

# The BTB/POZ-ZF Transcription Factor dPLZF Is Involved in Ras/ERK Signaling During *Drosophila* Wing Development

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In *Drosophila*, broad complex, tramtrack, bric à brac (BTB)/poxvirus and zinc finger (POZ) transcription factors are essential regulators of development. We searched the *Drosophila* genome for BTB/POZ-ZF domains and discovered an unknown *Drosophila* gene, *dPLZF*, which encodes an orthologue of human *PLZF*. We then characterized the biological function of the *dPLZF* via genetic interaction analysis. Ectopic expression of *dPLZF* in the wing induced extra vein formation during wing development in *Drosophila*. Genetic interactions between *dPLZF* and Ras or extracellular signal-regulated kinase (ERK) significantly enhanced the formation of vein cells. On the other hand, loss-of-function mutations in *dPLZF* resulted in a dramatic suppression of the extra and ectopic vein formation induced by elevated Ras/ERK signaling. Moreover, *dPLZF* activity upregulated the expression of *rhomboid* (*rho*) and *spitz*, which perform crucial functions in vein cell formation in the developing wing. These results indicate that *dPLZF* is a transcription factor controlled by the Ras/ERK signaling pathway, which is a prominent regulator of vein cell formation during wing development in *Drosophila*.

## INTRODUCTION

The broad complex (BR-C), tramtrack (*ttk*), and bric à brac (*bab*) (BTB) domain, also known as the poxvirus and zinc finger (POZ) domain, has been identified as a conserved motif in transcription regulators that have crucial roles in *Drosophila* development (Albagli et al., 1995). *Ttk*, a transcriptional repressor, is expressed in a complementary pattern to that of *fushi tarazu* (*ftz*) throughout early embryogenesis (Harrison and Travers, 1990). BR-C functions as a crucial regulator of gene activity at the onset of metamorphosis (DiBello et al., 1991; Karim and Rubin, 1998). *Bab* is required for pattern formation along the proximal-digital axis of the leg and antenna (Godt et

al., 1993). All these factors contain this motif and are involved in *Drosophila* development.

The BTB/POZ domain is absolutely conserved from *Drosophila* to mammals and has been identified in more than 200 proteins in the human genome (Bardwell and Treisman, 1994). In fact, the human promyelocytic leukemia zinc finger gene (*PLZF*) encodes a 673-amino acid transcription factor with a conserved BTB/POZ protein-protein interaction domain as well as a DNA-binding motif constructed from nine C<sub>2</sub>H<sub>2</sub> Krüppel-like type zinc fingers (ZFs). *PLZF* has been linked both directly and indirectly to tumorigenic and developmental processes, including pattern formation, metamorphosis, eye development, and limb development (Blair, 2007; Kelly and Daniel, 2006). Specifically, a transgenic *Drosophila* strain that ectopically expresses the human hepatocytic  $\Delta$ BTB-*PLZF*, a 45-kDa isoform of *PLZF* that lacks the BTB domain, showed phenotypic changes in eye and wing development via enhanced extracellular signal-regulated kinase (ERK) signaling (Ko et al., 2006). EOR-1, the *C. elegans* orthologue of human *PLZF*, also has been identified as a positive regulator of both the Ras and Wnt signaling pathways (Howard and Sundaram, 2002; Rocheleau et al., 2002). The endogenous biological functions of *PLZF*, however, have yet to be elucidated.

In *Drosophila*, Ras/ERK signaling has been implicated in the regulation of a variety of processes, including cell proliferation (Karim and Rubin, 1998), differentiation (Fortini et al., 1992), patterning (Schnorr and Berg, 1996), apoptosis (Bergmann et al., 1998; Kurada and White, 1998), cell migration (Lee et al., 1996), cellular growth, and wing development (Blair, 2007; O'Keefe et al., 2007). Ras activation results in the sequential activation of the highly conserved mitogen-activated protein kinase (MAPK) cascade, which includes Raf, MAP kinase kinase (MEK), and ERK. Among Ras regulators in *Drosophila*, *rho* activates epidermal growth factor receptor (Egfr) ligands and induces Ras activation via the transmembrane Egfr (Roch et al., 2002). The expression of *rho* in the primordial vein is the

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initial step in the process of vein cell fate determination. Therefore, Ras/ERK activation in a specific row of cells determines the fate of the cells as future wing veins. Consistent with this observation, loss-of-function mutations of the Ras/ERK pathway components result in loss of veins, whereas gain-of-function alleles and/or overexpression of these components induce ectopic vein development (Brunner et al., 1994; Kim et al., 2006; Prober and Edgar, 2000; Sturtevant and Bier, 1995). Additionally, phosphorylated cytoplasmic ERK promotes vein differentiation in the developing wing vein and margin cells during the larval and early pupal stage (Marenda et al., 2006). Thus, Ras/ERK is critical for the control of vein differentiation and proliferation in *Drosophila*.

In this study, we isolated *Drosophila* PLZF (*dPLZF*), a novel gene that contains the BTB/POZ-ZF domain, and investigated the role of *dPLZF* during development *in vivo*. In order to assess the effects of ectopic expression and knockdown of this gene, we generated a transgenic fly that overexpresses full-length *dPLZF* and two loss-of-function mutants of *dPLZF*. The ectopic expression of *dPLZF* in the wing phenocopied the overexpression of Ras/ERK pathway components. We also evaluated the epistatic interaction between *dPLZF* and components of the Ras/ERK pathway. Ras/ERK activity was enhanced by overexpression of *dPLZF* and was reduced in *dPLZF* loss-of-function mutants. Furthermore, we demonstrated a crucial function of *dPLZF* in larval development and survival. This study provides the first demonstration that *dPLZF* is an essential gene for wing vein formation and may function as a transcriptional regulator of the Ras/ERK pathway during wing development in *Drosophila*.

## MATERIALS AND METHODS

### *Drosophila* strains

The fly strains EP(2) 6792 (*dPLZF*), EP(2) 5575 (*Ken & Barbie*), EP(3) 6384 (*Cp190*), EP(X) 15725, EP(X) 32611, and EP(3) 6765 with an inserted EP (enhancer-promoter) element were obtained from GenExel. The *vg-Ras<sup>V12</sup>*, *UAS-Ras85D<sup>V12</sup>*, *UAS-Ras85D<sup>N17</sup>*, *UAS-Draf*, *UAS-ERK (rolled)*, *UAS-ERK<sup>S</sup> (r<sup>691</sup>)*, *P[42-3]/Cyo*, MS1096-Gal4, *engrailed-Gal4 (en-Gal4)*, and *vestigial-Gal4 (vg-Gal4)* lines and a variety of Gal4 and balancer lines were acquired from the Bloomington *Drosophila* Stock Center. The RNAi CG6792 (*dPLZF*) lines were acquired from the Vienna *Drosophila* RNAi Center. All *Drosophila* stocks were maintained and cultured with standard medium at 25°C unless noted otherwise.

### Generation of transgenic and null flies

To induce ectopic *dPLZF* expression (based on cDNA clone SD03430), we employed the UAS/GAL4 system. The *dPLZF* cDNAs were acquired from the *Drosophila* Genomics Resource Center. Transgenic flies were generated via injection of pUAST-*dPLZF* and a helper plasmid into embryos. Injections were conducted prior to pole cell formation in stage 5 using an IM-300 microinjector (Narishige) and an Axiovert 25 micromanipulator (Carl Zeiss). To generate *dPLZF* loss-of-function mutants, we employed an EP line from the GenExel fly library. This line harbored an EP-element insertion closest to the center of the gene. The P-element from *dPLZF* was excised by crossing the flies with flies harboring  $\Delta 2-3$  transposase. More than 200 excision lines were established and analyzed by PCR using primers flanking *dPLZF*.

### Analysis of phenotypes and measurement of survival rate

The wings of adult flies were dissected from euthanized flies

and mounted in 50% Canadian Balsam (Sigma) in methylsalicylate (Sigma). Adult wing phenotypes were observed via light microscopy (Leica CTR 6000). The number of extra veins per wing was counted for each genotype, and more than 200 wings for each genotype were employed for quantification. In order to assess the survival rate of *dPLZF* mutants from the embryo to adult stages, we calculated the percentage of embryos that survived to adulthood. Experiments were conducted in triplicate with 200 embryos per experiment. The average survival rate of three experiments is presented.

### RT-PCR, realtime PCR and genomic PCR analysis

Expression of *dPLZF* was analyzed via RT-PCR in *dPLZF*-positive or *dPLZF*-null flies. Total RNA was prepared with Trizol (Gibco/BRL) in accordance with the manufacturer's instructions. Messenger RNA was also isolated in accordance with the manufacturer's instructions (QIAGEN, Oligotex<sup>®</sup> mRNA). Quantitative realtime PCR was performed with a Rotor Gene 6000 (Cobett Research) and a SYBR Green reaction (Qiagen) according to the manufacturer's instructions. The *dPLZF* cDNA was amplified using the following primer set: 5'-GGACTTCTTCTACGAGGATTTGGTGTCC-3' and 5'-TTCTGTACCTGATAGCACTTGAAGTCGC-3'. Deletion sites within *dPLZF* were determined via genomic PCR analyses. The *dPLZF* mutants were detected with the following primer set: 5'-ACCCATGACGGTGTGTTGATATTCTGTAAAG-3' and 5'-AGGCAACGCATTTGTTTATTCTGAATAG-3'.

### Immunohistochemistry

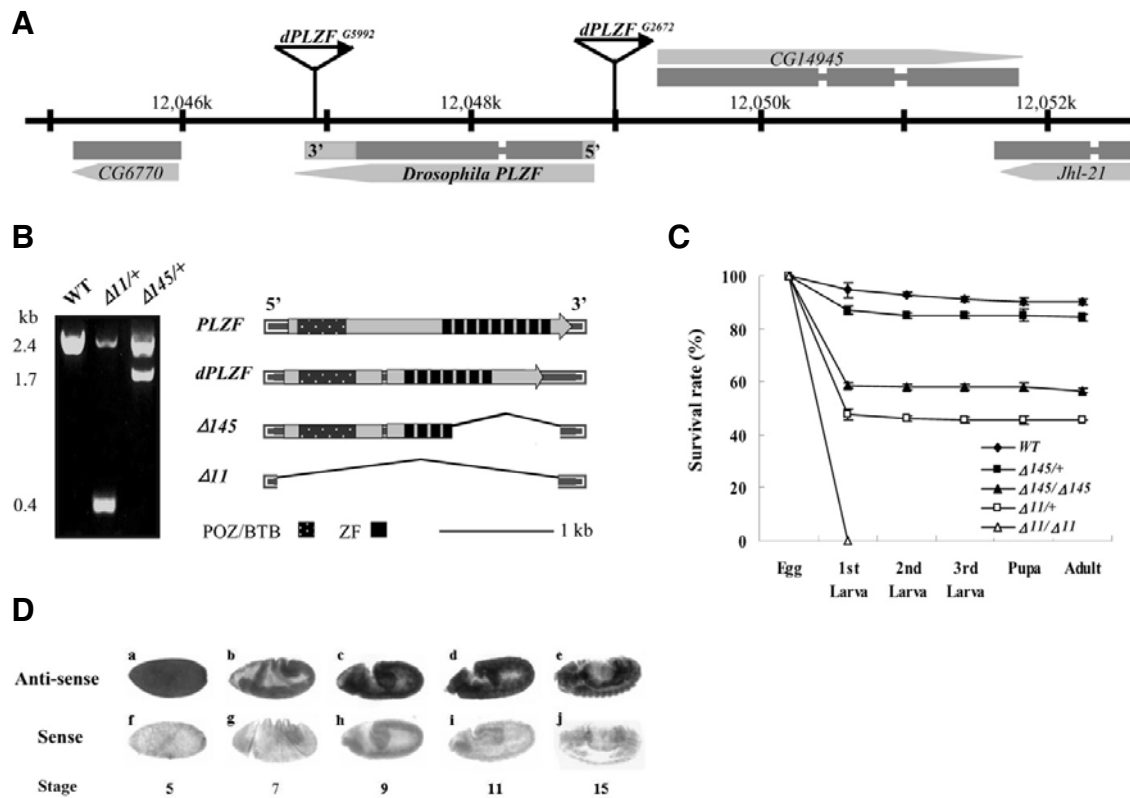
In order to detect the increase of rhomboid (*rho*) in wing imaginal disc, third-instar larval wing discs were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 1 h at room temperature and then incubated first with Rho antibody (1:200; Developmental Studies Hybridoma Bank) and subsequently with Alexa Fluor 488 goat anti-mouse immunoglobulin G secondary antibody (1:200; Invitrogen). The samples were examined under a fluorescence microscope.

## RESULTS

### *Drosophila* PLZF encodes a BTB/POZ domain with ZF motifs

To assess the roles of unknown genes containing BTB/POZ-ZF, we isolated the BTB/POZ-ZF-containing *Drosophila* genes CG6792 (*dPLZF*), *Ken & Barbie*, *Cp190*, CG15725, CG32611, and CG6765 in an EP line collection from GenExel and specifically overexpressed these genes in the eye and wing using the Gal4 system. Among the tested BTB/POZ-ZF genes, *dPLZF* induced phenotypic changes in wing development when overexpressed by the *en-Gal4* and MS1096-Gal4 drivers. The *dPLZF* gene encodes a 469-amino acid protein that contains an N-terminal BTB/POZ domain and seven C-terminal C<sub>2</sub>H<sub>2</sub>ZF motifs (Fig. 1B). The *Drosophila* PLZF (*dPLZF*), human PLZF, and *C. elegans* EOR-1 all harbor an N-terminal BTB/POZ domain (Supplementary Fig. S1a) and similarly spaced C<sub>2</sub>H<sub>2</sub>ZF Krüppel-type ZF motifs (Supplementary Fig. S1b). Human PLZF and *dPLZF* are 32% identical and 44% similar over those regions. Previously, we found that the wings of a transgenic fly that expressed the human PLZF under the control of MS1096-Gal4 exhibited additional ectopic veins (Ko et al., 2006). The wing phenotype of the *dPLZF* fly is similar to that of the human PLZF transgenic fly. These similarities indicate that *dPLZF* functions similarly to human PLZF. Therefore, *dPLZF* is an orthologue of human PLZF.

Mammalian PLZF expression has been reported in a variety



**Fig. 1.** *dPLZF* is essential for normal development. (A) Schematic of the genomic organization of the *dPLZF* locus in *Drosophila*. (B) Left panel: deletion size measurement by genomic DNA PCR. Right panel: Gene structures of human *PLZF*, *dPLZF*, and *dPLZF* mutants ( $\Delta 11$  and  $\Delta 145$ ). The gray box indicates coding regions. (C) Survival rates of wild-type flies and *dPLZF* mutants during development: egg, larva, pupa, and adult. (D) Whole-mount *in situ* hybridization of wild-type embryos was conducted at various stages using *dPLZF* anti-sense (A-E) or sense (F-J) RNA probes.

of tissues and developmental processes (Kelly and Daniel, 2006). In an effort to determine the expression patterns of *dPLZF* during the developmental process, we assessed mRNA levels during different developmental stages using polymerase chain reaction with reverse transcription (RT-PCR). We detected *dPLZF* mRNA in a variety of developmental stages, but the expression levels differed significantly. Expression of *dPLZF* gradually increased from the egg to the pupal stages but was present only at low levels in adult flies (Supplementary Fig. S2). In order to characterize the specific expression pattern of *dPLZF* during the early developmental stages, we visualized expression in several egg stages using *in situ* hybridization with digoxigenin-labeled *dPLZF* RNA probes. We found that *dPLZF* is expressed throughout the majority of egg stages (Fig. 1D). These results show that *dPLZF* is expressed from the egg to pupal stages, thereby reflecting the centrality of *dPLZF* in development.

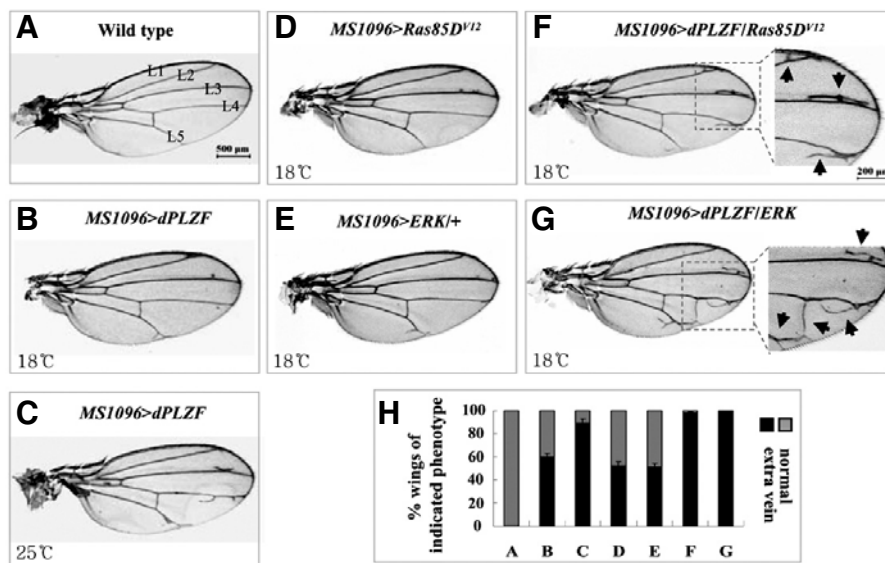
#### Characterization of *dPLZF* loss-of-function mutants

To further evaluate the physiological roles of *dPLZF*, we generated two *dPLZF* deletion-mutant lines ( $\Delta 11$  and  $\Delta 145$ ) via imprecise excision of the EP-element from the *dPLZF*<sup>G5992</sup> line (Fig. 1A). In the *dPLZF* mutant lines, either 2029 bp ( $\Delta 11$ , *Drosophila* chromosome 2L; from 12,047,078 bp to 12,049,106 bp) or 770 bp ( $\Delta 145$ , *Drosophila* chromosome 2L; from 12,047,078 bp to 12,047,847 bp) was deleted by the EP-element. Deletion sites were confirmed using PCR of genomic DNA (Fig. 1B) and

sequencing. The homozygous  $\Delta 11$  *dPLZF*-null flies completely lacked *dPLZF* transcripts and showed complete lethality in the egg stage (Fig. 1C). The  $\Delta 145$  *dPLZF*-mutant flies lacked only three ZF motifs (Fig. 1B), but no full-length *dPLZF* was detected in the homozygote (Fig. 4A). We verified that the homozygotes of  $\Delta 145$  *dPLZF*-mutant flies generated a small quantity of shortened transcripts (data not shown). Interestingly, in contrast to the  $\Delta 11$  *dPLZF*-null flies, approximately 60% of homozygote  $\Delta 145$  *dPLZF*-mutant flies survived to the adult stage (Fig. 1C). These data indicate that the BTB/POZ domains are crucial to development as the homozygous  $\Delta 11$  *dPLZF*-null flies, which lack the entire BTB/POZ domain, died during very early development whereas the homozygous  $\Delta 145$  *dPLZF*-mutant flies, which lack only a portion of this domain, did not.

#### *Drosophila* PLZF positively regulates wing vein differentiation

To determine the role of *dPLZF* in the fly, we analyzed *dPLZF* in developing transgenic flies using the GAL4/UAS system (Brand and Perrimon, 1993). We carefully observed the eyes, thorax, abdomen, legs, and wings of adult flies that expressed *dPLZF* under the control of a variety of Gal4 promoters. Notably, the overexpression of *dPLZF* driven by *engrailed*-Gal4 (*en*-Gal4), which drives the expression of the Gal4 activator in the wing posterior compartment, or MS1096-Gal4, which drives the expression of the Gal4 activator in the whole wing blade, generated extra and ectopic veins in various wing regions (Figs. 2B



**Fig. 2.** dPLZF upregulates the formation of wing veins via an interaction with Ras/ERK. (A) Wings from wild-type flies served as controls for comparison. (B-E) The overexpression of *Ras85D<sup>V12</sup>*, *ERK*, or *dPLZF* in *MS1096-Gal4* flies generated several extra wing veins and resulted in a wound edge on the wing. (F) Genetic interaction between *dPLZF* and *Ras85D<sup>V12</sup>* resulted in many extra veins, which were wider than the control veins. (G) Genetic interaction between *dPLZF* and *ERK* generated many extra veins that were wider than the control veins. Magnification is equivalent in (A-G). The fly lines shown in (B), (D), (E), (F), and (G) were grown at 18°C. (H) Quantification of flies with extra wing vein phenotype for each genotype (from A to G).  $n > 200$  *Drosophila* wings.

and 2C; Supplementary S3a) and also resulted in a distorted wing shape with expression of two copies of *dPLZF* (Supplementary Fig. S3b). These phenotypes are similar to the phenotypes induced by *Ras* or *ERK* overexpression in the wing of *Drosophila* (Figs. 2D, 2E, 3D, and 3G). The extra and ectopic veins generated by *dPLZF* overexpression, however, were reduced significantly by RNA interference (RNAi) of *dPLZF* (Figs. 4A and Supplementary Fig. S4).

We subsequently determined whether upregulation of Ras/ERK signaling enhanced the *dPLZF* overexpression phenotypes. The overexpression of both *dPLZF* and *Ras* (*Ras85D<sup>V12</sup>*) generated significantly enhanced extra and wide veins as compared to either *dPLZF* or *Ras* overexpression alone (Figs. 2B, 2D, 2F, and 3D). The overexpression of both *ERK* and *dPLZF* also resulted in the formation of many extra and wide veins, and this effect was greater than that induced by the expression of *dPLZF* or *ERK* alone (Figs. 2B, 2E, 2G, and 3G). These results demonstrated a more dramatic effect at the low temperature (18°C) than at room temperature (25°C). Another Ras pathway component *Draf*, which is downstream of Ras, was test for genetic interaction but, we were unable to assess the interaction between *dPLZF* and *Draf*, because the flies that co-expressed *Draf* and *dPLZF* died at the pupal stage due to wing malformation. The levels of wing phenotype variation by different genetic interactions were statistically analyzed and presented in Fig. 2H. These data are consistent with the argument that the two genes interact with each other and suggest that *dPLZF* promotes the differentiation of wing vein cells via the Ras/ERK signaling pathway.

#### Genetic interactions between the dPLZF and Ras/ERK pathways

To more clearly define the role of *dPLZF* in the *Drosophila* Ras/ERK signaling pathway, we tested a variety of genetic interactions between *dPLZF*-null flies ( $\Delta 11$  and  $\Delta 145$ ) and flies that overexpress Ras/ERK pathway components. We observed that the gain-of-function phenotypes of the Ras/ERK pathway components (*Ras*, *Draf*, and *ERK*) were reduced by crossing with *dPLZF*-null flies (Fig. 3; Supplementary Fig. S4). Wing phenotypes with extra veins induced by the ectopic expression of constitutively active Ras (Figs. 2D, 3A, and 3D) were partially

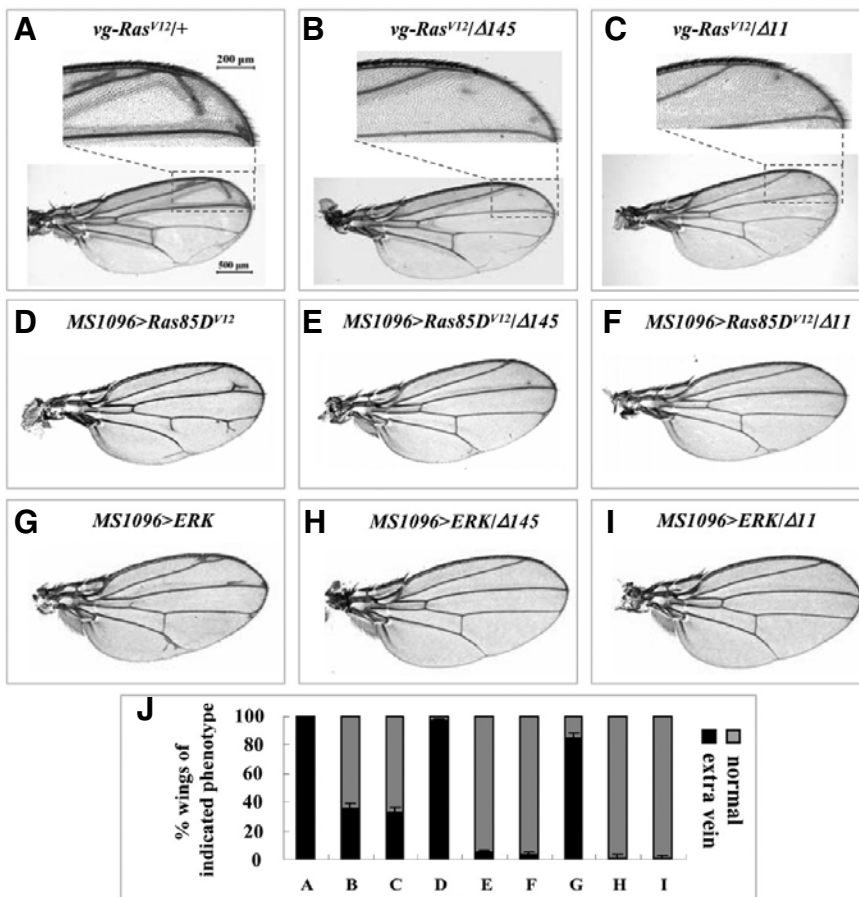
reduced in  $\Delta 145$  and  $\Delta 11$  *dPLZF*-null flies (Figs. 3B, 3C, 3E, and 3F). Furthermore, the gain-of-function phenotypes induced by *ERK* were abolished completely in the  $\Delta 145$  and  $\Delta 11$  *dPLZF*-null flies (Figs. 3H and 3I). The levels of wing phenotype variation by different genetic interactions were statistically analyzed and depicted as a graph in Fig. 3J.

The Ras signaling cascade is conserved in *Drosophila*, and both c-Jun N-terminal kinase (JNK) and ERK are downstream targets of the Ras cascade (Derjard et al., 1994). Therefore, we attempted to determine whether *dPLZF* is involved in the JNK signaling pathway via crossing the flies that overexpress *dPLZF* with dominant-negative JNK (*JNK<sup>DN</sup>*) flies; however, *JNK<sup>DN</sup>* exerted no effect on the extra vein formation induced by *dPLZF* overexpression (data not shown).

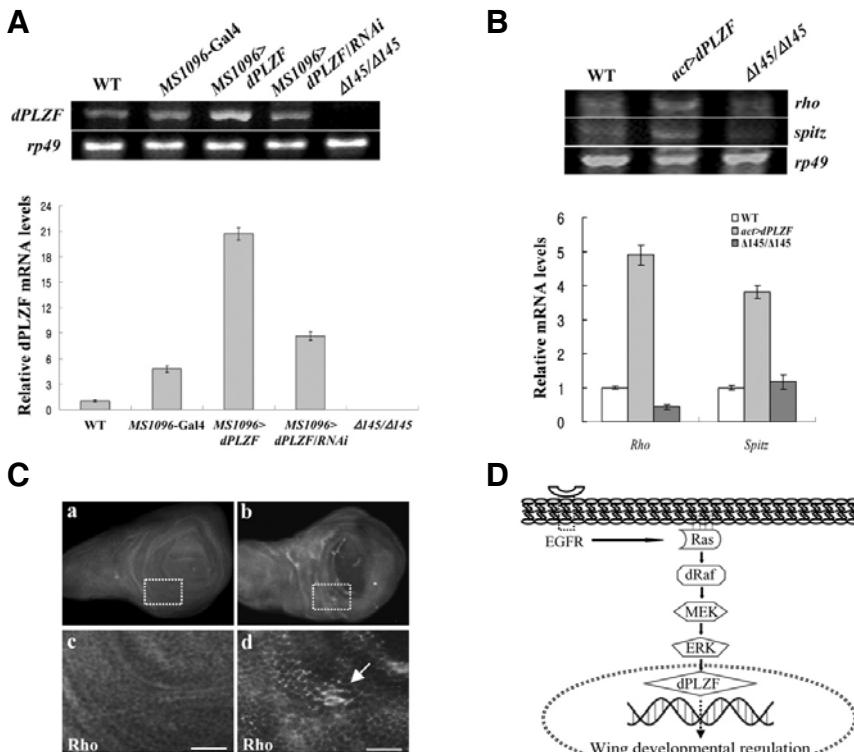
Furthermore, the *rhomboid* (*rho*), *decapentaplegic* (*dpp*), *spitz*, and *vein* (*vn*) genes perform crucial functions in vein specification during wing development (Blair, 2007). Indeed, *rho*-mediated hyperactivation of the MAPK pathway is required for vein formation throughout late larval and early pupal development. We determined whether these genes were regulated by *dPLZF* using RT-PCR analysis and real-time PCR for these genes from *dPLZF*-overexpressing flies and *dPLZF*-null flies. The expression levels of *rho* and *spitz* were induced more than three and a half times than normal in overexpressed *dPLZF* mutants and reduced in hypomorph *dPLZF* mutants (Fig. 4B). Additionally, the *rho* protein was highly expressed in the wing disc of *dPLZF*-overexpressing 3<sup>rd</sup> instar larva (Fig. 4C). The results of these experiments indicate that *dPLZF* modulates vein cell fate by functioning as a transcriptional regulator of the Ras/ERK pathway downstream targets, *rho* and *spitz*, during wing development in *Drosophila* (Fig. 4D).

#### DISCUSSION

BTB/POZ-ZF transcription factors perform critical functions in development and tumorigenesis via direct or indirect modulation of gene expression. The human genome harbors approximately 60 BTB/POZ-ZF proteins (van Roy and McCrea, 2005), including PLZF, Bcl-6, FAZF, ZBTB7, HIC-1, MIZ-1, and Kaiso as well as many other BTB/POZ-ZF proteins whose relevant molecular mechanisms have yet to be determined. Therefore,



**Fig. 3.** Extra wing veins induced by Ras/ERK activation can be down-regulated by haploinsufficiency of *dPLZF* mutants. (A) *vg-Ras<sup>V12</sup>/+* wing exhibited extra veins in the wing. (B, C) Extra wing vein phenotype reduced by haploinsufficiency of *dPLZF* mutants. (D) *Ras85D<sup>V12</sup>* flies contained the extra wing vein that exhibited Ras activity by *MS1096-Gal4*. (E, F) Ras activity was almost completely suppressed in *dPLZF* mutants. (G) The wing from flies that overexpressed ERK. (H, I) ERK activity was completely suppressed in the *dPLZF* mutants. (J) Quantified data from (A-I).  $n > 200$  *Drosophila* wings. Magnification is equivalent in A-C and D-I.



**Fig. 4.** *dPLZF* promotes wing vein-specific gene expression during wing development. (A) *dPLZF* mRNA levels in the wing imaginal disc from flies with *dPLZF* overexpression or knockdown. Upper panel: RT-PCR analysis of *dPLZF* expression. Lower panel: Real-time PCR analysis of *dPLZF* expression. (B) The mRNA levels of *rho* and *spitz* analyzed in the wing imaginal disc from flies with *dPLZF* overexpression or knockdown mutants. Upper panel: RT-PCR analysis of *rho* and *spitz* expression in flies that have *dPLZF* overexpression or knockdown. Lower panel: Real-time PCR analysis of *rho* and *spitz* expression. The *rp49* gene was used as a control. (C) Subcellular localization of rho in the wing imaginal disc. Arrow in (D) indicates exogenous rho protein. (A, C) show the wing imaginal disc from control *MS1096-Gal4* flies. (B, D) show the wing imaginal disc from *dPLZF* overexpression flies. Boxes indicate the magnified sections shown in (C) and (D). Scale bars: 20  $\mu$ m. (D) Model of the *dPLZF* role in wing vein development correlated with Ras/ERK signaling. EGFR/Ras/ERK signaling controls both vein and inter-vein cell fate in the *Drosophila* wing. Active genes are indicated with black arrows.

we screened the *Drosophila* genome for undefined BTB/POZ-ZF genes in order to identify orthologues of human genes. In this screen, we detected the *dPLZF* gene, which encodes an N-terminal BTB/POZ domain and seven C-terminal ZF motifs of the C<sub>2</sub>H<sub>2</sub> DNA-binding type. This dPLZF also harbors the (S/T)P motifs, T268 and S437, similar to the ERK phosphorylation site in the C-terminal region. The structure of dPLZF is similar to that of human PLZF (Fig. 1), and the wing phenotype generated by *dPLZF* overexpression is also quite similar to the wing phenotype of the transgenic fly that expresses human PLZF under the control of MS1096-Gal4 (Ko et al., 2006). These similarities indicated that *dPLZF* is an orthologue of human PLZF.

We hypothesized that *dPLZF* has an effect on developmental process, as this transcription factor is similar to other members of the BTB/POZ-ZF family. As might be expected of a BTB/POZ-ZF family member, *dPLZF* was expressed in a variety of developmental stages, including the egg, larval, and pupal stages but not during adulthood in *Drosophila* (Supplementary Fig. S2). Moreover, *dPLZF* markedly induced the generation of extra veins in the *Drosophila* wing, and this phenotype is similar to the *Ras* or *ERK* expression phenotype in the *Drosophila* wing (Fig. 2). Ras/ERK signaling is a critical determinant of vein cell fate, but the downstream effectors that control vein formation remain to be elucidated. Here, we provide evidence from experiments using null and overexpression *dPLZF* mutants that suggest that dPLZF performs a function in ectopic vein regulation. We noted that the co-expression of both *dPLZF* and *Ras/ERK* resulted in significantly enhanced extra and wide veins to a greater degree than was noted upon individual expression of either *dPLZF* or *Ras/ERK*. In contrast, the gain-of-function phenotypes induced by *Ras* or *ERK* were suppressed in flies crossed with the loss-of-function *dPLZF* mutant. No genetic interactions, however, were noted between *dPLZF* and JNK signaling. In addition, we demonstrated that *dPLZF* overexpression induces the expression of vein-specific genes, such as *rho* and *spitz*, but not *dpp* and *vn*, which are required for the specification of vein fate. Rho signaling is required for the maintenance of vein cell fate and activates *spitz* (Blair, 2007; Lee, 2010) as well as the canonical Ras/ERK pathway via the activation of Egr ligands (Roch et al., 2002). These results demonstrate that dPLZF is both a downstream component of the Ras/ERK pathway and an activator of Ras/ERK signaling during the development of the *Drosophila* wing.

In summary, we identified *dPLZF*, an orthologue of human PLZF and *C. elegans* EOR-1, that is involved in the Ras/ERK pathway. Remarkably, *dPLZF* not only regulates the Ras/ERK pathway but is also affected by the Ras/ERK pathway during vein development in the *Drosophila* wing. Moreover, transformed flies expressing *dPLZF* constitute a favorable model for the study of the genetic interactions and molecular properties of PLZF. Further genetic studies of *dPLZF* will provide us with greater insight into the function and regulation of this transcription factor during development.

*Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).*

## ACKNOWLEDGMENTS

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