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型態發生的分子機轉：以果蠅複眼感小體發生為模式(2/3)

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中文摘要：

形態發生是發育過程中非常重要的一個步驟，它可以決定細胞、組織或是器官最後的形態。目前有關發育過程形態發生的分子調控機轉尚不清楚，本研究計畫以果蠅複眼感小體的發育為研究模式以探討基因與形態發生之調控。果蠅複眼的感小體是位於感光細胞的頂部，感小體形態發生開始於蛹期，經由細胞劇烈的形狀改變後形成了成蟲時所見的感光細胞。過去的研究顯示細胞極性基因與感小體形態發生有密切的關係。果蠅的感小體相當於脊椎動物感光細胞之外節部，脊椎動物感光細胞之外節部的發育與維持對於感光細胞之功能有密切的影響，過去研究顯示若感光細胞之外節部的發育與維持受到阻礙則會導致視覺的喪失，因此，研究果蠅感小體的形態發生亦有助於我們瞭解脊椎動物感光細胞之外節部的發育與維持。本實驗室過去主要研究微管動蛋白 dynactin 與細胞極性基因 crumbs 對感小體形態發生的作用機轉，本計畫中我們發現一個新穎的基因可能與感小體的形態發生有關，利用 RNA Interference 的方式抑制 *Dmob2* 基因之表現，我們發現在感小體之發育變的不規則，而且極性蛋白(Dlt)之表現位置亦受到影響。此結果顯示 *Dmob2* 基因可能參與細胞極性基因之決定並進而影響感小體之發育。

英文摘要：

Morphogenesis is an important process in development to build a specific cell type, tissue or organ. Currently, the molecular regulation of the morphogeensis remains unclear. My lab uses *Drosophila* retina as system to study the genetic regulation of morphogenesis. Rhabdomere is a photosensitive structure localizes at the apical surface of the photoreceptor cells (PRCs). Rhabdomere morphogenesis occurs at the early pupal stage via turning and extension of the apical surface and establishment of the rhabdomere and stalk domain in the apical surface of PRCs. We have previously showed that dynactin (*Glued*), the dynein activator and the apical polarity gene, *crumbs* affect rhabdomere morphogenesis. In this study, we reported to identify a novel gene, *Dmob2*, plays a significant role in mediating rhabdomere morphogenesis. Using RNA interference to down-regulate the expression of *Dmob2*, we found the rhabdomere became irregular. In addition, the localization of apical marker, Dlt, was altered when expression of *Dmob2* was down-regulated. Together, we identified a novel gene, *Dmob2*, in mediating rhabdomere morphogenesis.

關鍵字：複眼，感小體，型態發生，細胞極性基因。

Keywords: compound eye, rhabdomere, morphogenesis, cell polarity, *Dmob2* gene

報告內容：

Morphogenesis is a developmental process to build specific cell type, tissue or organ in organism. The molecular mechanism regulating morphogenesis remains largely unclear. Rhabdomere is the photosensitive structure that always localizes at the center of apical surface in *Drosophila* photoreceptor cells. Rhabdomere is not born with this structure. Instead, it is gradually built during late stages of eye development. My lab is interested in study why rhabdomere is always formed at the center of photoreceptor cell. In *Drosophila*, photoreceptor cells are derived from a layer of polarized epithelium, the eye disc (Wolff and Ready, 1993). Early stages of eye development are mainly focused in cell differentiation and pattern formation. At about 35% of pupal dvelopment, the photoreceptor cells undergo dramatic changes in their shape by turning their apical surface toward the interrhabdomeral space and reorganize adherens junctions among photoreceptor cells (Longley and Ready, 1995; Fan, 2004). After this turning process, photoreceptor cells begin to build their rhabdomere, a specific domain that enrich with actin cytoskeleton and proteins associated with phototransduction cascade, at the apical surface (Kumar and Ready, 1995). Recently, molecular and genetic studies showed that cell polarity genes, such as *bazooka*, *crumbs*, *moesin*, and *stardust*, (Izaddoost et al., 2002; Pellikka et al., 2002; Karagiosis and Ready, 2004; Hong et al., 2003) play important roles in formation of rhabdomere. However, the molecular mechanism of apical polarity genes in mediating morphogenesis of photoreceptor cells remains to be elucidated. In this study, we reported a novel gene, *Dmob2*, may play a significant role in photoreceptor morphogenesis.

Result

Using EP screening to identify genes can modify *GMR>crb^{intramyc}* eye phenotype

Cell polarity gene, *crb*, plays a significant role in mediating photoreceptor morphogenesis (Izaddoost et al., 2002; Pellikka et al., 2002). The *crb* interacting genes, moesin (Karagiosis and Ready, 2004), and *stardust* (Hong et al., 2003) also have significant impact in photoreceptor morphogenesis. However, the downstream of *crb* in regulation of photoreceptor morphogenesis is unclear. In this study, we used EP modify screening (Rorth, 1996) to search for genes, which can modify *GMR-Gal4/ UAS-crb^{intarmyc}* (*GMR>crb^{intarmyc}*) eye phenotype (Fan et al., 2003). Totally, we screened about a thousand EP lines. Among these EP lines, we identified one of EP lines, which it activates a 57 kDa Mob2 protein. To further confirm *Dmob2* is indeed the gene could modify *GMR>crb ^{intramyc}* eye phenotype, we generated transgenic flies to express *Dmob2* gene under control of the UAS promoter. By crossing

UAS-Flag-Dmob2 to *GMR>crb* ^{intramyc} flies, we found *Dmob2* gene could mitigate the eye phenotype of *GMR>crb* ^{intramyc} fly. In *GMR>crb* ^{intramyc} adult flies (Fig. 1A), the eyes are small and disorganized. In *GMR>crb* ^{intramy}*/UAS-Flag-Dmob2* adult flies (Fig. 1B), the adult eyes became much better than *GMR>crb* ^{intramyc} eye. In *GMR>crb* ^{intramy}*/pWIZ-Dmob2-RNAi* (Fig. 1C), the adult eyes became even worse than in *GMR>crb* ^{intramyc} adult eyes. These results suggested that *Dmob2* may interact directly or indirectly to *crb* in regulation of photoreceptor morphogenesis.

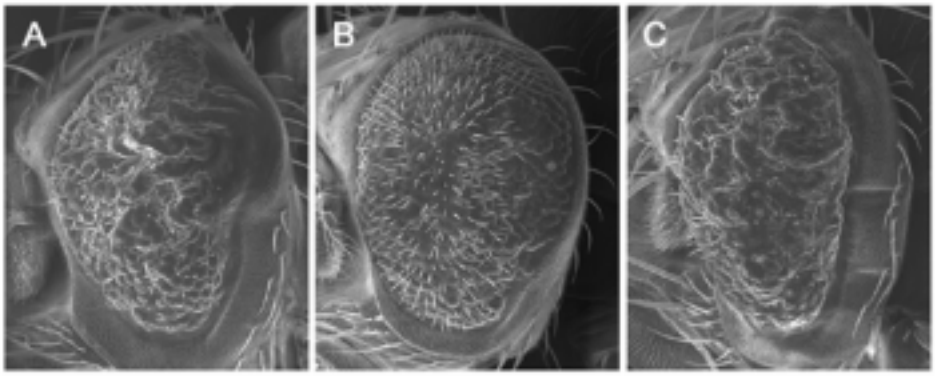

Fig. 1 Scanning micrographs show the eye phenotype in *GMR>crb* ^{intramyc}, *GMR>crb* ^{intramyc}*/Flag-Dmob2, GMR>crb* ^{intramyc}*/Dmob2-RNAi*.

Alternation of *Dmob2* expression caused defects in photoreceptor morphogenesis

Expression of *Dmob2* could mitigate *GMR>crb* ^{intramyc} eye phenotype suggesting that *Dmob2* may also involve in photoreceptor morphogenesis. To test this hypothesis, we generate transgenic fly that expresses double strand of *Dmob2* RNA and full-length *Dmob2* with Flag tag at its N-terminus. To test the transgenic flies were able to down-regulate or overexpress *Dmob2* gene, we crossed *pWIZ-Dmob2-RNAi* and *pUAST-Falg-Dmob2* transgenic flies to *hs-Gal4* activator. After two heat-shocks at 37 ℃, we isolated the total RNA from these flies and examined the expression of *Dmob2* transcripts using semi-quantitative RT-PCR. Results showed that activation of *pWIZ-Dmob2-RNAi* could down-regulate *Dmob2* expression while activation of *pUAST-Flag-Dmob2* could enhance the expression of *Dmob2* (Fig. 2). With these transgenic flies on hands, we then crossed these transgenic flies to eye specific activator, *GMR-Gal4*, to down-regulate or over-express *Dmob2* in developing eye. We then asked whether alternation of *Dmob2* expression could lead to abnormal eye development. The results indicated that down-regulation of *mob2* in developing eye caused severe eye phenotype. In wild type, the compound eyes comprise about 750 to 800 ommatidia and array as a regular pattern (Fig. 3A). In *GMR>Dmob-RNAi2* fly, the eye became irregular; usually the dome shape ommatidia became flat (Fig. 3B). In

GMR>Flag-Dmob2 fly, the orientation of ommatidia were irregular (Fig. 3C).

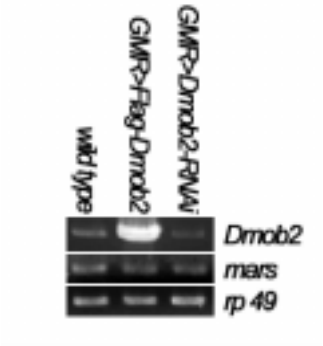

Fig. 2 RT-PCR indicates the expression of *Dmob2* transcripts in wild type, *GMR>Flag-Dmob2*, and *GMR>Dmob2-RNAi* flies.

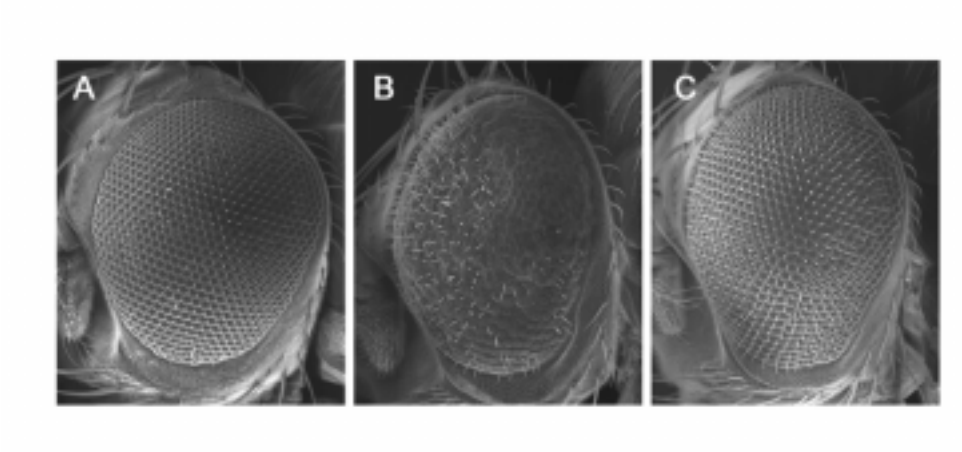

Fig. 3 Scanning electron micrographs demonstrates the eye phenotype in wild type (A), *GMR>Dmob-RNAi2* (B) and *GMR>Flag-Dmob2* fly (C).

Dmob2 plays a role in organization of ommatidia

To investigate the causes of eye phenotype in down-regulation of *Dmob2*, we stained the eyes at 55% of pupal development (pd) using rhodamine-phalloidin. We then compared the array of ommatidia between wild type and *GMR>Dmob2-RNAi* flies. The results showed that wild type eye contained regular array of ommatidia and each ommatidium contained seven photoreceptors in a single optical section. The pigment cells then enclosed the photoreceptors and formed an isolate unit eye (Fig. 4A). Down-regulation of *Dmob2* disrupted the regular array of ommatidia. The pigment cells did not differentiate well and thus the fused ommatidia were found frequently in pupal eyes. In addition, the number of photoreceptors in *GMR>Dmob2-RNAi* flies was often less than the wild type eye (Fig. 4B). When *Dmob2* was overexperssed, the array of ommatidia was also disturbed. The number of photoreceptors was also less than wild type eye occasionally (Fig. 4C).

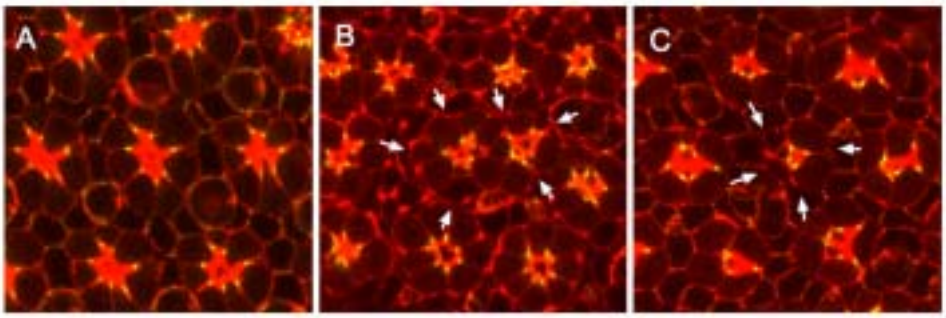

Fig. 4 Manipulation of *Dmob2* expression altered the ommatidial array. (A). Wild type eye has regular array of ommatidia. (B).Array of ommatidia in *GMR>Dmob2- RNA*i fly. (C). Array of ommatidia in *GMR>Flag-Dmob2* fly.

***Dmob2* is required to localize Crb and Dlt in the stalk domain of photoreceptors**

To study the function of Dmob2 protein in photoreceptor morphogenesis, we used immunocytochemistry to examine whether down-regulation of Dmob2 expression could alter the localization of cell polarity genes, such as, Crb, Dlt. To test this hypothesis, we stained the eyes with phalloidin and anti-Crb or anti-Dlt for stalk domain. In wild type eye, the Dlt demarcated the stalk domain specifically in 85% of pd (Fig. 5A). When Dmob2 was down-regulated, the Dlt failed to localize specifically at the stalk domain. Instead, it was often found that Dlt was localized at the apical rhabdomere domain (Fig. 5B). Together, these results suggest that Dmob2 is required to maintain the subcellular localization of Dlt.

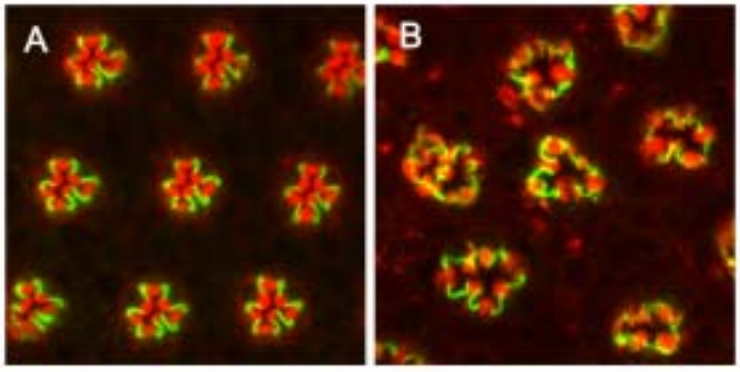

Fig. 5 Down-regulation of Dmob2 altered the subcellular localization of Dlt. (A). Wild type eye stained with anti-Dlt antibody (Green) and phalloidine (Red). (B). In *GMR>Dmob2-RNAi* fly, the subcellular localization of Dlt was altered.

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計畫自評：

The purpose of this study is to identify the genes that involve in rhabdomere morphogenesis. We and other labs have previously shown that mutation of cell polarity gene, *crumbs* affects rhabdomere morphogenesis. Using EP modified screen, we identified a novel *Dmob2* gene, which may interact with *crb* and plays significant

role in mediating rhabdomere morphogenesis. Using RNAi technique to down-regulate *Dmob2* expression in developing eye, we found the rhabdomere failed to develop normally suggesting the role of *Dmob2* in mediating rhabdomere morphogenesis. The results in this study are significant and promise, and are worth to be continuous.