almost ubiquitous expression. In the meantime approximately 30 vertebrate isoforms have been isolated, but their full range of physiological functions is still only gradually being revealed, whilst their gating mechanisms in most cases remain poorly defined. Although it is the most recently discovered major class of ion channel families, the TRP family has already been extensively studied, with many thousands of papers published in its relatively brief, recognized, lifetime. Probably no other ion channel family has been so extensively reviewed in recent years.

Transient receptor potential or TRP ion channels are a family of loosely related transmembrane ion channels that are relatively non-selectively permeable to cations, including sodium, calcium and magnesium. These TRP channels have polymodal activation properties, that is, they are activated by a huge variety of stimuli, including physical factors such as temperature, voltage and mechanical forces, chemical factors such as pH and ions, mainly Ca^{2+} and Mg^{2+}, and various intracellular signaling pathways [1, 2]. Changes in the cytosolic free Ca^{2+} concentration, ($[Ca^{2+}]i$), play a central role in

many fundamental cellular processes including muscle contraction, transmitter release, cell proliferation, gene transcription and cell death[3]. The family of transient receptor potential (TRP) channels contribute to changes in $[Ca^{2+}]i$ by providing Ca^{2+} entry pathways, by modulating the driving force for the Ca^{2+} entry, and very likely also by providing intracellular pathways for Ca^{2+} release from cellular organelles. Members of the TRP channels play important roles in sensory physiology. These include requirements for TRPs in vision, thermosensation, olfaction, hearing, and touch. They also mediate responses to nerve growth factors, pheromones, vasorelaxation of blood vessels and metabolic stress. In addition to facilitating an animal's capacity to perceive the external environment TRP channels promote the ability of individual cells to sense changes in the surrounding milieu, such as alterations in osmolarity and possibly fluid flow. Furthermore, mutations in members of the TRP family are responsible for several diseases, such as several tumors and neurodegenerative disorders.

TRP channels are conserved throughout animal phylogeny and there are distant relatives encoded in various fungal genomes.[4] Thus, these channels have ancient origins. Mammals, such as mice and humans, express 27 and 28 members respectively, while the worm and fly genomes encode 17 and 13 representatives each. Based on their amino acid sequence homologies and comparisons, these channels are subdivided into five group-1; TRPC (canonical or classical), TRPM (melastatin), TRPN (NOMPC), TRPV (vanilloid receptor), TRPA (ANKTM1), and two, more distantly related, group-2 subfamilies; TRPP (polycystin), and TRPML (mucolipin).fig(1). All these TRP subfamilies are represented in the *Drosophila* genome [4], and three of them were initially discovered in *Drosophila*. The pioneering research and basic concepts on the properties and function of the channels in two of them (i.e., TRP and NOMPC) were established in *Drosophila*. The three subfamilies are TRPC, which includes the founding member of the TRP superfamily (TRP); TRPA, which includes the temperature detectors painless [5] pyrexia [6], and ANKTM1 [7, 8]; and the TRPN subfamily, in which the no mechanical potential C (NOMPC) is the first member [9]. NOMPC currently includes only one vertebrate homolog that was found in Zebra fish [10]. The range of functions ascribed to mammalian and invertebrate TRP channels appear to be remarkably similar. This is especially apparent in comparisons between the mammalian and fly TRPs. Due to

functional similarities and the range of genetic tools available, the fruitfly represents a very appealing model organism for dissecting the functions of TRPs and the molecular pathways that lead to their activation.

To date, 9 out of the 13 Drosophila TRPs have been characterized in various degrees of detail. Most extensively studied is the founding member of this superfamily, Drosophila TRP, which is critically important in visual transduction. Loss-of-function mutations have been generated and characterized for eight Drosophila TRPs, while the roles of two others have been addressed using RNAi or dominant negative approaches (Fig 1). In addition, seven of the fly TRPs have been expressed functionally in vitro (Fig 1), providing parallels between the mechanisms leading to activation of the mammalian and Drosophila TRPs.

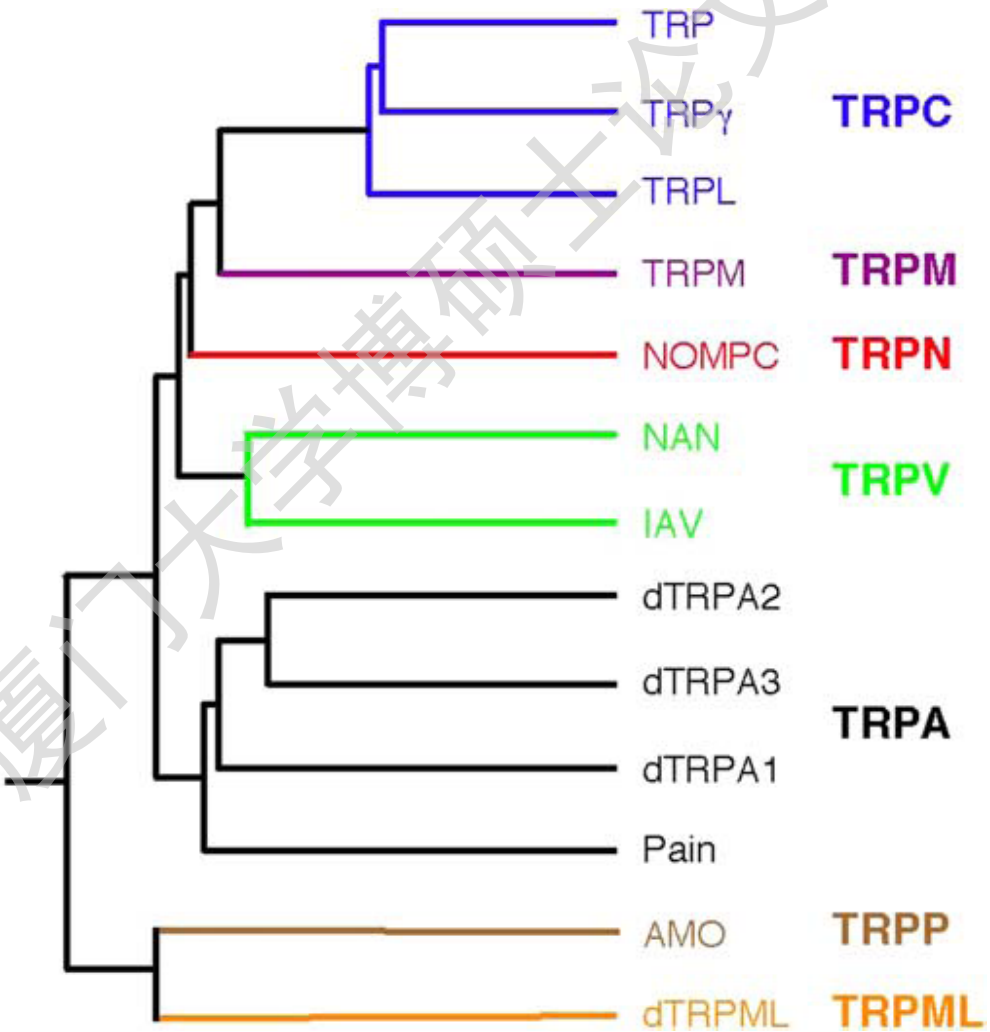

Fig. 1 Dendrogram showing the relatedness of the Drosophila transient receptor potential (TRP) proteins

1.2 Historical Background of TRP Channels

1.2.1 The *trp* Phenotype

The first ion channels to be sequenced, including the ACh receptor and voltage-gated Na^+ and Ca^{2+} channels, were isolated by the classic approach of protein purification and microsequencing. However, ion channels are often expressed at low levels, and therefore alternative approaches, which make no demands on protein abundance or high-affinity ligands, were required to identify most of the other major classes of ion channel. In this respect, genetic approaches have proved particularly powerful, as exemplified by the voltage-gated K^+ channel family, first identified by the cloning of the *Drosophila* behavioral mutant gene, *Shaker [11-13]*.

A spontaneously occurring *Drosophila* mutant was designated transient receptor potential (*trp*) by Minke et al. [14] because of its unique phenotype. Drosophila trp was the first mutation shown to cause a defect in the electroretinogram (ERG) recording, which is a simple assay for defects in the visual response. During exposure to constant light, wild-type flies display a sustained response. *trp* is referred to as the ‘‘transient receptor potential’’ mutant due to the transient response to light. In this mutant the response to prolonged illumination declines to baseline during light [14-16] (Fig. 2). *Drosophila* photoreceptors of wild-type and *trp* mutants have become a preparation of extensive research because of the ability to study TRP within the well characterized phototransduction cascade in the native tissue [17-20]. The *trp* phenotype was originally explained by the hypothesis that some critical factor, which is required for excitation and needs to be constantly replenished during illumination, is in short supply in the mutant and cannot be replenished fast enough due to a mutation in the *trp* gene. The *trp* gene was cloned in 1989 [21].Subsequently it was found that two additional homologous channels, TRP-like (TRPL) and TRPγ, are also involved in this process [22]. Double mutants lacking both TRP and TRPL are completely unresponsive to light, indicating that TRPγ cannot function independently of TRP and TRPL. TRPγ may function in combination with TRPL, since the light response is almost completely abolished in *trp* mutant flies expressing a dominant negative form of TRPγ.

Indeed, several lines of evidence indicate that the decline of the light response in *trp* mutants is due to exhaustion of excitation. Accordingly, the response to continuous intense light becomes equivalent to a response to dim light and then to darkness during illumination [14, 16]. The first clue that there is a defect in Ca^{2+} homeostasis in the trp mutant is that the light-dependent movement of the pigment granules, which is a Ca^{2+}-dependent phenomenon, is transient in trp flies. Moreover, light-induced Ca2+ influx is reduced about 10-fold in trp flies, raising the possibility that trp encodes a Ca^{2+}-permeable channel, or is required for the activity of such a channel. A correlation between the *trp* phenotype and exhaustion of cellular Ca^{2+} was provided by exposure of isolated *Drosophila* ommatidia to Ca^{2+} free medium for a long (1 h) period of time in cells buffered with EGTA to ~50 nM intracellular Ca^{2+}. The typical *trp* phenotype was obtained under such conditions [23]. Similar apparent cellular Ca^{2+} deprivation could be obtained in the isolated ommatidia of wild-type (WT) *Drosophila* during a critical period of development. At this developmental stage (P14), no response to light can be observed unless Ca^{2+} (μM) is applied by the whole cell recording pipette. Interestingly, the light response under such conditions has the typical characteristics of the *trp* phenotype [24]. Presumably under such conditions cellular Ca^{2+} is the limiting factor of excitation as in La^{3+}-treated or Ca^{2+} - deprived WT cells.

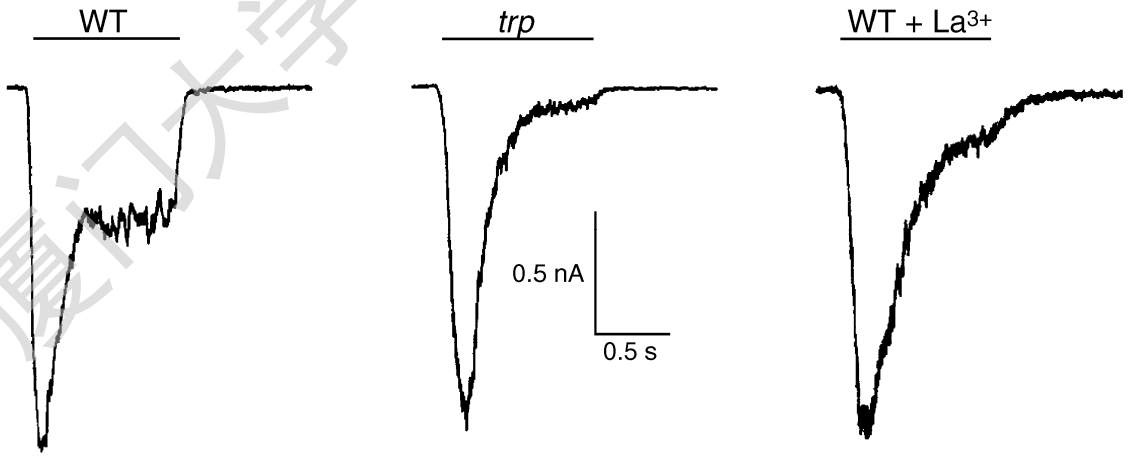

FIG. 2. The *trp* phenotype. Light-induced currents in response to prolonged intense orange lights were recorded in voltage-clamped photoreceptors of wild type (WT), the *trp* mutant, and WT treated with La^{3+}. A peak response and a plateau characterizes the light response of WT. The rapid peak-plateau transition is a manifestation of Ca^{2+}-dependent light adaptation. The response of the *trp* photoreceptor decays close to baseline during light due to exhaustion of excitation. A similar decay of the light response close to baseline