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EOR-1 and EOR-2 act independently of RAS and WNT signaling pathways in RMED/V neuron specification

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		Percentage of animals that do not express P_{unc-25}GFP in		N
		RMED	RMEV	
	WT	0	0	>100
RAS pathway	*lin-3(n1059)*	22	13	45
	lin-3(e1417)	0	0	51
	lin-3(n378)	0	0	41
	let-23(sy17)	0	0	57
	let-23(sy1)	0	0	40
	let-60(s1124)	4	9	46
	let-60(sy99)	0	0	45
	let-60(n1531)	0	5	40
	let-60(n1046dm)	0	0	50
	sem-5(n1619)	2	2	69
	sem-5(n2019)	0	0	53
	lin-45(n2018cs) at 15°C	15	21	86
	lin-45(n2506)	0	0	62
	lin-45(ku112)	0	2	42
	mek-2(n2678)	0	0	58
	mek-2(q425)	0	0	30
	mpk-1(ku1)	0	0	56
	mpk-1(n2521)	0	0	60
	lin-25(n545ts) at 25°C	0	0	84
	lin-25(e1446)	0	0	42
WNT pathway	*egl-20(n585)*	0	0	30
	egl-20(mu39)	0	0	88
	pry-1(mu38)	0	0	62
	pry-1(nc1)	0	0	35
	bar-1(ga80)	0	0	100
	bar-1(mu63)	0	0	47

Table 1: RAS-ERK pathway and the canonical WNT signaling are likely not involved in RMED/V cell specification.P_{unc-25}GFP expression in RMED/V cells in mutations in RAS or WNT signaling pathway components.

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Description

We found that loss of either *eor-1* or *eor-2* function results in identical differentiation defects in RMED/V neurons (Huang and Jin, 2019a; Huang and Jin, 2019b). EOR-1 and EOR-2 are thought to positively regulate RAS and WNT signaling pathways in vulval cell induction and in P12 cell fate specification (Howard and Sundaram, 2002). Genetic double mutant analysis suggests that *eor-1* and *eor-2* function redundantly with the Mediator complex proteins *sur-2* and *lin-25* (Howard and Sundaram, 2002). We wished to test whether RAS and WNT signaling pathways are involved in RMED/V differentiation. We examined P_{unc-25}GFP expression in several RAS and WNT mutants (Huang et al., 2004). In the canonical RAS signaling pathway, the EGF-like growth factor LIN-3 binds its receptor LET-23, which then activates LET-60/ras and the MAP kinase cascade that includes LIN-45/raf (MAPKKK),MEK-2/MEK (MAPKK) and MPK-1/ERK (MAPK). We examined strong loss-of-function or putative null mutations in these genes. We detected mild defects in RMED/V cells in *lin-3(n1059)* and *lin-45(n2018cs)* mutant animals. Twenty-two percentage of *lin-3(n1059)* mutants lost P_{unc-25}GFP expression in RMED, and 13% lost the expression in RMEV (N=45). Fifteen percentage and 21% of *lin-45(n2018cs)* animals at non-permissive temperature did not express P_{unc-25}GFP in RMED and RMEV, respectively (N=86) (Table 1). However, similar phenotypes were not found in several other alleles of *lin-3* and *lin-45* (Table 1). In addition, mutations in LET-23/EGFR, SEM-5, an adaptor protein, MEK-2/MAPKK and MPK-1/MAPK, had little or no effects on P_{unc-25}GFP expression in RMED/V (Table 1). The *let-60(n1046)* dominant mutation also did not affect RMEs. *lin-25* has been shown to act in parallel to *eor-1* and *eor-2* in vulva induction, and also did not show any effects on RME. We observed similar results in mutants for the canonical WNT signaling genes including *egl-20/WNT*, *pry-1/Axin* and *bar-1/b-catenin* (Table 1). Therefore, these data suggest that the function of EOR-1 and EOR-2 in RMED/V neurons is likely independent of canonical RAS and WNT pathways.

Reagents

The mutations used are listed below: Linkage group LGI: *mek-2(n2678)*, *mek-2(q425)*; LGII: *let-23(sy17)*, *let-23(sy1)*; LGIII: *mpk-1(n2521)*, *mpk-1(ku1)*; LGIV: *lin-3(n1059)*, *lin-3(e1417)*, *lin-3(n378)*, *eor-1(cs28)*, *eor-1(ju198)*, *lin-45(n2018)*, *lin-45(n2506)*, *lin-45(ku112)*, *let-60(s1124)*, *let-60(sy99)*, *let-60(n1531)*, *let-60(n1046)*, *egl-20(n585)*, *egl-20(mu39)*; LGV: *lin-25(n545)*, *lin-25(e1446)*, *pry-1(mu38)*, *pry-1(nc1)*, *daf-21(nr2081)*, *daf-21(p673)*; LGX: *sem-5(n1619)*, *sem-5(n2019)*, *eor-2(cs42)*, *eor-2(ju190)*, *bar-1(ga80)*, *bar-1(mu63)*.

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