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Hyperactivation of the G_{12}-Mediated Signaling Pathway in *Caenorhabditis elegans* Induces a Developmental Growth Arrest via Protein Kinase C

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Summary

The G_{12} type of heterotrimeric G-proteins play an important role in development and behave as potent oncogenes in cultured cells [1–5]. However, little is known about the molecular nature of the components that act in the G_{12}-signaling pathway in an organism. We characterized a *C. elegans* Gα subunit gene, *gpa-12*, which is a homolog of mammalian $G_{12}/G_{13}\alpha$, and found that animals defective in *gpa-12* are viable. Expression of activated GPA-12 (G_{12}QL) results in a developmental growth arrest caused by a feeding behavior defect that is due to a dramatic reduction in pharyngeal pumping. To elucidate the molecular nature of the signaling pathways in which G_{12} participates, we screened for suppressors of the G_{12}QL phenotype. We isolated 50 suppressors that contain mutations in *tpa-1*, which encodes two protein kinase C isoforms, TPA-1A and TPA-1B, most similar to PKCθ/δ. TPA-1 mediates the action of the tumor promoter PMA [6]. Expression of G_{12}QL and treatment of wild-type animals with PMA induce an identical growth arrest caused by inhibition of larval feeding, which is dependent on TPA-1A and TPA-1B function. These results suggest that TPA-1 is a downstream target of both G_{12} signaling and PMA in modulating feeding and growth in *C. elegans*. Taken together, our findings provide a potential molecular mechanism for the transforming capability of G_{12} proteins.

Results and Discussion

The *C. elegans* homolog of mammalian $G_{12}/G_{13}\alpha$, GPA-12, is expressed throughout development in pharyngeal muscle cells that control pumping of the pharynx (Figure 1A) and in hypodermal cells (Figure 1B). We isolated a loss-of-function *gpa-12(pk322)* mutant [7] in which 2 kb of the *gpa-12* coding sequence is deleted. Since the deletion includes the regions needed for GTP binding and hydrolysis, *pk322* is likely a null allele. Unlike the $G_{12}/G_{13}\alpha$ homolog Concertina in *Drosophila* [4] and $G_{13}\alpha$ in mice [5], the inactivation of *gpa-12* does not cause embryonic lethality. This raises the possibility that GPA-12 is redundant with other Gα subunits of *C. elegans*, as demonstrated for $G_{12}\alpha$ and $G_q\alpha$ in mice [8]. Since GPA-12 is highly expressed in the pharynx, we quantified the pharyngeal pumping rate in *gpa-12* mutants. Mutations that affect pumping cause feeding behavior defects and a delay in growth [9]. However, we observed no significant difference in the rate of pumping (Figure 2A) or growth rate (data not shown) of *gpa-12* mutants compared to wild-type. Furthermore, no disorganization of the hypodermis is seen in *gpa-12* mutants, as demonstrated by normal expression of a *jam-1::gfp* reporter construct in hypodermal cells [10] (data not shown).

Because no phenotypic defects are observed in *gpa-12* mutants, we analyzed the effect of *gpa-12* gene dosage on behavior. Therefore, we generated transgenic animals that overexpress either wild-type or activated GPA-12. To construct the activated form of GPA-12, we changed a glutamine (Q) at position 205 to a leucine (L), which is analogous to the Q229L mutation in mammalian activated $G_{12}\alpha$. Overexpression was accomplished by using transgenes that carry a multicopy array of the *gpa-12* wild-type gene or the constitutively active *gpa-12* mutant gene under control of its endogenous promoter or a heat shock promoter (*hsp*). Interestingly, when we heat shocked animals that carry a chromosomal integrated transgene overexpressing activated GPA-12 (*pkIs1330*[*hsp::gpa-12^{QL}*]) for 2 hr at an early larval stage, we found that all animals arrest their developmental growth (Figure 2B) but eventually recover. Similar results were found for another independently integrated transgene, *pkIs1355*[*hsp::myc:gpa-12^{QL}*], which contains a Myc-tag fused to the active form of GPA-12 (data not shown). Also, animals carrying an extrachromosomal array of the constitutively activated *gpa-12* gene under control of its endogenous promoter, *pkEx1467*, arrest their growth (see the Supplemental Experimental Procedures available with this article online). In contrast to *pkIs1330* and *pkIs1355* animals, these developmentally arrested larvae could not recover to adulthood and ultimately died. No growth arrest or reduced pumping (see below; Figure 2C) was observed in animals overexpressing wild-type GPA-12 from its endogenous promoter (*gpa-12::gpa-12^{WT}*) or a heat shock promoter (*hsp::gpa-12^{WT}*). Thus, while GPA-12 is not essential, overexpression of activated GPA-12 causes an arrest of larval growth.

Based on the expression of GPA-12 in the pharynx, we determined whether reduced pharyngeal pumping causes the growth arrest induced by activated GPA-12 (G_{12}QL). As shown in Figure 2C, heat-shocked *pkIs1330* [*hsp::gpa-12^{QL}*] transgenic larvae exhibit slow and irregular pumping, whereas wild-type larvae pump normally after heat shock. Slow pumping is not caused by structurally defective pharyngeal muscle, because these *pkIs1330* animals completely recover from the growth arrest and do not show any pharyngeal defects. As a result of slow pumping, almost no food is taken up by the pharynx in *pkIs1330* larvae, as can be visualized by staining bacteria in the gut of these animals with the lipophilic fluorescent dye (DiO) [11] (Figure S1). Taken together, these data indicate that the arrest of larval growth is caused by a feeding defect.

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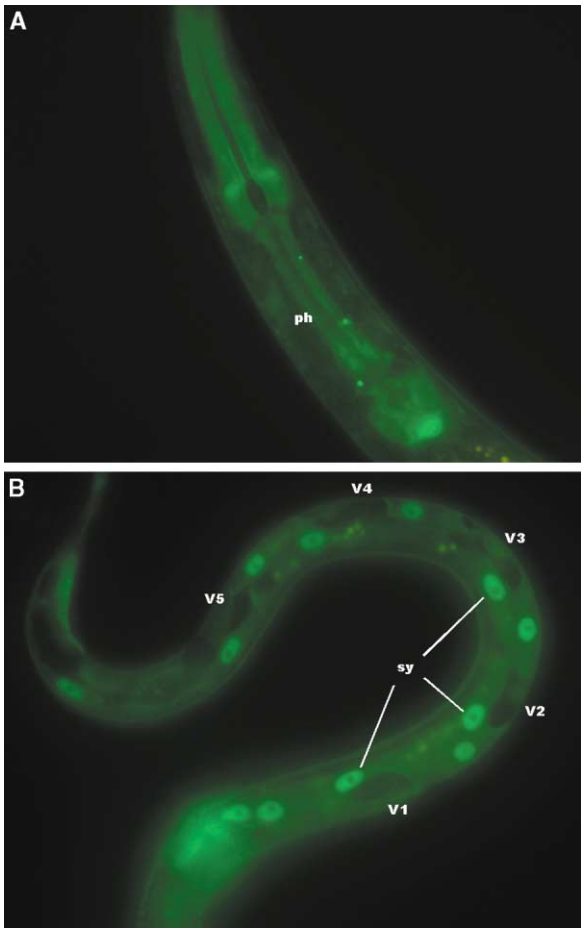

Figure 1. Expression of *gpa-12* in *C. elegans* as Detected by Using a Translational GFP Gene Fusion

(A and B) pRP2018 contains the full-length wild-type *gpa-12* sequence fused to *gfp*. (A) The head region of an L2-staged larva showing expression of GPA-12 in the pharyngeal muscle cells (ph). (B) L1 larva showing expression in the cytoplasm and nuclei of the hyp7 syncytium (sy), which comprises the main body hypodermis, and in additional cells, which are presumably the P cells (precursors of motorneurons in the ventral nerve cord). No expression is detected in hypodermal seam cells (V1–V6 cells). The nuclear localization of the *gpa-12::gfp* reporter construct (pRP2018) may reflect the endogenous GPA-12 expression, since the *gfp*-reporter vector used does not contain a nuclear localization signal. We observed an identical expression pattern of GPA-12 by using another *gpa-12::gfp* reporter construct (pRP2015; see the Supplemental Experimental Procedures), including nuclear localization.

To study the molecular nature of the components involved in G_{12}-mediated signaling, we performed genetic screens to isolate extragenic mutations that suppress the activated GPA-12 (G_{12}QL)-induced growth arrest. In total, 50 independent mutations were isolated that suppress the G_{12}QL-induced growth arrest after heat shock treatment (2 hr at 33°C); 44 mutations were caused by the use of ethylmethane-sulfonate (EMS) as a mutagen, and 6 were caused by using a transposon insertion method [12]. By means of the latter approach, we found that four suppressors contained a Tc1 transposon insertion in the coding region of the *tpa-1* gene. Sequence analyses revealed that all EMS-derived suppressors also contain a mutation in the *tpa-1* gene; most mutations change conserved amino acid residues or result in premature stops (Figure 3A; Table S1). *tpa-1* encodes two isoforms of protein kinase C (PKC), TPA-1A and TPA-1B, which are most similar to the "novel" PKCθ/δ [13]. All isolated mutations disrupt both isoforms of TPA-1, whereas none are located in the first four exons of *tpa-1A*. In a separate mutagenesis screen in which shorter heat shock treatment (5 min) was used, we isolated two alleles of *tpa-1*, *pk1584* and *pk1585*, that have a stop in *tpa-1A*, thereby disrupting TPA-1A, but not TPA-1B (Figure 3A). These mutations only moderately suppress the growth arrest induced by 2 hr of heat shock (Table S1). Introduction of *tpa-1* wild-type genomic sequence rescued *tpa-1(pk1401)* mutants, resulting again in developmentally arrested animals after the heat shock-induced G_{12}QL expression of *pkIs1330*. Moreover, reduced pumping induced by G_{12}QL is completely suppressed by *tpa-1(pk1401)* mutants (Figure 2C). Importantly, *tpa-1* mutations do not affect GPA-12 expression, but rather act on GPA-12 signaling (see the Supplemental Experimental Procedures). Together, these data indicate that both TPA-1A and TPA-1B are involved in the growth arrest induced by G_{12}QL.

Mutations in *tpa-1* were first identified as suppressors of the developmental growth arrest induced by the tumor-promoting phorbol ester PMA [6]. To further investigate whether the *tpa-1* mutations isolated in this study are able to suppress the growth arrest induced by PMA, we analyzed all suppressors of the G_{12}QL phenotype in the presence of 0.1–1 μg/ml PMA, a concentration range commonly used to activate PKCs. Most homozygous mutations in *tpa-1* that disrupt both isoforms of TPA-1 cause resistance to the PMA-induced growth arrest compared to wild-type and non-heat-shocked *pkIs1330* [*hsp::gpa-12*^{QL}] control animals (Figure 4; Table S1). Only 2 *tpa-1* alleles, *pk1478* and *pk1517*, of the 48 isolated *tpa-1* suppressor mutations are sensitive to 1 μg/ml PMA but are resistant to lower PMA concentrations compared to non-heat-shocked *pkIs1330* control animals (Figure 4). Animals with mutations in *tpa-1* that disrupt TPA-1A, but not TPA-1B (*pk1584* and *pk1585*), are moderately resistant to the developmental growth arrest induced by 1 μg/ml PMA (Table S1). These data suggest that both TPA-1A and TPA-1B are required for the growth arrest induced by PMA.

We observed that both PMA and activated GPA-12 (G_{12}QL) induce a growth arrest that is mediated by TPA-1A and TPA-1B. After treatment with PMA for 6 hr, wild-type larvae pump slowly (94 $\pm$ 8 pumps/min), as they do following heat shock-induced expression of G_{12}QL, and this is suppressed by *tpa-1(pk1401)* (206 $\pm$ 9 pumps/min). This suggests that both PMA and GPA-12 act via TPA-1 in the pharynx to induce a growth arrest. To test this hypothesis, we cloned *tpa-1A* and *tpa-1B* cDNA under the control of the *myo-2* promoter; *myo-2* encodes a myosin heavy chain that is specifically expressed in the pharyngeal muscle. Indeed, specific expression of wild-type TPA-1A and TPA-1B in the pharynx in a *tpa-1* background results in the inhibition of larval growth after heat shock-induced G_{12}QL expression of *pkIs1330*[*hsp::gpa-12*^{QL}] animals or treatment with PMA (Table 1). Transgenic animals carrying *tpa-1B* cDNA un-

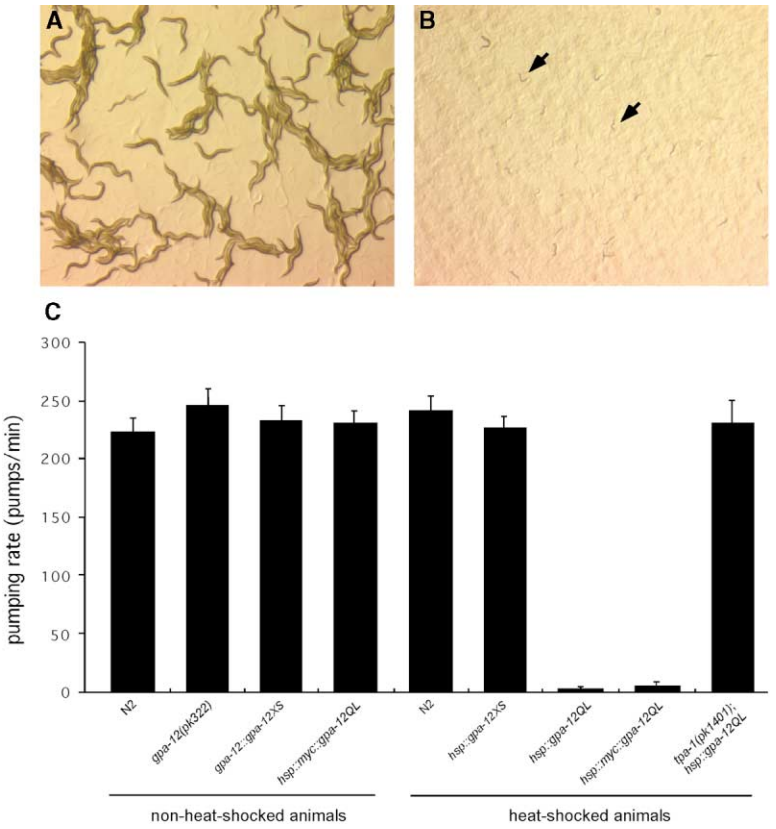

Figure 2. Transgenic Overexpression of Activated GPA-12 Induces Severely Reduced Pumping that Causes a Developmental Growth Arrest

(A and B) (A) In the presence of a heat shock treatment, growth of N2 (wild-type) animals is not inhibited and all larvae reach adulthood, (B) whereas *pkIs1330* transgenic animals that overexpress G_{12}QL show a developmental growth arrest, as indicated by the young larvae (arrows). Animals were synchronized, heat shocked for 2 hr at the L1 larval stage, and photographed 3 days later. Pictures were taken at the same magnification.

(C) The pharyngeal pumping rate is decreased to almost zero in animals after heat shock-induced expression of G_{12}QL using two independently integrated transgenes: *pkIs1330* [*hsp::gpa-12*^{QL}] and *pkIs1355*[*hsp::myc::gpa-12*^{QL}]. Both *pkIs1330* and *pkIs1355* contain multiple copies of the constitutive active *gpa-12* gene under control of a heat shock promoter (*hsp*); *pkIs1355* also contains a Myc-tag fused to the active form of GPA-12. The pumping activity is not affected in *gpa-12(pk322)* mutants compared to N2 (wild-type) animals. Normal pumping activity is also observed in multiple transgenic lines that overexpress wild-type GPA-12 from a transgene carrying a multicopy array, either extrachromosomal or integrated, of the wild-type *gpa-12* gene under control of its endogenous (*gpa-12::gpa-12*^{WT}) or heat shock promoter (*hsp:::gpa-12*^{WT}); in each case, data is shown for one transgenic line. Mutations in *tpa-1* suppress the pharyngeal pumping defect after heat shock treatment of *pkIs1330* animals. The rate of pumps is quantified in at least 20 larvae with or without heat shock treatment for each strain (see the Supplemental Experimental Procedures). The error bars indicate the 95% confidence interval of the mean.

der control of a hypodermal promoter (*dpy-7*) did not show a growth arrest, but resulted in cuticle defects (data not shown). Thus, the growth arrest is caused by activation of TPA-1 in the pharynx. This is further confirmed by the fact that expression of G_{12}QL from the *myo-2* promoter (*myo-2::gpa-12*^{QL}) is also able to induce a developmental growth arrest (see the Supplemental Experimental Procedures). The growth arrest of *myo-2::gpa-12*^{QL} transgenic animals is restored in a *tpa-1* suppressor background. We found one homozygous wild-type animal of the progeny of heterozygous *tpa-1* suppressor animals that contained the *myo-2::gpa-12*^{QL} transgenic array and survived to adulthood. Interestingly, this mosaic transgenic animal did not express the coinjected *gpb-2::gfp* reporter construct in the pharynx, where it is normally expressed [14], indicating that the *myo-2::gpa-12*^{QL} array has been lost in this lineage. Altogether, these data support a role for PMA and activated GPA-12 in the pharynx in regulating feeding behavior and therefore indirectly the growth of *C. elegans*.

Since the phorbol ester PMA directly interacts with and mediates the action of TPA-1, we examined whether the effects of PMA require the function of GPA-12. However, unlike *tpa-1* mutants, *gpa-12* mutants are sensitive to the PMA-induced developmental growth arrest like wild-type animals (Figure 4), suggesting that wild-type GPA-12 function is not required to cause PMA sensitivity. Therefore, we examined whether PMA sensitivity is affected in animals overexpressing activated GPA-12 (G_{12}QL). In the absence of heat shock-induced expression of G_{12}QL, two independent transgenic lines, *pkIs1330* and *pkIs1355*, are hypersensitive to PMA compared to wild-type (Figure 4). This result can be explained by a leaky expression of the heat shock promoter used. After heat shock-induced expression of G_{12}QL, the sensitivity of both transgenic lines toward PMA becomes even higher. As a consequence, these animals died at an early larval stage (100%). However, when we tested several transgenic lines that overexpress wild-type GPA-12 from its endogenous promoter or a heat shock promoter, no hypersensitivity toward PMA is observed (data not shown). These findings suggest that excessive activated GPA-12, and not loss or overexpression of wild-type GPA-12 function, affects the PMA-induced developmental sensitivity.

The genetic experiments presented here support a model in which TPA-1, most similar to nPKCθ or δ subunits, functions as a direct or indirect downstream effector of activated GPA-12. The two other subclasses of PKC isoforms, the "conventional" and "atypical" PKCs, as well as another member of the nPKC class, appear not to be involved in the activated GPA-12- and PMA-induced growth arrest (Table S2). Our experiments do not conclusively demonstrate that wild-type GPA-12 regulates TPA-1 activity, since we were unable to detect any phenotype (in particular a growth arrest) for either

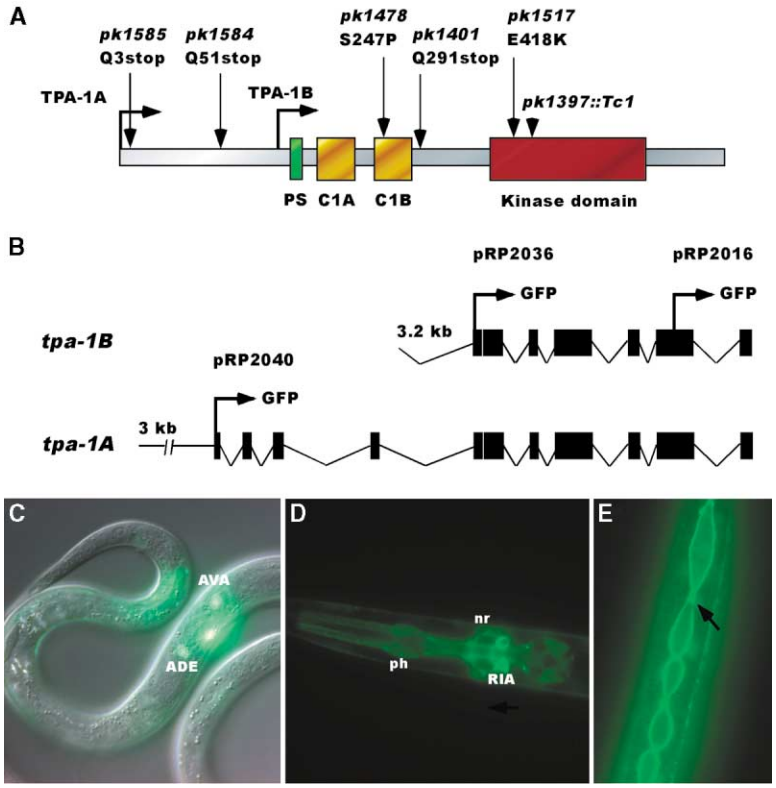

Figure 3. The Gene Structure of *tpa-1* and the Expression Pattern of *tpa-1A* and *tpa-1B* in *C. elegans*

(A) Structure of TPA-1 containing the pseudosubstrate region (PS), the cysteine-rich domains (C1A and C1B), and the kinase domain. Shown are the different mutations in *tpa-1*A and *tpa-1B* mentioned in the results section. We found 15 independent mutations in *tpa-1* that cause a premature termination of TPA-1, including 3 that contain an identical mutation (see Table S1 for the properties of all 50 *tpa-1* alleles).

(B) Genomic organization of the *tpa-1A* and *tpa-1B* gene. Solid boxes represent the coding sequence. *gfp*-reporter constructs of *tpa-1B* that only show expression of TPA-1B are made by using the intronic promoter present in the fourth intron of *tpa-1*. pRP2016 includes 3.2 kb of the upstream *tpa-1B* sequence and contains most of the coding sequence of *tpa-1B*, pRP2036 includes 3.2 kb of the upstream sequence of *tpa-1B*, and pRP2040 includes 3 kb of the upstream sequence of *tpa-1A*.

(C) Expression of *tpa-1A* in various neurons of the head, including AVA and ADE of an L1-staged larva using the *gfp*-reporter construct pRP2036.

(D and E) Animals that carry an integrated *tpa-1B::gfp* transgene (pRP2016) show expression of *tpa-1B* in the pharyngeal muscle cells (ph) (D), in neurons in the head (nr), including RIA (D), and in the membrane surrounding the hypodermal seam cells (E). More intense expression of *tpa-1B* is seen at the boundary between two seam cells ([E], arrow).

animals that overexpress wild-type GPA-12 or lack GPA-12 function. This is, however, in agreement with data on TPA-1 isoforms, because animals overexpressing TPA-1A or TPA-1B also do not show a discernible phenotype (data not shown). In *tpa-1* null mutants, the only detectable phenotypes relate to tissues in which GPA-12 is not expressed [15, 16]. Furthermore, the finding that activated GPA-12 and PMA induce an identical arrest of larval growth caused by their action in the pharynx suggests that the apparent activation of TPA-1 by activated GPA-12 reflects the actual signaling pathway normally used by wild-type GPA-12. In this pathway, PMA can bypass GPA-12 function by directly interacting with TPA-1, since animals lacking GPA-12 are not resistant to the developmental defects induced by PMA. The data do not support a model in which GPA-12 serves as a substrate for TPA-1, such that GPA-12 is inactivated by TPA-1. This is emphasized by the finding that animals overexpressing activated GPA-12 in the presence of PMA show a more severe phenotype (lethality at an early larval stage), which is probably due to an excessive activation and recruitment of TPA-1. We also do not believe that TPA-1 acts as positive regulator of GPA-12 in a feedback loop, since animals overexpressing wild-type GPA-12 are not hypersensitive to PMA (data not shown).

The discovery that PKCs function as receptors for phorbol ester tumor promoters established a key role for PKCs in tumorigenesis. In addition, constitutively active mutants of members of the G_{12} family are capable of malignant transformation of human cells. Therefore, it is an attractive model that the transforming capability of activated G_{12} is due (at least in part) to their ability to activate PKCs. Several biochemical studies performed in mammalian cells have shown that PKCs are activated by diacylglycerol (DAG) or other lipids (for review, see [17, 18]). In line with these data, GPA-12 may activate TPA-1 via lipid intermediates that can be generated by, for instance, phospholipases. Mutations in *egl-8* encoding a phospholipase Cβ isoform (PLCβ) do not suppress the growth arrest induced by G_{12}QL or PMA (data not shown). This finding suggests that there should be other lipid-mediated signal transducing proteins, like phospholipase D or A2, that activate TPA-1 through the production of DAG. All 44 EMS-mutagenized mutants that were identified as suppressors of the G_{12}QL-induced growth arrest by using 2 hr of heat shock have mutations in *tpa-1*. Components that act between GPA-12 and TPA-1 may not be identified in our suppressor screen, because they are either redundant or essential. Initial studies on suppressors isolated after 5 min of heat shock suggest that loci other than *tpa-1* are involved in the G_{12}QL-induced phenotype. Further characterization of these loci should uncover more components in the G_{12} pathway and give insight in the molecular mechanism with which activated G_{12} and TPA-1 induce a growth arrest.

Supplemental Data

Supplemental Data including the Experimental Procedures as well as Figure S1 and Tables S1 and S2 are available at http://images.cellpress.com/supmat/supmatin.htm.

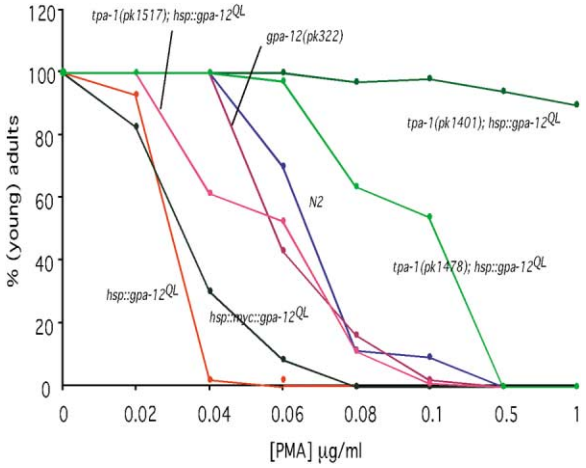

Figure 4. Developmental Growth Sensitivity in the Presence of the Phorbol Ester PMA

Loss-of-function *gpa-12(pk322)* animals are sensitive to PMA like N2 (wild-type) animals. In the absence of a heat shock treatment, *pkIs1330*[*hsp::gpa-12*^{QL}] and *pkIs1355*[*hsp::myc::gpa-12*^{QL}] transgenic animals are hypersensitive to PMA, whereas they die when a heat shock is applied (larvae showed pharyngeal defects and vacuoles in their hypodermis). Both *pkIs1330* and *pkIs1355* animals bear multiple copies of the constitutive active *gpa-12* gene under control of a heat shock promoter (*hsp*); *pkIs1355* also contains a Myc-tag fused to the active form of GPA-12. Three *tpa-1* mutants (*pk1401*, *pk1487*, and *pk1517*) in a *pkIs1330* background suppress the PMA-induced developmental sensitivity compared to non-heat-shocked *pkIs1330* control animals. *tpa-1(pk1401)* is a putative null allele. *tpa-1 (pk1478*) contains a S247P amino acid substitution in the first C1A region, which is responsible for binding of phorbol esters [19], indicating that this mutation in *tpa-1* may lower its affinity for PMA. In *tpa-1(pk1517)*, a conserved glutamic acid (E) at position 418 in the kinase domain is changed into a lysine (K), which presumably lowers the catalytic activity of TPA-1. Inhibition of larval growth induced by PMA is quantified by measuring the number of young adults at different concentrations of PMA after 5 days (see the Supplemental Experimental Procedures).

Acknowledgments

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References

1. Chan, A.M., Fleming, T.P., McGovern, E.S., Chedid, M., Miki, T., and Aaronson, S.A. (1993). Expression cDNA cloning of a transforming gene encoding the wild-type Gα_{12} gene product. Mol. Cell. Biol. *13*, 762–768.
2. Voyno-Yasenetskaya, T.A., Pace, A.M., and Bourne, H.R. (1994). Mutant α-subunits of G_{12} and G_{13} proteins induce neoplastic transformation of Rat-1 fibroblasts. Oncogene *9*, 2559–2565.
3. Xu, N., Voyno-Yasenetskaya, T., and Gutkind, J.S. (1994). Potent transforming activity of the Gα_{13} subunit defines a novel family of oncogenes. Biochem. Biophys. Res. Commun. *201*, 603–609.
4. Parks, S., and Wieschaus, E. (1991). The *Drosophila* gastrulation gene *concertina* encodes a Gα-like protein. Cell *64*, 447–458.
5. Offermanns, S., Mancino, V., Revel, J.P., and Simon, M.I. (1997). Vascular system defects and impaired cell chemokinesis as a result of Gα_{13} deficiency. Science *275*, 533–536.
6. Tabuse, Y., Nishiwaki, K., and Miwa, J. (1989). Mutations in a protein kinase C homolog confer phorbol ester resistance on *Caenorhabditis elegans*. Science *243*, 1713–1716.
7. Jansen, G., Thijssen, K.L., Werner, P., van der Horst, M., Hazendonk, E., and Plasterk, R.H. (1999). The complete family of genes encoding G proteins of *Caenorhabditis elegans*. Nat. Genet. *21*, 414–419.
8. Gu, J.L., Muller, S., Mancino, V., Offermanns, S., and Simon, M.I. (2002). Interaction of Gα_{12} with Gα_{13} and Gα_q signaling pathways. Proc. Natl. Acad. Sci. USA *99*, 9352–9357.
9. Avery, L. (1993). The genetics of feeding in *Caenorhabditis elegans*. Genetics *133*, 897–917.
10. Mohler, W.A., Simske, J.S., Williams-Masson, E.M., Hardin, J.D., and White, J.G. (1998). Dynamics and ultrastructure of developmental cell fusions in the *Caenorhabditis elegans* hypodermis. Curr. Biol. *8*, 1087–1090.
11. Hedgecock, E.M., Culotti, J.G., Thomson, J.N., and Perkins, L.A. (1985). Axonal guidance mutants of *Caenorhabditis elegans* identified by filling sensory neurons with fluorescein dyes. Dev. Biol. *111*, 158–170.
12. Wicks, S.R., de Vries, C.J., van Luenen, H.G., and Plasterk, R.H. (2000). CHE-3, a cytosolic dynein heavy chain, is required for sensory cilia structure and function in *Caenorhabditis elegans*. Dev. Biol. *221*, 295–307.
13. Sano, T., Tabuse, Y., Nishiwaki, K., and Miwa, J. (1995). The *tpa-1* gene of *Caenorhabditis elegans* encodes two proteins similar to Ca^{2+}-independent protein kinase Cs: evidence by

Table 1. Expression of *tpa-1A* and *tpa-1B* in the Pharyngeal Muscle Rescues the Activated GPA-12- and PMA-Induced Developmental Growth Arrest

Genotype	Transgene	Noninduced Animals (Percentage of the Total Number of Young Adults)	Growth after Expression of G_{12}QL (Percentage of the Total Number of Young Adults)	Growth in the Presence of PMA (Percentage of the Total Number of Young Adults)
tpa-1(pk1397)	none	99 (n = 368)	98 (n = 328)	94 (n = 317)
tpa-1(pk1397); hsp::gpa-12^{QL}	none	95 (n = 433)	96 (n = 384)	95 (n = 459)
tpa-1(pk1397)	*myo-2::tpa-1A*	97 (n = 413)	96 (n = 392)	7 (n = 229)
tpa-1(pk1397); hsp::gpa-12^{QL}	*myo-2::tpa-1A*	91 (n = 280)	5 (n = 193)	3 (n = 177)
tpa-1(pk1397)	*myo-2::tpa-1B*	98 (n = 358)	95 (n = 368)	8 (n = 296)
tpa-1(pk1397); hsp::gpa-12^{QL}	*myo-2::tpa-1B*	82 (n = 136)	2 (n = 144)	4 (n = 97)

Assays are described in the Supplemental Experimental Procedures. For each injected construct, we generated at least two extrachromosomal transgenic lines and crossed these lines in a *pkIs1330*[*hsp::gpa12*^{QL}] background. *pkIs1330* contains multiple copies of the constitutive active *gpa-12* gene under control of a heat shock promoter (*hsp*). Growth after expression of G_{12}QL (2 hr at 33°C) or in the presence of PMA (1.0 µg/ml) was determined by counting the number of animals (n) after 4 days and calculating the percentage of young adults.

complete genomic and complementary DNA sequences of the *tpa-1* gene. J. Mol. Biol. *251*, 477–485.

14. van der Linden, A.M., Simmer, F., Cuppen, E., and Plasterk, R.H. (2001). The G-protein β-subunit GPB-2 in *Caenorhabditis elegans* regulates the $G\alpha_o$-$G\alpha_q$ signaling network through interactions with the regulator of G-protein signaling proteins EGL-10 and EAT-16. Genetics *158*, 221–235.
15. Waggoner, L.E., Zhou, G.T., Schafer, R.W., and Schafer, W.R. (1998). Control of alternative behavioral states by serotonin in *Caenorhabditis elegans*. Neuron *21*, 203–214.
16. Waggoner, L.E., Dickinson, K.A., Poole, D.S., Tabuse, Y., Miwa, J., and Schafer, W.R. (2000). Long-term nicotine adaptation in *Caenorhabditis elegans* involves PKC-dependent changes in nicotinic receptor abundance. J. Neurosci. *20*, 8802–8811.
17. Nishizuka, Y. (1992). Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science *258*, 607–614.
18. Ron, D., and Kazanietz, M.G. (1999). New insights into the regulation of protein kinase C and novel phorbol ester receptors. FASEB J. *13*, 1658–1676.
19. Ono, Y., Fujii, T., Igarashi, K., Kuno, T., Tanaka, C., Kikkawa, U., and Nishizuka, Y. (1989). Phorbol ester binding to protein kinase C requires a cysteine-rich zinc-finger-like sequence. Proc. Natl. Acad. Sci. USA *86*, 4868–4871.