
Canonical RTK-Ras-ERK signaling and related alternative pathways^{*}

Meera V. Sundaram[§]

Dept. of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia PA 19104-6145, USA

Table of Contents

1. Introduction	2
2. Overview of the canonical RTK-Ras-ERK signal transduction pathway	7
3. Growth factors and RTKs that signal through LET-60/Ras	7
4. Alternatives to the canonical pathway	8
5. Biological roles of Ras signaling in <i>C. elegans</i>	9
6. Ras-independent roles of LET-23/EGFR, EGL-15/FGFR or the Raf-MEK-ERK cascade	12
7. Screens used to identify RTK-Ras-ERK pathway components, targets and regulators	13
8. Regulators of growth factor ligand expression	13
9. Regulators of growth factor ligand activity	14
10. Regulators and targets of RTK activity	15
11. Regulators of LET-60/Ras activity or expression	16
12. Regulators of the Raf-MEK-ERK kinase cascade	17
13. Targets of MPK-1/ERK, and other factors influencing downstream responses	19
14. Interactions between the RTK-Ras-ERK pathway and other signaling pathways	23
15. Summary and relevance to human health	24
16. Acknowledgements	24
17. References	25

Abstract

Receptor Tyrosine Kinase (RTK)-Ras-Extracellular signal-regulated kinase (ERK) signaling pathways control many aspects of *C. elegans* development and behavior. Studies in *C. elegans* helped elucidate the basic framework of the RTK-Ras-ERK pathway and continue to provide insights into its complex regulation, its biological roles, how it elicits cell-type appropriate responses, and how it interacts with other signaling pathways to do so. *C. elegans* studies have also revealed biological contexts in which alternative RTK- or Ras-dependent pathways are used instead of the canonical pathway.

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[§]To whom correspondence should be addressed. E-mail: sundaram@mail.med.upenn.edu

1. Introduction

The small GTPase Ras is a central player in multiple different signaling pathways, but one of the most important and well conserved of these is the RTK-Ras-ERK pathway (Malumbres and Barbacid, 2002; Yoon and Seger, 2006; Karnoub and Weinberg, 2008; Lemmon and Schlessinger, 2010; Udell et al., 2011) (Figure 1A). RTK-Ras-ERK signaling is used repeatedly during animal development to control many different biological processes, and mutations affecting RTK-Ras-ERK signaling cause many human syndromes and diseases, including Noonan syndrome, Costello syndrome and many types of cancers (Karnoub and Weinberg, 2008; Tidyman and Rauen, 2009).

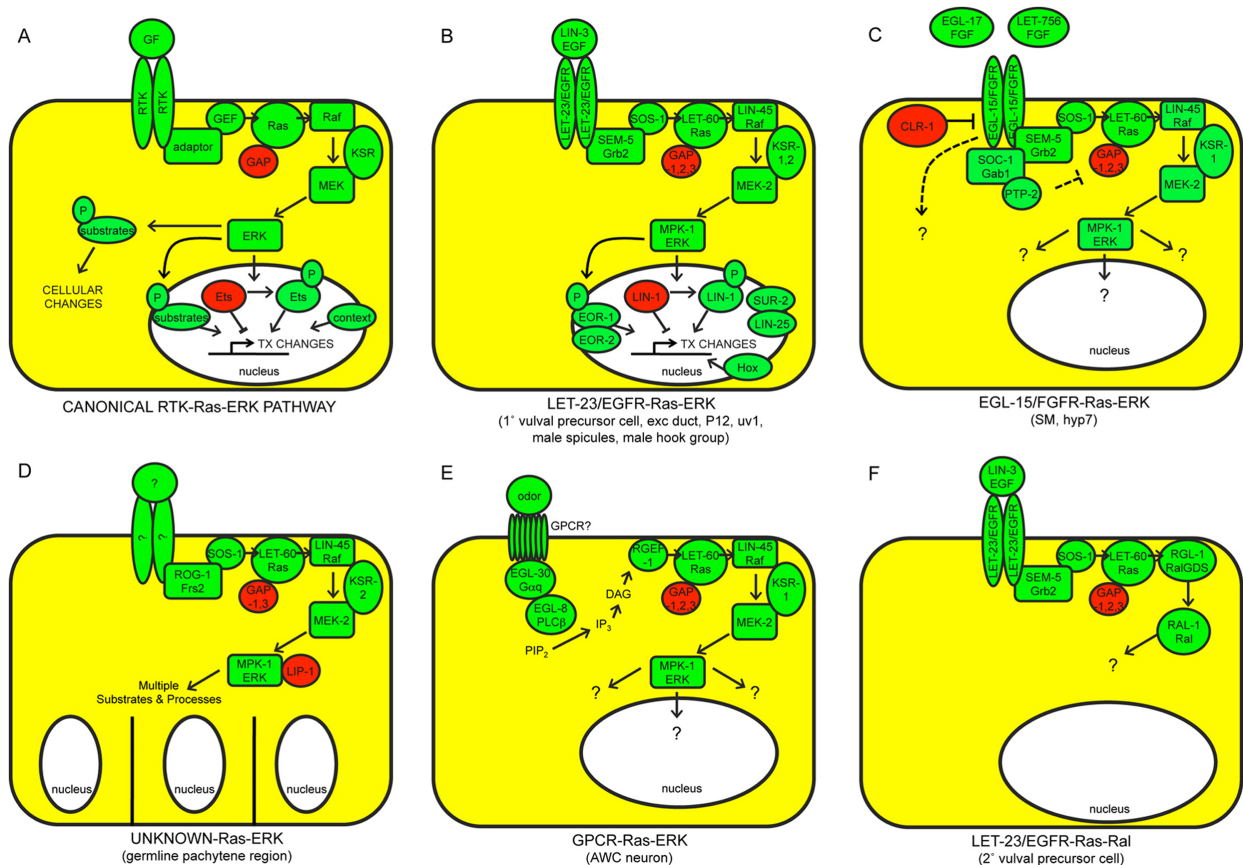


Figure 1. The canonical RTK-Ras-ERK signaling pathway and alternative Ras-dependent pathways in *C. elegans*. A) The canonical pathway. Positively acting factors are shown in green; negatively acting factors are in red. Only the most widely required and conserved factors are shown. GF, growth factor. TX, transcription. B) LET-23/EGFR and C) EGL-15/FGFR both act in part through the canonical pathway, but they are activated by different ligands and control different sets of developmental processes. Included in B) are nuclear proteins, including two MPK-1/ERK substrates (LIN-1/ETS and EOR-1), that are jointly important for most LET-23-dependent somatic cell fates. Downstream targets of EGL-15-Ras-ERK signaling are not yet known. D) Ras-ERK functions to promote meiotic pachytene progression and various other aspects of germline development; the receptors that activate Ras-ERK for these functions are not currently known. MPK-1/ERK utilizes more than 30 different substrates in the germline. E) A G-protein coupled receptor (GPCR) may activate Ras-ERK signaling in some contexts. F) LET-60/Ras can signal through the alternative Ral pathway. See text for additional details and references.

This review summarizes work on RTK-Ras-ERK signaling in *C. elegans*. In an attempt to make this review accessible to a broad audience, I use the more familiar names of the mammalian proteins in conjunction with, or often in place of, the *C. elegans* nomenclature. For example, I refer to the *C. elegans* Ras protein as LET-60/Ras or simply Ras. See Figure 1 and Table 1 for corresponding names of core signaling proteins in *C. elegans* vs. mammals.

Table 1. Core components and selected regulators and targets of the *C. elegans* RTK-Ras-ERK signaling pathway. Core components are shown in black, positive regulators or targets are shown in green, and negative regulators or targets are shown in red.

Gene Product	Mammalian Relative(s)	Molecular Function	Ras-related Phenotypes	Reference(s)
ARK-1 (Ack-Related Kinase)	Ack	Cytoplasmic tyrosine kinase	~WT. Muv in combination with <i>sli-1</i> , others.	(Hopper et al., 2000)
CDF-1 (Cation Diffusion Facilitator)	ZnT1	Zinc transporter	~WT. Suppresses <i>let-60 ras(gf)</i> Muv.	(Bruinsma et al., 2002; Jakubowski and Kornfeld, 1999)
CGR-1 (CRAL/TRIO and GOLD domain suppressor of activated Ras)	SPF/TAP-1	CRAL/TRIO and GOLD domains	~WT. Suppresses <i>let-60(gf)</i> Muv.	(Goldstein et al., 2006)
CLR-1 (CLeaR)	LAR family	Receptor Tyrosine Phosphatase	Lethal fluid accumulation, Suppressed by <i>egl-15(rf)</i>	(Kokel et al., 1998)
CNK-1 (Connector/eNhancer of Ksr)	CNK1,2,3	Scaffold protein	~WT. Suppresses <i>let-60 ras(gf)</i> Muv. Enhances Vul and rod-like lethal phenotypes of <i>lin-45</i> , others.	(Rocheleau et al., 2005)
DAB-1 (DisABled homolog)	DAB2	Adaptor. Required for EGL-17 secretion.	Abnormal sex myoblast positions.	(Kamikura and Cooper, 2003)
DEP-1 (Density Enhanced Phosphatase)	Dep1	Receptor Tyrosine Phosphatase	~WT. Muv and excretory duct duplication in combination with <i>lip-1</i> , others.	(Berset et al., 2005)
DPY-23 (DumPY)	AP-50	Clathrin Adaptor subunit	~WT. Muv in combination with <i>gap-1</i> .	(Yoo et al., 2004)
EGL-15 (EGg Laying defects)	FGFR	Receptor Tyrosine Kinase	Larval lethal. Abnormal sex myoblast positions. Axon guidance defects.	(Bulow et al., 2004; DeVore et al., 1995; Goodman et al., 2003; Stern and Horvitz 1991)
EGL-17 (EGg-Laying defects)	FGF8	Ligand for EGL-15 RTK	Abnormal sex myoblast positions.	(Burdine et al., 1998; Stern and Horvitz 1991)
EOR-1 (Egl-1 suppressor/diO uptake defective/Raf enhancer)	PLZF	BTB/Zinc finger protein, probable transcriptional regulator and MPK-1 substrate	Partial rod-like lethal and P12→P11 fate changes. Strong rod-like lethal in combination with <i>sur-2</i> , <i>lin-25</i> or <i>lin-1</i> . Reduced age-dependent ubiquitination.	(Howard and Sundaram, 2002; Howell et al., 2010; Liu et al., 2011; Rocheleau et al., 2002)
EOR-2 (Egl-1 suppressor/diO uptake defective/Raf enhancer)	NP_079001.2	Novel nuclear protein, functions with EOR-1	Partial rod-like lethal and P12→P11 fate changes. Strong rod-like lethal in combination with <i>sur-2</i> , <i>lin-25</i> or <i>lin-1</i> . Reduced age-dependent ubiquitination.	(Howard and Sundaram, 2002; Howell et al., 2010; Liu et al., 2011; Rocheleau et al., 2002)

EPS-8 (Egfr Protein Substrate 8)	EPS8	EGFR substrate and regulator of EGFR trafficking	~WT. Enhances <i>lin-3(rf)</i> or <i>let-23(rf)</i> Vul.	(Stetak et al., 2006)
GAP-1 (GTPase Activating Protein)	GAP-1	Ras GAP	~WT. Muv and Clr in combination with <i>gap-3</i> . Excretory duct duplication in combination with <i>gap-2</i> .	(Hajnal et al., 1997; Stetak et al., 2008)
GAP-2 (GTPase Activating Protein)	synGAP	Ras GAP	~WT. Excretory duct duplication in combination with <i>gap-1</i> .	(Hayashizaki et al., 1998; Stetak et al., 2008)
GAP-3 (GTPase Activating Protein)	p120GAP	Ras GAP	Premature pachytene exit. Muv and Clr in combination with <i>gap-1</i> .	(Stetak et al., 2008)
GCK-1 (Germinal Center Kinase)	GCK III	Serine/Threonine kinase, binds MPK-1	Increased germline apoptosis, oocyte defects	(Schouest et al., 2009)
GLA-3 (GermLine Apoptosis)	Tis11	CCCH zinc finger protein, binds MPK-1	Increased germline apoptosis, oocyte defects	(Kritikou et al., 2006)
GPA-5 (G-Protein, Alpha subunit)	GNAZ	G α	Increased chemotaxis. Suppresses <i>let-60(dn)</i> Vul.	(Battu et al., 2003)
KSR-1 (Kinase Suppressor of Ras)	KSR1, 2	Raf-related MEK-binding protein, scaffold for Raf/MEK/ERK	Abnormal sex myoblast positions. Suppresses <i>let-60 ras(gf)</i> Muv. Rod-like lethal and Vul in combination with <i>ksr-2</i> , others.	(Kornfeld et al., 1995b; Sundaram and Han, 1995)
KSR-2 (Kinase Suppressor of Ras)	KSR1, 2	Raf-related MEK-binding protein, scaffold for Raf/MEK/ERK	Sterile. Rod-like lethal and Vul in combination with <i>ksr-1</i> .	(Ohmachi et al., 2002)
LET-23 (LEThal)	EGFR	Receptor Tyrosine Kinase	Rod-like larval lethal, Vul, sterile, etc.	(Aroian et al., 1990)
LET-60 (LEThal)	K-Ras	Small GTPase	Rod-like larval lethal, Vul, sterile, etc.	(Beitel et al., 1990; Han et al., 1990; Han and Sternberg, 1990)
LET-92 (LEThal)	PPP2CB	Catalytic subunit of Protein Phosphatase 2A	Larval lethal. Dominantly enhances <i>let-60(dn)</i> Vul	(Kao et al., 2004)
LET-756 (LEThal)	FGF9	Ligand for EGL-15 RTK	Larval lethal. Axon guidance defects.	(Bulow et al., 2004; Popovici et al., 2004; Roubin et al., 1999)
LIN-1 (abnormal cell LINeage)	Elk1	Ets domain transcription factor. Substrate of MPK-1.	Muv, excretory duct duplication, etc. Rod-like lethal in combination with <i>eor-1</i> or <i>eor-2</i> .	(Beitel et al., 1995; Jacobs et al., 1998; Howard and Sundaram, 2002)
LIN-2 (abnormal cell LINeage)	CASK	Membrane-associated guanylate kinase, required for basal localization of LET-23	Vul	(Hoskins et al., 1996; Kaech et al., 1998)

LIN-3 (abnormal cell LIneage)	EGF	Ligand for LET-23 RTK	Rod-like larval lethal, Vul, Sterile, etc.	(Hill and Sternberg, 1992; Dutt et al., 2004)
LIN-7 (abnormal cell LIneage)	Lin-7	PDZ and PTB-domain protein, required for basal localization of LET-23	Vul	(Kaech et al., 1998; Simske et al., 1996)
LIN-10 (abnormal cell LIneage)	Mint1, 2, 3	PDZ protein, required for basal localization of LET-23	Vul	(Kaech et al., 1998; Whitfield et al., 1999)
LIN-15A and LIN-15B (abnormal cell LIneage)	-	Adjacent <i>SynMuvA</i> and <i>SynMuv B</i> genes encoding nuclear proteins that redundantly inhibit <i>lin-3</i> expression	double mutant <i>Muv</i> , excretory duct duplication, etc.	(Ferguson and Horvitz, 1989; Huang et al., 1994; Cui et al., 2006)
LIN-25 (abnormal cell LIneage)	-	Novel nuclear protein, functions with SUR-2	Vul. Rod-like lethal in combination with <i>eor-1</i> , others.	(Nilsson et al., 1998; Nilsson et al., 2000; Tuck and Greenwald, 1995; Howard and Sundaram, 2002)
LIN-31 (abnormal cell LIneage)	FoxB2	Winged helix transcription factor. Target of MPK-1.	Mixed Vul and <i>Muv</i>	(Miller et al., 1993; Tan et al., 1998)
LIN-45 (abnormal cell LIneage)	B-Raf	Serine/Threonine Kinase. Binds Ras-GTP, phosphorylates MEK.	Sterile Vul (maternal rescue of rod-like lethal), etc.	(Han et al., 1993; Hsu et al., 2002)
LIP-1 (Lateral signal Induced Phosphatase)	MKP1	MAPK phosphatase	Partial sterile. <i>Muv</i> in combination with <i>gap-1</i> , others.	(Berset et al., 2001; Hajnal and Berset, 2002)
LPR-1 (LiPocalin Related)	lipocalin family	secreted protein	rod-like lethal	(Stone et al., 2009)
LRP-1 (low-density Lipoprotein Receptor-related Protein)	LRP1	Lipoprotein Receptor-related protein. Required for EGL-17 secretion.	Abnormal sex myoblast positions.	(Kamikura and Cooper, 2003)
LST-1 (Lateral Signaling Target)	-	Novel MPK-1 binding protein	WT. <i>Muv</i> in combination with <i>gap-1</i> .	(Yoo et al., 2004)
LST-2 (Lateral Signaling Target)	Zinc finger FYVE domain containing protein 28	FYVE domain protein	WT. <i>Muv</i> in combination with <i>gap-1</i> .	(Yoo et al., 2004)
LST-3 (Lateral Signaling Target)	CCAR1	SAF-A/B, Acinus and PIAS domain protein	WT. <i>Muv</i> in combination with <i>gap-1</i> .	(Yoo et al., 2004)

LST-4 (Lateral Signaling Target)	NM_153271	Sorting nexin, may promote LET-23 degradation	WT. Muv in combination with <i>gap-1</i> .	(Yoo et al., 2004)
MEK-2 (Map kinase kinase or Erk Kinase)	MEK1, 2	Dual Specificity kinase, phosphorylates ERK	Sterile Vul (maternal rescue of rod-like lethal), etc.	(Kornfeld et al., 1995a; Wu et al, 1995)
MPK-1/SUR-1 (MaP Kinase/ SUPpressor of Ras)	ERK1, 2	Serine/Threonine kinase	Sterile Vul (maternal rescue of rod-like lethal), etc.	(Lackner and Kim, 1998; Lackner et al., 1994; Wu and Han, 1994)
PAR-1 (abnormal embryonic PARTitioning of cytoplasm)	MARK2/ C-TAK1	Serine/Threonine kinase	Weak Muv. Reverses <i>sur-6</i> suppressor phenotype.	(Kao et al., 2004; Yoder et al., 2004)
PTP-2 (Protein Tyrosine Phosphatase)	Shp-2	Tyrosine phosphatase	Sterile and maternal-effect lethal. Suppressed by <i>let-60 ras(gf)</i> . Suppresses <i>clr-1</i> .	(Gutch et al., 1998; Schutzman et al., 2001)
RAB-7 (RAB GTPase)	Rab7	Rab GTPase	WT. Muv in combination with <i>ark-1</i> or <i>sli-1</i> .	(Skorobogata and Rocheleau, 2012)
RGEF-1 (Ras Guanine nucleotide Exchange Factor)	RasGRP	Guanine Nucleotide Exchange Factor	Chemotaxis-defective	(Chen et al., 2011)
ROM-1 (RhOMboid-related)	RHBDL2	7-pass transmembrane serine protease	WT. Suppresses <i>let-60 ras(gf)</i> Muv.	(Dutt et al., 2004)
SEM-5 (SEx Muscle abnormality)	Grb2	RTK-binding adaptor	Rod-like larval lethal, Vul, etc.	(Clark et al., 1992)
SLI-1 (Suppressor of LIneage defect)	Cbl	E3 ubiquitin ligase, Involved in LET-23 endocytosis/ degradation	WT. Muv in combination with <i>unc-101</i> , others.	(Jongeward et al., 1995; Yoon et al., 1995)
SOC-1 (Suppressor Of Clr-1)	Gab1	RTK-binding adaptor, Promotes EGL-15 signaling	Scrawny. Suppresses <i>clr-1</i> lethality.	(Schutzman et al., 2001)
SOC-2/SUR-8 (SUPpressor of Ras, SUPpressor Of Clr-1)	Shoc2/Sur-8	Leucine-rich repeat protein, binds Ras and Raf	Scrawny. Suppresses <i>let-60 ras(gf)</i> Muv and <i>clr-1</i> lethality. Enhances Vul and rod-like lethal phenotypes of <i>lin-45</i> hypomorphs, others.	(Selfors et al., 1998; Sieburth et al., 1998)
SOS-1/LET-341 (Son Of Sevenless/ LEThal)	Sos-1	Guanine Nucleotide Exchange Factor	Rod-like larval lethal, Vul, Sterile, etc.	(Chang et al., 2000)
SRA-13 (Serpentine Receptor, A class)	-	G-protein coupled receptor	Increased chemotaxis. Suppresses <i>let-60(dn)</i> Vul.	(Battu et al., 2003)
SUR-2 (SUPpressor of Ras)	Med23/Sur-2	Mediator subunit	Vul. Rod-like lethal in combination with <i>eor-1</i> , others.	(Singh and Han, 1995; Howard and Sundaram, 2002)
SUR-5 (SUPpressor of Ras)	NM_023928	Acetyl coenzyme A synthetase	WT. Suppresses <i>let-60(dn)</i> Vul.	(Gu et al., 1998)
SUR-6 (SUPpressor of Ras)	PPP2R2A	PR55/B Regulatory subunit of Protein Phosphatase 2A	Suppresses <i>let-60 ras(gf)</i> Muv. Enhances Vul and rod-like lethal phenotypes of <i>lin-45</i> hypomorphs, others.	(Kao et al., 2004; Sieburth et al., 1999)

SUR-7 (SUppressor of Ras)	-	Zinc transporter	Suppresses <i>let-60 ras(gf)</i> Muv.	(Yoder et al., 2004)
SYNMUV GENES (SYNthetic MULtiVulva)	Rb, E2F, NuRD, SETDB1, HP1, Tip60/NuA4, etc.	inhibitors of <i>lin-3</i> gene expression	Muv in combination with appropriate other SynMuv mutations	(Ferguson and Horvitz, 1989; Cui et al., 2006; Fay and Yochem, 2007)
UNC-101 (UNCoordinated)	AP-47	Clathrin adaptor subunit	~WT. Muv in combination with <i>sli-1</i> , others.	(Lee et al., 1994)

2. Overview of the canonical RTK-Ras-ERK signal transduction pathway

A general model for the canonical RTK-Ras-ERK signaling pathway is shown in Figure 1A, and canonical pathways in *C. elegans* are shown in Figures 1B-C. Upon growth factor binding, an RTK dimerizes and autophosphorylates its C-terminal region (Lemmon and Schlessinger, 2010). The resulting phospho-tyrosine residues serve as docking sites for adaptor proteins such as Grb2. These adaptors recruit the Guanine Nucleotide Exchange Factor (GEF) Sos to activate the small GTPase Ras (Karnoub and Weinberg, 2008). Ras-GTP then binds to Raf and promotes its stable association with the plasma membrane and/or endomembranes, where other events then activate Raf kinase activity (Udell et al., 2011). The scaffold protein KSR assists in Raf activation, but also promotes further signal transmission by bringing together different components of the Raf-MEK-ERK kinase cascade (Udell et al., 2011). Raf phosphorylates and activates MEK, MEK phosphorylates and activates ERK, and ERK then phosphorylates and either activates or inactivates various substrates, depending on the cell-type and context (Yoon and Seger, 2006). In many cases, ERK phosphorylates nuclear transcription factors such as Ets domain proteins, thus leading to changes in gene expression. ERK can also phosphorylate a broad array of other, non-transcription factor substrates to affect other cell biological processes.

This model draws on a large body of data from multiple systems, including *C. elegans*, *Drosophila* and mammalian cells. *C. elegans* genetics has been particularly powerful for identifying the gene products required for particular signaling events and determining their order of action; for example, studies in *C. elegans* were among the first to show that Ras acts downstream of RTKs (Aroian et al., 1990) and upstream of Raf (Han et al., 1993), and to identify key pathway components such as SEM-5/Grb2 (Clark et al., 1992) and KSR (Kornfeld et al., 1995b; Sundaram and Han, 1995). In some cases, physical interactions and/or phosphorylation events have been demonstrated for the worm proteins, but in other cases such interactions are inferred based on biochemical studies of related proteins in mammalian cells. The canonical RTK-Ras-ERK pathway (and much of its regulation) appears highly conserved between *C. elegans* and mammals (Figure 1A-C; Table 1).

3. Growth factors and RTKs that signal through LET-60/Ras

The *C. elegans* genome contains 33 predicted RTKs, with about half of these belonging to two nematode-specific RTK families (Popovici et al., 1999; see Genomic overview of protein kinases). Of the RTKs that have been characterized, only the Epidermal Growth Factor (EGF) receptor relative LET-23 and the Fibroblast Growth Factor (FGF) receptor relative EGL-15 clearly signal through LET-60/Ras (Aroian et al., 1990; DeVore et al., 1995), and signaling by LET-23 and EGL-15 can account for most (but not all) of the LET-60/Ras-dependent processes described below. The insulin-like RTK DAF-2 may also signal in part through LET-60/Ras (Nanji et al., 2005), but acts primarily through a separate PI3-kinase-Akt pathway (see Dauer). The ephrin RTK VAB-1 negatively regulates MPK-1/ERK activation (Miller et al., 2003). Several RTKs act in other characterized pathways; for example, VAB-1 acts upstream of PLC-3/phospholipase C-gamma (PLC γ) and inositol 1,4,5-triphosphate (IP $_3$) (Yin et al., 2004), SCD-2/ALK acts upstream of the SMA-5 MAP kinase (Reiner et al., 2008), and LIN-18/Ryk and CAM-1/Ror act in Wnt signaling pathways (Inoue et al., 2004; Green et al., 2007, Green et al., 2008). Although mutations are now available for most *C. elegans* RTKs (www.wormbase.org), the biological roles of many of these RTKs remain unknown.

In this review, I focus on LET-23/EGFR and EGL-15/FGFR, the two RTKs that signal widely (though not exclusively) through Ras-ERK. LET-23 has one known ligand, LIN-3, the sole EGF-related protein in *C. elegans* (Hill and Sternberg, 1992) (Figure 1B). EGL-15 has two different FGF-related ligands, EGL-17 and LET-756, that

are used in different biological contexts (Burdine et al., 1997; Roubin et al., 1999; Birnbaum et al., 2005; Huang and Stern, 2005) (Figure 1C). EGL-17 and LET-756 are most related to the vertebrate FGF8 and FGF9 families, respectively (Popovici et al., 2004; Birnbaum et al., 2005).

4. Alternatives to the canonical pathway

As in other organisms, several alternative signaling pathways in *C. elegans* utilize only part of the core RTK-Ras-ERK pathway.

Alternative receptors: Unknown receptors, potentially not RTK-related, can stimulate Ras-ERK signaling (Figure 1D, 1E). In particular, there is evidence for G-protein-coupled receptor (GPCR)-dependent activation of Ras in neurons (Chen et al., 2011) (Figure 1E).

Alternative Ras targets: LET-60/Ras can act independently of Raf-MEK-ERK to stimulate an alternative pathway involving RGL-1 (related to mammalian Ral guanine nucleotide dissociation stimulator or RalGDS) and the RAL-1 GTPase (Zand et al., 2011) (Figure 1F). Evidence for LET-60/Ras-dependent regulation of other well-known mammalian Ras effectors, such as PI-3 kinase, is so far lacking in *C. elegans*, but LET-60/Ras can bind to other candidate effectors such as AFD-1/AF-6 (Watari et al., 1998) and PLC-1/PLC ϵ (Shibatohge et al., 1998). Whether Ras acts through Raf-MEK-ERK or through alternative pathways has not yet been established for all known Ras-dependent events (particularly those involving the EGL-15 RTK). There is at least one situation in which the Raf-MEK-ERK cascade appears to act at least partly independently of LET-60/Ras (Nicholas and Hodgkin, 2004; McMullan et al., 2012).

Alternative RTK targets: LET-23/EGFR can stimulate other, Ras-independent pathways such as PLC-3/PLC γ and IP $_3$ -dependent release of intracellular calcium stores (Clandinin et al., 1998; Yin et al., 2004) (Figure 2A), or PLC-3 and diacylglycerol (DAG)-dependent release of synaptic vesicles (Van Buskirk and Sternberg, 2007) (Figure 2B). EGL-15/FGFR also may act through additional, unknown pathways (Figure 1C), since constitutive LET-60/Ras cannot bypass all *egl-15* requirements (DeVore et al., 1995; Sundaram et al., 1996; Schutzman et al., 2001). Possible direct substrates of EGL-15 kinase activity include the planar polarity protein VANG-1 (Hoffmann et al., 2010) and the cell adhesion molecule LAD-1/LICAM (Chen et al., 2001). Furthermore, the extracellular domain of EGL-15A can act in a kinase-independent manner to affect axon positions (Bulow et al., 2004). LET-23 appears to signal through Ras-dependent vs. Ras-independent pathways in different cell types, with Ras-dependent signaling promoting transcriptional changes (Figure 1B), and Ras-independent signaling promoting other, non-transcriptional responses (Figure 2A, B). EGL-15 may signal through both Ras-dependent and Ras-independent pathways within the same cell (Figure 1C).

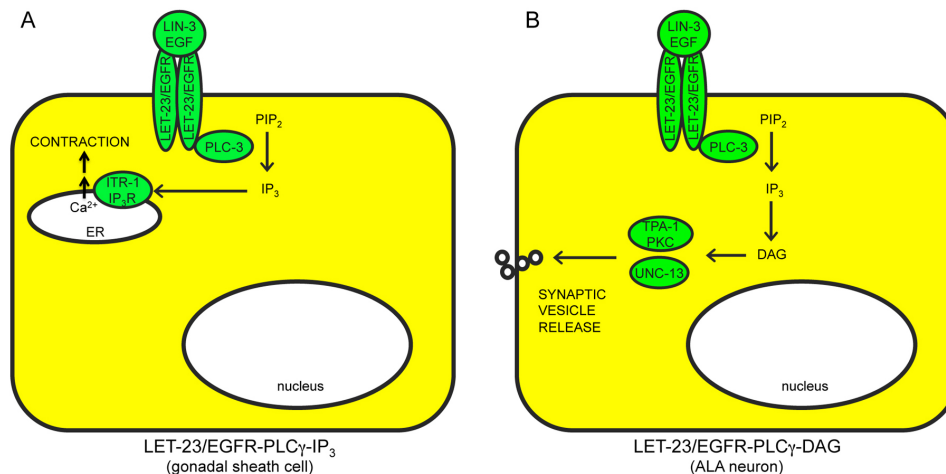


Figure 2. Ras-independent pathways downstream of LET-23/EGFR. A) LET-23-PLC γ -IP $_3$ stimulates calcium release from intracellular stores. ER, endoplasmic reticulum. B) LET-23-PLC γ -DAG stimulates synaptic vesicle release. Positively acting factors are shown in green. See text for details and references.

Biological roles of both the canonical pathway and known alternative pathways are summarized in Figure 3 and discussed below.

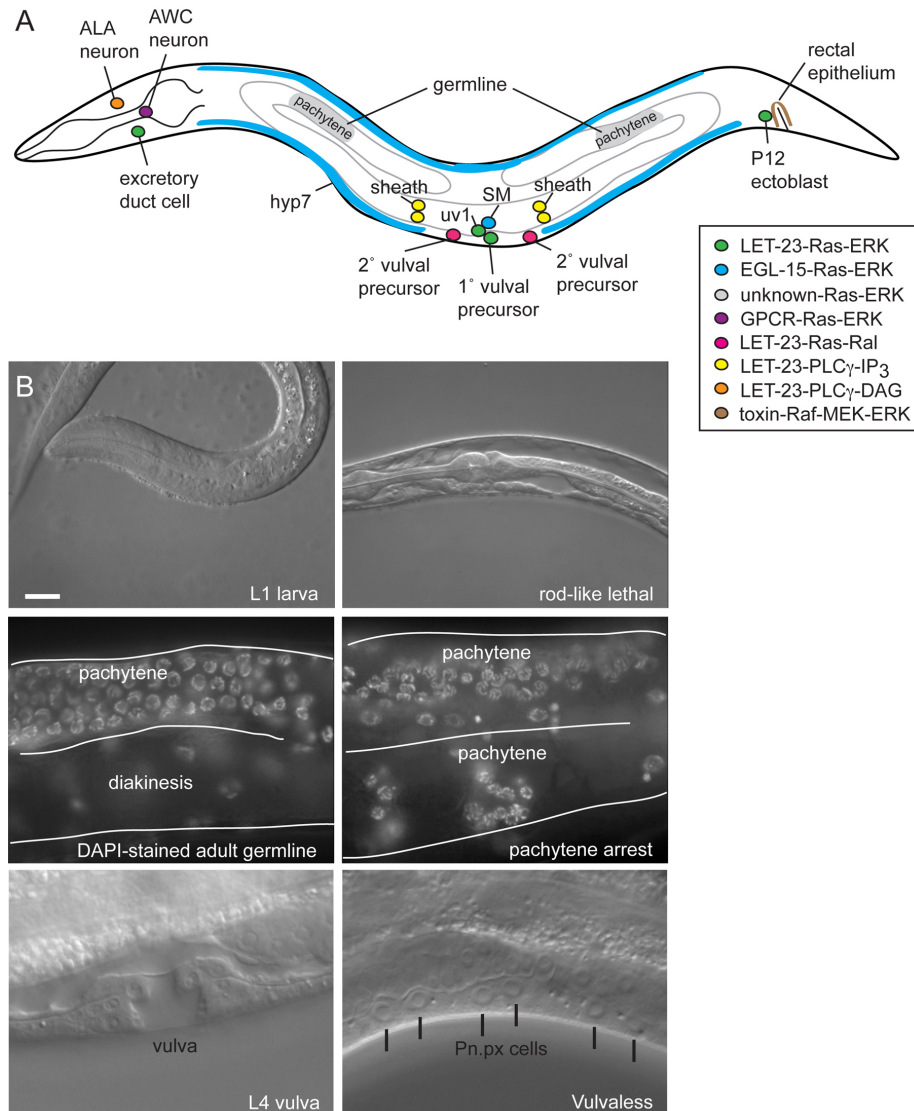


Figure 3. Biological roles of canonical RTK-Ras-ERK signaling and alternative pathways. A) Cell types in the hermaphrodite that require LET-23/EGFR, EGL-15/FGFR and/or LET-60/Ras-dependent signaling are colored according to the type of pathway involved. Not all biological roles are included here due to limited information about the relevant pathway or site of action. Note that many signaling events actually occur during embryogenesis or during larval development, but the locations of relevant cells (or their descendants) in the adult are shown here. B) Photomicrographs of selected mutant phenotypes observed in Ras pathway mutants. Left column, wild type; Right column, mutants. See text for details and references. Scale bar, 10 microns.

5. Biological roles of Ras signaling in *C. elegans*

Ras signaling is not required for cellular viability or proliferation in *C. elegans*; in a mosaic analysis, loss of *let-60*/Ras from large portions of the cell lineage was still compatible with continued cell division and organismal viability, albeit with specific developmental alterations (Yochem et al., 1997). Similarly, increased Ras activity does not promote somatic cell proliferation in *C. elegans*. The absence of cell-cycle related phenotypes has facilitated studies of Ras requirements in other specific developmental, behavioral and physiological processes.

A *let-60*/Ras reporter is widely expressed during development (Dent and Han, 1998), and mutations affecting Ras signaling can cause many different phenotypes, including lethality, reflecting the many biological roles of Ras in *C. elegans* (Figure 3; Table 1). Null mutations in *lin-3*/EGF, *let-23*/EGFR, *sem-5*/Grb2, *sos-1*/Sos, and *let-60*/Ras cause “rod-like” L1 larval lethality due to defects in excretory duct cell development (Ferguson and Horvitz, 1985;

Yochem et al., 1997; Abdus-Saboor et al., 2011) (Figure 3B). Null mutations in *let-756/FGF* and *egl-15/FGFR* cause a distinct (non-rod-like) L1 lethality due to hypodermal defects (DeVore et al., 1995; Roubin et al., 1999; Bulow et al., 2004). Null mutants for *lin-45/Raf*, *mek-2/MEK* or *mpk-1/ERK* are maternally-rescued for earlier essential functions and display an adult sterile phenotype due to germline defects (Church et al., 1995; Lackner and Kim, 1998; Hsu et al., 2002; Lee et al., 2007) (Figure 3B). *ksr-2* null mutants show a similar sterile phenotype (Ohmachi et al., 2002). Because of the lethal or sterile phenotypes of null mutants, studies of other biological processes often require the use of conditional or non-null alleles or RNAi.

Excretory duct cell fate and differentiation: LIN-3-LET-23 signaling via Ras-ERK promotes multiple aspects of excretory duct cell development, including excretory duct vs. excretory pore fate specification and other aspects of duct tube morphogenesis and differentiation (Yochem et al., 1997; Abdus-Saboor et al., 2011). The excretory duct cell is a tubular component of the worm's renal-like organ and is required for osmoregulation (Nelson et al., 1983; Nelson and Riddle, 1984). Therefore, *let-60/Ras* loss-of-function mutants, which lack the excretory duct cell, die as “rod-like” L1 larvae with a turgid, fluid-filled appearance (Figure 3B). *let-60/Ras* gain-of-function mutants (which are viable) have two fused excretory duct cells (Yochem et al., 1997; Abdus-Saboor et al., 2011). The excretory duct cell was the sole lethal focus identified in a mosaic analysis of *let-60 ras* (Yochem et al., 1997).

A mosaic analysis of *let-23/EGFR* identified two lethal foci, one corresponding to the ABpl lineage (which generates the excretory duct) and another corresponding to the ABa lineage (Koga and Ohshima, 1995). Therefore, *let-23* appears to have an additional, as yet unknown, essential role.

Hypodermal fluid homeostasis: LET-756-EGL-15 signaling via Ras-ERK also appears to have essential roles in fluid homeostasis, but in the hypodermis. *egl-15/FGFR* mutant lethality can be rescued by constitutive activation of LET-60/Ras in the hypodermis (DeVore et al., 1995; Bulow et al., 2004). The fact that mosaic analysis of *let-60* (Yochem et al., 1997) did not identify a discrete cellular focus for this lethal phenotype is consistent with a requirement for signaling in hyp7, a syncytium that derives from multiple cell lineages (Sulston et al., 1983). The physiological basis for *egl-15/FGFR* lethality or for the related “scrawny” phenotype has not been determined, but it has been speculated to involve desiccation and thus be the converse of the Clr phenotype described below (Borland et al., 2001).

Hyperactivation of *egl-15/FGFR* or *let-60/Ras*, as seen in *clr-1* mutants, can result in fluid accumulation and lethality with a “Clear” (Clr) appearance, and these defects are suppressed by loss of *mpk-1* or other downstream genes (the Suppressor of Clr or “Soc” phenotype) (Kokel et al., 1998; Schutzman et al., 2001). Expression studies, tissue-specific transgenes, and mosaic experiments suggest that the Clr phenotype is caused by hypodermal defects rather than excretory system defects (Huang and Stern, 2004). Indeed, the hypodermis is one major site of osmosensitive gene regulation (Lamitina et al., 2006). EGL-15 apparently plays a physiological role in osmoregulation and not a developmental role, since hyperactivation of EGL-15 at any larval stage leads to the Clr phenotype.

Germline development: *mpk-1/ERK* has genetically separable roles in multiple aspects of germline development, as outlined below. *mpk-1* acts cell autonomously in the germline for these functions, and the pattern of MPK-1 phosphorylation in the germline is dynamic, consistent with these multiple roles (Lee et al., 2007). The entire Ras-ERK cascade is involved in many cases, as indicated, but in other cases the evidence is incomplete. Note that the relevant receptors that promote LET-60/Ras activation in the germline are unknown, although requirements for an adaptor protein, ROG-1, suggest that RTK(s) are involved at least in part (Matsubara et al., 2007) (Figure 1D).

meiotic pachytene progression: Ras-ERK signaling promotes progression through the pachytene stage of meiosis I prophase (Church et al., 1995). Null mutants have germ cells arrested at mid-pachytene (Lee et al., 2007) (Figure 3B).

pachytene cellular organization: Ras-ERK signaling promotes proper germ cell membrane organization (Lee et al., 2007; Lin and Reinke, 2008; Arur et al., 2011). Null mutants have arrested meiotic pachytene nuclei that are clumped together and lack the normal honeycomb pattern of membrane organization (Figure 3B).

germ cell sexual fate: Raf-ERK signaling promotes the male germ cell fate (Lee et al., 2007). *lin-45/Raf*, *mek-2/MEK* and *mpk-1/ERK* null mutants lack sperm and have a feminized germline in which all germ cells adopt a female or oocyte fate. However, these mutants fail to form normal oocytes due to the pachytene arrest and membrane disorganization phenotypes described above. Note that *let-60/Ras* and *ksr-2* mutants do make sperm

Ohmachi et al., 2002; Lee et al., 2007). This suggests a potential Ras-independent role of Raf-MEK-ERK, although maternal rescue cannot be ruled out.

oocyte growth and differentiation: ERK signaling promotes oocyte differentiation and restrains oocyte growth (Lee et al., 2007). Mutants with reduced ERK signaling have oocytes with abnormal positions, shapes and sizes and/or oocytes that have multiple or abnormally positioned nuclei.

oocyte maturation: ERK signaling promotes sperm-dependent oocyte maturation (i.e., the transition from diakinesis to metaphase of meiosis I) (McCarter et al., 1999; Han et al., 2010; Kim et al., 2012). Maturation occurs just prior to ovulation (movement of oocytes into the spermatheca for fertilization). Mutants with reduced ERK signaling show a failure in maturation and ovulation or a significant delay in maturation, with ovulation following at the normal time (Lee et al., 2007). Note that while LIN-3-LET-23 do not function in maturation, they act by a distinct mechanism to promote ovulation (see below).

germline apoptosis: Ras-ERK signaling appears to affect germline apoptosis in multiple ways. Null mutants do not undergo physiological germline apoptosis (Gumienny et al., 1999; Kritikou et al., 2006), but show meiotic arrest prior to the late pachytene stage, when apoptosis normally occurs (Lee et al., 2007). Increasing or partially reducing MPK-1 suggests roles both in promoting (Gumienny et al., 1999; Kritikou et al., 2006) and inhibiting (Arur et al., 2009) physiological apoptosis. MPK-1 also promotes DNA-damage induced apoptosis (Rutkowski et al., 2011).

mitosis to meiosis transition: ERK signaling also appears to affect the transition from mitosis to meiosis in multiple ways. RNAi against *mpk-1* leads to an expanded germline mitotic zone and a decreased early meiotic transition zone, suggesting that signaling promotes meiotic entry (Lee et al., 2006; Lee et al., 2007). On the other hand, *mpk-1* null mutants have a slightly decreased mitotic zone and enhance *glp-1*/Notch defects (Lee et al., 2007). Note that phosphorylated MPK-1 has not been detected in the distal germline, so some of these effects could be indirect (Lee et al., 2007).

Vulval cell fates: LIN-3-LET-23 signaling via Ras cooperates with LIN-12/Notch and Wnt signaling to promote hermaphrodite vulval development (see [Vulval development](#)). Ras acts via the Raf-MEK-ERK cascade to promote the 1° vulval fate and acts via the RalGDS pathway as well as non-autonomously via Notch signaling to promote the 2° vulval fate (Simske and Kim, 1995; Chen and Greenwald, 2004; Zand et al., 2011). Reduced Ras signaling causes a vulvaless or Vul phenotype (Figure 3B), whereas increased Ras signaling leads to a multivulva or Muv phenotype. Vulvaless animals cannot lay eggs, leading to a “bag of worms” phenotype in which progeny hatch internally.

Uterine uv1 cell fate: LIN-3-LET-23 signaling via Ras-ERK promotes the uterine uv1 vs. utse cell fate (Chang et al., 1999). uv1 is important for establishing a proper vulval-uterine connection, and in its absence, hermaphrodites cannot lay eggs.

P12 ectoblast cell fate: LIN-3-LET-23 signaling via Ras-ERK cooperates with Wnt signaling to promote the P12 vs. P11 ectoblast cell fate (Fixsen et al., 1985; Jiang and Sternberg, 1998). The P11 and P12 ectoblasts are neighboring cells that give rise to slightly different types of hypodermal and neuronal descendants (Sulston and Horvitz, 1977). Reduced Ras signaling causes P12 → P11 cell fate transformations, while increased signaling causes P11 → P12 fate transformations.

Male spicule fates: LIN-3-LET-23 signaling via Ras-ERK promotes several different male spicule fates that derive from the B ectoblast (Chamberlin and Sternberg, 1994; Seah and Sternberg, 2009). B.a gives rise to four anterior-posterior pairs of cells that form equivalence groups (Sulston et al., 1980). Reduced Ras signaling causes anterior-to-posterior fate transformations within each of these equivalence groups (Chamberlin and Sternberg, 1994). The resulting spicule defects prevent males from mating effectively.

Male hook competence group fates: LIN-3-LET-23 signaling via Ras-ERK cooperates with Wnt signaling to promote the P11.p fate in the male hook competence group (HCG) (Yu et al., 2009). The HCG consists of P9.p (3° fate), P10.p (2° fate) and P11.p (1° fate) (Sulston and White, 1980). Reduced Ras signaling does not appear to affect HCG fates on its own, but can enhance the P11.p fate defects of *lin-17*/Frizzled mutants (Yu et al., 2009).

Aging: Ras signaling promotes longevity, potentially via multiple mechanisms. First, as animals age, LIN-3-LET-23 signaling acts via Ras-ERK and EOR-1 to upregulate *skr-5* and other genes involved in the ubiquitin

proteasome system (UPS), thereby promoting proper protein homeostasis (Liu et al., 2011). This pathway appears to operate broadly in epithelial cells such as the intestine and hypodermis. Second, the Raf-MEK-ERK cascade stimulates the activity of the SKN-1 transcription factor to repress insulin-like peptide expression (Okuyama et al., 2010). Whether Ras acts upstream in this process is unknown. Third, EGL-17 and EGL-15A also promote longevity, but whether they do so through Ras is unknown (Chateau et al., 2010). Loss of *egl-17*/FGF or *egl-15A*/FGFR function or RNAi knockdown of *lin-45*/Raf, *mek-2*/MEK or *mpk-1*/ERK shortens adult lifespan, while RNAi of the MPK-1 phosphatase gene *lip-1* increases lifespan (Chateau et al., 2010; Okuyama et al., 2010). LET-23 also plays Ras-independent roles in aging (Iwasa et al., 2010) (see below).

Sex myoblast migration: EGL-17-EGL-15 signaling via Ras-ERK in sex myoblasts helps to specify the proper endpoint of sex myoblast migration (DeVore et al., 1995; Sundaram et al., 1996; Burdine et al., 1997). Two different splice isoforms of EGL-15 mediate chemoattractive and chemorepulsive mechanisms that position the SMs with respect to the gonad and vulva (Stern and Horvitz, 1991; Branda and Stern, 2000; Lo et al., 2008). In *egl-15* or *let-60*/Ras null mosaics, sex myoblasts adopt a broadened range of final positions, whereas in *egl-15A*-specific mutants (which perturb chemoattraction), sex myoblasts are displaced more posteriorly (Sundaram et al., 1996; Lo et al., 2008). The resulting defects in sex muscle position can affect egg-laying.

Sex muscle differentiation: The sex myoblasts divide to generate the sex muscles, which attach to the vulva and control egg-laying (Sulston and Horvitz, 1977). Although reduced EGL-15-Ras signaling does not perturb sex muscle differentiation, hyperactive signaling by EGL-15A prevents terminal differentiation and thus causes a strong egg-laying defect (Sasson and Stern, 2004).

Body muscle maintenance: In addition to causing the Clr phenotype, hyperactivating EGL-15-Ras-ERK in adults induces body muscle wasting and failure to express a muscle gene reporter (Szewczyk et al., 2002; Szewczyk and Jacobson, 2003). This has been proposed to be a direct effect in muscle.

Axon outgrowth: LET-756-EGL-15 signaling via Ras in the hypodermis influences axon extension and pathfinding by certain ventral cord neurons that migrate over the hypodermis (Bulow et al., 2004). Whereas in wild-type these neurons extend axons along one side of the ventral midline, in mutants the axons are shorter than normal and/or wander across the midline. Whether Ras acts through Raf-MEK-ERK or through another pathway has not been determined.

Body muscle membrane extension: LET-756-EGL-15 signaling via Ras in body muscles restrains the outgrowth of muscle membrane extensions at neuro-muscular junctions (NMJs) (Dixon et al., 2006). Reduced signaling leads to ectopic muscle extensions, while increased signaling leads to fewer muscle extensions. Whether Ras acts through Raf-MEK-ERK or through another pathway has not been determined.

Acetylcholine receptor (AChR) synaptic clustering: EGL-15-Ras signaling in body muscles promotes AChR clustering and/or maintenance at NMJs (Gottschalk et al., 2005). Reduced signaling leads to reduced AChR expression at NMJs and confers moderate resistance to AChR agonists such as nicotine and levamisole. The ligand involved, and whether Ras acts through Raf-MEK-ERK or through another pathway, has not been determined.

Olfaction: Ras-ERK signaling in the mature AWC neuron is required for sensitivity to volatile attractants (Hirotzu et al., 2000). Whereas wild-type *C. elegans* will chemotax toward volatile attractants such as isoamylalcohol and diacetyl, mutants with reduced Ras signaling fail to chemotax towards such attractants. The receptor mediating this response is unknown, but it appears to be a G-protein coupled receptor that acts through EGL-30/G α q to stimulate EGL-8/PLC β and DAG production, activating the Ras GEF RGEF-1 (Chen et al., 2011) (Figure 1F).

6. Ras-independent roles of LET-23/EGFR, EGL-15/FGFR or the Raf-MEK-ERK cascade

As mentioned above, there are several contexts in which LET-23/EGFR acts via alternative Ras-independent signaling pathways (Figure 2). There also appears to be a Ras-independent mechanism for activating Raf-MEK-ERK.

Ovulation: LIN-3 and LET-23 signal through the PLC γ -IP $_3$ pathway to promote ovulation (Clandinin et al., 1998; McCarter, 1998; Bui and Sternberg, 2002; Miller et al., 2003) (Figure 2A). LIN-3 is required in the oocyte while LET-23 is required in the somatic gonadal sheath and spermathecal cells for dilation of the distal spermatheca

and contraction of the myoepithelial sheath cells necessary for ovulation. When *lin-3* or *let-23* activity is reduced or absent, oocytes mature but somatic gonadal function is abnormal, resulting in failed ovulation into the spermatheca, with the unfertilized oocytes undergoing endoreduplication (the Emo phenotype).

Healthy aging: LET-23 signaling through the PLC γ -IP₃ pathway also promotes healthy aging (Iwasa et al., 2010). As animals age, their locomotion and swimming rates decline; this age-related decline is delayed in animals with increased LET-23 signaling and accelerated in animals with decreased signaling. The site of action of this signaling event is still unclear.

Behavioral quiescence: Over-expression of *lin-3*/EGF induces behavioral quiescence, a reversible, sleep-like state in which feeding and movement cease (Van Buskirk and Sternberg, 2007). This involves another Ras-independent pathway consisting of LET-23, PLC-3/PLC γ and diacylglycerol, which stimulates synaptic vesicle release in the ALA neuron (Figure 2B). *let-23*/EGFR and *plc-3*/PLC γ mutants show reduced behavioral quiescence during lethargus.

Axon maintenance: EGL-15A has a kinase- and presumably Ras-independent function in maintaining axon positions after outgrowth (Bulow et al., 2004). This function was revealed by the ability of truncated EGL-15 transgenes to rescue axon positioning defects of *egl-15* mutants.

Resistance to Microbacterium nematophilum-induced swelling: *ksr-1*, *lin-45*/Raf, *mek-2*/MEK and *mpk-1*/ERK are required for the rectal swelling response to infection by *M. nematophilum* (Nicholas and Hodgkin, 2004). *let-60*/Ras is only partly required for this response, and appears to cooperate with *rho-1* to activate the Raf-MEK-ERK cascade (McMullan et al, 2012).

7. Screens used to identify RTK-Ras-ERK pathway components, targets and regulators

A major strength of *C. elegans* as a model system is the ease of conducting unbiased forward genetic screens to identify mutants and genes relevant to a biological process of interest. Many studies on RTK-Ras-ERK signaling have focused on the role of the LET-23/EGFR pathway in promoting vulval development (see Vulval development), and many core components of the pathway were first identified through forward genetic screens for mutants with Vul or Muv mutant phenotypes (Ferguson and Horvitz, 1985). Some components of the pathway were originally identified based on other mutant phenotypes described above such as sex myoblast migration defects (Stern and Horvitz, 1991) or germline meiosis defects (Church et al., 1995; Hsu et al., 2002; Ohmachi et al., 2002). Many relevant regulators and targets of the signaling pathway were identified as genetic modifiers (suppressors or enhancers) of other mutant phenotypes in forward genetic screens or reverse genetic tests. The genes identified in these types of genetic screens have revealed many mechanisms that influence the efficiency and spatial dynamics of RTK-Ras-ERK signaling, or that mediate its downstream responses, as discussed in the following sections.

Note that for many genes described below, single mutants have relatively mild phenotypes, yet can confer strong phenotypes in certain sensitized genetic backgrounds (Table 1). Given the many detrimental effects of misregulating Ras signaling, it is perhaps not surprising that, for most steps of the signaling pathway, there are multiple layers of regulation that function semi-redundantly.

8. Regulators of growth factor ligand expression

Transcriptional regulation of growth factor ligand expression is an important determinant of when and where RTK signaling occurs in *C. elegans* (Figure 4). *lin-3*/EGF, *egl-17*/FGF and *let-756*/FGF all show relatively restricted patterns of gene expression. For example, *lin-3* is expressed in the gonadal anchor cell during vulval development (Hill and Sternberg, 1992), in primary vulval descendants during uterine development (Chang and Sternberg, 1999), and in the excretory canal cell during excretory duct development (Abdus-Saboor et al., 2011). *egl-17* is expressed in the gonad and in the primary vulval precursor cell P6.p during sex myoblast migration (Burdine et al., 1998; Branda and Stern, 2000). *let-756* is expressed in body muscles and some neurons (Bulow et al., 2004). *egl-17* expression in P6.p and *lin-3* expression in P6.p descendants both depend on LET-23/EGFR and LET-60/Ras-dependent cell fate specification (Burdine et al., 1998; Chang and Sternberg, 1999; Cui and Han, 2003). Simple overexpression of *lin-3*(+) is sufficient to cause gain-of-function phenotypes (Katz et al., 1995), and multiple genes that restrict or promote *lin-3* expression have been identified.

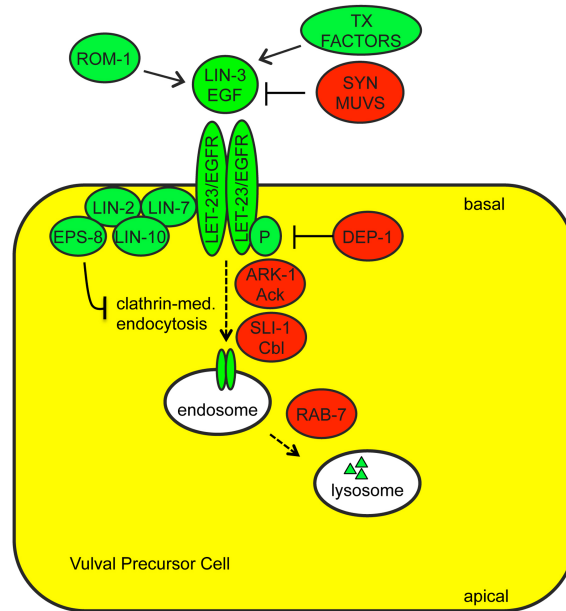


Figure 4. Regulation of LIN-3/EGF and LET-23/EGFR. *lin-3* transcription, LIN-3 proteolysis and LET-23 endocytosis are known or suspected points of regulation. Green indicates positively acting factors; red indicates negatively acting factors. See text for details and references.

Syn Muv genes: The “synthetic multivulva” (SynMuv) genes encode three sets of nuclear factors that function redundantly to inhibit ectopic *lin-3* expression (Ferguson and Horvitz, 1989; Cui et al., 2006; Fay and Yochem, 2007). One set, the SynMuv B genes, encodes orthologs of a conserved transcriptional repression cascade including LIN-35/Rb (Lu and Horvitz 1998), DPL-1/DP and EFL-1/E2F (Ceol and Horvitz, 2001), a NuRD-like histone deacetylase complex including HDA-1/HDAC1 (Solari and Ahringer, 2000), an H3K9 histone methyltransferase MET-2/SETDB1 (Andersen and Horvitz, 2007) and the chromodomain protein HPL-2/HP1 (Couteau et al., 2002). The SynMuv C genes encode components of a Tip60/NuA4-like histone acetyltransferase complex (Ceol and Horvitz, 2004), while the SynMuv A genes include several THAP-domain proteins that are thought to act as transcriptional repressors (Huang et al., 1994; Davison et al., 2005; Davison et al., 2011). A point mutation in the *lin-3* gene promoter behaves like a SynMuv A mutation, suggesting that synMuv A proteins may act directly on the *lin-3* promoter to repress ectopic *lin-3* expression (Saffer et al., 2011). It is not clear if the other SynMuv complexes act directly or indirectly on *lin-3*. Although single mutants for any given SynMuv gene have relatively normal levels of *lin-3* expression, double mutants for a SynMuv A and a SynMuv B mutation have increased levels of *lin-3* expression and several corresponding Ras(gf)-like phenotypes such as Muv (Cui et al., 2006). Single molecule RNA FISH demonstrated that SynMuv mutants have ectopic *lin-3* mRNA in most cells of the body, but that elevation in *lin-3* mRNA levels is quite modest on a per cell basis, highlighting the sensitivity of signaling to ligand levels (Saffer et al., 2011). Functional analysis suggests that the Muv phenotype primarily results from derepression of *lin-3* in *hyp7* (Myers and Greenwald, 2005; Cui et al., 2006).

Transcription factors that promote *lin-3* expression: In the anchor cell, *lin-3* expression requires the E-protein/daughterless homolog HLH-2 and an unidentified nuclear hormone receptor (NHR); a 59 bp *lin-3* enhancer element containing predicted E-box and NHR binding sites is both necessary and sufficient for anchor cell expression (Hwang and Sternberg, 2004). In the primary vulval lineage, *lin-3* expression requires the Pax family transcription factor EGL-38 (Chang and Sternberg, 1999).

9. Regulators of growth factor ligand activity

Proteolytic processing and secretion of ligands and interaction of ligands with receptors are additional steps at which signaling can be regulated (Figure 4).

LIN-3/EGF proteolysis and signaling: Like other ligands of the EGF family, LIN-3 is synthesized initially as a transmembrane protein but may be subsequently cleaved proteolytically to generate a diffusible form (Hill and Sternberg, 1992; Blobel et al., 2009). There are at least four different splice isoforms of LIN-3 that differ in their juxta-membrane regions, potentially affecting proteolysis (Dutt et al., 2004; Van Buskirk and Sternberg, 2007).

Biological activity of one specific isoform, LIN-3L, appears to require proteolysis by the Rhomboid ortholog ROM-1 (Dutt et al., 2004). *rom-1* mutants have only subtle phenotypes, suggesting either that other LIN-3 proteases exist or that other LIN-3 isoforms can function effectively in an unprocessed, transmembrane form.

In *Drosophila*, various extracellular factors influence EGF-like ligand diffusion and interactions with receptors (Klein et al., 2004; Miura et al., 2006; Pizette et al., 2009). There is emerging evidence for this type of regulation in *C. elegans* as well. The secreted proteins HPA-1 and HPA-2 limit LIN-3 signaling during adult aging, potentially by LIN-3 sequestration (Iwasa et al., 2010). The secreted lipocalin LPR-1 facilitates LIN-3 signaling during excretory duct cell development (C. Stone and M. Sundaram, in preparation).

FGF secretion and signaling: Both *C. elegans* FGF-like ligands utilize unusual pathways for secretion. EGL-17 is secreted via a mechanism that requires the lipoprotein receptor-related proteins LRP-1 and LRP-2 and the Disabled-related adaptor DAB-1 (Kamikura and Cooper, 2003). LET-756 lacks a classical signal peptide, and like mammalian FGF9, its secretion depends on an internal hydrophobic domain (Popovici et al., 2004). Also like FGF9, LET-756 shows partial localization to the nucleus (Popovici et al., 2006), though the significance of this localization remains unknown.

In *C. elegans*, as in other organisms, FGF signaling can be influenced by the extracellular matrix (Bulow and Hobert, 2006). Heparan sulfate polysaccharides facilitate EGL-17-dependent axon outgrowth (Tornberg et al., 2011). The matrix component *unc-52*/perlecan genetically interacts with *egl-17* to influence distal tip cell migration (Merz et al., 2003). The ability of EGL-17 vs. LET-756 to signal through EGL-15/FGFR also depends to some degree on alternative splicing of *egl-15* (see below).

10. Regulators and targets of RTK activity

LET-23/EGFR basolateral localization: Factors that regulate LET-23/EGFR trafficking and localization are important modulators of signaling strength during vulva development (Figure 4). A complex consisting of three PDZ-domain proteins, LIN-2, LIN-7 and LIN-10, facilitates signaling by localizing LET-23 to the basolateral membrane of vulval precursor cells, adjacent to the source of LIN-3 ligand (Simske et al., 1996; Kaech et al., 1998; Whitfield et al., 1999). EPS-8 (EGFR substrate protein -8) interacts with this LIN-2-LIN-7-LIN-10 complex to further enhance basolateral LET-23 levels in the primary vulval precursor cell (Stetak et al., 2006). The E3 ubiquitin ligase SLI-1/Cbl (Jongeward et al., 1995; Yoon et al., 1995), the Ack-related tyrosine kinase ARK-1 (Hopper et al., 2000), the clathrin adaptors UNC-101 (Lee et al., 1994) and DPY-23 (Yoo et al., 2004), the sorting nexin LST-4 (Yoo et al., 2004), the RAB-7 GTPase and several members of the ESCRT complex (Skorobogata and Rocheleau, 2012) all negatively regulate signaling, probably by promoting LET-23 endocytosis and lysosomal degradation.

EGL-15/FGFR alternative splicing and glycosylation: EGL-15/FGFR activity is regulated in part via alternative splicing and other modifications to its extracellular domain that may affect ligand binding. The EGL-15(A) and EGL-15(B) splice isoforms differ in a *C. elegans*-specific region of the extracellular domain, and EGL-15(A) primarily mediates EGL-17/FGF-dependent signaling, while EGL-15(B) primarily mediates LET-756/FGF-dependent signaling (Goodman et al., 2003; Lo et al., 2008). However, these functional distinctions are not complete, and both ligands can act to some degree through both receptor isoforms (Goodman et al., 2003; Bulow et al., 2004; Dixon et al., 2006). At least five other alternative splice forms differ in the EGL-15 extreme C-terminus, potentially affecting interactions with downstream targets. EGL-15 is N-glycosylated *in vivo* and this modification appears to negatively regulate its activity, potentially by interfering with ligand binding or self-association (Polanska et al., 2009). EGL-15A activity may be positively regulated by two *C. elegans* orthologs of Klotho, a mammalian FGFR binding protein and co-receptor (Chateau et al., 2010; Polanska et al., 2011).

Adaptor Proteins that bind RTKs: RTKs transduce signals by autophosphorylation and subsequent binding to cytosolic phospho-tyrosine binding adaptor proteins, which then recruit other factors (Lemmon and Schlessinger, 2010). LET-23 acts primarily through the adaptor SEM-5 (related to vertebrate Grb2) (Clark et al., 1992), which recruits SOS-1 (Downward, 1994), but also in part through SOC-1 (related to mammalian Gab1), which recruits PTP-2/Shp2 (Hopper, 2006) (Figure 1B). EGL-15 acts through both SEM-5 and SOC-1-PTP-2 (Schutzman et al., 2001) (Figure 1C). Whether these RTKs interact directly or indirectly with SOC-1 (via SEM-5) has not been established. The SOC-1 adaptor also acts with the RTK SCD-2/ALK (Reiner et al., 2008). The ROG-1 adaptor (related to vertebrate FRS2) does not seem to act downstream of LET-23 or EGL-15, but may act downstream of another unknown RTK to control Ras-ERK-dependent meiotic progression in the germline (Matsubara et al., 2007; Lo et al., 2010) (Figure 1D). Adaptors can influence the relative use of different RTK-dependent signaling pathways, as SEM-5 and SOC-1 promote LET-23-mediated activation of LET-60 Ras, but inhibit LET-23-mediated activation of the PLC γ -IP $_3$ pathway (Hopper et al., 2000; Hopper, 2006).

Receptor Tyrosine Phosphatases: Factors that regulate RTK phosphorylation status could obviously modulate adaptor binding and signaling strength. The receptor tyrosine phosphatases (RTPs) *CLR-1* and *DEP-1* negatively regulate *EGL-15* and *LET-23* RTK activity, respectively, most likely through direct dephosphorylation of key tyrosine residues (Kokel et al., 1998; Berset et al., 2005). *CLR-1* is related to the LAR RTP family, and is required in the hypodermis to prevent *EGL-15*/FGFR hyperactivation (Kokel et al., 1998; Huang and Stern, 2004) (Figure 1C). *DEP-1* is the *C. elegans* ortholog of the mammalian tumor suppressor Density Enhanced Phosphatase-1, which inhibits EGFR and multiple other RTKs (Ruivenkamp et al., 2002; Tarcic et al., 2009). *DEP-1* acts redundantly with a variety of other inhibitors to dampen *LET-23*/EGFR signaling (Berset et al., 2005) (Figure 4).

Cytosolic phosphatases and kinases: The cytosolic tyrosine phosphatase *PTP-2*/Shp2 positively regulates *LET-23* and *EGL-15* RTK signaling (Gutch et al., 1998; Schutzman et al., 2001), and the cytosolic tyrosine kinase *ARK-1* (Ack-related) negatively regulates *LET-23* RTK signaling (Hopper et al., 2000). The substrates of *PTP-2* and *ARK-1* are still unclear, but these proteins can bind to the adaptors *SOC-1* and *SEM-5*, respectively, and appear to act at a step immediately downstream of the RTKs to influence the strength or kinetics of signaling. In the germline, *PTP-2* antagonizes the activity of *GAP-2* and *GAP-3*, suggesting these could be relevant *PTP-2* substrates (Yang et al., 2010). Evidence from mammalian cells suggests that Ack tyrosine kinases promote EGFR internalization and degradation (Shen et al., 2007).

11. Regulators of *LET-60*/Ras activity or expression

GEFs and GAPs: As is the case for other small GTPases, the activity of *LET-60*/Ras is controlled by Guanine Nucleotide Exchange Factors (GEFs), which activate Ras by stimulating conversion of Ras-GDP to Ras-GTP, and by GTPase activating proteins (GAPs), which inactivate Ras by stimulating conversion of Ras-GTP to Ras-GDP (Malumbres and Barbacid, 2002; Karnoub and Weinberg, 2008) (Figure 5). Gain-of-function (gf) mutations lock Ras in the active, GTP-bound state; the *let-60(gf)* allele *n1046* (G13E) has been widely used for genetic analyses (Han and Sternberg, 1990). Dominant-negative (dn) mutations lock Ras in the inactive, GDP-bound state, causing it to bind stably to and titrate out GEFs; a variety of *let-60(dn)* mutations have been described (Han and Sternberg, 1991).

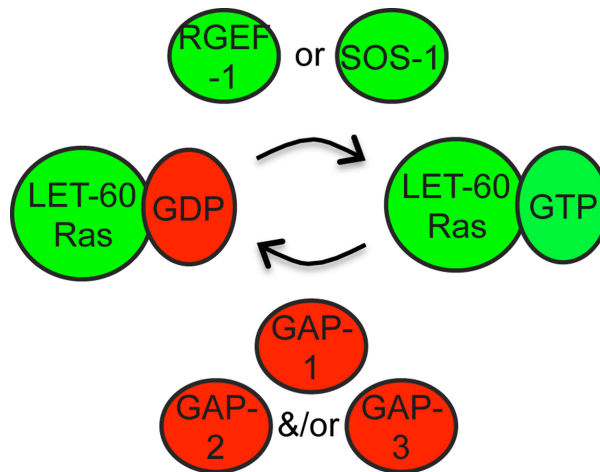


Figure 5. Regulation of *LET-60*/Ras by GEFs and GAPs. Ras GTPase regulation by GEFs and GAPs has been extensively studied in other systems (Malumbres and Barbacid, 2002). The GEF *SOS-1* has widespread requirements in *LET-60*/Ras signaling (Chang et al., 2000). The alternative *RGEF-1* is used in AWC (Chen et al., 2011). There are three *C. elegans* GAP proteins that show varying tissue specificity and function in a semi-redundant manner (Stetak et al., 2008).

The GEF *SOS-1* is required for most Ras-mediated developmental events (Chang et al., 2000). A temperature-sensitive allele, *sos-1(cs41)*, is a useful tool for conditional inactivation of Ras signaling (Rocheleau et al., 2002). A different GEF, *RGEF-1* (related to mammalian RasGRPs), regulates *LET-60* Ras in AWC neurons during olfaction (Chen et al., 2011).

Three GAPs, *GAP-1*, *GAP-2* and *GAP-3*, function redundantly to negatively regulate Ras signaling, with each required to different degrees in different tissues. *GAP-1* plays a predominant role during vulval and P12 fate specification, while *GAP-3* predominates in the germline (Hajnal et al., 1997; Hayashizaki et al., 1998; Stetak et al., 2008).

Post-translational modifications of Ras: In other systems, a variety of post-translational modifications can modulate Ras trafficking and localization (Ahearn et al., 2012). For example, farnesylation of the Ras C-terminus promotes plasma membrane attachment. Chemical farnesyltransferase inhibitors suppress the Muv phenotype of *let-60 ras(gf)* mutants, suggesting that farnesylation promotes LET-60/Ras activity (Hara and Han, 1995). A negative regulator of LET-60/Ras is SUR-5, a protein of unknown function that resembles acetyl coenzyme A synthetases (Gu et al., 1998).

miRNA-dependent regulation of Ras: The overall level of *let-60 ras* expression can also influence Ras signaling activity, as overexpression of wild-type LET-60 leads to a Muv phenotype (Sundaram and Han, 1995). In vulval precursor cells and hypodermal seam cells, normal levels of *let-60 ras* depend on negative regulation by the *let-7* family microRNAs *mir-84* and *let-7* (Johnson et al., 2005). These microRNAs appear to act directly on the *let-60* 3' UTR, and this mode of regulation is conserved in mammalian *ras* genes (Johnson et al., 2005).

12. Regulators of the Raf-MEK-ERK kinase cascade

Raf, MEK and ERK activities depend in large part on their phosphorylation status (Udell et al., 2011). For example, Raf must be de-phosphorylated at certain sites and phosphorylated at other sites in order to become active (Chong et al., 2001) (Figure 6A). Transgenic, hyperactive forms of LIN-45/Raf, MEK-2/MEK and MPK-1/ERK were generated by mutating relevant sites to prevent or mimic phosphorylation; such transgenes have been useful for epistasis experiments (Lackner and Kim, 1998; Chong et al., 2001; Kao et al., 2004; Yoder et al., 2004; Rocheleau et al., 2005). Commercially available antibodies also recognize phosphorylated MPK-1 by Western analyses and (in some cases) immunofluorescence, and have been very useful for molecular epistasis (Hirotsu et al., 2000; Page et al., 2001; Ohmachi et al., 2002; Lee et al., 2007; Chen et al., 2011).

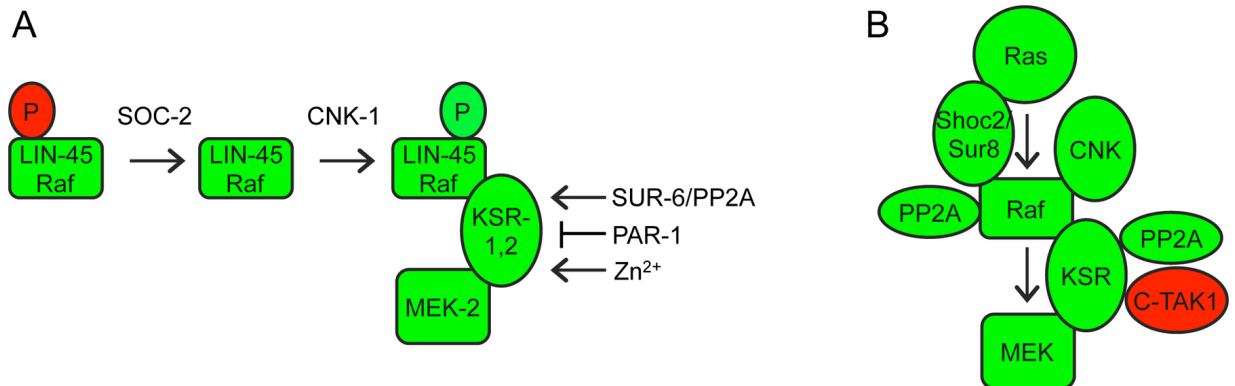


Figure 6. Regulation of Raf-MEK-ERK by scaffolds and other associated proteins. A) Phosphorylation (P) at different sites inhibits (red) or activates (green) LIN-45 and other Raf proteins (Chong et al., 2001; Udell et al., 2011). Epistasis experiments with LIN-45/Raf phosphorylation site variants demonstrated that SOC-2 is required upstream or during inhibitory phosphorylation removal (Yoder et al., 2004), while CNK-1 is required downstream of inhibitory phosphate removal but upstream of activating phosphorylation addition (Rocheleau et al., 2005). KSR-1 and SUR-6 are required downstream of both events (Kao et al., 2004; Yoder et al., 2004; Rocheleau et al., 2005). B) Protein interactions of related scaffold proteins in mammalian cells (Udell et al., 2011). C-TAK1 is a mammalian homolog of the PAR-1 kinase (Muller et al., 2001). See text for additional details and references.

KSR and other scaffold proteins: Positive regulators of Raf-MEK-ERK include so-called “scaffold” proteins (Figure 6A) - KSR-1 (Kornfeld et al., 1995b; Sundaram and Han, 1995), KSR-2 (Ohmachi et al., 2002), CNK-1 (Rocheleau et al., 2005) and SOC-2 (also known as SUR-8) (Selfors et al., 1998; Sieburth et al., 1998). These proteins have no obvious enzymatic activity, but bind to one or more core kinases in the cascade and are thought to bring different components together at the right place and time (Udell et al., 2011). KSR-1 and KSR-2 are required downstream of activating Raf phosphorylation but upstream of MEK-ERK (Ohmachi et al., 2002; Rocheleau et al., 2005). CNK-1 is required upstream of Raf activating phosphorylation (Rocheleau et al., 2005), while SOC-2 is required upstream of (or during) Raf inhibitory phosphate removal (Yoder et al., 2004). These epistasis results fit well with the observed protein interactions of the mammalian scaffolds (Figure 6B): Mammalian KSR binds constitutively to MEK and variably to Raf and ERK (Xing et al., 1997; Yu et al., 1998; Stewart et al., 1999), while CNK binds Raf (Yao et al., 2000; Lanigan et al., 2003), and SOC-2 binds to Ras and Raf (Li et al., 2000). In *C. elegans*, the KSR scaffold is a core pathway component but encoded by two paralogs: KSR-1 and KSR-2 are individually required for a few developmental processes, but redundantly required for many others (Ohmachi et al., 2002). CNK-1 and SOC-2 are not individually required under standard laboratory conditions, but help buffer

signaling against other perturbations (Selfors et al., 1998; Sieburth et al., 1998; Rocheleau et al., 2005). Another positive regulator of Raf-MEK-ERK signaling is CGR-1, a CRAL/TRIO and GOLD domain protein that promotes signaling in the vulva, possibly by modulating Raf activation (Goldstein et al., 2006; Johnson and Kornfeld, 2010).

Regulators of the KSR scaffold: Several gene products appear to influence KSR scaffold activity (Figure 6). The PAR-1 kinase inhibits Raf-MEK-ERK signaling (Kao et al., 2004; Yoder et al., 2004) and is homologous to mammalian C-TAK1, which directly phosphorylates KSR to affect its localization (Ory et al., 2003). SUR-6, a PR55/B-type regulatory subunit of Protein Phosphatase 2A (PP2A-B), promotes Raf-MEK-ERK signaling (Sieburth et al., 1999) and has been suggested to dephosphorylate and activate both Raf and KSR (Ory et al., 2003; Kao et al., 2004; Yoder et al., 2004). Finally, the zinc transporter proteins CDF-1 and SUR-7 also promote Raf-MEK-ERK signaling in the vulva (Bruinsma et al., 2002; Yoder et al., 2004). High levels of zinc can influence KSR phosphorylation in mammalian cells, suggesting that zinc modulates the activity of a KSR kinase (Yoder et al., 2004).

MPK-1/ERK inhibitors: Several inhibitors of MPK-1/ERK have been described. The MAP kinase phosphatase LIP-1 dephosphorylates and inhibits MPK-1 in both the soma and the germline (Berset et al., 2001; Hajnal and Berset, 2002; Lee et al., 2006; Rutkowski et al., 2011). The CCCH zinc finger protein GLA-3 binds to and inhibits MPK-1 without altering MPK-1 phosphorylation (Kritikou et al., 2006). The Germinal Center Kinase GCK-1 also binds directly to MPK-1 and inhibits MPK-1 phosphorylation in the pachytene region of the germline (Schouest et al., 2009). A gap junction-dependent mechanism and a VAB-1 RTK-dependent mechanism inhibit MPK-1 phosphorylation in oocytes (Miller et al., 2003; Govindan et al., 2006; Govindan et al., 2009; Kim et al., 2012) (Figure 7). Repression of *mpk-1* expression by the RNA binding proteins FBF-1, FBF-2 and LARP-1 also helps shape the distribution of phosphorylated MPK-1 in the germline (Lee et al., 2007; Nykamp et al., 2008).

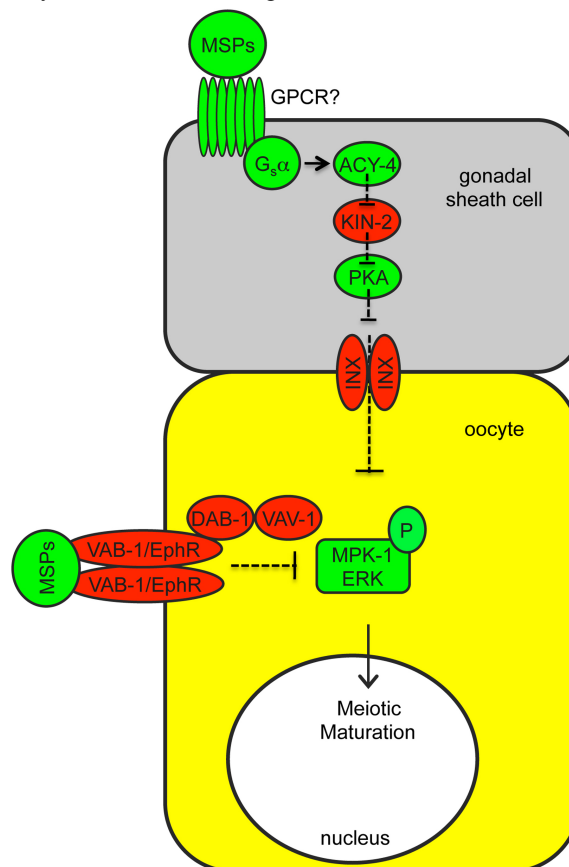


Figure 7. Regulation of MPK-1/ERK phosphorylation during oocyte maturation. Sperm, via the Major Sperm Protein (MSP), promotes MPK-1/ERK phosphorylation and oocyte maturation (McCarter et al., 1999; Miller et al., 2001; Miller et al., 2003). Somatic gonadal sheath cells signal adjacent oocytes via innexin (INN)-based gap junctions, antagonizing MPK-1/ERK activation (Govindan et al., 2006; Govindan et al., 2009; Kim et al., 2012). When sperm are present, MSP relieves gap junctional inhibition by binding to unknown G-protein coupled receptors (GPCRs) on sheath cells that act through G α and ACY-4 adenylate cyclase signaling and Protein Kinase A (PKA). The VAB-1 RTK in oocytes also antagonizes MPK-1/ERK activation via a mechanism that involves DAB-1/Disabled and VAV-1/Vav, and this is also relieved by MSP (Miller et al., 2003; Govindan et al., 2006). It is currently unknown what pathway directly activates MPK-1 in oocytes or at what step gap junction-dependent and VAB-1 signaling antagonize MPK-1 activation.

Cross-tissue interactions: There is evidence that some genes may influence Raf-MEK-ERK signaling indirectly, by affecting neighboring cells. Some of the clearest examples involve genes that function within gonadal sheath cells to affect gap-junction dependent inhibition of MPK-1/ERK phosphorylation in oocytes (Kim et al., 2012) (Figure 7). Also, the $G\alpha_q$ protein EGL-30 and the voltage-gated calcium channel EGL-19 function in neurons and muscle, respectively to promote Ras-mediated vulval induction (Moghal et al., 2003). The G-protein coupled receptor SRA-13 and its $G\alpha$ target GPA-5 inhibit Ras-mediated vulval induction, but it is unknown whether these genes function in vulval cells or in neurons (Battu et al., 2003). The zinc transporter CDF-1 can influence vulval induction when expressed in either the vulva or the intestine (Bruinsma et al., 2002). The mechanisms underlying these other cross-tissue effects are still unclear.

13. Targets of MPK-1/ERK, and other factors influencing downstream responses

The specific outcomes of RTK-Ras-ERK signaling in different tissues are thought to depend on the availability and use of different ERK substrates and on combinatorial action of ERK substrates with other tissue-specific or signal-regulated factors (Tan and Kim, 1999; Flores et al., 2000; Halfon et al., 2000; Barolo and Posakony, 2002; Arur et al., 2009). In *C. elegans* somatic cell fate decisions, the combinatorial mechanism appears quite relevant, since two identified MPK-1/ERK targets (LIN-1 and EOR-1) are widely expressed and required for many different outcomes (Beitel et al., 1995; Jacobs et al., 1998; Howard and Sundaram, 2002; Rocheleau et al., 2002; Howell et al., 2010) (Figure 1B; Table 2). LIN-1 and EOR-1 are both nuclear transcription factors, consistent with the idea that transcriptional control of gene expression is the critical mechanism for cell fate specification. The situation is different in the germline, where there are many identified targets of MPK-1, few of which are transcription factors and each of which affects only a small subset of MPK-1-dependent processes (Arur et al., 2009) (Figure 1D; Table 2). In both the soma and the germline, a common theme is redundancy among different MPK-1/ERK substrates.

Table 2. Known MPK-1/ERK substrates. Positive targets are shown in green, and negative targets are shown in red.

Gene Product	Mammalian Relative(s)	Molecular Function	Ras-related Phenotypes	Reference(s)
Soma				
EOR-1 (Egl-1 suppressor/diO uptake defective/Raf enhancer)	PLZF	BTB/Zinc finger protein, probable transcriptional regulator	Partial rod-like lethal and P12→P11 fate changes. Strong rod-like lethal in combination with <i>sur-2</i> , <i>lin-25</i> or <i>lin-1</i> . Reduced age-dependent ubiquitination.	(Howard and Sundaram, 2002; Howell et al., 2010; Liu et al., 2011; Rocheleau et al., 2002)
LIN-1 (abnormal cell LINeage)	Elk1	Ets domain transcription factor	Muv, excretory duct duplication, etc. Rod-like lethal in combination with <i>eor-1</i> or <i>eor-2</i> .	(Beitel et al., 1995; Jacobs et al., 1998; Howard and Sundaram, 2002)
LIN-31 (abnormal cell LINeage)	FoxB2	Winged helix transcription factor	Mixed Vul and Muv	(Miller et al., 1993; Tan et al., 1998)
Germline				
CDC-48.2 (Cell Division Cycle related)	p97	AAA ATPase	Enhances <i>mpk-1(rf)</i> pachytene organization and oocyte differentiation defects	(Arur et al., 2009)
CDK-7 (Cyclin-Dependent Kinase)	CDK7/CAK	Kinase	Enhances <i>let-60(gf)</i> small oocyte defect	(Arur et al., 2009)
CDTL-7 CDc-Two-Like)	CRKRS/CDTL7	Kinase	Enhances <i>mpk-1(rf)</i> pachytene progression and apoptosis defects	(Arur et al., 2009)

CGH-1 (Conserved Germline Helicase)	DDX6	ATP-dependent RNA Helicase	Enhances <i>mpk-1(rf)</i> pachytene progression and apoptosis defects	(Arur et al., 2009)
CYA-1 (CYclinA)	CYA1	Cyclin A	Enhances <i>mpk-1(rf)</i> oocyte differentiation defects	(Arur et al., 2009)
DCR-1 (DiCeR)	DICER	small RNA synthesis	Enhances <i>let-60(gf)</i> small oocyte defect	(Arur et al., 2009)
DIS-3 (DISjunction abnormal)	DIS3	exosome catalytic subunit	Enhances <i>let-60(gf)</i> small oocyte defect	(Arur et al., 2009)
DPL-1 (DP-Like)	DP	component of E2F transcription factor	Suppresses <i>mpk-1(lf)</i> pachytene disorganization defect	(Lin and Reinke, 2008)
DRSH-1 (DRoSHa)	DROSHA	small RNA synthesis	Enhances <i>let-60(gf)</i> small oocyte defect	(Arur et al., 2009)
DDX-19 (DeaD boX helicase)	DDX19	Dead box helicase	Enhances <i>mpk-1(rf)</i> pachytene organization and oocyte differentiation defects	(Arur et al., 2009)
EIF-3.D (Eukaryotic Initiation Factor)	EIF3	Translation initiation Factor	Enhances <i>let-60(gf)</i> oocyte maturation defect	(Arur et al., 2009)
GSK-3 (Glycogen Synthase Kinase)	GSK3B	Serine/Threonine Kinase	Enhances <i>let-60(gf)</i> small oocyte defect	(Arur et al., 2009)
MRPS-5 (Mitochondrial Ribosomal Protein, Small)	MRPS5	Ribosomal Protein S5	Enhances <i>mpk-1(rf)</i> pachytene progression and oocyte differentiation defects	(Arur et al., 2009)
MTR-4 (MRNA Transporter)	SKIV-2L2	Nuclear exosomal RNA helicase	Enhances <i>mpk-1(rf)</i> pachytene progression and pachytene organization defects	(Arur et al., 2009)
NOS-3 (NanOS-related)	-	RNA binding protein	Suppresses <i>mpk-1(lf)</i> pachytene disorganization defect	(Arur et al., 2011)
PAC-1 (PAR-6 At Contacts)	ARHGAP21	Rho GAP	Enhances <i>mpk-1(rf)</i> pachytene progression and oocyte differentiation defects	(Arur et al., 2009)
PAR-5 (abnormal embryonic PARTitioning of cytoplasm)	14-3-3-zeta	14-3-3 family chaperone	Enhances <i>mpk-1(rf)</i> pachytene progression and pachytene organization defects	(Arur et al., 2009)
POLE-2 (DNA POLymerase Epsilon)	POLE2	DNA Polymerase epsilon, subunit B	Enhances <i>mpk-1(rf)</i> oocyte growth defects	(Arur et al., 2009)
RAB-5 (RAB family)	RAB5	Rab GTPase	Enhances <i>let-60(gf)</i> small oocyte defect	(Arur et al., 2009)
RBA-1 (RBAp48-related)	CAF1/TBAP48	Rb-binding protein	Enhances <i>let-60(gf)</i> small oocyte defect	(Arur et al., 2009)

RHA-2 (RNA HelicAse)	DHX37	RNA helicase	Enhances <i>mpk-1(rf)</i> pachytene progression and oocyte growth defects	(Arur et al., 2009)
ROP-1 (RO ribonucleoProtein)	R(O) RNP	RNA binding protein	Enhances <i>let-60(gf)</i> small oocyte defect	(Arur et al., 2009)
RSKD-1 (Ribosomal protein S6 Kinase Delta)	RPS6K	Ribosomal Protein S6 Kinase	Enhances <i>mpk-1(rf)</i> maturation defects	(Arur et al., 2009)
RSKN-1 (RSK-pNinety)	RSK4	Ribosomal Protein S6 Kinase	Enhances <i>mpk-1(rf)</i> pachytene progression and oocyte differentiation defects	(Arur et al., 2009)
SEL-11/HRD-1 (Suppressor/Enhancer of LIN-12)	SYVN1	E3 ubiquitin ligase	Enhances <i>mpk-1(rf)</i> pachytene organization and oocyte differentiation defects	(Arur et al., 2009)
TOE-1 (Target of ERK kinase MPK-1)	HEAT1	HEAT-repeat protein	Enhances <i>mpk-1(rf)</i> pachytene organization and oocyte defects	(Arur et al., 2009)
TOE-2 (Target of ERK kinase MPK-1)	DEPDC1B	DEP-domain protein	Enhances <i>mpk-1(rf)</i> apoptosis defects	(Arur et al., 2009)
TOE-4 (Target of ERK kinase MPK-1)	RNF44	RING finger protein, predicted E3 ubiquitin ligase	Enhances <i>mpk-1(rf)</i> oocyte differentiation defect	(Arur et al., 2009)
TOE-5 (Target of ERK kinase MPK-1)	HMMR	F-box protein	Enhances <i>mpk-1(rf)</i> apoptosis defects	(Arur et al., 2009)
TTBK-2 (Tau TuBulin Kinase)	TTBK2	Casein kinase	Enhances <i>mpk-1(rf)</i> apoptosis defects	(Arur et al., 2009)
USP-46 (Ubiquitin Specific Protease)	USP46	ubiquitin protease	Enhances <i>mpk-1(rf)</i> oocyte differentiation defect	(Arur et al., 2009)
ZIM-2 (Zinc Finger in Meiosis)	ZFP62	C2H2 zinc finger protein	Enhances <i>mpk-1(rf)</i> germline sex determination defects	(Arur et al., 2009)

LIN-1/Ets: The Ets domain transcription factor LIN-1 is arguably the most important MPK-1 substrate in the soma (Beitel et al., 1995; Jacobs et al., 1998) (Figure 1B; Table 2). LIN-1 is related to the mammalian ERK target Elk1 (Sharrocks, 2002; Yang et al., 2003). LIN-1 is a direct substrate of ERK *in vitro*, and studies of *lin-1* gain-of-function mutations first identified the FXF ERK docking site, which confers high affinity binding between ERK and many of its substrates, including Elk1 (Jacobs et al., 1998; Jacobs et al., 1999; Sharrocks et al., 2000). *lin-1* null mutants have phenotypes resembling those of *let-60 ras* gain-of-function mutants and are epistatic to *mpk-1*, suggesting that Ras-ERK signaling antagonizes LIN-1 activity (Beitel et al., 1995; Lackner and Kim, 1998). However, some evidence suggests that LIN-1 has an additional positive role in Ras signaling, since removal of *lin-1* in certain genetic backgrounds can cause Ras loss-of-function phenotypes (Howard and Sundaram, 2002; Tiensuu et al., 2005; Abdus-Saboor et al., 2011) (see below). The biochemical basis for these opposite roles is not yet clear. One simple model consistent with studies of Elk1 (Boyer et al., 1999; Yang et al., 2003) is that MPK-1/ERK phosphorylation converts LIN-1 from a transcriptional repressor to a transcriptional activator in order to turn on signal-dependent gene expression (Figure 1B). Alternatively, MPK-1/ERK phosphorylation may simply inactivate LIN-1, and unphosphorylated LIN-1 may repress or activate different targets. The dual positive and negative roles of LIN-1 could indicate that LIN-1 has some targets that have positive roles and some targets that have negative roles.

There is evidence for LIN-1 having repressor activity in cell culture and *in vivo* (Leight et al., 2005). The repressive role of LIN-1 requires sumoylation, which then allows a SUMO-dependent interaction with MEP-1, a component of the *C. elegans* NuRD-like repressor complex (Leight et al., 2005). LIN-1 also physically associates with the NuRD-like component LET-418/Mi-2 (Guerry et al., 2007). Two directly repressed targets of LIN-1 in the vulva are the Hox gene *lin-39* (Wagmaister et al., 2006b; Guerry et al., 2007) and the Notch pathway ligand gene *lag-2* (Zhang and Greenwald, 2011).

Evidence for a LIN-1 activator function is more limited, but unphosphorylated LIN-1 appears to directly activate the Rho kinase gene *let-502* during vulva development (Farooqui et al., 2012). Ras-dependent upregulation of *lag-2* appears to occur solely through relief of LIN-1-dependent repression (Zhang and Greenwald, 2011).

The Mediator complex: Two widely important positive factors that act in conjunction with LIN-1/Ets are the Mediator subunit SUR-2/Med23 and its partner LIN-25 (Singh and Han, 1995; Tuck and Greenwald, 1995; Nilsson et al., 1998) (Figure 1B). The conserved Mediator complex links certain sequence-specific DNA binding proteins to the general RNA Polymerase II transcriptional machinery (Borggreffe and Yue, 2011). SUR-2 and LIN-25 function together downstream or parallel to MPK-1, and they have very specific Ras-related phenotypes including a partial Vul phenotype and low penetrance defects in excretory duct and P12 development (Singh and Han, 1995; Tuck and Greenwald, 1995; Nilsson et al., 1998; Nilsson et al., 2000). Mammalian Sur2 binds to ERK-phosphorylated Elk1 to recruit Mediator and allow for transcriptional activation of target genes (Boyer et al., 1999; Stevens et al., 2002). The genetics of *C. elegans* *sur-2* and *lin-1*/Ets are compatible with such a model, though physical interactions between the worm proteins have not been demonstrated and no directly activated targets of phosphorylated LIN-1 or SUR-2 have been identified to date. Analyses of the Ras-responsive *lag-2* promoter suggest that, in addition to (or instead of) acting with LIN-1, SUR-2 must function with other unidentified transcription factors (Zhang and Greenwald, 2011).

Another component of the Mediator complex, DPY-22/Med12, functions antagonistically to LET-23/EGFR-Ras-ERK during vulval development, though the precise point at which it acts has not been determined (Moghal and Sternberg, 2003).

EOR-1 and EOR-2: Two other widely important positive factors downstream of MPK-1 are the BTB/Zinc finger protein EOR-1 and its binding partner EOR-2 (Howard and Sundaram, 2002; Howell et al., 2010) (Figure 1B; Table 2). EOR-1 contains two “D-domain” type ERK docking sites and is an excellent substrate of ERK *in vitro*, and mutation of one of these docking sites or of several potential phosphoacceptors reduces EOR-1 function *in vivo* (Howell et al., 2010). EOR-1 is related to known transcriptional activators and repressors, including *Drosophila* GAGA factor and the mammalian oncoproteins PLZF and BCL-6 (Collins et al., 2001; Barna et al., 2002), but it is not known if it acts primarily as an activator or a repressor (or both) or how phosphorylation affects its activity. *eor-1* and *eor-2* mutants have incompletely penetrant *let-60 ras*-like defects in excretory duct and P12 development (Rocheleau et al., 2002) and strong defects in age-dependent ubiquitination (Liu et al., 2011), as well as several phenotypes that are apparently unrelated to Ras signaling (Hoepfner et al., 2004). EOR-1 and EOR-2 act redundantly with SUR-2 and LIN-25, such that *eor-1; sur-2* double mutants have multiple strong *let-60 ras*-like loss-of-function phenotypes (Howard and Sundaram, 2002). EOR-1 and EOR-2 also function redundantly with the cryptic positive function of LIN-1, such that *lin-1 eor-1* double mutants also have strong *let-60 ras*-like loss-of-function phenotypes (Howard and Sundaram, 2002; Abdus-Saboor et al., 2011). Thus, together, LIN-1 and EOR-1 appear to mediate many of the somatic effects of Ras signaling.

Hundreds of potential EOR-1 targets have been identified by the modencode project using ChIP-Seq (Niu et al., 2010), but the biological relevance of these targets is still unclear.

LIN-31: The forkhead transcription factor LIN-31 functions downstream of MPK-1 during vulval development (Miller et al., 1993; Tan et al., 1998) and male spicule development (Seah and Sternberg, 2009). LIN-31 appears to be a tissue-specific MPK-1 substrate since it contains a potential ERK docking site (Swathi Arur and Tim Schedl, pers. comm.) and mutation of putative ERK phosphorylation sites in LIN-31 alters function in transgenic assays (Tan et al., 1998). *lin-31* mutants have a mixed Vul and Muv phenotype, suggesting that LIN-31 both promotes and inhibits vulval development (Miller et al., 1993). Unphosphorylated LIN-31 appears to bind LIN-1 and inhibit vulval development, while phosphorylated LIN-31 promotes vulval development (Tan et al., 1998). *lin-31* has a relatively restricted expression pattern, and ectopic expression of LIN-31 can cause other tissues to adopt some vulval-like characteristics, supporting the model that tissue-specific availability of ERK substrates is one key determinant of tissue-specific responses (Tan et al., 1998; Tan and Kim, 1999).

Hox genes: A common theme among many LET-23/EGFR-Ras-ERK-dependent cell fates is a requirement for specific Hox genes (Figure 1B). In *C. elegans*, as in other animals, different Hox genes are expressed along the anterior-posterior axis (Aboobaker and Blaxter, 2003). The Hox genes *lin-39*, *egl-5*, *mab-5* and *ceh-13* are upregulated by Ras signaling and/or are required for Ras-dependent induction of vulval, P12, male hook and male spicule fates, respectively (Clandinin et al., 1997; Eisenmann et al., 1998; Maloof and Kenyon, 1998; Wagmaister et al., 2006a; Seah and Sternberg, 2009; Yu et al., 2010). Furthermore, ectopic expression of some Hox genes can change how cells respond to Ras activation (Clandinin et al., 1997; Maloof and Kenyon, 1998; Yu et al., 2010). It therefore appears that the Hox code of a given cell is one important contextual factor that determines tissue-specific outcomes of Ras signaling in the soma.

Germline MPK-1 substrates: In the germline, MPK-1 utilizes a completely different and much broader set of substrates than those described above (Figure 1D; Table 2). Arur et al., (2009) used known ERK docking site sequences (Sharrocks et al., 2000) to identify candidate MPK-1 substrates and then systematically tested their requirements in the germline by RNAi. These studies identified 30 likely MPK-1 substrates that control specific steps of germline development. In general, multiple substrates influenced each step examined, and depletion of individual substrates caused only mild phenotypes, suggesting a high level of substrate redundancy. Many germline MPK-1 substrates also influenced the spatial pattern of MPK-1 phosphorylation, suggesting that they participate in positive or negative feedback loops.

Germline MPK-1 substrates include many different types of proteins (Table 2). MPK-1 substrates relevant to pachytene progression include several RNA helicases, the kinase CDTL-7 and the Rho GAP PAC-1 (Arur et al., 2009). MPK-1 substrates relevant to pachytene cell membrane organization include the translational regulator NOS-3 (related to *Drosophila* Nanos) (Arur et al., 2011), the DEAD-box helicase DDX-19 (Arur et al., 2009), and the transcription factor DPL-1/DP (Lin and Reinke, 2008). DDX-19 also affects oocyte differentiation (Arur et al., 2009). One of several MPK-1 substrates relevant to oocyte growth control is the kinase GSK-3 (Arur et al., 2009). A potential substrate relevant to DNA-damage induced germline apoptosis is the p53 ortholog CEP-1 (Rutkowski et al., 2011).

14. Interactions between the RTK-Ras-ERK pathway and other signaling pathways

The RTK-Ras-ERK pathway often interacts with other signaling pathways to control downstream responses.

Ras-Wnt cooperation: The LET-23/EGFR-Ras-ERK pathway cooperates with a Wnt pathway (see Wnt signaling) to specify vulval fates (Eisenmann et al., 1998; Gleason et al., 2002), P12 fates (Jiang and Sternberg, 1998), and male hook group (Yu et al., 2009) and spicule (Seah and Sternberg, 2009) fates (Figure 8A). The relative importance of Ras vs. Wnt signaling varies among these different tissues—for example, Ras is more important in the vulva and Wnt is more important in the male hook group—but hyperactivation of the “minor” pathway can often bypass the requirement for the “major” pathway, suggesting that both pathways converge on common targets (Gleason et al., 2002; Yu et al., 2009). Some of these common targets may include Hox genes. Interestingly, the relative importance of Ras vs. Wnt signaling also varies between different nematode species - in *Pristionchus pacificus*, vulval development depends primarily on Wnt signaling (Tian et al., 2008).

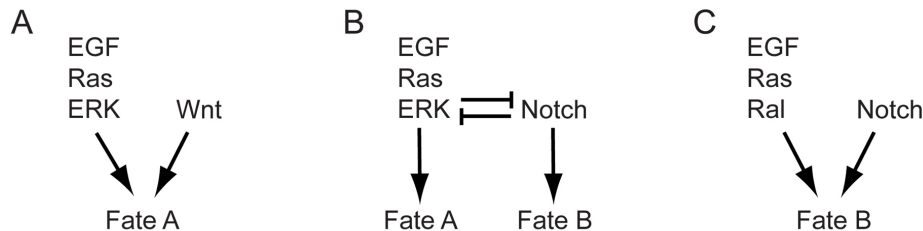


Figure 8. Interactions between EGF-Ras-ERK and other signaling pathways. A) EGF-Ras-ERK and Wnt signaling cooperate to promote several cell fates. B) EGF-Ras-ERK and Notch signaling function antagonistically to promote distinct cell fates. C) The alternative EGF-Ras-Ral pathway and Notch cooperate to promote the secondary vulval fate. See text for details and references.

Ras-Notch sequential signaling: The LET-23/EGFR-Ras-ERK pathway acts sequentially with a Notch pathway (see LIN-12/Notch signaling in *C. elegans*) to promote different cell fates during development of the vulva (see Vulval development), uterus (Chang et al., 1999), male hook (Yu et al., 2009) and excretory system (Abdus-Saboor et al., 2011). In the uterus, LIN-12/Notch signaling induces the fate of a progenitor cell (the π cell)

Newman et al., 1995) whose descendants later require Ras signaling to adopt their final fate (uv1) (Chang et al., 1999). In several cases, signaling by one pathway controls the fate of a cell type that expresses a ligand for the other pathway. For example, Ras signaling induces the primary vulval cell type, which then expresses Notch pathway ligands to induce the secondary cell fate (Simske and Kim, 1995; Chen and Greenwald, 2004). LIN-12/Notch signaling inhibits generation of the anchor cell (Greenwald et al., 1983) and LIN-12 and GLP-1/Notch signaling promotes generation of the excretory canal cell (Lambie and Kimble, 1991; Moskowitz and Rothman, 1996), both of which express LIN-3/EGF to induce other cell fates (Hill and Sternberg, 1992; Abdus-Saboor et al., 2011). In these various cases, sequential signaling allows for the properly patterned generation of multiple cell types that make up a functional organ.

Ras-Notch antagonism: When activated within the same cell, LET-23/EGFR-Ras-ERK signaling and Notch signaling tend to function antagonistically (Figure 8B). In primary vulval cells, LET-23/EGFR-Ras-ERK signaling antagonizes LIN-12/Notch signaling by triggering LIN-12 endocytosis (Shaye and Greenwald, 2002). In secondary vulval cells, LIN-12/Notch signaling antagonizes Ras-ERK signaling by stimulating the transcription of various negative regulators such as *lip-1* and *lst-1-4* (Berset et al., 2001; Yoo et al., 2004). LIN-12/Notch-dependent inhibition of Ras-ERK signaling may allow the alternative Ras-RalGDS pathway to become active, and Notch and Ras-RalGDS can then cooperate to promote the secondary vulval fate (Zand et al., 2011) (Figure 8C).

Coordination of related developmental events: In addition to the Ras-Notch sequential signaling events described above, there are several additional examples where different RTK- or Ras-dependent pathways act together to coordinate related developmental events. For example, after LET-23/EGFR-Ras-ERK-dependent specification of the 1° vulval cell fate, 1° vulval descendants then express EGL-17/FGF and LIN-3/EGF to coordinate sex muscle positioning and uterine development with vulval development (Burdine et al., 1998; Chang et al., 1999). ERK signaling in oocytes and LET-23-PLC γ -IP₃ signaling in somatic gonadal cells act together to coordinate oocyte maturation and ovulation (Kim et al., 2012).

15. Summary and relevance to human health

Studies in *C. elegans* have been instrumental in elucidating the basic framework of the RTK-Ras-ERK pathway and many aspects of its regulation and cross-talk with other pathways. It is now clear that each step of the RTK-Ras-ERK pathway is subject to multiple regulatory mechanisms that facilitate or dampen signaling. Many of these mechanisms appear redundant under standard laboratory conditions, such that loss of a single regulatory gene has few phenotypic consequences, and some vary in importance between tissue types. There is also significant redundancy and tissue-specificity among downstream ERK targets. Although much has been learned, many questions remain about how regulatory mechanisms actually work and how use of different ERK targets ultimately leads to the many different and interesting biological outcomes observed.

Importantly, many of the regulatory mechanisms and targets described thus far in *C. elegans* are widely conserved in other organisms, including humans (Tables 1, 2). Therefore, these represent possible points of intervention in the treatment of diseases that involve aberrant RTK-Ras-ERK signaling. For example, given that hyperactivation of RTKs or Ras is a leading cause of many cancers (Malumbres and Barbacid, 2002; Yoon and Seger, 2006; Karnoub and Weinberg, 2008; Lemmon and Schlessinger, 2010; Udell et al., 2011), drugs targeting positive regulators of the signaling pathway could be one promising approach for cancer therapy. Indeed, antisense oligonucleotides targeting KSR1 have been effective in treating mouse models of pancreatic cancer, effectively suppressing Ras-dependent tumorigenesis with minimal toxicity to wild-type cells (Xing et al., 2003). There are still many challenges to developing clinically-effective drugs, but studies in *C. elegans* have contributed to a long list of potentially useful targets.

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