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Torso signalling regulates terminal patterning in *Drosophila* by antagonising Groucho-mediated repression

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SUMMARY

Patterning of the non-segmental termini of the *Drosophila* embryo depends on signalling via the Torso receptor tyrosine kinase (RTK). Activation of Torso at the poles of the embryo triggers restricted expression of the zygotic gap genes *tailless* (*tll*) and *huckebein* (*hkb*). In this paper, we show that the Groucho (Gro) corepressor acts in this process to confine terminal gap gene expression to the embryonic termini. Embryos lacking maternal *gro* activity display ectopic *tll* and *hkb* transcription; the former leads, in turn, to lack of abdominal expression of the *Krüppel* and *knirps* gap genes. We show that *torso* signalling permits terminal gap gene expression by antagonising Gro-mediated repression. Thus, the corepressor Gro is employed in diverse developmental contexts and, probably, by a variety of DNA-binding repressors.

Key words: *Drosophila*, repression, *groucho*, Torso signalling, terminal patterning, *tailless*, gap genes

INTRODUCTION

The development of complex, multicellular organisms requires that transcription of many genes be spatially restricted. Much emphasis has been placed on mechanisms for achieving tissue-specific activation of gene expression, and on factors that promote transcription. Less attention has been focused on studies of transcriptional repression, although this latter mechanism is likely to be equally important in contributing to spatially organised gene expression.

Recent work has highlighted several different mechanisms for preventing gene transcription (reviewed in Levine and Manley, 1989; Johnson, 1995; Hanna-Rose and Hansen, 1996). One class of repressors function as transcriptional 'poisons', forming protein complexes with activators to retain them in the cytoplasm, or rendering them unable to bind DNA. Other repressors act by competing for common or overlapping DNA target sites, thereby excluding activators from access to target promoters.

A third class of negative transcriptional regulators also binds DNA but appears to act in a more instructive manner. Some such repressors (e.g. Snail, Krüppel (Kr), Knirps (Kni)) act at short-range to mask adjacent, proximally bound activators (Gray et al., 1994; Arnosti et al., 1996; Gray and Levine, 1996). Others act over long-range (e.g. Hairy: Barolo and Levine, 1997; Dorsal: Huang et al., 1993; Cai et al., 1996), probably by directly interfering with the general transcriptional machinery or by altering local chromatin structure to occlude promoter sites. This latter class of DNA-bound repressors is likely to be assisted by general cofactors, by analogy to transcriptional activators that recruit co-activators to target gene promoters. Consistent with this idea, a novel class of nuclear cofactors, termed corepressors, have recently been identified, which bind to negative transcriptional regulators and are required for them to silence downstream target genes (Keleher et al., 1992; Wang and Stillman, 1993; Ayer et al., 1995; Chen and Evans, 1995; Horlein et al., 1995). Indeed, it has been shown that some corepressors are even able to bind to positive regulators and convert them into transcriptional repressors (Weintraub et al., 1992; Jiang et al., 1993; Kirov et al., 1993; Lehming et al., 1994).

The Groucho (Gro) protein is a putative corepressor. It is a ubiquitously expressed nuclear protein that lacks a known DNA-binding domain, but which includes multiple WD repeats that are believed to mediate protein-protein interactions (Hartley et al., 1988; Delidakis et al., 1991; Tata and Hartley, 1993; Neer et al., 1994). We have previously demonstrated physical associations between Gro and a subfamily of related basic-helix-loop-helix (bHLH) repressor proteins encoded by *hairy* (*h*), the *Enhancer-of-split* (*E(spl)*) Complex and *deadpan* (Klämbt et al., 1989; Rushlow et al., 1989; Bier et al., 1992; Delidakis and Artavanis-Tsakonas, 1992; Knust et al., 1992; Schrons et al., 1992; Paroush et al., 1994). These Hairy-related bHLH proteins all show similarities in their bHLH domains (required for dimerisation and DNA-binding; Murre et al., 1989; Ferre-D'Amare et al., 1993) and in their Orange domains (implicated in defining target gene specificity; Dawson et al., 1995). In addition, all terminate in a specific tetrapeptide motif, WRPW, that has been shown to be necessary and sufficient for binding to Gro (Paroush et al., 1994; Fisher et al., 1996), and to be required for the biological activity of this class of bHLH proteins (Wainwright and Ish-Horowicz, 1992). In embryos lacking maternal *gro* activity, there is a failure to repress the cognate target genes

of Hairy, E(spl) and Deadpan, and segmentation, neurogenesis and sex-determination, the developmental processes regulated by the Hairy-related bHLH proteins, are defective (Ingham et al., 1985; Delidakis et al., 1991; Schrons et al., 1992; Younger-Shepherd et al., 1992; Paroush et al., 1994). These experiments indicated that WRPW/Gro interactions are indeed important in vivo, leading us to propose that Gro is a transcriptional corepressor that is recruited to specific target genes by bHLH DNA-bound regulators that structurally resemble Hairy (Paroush et al., 1994).

Repression appears to be a major mechanism for achieving the restricted transcription of segmentation genes that underlies embryonic patterning in *Drosophila*. Many of the segmentation genes themselves act genetically as negative transcriptional regulators (e.g. Carroll, 1990; Ip et al., 1991; Small et al., 1991; Manoukian and Krause, 1992; Jäckle and Sauer, 1993; Gray et al., 1994; Arnosti et al., 1996; Gray and Levine, 1996; Jiménez et al., 1996) and are tightly regulated both temporally and spatially. Thus, they may use cofactors that are more generally expressed. Hence, although Gro could be dedicated to the Hairy-related repressors, it is 719 aminoacids long, and should be capable of interacting with a variety of proteins. Here, we describe a requirement for Gro in a developmental setting that does not involve any of the known Hairy-related bHLH proteins, consistent with the idea that Gro mediates the action of other repressors.

Specification of cell-fates at the termini of *Drosophila* embryos is under the control of the maternal Torso (Tor) receptor tyrosine kinase (RTK; reviewed by St Johnston and Nüsslein-Volhard, 1992; Lu et al., 1993; Pankratz and Jäckle, 1993; Duffy and Perrimon, 1994). The Tor receptor is uniformly distributed on the blastoderm membrane but is activated by a localised ligand only at the poles of the embryo (Casanova and Struhl, 1989; Sprenger et al., 1989; Sprenger and Nüsslein-Volhard, 1992). Where activated, Tor initiates a signal-transduction pathway mediated by Ras1, D-raf, MAP-kinase (MAPK) and other effectors (reviewed in Lu et al., 1993; Duffy and Perrimon, 1994). This developmental pathway ultimately drives localised transcription of the zygotic terminal gap genes *tailless* (*tll*) and *huckebein* (*hkb*) at the poles of the embryo (Bronner and Jäckle, 1991; Pignoni et al., 1992). In this paper, we demonstrate that Gro is required to restrict expression of *tll* and *hkb* during terminal patterning, and that local *torso* signalling acts to relieve general, Gro-mediated repression of these gap genes. In the absence of maternal *gro*, expansion of the *tll* domain leads to loss of the expression of the abdominal gap genes *Kr* and *kni*. We discuss the implications of these results for mechanisms of terminal patterning.

MATERIALS AND METHODS

Fly culture

Flies were cultured and crossed on yeast-cornmeal-molasses-malt extract-agar medium at 25°C.

In situ staining of *Drosophila* embryos

1-3.5 hour collections of wild-type or mutant *Drosophila* embryos were dechorionated in bleach and fixed in 4% formaldehyde for 20 minutes. Expression patterns were visualised by in situ hybridisation using antisense RNA probes labelled with digoxigenin-UTP and anti-digoxigenin antibodies conjugated to alkaline phosphatase (Boehringer Mannheim) (Tautz and Pfeifle, 1989; Klingler and Gergen, 1993).

Fly stocks and germline clones

gro^{mat-} embryos

Embryos were derived from mosaic females with either *gro^{E48}* or *gro^{BX22}* mutant germlines, obtained by using the FLP-DFS technique (Chou et al., 1993). Briefly, males carrying the *FRT[82B] ovo^{D1}* chromosome (gift of Norbert Perrimon) and an X-linked *hs-FLP1* chromosome were crossed to females carrying a *FRT[82B] gro* chromosome. Progeny of this cross were heat shocked (37°C/4 hours) on each of days 3, 4 and 5 after egg lay, and allowed to develop at 25°C. Non-heat-shocked control females were sterile (100% penetrance), and all eggs laid by fertile females displayed a severe neurogenic phenotype, as expected for *gro^{mat-}* embryos (Schrons et al., 1992; Paroush et al., 1994). Both *gro* genotypes generated eggs with similar phenotypes.

tor^{D} embryos

Strong gain-of-function *tor^{D}* embryos were generated from *tor^{Y9}/tor^{4021}* females (Klingler et al., 1988).

gro^{mat-} tll embryos

An *FRT[82B] gro^{E48} tll^{L10}* chromosome was generated by recombination and the FLP-DFS system used to generate females with *gro^{mat-} tll* germ-lines. These were crossed to *Df(3R)tll^{e}/TM3, Sb hb-lacZ* males. Embryos were analysed by in situ hybridisation with mixed probes: *Kr+lacZ* or *kni+lacZ*. *gro^{mat-} tll* embryos were recognised by the absence of anterior *lacZ* staining. Control *tll* embryos were homozygous for *Df(3R)tll^{e}*.

tsl gro^{mat-} embryos

tsl is required somatically and lies on the same chromosome arm as *gro*. Females homozygous for *tsl* and heterozygous for *gro* were used to generate clones of *gro^{mat-}* germ cells in a *tsl^{691}* soma. An *FRT[82B] tsl^{691} gro^{E48}* chromosome was obtained by recombination, and an *FRT[82B] tsl^{691} ovo^{D1}* chromosome was constructed by γ-ray-induced male recombination of 24-48 hour *FRT[82B] tsl^{691} /FRT[82B] ovo^{D1}* larvae (1000rad). Four putative recombinant males were collected, two of which were fertile and included both *tsl* and *ovo^{D1}* mutations. These were used to generate *hs-FLP1/+; FRT[82B] tsl^{691} gro^{E48}/FRT[82B] tsl^{691} ovo^{D1}* females which were heat shocked to generate *gro^{-}* germ cells in flies whose soma was mutant for *tsl*. *tsl gro^{mat-} tll* embryos were generated by introducing *tll* onto the *FRT[82B] tsl gro^{mat-}* chromosome.

RESULTS

Abdominal *Krüppel* and *knirps* expression depends on maternal *groucho*

We have previously shown that *gro* is required for embryonic segmentation and that pair-rule and segment polarity gene expression is aberrant in *gro^{mat-}* embryos (embryos generated from *gro^{-}* female germ-cells). For example, in *gro^{mat-}* embryos, striped expression of the *h* pair-rule gene fails; instead, it is strongly expressed in two broad domains that are separated by a domain of weaker expression (Paroush et al., 1994). Also, the anterodorsal *h* domain is shifted anteriorly and ventrally to the extreme tip of the embryo. Together, these alterations indicate that *gro* acts prior to the establishment of pair-rule periodicity, and that it is required for patterning of both trunk and the non-segmented termini.

The abnormal pattern of *h* expression suggests that *gro* is

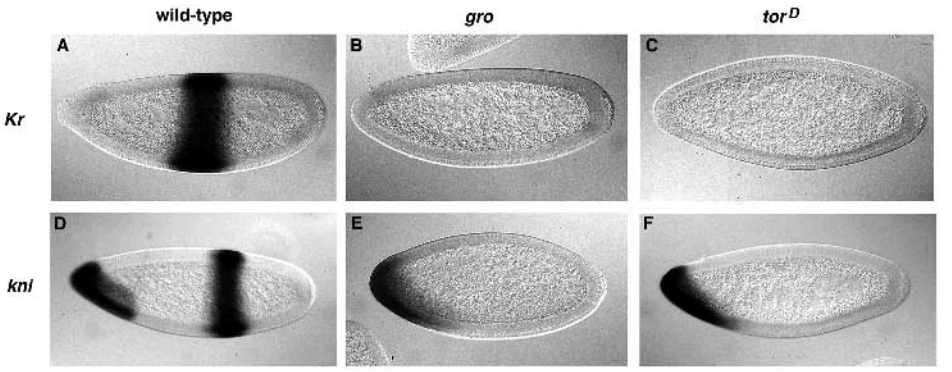

Fig. 1. *Kr* and *kni* are not expressed in gro^{mat-} and tor^{D} embryos. *Kr* (A-C) and *kni* (D-F) transcripts are lost in the trunk domains of gro^{mat-} (B,E) and tor^{D} (C,F) blastoderm stage 14 embryos (cf. wild-type embryos; A,D). *Kr* and *kni* expression in the head arise later and are under different control, so are not considered further in this paper. In this and following figures, embryos are oriented anterior to the left and dorsal up.

required for the activity of genes upstream of *h*. Further examination reveals that gro^{mat-} embryos completely lack expression of the central gap genes *Kr* and *kni* in their respective trunk domains (Fig. 1B,E). *Kr* and *kni* are still transcribed normally elsewhere in the embryo (e.g., *kni* head patch), indicating that Gro is selectively required for correct gap gene expression in the prospective abdomen. Although Gro could be acting as a direct activator of gap gene expression, its role as a negative regulator in other developmental contexts led us to investigate a model in which it acts indirectly, by repressing a repressor of *Kr* and *kni* transcription.

groucho restricts *tailless* and *huckebein* expression to the termini

We were particularly struck by the similar effects of gro^{mat-} and dominant gain-of-function *torso* mutations (tor^{D}) on gap and pair-rule gene expression: in both cases, *Kr* and *kni* are not expressed (Fig. 1C,F), and pair-rule genes are transcribed in broad central domains rather than in stripes (Klingler et al., 1988; Pankratz et al., 1989; Weigel et al., 1990; Paroush et al., 1994). In tor^{D} embryos, ligand-independent constitutive signalling from the Tor^{D} RTK drives ectopic expression of terminal genes which, in turn, repress trunk expression of *Kr* and *kni* (Klingler et al., 1988; Casanova and Struhl, 1989; Bronner and Jäckle, 1991; Steingrímsson et al., 1991; Hoch et al., 1992; Sprenger and Nüsslein-Volhard, 1992). Therefore, we tested whether deregulated terminal gene expression might also explain the loss of *Kr* and *kni* in gro^{mat-} embryos. Figure 2 shows that *tll* and *hkb* expression are indeed ectopically expressed in gro^{mat-} embryos: terminal domains of strong expression are expanded and weaker expression extends towards the centre of the embryo. Thus, Gro must normally be required to prevent *tll* and *hkb* from being expressed in trunk regions of wild-type embryos.

Groucho counteracts repression of *Krüppel* and *knirps* by *tailless*

Tll is a known repressor of *Kr* and *kni* (Pankratz et al., 1989; Steingrímsson et al., 1991; Hoch et al., 1992), so its ectopic expression is a likely explanation for the failure of trunk gap-gene expression in gro^{mat-} embryos. To test this idea, we examined gro^{mat-} embryos that are also mutant for *tll*. *Kr* and *kni* expression is restored in such gro^{mat-} *tll* double-mutant embryos (*tll* embryos from gro^{-} germ cells; Fig. 3A,C), demonstrating that the failure to express trunk gap genes in gro^{mat-} embryos is due to repression by ectopic *tll*. The above results confirm that Gro plays a repressive role during gap gene patterning. Regulation of *tll* transcription does not involve a known Hairy-related bHLH protein, so Gro may function in this context in collusion with a partner protein belonging to a different class of DNA-binding transcription factors (see Discussion).

Groucho-mediated repression of *tailless* is relieved by the *torso* pathway

During embryonic patterning, the Tor RTK is activated by an extracellular ligand, and signals via the highly conserved Ras/Raf/MAPK transduction pathway. A putative maternal transcription factor 'Y' has been proposed to explain how phosphorylation by MAPK drives *tll* transcription; Y should be generally distributed but only activated by MAPK modification that occurs selectively at the poles of the embryo (St Johnston and Nüsslein-Volhard, 1992; Lu et al., 1993; Pankratz and Jäckle, 1993; Perrimon, 1993; Duffy and Perrimon, 1994).

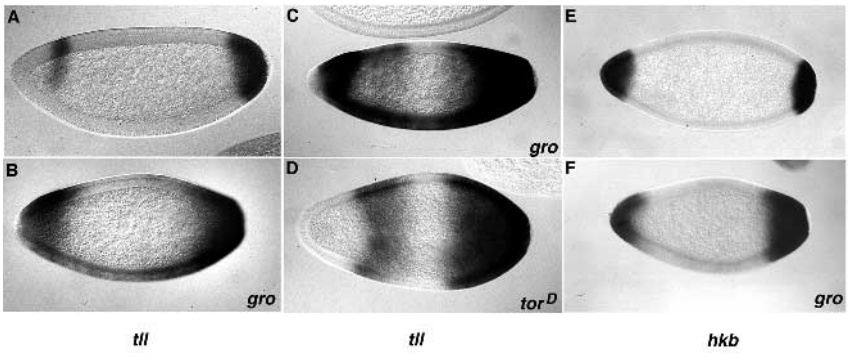

Fig. 2. Terminal gap gene expression is expanded in gro^{mat-} embryos. (A-D) Embryos stained for *tll* transcripts. *tll* is expressed strictly at the poles of wild-type embryos (A), but is ectopically expanded towards the centre of gro^{mat-} (B,C) and tor^{D} (D) embryos. Embryos A and B were stained similarly; the embryo in C is stained longer to show that ectopic *tll* transcripts extend centrally into the embryo. Wild-type (E) and gro^{mat-} (F) embryos stained for *hkb* transcripts showing that the posterior domain is expanded in the latter. The anterior domain is not under *tor* control (Bronner and Jäckle, 1991; not shown).

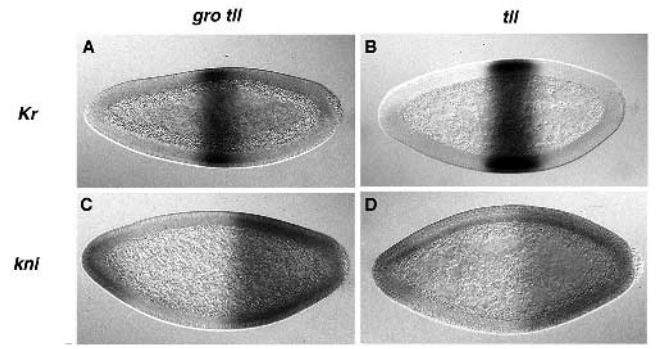

Fig. 3. Loss of *Kr* and *kni* expression in *gro*$^{mat-}$ embryos is due to ectopic *tll* expression. Staining for *Kr* (A,B) and *kni* (C,D) transcripts in *gro*$^{mat-}$ *tll* (A,C) and *tll* (B,D) embryos. Expression of both *Kr* and *kni* is restored in *gro*$^{mat-}$ *tll* embryos (cf. Fig. 3A,C with Fig 1B,E, respectively), indicating that the expanded *tll* expression in *gro*$^{mat-}$ is responsible for repressing the central gap genes. *kni* expression extends to the posterior pole in both *tll* (D; Pankratz et al., 1989) and *gro*$^{mat-}$ *tll* embryos (C).

However, previous analysis of the *tll* promoter suggested an alternative explanation, that the *tor* RTK-signalling pathway allows local *tll* expression by relief of repression (Liaw et al., 1995). Our results are consistent with this latter model and strongly suggest that Gro mediates the general repression which is overridden by Tor signalling (Fig. 5A).

The former model predicts that *tor* signalling is positively required to activate *tll* expression. In contrast, the latter model implies that activation of *tll* should be independent of *tor* signalling in embryos lacking *gro* activity. We therefore examined *gro*$^{mat-}$ flies that are unable to activate *tor* signalling. We made use of mutations in the *torso-like* (*tsl*) gene, which is required in somatic ovarian cells for presentation or maturation of the ligand for Tor (Stevens et al., 1990; Savant-Bhonsale and Montell, 1993; Martin et al., 1994). In *tsl* embryos, posterior *tll* expression is lost (Fig. 4B; Pignoni et al., 1992), because *tor* signalling is not activated. In contrast, *tll* is still transcribed in *tsl gro*$^{mat-}$ embryos (Fig. 4A), showing that *tor* signalling is not a prerequisite for *tll* expression, but is only needed to overcome *gro*-dependent repression, as in the second model. *hkb* is similarly expressed in *tsl gro*$^{mat-}$ embryos (data not shown).

The lack of *Kr* and *kni* expression is solely due to ectopic *tll*. *Kr* and *kni* are not expressed in the trunk of *tsl gro*$^{mat-}$ embryos (not shown), which do express *tll*, but are restored in *tsl gro*$^{mat-}$ *tll* embryos (Fig. 4C). Thus Gro appears to act as a negative regulator in terminal patterning, as it does in other developmental contexts.

DISCUSSION

We and others have previously shown that the Gro corepressor functions during sex determination and neurogenesis, and biochemical and genetic analysis implicate it in Hairy function during segmentation (Delidakis et al., 1991; Schrons et al., 1992; Paroush et al., 1994; Dawson et al., 1995; Fisher et al., 1996). In this paper, we have shown that *tll* and *hkb* are ectopically expressed in *gro*$^{mat-}$ embryos, independently of *tor* signalling, arguing that maternal Gro is also required in terminal patterning, and that expression of both terminal gap genes is under negative control. Gro is also required for dorsoventral patterning, which is disrupted in *gro*$^{mat-}$ embryos: the anterodorsal domain of *h* expression becomes dorsoventrally symmetrical (Paroush et al., 1994), and ventral furrow formation is abnormal (Z. P., unpublished data), indicative of a failure of ventral repression. Thus, there are repeated requirements for Gro-mediated repression in the course of early fly development.

Spatial regulation of *tailless* and *huckebein* expression

Our findings support the following model for regulation of *tll* expression by generally distributed maternal factors (Fig. 5A): Gro acts in conjunction with a DNA-binding protein ('X') to repress *tll* expression throughout the embryo, thereby permitting transcription of posterior and central gap genes. *tor* signalling at the poles of the embryo relieves Gro-mediated repression, allowing other maternal factors to activate *tll* transcription locally. In combination, these elements lead to selective activation of *tll* transcription at the embryo termini. *hkb* is affected similarly to *tll* in *gro*$^{mat-}$ and *tsl gro*$^{mat-}$ embryos (unpublished observations), indicating that it is regulated like *tll*. In *tor*^{D} embryos, constitutive Tor signalling inactivates repression even in the centre of the embryo, leading to ectopic *tll* transcription, and silencing of *Kr* and *kni*. This model also offers a plausible explanation of how Tor signalling generates different domains of *tll* and *hkb* expression: if activation of *tll* and *hkb* is differentially sensitive to repression, they will be activated by different signalling thresholds (Fig. 5B).

Our model predicts that proteins that activate and repress *tll* and *hkb* are ubiquitously distributed; in the middle of the embryo, where the *tor* RTK is inactive, repression prevails. Thus, the key activity modulated by the Tor RTK pathway is a repressor present throughout the embryo. Indeed, a manifestation of this unique mode of regulation is present in the *tll* upstream regulatory region. A comprehensive analysis of the *tll* promoter using a battery of reporter-gene fusion constructs

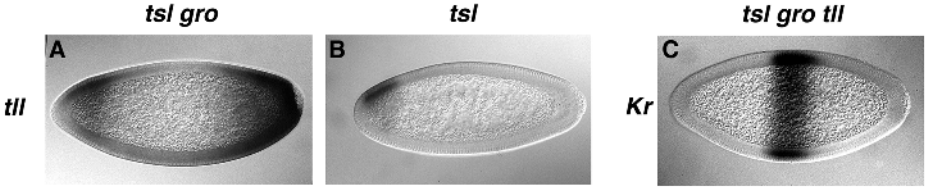

Fig. 4. Activation of *tll* is independent of *tor* signalling in *gro*$^{mat-}$ embryos. *tll* is strongly expressed at the posterior of *tsl*^{691} *gro*^{E48} embryos, as in *gro*$^{mat-}$ embryos (A), but not expressed at the posterior of *tsl*^{691} mutant embryos (B). (Posterior *tll* expression is under the regulation of the terminal system, whereas anterior *tll* expression is also controlled by *bcd* and *dl* (Liaw and Lengyel, 1992; Pignoni et al., 1992), and is not considered in this paper.) Thus, *tor* signalling is not required to activate *tll* expression directly, but rather to override general repression of *tll*. (C) *Kr* expression (and that of *kni*, not shown) is restored in a *tsl gro*$^{mat-}$ *tll* embryo.

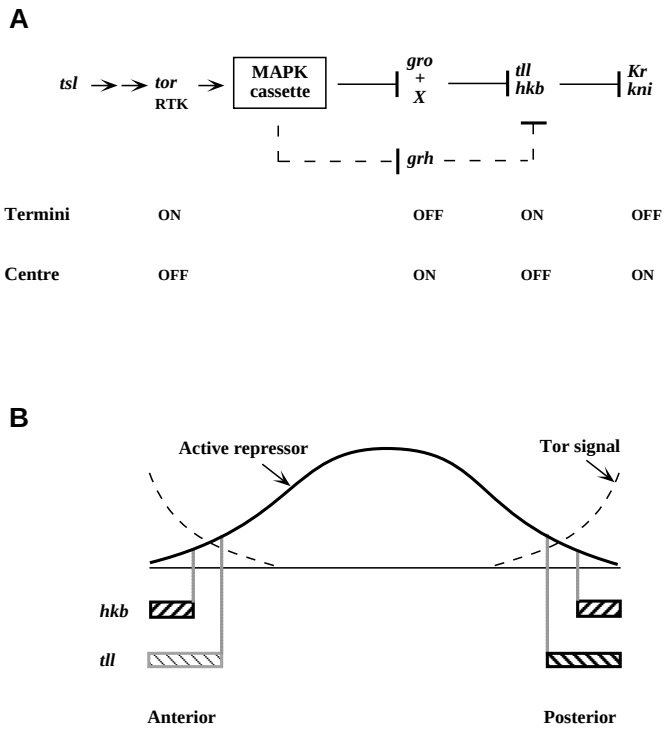

Fig. 5. (A) Model for activation of *tll* and *hkb* expression by *tor* signalling. At the termini, an extracellular ligand (for whose function *tsl* activity is required) locally activates the Tor RTK which, in turn, activates the Ras/Raf/MAPK signalling cascade. MAPK presumably modifies a currently uncharacterised DNA-binding partner (X) for Gro, preventing Gro-complexes from repressing *tll* and *hkb* expression at the poles. Elsewhere in the embryo, Gro+X repress terminal gap gene expression, thereby allowing *Kr* and *kni* expression. Tor signalling may also lead to modification of the Grh protein, which could mediate a parallel pathway of repressing *tll* (Liaw et al., 1995; see text). (B) Model illustrating how differential repression by Gro of *tll* and *hkb* can lead to different transcriptional domains. Graded activation of Tor leads to a reduction of active Gro-mediated repression. The anterior *tll* domain is denoted by a lightened box because it is normally repressed by the action of Bicoid.

has identified regulatory elements capable of driving transcriptional activation throughout the embryo and has defined *cis*-acting regulatory elements that mediate repression (Liaw et al., 1995). Our results are consistent with and extend this analysis, by providing direct evidence that the endogenous *tll* gene is indeed regulated by relief of repression and by implicating Gro in the process.

Groucho may act in conjunction with repressors unrelated to Hairy

Gro apparently lacks inherent sequence-specific DNA-binding activity, so its function during terminal patterning should require a DNA-binding partner protein which, like Gro, is expressed maternally and distributed throughout the embryo. We favour the notion that this partner is not a Hairy-related bHLH protein and that Gro is able to bind to and mediate repression by non-bHLH repressors. Of the known Hairy-related bHLH proteins, only one (E(spl)-m3) is contributed to the egg maternally (Knust et al., 1987). However, embryos lacking maternal *m3* do not show defects in terminal patterning (S. M. Parkhurst, personal communication).

tll promoter analysis has defined the torso Response Element (tor-RE), a *cis*-acting element through which a *tor*-sensitive negative regulator of *tll* transcription acts (Liaw et al., 1995). Deletion of the tor-RE brings about the uniform expression of a reporter gene resembling the expanded *tll* pattern in tor^{D} and gro^{mat-} embryos (Liaw et al., 1995). Significantly, this sequence contains neither a canonical E-box nor the variant sites that are bound by Hairy-related bHLH proteins in vitro (Ohsako et al., 1994; van Doren et al., 1994). Further analysis of the tor-RE has highlighted an internal 11 bp sequence, which is required for repression and to which a putative negative regulator binds (Liaw et al., 1995). It was shown biochemically that the transcription factor NTF-1 (Dynlacht et al., 1989), encoded by the *grainyhead* (*grh*) gene (Bray and Kafatos, 1991), binds to and footprints the wild-type Tor-RE, but not a mutated, inactive form of the element (Liaw et al., 1995). *tll* expression is somewhat derepressed in embryos devoid of maternal *grh* (grh^{mat-} embryos) suggesting that Grh/NTF-1 plays a role in restricting expression of terminal gap genes (Liaw et al., 1995). Moreover, Grh contains a single putative MAPK phosphorylation site and can be phosphorylated in vitro by ERK2 (the mammalian homologue of the MAPK activated by the terminal system) (Liaw et al., 1995). Together, these results implicate Grh as an in vivo target of the *tor* signalling pathway.

Grh could, therefore, be the protein X that recruits Gro to the *tll* promoter. However, protein-protein interaction assays fail to detect Gro/Grh associations in vitro (Dubnicoff et al., unpublished). Lack of either *gro* or *grh* each causes only partial *tll* derepression (Fig. 2b; Liaw et al., 1995), implying that *tll* is subject to at least two parallel repressor systems that are sensitive to *tor* signalling (Fig. 5A). However, the effects on *tll* expression of removing maternal *gro* are significantly more severe that those of removing maternal *grh* (G.-J. Liaw and J. Lengyel, personal communication), suggesting that repression via Gro is independent of and, probably, more effective than via Grh.

It has recently been shown that Hairy can act as a dominant, long-range repressor that may directly interact with one or more components of the basal transcriptional machinery of the cell (Barolo and Levine, 1997). This activity should require Gro, so one would expect Gro-mediated repression of *tll* to be similar mechanistically. Indeed, the tor-RE is able to direct long-range *tor*-dependent repression, even when 2.5 kb upstream of a heterologous promoter (Liaw et al., 1995). As a long-range corepressor, Gro is likely either to interfere directly with the transcriptional machinery, or with the accessiblity of the promoter to activating factors. Our experiments do not distinguish between these mechanisms. Indeed, other mechanisms remain possible. It has recently been shown that the SIN3 co-repressor acts in both yeast and mammalian cells by directing histone deacetylation, which modulates chromatin folding and accessibility (Alland et al., 1997; Heinzel et al., 1997). Thus, Gro may effect repression by regulating chromatin conformation.

In the course of the above studies, we have also shown that Tll repression of *Kr* and *kni* is Gro-independent. Previous work has indicated that Tll represses transcription by competing with activators for DNA-binding sites (Hoch et al., 1992). Alter-

natively, it is possible that Tll acts in association with other, as yet unknown, corepressors

Gro-complexes as nuclear targets for MAPK signalling

Many RTKs use the Ras/Raf/MAPK signal transduction pathway to relay information into the nucleus. The unique effects of RTK activation in each case may be due to differential phosphorylation of distinct nuclear targets. Significantly, the transcription factors that are known to be modified by MAPK in the fly – Pointed, Yan and DJun – are all key regulatory factors in development (Brunner et al., 1994a,b; O'Neill et al., 1994; Rebay and Rubin, 1995). Of these, Yan is an Ets-like DNA-binding repressor inactivated by direct MAPK phosphorylation. Yan lies downstream of the *sevenless* RTK and is required to maintain nascent photoreceptor cells in an undifferentiated state. Phosphorylation of Yan by MAPK brings about a dramatic shift in its distribution from nucleus to cytoplasm, downregulating its activity and leading to its rapid degradation (Rebay and Rubin, 1995).

Either Gro or its partner could be the targets for inactivation by MAPK phosphorylation. Although Gro itself contains several putative MAPK phosphorylation sites, we do not favour its being the direct target of MAPK signalling. tor^D embryos (in which *tll* is not repressed because Gro-complexes are presumably inactive) appear to retain Gro-repressive activity. Evidence for this view comes from examining Sex-*lethal* (*Sxl*) expression in male tor^D embryos. *Sxl* is repressed in normal males (Keyes et al., 1992), and this repression requires *gro* (Paroush et al., 1994). Nevertheless, we find that male tor^D embryos do not express ectopic *Sxl* (not shown), implying that they retain *gro* activity. Moreover, Gro is still present and nuclear at the termini of wild-type embryos (where tor signalling is active; Klingler et al., 1988) and in tor^D embryos (unpublished observations). Thus, it is more probable that Tor-driven MAPK phosphorylation inactivates a DNA-binding partner protein for Gro in *tll* regulation. Similarly, *tor*-mediated inactivation of a Dorsal-mediated repression complex may allow *zen* transcription at the embryo termini (Rusch and Levine, 1994). An understanding of exactly how the Torso pathway counteracts Gro-mediated general repression will have to await the identification of the protein(s) that recruit Gro to the *tll* and *hkb* promoters.

Many genes and regulatory pathways have been broadly conserved in eukaryotes. In particular, vertebrates include several *gro*-homologous genes (Stifani et al., 1992; Schmidt and Sladek, 1993; Mallo et al., 1994). Thus, it is possible that Ras signalling in some vertebrate contexts may also activate gene expression by antagonising Gro activity.

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