Down-regulation of Drosophila Egf-r mRNA levels following hyperactivated receptor signaling

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SUMMARY

Internalization of ligand-receptor complexes is a well-documented mechanism for limiting the duration and magnitude of a signaling event. In the case of the EGF-Receptor (EGF-R), exposure to EGF or TGF-α results in internalization of up to 95% of the surface receptor pool within 5 minutes of exposure to ligand. In this report, we show that levels of Drosophila Egf-r mRNA are strongly down-regulated in epidermal cells likely to have recently undergone high levels of EGF-R signaling. The cells in which Egf-r mRNA levels are down-regulated express the rhomboid gene, which is thought to locally amplify EGF-R signaling. Widespread Egf-r mRNA down-regulation can be induced by ubiquitous expression of rhomboid or by eliminating the Gap1 gene. These results suggest that cells engaged in intense EGF-R/RAS signaling limit the duration of the signal through a combination of short-acting negative feedback mechanisms such as receptor internalization followed by a longer lasting reduction in receptor transcript levels. Control of Egf-r mRNA levels by altering transcription or mRNA stability is a new tier of regulation to be considered in analysis of EGF-R signaling during development.

Key words: EGF-R, down-regulation, mRNA stability, transcription, negative feedback, rhomboid, Drosophila

INTRODUCTION

Loss-of-function mutations in a small group of Drosophila genes known as the ventrolateral or spitz group genes (including rhomboid, spitz and Star) result in remarkably similar embryonic phenotypes suggesting that these genes function in a common pathway (Mayer and Nüsslein-Volhard, 1988; Bier et al., 1990; Rutledge et al., 1992; Kim and Crews, 1993). Defects in ventrolateral group mutants are complex, but primarily affect the formation or differentiation of epidermal structures. A variety of evidence suggests that the ventrolateral group genes are required for hyperactivation of EGF-R signaling. The most direct support for this view is that embryos carrying moderate alleles of a gene encoding a Drosophila homolog of the vertebrate EGF-R family (Egf-r) have similar phenotypes to ventrolateral group mutants (Clifford and Schüpbach, 1992; Raz and Shilo, 1992, 1993; J. O’Neill and E. Bier, unpublished data). Further evidence implicating the ventrolateral group in EGF-R signaling is that the spitz (spi) gene encodes an EGF/TGF-α-like predicted protein (Rutledge et al., 1992). Finally, genetic interactions between rho and genes encoding components of the EGF-R/RAS signaling pathway, as well as between rho and other genes of the ventrolateral group, support models in which rho amplifies EGF-R signaling during wing development (Sturtevant et al., 1993; Noll et al., unpublished data). Similar genetic interactions suggest that Egf-r works in concert with ventrolateral group genes during embryogenesis (Raz and Shilo, 1993).

The genes encoding the EGF-Receptor and putative Spi ligand are expressed broadly throughout development (Kammermeyer and Wadsworth, 1987; Zak et al., 1990; Katzen et al., 1991; Zak and Shilo, 1992; Rutledge et al., 1992), while expression of the rhomboid (rho) gene is highly localized and predominantly limited to cells requiring the activity of the ventrolateral pathway to differentiate (Bier et al., 1990; Sturtevant et al., 1993). Localized expression of rho is important for restricting hyperactivation of EGF-R signaling to appropriate cells since ectopic expression of rho leads to overproduction of cells that normally depend on the activity of the ventrolateral group genes (Sturtevant et al., 1993; Ruohola-Baker et al., 1993; J. W. O’Neill and E. Bier, unpublished data). For example, in the case of wing development, localized expression of rho is required for development of the normal pattern of veins. Flies homozygous for the viable rho* promoter mutation specifically lack expression of rho in wing vein primordia and as a consequence have truncated wing veins (Sturtevant et al., 1993). Conversely, ubiquitous expression of rho at any stage of vein development leads to the production of extra veins (Sturtevant et al., 1993; M. A. Sturtevant and E. Bier, unpublished data). Similar requirements for localized expression of rho have been observed during oogenesis (Ruohola-Baker et al., 1993) and during embryogenesis (J. W. O’Neill and E. Bier, unpublished data). These data support models in which localized expression of rho leads to spatially and temporally regulated hyperactivation of EGF-R signaling during development.
In this report, we show that, while the Egf-r gene is ubiquitously expressed during much of embryogenesis and larval imaginal disc development, Egf-r mRNA levels are down-regulated in cells expressing rho. We also show that down-regulation of Egf-r transcripts is dependent on rho function during wing development and that ubiquitous expression of rho leads to ectopic down-regulation of Egf-r mRNA levels in ectodermal cells.

MATERIALS AND METHODS

Fly stocks

All genetic markers and chromosome balancers used are described in Lindsay and Grell (1968) and Lindsay and Zimm (1992). Fly stocks were obtained from the Bloomington, Indiana Drosophila Stock Center and the Bowling Green, Ohio Stock Center.

Mounting fly wings

Wings from adult flies were dissected in isopropanol and mounted in Canadian Balsam mounting medium (Gary’s Magic Mount) following the protocol of Lawrence et al. (in Roberts, 1986). Mounted wings were photographed under Nomarski optics with a 4× lens on a compound microscope. Alternatively, whole flies or portions of flies were photographed through a dissection microscope.

In situ hybridization to whole-mount embryos or discs

In situ hybridization to whole-mount discs and embryos was performed using digoxigenin-labeled RNA probes (Boehringer-Mannheim, 1093 657) based on the method of Tautz and Pfeiffle (1989) and modified according to Sturtevant et al. (1993) using 4 μg/ml Proteinase K instead of 40 μg/ml as required for digoxigenin-labeled DNA probes.

Antibody labeling

Antibody labeling was performed according to Bier et al. (1990). The anti-Rho antiserum was raised in rabbits against a 30 amino acid amino terminal peptide of the predicted Rho protein conjugated through a carboxyl terminal cysteine to KLH using the Pierce sulfide linkage kit. Anti-EGF-R antibody was kindly provided by Dr B. Shilo (Weizmann Institute).

Double labeling of embryos or discs

Embryos were fixed for 15 minutes in 4% formaldehyde in PBS and discs were fixed for 30 minutes in 0.1 M Pipes (pH 6.9), 2 mM MgSO4, 1 mM EGTA, 1% Triton X-100 and 4% formaldehyde. After washing 5× 10 minutes in an incubation buffer containing 50 mM Tris (pH 6.8), 150 mM NaCl and 0.5% NP-40, the tissues were then blocked for 2 hours at 4°C in incubation buffer with 5 mg/ml BSA. Subsequent incubations and washes for antibody staining were done in the incubation buffer with 1 mg/ml BSA. Anti-Rho antibody (1:1,000), which was preabsorbed against fixed embryos, was incubated with preparations overnight at 4°C. After 5× 10 minutes washes, biotinylated goat anti-rabbit secondary antibodies (Vector) were added at 1:200 for 1 hour and then washed 5× for 10 minutes in PBT. At this point, the antibody-staining protocol was suspended and discs were refixed in PBS with 50 mM EGTA and 4% formaldehyde overnight to insure that the biotinylated secondary antibody remained in place during the subsequent whole-mount in situ protocol. The preparations were then washed and processed according to the standard whole-mount in situ protocol. Following development of the alkaline phosphatase reaction from the whole-mount in situ protocol, we resumed the antibody staining protocol at the Avidin-Biotinylated-HRP addition step and proceeded with the Vectastain ABC elite kit according to instructions.

Other molecular techniques

RNA probe synthesis was performed according to Boehringer-Mannheim protocols and other cloning techniques followed standard procedures, as in Maniatis et al. (1982).

RESULTS

The pattern of Egf-r expression during embryogenesis is complex and often nearly uniform in the epidermis (Kammermeyer and Wadsworth, 1987; Zak et al., 1990; Katzen et al., 1991; Zak and Shilo, 1992; Rutledge et al., 1992; Sturtevant et al., unpublished data); however, there are stages when Egf-r expression is spatially modulated. The regions of lower Egf-r expression are remarkably similar to the pattern of rho expression. rho is expressed in a complex pattern during embryogenesis including a ventrolateral strip of cells at the cellular blastoderm, the mesectoderm during germ band extension, and lateral stretch receptor organ precursor cells and tracheal pits at full germ band extension (Bier et al., 1990). Because of the intimate link between rho and Egf-r signaling, the rho expression pattern serves as a marker for hyperactivated EGF-R signaling. Therefore, the pattern of Egf-r down-regulation largely recapitulates the pattern of intense EGF-R signaling but with a slight temporal delay.

To pursue the possible connection between amplified EGF-R signaling and subsequent Egf-r down-regulation, we closely examined the relation of Rho protein and Egf-r mRNA expression during various stages of embryogenesis in double-labeling experiments (Fig. 1). The repeating theme from this double-label analysis is that Egf-r and Rho expression domains initially overlap (consistent with the hypothesis that cells expressing Rho undergo high levels of EGF-R signaling) and then with a short delay, Egf-r mRNA levels are strongly down-regulated. For example, during formation of the cellular blastoderm, Egf-r is initially co-expressed with Rho in a ventrolateral strip of cells (Fig. 1A; arrow). Egf-r expression is even somewhat elevated in these ventrolateral cells. 15 minutes later, Egf-r transcripts begin to fade from the most ventral territory of Rho expression (Fig. 1B; arrow). Within another 15 minutes, Egf-r mRNA levels are extremely reduced in all ventrolateral cells expressing Rho (Fig. 1C; arrow), leading to a virtually complementary pattern of Egf-r and Rho expression.

Down-regulation of Egf-r mRNA in ventrolateral cells persists for more than an hour after rho mRNA (Bier et al., 1990) and Rho protein have become undetectable in these cells (Fig. 1D; arrows). Later, during germ band extension Egf-r and Rho are co-expressed in tracheal pit precursor cells (Fig. 1E; bracket). At this higher magnification, rho-expressing cells can be unambiguously identified by the presence of dark punctate plaques of Rho (Fig. 1E,G,H). Within 30 minutes Egf-r transcripts virtually disappear from Rho-expressing cells (Fig. 1F,G; brackets) and from single isolated lateral stretch receptor organ precursor cells (Fig. 1G; arrow). Similarly, following the reappearance of Egf-r mRNA in all ventral cells during germ band retraction, it is down-regulated in midline cells expressing Rho so that by early germ band retraction the pattern of Egf-r mRNA is essentially complementary to Rho (Fig. 1H).

We have also compared expression of rho and Egf-r during wing vein development. In the early pupal wing disc, Egf-r
Fig. 1. Rho protein expression and Egfr mRNA down-regulation during embryogenesis. Rho protein (brown punctate staining) was detected with a rabbit anti-Rho antibody (J.W. O’Neill and E. Bier, unpublished data) and visualized with a peroxidase reaction. Egfr mRNA (blue staining) was detected by in situ hybridization with a digoxigenin-labeled Egfr anti-sense RNA probe and visualized with an alkaline phosphatase reaction. The Egfr probe was synthesized to a full-length cDNA clone (kindly provided by B. Shilo) and therefore detects the expression of all Egfr transcripts. (A) Egfr and Rho expression overlap during the early cellular blastoderm (arrow). (B) Within 15-20 minutes Egfr expression begins to fade from Rho-expressing cells (arrow). (C) Approximately 15 minutes later the patterns of Egfr and Rho (arrow) are mutually exclusive. (D) Egfr down-regulation in the ventral ectoderm persists during germband extension. By this stage, Rho expression is confined to the midline (arrowhead), but Egfr expression is still excluded from ventral ectodermal cells which expressed Rho during the blastoderm stage (arrows). (E) When Rho is first detected in tracheal pit precursor cells (punctate plaques above bracket), Egfr expression is fairly uniform in the lateral ectoderm. High magnification view of three segments. (F) As the tracheal pits (bracket) exhibit the first signs of invagination, Egfr transcripts fade from these cells. (G) Double-labeled Egfr mRNA and Rho protein patterns at the same stage as in panel F. High magnification view of three segments showing essentially complementary Egfr mRNA and Rho protein. Expression of Egfr also appears substantially reduced in the Rho-expressing stretch receptor organ precursor cell (arrow). Note the punctate plaques of Rho. (H) The pattern of Egfr mRNA-(arrow) and Rho-(arrowhead) expressing cells alternate along the midline of early germband retracted embryos. Again, note the punctate plaques of Rho.
Fig. 2. *Egf-r* down-regulation during wing development.

(A) *Egf-r* mRNA expression 2-4 hours AP in early everting prepupal wing discs.
(B) *rho* expression 2-4 hours AP. Labels are as follows: M, future wing margin; L1-L5, longitudinal vein primordia.
(C) *Egf-r* mRNA expression 6-8 hours AP. Dorsal and ventral surfaces of the wing have come together and *Egf-r* down-regulation is first apparent. (D) Double label of *Egf-r* and *rho* expression 6-8 hours AP. Note the slight gap between *Egf-r* and *rho*-expressing cells. Intersections of longitudinal veins L2-L5 with the margin are indicated.
(E) *Egf-r* expression 18 hours AP. (F) Double label of *Egf-r* and *rho* mRNA expression 18 hours AP. There is a slight gap between *Egf-r* and *rho*-expressing cells. The intersection of L4 with the margin is marked with an asterisk. Inset: higher magnification view of L4 vein.
(G) EGF-R protein expression at about 25 hours AP. Close examination reveals that EGF-R expression is not totally absent from these cells. A reproducible feature of *Egf-r* RNA or protein expression at this stage is that cells expressing elevated levels of EGF-R immediately flank the strip of low expressing cells (arrow). The intersection of L4 with the margin is marked with an asterisk. Inset: higher magnification view of L4 vein.
(H) Double label of *Egf-r* and *rho* mRNA expression at about 25 hours AP. A slight gap is still present between EGF-R and *rho*-expressing cells. (I) *Egf-r* mRNA expression at about 30 hours AP. (J) Double label of *Egf-r* and *rho* mRNA expression at about 30 hours AP. The two patterns of staining are entirely complementary by this stage as judged by the absence of unstained cells. The intersection of L4 with the margin is marked with an asterisk. Inset: higher magnification view of L4 vein.
expression is virtually uniform (Fig. 2A). At this same stage, rho is expressed in a pattern of intersecting stripes representing the future longitudinal veins and wing margin (Fig 2B). During early pupariation after the wing disc has evverted to bring the dorsal and ventral surfaces of the wing together (approximately 8 hours after pupariation (AP)), down-regulation of Egf-r transcripts becomes apparent (Fig. 2C). Double-label experiments reveal that domains of Egf-r mRNA down-regulation are slightly broader than those expressing rho (Fig. 2D). The pattern of Egf-r down-regulation becomes more sharply defined between 18 hours AP (Fig. 2E) and 25 hours AP (Fig. 2G). Double-label experiments demonstrate that Egf-r and rho expression remain nearly complementary throughout this period (Fig. 2F,H). Examination of these preparations at higher magnification (inset in Fig. 2F) suggests that cells expressing high levels of Egf-r mRNA are typically separated from rho-expressing cells by a row of one or two low-Egf-r-expressing cells. By 30 hours AP, Egf-r (Fig. 2I) and rho are expressed in essentially complementary patterns, as no gaps of unlabeled cells are observed in double-labeled wings (Fig. 2J). Although Egf-r is expressed in all intervein cells between 20 and 30 hours AP, expression is strongest in cells flanking developing veins (see inset in Fig. 2G). It is worth noting that ectopic expression of rho during comparable pupal stages leads to profound ectopic wing vein formation, frequently in regions flanking existing veins (M. A. Sturtevant and E. Bier, unpublished data) demonstrating that all components of the Egf-r signaling cascade are present in intervein cells. Other examples of localized Egf-r down-regulation following rho expression include cells behind the morphogenetic furrow in the eye (Zak et al., 1990) and cells forming adult external sensory organs (data not shown).

The clear reiterated theme during both embryogenesis and larval development is that cells initially express both Egf-r and rho and then with a brief delay, Egf-r mRNA levels are sharply down-regulated in rho-expressing cells. One possible explanation for this sequence of events is that Rho activity leads to subsequent Egf-r down-regulation by some negative feedback mechanism. Such negative feedback could be mediated by heightened EGF-R signaling or by a separate action of Rho. Alternatively, it is possible that control of Egf-r down-regulation is independent of rho activity. Fully resolving this important question is beyond the scope of this report; however, data described below suggest that Egf-r down-regulation is linked to rho function.

To address whether rho expression is coupled to Egf-r mRNA down-regulation, we examined the effect of ubiquitous rho expression during embryogenesis and wing development, as there are phenotypic consequences to ectopic rho expression during these stages that are opposite to phenotypes resulting from loss of rho function. For example, heat induction of embryos carrying a heat-shock rho construct (HS-rho) at germband extension leads to the production of supernumerary stretch receptor organ precursor cells (J.W. O'Neill and E. Bier, unpublished data), and heat inductions performed throughout wing vein development lead to the formation of ectopic veins (Sturtevant et al., 1993; M. A. Sturtevant and E. Bier, unpublished data). During the germband extended stage Egf-r is normally strongly expressed in the mesoderm and epidermis, but not in the neuroblast layer, leading to a railroad track staining pattern in sagittal section (Fig. 3A). Ubiquitous rho expression resulting from brief induction of the HS-rho construct followed by an hour of recovery leads to dramatic global down-regulation of Egf-r expression in the epidermis in greater than 90% of embryos, but does not reduce expression in the mesoderm (Fig. 3B). The lack of down-regulation in the mesoderm, perhaps due to the absence of some necessary component present in epidermal cells, provides an internal control for the in situ staining reaction at this particular stage. HS-rho induced Egf-r down-regulation in the epidermis is due to ectopic rho expression since uninduced embryos carrying the HS-rho construct (Fig. 3A) or heat-shocked wild-type embryos (Fig. 3C) have the normal railroad track pattern of epidermal and mesodermal Egf-r expression. To eliminate a potential role of mis-expressing rho during oogenesis, we examined Egf-r expression in embryos collected from a cross of heterozygous HS-rho male flies to wild-type females. As expected, approximately half of these embryos exhibit the broad epidermal down-regulation phenotype. Down-regulation of Egf-r mRNA levels is not a general feature of all epidermal transcripts since expression of spitz, which is also strongly expressed in the epidermis and mesoderm but not in the neuronal layer, is unaltered in heat-shocked HS-rho embryos (data not shown). Because embryos lacking zygotic Gap1 activity exhibit a similar pattern of Egf-r down-regulation (Fig. 3D), rho-induced Egf-r mRNA down-regulation may be mediated by a tyrosine kinase signaling pathway.

rho is also involved in Egf-r mRNA down-regulation during wing development. Heat induction of the HS-rho stock 15-16 hours AP leads to peak formation of excess veins and blistered wings (data not shown). Wild-type or uninduced HS-rho wings at this stage show clear signs of Egf-r down-regulation in vein territories (Fig. 3E). Egf-r down-regulation is expanded to occupy the entire wing blade following HS-rho induction (Fig. 3F). Expression along the margin, which is unaffected by ectopic rho expression, serves as an internal staining control. Control heat-shocked wild-type flies do not exhibit this specific down-regulation, although levels of RNA are reduced somewhat in all cells (data not shown). HS-rho induced Egf-r RNA down-regulation is also obvious earlier (6-8 hours AP) during prepupal stages (data not shown). A further link between rho function and Egf-r RNA down-regulation is revealed by examining Egf-r expression in developing rave mutant wings (Fig. 3I), which lack distal portions of wing veins (Fig. 3G), and in a HS-rho transformant line (Fig. 3J), which constitutively forms excess veins (Fig. 3H). Egf-r down-regulation is not observed in distal regions of developing rave wings (Fig. 3I), while down-regulation is observed in more cells than normal in the HS-rho line (Fig. 3J). Thus, the pattern of Egf-r down-regulation in both of these cases prefigures the final vein phenotype.

**DISCUSSION**

The results described in this study suggest an intimate link between rho expression and Egf-r mRNA down-regulation. Down-regulation of Egf-r mRNA levels in developing wings requires rho function and ubiquitous expression of rho during embryogenesis and wing development leads to ectopic Egf-r mRNA down-regulation in epidermal cells. The short time between ectopic rho expression and strong Egf-r mRNA down-
regulation suggests that this link may be fairly direct. Other pathways in addition to that mediated by Rho, however, must also contribute to Egf-r down-regulation during certain developmental stages. For example, Egf-r transcripts are down-regulated in embryos homozygous for a null rho point mutant, albeit not as sharply as in wild type (data not shown). This is
not too surprising since other genes are likely to function in parallel with \( \text{rho} \) during embryogenesis (Bier et al., 1990) and wing vein formation (Sturtevant et al., 1993). A role for EGF-R/RAS signaling in \( Egf-r \) down-regulation is suggested by the ectopic down-regulation observed in heat-induced HS-\( \text{rho} \) embryos and in embryos lacking the \( \text{Gap}1 \) gene. \( Egf-r \) function is not required for down-regulation; however, since down-regulation is initiated normally in three different strong \( Egf-r \) point mutants, but is not as sharply defined or as long lasting as in wild-type embryos (data not shown). As GAP proteins have been proposed to function as effectors as well as regulators of RAS (Martin et al., 1992; Medema et al., 1992; Al-Alawi et al., 1993; Declue et al., 1993; Grunicke and Maly, 1993; Marshal, 1993), it is possible that an effector function of GAP plays a role in down-regulating \( Egf-r \). This possibility is consistent with the fact that \( \text{rho} \) interacts genetically with \( \text{Gap}1 \) but not with \( \text{sos} \) or \( \text{drk} \) (GRB2) in wing vein formation (Sturtevant et al., 1993). Thus, \( \text{Rho} \) may induce ectopic \( Egf-r \) down-regulation via its role in promoting EGF-R/RAS signaling or by some other means such as a RAS-independent activity of GAP (Martin et al., 1992). It is also possible in some cases that \( Egf-r \) down-regulation is due to an indirect cell non-autonomous action of \( \text{rho} \). For example, during much of pupal development, the strips of cells that down-regulate \( Egf-r \) are slightly broader than the presumptive vein cells expressing \( \text{rho} \) (Fig. 2C,D and E,F). Further experiments will be required to determine the role that \( \text{rho} \) and other genes, such as those encoding components of the EGF-R signaling pathway, play in \( Egf-r \) down-regulation.

\( Egf-r \) mRNA down-regulation could be due to decreased \( Egf-r \) transcription or decreased mRNA stability. As the probe that we used should detect all \( Egf-r \) transcripts, it is likely that transcripts derived from both \( Egf-r \) promoters are affected. EGF stimulation of various cell types can induce alterations in either mRNA stability (Le et al., 1991) or in the transcription rate of a variety of target genes (Hopkin et al., 1991), including \( EGF-R \) itself (Kesavan et al., 1990; Joh et al., 1992). Further investigation, such as constructing \( Egf-r \) promoter lacZ fusions, will be required to distinguish between these possibilities. In either case, down-regulation of \( Egf-r \) mRNA levels provides a novel mechanism for extending the period of low EGF-R activity following transient down-regulation via receptor internalization (Haigler et al., 1978; Lund et al., 1990; Wiley et al., 1990) and reduction of high affinity receptor activity due to protein kinase C phosphorylation of the receptor (Welsh et al., 1991).

The observation that \( Egf-r \) activity is decreased following receptor activation is similar to genetic observations that have been made with respect to regulation of EGF-R activity in \( C. \ elegans \). A category of loss-of-function \( \text{let-23} \) (\( Egf-r \)) alleles cause a hyperinduced (Hin) phenotype resulting, paradoxically, in elevated EGF-R activity (Aroian and Sternberg, 1991). These Hin alleles in \textit{trans} to \( Egf-r \) null alleles exhibit loss-of-function phenotypes (e.g. vulvalless) and combinations of \( \text{let-23} \) Hin alleles with similar alleles at two other distinct loci (\textit{lin-2} and \textit{lin-7}) also result in vulvalless phenotypes. It has been proposed that these mutants when individually homozygous disrupt some aspect of negative regulation of \textit{let-23} more than they reduce signaling activity of the receptor, but that when Hin alleles are combined with stronger loss-of-function alleles signaling is also compromised leading to the vulvalless phenotype (Aroian and Sternberg, 1991; Sternberg, 1993). Although \textit{Drosophila} \( Egf-r \) alleles corresponding to \( C. \ elegans \) Hin alleles have not been reported, mild \( Egf-r \) loss-of-function alleles (\( Egf-r^{<}\)) exhibit a slight gain-of-function phenotype in the wing (e.g. distal vein branching) and some combinations of HS-\( \text{rho} \) constructs with the gain-of-function \( Egf-r^{<}\) allele lead to a serrated margin phenotype. Serrated wing margin phenotypes are typical of genes antagonizing \( Egf-r \) and \( \text{rho} \) activity (Sturtevant et al., 1993; Sturtevant and Bier, unpublished data). The existence of other genes behaving genetically as coactivators of EGF-R (i.e. \textit{lin-2} and \textit{lin-7}), which also participate in negative regulation of the receptor, is formally similar to the role played by \( \text{rho} \) in \textit{Drosophila}. Whether any of the four pathways that negatively regulate \textit{let-23} in \( C. \ elegans \) (Sternberg, 1993) involve down-regulation of EGF-R protein or mRNA levels remains to be determined.

It should be noted that the systematic down-regulation of \( Egf-r \) mRNA levels following heightened EGF-R signaling, which we report here, contrasts with the transient increase in \( Egf-r \) mRNA levels observed in several mammalian cell culture studies (Clark et al., 1985; Earp et al., 1986; Bjorge and Kudlow, 1987; Thompson and Rosner, 1989). This may reflect a fundamental difference between uncommitted embryonic cells undergoing a series of distinct cell fate choices during development and more terminally differentiated cells, which are primarily concerned with whether to proliferate in response to exogenous cues. Another potentially relevant difference is that cells in culture generally respond to soluble ligands, whereas embryonic cells are in direct contact with other cells. It is possible that direct cell-cell contact is required for the kind of regulation we have observed.

Since failure to down-regulate EGF-R activity by interfering with EGF-R internalization leads to tumor formation in mice (Masui et al., 1991) and overexpression of EGF-R or the highly related \( \text{neu} \) receptor are associated with aggressive forms of cancer (Neal et al., 1985; Santon et al., 1986; Slamon et al., 1987), understanding transcriptional control of \( Egf-r \) is relevant to oncogenesis. Down-regulation of \( Egf-r \) mRNA levels in specific differentiating \textit{Drosophila} epidermal cells may play a similar role to down-regulation of FGF (Velcich et al., 1989; Moore et al., 1991) and FGF-R (Moore et al., 1991) expression in differentiating myogenic cells. Determining the mechanism of \( Egf-r \) transcriptional down-regulation and whether this regulation plays an important role in development are key issues to resolve in future experiments.

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REFERENCES


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