Note

Caenorhabditis elegans unc-37/groucho Interacts Genetically With Components of the Transcriptional Mediator Complex

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ABSTRACT

Groucho functions as a general corepressor by modulating chromatin structure and has a widespread role in many developmental processes. Here we show that Groucho may also interact with the basal transcriptional machinery. Mutations in *Caenorhabditis elegans groucho* interact with mutations in components of the transcriptional Mediator complex, resulting in synthetic lethality and loss of male sensory neurons.

TRANSCRIPTION initiation in eukaryotes requires the recruitment and assembly of a multifactor complex on the promoter, including chromatin remodeling factors, gene-specific DNA-binding proteins, general transcription factors (TAFs), the Mediator complex, and core RNA polymerase (Björklund *et al.* 1999). The Mediator complex is one component of the holoenzyme that is thought to contain targets of regulatory factors (Carlson 1997). How the transcription initiation apparatus integrates multiple inputs to specify gene expression during cell fate determination in multicellular development is a major question in developmental biology.

The postembryonic development of *Caenorhabditis elegans* male rays provides an opportunity to study the problem of how regulation of a transcriptional cascade leads to the differentiation of defined cell types at predetermined sites in the body. There are nine pairs of rays in the adult male tail (Figure 1, A and B), which collectively develop from three bilateral pairs of embryonic seam cells, V5, V6, and T. Male-specific postembryonic proliferation of V6 initiates with expression of the *caudal* homolog *pal-1*, which acts cell autonomously to turn on the expression of Hox gene *mab-5* (Hunter *et al.* 1999). MAB-5 in turn directly or indirectly activates the expression of another Hox gene *egl-5* and bHLH protein *lin-32* (Emmons 1999; Ferreira *et al.* 1999).

In wild type, *pal-1* expression in V6 requires the function of a *cis*-regulatory element lying within an intron (Zhang and Emmons 2000). We showed previously that *pal-1* can also be activated by an alternate pathway that under normal circumstances is repressed by two proteins, SOP-1 and SOP-3, both putative components of the transcriptional Mediator complex. *pal-1* expression in V6 is blocked if the *cis*-regulatory element is mutated [in the mutant *pal-1(e2091)*]. Expression can be restored via the alternate pathway if the activity of either SOP-1 or SOP-3 is reduced or eliminated (Zhang and Emmons 2000, 2001).

Unlike the normal pathway, the alternate pathway is stimulated by *bar-1*, which encodes a β-catenin homolog (Eisenmann *et al.* 1998; Zhang and Emmons 2000, 2001). β-Catenin is a signal transduction component of the Wnt pathway that acts together with a high mobility group DNA-binding protein of the TCF/LEF family (POP-1 in *C. elegans*) to promote gene activation (Korswagen *et al.* 2000; Polakis 2000). Mutations in *bar-1* have no effect on ray development in an otherwise wild-type genetic background. Stimulation of *pal-1(e2091)* gene activity by *bar-1* in *sop-1* and *sop-3* mutants suggests that *sop-1* and *sop-3* act in part by blocking action of the Wnt pathway under inappropriate circumstances.

In Drosophila and vertebrates, negative gene regulation, including negative regulation by the Wnt pathway, often requires proteins of the Groucho/transducin-like Enhancer of split (Gro/TLE) family, which act as corepressors (Cavallo *et al.* 1998; Fisher and Caudy 1998; Levanon *et al.* 1998; Roose *et al.* 1998; Mannervik *et al.* 1999; Chen and Courey 2000). A similar corepressor function has been demonstrated for the *C. elegans groucho* homolog, *unc-37* (Pflugrad *et al.* 1997). We therefore examined the effects of *unc-37* mutations on ray development, alone and in combination with other mutations, to determine whether it played a role in negative regulation, possibly by the Wnt pathway.

Positive requirement for *unc-37* in expression of *pal-1* activity: *unc-37* has one or more essential zygotic func-

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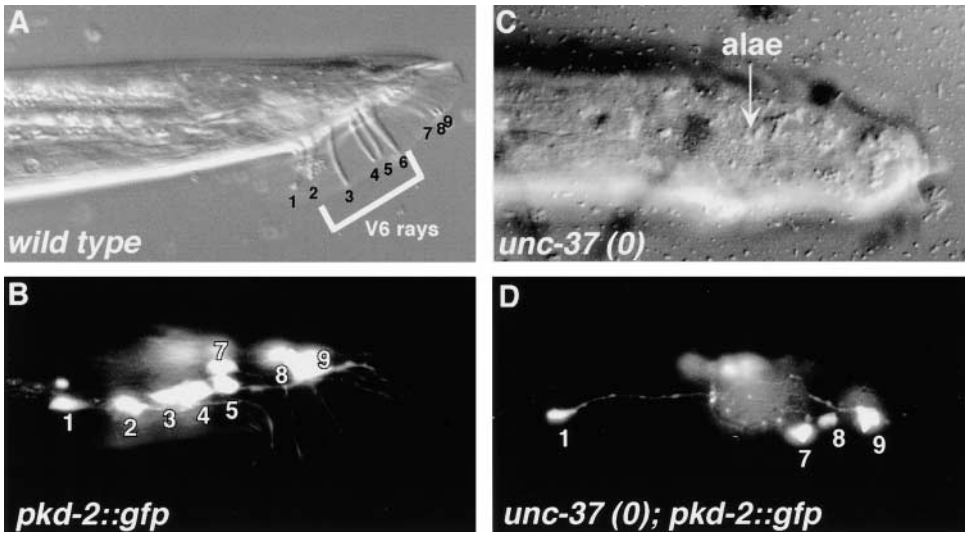

FIGURE 1.—An *unc-37(0)* male has a Pal phenotype. (A) Nomarski photomicrograph of a wild-type adult male, lateral view. Each side has nine rays. Ray 1 is derived from seam cell V5, rays 2–6 are derived from seam cell V6, rays 7–9 are derived from seam cell T. (B) Expression of a *pkd-2::gfp* reporter gene in wild-type male rays. *pkd-2* encodes the homolog of human polycystic kidney disease gene (BARR and STERNBERG 1999) and is expressed in all nine rays as well as ray axons (L. JIA and S. W. EMMONS, unpublished data). The reporter is expressed in 10% of ray 6 in the integrated line used in this work [EM773: *bxIs14(pkd-2::gfp, pha-1)* was derived from PS32- 28A by integration of the transgene]. (C) V6 produces alae instead of rays in an *unc-37* null (*unc-37[wd17dm wd22]*) male. Alae are absent both anterior and posterior of V6, suggesting that rays from V5 and T are still produced, though they are not visible due to abnormal tail morphogenesis. (D) Expression of a *pkd-2::gfp* reporter gene in an *unc-37* null (*unc-37[wd17dm wd22]*) male. The reporter shows that V6 rays are missing whereas V5 and T rays are still generated.

tions during *C. elegans* development; a large fraction of the homozygous null progeny of an *unc-37(0/+)* mutant die as embryos or larvae (PFLUGRAD *et al.* 1997). We examined the tails of adult male escapers of this embryonic and larval lethality and found that they had a Pal phenotype (Table 1, line 2; Figure 1, C and D). In Pal phenotype males, rays are replaced by *p*osterior *al*ae as a result of a cell fate transformation in which the posterior seam cell V6 has taken the fate appropriate to anterior analogs and has generated cuticular ridges called alae instead of rays (WARING and KENYON 1990). *pal-1* and the Hox gene *mab-5* are both required for the rays *vs.* alae cell fate choice (KENYON 1986). In *mab-5* mutants, the rays descended from two seam cells, V5 (ray 1) and V6 (rays 2–6), are affected and transformed to alae, whereas in the *pal-1(e2091)* mutant, only those rays descended from V6 are transformed to alae (Table 1, line 3). In *unc-37(0)* mutants, ray 1 was still generated, and rays 7–9, descended from T, were also unaffected (Figure 1, C and D). This suggested that the Pal phenotype in *unc-37(0)* escapers was the specific result of lack of *pal-1* activity in V6 (HUNTER *et al.* 1999).

In this experiment, the alternate pathway is presumptively blocked by the wild-type activities of SOP-1 and SOP-3. Thus *pal-1* activation via the normal pathway (requiring the intronic enhancer) requires the function of *unc-37*. Since neither pathway is active, we also conclude that *unc-37* is not necessary for repression of the alternate pathway. Thus UNC-37 is not acting as a cofactor of the Wnt pathway in maintaining repression of the alternate pathway.

Positive requirement for *unc-37* in activation of *pal-1* by an alternate pathway: Not only is UNC-37 not necessary to block *pal-1* activation via the alternate pathway, it is positively required for generation of *pal-1* activity via this pathway, just as it is for generation of *pal-1* activity via the normal pathway. To address this issue, we used the viable *unc-37(e262)* allele (a missense mutation), because null mutations in *unc-37* cause severe lethality. We found that, as concluded above, not only was *unc-37* not required to repress the alternate pathway, that is, *unc-37(e262)* was not a *pal-1(e2091)* suppressor (Table 1, line 5), but the frequency of animals with posterior alae increased rather than decreased when *unc-37(e262)* was introduced into *pal-1(e2091); sop-1* or *sop-3; pal-1 (e2091)* backgrounds (Table 1, lines 6–9). Thus *unc-37* is required for generation of rays via the alternate pathway as it is for the normal pathway.

***unc-37* interacts synergistically with components of the Mediator complex at one or more later steps to promote ray development:** Since *unc-37(e262)* alone had little effect on activation of the V6 ray developmental program in a *pal-1(+)* background [none of the animals are Pal (Table 1, line 4)], it was possible to use this mutation to test whether *unc-37* interacted synergistically with Mediator components during later steps of the ray transcription factor cascade. The conclusion that *unc-37* was required at some later step in ray generation was suggested by the observation that a small percentage of V6 rays was missing in *unc-37(e262)* males (Table 1, line 4). This percentage was greatly increased by mutations or RNAi of *sop-1* (Table 1, lines 16 and 23), *sop-3* (Table 1, line 17), or another known component of the Mediator complex, *sur-2* (Table 1, line 18). SUR-2 interacts with transcription factors targeted by the Ras/MAPK pathway (SINGH and HAN 1995; BOYER *et al.* 1999). *sur-2* does not function in the same pathway as *sop-1* and *sop-3* in regulation of *pal-1*, since a mutation in

TABLE 1

Genetic interactions between *unc-37*, *sop-1*, *sop-3*, and *sur-2*

Genotype[a]	% sides with Pal phenotype[b]	% sides with missing rays (average no. of rays missing per non-wild-type side)[c]	No. of sides	% inviable ($n > 500$)[d]
1. Wild type	0	0	>1000	9
2. *unc-37(wd17dm wd22)*	100		81	>90
3. *pal-1(e2091)*	95[f]		232	13
4. *unc-37(e262)*	0	25 (1.1)	289	
5. *unc-37(e262);pal-1(e2091)*	97		200	
6. *pal-1(e2091); sop-1(bx103)*	38[g]		412	
7. *unc-37(e262); pal-1(e2091); sop-1(bx103)*	87		458	69
8. *sop-3(bx96); pal-1(e2091)*	15[f]		1968	
9. *unc-37(e262) sop-3(bx96); pal-1(e2091)*	95		19	>95
10. *sop-1(bx92)*		0	112	
11. *sop-1(bx103)*		0	86	
12. *sop-3(bx96)*		3 (1)	415	
13. *sur-2(ku9)*		7 (1.5)	217	
14. *sop-1(RNAi)*		29 (1.6)	238	
15. *sop-3(RNAi)*		14 (1.9)	210	
16. *unc-37(e262); sop-1(RNAi)*		100 (4.5)	7	97
17. *unc-37(e262); sop-3(RNAi)*		100 (2.8)	149	48
18. *unc-37(e262) sur-2(ku9)*		100 (3.3)	71	51
19. *sop-3(RNAi); sop-1(RNAi)*		100 (2.7)	269	63
20. *sur-2(ku9); sop-1(RNAi)*		100 (3.6)	14	ND
21. *sur-2(ku9) sop-3(RNAi)*		100 (2.5)	25	ND
22. *unc-37(e262); sop-1(bx92)*		ND		100
23. *unc-37(e262); sop-1(bx103)*		73 (2.6)	80	61
24. *unc-37(e262) sop-3(bx96)*		ND		>95
25. *sop-3(bx96); sop-1(bx92)*		26 (1.3)	128	89[f]
26. *sur-2(ku9); sop-1(bx92)*		25 (2.1)	48	78[f]
27. *sop-3(bx96) sur-2(ku9)*		26 (2.2)	15	38[f]
28. *pal-1(e2091); lin-22(mu2)*[e]	0.5		212	
29. *unc-37(e262); pal-1(e2091); lin-22(mu2)*[e]	1		179	

The templates for synthesizing RNA for RNAi experiments were as described previously (Zhang and Emmons 2000, 2001). The RNA was synthesized using MEGAscript T3 and T7 kit (Ambion, Austin, TX).

[a] Rays on each side of the body were scored independently. All the strains in this work carried the *him-5 (e1490)* mutation, which gives a high frequency of males in the selfing populations.

[b] The Pal phenotype is not observed in the strains listed from line 10 to 27.

[c] The missing ray phenotype is not scored in the strains carrying *pal-1(e2091)*.

[d] Data are not shown for those strains where there is no significant difference from either *him-5* or *pal-1; him-5*.

[e] In *pal-1; lin-22* mutants, 74.5% of V6 produce two rays, 25% of V6 produce normal five rays. In *unc-37(e262); pal-1(e2091); lin-22(mu2)* mutants, 74.5% of V6 produce two rays, 24.5% of V6 produce five rays.

[f] Data from Zhang and Emmons (2001).

[g] Data from Zhang and Emmons (2000).

sur-2 cannot suppress the Pal phenotype of *pal-1(e2091)* (Zhang and Emmons 2001). Whereas singly, mutation or RNAi of each of these Mediator genes resulted in a small percentage of V6 ray loss similar to *unc-37(e262)* (Table 1, lines 10–15), indicating a weak positive requirement for ray generation, in combination with *unc-37(e262)* there was extensive ray loss (Table 1, lines 16–18).

All the mutations tested in combinations in these experiments are nonnull. Synergistic interaction suggested that these genes could act in a single pathway to promote ray development. Alternatively, they might act in parallel pathways with additive effects. Consistent with either interpretation, *sop-1*, *sop-3*, and *sur-2* mutations also resulted in extensive synthetic V6 ray loss in combinations with each other (Table 1, lines 19–21). Absence of an effect on generation of rays 1 and 7–9 indicated that *unc-37*, *sop-1*, *sop-3*, and *sur-2* were required specifically for one or more steps of the transcription factor cascade in the V6 cell lineage.

Synthetic lethal interactions between *unc-37* and components of the Mediator complex: *unc-37(e262)* interacted with *sop-1*, *sop-3*, and *sur-2* not only for generation of V6 rays, but also for viability. This indicated that the

putative pathway or pathways involving these genes were required for one or more essential steps during embryogenesis or postembryonic development, as well as for ray development. Whereas single alleles had little effect on viability, in combination with *unc-37(e262)* there was a large lethal sector (Table 1, lines 16–18 and 22–24). Once again consistent with all these genes acting either in a single pathway or in parallel pathways with a common effect, double mutants among *sop-1*, *sop-3*, and *sur-2* also showed synthetic lethality, as we demonstrated previously (Zhang and Emmons 2001; Table 1, lines 19 and 25–27).

In Drosophila and vertebrates, Groucho functions as a corepressor for Hairy family proteins (Fisher and Caudy 1998; Mannervik *et al.*, 1999). *lin-22* encodes the *C. elegans* Hairy/E(spl) homolog. In contrast to *unc-37*, mutations in *lin-22* suppress the Pal phenotype of *pal-1(e2091)* (Waring and Kenyon 1990; Wrischnik and Kenyon 1997). Thus, *unc-37* is not a *lin-22* corepressor in regulation of *pal-1*. Consistent with this conclusion, LIN-22 does not contain the WRPW (Trp, Arg, Prv, Trp) domain that mediates protein-protein interactions between Hairy family proteins and Groucho (Wrischnik and Kenyon 1997). Unlike *sop-1*, *sop-3*, and *sur-2*, the V6 rays in *lin-22* or *pal-1; lin-22* mutants are not affected by introduction of *unc-37(e262)* mutation (Table 1, lines 28 and 29), suggesting that *lin-22* acts downstream of *sop-1* and *sop-3* to block ray development or blocks an independent activation pathway. *unc-37* might promote V6 ray development by repressing *lin-22* expression or function in V6. In activating an independent pathway, *lin-22* mutation might result in activation of a ray developmental program normally expressed only in V5. In support of this conjecture, in *lin-22* mutants, seam cells V1–V4 and V6 express the same cell lineage and cell fates as V5 (Horvitz *et al.* 1983).

UNC-37, like other proteins of the Gro/TLE family, has been suggested to act as a corepressor. It directly interacts with homeodomain-containing protein UNC-4 to specify the identity of VA-type motor neurons by preventing the expression of VB-type motor neuron-specific genes (Winnier *et al.* 1999). Here we demonstrated that *unc-37* is required for the activation of the transcription factor cascade leading to the generation of V6 rays and this requirement is sensitized by mutations in the components of the Mediator complex *sop-1*, *sop-3*, and *sur-2*. This observation would be explained if, in the ray pathway, *unc-37* functions as a coactivator rather than as a corepressor. Alternatively, *unc-37* might act to repress the expression of a *pal-1* repressor. One candidate repressor gene, mentioned above, is *lin-22*. An alternate candidate repressor is POP-1, the *C. elegans* TCF/LEF homolog. POP-1 might repress *pal-1* in V6, and UNC-37 might function to repress expression of POP-1. Later in the ray cell lineage, a higher level of POP-1 in the *unc-37* background might result in a more stringent requirement for SOP-1 and SOP-3, acting as positive activators, thus accounting for the later synergistic interaction between mutations in these genes and *unc-37*.

Given the structural and functional similarity between Groucho and yeast transcription corepressor Tup1, it is likely that Groucho and Tup1 function through common mechanisms (Fisher and Caudy 1998). Tup1 interacts directly with histones H3, H4, and histone deacetylase (Edmondson *et al.* 1996; Watson *et al.* 2000). In addition, at least seven genes encoding Mediator proteins, including Srb8, -9, -10, -11, Sin4, Rgr1, and Rox3, are genetically required for Tup1 to repress gene expression (Wahi *et al.* 1998). Therefore, Tup1 represses transcription both by modifying chromatin structure and by interacting with the basal transcriptional machinery. Drosophila Groucho also directly interacts with histones H1, H3, and histone deacetylase Rpd3. Mutations in *groucho* and *rpd3* result in synergistic effects on embryonic viability (Chen *et al.* 1999). Therefore, Groucho may repress transcription by modulating local chromatin structure. Additional unknown mechanisms have been implicated for the repression mediated by Groucho (Chen *et al.* 1999). The data presented here provide genetic evidence that UNC-37/Groucho also interacts with the basal transcriptional machinery to regulate gene activity.

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