**Chapter 1**

**Introduction:**

**Groucho – A Multifunctional Regulator of Drosophila Development**

**Introduction**

*The Groucho/TLE family of corepressors are ubiquitous regulators of animal development*

The Groucho/TLE (Gro) family of corepressors play crucial roles in the interpretation and integration of multiple spatial, temporal, and signaling inputs during development in higher eukaryotes. Groucho, the sole *Drosophila melanogaster* member of this protein family, was first discovered in the context of a slight hypomorphic allele which resulted in the formation of extra supraorbital bristles reminiscent of the bushy eyebrows of Groucho Marx (Lindsley, 1968). Subsequent research on Gro in *Drosophila* has served to characterize this factor’s central importance to developmental gene regulation in response to multiple developmental programs and signaling pathways. As a corepressor, Groucho has no documented ability to bind DNA directly in a sequence-specific manner, instead relying on recruitment to genomic loci through interactions with a diverse array of transcriptional repressors (Mannervik, 2014). Through its interactions with these repressors, it is essential to nearly all aspects of embryonic and imaginal *Drosophila* development (Paroush et al., 1994). In humans, Gro/TLE family proteins are involved in such processes as organ development, adipogenesis, neurogenesis, hematopoiesis, and osteogenesis (Bajoghli et al., 2005; Javed et al., 2000; Metzger et al., 2012; Villanueva et al., 2011).

Groucho consists of five domains, two of which are highly conserved throughout higher eukaryotes (Chen and Courey, 2000; Turki-Judeh and Courey, 2012a). A great body of work has arisen documenting the contributions of each domain to the overall function and regulation of Groucho. While much of this work has focused on the N- and C- terminal domains, as they are more conserved and more sensitive to point mutagenesis (Jennings et al., 2006; Jennings et al., 2007), the central domains of Groucho have been investigated for their roles in Groucho activity through interaction with a number of regulatory targets, including protein kinases, histones, and histone modifying enzymes (Turki-Judeh and Courey, 2012a).

Homologs of Groucho with similar roles in developmental decision making have been identified throughout metazoans (Fig. 1.1) (Paroush et al., 1994). Homologs have been identified and characterized in rats (Schmidt and Sladek, 1993), nematodes (Pflugrad et al., 1997), frogs (Choudhury et al., 1997), zebrafish (Wulbeck, 1997), mice (Mallo et al., 1993), and humans (Stifani et al., 1992). While the *Drosophila* and *C. elegans* genomes each encode single Gro family genes, the mouse, chick, and human genomes each encode four members, while zebrafish and medaka each encode six members (Li, 2000). The full-length human Gro orthologs, termed transducin-like Enhancer of Split 1-4 (TLE1-4) (Miyasaka et al., 1993), are expressed combinatorially during cell differentiation and have non-redundant roles during development (Stifani et al., 1992; Yao et al., 1998).

Mammalian genomes additionally encode two truncated Gro homologs, *Amino Enhancer of Splt (AES)*, which is homologous to the two N-terminal domains of Groucho (Gasperowicz and Otto, 2005), and *Tle6/Grg6,* which possesses a poorly conserved N-terminal region and a C-terminal WD-repeat domain (Dang et al., 2001). Both factors are thought to antagonize the activity of full-length TLE family members. AES may function by directly binding to TLE proteins through Q-domain interactions (Brantjes et al., 2001) or by interacting with a subset of TLE-dependent repressors (Muhr et al., 2001). Similarly, TLE6/Grg6 has been shown to interact with repressors to block recruitment of full-length TLE family proteins and thereby alleviate repression (Marcal et al., 2005). More distantly related Gro homologs have been identified in yeast (Tup1) and plants (TOPLESS) (Courey and Jia, 2001; Lee and Golz, 2012; Smith and Johnson, 2000).

*The domain architecture of Groucho/TLE family proteins*

The N-terminal Q (glutamine rich) domain is one of the two highly conserved domains and is responsible for the formation of tetramers and potentially higher-order oligomers of Gro (Chen et al., 1998). Additionally, the Q-domain mediates a subset of interactions with transcriptional repressors, including the Tcf/Lef family of proteins (Brantjes et al., 2001). The structure of the Q-domain of TLE1, a human homologue of Gro, was recently solved, revealing that the domain forms a dimer of dimers consisting of two coiled-coils interdigitated in a head-to-head complex (Chodaparambil et al., 2014a). The resulting structure provides an elegant explanation of the mechanics of tetramerization, and corroborates the large frictional coefficient measured in hydrodynamic studies of the purified Q-domain, as the predicted structure is thin and rod-like (Kuo et al., 2011).

The ability of the Q domain to direct the formation of high-order oligomers has been proposed to mediate the spreading of Gro along chromatin allowing for the establishment of large transcriptionally silent domains. This might explain the documented ability of Gro to direct long-range repression in which entire loci are organized into transcriptionally silent states. In support of this idea, assays involving Grg3, a mouse homolog of Gro, on *in vitro* chromatin arrays showed that oligomerization mediated through the Q-domain is not required for recruitment of Gro to chromatin but is required for subsequent aggregation of chromatinized fragments into a form that was resistant to transcription (Sekiya and Zaret, 2007).

Contrary to the idea that the Q domain could mediate spreading, chromatin immunoprecipitation (ChIP) assays in cell culture revealed that oligomerization-deficient mutants of *Drosophila* Gro exhibited similar median peak widths to wild-type Gro (Kaul et al., 2014). The interpretation of this result is somewhat complicated by the fact that binding data was generated from two *Drosophila* cell lines depleted of endogenous Groucho via RNAi and overexpressing either GFP-tagged wild-type or oligomerization-deficient Groucho. The authors showed a significant reduction of endogenous Gro that nonetheless remained detectable by immunoblot. Thus, it remains a possibility that low levels of endogenous Groucho were contributing to peak formation or spreading in both contexts.

Regardless of the role of oligomerization in the definition of the size of Groucho binding domains, loss of oligomerization does result in significant differences in the recruitment patterns of overexpressed wild-type and oligomerization-deficient mutants. Of the approximately 3000 distinct Groucho binding sites identified in Kc167 cells expressing wild-type or oligomerization-deficient Gro, 48% are unique to a single condition (Kaul et al., 2014). Loss of oligomerization potential therefore, while preserving some aspects of wild-type Gro binding patterns, does disrupt Groucho association with chromatin in some contexts, the nature of which remains unexplained.

The WD-domain is the second conserved domain of Gro and comprises the C-terminal 329 amino acids of the protein. The WD-domain consists of a seven-bladed β-propeller domain and is responsible for the majority of Groucho interactions with DNA-binding repressors (Table 1-1) (Pickles et al., 2002). The majority of these interactions are mediated through binding of the WD-domain to short peptide motifs (Jennings et al., 2006), which are recognized by the central pore of the propeller domain. Several such peptide motifs have been identified in Groucho-interacting proteins. The majority of these peptide motifs fall into one of two categories. C-terminal WRPW/Y recognition sequences have been found in Hairy/Enhancer of split (HES) and Runt family transcription factors (Aronson et al., 1997; Canon and Banerjee, 2003; Fisher et al., 1996; Jimenez et al., 1997; Paroush et al., 1994). And the engrailed homology domain-1 (eh1) motif is an internal peptide motif with the consensus sequence FxIxxIL that is found in Engrailed, Dorsal, Odd-skipped, and Goosecoid, among others (Copley, 2005; Dubnicoff et al., 1997; Jiménez et al., 1997; Jimenez et al., 1999; Smith and Jaynes, 1996; Tolkunova et al., 1998). The WD domain binds to these motifs with differing affinities. These differences in affinity are utilized to control the recruitment of Groucho to specific factors. For example, the affinity of Groucho for binding the eh1-like motif of Dorsal is relatively weak (Flores-Saaib and Courey, 2000), necessitating the assistance of additional factors in facilitating a stable interaction between the two proteins. This weak affinity of the Dorsal/Groucho interaction is crucial to allowing Dorsal to function as a bifunctional transcription factor, as mutation of this motif to a higher-affinity sequence abolishes Dorsal’s ability to activate genes in the embryo due to constitutive recruitment of Groucho (Ratnaparkhi et al., 2006).

The WD-repeat domain may be involved in additional protein interactions. Studies of Grg3, a mouse Gro/TLE family member, have shown that the WD domain is critical for binding to histone arrays *in vitro* as well as condensation of these arrays(Sekiya and Zaret, 2007). The observation that the Q domain is also capable of strong interaction with K20 methylated H4 tails suggests multiple levels of interaction between Gro/TLE proteins and histones, and may contribute to the protein’s ability to associate with histones both locally, at its recruitment site, and distantly, through association with non-contiguous stretches of chromatin (Chodaparambil et al., 2014b).

The central region of Groucho is divided into three domains, the GP, CcN, and SP domains. The GP domain binds to a histone deacetylase (HDAC1/Rpd3), which is involved with some but not all Groucho-repressive activity (Chen et al., 1999). The CcN domain is involved in Groucho regulation, containing multiple Ck2 and Cdc2 phosphorylation sites (Nuthall et al., 2002). The SP domain contains multiple sites phosphorylated in response to MAPK signaling, resulting in down-regulation of Groucho activity (Hasson et al., 2005). There is evidence that the central regions of Groucho are intrinsically disordered (Turki-Judeh and Courey, 2012b), which has emerged as a common strategy among eukaryotic proteins to facilitate participation in diverse protein-protein interactions, expose signaling motifs, and/or accept posttranslational modifications (Dunker et al., 2008).

*Groucho integrates multiple signaling pathways to generate specific cellular responses and fates*

In *Drosophila,* Groucho’s roles in responses to signaling pathways are well documented. The factor participates in Ras/MAPK, Notch, Decapentapletic (Dpp/BMP), and Wingless/Wnt signaling, among others. Groucho activity is down-regulated via the Ras/MAPK pathway in response to signals initiated at multiple receptor tyrosine kinases (RTKs) such as EGFR, FGFR, and Torso (Cinnamon and Paroush, 2008; Hasson et al., 2005). The resulting relief of Groucho-mediated repression is critical to the cellular response to RTK signaling and is thought to precipitate in cellular memory, whereby the attenuation of Groucho activity persists after loss of signaling (Cinnamon and Paroush, 2008; Helman et al., 2011).

In the absence of Notch signaling, Groucho represses *E(spl)* complex genes through interactions with Hairy, which is itself associated with Su(H), a sequence-specific transcription factor that targets Notch-responsive genes (Delidakis et al., 1991). Recruitment of a Notch ligand to Notch transmembrane receptors activates the pathway, leading to proteolytic cleavage of the receptor and subsequent release of the Notch Intracellular Domain (Notch ICD). The Notch ICD rapidly enters the nucleus, where it displaces Hairy binding at Su(H) sites, relieving Groucho repression and initiating expression of *E(spl)* genes. Groucho then interacts with newly expressed E(spl) family proteins to repress a number of proneural genes (Preiss et al., 1988; Wurmbach et al., 1999). This repressive activity is alleviated by MAPK signaling, which results in the phosphorylation of Gro, negatively affecting its ability to repress these proneural genes in cooperation with E(spl) members (Andersson et al., 2011). The partial or complete negation of Notch signaling through the activation of the MAPK pathway thus represents a Groucho-mediated point of crosstalk between the two pathways (Hasson et al., 2005).

Groucho is also critical to signaling via Decapentaplegic (dpp), a *Drosophila* TGF-β homolog whose diffusion over long distances is essential to patterning during embryogenesis and later during appendage development (Upadhyai and Campbell, 2013). The Dpp morphogen is expressed dorsally in the embryo and is required for the definition of cell-fate along the dorsal-ventral axis (Ferguson and Anderson, 1992). Groucho, through interaction with Dorsal, represses ventral expression of *dpp*, meaning that Gro is involved in both the spatiotemporal definition and interpretation of dpp signaling(Schwyter et al., 1995)*.* In the absence of Dppsignaling, Brinker (Brk) represses a subset of dpp target genes through two independent repressive mechanisms, one involving dCtBP (a short-range corepressor), and the other involving Gro (Hasson et al., 2001). Upon activation of Dpp signaling, Brinker becomes repressed by Schnurri in dorsal regions of the embryo, while continuing to be expressed in ventrolateral regions (Marty et al., 2000).

Finally, Groucho participates in Wingless/Wnt signaling, through interactions with Tcf/Lef family proteins, to regulate cell-fate choice (Cavallo et al., 1998)(Roose and Clevers, 1999). In unstimulated cells, Groucho assists in repressing Tcf/Lef target genes through interactions with the Q-domain (Clevers, 2006). Upon Wnt activation, nuclear beta-catenin (Armadillo) concentration increases, which binds to Tcf, releasing Groucho and leading to gene activation. In this context, Groucho is essential in guarding against spurious activation of Wnt target genes in unstimulated cells (Daniels and Weis, 2005).

While there are hundreds of cell types in the adult fly, far fewer developmental signaling pathways have been documented (Perrimon et al., 2012). To generate this cellular complexity, informational content from multiple extracellular signals must be interpreted within each cell’s specific spatial and temporal context (Hsueh et al., 2009). Even with this ability to simultaneously respond to multiple signals, the high number of discrete transcriptional states required during development necessitates that these signals are integrated non-additively (Housden and Perrimon, 2014). Factors that participate in multiple signaling pathways, such as Groucho, are a necessary component of a non-additive response. Groucho therefore presents a convenient node through which a cell can process limited combinations of inputs to produce a larger number of outcomes.

*Groucho is an essential component of the embryonic axial patterning network*

It is primarily through the spatially and temporally controlled regulation of gene transcription that Groucho becomes fundamental to embryonic patterning. Many early embryonic patterning proteins can be divided into effectors of the dorsal-ventral and anterior-posterior programs, though these processes are complex and highly interconnected (Jaeger et al., 2012), requiring the coordinated regulation of dozens of transcriptional activators, repressors, and co-regulators (Mannervik, 2014). Definition of the dorsal-ventral axis, which is critical to germ layer development, is carried out by the maternally-contributed gradient of nuclear Dorsal along this axis (Roth et al., 1989). Dorsal is a sequence-specific transcription factor, and the strength, spacing, and grouping of Dorsal binding sites, along with the distribution of adjacent binding sites for other interacting factors modulate Dorsal binding and cofactor recruitment in order to correctly interpret the Dorsal gradient (Zeitlinger et al., 2007).

On the ventral side of the embryo, high concentrations of nuclear Dorsal initiate transcriptional programs that determine the mesoderm (Gonzalez-Crespo and Levine, 1993). In ventrolateral regions, modest Dorsal concentrations help direct a neuroectodermal fate (Ip et al., 1992). Dorsal also acts as a repressor of dorsal ectodermal genes and, by keeping them off in ventral and ventrolateral region, it restricts their expression to the dorsal ectodermal primordium {Zeitlinger, 2007 #3025}. Groucho is required for this repression and plays a critical role in switching Dorsal from an activator to a repressor (Dubnicoff et al., 1997).

In addition to its roles in dorsal/ventral patterning, Groucho has multiple roles in anterior/posterior pattern formation. For example, it is required for repression by numerous segmentation gene products such as Hairy, Runt, and Engrailed (Levine, 2008). Groucho is also required for the patterning of the anterior and posterior terminal domains by the Torso RTK through its interaction with Capicua (Ajuria et al., 2011), a process regulated by Ras/MAPK signaling (Chen et al., 2009; Paroush et al., 1997). Capicua recruits Gro to *tailless* and *huckebein* throughout the embryo maintaining these genes in an off state. Torso RTK then activates Ras/MAPK signaling at the termini leading to the phosphorylation and consequent inactivation of both Capicua and Gro at the embryonic termini allowing the expression of *tll* and *hkb* as required for specification of terminal fate (Winkler et al., 2010).

*Groucho is capable of both short- and long-range repression*

Transcriptional repressors in *Drosophila* can be classified as acting as either short- or long-range repressors dependent on their ability to counteract the regulatory potential of local (within ~100 bp) or distal (thousands of bp away or more) activating elements or promoters (Gray and Levine, 1996; Gray et al., 1994). Some repressors are specific for one type of repression, while others can adopt a short- or long-range repressive activity through association with multiple corepressors operating via distinct mechanisms of repression (Courey and Jia, 2001). Groucho was originally considered a long-range co-repressor recruited exclusively by long-range repressors such as Hairy and Dorsal (Cai et al., 1996; Dubnicoff et al., 1997). CtBP, in contrast, is a well-studied corepressor capable of short-range repression when recruited by such short-range repressors as Kruppel, Giant, and Snail (Nibu and Levine, 2001; Nibu et al., 1998). Evidence that Groucho could oligomerize and potentially crosslink non-contiguous regions of chromatin provides a mechanistic explanation for its ability to quench distant regulatory elements.

More recently, it was found that in some contexts Groucho behaves as a short-range corepressor. Groucho appears to be recruited by Knirps, a short-range repressor capable of interacting with CtBP, to repress the expression of *even-skipped* (Payankaulam and Arnosti, 2009)*.* Sloppy-paired 1 (Slp1), a Groucho-interacting repressor, is involved in the short-range repression of regulatory elements controlling the expression of multiple pair-rule genes (Andrioli et al., 2004). If Groucho is in fact commonly utilized as both a short- and long-range repressor, this sheds light on the observation that Groucho oligomerization is required in a context-dependent manner *in vivo* (Jennings et al., 2007), suggesting a mechanism whereby Groucho oligomerization is necessary for long-range repression but dispensable for short-range. Likely the classification of repressors as short- and long-range actors, while a useful abstraction when classifying repressors, masks much of the complexity of repressive activity that would be provided by a thorough understanding of repressive mechanisms.

*The mechanism of Groucho-mediated repression*

While a great deal is known about the developmental participation and interactors of Gro, details of the mechanism by which Gro achieves repression have remained elusive. Multiple models have been proposed to explain Groucho’s ability to fully and reversibly initiate and maintain both short- and long-range repression, yet a full picture, able to account for all observations of Groucho behavior, has yet to emerge.

Much of the speculation surrounding Groucho activity centers on the possibility that oligomeric structures of Gro form *in vivo*, how these structures interact with chromatin, and what relevance these structures have to repression. Early evidence showed that Groucho tetramerizes *in vitro* via the Q-domain (Chen et al., 1998)(Song et al., 2004). In another experiment, Groucho was found to be associated with chromatin over 2 kb away from its recruitment site, leading to the hypothesis that Groucho spreads from its recruitment site, analogous to the spreading activity of Sir family corepressors (Pirrotta and Gross, 2005). Experiments on a mouse Gro homolog showed that while tetramerization is not required for recruitment to chromatin, it is necessary for the aggregation of nucleosomal arrays in vitro (Sekiya and Zaret, 2007). Monomeric forms of the protein successfully bind to and increase the density of dinucleosomes *in vitro* (Sekiya and Zaret, 2007)*.*  *In vivo,* the loss of tetramerization is lethal but does not entirely abolish Gro-mediated repression (Jennings et al., 2007). More recent evidence in cell culture has shown that Gro binds in discrete peaks, though longer stretches of binding do occur (Kaul et al., 2014).

Gro preferentially associates with histone tails and can do so without the involvement of additional DNA-binding interacting factors (Flores-Saaib and Courey, 2000; Sekiya and Zaret, 2007). Additionally, Gro associates with a histone deacetylase, HDAC1/Rpd3 (Chen et al., 1999). This association accounts for some but not all of Groucho’s repressive ability *in vivo*, where Groucho binding is associated with decreased acetylation of the tails of histones H3 and H4, as well as increased nucleosome density(Winkler et al., 2010). Colocalization of Gro and Rpd3 is prevalent in Kc167 cells (a cell line derived from *Drosophila* embryos), with over half of Groucho binding sites found to overlap Rpd3 binding (Kaul et al., 2014).

Given the many gaps in our knowledge regarding the mechanisms of Gro-mediated repression, I carried out a genome-wide analysis of Gro function in hopes of filling in some of these gaps. Experiments described in Chapter 2, employing a combination of Gro-ChIP-seq on staged wild-type embryos and RNA-seq on staged embryos expressing different levels of Gro show that Groucho associates with chromatin in discrete < 1 kilobase peaks, often clustered closely upstream or within regulated genes. This data was used to generate a set of high-confidence Groucho targets at multiple developmental stages. Experiments described in Chapter 3, employing nascent-seq on staged wild-type embryos show that Groucho-regulated genes are enriched for promoter-proximal paused polymerase, suggesting a possible role for PolII stalling in Groucho-mediated gene repression. Chapter 4 is a published paper in which we identified the Gro interactome as a way of illuminating mechanisms of Gro-mediated repression.

**Figure 1-1. Groucho/TLE family proteins are partially conserved throughout metazoans.** The Gro/TLE family of corepressors are typified by five domains defined based on function and sequence. Domain-wise homology to the *D. melanogaster* Groucho is indicated by percentages, when significant. Two domains, the N-terminal Q domain and the C-terminal WD-repeat domain are well conserved while the central region, consisting of the GP, CcN, and SP domains shares little sequence homology between species. The Q domain is involved in association with repressor and the formation of homo-oligomeric Groucho complexes. The WD domain is additionally involved in repressor association. The central region is predicted to be intrinsically disordered and serves as a scaffold for a number of protein interactions, notably with Rpd3, a histone deacetylase involved in some aspects of Groucho-mediated repression. The central regions also serve as a regulatory region of Groucho via being target for multiple post-translational modifications.

**Fig. 1-1ch1_introduction.figures/ch1_introduction.figures.split.1.pdf**

**Table 1-1. Groucho-interacting transcription factors**

|  |  |  |
| --- | --- | --- |
| **Interacting Protein** | **Biological Role** | **Citation** |
| Capicua | RTK signaling; embryonic terminal gene expression | (Jimenez et al., 2000) |
| Huckebein | Embryonic terminal gene expression | (Goldstein et al., 1999) |
| Hairy | Segmentation/ Anterior-posterior patterning | (Paroush et al., 1994) |
| Runt | Segmentation/ Anterior-posterior patterning | (Aronson et al., 1997) |
| Even-skipped | Segmentation/ Anterior-posterior patterning | (Kobayashi et al., 2001) |
| Odd-skipped | Segmentation/ Anterior-posterior patterning | (Goldstein et al., 2005) |
| Sloppy-paired 1 | Segmentation/ Anterior-posterior patterning | (Andrioli et al., 2004) |
| Engrailed | Segmentation/ Anterior-posterior patterning | (Jimenez et al., 1997) |
| Knirps | Segmentation/ Anterior-posterior patterning | (Payankaulam and Arnosti, 2009) |
| Goosecoid | Segmentation/ Anterior-posterior patterning | (Jimenez et al., 1999) |
| Dorsal | Dorsal-ventral patterning | (Dubnicoff et al., 1997) |
| Brinker | Dorsal-ventral patterning | (Zhang et al., 2001) |
| Ind | Dorsal-ventral patterning | (Von Ohlen et al., 2007) |
| Vnd | Dorsal-ventral patterning | (Cowden and Levine, 2003) |
| Su(H) | Notch signaling | (Barolo et al., 2002) |

**References**

Ajuria, L., Nieva, C., Winkler, C., Kuo, D., Samper, N., Andreu, M.J., Helman, A., Gonzalez-Crespo, S., Paroush, Z., Courey, A.J.*, et al.* (2011). Capicua DNA-binding sites are general response elements for RTK signaling in Drosophila. Development (Cambridge, England) *138*, 915-924.

Andersson, E.R., Sandberg, R., and Lendahl, U. (2011). Notch signaling: simplicity in design, versatility in function. Development *138*, 3593-3612.

Andrioli, L.P., Oberstein, A.L., Corado, M.S., Yu, D., and Small, S. (2004). Groucho-dependent repression by sloppy-paired 1 differentially positions anterior pair-rule stripes in the Drosophila embryo. Dev Biol *276*, 541-551.

Aronson, B.D., Fisher, A.L., Blechman, K., Caudy, M., and Gergen, J.P. (1997). Groucho-dependent and -independent repression activities of Runt domain proteins. Mol Cell Biol *17*, 5581-5587.

Bajoghli, B., Aghaallaei, N., and Czerny, T. (2005). Groucho corepressor proteins regulate otic vesicle outgrowth. Dev Dyn *233*, 760-771.

Barolo, S., Stone, T., Bang, A.G., and Posakony, J.W. (2002). Default repression and Notch signaling: Hairless acts as an adaptor to recruit the corepressors Groucho and dCtBP to Suppressor of Hairless. Genes Dev *16*, 1964-1976.

Brantjes, H., Roose, J., van De Wetering, M., and Clevers, H. (2001). All Tcf HMG box transcription factors interact with Groucho-related co-repressors. Nucleic Acids Res *29*, 1410-1419.

Cai, H.N., Arnosti, D.N., and Levine, M. (1996). Long-range repression in the Drosophila embryo. Proc Natl Acad Sci U S A *93*, 9309-9314.

Canon, J., and Banerjee, U. (2003). In vivo analysis of a developmental circuit for direct transcriptional activation and repression in the same cell by a Runx protein. Genes Dev *17*, 838-843.

Cavallo, R.A., Cox, R.T., Moline, M.M., Roose, J., Polevoy, G.A., Clevers, H., Peifer, M., and Bejsovec, A. (1998). Drosophila Tcf and Groucho interact to repress Wingless signalling activity. Nature *395*, 604-608.

Chen, G., and Courey, A.J. (2000). Groucho/TLE family proteins and transcriptional repression. Gene *249*, 1-16.

Chen, G., Fernandez, J., Mische, S., and Courey, A.J. (1999). A functional interaction between the histone deacetylase Rpd3 and the corepressor groucho in Drosophila development. Genes Dev *13*, 2218-2230.

Chen, G., Nguyen, P., and Courey, A. (1998). A role for Groucho tetramerization in transcriptional repression. Molecular and Cellular Biology *18*, 7259.

Chen, Y.C., Lin, S.I., Chen, Y.K., Chiang, C.S., and Liaw, G.J. (2009). The Torso signaling pathway modulates a dual transcriptional switch to regulate tailless expression. Nucleic Acids Res *37*, 1061-1072.

Chodaparambil, J.V., Pate, K.T., Hepler, M.R., Tsai, B.P., Muthurajan, U.M., Luger, K., Waterman, M.L., and Weis, W.I. (2014a). Molecular functions of the TLE tetramerization domain in Wnt target gene repression. EMBO J *33*, 719-731.

Chodaparambil, J.V., Pate, K.T., Hepler, M.R.D., Tsai, B.P., Muthurajan, U.M., Luger, K., Waterman, M.L., and Weis, W.I. (2014b). Molecular functions of the TLE tetramerization domain in Wnt target gene repression. The EMBO Journal *33*, 719-731.

Choudhury, B.K., Kim, J., Kung, H.F., and Li, S.S. (1997). Cloning and developmental expression of Xenopus cDNAs encoding the Enhancer of split groucho and related proteins. Gene *195*, 41-48.

Cinnamon, E., and Paroush, Z. (2008). Context-dependent regulation of Groucho/TLE-mediated repression. Current opinion in genetics &amp; development *18*, 435-440.

Clevers, H. (2006). Wnt/beta-catenin signaling in development and disease. Cell *127*, 469-480.

Copley, R.R. (2005). The EH1 motif in metazoan transcription factors. BMC Genomics *6*, 169.

Courey, A.J., and Jia, S. (2001). Transcriptional repression: the long and the short of it. Genes Dev *15*, 2786-2796.

Cowden, J., and Levine, M. (2003). Ventral dominance governs sequential patterns of gene expression across the dorsal-ventral axis of the neuroectoderm in the Drosophila embryo. Dev Biol *262*, 335-349.

Dang, J., Inukai, T., Kurosawa, H., Goi, K., Inaba, T., Lenny, N.T., Downing, J.R., Stifani, S., and Look, A.T. (2001). The E2A-HLF oncoprotein activates Groucho-related genes and suppresses Runx1. Mol Cell Biol *21*, 5935-5945.

Daniels, D.L., and Weis, W.I. (2005). Beta-catenin directly displaces Groucho/TLE repressors from Tcf/Lef in Wnt-mediated transcription activation. Nat Struct Mol Biol *12*, 364-371.

Delidakis, C., Preiss, A., Hartley, D.A., and Artavanis-Tsakonas, S. (1991). Two genetically and molecularly distinct functions involved in early neurogenesis reside within the Enhancer of split locus of Drosophila melanogaster. Genetics *129*, 803-823.

Dubnicoff, T., Valentine, S.A., Chen, G., Shi, T., Lengyel, J.A., Paroush, Z., and Courey, A.J. (1997). Conversion of dorsal from an activator to a repressor by the global corepressor Groucho. Genes &amp; Development *11*, 2952-2957.

Dunker, A.K., Oldfield, C.J., Meng, J., Romero, P., Yang, J.Y., Chen, J.W., Vacic, V., Obradovic, Z., and Uversky, V.N. (2008). The unfoldomics decade: an update on intrinsically disordered proteins. BMC Genomics *9 Suppl 2*, S1.

Ferguson, E.L., and Anderson, K.V. (1992). Decapentaplegic acts as a morphogen to organize dorsal-ventral pattern in the Drosophila embryo. Cell *71*, 451-461.

Fisher, A.L., Ohsako, S., and Caudy, M. (1996). The WRPW motif of the hairy-related basic helix-loop-helix repressor proteins acts as a 4-amino-acid transcription repression and protein-protein interaction domain. Mol Cell Biol *16*, 2670-2677.

Flores-Saaib, R., and Courey, A. (2000). Analysis of Groucho–histone interactions suggests mechanistic similarities between Groucho-and Tup1-mediated repression. Nucleic Acids Research *28*, 4189.

Gasperowicz, M., and Otto, F. (2005). Mammalian Groucho homologs: redundancy or specificity? J Cell Biochem *95*, 670-687.

Goldstein, R.E., Cook, O., Dinur, T., Pisante, A., Karandikar, U.C., Bidwai, A., and Paroush, Z. (2005). An eh1-like motif in odd-skipped mediates recruitment of Groucho and repression in vivo. Mol Cell Biol *25*, 10711-10720.

Goldstein, R.E., Jimenez, G., Cook, O., Gur, D., and Paroush, Z. (1999). Huckebein repressor activity in Drosophila terminal patterning is mediated by Groucho. Development *126*, 3747-3755.

Gonzalez-Crespo, S., and Levine, M. (1993). Interactions between dorsal and helix-loop-helix proteins initiate the differentiation of the embryonic mesoderm and neuroectoderm in Drosophila. Genes Dev *7*, 1703-1713.

Gray, S., and Levine, M. (1996). Short-range transcriptional repressors mediate both quenching and direct repression within complex loci in Drosophila. Genes Dev *10*, 700-710.

Gray, S., Szymanski, P., and Levine, M. (1994). Short-range repression permits multiple enhancers to function autonomously within a complex promoter. Genes Dev *8*, 1829-1838.

Hasson, P., Egoz, N., Winkler, C., Volohonsky, G., Jia, S., Dinur, T., Volk, T., Courey, A.J., and Paroush, Z. (2005). EGFR signaling attenuates Groucho-dependent repression to antagonize Notch transcriptional output. Nat Genet *37*, 101-105.

Hasson, P., Muller, B., Basler, K., and Paroush, Z. (2001). Brinker requires two corepressors for maximal and versatile repression in Dpp signalling. EMBO J *20*, 5725-5736.

Helman, A., Cinnamon, E., Mezuman, S., Hayouka, Z., Von Ohlen, T., Orian, A., Jiménez, G., and Paroush, Z.a.e. (2011). Phosphorylation of Groucho Mediates RTK Feedback Inhibition and Prolonged Pathway Target Gene Expression. Current Biology *21*, 1102-1110.

Housden, B.E., and Perrimon, N. (2014). Spatial and temporal organization of signaling pathways. Trends Biochem Sci *39*, 457-464.

Hsueh, R.C., Natarajan, M., Fraser, I., Pond, B., Liu, J., Mumby, S., Han, H., Jiang, L.I., Simon, M.I., Taussig, R.*, et al.* (2009). Deciphering signaling outcomes from a system of complex networks. Sci Signal *2*, ra22.

Ip, Y.T., Park, R.E., Kosman, D., Bier, E., and Levine, M. (1992). The dorsal gradient morphogen regulates stripes of rhomboid expression in the presumptive neuroectoderm of the Drosophila embryo. Genes Dev *6*, 1728-1739.

Jaeger, J., Manu, and Reinitz, J. (2012). Drosophila blastoderm patterning. Curr Opin Genet Dev *22*, 533-541.

Javed, A., Guo, B., Hiebert, S., Choi, J.Y., Green, J., Zhao, S.C., Osborne, M.A., Stifani, S., Stein, J.L., Lian, J.B.*, et al.* (2000). Groucho/TLE/R-esp proteins associate with the nuclear matrix and repress RUNX (CBF(alpha)/AML/PEBP2(alpha)) dependent activation of tissue-specific gene transcription. J Cell Sci *113 ( Pt 12)*, 2221-2231.

Jennings, B.H., Pickles, L.M., Wainwright, S.M., Roe, S.M., Pearl, L.H., and Ish-Horowicz, D. (2006). Molecular recognition of transcriptional repressor motifs by the WD domain of the Groucho/TLE corepressor. Mol Cell *22*, 645-655.

Jennings, B.H., Wainwright, S.M., and Ish-Horowicz, D. (2007). Differential in vivo requirements for oligomerization during Groucho-mediated repression. EMBO reports *9*, 76-83.

Jimenez, G., Guichet, A., Ephrussi, A., and Casanova, J. (2000). Relief of gene repression by torso RTK signaling: role of capicua in Drosophila terminal and dorsoventral patterning. Genes Dev *14*, 224-231.

Jimenez, G., Paroush, Z., and Ish-Horowicz, D. (1997). Groucho acts as a corepressor for a subset of negative regulators, including Hairy and Engrailed. Genes Dev *11*, 3072-3082.

Jiménez, G., Paroush, Z., and Ish-Horowicz, D. (1997). Groucho acts as a corepressor for a subset of negative regulators, including Hairy and Engrailed. Genes &amp; Development *11*, 3072.

Jimenez, G., Verrijzer, C.P., and Ish-Horowicz, D. (1999). A conserved motif in goosecoid mediates groucho-dependent repression in Drosophila embryos. Mol Cell Biol *19*, 2080-2087.

Kaul, A., Schuster, E., and Jennings, B.H. (2014). The Groucho Co-repressor Is Primarily Recruited to Local Target Sites in Active Chromatin to Attenuate Transcription. PLoS Genetics *10*, e1004595.

Kobayashi, M., Goldstein, R.E., Fujioka, M., Paroush, Z., and Jaynes, J.B. (2001). Groucho augments the repression of multiple Even skipped target genes in establishing parasegment boundaries. Development *128*, 1805-1815.

Kuo, D., Nie, M., De Hoff, P., Chambers, M., Phillips, M., Hirsch, A.M., and Courey, A.J. (2011). A SUMO-Groucho Q domain fusion protein: characterization and in vivo Ulp1-mediated cleavage. Protein expression and purification *76*, 65-71.

Lee, J.E., and Golz, J.F. (2012). Diverse roles of Groucho/Tup1 co-repressors in plant growth and development. Plant Signaling &amp; Behavior *7*, 86-92.

Levine, M. (2008). A systems view of Drosophila segmentation. Genome Biol *9*, 207.

Li, S.S. (2000). Structure and function of the Groucho gene family and encoded transcriptional corepressor proteins from human, mouse, rat, Xenopus, Drosophila and nematode. Proc Natl Sci Counc Repub China B *24*, 47-55.

Lindsley, D.L., Zimm, Georgianna G. (1968). Genetic Variations of Drosophila melanogaster, Vol 627 (Carnegie Institution of Washingtion Publication).

Mallo, M., Franco del Amo, F., and Gridley, T. (1993). Cloning and developmental expression of Grg, a mouse gene related to the groucho transcript of the Drosophila Enhancer of split complex. Mech Dev *42*, 67-76.

Mannervik, M. (2014). Control of Drosophila embryo patterning by transcriptional co-regulators. Experimental cell research *321*, 47-57.

Marcal, N., Patel, H., Dong, Z., Belanger-Jasmin, S., Hoffman, B., Helgason, C.D., Dang, J., and Stifani, S. (2005). Antagonistic effects of Grg6 and Groucho/TLE on the transcription repression activity of brain factor 1/FoxG1 and cortical neuron differentiation. Mol Cell Biol *25*, 10916-10929.

Marty, T., Muller, B., Basler, K., and Affolter, M. (2000). Schnurri mediates Dpp-dependent repression of brinker transcription. Nat Cell Biol *2*, 745-749.

Metzger, D.E., Gasperowicz, M., Otto, F., Cross, J.C., Gradwohl, G., and Zaret, K.S. (2012). The transcriptional co-repressor Grg3/Tle3 promotes pancreatic endocrine progenitor delamination and  -cell differentiation. Development (Cambridge, England) *139*, 1447-1456.

Miyasaka, H., Choudhury, B.K., Hou, E.W., and Li, S.S. (1993). Molecular cloning and expression of mouse and human cDNA encoding AES and ESG proteins with strong similarity to Drosophila enhancer of split groucho protein. Eur J Biochem *216*, 343-352.

Muhr, J., Andersson, E., Persson, M., Jessell, T.M., and Ericson, J. (2001). Groucho-mediated transcriptional repression establishes progenitor cell pattern and neuronal fate in the ventral neural tube. Cell *104*, 861-873.

Nibu, Y., and Levine, M.S. (2001). CtBP-dependent activities of the short-range Giant repressor in the Drosophila embryo. Proc Natl Acad Sci U S A *98*, 6204-6208.

Nibu, Y., Zhang, H., and Levine, M. (1998). Interaction of short-range repressors with Drosophila CtBP in the embryo. Science *280*, 101-104.

Nuthall, H.N., Joachim, K., Palaparti, A., and Stifani, S. (2002). A role for cell cycle-regulated phosphorylation in Groucho-mediated transcriptional repression. J Biol Chem *277*, 51049-51057.

Paroush, Z., Finley, R.L., Jr., Kidd, T., Wainwright, S.M., Ingham, P.W., Brent, R., and Ish-Horowicz, D. (1994). Groucho is required for Drosophila neurogenesis, segmentation, and sex determination and interacts directly with hairy-related bHLH proteins. Cell *79*, 805-815.

Paroush, Z., Wainwright, S.M., and Ish-Horowicz, D. (1997). Torso signalling regulates terminal patterning in Drosophila by antagonising Groucho-mediated repression. Development *124*, 3827-3834.

Payankaulam, S., and Arnosti, D.N. (2009). Groucho corepressor functions as a cofactor for the Knirps short-range transcriptional repressor. Proceedings of the National Academy of Sciences *106*, 17314-17319.

Perrimon, N., Pitsouli, C., and Shilo, B.Z. (2012). Signaling mechanisms controlling cell fate and embryonic patterning. Cold Spring Harb Perspect Biol *4*, a005975.

Pflugrad, A., Meir, J.Y., Barnes, T.M., and Miller, D.M., 3rd (1997). The Groucho-like transcription factor UNC-37 functions with the neural specificity gene unc-4 to govern motor neuron identity in C. elegans. Development *124*, 1699-1709.

Pickles, L.M., Roe, S.M., Hemingway, E.J., Stifani, S., and Pearl, L.H. (2002). Crystal structure of the C-terminal WD40 repeat domain of the human Groucho/TLE1 transcriptional corepressor. Structure *10*, 751-761.

Pirrotta, V., and Gross, D.S. (2005). Epigenetic silencing mechanisms in budding yeast and fruit fly: different paths, same destinations. Mol Cell *18*, 395-398.

Preiss, A., Hartley, D.A., and Artavanis-Tsakonas, S. (1988). The molecular genetics of Enhancer of split, a gene required for embryonic neural development in Drosophila. EMBO J *7*, 3917-3927.

Ratnaparkhi, G.S., Jia, S., and Courey, A.J. (2006). Uncoupling dorsal-mediated activation from dorsal-mediated repression in the Drosophila embryo. Development *133*, 4409-4414.

Roose, J., and Clevers, H. (1999). TCF transcription factors: molecular switches in carcinogenesis. Biochim Biophys Acta *1424*, M23-37.

Roth, S., Stein, D., and Nüsslein-Volhard, C. (1989). A gradient of nuclear localization of the dorsal protein determines dorsoventral pattern in the Drosophila embryo. Cell *59*, 1189-1202.

Schmidt, C.J., and Sladek, T.E. (1993). A rat homolog of the Drosophila enhancer of split (groucho) locus lacking WD-40 repeats. J Biol Chem *268*, 25681-25686.

Schwyter, D.H., Huang, J.D., Dubnicoff, T., and Courey, A.J. (1995). The decapentaplegic core promoter region plays an integral role in the spatial control of transcription. Mol Cell Biol *15*, 3960-3968.

Sekiya, T., and Zaret, K.S. (2007). Repression by Groucho/TLE/Grg Proteins: Genomic Site Recruitment Generates Compacted Chromatin In Vitro and Impairs Activator Binding In Vivo. Molecular Cell *28*, 291-303.

Smith, R.L., and Johnson, A.D. (2000). Turning genes off by Ssn6-Tup1: a conserved system of transcriptional repression in eukaryotes. Trends Biochem Sci *25*, 325-330.

Smith, S.T., and Jaynes, J.B. (1996). A conserved region of engrailed, shared among all en-, gsc-, Nk1-, Nk2- and msh-class homeoproteins, mediates active transcriptional repression in vivo. Development *122*, 3141-3150.

Song, H., Hasson, P., Paroush, Z.a.e., and Courey, A.J. (2004). Groucho oligomerization is required for repression in vivo. Molecular and Cellular Biology *24*, 4341-4350.

Stifani, S., Blaumueller, C.M., Redhead, N.J., Hill, R.E., and Artavanis-Tsakonas, S. (1992). Human homologs of a Drosophila Enhancer of split gene product define a novel family of nuclear proteins. Nat Genet *2*, 119-127.

Tolkunova, E.N., Fujioka, M., Kobayashi, M., Deka, D., and Jaynes, J.B. (1998). Two distinct types of repression domain in engrailed: one interacts with the groucho corepressor and is preferentially active on integrated target genes. Mol Cell Biol *18*, 2804-2814.

Turki-Judeh, W., and Courey, A.J. (2012a). Groucho: A Corepressor with Instructive Roles in Development. In (Elsevier), pp. 65-96.

Turki-Judeh, W., and Courey, A.J. (2012b). The Unconserved Groucho Central Region Is Essential for Viability and Modulates Target Gene Specificity. PLoS ONE *7*, e30610.

Upadhyai, P., and Campbell, G. (2013). Brinker possesses multiple mechanisms for repression because its primary co-repressor, Groucho, may be unavailable in some cell types. Development (Cambridge, England) *140*, 4256-4265.

Villanueva, C.J., Waki, H., Godio, C., Nielsen, R., Chou, W.-L., Vargas, L., Wroblewski, K., Schmedt, C., Chao, L.C., Boyadjian, R.*, et al.* (2011). TLE3 Is a Dual-Function Transcriptional Coregulator of Adipogenesis. Cell Metabolism *13*, 413-427.

Von Ohlen, T., Syu, L.J., and Mellerick, D.M. (2007). Conserved properties of the Drosophila homeodomain protein, Ind. Mech Dev *124*, 925-934.

Winkler, C.J., Ponce, A., and Courey, A.J. (2010). Groucho-Mediated Repression May Result from a Histone Deacetylase-Dependent Increase in Nucleosome Density. PLoS ONE *5*, e10166.

Wulbeck, C.C.-O., J. A. (1997). Two zebrafish homologues of the Drosophila neurogenic gene groucho and their pattern of transcription during early embryogenesis. Dev Genes Evol *207*, 156-166.

Wurmbach, E., Wech, I., and Preiss, A. (1999). The Enhancer of split complex of Drosophila melanogaster harbors three classes of Notch responsive genes. Mech Dev *80*, 171-180.

Yao, J., Liu, Y., Husain, J., Lo, R., Palaparti, A., Henderson, J., and Stifani, S. (1998). Combinatorial expression patterns of individual TLE proteins during cell determination and differentiation suggest non-redundant functions for mammalian homologs of Drosophila Groucho. Dev Growth Differ *40*, 133-146.

Zeitlinger, J., Zinzen, R.P., Stark, A., Kellis, M., Zhang, H., Young, R.A., and Levine, M. (2007). Whole-genome ChIP-chip analysis of Dorsal, Twist, and Snail suggests integration of diverse patterning processes in the Drosophila embryo. Genes &amp; Development *21*, 385-390.

Zhang, H., Levine, M., and Ashe, H.L. (2001). Brinker is a sequence-specific transcriptional repressor in the Drosophila embryo. Genes Dev *15*, 261-266.