RTK/Ras/MAPK signaling*

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Abstract

Receptor Tyrosine Kinase (RTK)/Ras GTPase/MAP kinase (MAPK) signaling pathways are used repeatedly during metazoan development to control many different biological processes. In the nematode *Caenorhabditis elegans*, two different RTKs (LET-23/EGFR and EGL-15/FGFR) are known to stimulate LET-60/Ras and a MAPK cascade consisting of the kinases LIN-45/Raf, MEK-2/MEK and MPK-1/ERK. This Ras/MAPK cascade is required for multiple developmental events, including induction of vulval, uterine, spicule, P12 and excretory duct cell fates, control of sex myoblast migration and axon guidance, and promotion of germline meiosis. Studies in *C. elegans* have provided much insight into the basic framework of this RTK/Ras/MAPK signaling pathway, its regulation, how it elicits cell-type specific responses, and how it interacts with other signaling pathways such as the Wnt and Notch pathways.

1. Introduction

Receptor Tyrosine Kinase (RTK)/Ras GTPase/MAP kinase (MAPK) signaling pathways are used repeatedly during metazoan development to control many different biological processes (Schlessinger, 2000). Mutations

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affecting RTK/Ras/MAPK signaling cause many human syndromes and diseases, including cancer (Malumbres and Barbacid, 2002). Studies in *C. elegans* have provided much insight into the basic framework of this canonical type of Ras pathway, its regulation, how it elicits cell-type specific responses, and how it interacts with other signaling pathways (for recent reviews see Moghal and Sternberg, 2003; Sundaram, 2004; Tan and Kim, 1999; Wang and Sternberg, 2001).

C. elegans Ras is called LET-60 (Han and Sternberg, 1990). LET-60 Ras acts downstream of at least two different RTKs, LET-23 (related to the Epidermal Growth Factor Receptor or EGFR; Aroian et al., 1990) and EGL-15 (related to the Fibroblast Growth Factor Receptor or FGFR; DeVore et al., 1995). The only known role of LET-60 Ras is to stimulate a MAPK cascade consisting of the kinases LIN-45 (Raf; Han et al., 1993), MEK-2 (MEK; Church et al., 1995; Kornfeld et al., 1995; Wu et al., 1995) and MPK-1 (ERK/MAPK; Lackner et al., 1994; Wu and Han, 1994). LET-60 Ras signaling is required for multiple developmental events, the best studied of which is vulval induction (see Vulval development). Genetic screens based on various *let-60* mutant phenotypes have identified many generally-acting "core" components of the Ras pathway as well as numerous regulators or targets of the pathway (Figure 1; Table 1). Indeed, many important Ras pathway genes were first identified in the worm.

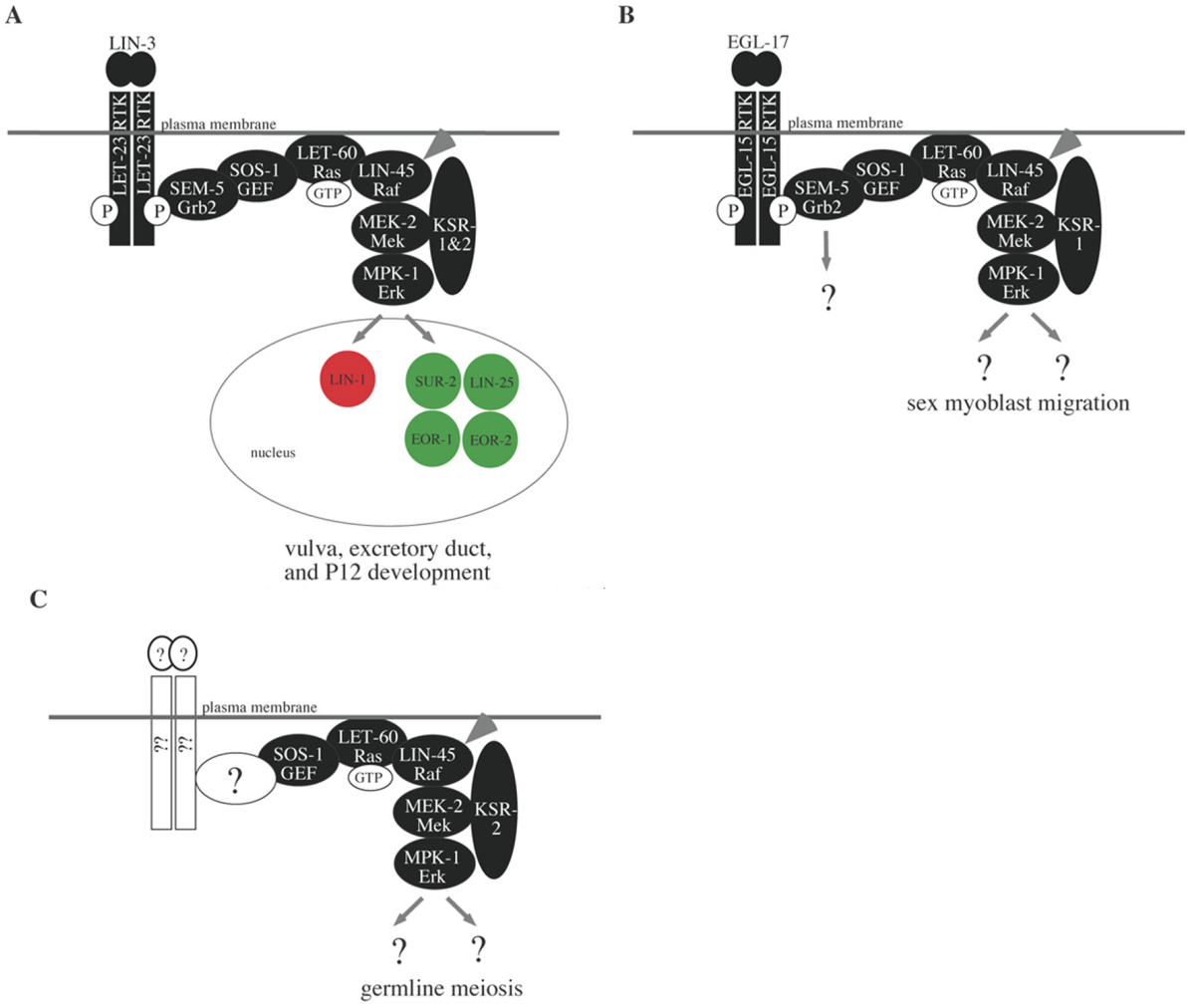

Figure 1. Variations of the RTK/Ras/MAPK signaling pathway controlling different developmental processes in *C. elegans*. The RTKs LET-23 (EGFR) and EGL-15 (FGFR) are activated by different ligands and control different sets of developmental processes. A) LIN-3/LET-23-dependent processes include vulval, excretory duct and P12 development; B) EGL-17/EGL-15-dependent processes include sex myoblast migration; C) Neither LET-23 nor EGL-15 control pachytene progression during germline meiosis. Both RTKs signal through the adaptor SEM-5 (Grb2) to activate the same core Ras/MAPK pathway. EGL-15 and SEM-5 may also activate additional pathways during sex myoblast migration. The scaffold proteins KSR-1 and KSR-2 assist in LIN-45 (Raf) and/or MEK-2 activation; different processes have different requirements for these two KSR proteins. Shown in A) are five nuclear proteins (LIN-1, SUR-2, LIN-25, EOR-1, EOR-2) that are jointly important for vulval, excretory duct and P12 cell fates. Downstream targets of MPK-1 during sex myoblast migration and germline meiosis are not yet known. See text and Table 1 for details and references.

Table 1. Core components, regulators and targets of the *C. elegans* RTK/Ras/MAPK 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	Tyrosine kinase	~WT. Muv in combination with *sli-1*, others.	Hopper et al., 2000
CDF-1	Cation Diffusion Facilitator	ZnT1	Zinc transporter.	~WT. Suppresses *let-60 ras(gf)* Muv.	Bruinsma et al., 2002; Jakubowski and Kornfeld, 1999
CNK-1	Connector/eNhancer of Ksr	CNK1,2,3	Putative Raf-binding adaptor/scaffold.	~WT. Suppresses *let-60* ras(gf) Muv. Enhances Vul and rod-like lethal phenotypes of *lin-45*, others.	Rocheleau et al., 2005
DAB-1	DisABled homolog	DAB2	Adaptor. Required for EGL-17 secretion.	Abnormal sex myoblast positions.	Kamikura and Cooper, 2003
DPY-22/ SOP-1	DumPY/Suppressor Of Pal-1	TRAP230	Mediator subunit.	~WT. Enhances *let-23(gf)* Muv.	Moghal and Sternberg, 2003
DPY-23	DumPY	AP-50	Clathrin adaptor subunit	~WT. Muv in combination with *gap-1*.	Yoo et al., 2004
EGL-5	EGg-Laying defects	Hox9-13	Hox transcription factor. Upregulated by Ras signaling.	P12→P11 fate transformations.	Chisholm, 1991; Jiang and Sternberg, 1998
EGL-18	EGg-Laying defects	GATA4,5,6	GATA transcription factor	Partial Vul. Strong Vul in combination with *elt-6*.	Koh et al., 2002; Koh 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	FGF	Ligand for EGL-15 RTK	Abnormal sex myoblast positions.	Burdine et al., 1997; Stern and Horvitz 1991
EGL-19	Egg-Laying defects	Cav1.2	Alpha subunit of L-type voltage-gated calcium channel	Suppresses *egl-30(gf)* effects on vulva	Moghal and Sternberg, 2003
EGL-30	EGg-Laying defects	Gαq/Gα11	Galphaq subunit of heterotrimeric G protein	gf allele suppresses *let-23* and *let-60(dn)* Vul	Moghal and Sternberg, 2003
ELT-6	Erythroid-Like Transcription factor family	GATA4,5,6	GATA transcription factor	~WT. Vul in combination with *egl-18*.	Koh et al., 2002; Koh et al., 2004
EOR-1	Egl-1 suppressor/diO uptake defective/ Rafenhancer	PLZF	BTB/Zinc finger protein, probable transcriptional regulator	Partial rod-like lethal and P12→P11 fate changes. Strong	Howard and Sundaram, 2002; Rocheleau et al., 2002

Gene product		Mammalian relative(s)	Molecular function	Ras-related phenotypes	Reference(s)
				rod-like lethal in combination with *sur-2*, *lin-25* or *lin-1*.	
EOR-2	Egl-1 suppressor/diO uptake defective/ Rafenhancer	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 *sur-2*, *lin-25* or *lin-1*.	Howard and Sundaram, 2002; Rocheleau et al., 2002
GAP-1	GTPase Activating Protein	GAP-1	Ras GAP	~WT. Suppresses *let-23* Vul. Muv in combination with *lip-1*, others.	Hajnal et al., 1997
GAP-2	GTPase Activating Protein	p120GAP	Ras GAP	~WT. Suppresses *let-23* rod-like lethality.	Hayashizaki et al., 1998
GPA-5	G-Protein, Alpha subunit	GNAZ	Ga	Increased chemotaxis. Suppresses *let-60(dn)* 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 *let-60 ras(gf)* Muv. Rod-like lethal and Vul in combination with *ksr-2*, 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 *ksr-1*.	Ohmachi et al., 2002
LET-23	LEThal	EGFR	Receptor tyrosine kinase	Rod-like larval lethal, Vul, 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	Dominantly enhances *let-60(dn)* 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. Target of MPK-1.	Muv	Beitel et al., 1995; Jacobs et al., 1998
LIN-2	Abnormal cell	CASK	Membrane-associated	Vul	Hoskins et al., 1996;

Gene product		Mammalian relative(s)	Molecular function	Ras-related phenotypes	Reference(s)
	LINeage		Guanylate kinase, required for basal localization of LET-23		Kaech et al., 1998
LIN-3	Abnormal cell LINeage	EGF	Ligand for LET-23 RTK	Rod-like larval lethal, Vul, 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-25	Abnormal cell LINeage	-	Novel nuclear protein, functions with SUR-2	Vul	Nilsson et al., 1998; Nilsson et al., 2000; Tuck and Greenwald, 1995
LIN-31	Abnormal cell LINeage	FoxB2	Winged helix transcription factor. Target of MPK-1.	Mixed Vul and Muv	Miller et al., 1993; Tan et al., 1998
LIN-39	Abnormal cell LINeage	HoxB5	Hox transcription factor. Upregulated by Ras signaling.	Vul	Clark et al., 1993; Maloof and Kenyon, 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. Muv in combination with *gap-1*, others.	Berset et al., 2001
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. Muv in combination with *gap-1*.	Yoo et al., 2004
LST-2	Lateral Signaling Target	Zinc finger FYVE domain containing protein 28	FYVE domain protein	WT. Muv in combination with *gap-1*.	Yoo et al., 2004
LST-3	Lateral Signaling Target	CCAR1	SAF-A/B, Acinus and PIAS domain protein	WT. Muv in combination with *gap-1*.	Yoo et al., 2004
LST-4	Lateral Signaling Target	NM_153271	Sorting nexin, may promote LET-23 degradation	WT. Muv in combination with *gap-1*.	Yoo et al., 2004
MEK-2	Map kinase kinase or Erk Kinase	MEK1, 2	Dual specificity kinase,	Sterile Vul (maternal rescue of	Kornfeld et al., 1995a; Wu et al,

Gene product		Mammalian relative(s)	Molecular function	Ras-related phenotypes	Reference(s)
			phosphorylates ERK	rod-like lethal), etc.	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 *sur-6* 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 *let-60 ras(gf)*. Suppresses *clr-1*.	Gutch et al., 1998; Schutzman et al., 2001
ROM-1	RhOMboid-related	RHBDL2	7-pass transmembrane serine protease	WT. Suppresses *let-60 ras(gf)* Muv.	Dutt et al., 2004
SEM-4	SEx Muscle abnormality	Sal1	C2H2 zinc finger transcription factor	Partial Vul.	Grant et al., 2000
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 *unc-101*, 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 *clr-1* lethality.	Schutzman et al., 2001
SOS-1/ LET-341	Son Of Sevenless/ LEThal	Sos-1	Guanine Nucleotide Exchange Factor	Rod-like larval lethal, Vul, etc.	Chang et al., 2000
SRA-13	Serpentine Receptor, A class	-	G-protein coupled receptor	Increased chemotaxis. Suppresses *let-60(dn)* Vul.	Battu et al., 2003
SUR-2	SUppressor of Ras	Sur-2	Mediator subunit	Vul.	Singh and Han, 1995
SUR-5	SUppressor of Ras	NM_023928	Acetyl coenzyme A synthetase	WT. Suppresses *let-60(dn)* Vul.	Gu et al., 1998
SUR-6	SUppressor of Ras	PPP2R2A	PR55/B Regulatory subunit of Protein Phosphatase 2A	Suppresses *let-60 ras(gf)* Muv. Enhances Vul and rod-like lethal phenotypes of *lin-45* hypomorphs, others.	Kao et al., 2004; Sieburth et al., 1999
SUR-7	SUppressor of Ras	-	Zinc transporter	Suppresses *let-60 ras(gf)* Muv.	Yoder et al., 2004
SUR-8/ SOC-2	SUppressor of Ras, Suppressor Of Clr-1	Sur-8	Ras-binding Leucine-rich repeat protein	Scrawny. Suppresses *let-60 ras(gf)* Muv and *clr-1* lethality.	Selfors et al., 1998; Sieburth et al., 1998

Gene product		Mammalian relative(s)	Molecular function	Ras-related phenotypes	Reference(s)
				Enhances Vul and rod-like lethal phenotypes of *lin-45* hypomorphs, others.	
UNC-101	UNCoordinated	AP-47	Clathrin adaptor subunit	WT. Muv in combination with *sli-1*, others.	Lee et al., 1994

2. Overview of the core RTK/Ras/MAPK signal transduction pathway

A general model for the core *C. elegans* RTK/Ras/MAPK signaling pathway (and various tissue-specific variations) is shown in Figure 1 (Moghal and Sternberg, 2003; Schlessinger, 2000). Upon growth factor binding, an RTK such as LET-23 or EGL-15 dimerizes and autophosphorylates its C-terminal region. The resulting phospho-tyrosine residues serve as docking sites for adaptor proteins such as SEM-5 (Grb2) or SOC-1 (similar to Gab1). These adaptors recruit the Guanine Nucleotide Exchange Factor SOS-1 to activate the small GTPase LET-60 Ras. LET-60-GTP then binds to LIN-45 Raf and promotes its stable association with the plasma membrane and/or endomembranes, where other events then activate LIN-45 kinase activity (Chong et al., 2003). The scaffold protein KSR may assist in LIN-45 activation, but also promotes further signal transmission by bringing together different components of the MAPK cascade (Morrison and Davis, 2003). LIN-45 phosphorylates and activates MEK-2, MEK-2 phosphorylates and activates MPK-1, and MPK-1 then phosphorylates and either activates or inactivates various target proteins. In many cases MPK-1 may move into the nucleus to phosphorylate transcription factors such as the Ets domain protein LIN-1, thus leading to changes in gene expression.

This model draws on a large body of data from multiple systems. *C. elegans* genetics has been most useful for identifying the genes involved in particular signaling events and determining their order of action and cellular focus. In some cases, physical interactions and/or phosphorylation events have been demonstrated for the worm proteins (e.g., Jacobs et al., 1999; Sieburth et al., 1998; Wu et al., 1995), but in many cases such interactions are inferred based on biochemical studies of related proteins in vertebrate cells. Because the core Ras pathway (and much of its regulation) appears highly conserved between *C. elegans* and vertebrates (Table 1), current models draw on the combined data from these different systems.

3. Phenotypes of Ras pathway mutants

Ras signaling is not required for cellular viability in *C. elegans* (Yochem et al., 1997), but it is required for organismal viability and for many different developmental processes. Because of the widespread roles of Ras signaling during development, mutations affecting the Ras pathway can cause many different pleiotropic defects (Figure 2).

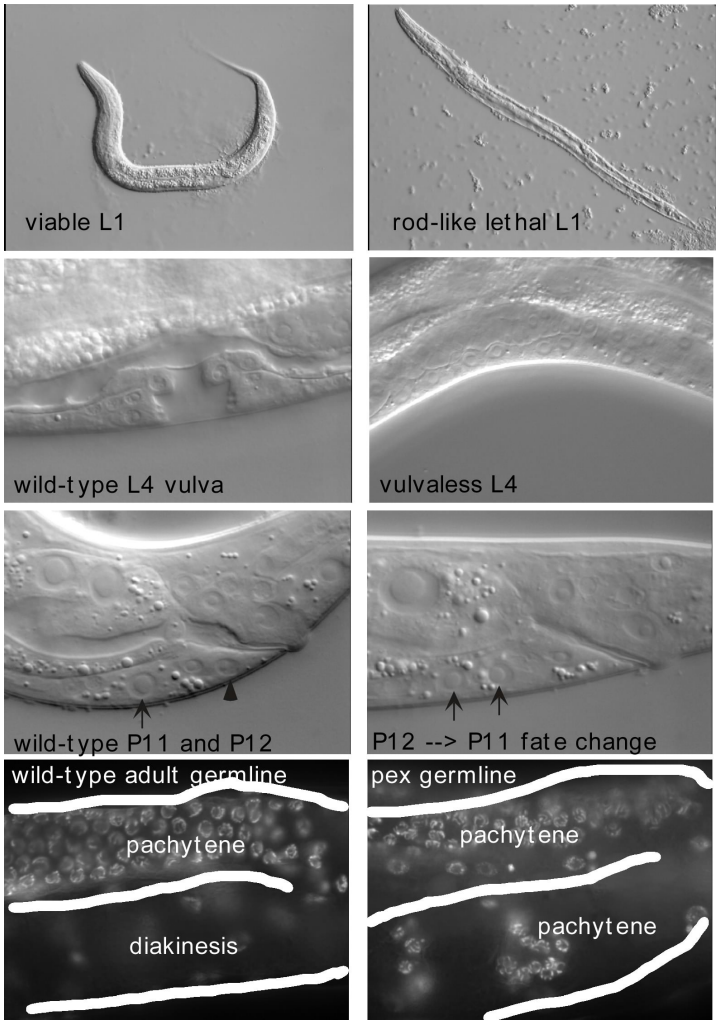

Figure 2. *let-60* ras mutant phenotypes. Wild-type phenotypes are shown on the left, Ras pathway mutant phenotypes on the right. See text for details.

Lethality: Ras signaling promotes the excretory duct cell fate, and mosaic analysis suggested that loss of this one specific cell can account for the zygotic lethality of *let-60* null mutants (Yochem et al., 1997). The excretory duct cell is required for osmoregulation (Nelson and Riddle, 1984). *let-60* loss-of-function mutants, which lack the excretory duct cell, die as “rod-like” larvae with a fluid-filled appearance (Figure 2). *let-60* gain-of-function mutants sometimes have two excretory duct cells (Yochem et al., 1997). Ras signaling may have other (maternally-rescued) essential roles in addition to excretory duct formation, since mutations in *let-23*/EGFR, *egl-15*/FGFR and *ptp-2*/SHP-2 cause distinct “scrawny” and lethal defects that are rescued by constitutive forms of LET-60 Ras (DeVore et al., 1995; Gutch et al., 1998; Koga and Ohshima, 1995).

Hyperactivation of *egl-15*/FGFR or *let-60*/Ras can also lead to lethality with a “Clear”, fluid-filled appearance (Kokel et al., 1998; Schutzman et al., 2001). Mosaic analysis suggests that the Clear phenotype is caused by hypodermal defects (Huang and Stern, 2004).

Vulval defects: Ras signaling cooperates with Notch and Wnt signaling to promote hermaphrodite vulval development (see Vulval development). *let-60* loss-of-function mutants lack a vulva (Vulvaless or Vul phenotype; Figure 2) whereas *let-60* gain-of-function mutants have extra vulval tissue (Multivulva or Muv phenotype).

Uterine defects: Ras signaling promotes the uterine uv1 fate, which is important for establishing a proper vulval-uterine connection (Chang et al., 1999). In the absence of uv1, hermaphrodites cannot lay eggs.

P11 and P12 ectoblast defects: Ras signaling cooperates with Wnt signaling to promote the P12 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; Hermaphrodite cell fate specification). Reduced Ras signaling causes P12-> P11 cell fate transformations (Figure 2), while increased signaling causes P11-> P12 fate transformations.

Male spicule defects: Ras signaling promotes male spicule fates (Chamberlin and Sternberg, 1994; Male development). Reduced Ras signaling causes spicule defects and prevents males from mating.

Sex myoblast migration defects: Ras signaling helps to specify the proper endpoint of sex myoblast migration (Sundaram et al., 1996). In *let-60* loss-of-function mutants, sex myoblasts adopt a broadened range of final positions. This defect can affect egg-laying.

Axon guidance defects: Ras signaling controls the paths of certain ventral cord neurons relative to the ventral midline (Bulow et al., 2004). Whereas in wild-type these neurons extend axons along one side of the midline, in *let-60* loss-of-function mutants the neurons wander across the midline.

Sterility due to pachytene exit (Pex) defects: Ras signaling is required for progression through the pachytene stage of meiosis (Church et al., 1995; Somatic sex determination). *let-60* loss-of-function mutants are sterile because germ cells arrest in pachytene (Figure 2).

Olfaction defects: Ras signaling is required for sensitivity to volatile attractants (Hirotsu et al., 2000). Whereas wild-type *C. elegans* will chemotax toward volatile attractants such as isoamylalcohol and diacetyl, *let-60* loss-of-function mutants fail to chemotax towards such attractants.

Resistance to Microbacterium nematophilum-induced swelling: *ksr-1*, *lin-45*, *mek-2* and *mpk-1* are required for the swelling response to infection by *M. nematophilum* (Nicholas and Hodgkin, 2004). Interestingly, *let-60* does not appear to be important for this response, suggesting that a bacterial toxin may directly activate the Raf/MEK/ERK cascade.

4. Screens used to identify Ras pathway components

The vast majority of studies on RTK/Ras/MAPK signaling have focused on the role of the Ras pathway in promoting vulval development (see Vulval development), and many known components of the pathway have been identified through forward genetic screens for mutants with Vul or Muv mutant phenotypes (Ferguson and Horvitz, 1985). Some components of the pathway have been identified based on other mutant phenotypes such as sex myoblast migration defects (Stern and Horvitz, 1991) or germline meiosis defects (Church et al., 1995; Ohmachi et al., 2002). Some have been identified through reverse genetic approaches (Dutt et al., 2004; Gutch et al., 1998; Kamikura and Cooper, 2003; Yoo et al., 2004). Finally, some core Ras pathway components as well as a large set of other genes that influence Ras signaling have been identified through genetic suppressor or enhancer screens (Sternberg and Han, 1998). Many modifier genes have essentially wild-type null phenotypes (Table 1), indicating a significant and surprising amount of redundancy amongst Ras pathway regulators.

One especially productive modifier screen involved looking for suppressors of the *let-60* gain-of-function Muv phenotype. This screen identified alleles of the downstream kinase genes *lin-45*/Raf (Hsu et al., 2002), *mek-2*/MEK (Kornfeld et al., 1995; Wu et al., 1995) and *mpk-1*/ERK (Lackner et al., 1994; Wu and Han, 1994), revealing that LET-60 Ras signals through the MAPK cascade. This screen also identified numerous positive regulators of this cascade such as *cdf-1* (Jakubowski and Kornfeld, 1999), *ksr-1* (Kornfeld et al., 1995; Sundaram and Han, 1995), *sur-6* (Sieburth et al., 1999), *sur-7* (Yoder et al., 2004), and *sur-8* (Sieburth et al., 1998; see below), as well as a key downstream factor, *sur-2* (Singh and Han, 1995; see below). Another very productive type of screen has been to look for enhancers of lethal or vulval defects in the background of a very mildly affected or "phenotype-less" mutant. For example, screens for enhancers of *lin-45* lethality identified the downstream factors *eor-1* and *eor-2* (Howard and Sundaram, 2002; Rocheleau et al., 2002), while screens for enhancers of the *gap-1* Muv phenotype identified the MAPK phosphatase *lip-1* (Berset et al., 2001). These types of genetic modifier screens have allowed the identification of genes with very subtle null phenotypes.

The power of genetic screening approaches in *C. elegans* is reflected in the large number of conserved genes whose involvement in RTK/Ras/MAPK signaling was first discovered in the worm; these include *ark-1* (Hopper et al., 2000), *cdf-1/ZnT1* (Jakubowski and Kornfeld, 1999), *ksr-1* (Kornfeld et al., 1995; Sundaram and Han, 1995),

lin-2/CASK, *lin-7*and *lin-10*/Mint (Ferguson and Horvitz, 1985), *sem-5*/Grb2 (Clark et al., 1992), *sli-1*/Cbl (Jongeward et al., 1995), *sur-2* (Singh and Han, 1995), *sur-5* (Gu et al., 1998), *sur-7* (Yoder et al., 2004), and *sur-8*/*soc-2* (Selfors et al., 1998; Sieburth et al., 1998).

5. Growth factors and RTKs that signal through Ras/MAPK

The *C. elegans* genome contains twenty-eight predicted RTKs (Popovici et al., 1999), only a few of which have been characterized mutationally (see Genomic overview of protein kinases). Of these characterized RTKs, only the EGF receptor LET-23 and the FGF receptor EGL-15 are known to signal positively through Ras/MAPK (see below). The ephrin receptor VAB-1 negatively regulates MAPK activation during oocyte maturation (Miller et al., 2003). Notably, the insulin-like RTK DAF-2 does not appear to signal through Ras/MAPK (G. Ruvkun, personal communication), but instead signals through a PI3-kinase/Akt pathway (see Signaling in the immune response).

Signaling by the LET-23 and EGL-15 RTKs can account for many but not all LET-60 Ras-dependent developmental events. The EGF-related ligand LIN-3 signals through LET-23/EGFR and LET-60 to control specification of hermaphrodite vulval and uterine fates, male spicule cell fates, the P12 ectoblast cell fate, and most likely the excretory duct cell fate (Aroian and Sternberg, 1991; Chang et al., 1999; Figure 1A). The FGF-related ligand EGL-17 signals through EGL-15/FGFR and LET-60 to control sex myoblast migration (Figure 1B), while the FGF-related ligand LET-756 signals through EGL-15 and LET-60 to promote axon guidance and viability (Bulow et al., 2004; Burdine et al., 1997; DeVore et al., 1995; Goodman et al., 2003; Roubin et al., 1999; Sundaram et al.,1996). In addition, LET-60 acts independently of any known RTK to control olfaction (Hirotsu et al., 2000) and the progression of germline meiosis (Church et al., 1995; Figure 1C). Furthermore, LIN-45, MEK-2 and MPK-1 act independently of known RTKs or LET-60 to mediate responses to *Microbacterium nematophilum* infection (Nicholas and Hodgkin, 2004).

In some cases the LET-23 and EGL-15 RTKs can also act through LET-60 Ras-independent pathways. LIN-3 and LET-23 control oocyte maturation via PLC gamma and an inositol polyphosphate signaling pathway (Bui and Sternberg, 2002; Clandinin et al., 1998; Yin et al., 2004). EGL-17 and EGL-15 may act through additional, unknown pathways to control sex myoblast migration and osmoregulation (DeVore et al., 1995; Schutzman et al., 2001; Sundaram et al., 1996).

6. Regulators of LIN-3/EGF and EGL-17/FGF

Reverse genetic approaches have identified several factors important for ligand processing and secretion. The ligand LIN-3 exists in both trans-membrane (locally-acting) and diffusible forms (Hill and Sternberg, 1992; Thomas et al., 1990); generation of the diffusible form in vulval cells appears to require cleavage by the Rhomboid ortholog ROM-1 (Dutt et al., 2004). The ligand 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).

7. Regulators and targets of LET-23/EGFR and EGL-15/FGFR

Factors that regulate LET-23 RTK trafficking and localization are important modulators of signaling strength. A complex consisting of three PDZ-domain proteins, LIN-2, LIN-7 and LIN-10, positively regulates signaling by localizing LET-23 to the basolateral membrane of vulval precursor cells, adjacent to the source of LIN-3 ligand (Kaech et al., 1998; Simske et al., 1996). The E3 ubiquitin ligase SLI-1 (Cbl; Jongeward et al., 1995; Yoon et al., 1995), the clathrin adaptors UNC-101 (Lee et al., 1994) and DPY-23 (Yoo et al., 2004), and the sorting nexin LST-4 (Yoo et al., 2004) all negatively regulate signaling, probably by promoting LET-23 endocytosis and/or degradation.

RTKs transduce signals by autophosphorylation and subsequent binding to phospho-tyrosine binding adaptor proteins (Schlessinger, 2000). LET-23 seems to act primarily through the adaptor SEM-5 (Clark et al., 1992), while EGL-15 acts through both SEM-5 and the adaptor SOC-1 (Schutzman et al., 2001). Factors that regulate RTK phosphorylation status could obviously modulate signaling strength. For example, the receptor tyrosine phosphatase CLR-1 negatively regulates EGL-15 RTK activity, most likely through direct dephosphorylation of key tyrosine residues (Kokel et al., 1998). On the other hand, the cytosolic tyrosine phosphatase PTP-2 (Shp-2) positively regulates LET-23 and EGL-15 RTK signaling (Gutch et al., 1998; Schutzman et al., 2001), and the tyrosine kinase ARK-1 (Ack-related) negatively regulates LET-23 RTK signaling (Hopper et al., 2000). The substrates and mechanisms of action 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.

8. Regulators of Ras activity

As is the case for other small GTPases (see 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 (Figure 3). The GEF SOS-1 appears necessary for most Ras-mediated developmental events (Chang et al., 2000). The GAPs GAP-1 and GAP-2 negatively regulate Ras signaling during vulval development and excretory duct development, respectively (Hajnal et al., 1997; Hayashizaki et al., 1998). Another negative regulator of Ras is SUR-5, a protein of unknown function that resembles acetyl coenzyme A synthetases (Gu et al., 1998).

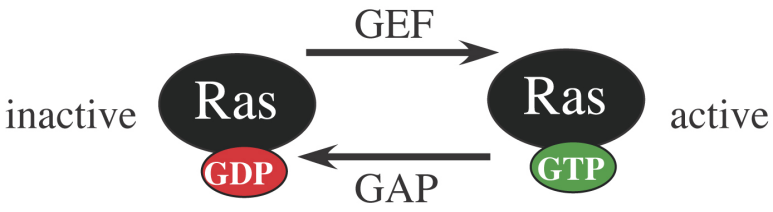

Figure 3. Regulation of Ras proteins. Ras GTPase regulation has been extensively studied in other systems (Malumbres and Barbacid, 2002). Ras-GDP is inactive, whereas Ras-GTP is active and can bind to effectors such as Raf. Guanine nucleotide exchange factors (GEFs) such as SOS-1 positively regulate Ras by promoting GDP dissociation. GTPase activating proteins (GAPs) negatively regulate Ras by stimulating Ras' intrinsic GTP hydrolyzing activity. 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 (Beitel et al., 1990; 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).

9. Regulators of the Raf/MEK/ERK kinase cascade

Genetic modifier screens have identified a large set of genes that promote or inhibit signaling through the MAPK cascade, but that are not individually required for normal development in most tissues. In general, the roles of these genes can only be detected as suppressor or enhancer effects in appropriate double mutant combinations. Raf/MEK/ERK signaling is apparently subject to many different levels of regulation that individually have modest effects on signaling strength.

Positively-acting gene products in this category include so-called "scaffold" proteins - CNK-1 (Rocheleau et al., 2005), KSR-1 (Kornfeld et al., 1995; Sundaram and Han, 1995), KSR-2 (Ohmachi et al., 2002), and SUR-8/SOC-2 (Selfors et al., 1998; Sieburth et al., 1998) - that bind to one or more core components of the signaling cascade and are thought to bring different components together at the right place and time (Morrison and Davis, 2003). KSR-1 and KSR-2 are individually required for some developmental processes, but redundantly required for many others (Ohmachi et al., 2002; Figure 1). Also in this category is SUR-6, a PR55/B-type regulatory subunit of Protein Phosphatase 2A (Sieburth et al., 1999), which has been suggested to dephosphorylate and activate both Raf and KSR (Kao et al., 2003; Ory et al., 2003). Finally, the zinc transporter proteins CDF-1 and SUR-7 also positively regulate signaling, suggesting that high levels of intracellular zinc inhibit Ras signaling (Bruinsma et al., 2002; Yoder et al., 2004).

Negatively acting gene products in this category include the G-protein coupled receptor SRA-13 and its Gα target GPA-5, which may modulate Ras signaling in response to environmental conditions such as food availability (Battu et al., 2003). Also in this category are the kinase PAR-1 (which is thought to modulate KSR localization; Kao et al., 2003; Muller et al., 2001; Yoder et al., 2004), the MPK-1-binding protein LST-1 (Yoo et al., 2004), and the MAP kinase phosphatase LIP-1 (Berset et al., 2001).

It is important to note that some regulatory proteins may influence Raf/MEK/ERK signaling indirectly, by affecting neighboring cells. For example, the Gαq protein EGL-30 and the voltage-gated calcium channel EGL-19 promote vulval induction, but function in neurons and muscle, respectively (Moghal et al., 2003). Similarly, SRA-13 and GPA-5 influence vulval induction but it is unknown whether these genes function in vulval cells or in neurons (Battu et al., 2003). Also, the zinc transporter CDF-1 can influence vulval induction when expressed in either the vulva or the intestine (Bruinsma et al., 2002). How tissues such as the intestine, neurons and muscle can influence Raf/MEK/ERK activity in vulval cells is still unclear.

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

No single downstream target of MPK-1 can account for all of the effects of RTK/Ras/MAPK signaling. Rather, different tissues seem to require different subsets of potential targets, and the availability of certain targets may control tissue-specific responses.

One widely important MPK-1 target is the Ets domain transcription factor LIN-1 (Beitel et al., 1995; Figure 1A). LIN-1 antagonizes Ras signaling and appears to be negatively regulated by MPK-1 phosphorylation (Jacobs et al., 1998). However, some evidence suggests an additional positive role in Ras signaling (Howard and Sundaram, 2002). Like other Ets domain factors (Yordy and Muise-Helmericks, 2000), LIN-1 may have both transcriptional repressor and transcriptional activator functions.

Four widely important positive factors are the Mediator subunit SUR-2, the BTB/Zinc finger protein EOR-1, and the novel nuclear proteins LIN-25 and EOR-2 (Howard and Sundaram, 2002; Singh and Han, 1995; Tuck and Greenwald, 1995; Figure 1A). None of these proteins are known to be direct targets of MPK-1, but their functions are important for downstream cellular responses. SUR-2 is a conserved component of the Mediator complex, which links certain sequence-specific DNA binding proteins (such as Ets proteins) to the general RNA Polymer*as*e II transcriptional machinery (Boyer et al., 1999; Stevens et al., 2002). SUR-2 and LIN-25 appear to function together, and they have strong effects on vulval development and weaker effects on excretory duct and P12 development (Nilsson et al., 1998; Nilsson et al., 2000; Singh and Han, 1995; Tuck and Greenwald, 1995). EOR-1 is related to known transcriptional activators and repressors (Barna et al., 2002; Collins et al., 2001). EOR-1 and EOR-2 appear to function together, and they have moderate effects on excretory duct and P12 development and weaker effects on vulval development (Howard and Sundaram, 2002). EOR-1 and EOR-2 appear to act redundantly with SUR-2 and LIN-25 (Howard and Sundaram, 2002).

Several transcription factors are required for cell-type specific responses to RTK/Ras/MAPK signaling, and are candidate MPK-1 targets. These include the forkhead transcription factor LIN-31 (Miller et al., 1993; Tan et al., 1998), which both promotes and inhibits vulval development, the Hox protein LIN-39 (Clark et al., 1993; Eisenmann et al., 1998; Maloof and Kenyon, 1998), the zinc-finger protein SEM-4 (Grant et al., 2000) and the GATA factors EGL-18 and ELT-6 (Koh et al., 2004; Koh et al., 2002), which promote vulval development, and the Hox protein EGL-5 (Chisholm, 1991; Jiang and Sternberg, 1998), which promotes P12 development. Ectopic expression of LIN-31 or LIN-39 can cause other tissues to adopt vulval-like characteristics in response to RTK/Ras/MAPK signaling, suggesting that the presence of these distinct transcription factors may be a key determinant of tissue-specific responses to Ras signaling (Maloof and Kenyon, 1998; Tan et al., 1998).

Not all targets of MPK-1 need be transcription factors. For example, none of the widely important transcriptional regulators seem to be involved in controlling germline meiosis or sex myoblast migration (Church et al., 1995; Sundaram et al., 1996; R. Howard and M. Sundaram, unpublished observations; Figure 1B and C). It is likely that MPK-1 targets in these tissues include factors more directly involved in meiosis and motility.

11. Interactions between the RTK/Ras/MAPK pathway and other signaling pathways

The RTK/Ras/MAPK pathway often interacts with other signaling pathways to control cell fates. For example, the Ras pathway cooperates with a Wnt pathway (see Wnt signaling) to specify P12 fates and vulval fates, possibly by convergent upregulation of common targets such as Hox genes (Eisenmann et al., 1998; Gleason et al., 2002). During vulval development (see Vulval development), the Ras pathway also acts sequentially with a Notch pathway (see LIN-12/Notch signaling in *C. elegans*) to induce the proper pattern of vulval fates (Simske and Kim, 1995; Sundaram, 2004). Ras signaling affects Notch signaling in at least two ways. First, Ras stimulates LIN-12/Notch endocytosis to downregulate Notch signaling in the same cell (Shaye and Greenwald, 2002). Second, Ras stimulates the transcription of Notch ligand genes to upregulate Notch signaling in adjacent cells (Chen and Greenwald, 2004). Notch signaling also antagonizes Ras 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).

RTK/Ras/MAPK signaling in both P12 and the vulva is antagonized by LIN-35 (Rb), EFL-1 (E2F) and other “Synthetic Multivulva” (SynMuv) gene products (Ceol and Horvitz, 2001; Fay and Han, 2000; Jiang and Sternberg, 1998; Lu and Horvitz, 1998). There are three classes of SynMuv genes, A, B and C, that function redundantly (Ceol and Horvitz, 2004; Ferguson and Horvitz, 1989). Many of these genes encode nuclear proteins with apparent roles in

chromatin remodeling and transcriptional regulation (Fay and Han, 2000). Some SynMuv genes seem to function cell autonomously and could antagonize Ras signaling at the level of downstream transcriptional output (Thomas and Horvitz, 1999). However, other SynMuv genes (including *lin-35*/Rb) appear to function cell non-autonomously in the syncytial hypodermis hyp7, suggesting that hyp7-derived signals can influence LET-23 RTK activity (Herman and Hedgecock, 1990; Myers and Greenwald, 2005).

12. Conclusions and future prospects

Although the basic framework of the RTK/Ras/MAPK pathway is well characterized, the pathway is subject to complex regulation that we are only just beginning to understand. A most surprising finding in *C. elegans* has been the large number of regulatory proteins that have only modest individual effects on signaling. Furthermore, some regulatory proteins (such as LIN-2/LIN-7/LIN-10) have very cell-type specific effects. Downstream targets of MPK-1 also appear to be cell-type specific, and likely control how a particular cell responds to the same basic signaling pathway. Although vulval development has been and will continue to be a powerful model for studying RTK/Ras/MAPK signaling, ongoing studies of other Ras-mediated developmental processes will surely reveal new and different types of regulatory mechanisms and targets.

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