REVIEW

Transcriptional repression: the long and the short of it

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Gene-specific repression of transcription plays a central role in gene regulation. This is true for the spatial control of gene activity in development, during which boundaries of gene expression are often determined by the spatially restricted localization or activity of transcriptional repressors (Mannervik et al. 1999). It is also true for the control of gene expression by extracellular signals, in which genes are often maintained in an off state by repressor proteins until signal transduction alleviates the repression (e.g., Roose and Clevers 1999).

One of the most useful ways of categorizing repressors is according to whether they mediate long-range or short-range repression (Gray and Levine 1996b). In long-range repression, a repressor makes a promoter resistant to the influence of all enhancers, even if those enhancers are located thousands of base pairs from the repressor binding site. This kind of repression is often referred to as silencing because an entire chromosomal locus is inactivated. In contrast, short-range repressors function in a less general manner. Rather than interfering with all transcription at a locus, they block the function of nearby DNA-bound activators while not interfering with more distantly bound activators.

In this review, we discuss examples of both long-range and short-range repression, showing that long-range repression may often involve the assembly of a multiprotein complex termed a repressosome that is analogous in many ways to the enhanceosomes known to mediate activation. Furthermore, we discuss how both long-range and short-range repression may involve the recruitment of histone deacetylases to the template and discuss models that may allow these enzymes to mediate both types of repression. Finally, we consider the possibility that interactions between repressors and the basal machinery as well as between repressors and activators play roles in long-range and short-range repression.

Groucho/Tup1 superfamily proteins and Sir proteins as paradigms for long-range corepressors

One form of long-range repression that has been extensively studied is Groucho-mediated repression (Fisher and Caudy 1998; Parkhurst 1998; Mannervik et al. 1999; Chen and Courey 2000). As a corepressor, the Groucho protein does not bind to DNA directly, but is recruited to the template by protein–protein interactions with a variety of DNA-bound repressors. Groucho was first characterized in *Drosophila*, and Groucho orthologs are found in all metazoan organisms. Human Groucho family proteins are called transducin-like enhancer-of-split (TLE) proteins. Groucho family members are characterized by a highly conserved N-terminal tetramerization domain (sometimes termed the Q domain because of an abundance of glutamine residues), a weakly conserved central region, and a highly conserved C-terminal seven-WD-repeat domain. WD repeats are ~40 amino acid motifs that occur as tandem repeats in a wide variety of proteins, and frequently mediate protein–protein interactions (Neer et al. 1994).

Although corepressors containing both the conserved Q and WD-repeat domains are found in all metazoans, they are absent in fungi (Chen and Courey 2000). However, a variety of considerations suggest that Tup1 may be a yeast homolog of Groucho. Like Groucho, Tup1 is a widely used corepressor protein that forms a tetramer and contains a C-terminal seven-WD-repeat domain, although it lacks a region with obvious homology to the Q domain (Keleher et al. 1992; Jabet et al. 2000). A careful sequence analysis of the Groucho and Tup1 WD-repeat domains reveals serial homology, that is, when one pairs each repeat in Tup1 with the repeat it most closely resembles in Groucho, the repeats in the two proteins are found to fall in approximately the same order (Flores-Saaib and Courey 2000). Because of the similarities between Tup1 and the Groucho family, we collectively refer to these proteins as the Groucho/Tup1 superfamily.

The available evidence suggests that Groucho family proteins are long-range corepressors that silence transcription of linked promoters in a relatively indiscriminate manner (Barolo and Levine 1997). In particular, binding sites for Groucho-dependent repressors have been found to block promoter function in a distance- and orientation-independent manner. Tup1-dependent repressors also seem to work indiscriminately on nearby promoters in an orientation-independent manner (Smith and Johnson 2000). It should be noted, however, that in yeast, genes and enhancers are packed together much more tightly than they are in multicellular eukaryotes.

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Therefore, rarely do repressors need to work over distances of more than a few hundred base pairs. Nonetheless, because of the striking structural and functional similarities between Groucho and Tup1, we assume that lessons learned from studies of Tup1-mediated repression are at least partially applicable to Groucho-mediated repression.

Groucho recruitment by short peptide motifs

Groucho was initially recognized as a corepressor by virtue of its role in repression by Hairy family basic-helix–loop–helix domain-containing repressors (Paroush et al. 1994; Chen and Courey 2000). All Hairy family factors are characterized by a WRPW tetrapeptide motif at the C terminus that is necessary and sufficient for recruitment of Groucho. Groucho has since been shown to mediate repression by a variety of DNA-binding repressor proteins in addition to Hairy family factors. Many of these factors recruit Groucho using a variation on the Hairy family WRPW motif. For example, Runt family proteins contain a C-terminal WRPY motif that mediates Groucho recruitment and transcriptional repression. At least one additional short peptide motif, which appears to be unrelated to the WRPW motif, is involved in Groucho recruitment. This is the Engrailed homology-1 (eh1) motif found in Engrailed family transcriptional repressors, and also in the *Drosophila* repressor protein Goosecoid. Recent analysis of another Groucho-dependent repressor, Dorsal, reveals that it may contain a degenerate version of the eh1 motif (Flores-Saaib et al. 2001).

The Groucho repressosome

Eukaryotic enhancers often consist of adjacent binding sites for multiple activator proteins that work together in a synergistic fashion (Carey 1998; Merika and Thanos 2001). Activators bound to these adjacent sites are believed to form platforms for the cooperative recruitment of coactivators and/or for cooperative interactions with the general transcriptional machinery. These cooperatively assembling nucleoprotein complexes, consisting of enhancer DNA packaged into chromatin, activators, coactivators, and perhaps even components of the general machinery, are often referred to as enhanceosomes (Fig. 1). One hallmark of enhanceosomes is the need for the stereospecific alignment of DNA-binding sites. Binding sites must be spaced such that adjacently bound factors are aligned with one another on the same face of the helix, thereby allowing for the cooperative recruitment of coactivators. Changing the spacing between binding sites by a half-integral multiple of the DNA helical repeat length often has much more deleterious effects than does changing the spacing by an integral multiple of the helical repeat length. A second hallmark of enhanceosomes is the requirement for so-called architectural factors. These factors facilitate enhanceosome formation, in some cases by altering the curvature of the DNA to allow cooperative interactions between other enhanceosome components.

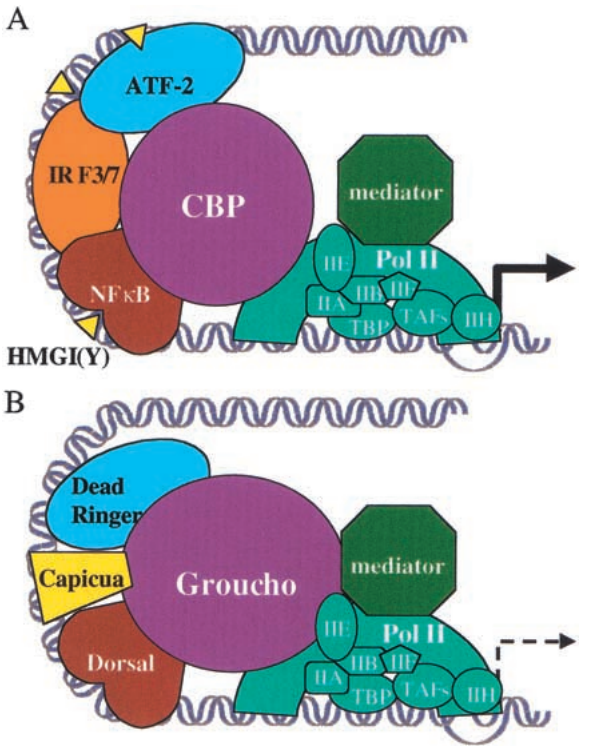

Figure 1. Groucho-mediated repression may be directed by a repressosome. (*A*) The β-interferon enhanceosome. Enhanceosome formation involves the cooperative assembly of a multiprotein complex containing the activators of NFκB, IRF3, IRF7, and ATF-2, the architectural factor HMGI(Y), and the coactivator CBP. CBP then activates transcription by multiple mechanisms including recruitment of the Pol II holoenzyme and covalent modification of chromatin through its function as a histone acetyl transferase. (*B*) The Groucho repressosome. The *zen* ventral repression region shown here contains binding sites for multiple DNA-binding proteins including Dorsal and Dead ringer, which may function together to cooperatively recruit Groucho to the template. Formation of the repressosome may be facilitated by Capicua, a possible architectural factor. Groucho may block the formation or function of the basal transcription complex by interacting with TFIIE, or as suggested by experiments on yeast Tup1, with components of the mediator complex.

Studies of Groucho-mediated repression suggest that large nucleoprotein complexes, in this case termed repressosomes, may also mediate repression. Strong although still circumstantial evidence that this might be the case comes from studies of Dorsal, a Groucho-dependent repressor that can also function as an activator of transcription (Dubnicoff et al. 1997). During the early development of the *Drosophila* embryo, Dorsal serves to activate some target genes (e.g., *twist* and *snail*) and repress others (e.g., *zerknüllt* [*zen*] and *decapentaplegic*) in the exact same cells (Ray et al. 1991). The ability of Dorsal to discriminate between targets that it should activate and targets that it should repress depends on the context of the Dorsal binding sites in any given target gene. For example, the *zen* gene contains a silencer in its 5′ flanking region, termed the ventral repression region

(VRR), that contains multiple Dorsal binding sites (Ip et al. 1991). In addition, this region contains other evolutionarily conserved elements (AT-rich elements) that are also required for repression. When the Dorsal sites are mutagenized, the VRR becomes nonfunctional (Jiang et al. 1992). However, when the AT-rich sites are mutagenized, the VRR becomes a Dorsal-dependent enhancer rather than a Dorsal-dependent silencer (Jiang et al. 1993; Kirov et al. 1993). Furthermore, as with enhancers that function through enhanceosomes, the spacing between the regulatory elements appears to be critical. Changing the distance between the AT-rich and Dorsal sites by a nonintegral multiple of the helical repeat distance prevents repression (Cai et al. 1996). These findings suggest that the assembly of a repressosome including Dorsal, Groucho, and other factors may be required to convert Dorsal from an activator to a silencer.

Further evidence for this Groucho repressosome comes from studies in which it was found that Dead ringer, an ARID-domain-containing transcription factor, binds some of the AT-rich sites in the *zen* VRR (Valentine et al. 1998). It was further shown that repression by a minimal *zen* VRR requires the function of the *dead ringer* gene. Finally, it was found that both Dorsal and Dead ringer bind to Groucho and that the two proteins function together in vitro in a greater than additive manner to recruit Groucho to the *zen* VRR. These findings suggest that repression by the VRR involves the action of multiple DNA-bound repressor proteins working together to cooperatively recruit Groucho to the template (Fig. 1).

In addition to the proteins mentioned above, the Groucho repressosome is likely to contain a protein called Capicua (Jimenez et al. 2000). The role of this protein in Groucho-mediated repression was discovered through studies of the *Drosophila* terminal pattern-forming system, which includes the Torso receptor tyrosine kinase (St. Johnston and Nusslein-Volhard 1992). When activated by an extraembryonic ligand present only at the poles of the embryo, Torso alleviates the repression of zygotically active genes such as *tailless* required for specification of the extreme anterior and posterior portions of the blastoderm fate map (Liaw et al. 1995). Genetic analysis indicates that both Capicua and Groucho are required for the repression of *tailless* in the absence of activated Torso (Paroush et al. 1997; Jimenez et al. 2000). In addition, like Groucho, Capicua is also required for Dorsal-mediated repression (Jimenez et al. 2000). Molecular analysis of the *capicua* gene shows that it encodes an HMG-box-containing transcription factor. Given that other proteins in this family (e.g., Lef1, HMG1, and HMG2) have been found to serve as architectural factors in enhanceosomes (Grosschedl et al. 1994), it is possible that Capicua serves an architectural role in the Groucho repressosome.

As mentioned above, Dorsal can function as either an activator or a repressor. In contrast, certain other Groucho-dependent repressors, such as Hairy and Engrailed, appear to be dedicated repressors. The difference between Dorsal on the one hand, and Hairy and Engrailed on the other hand, may be the affinity of the interaction with Groucho. As noted earlier, Dorsal contains a motif with homology to the eh1 motif originally characterized in Engrailed as being involved in Groucho-recruitment (Flores-Saaib et al. 2001). However, the Dorsal eh1 motif is a poor match for the consensus in that it is missing a critical phenylalanine residue. It is possible that this mutation in the Dorsal eh1 motif reduces the affinity for Groucho to such an extent that Dorsal can no longer recruit Groucho without assistance from the repressosome. In this regard, it is interesting to note that Capicua (the putative architectural component of the Groucho repressosome) is required for repression by Dorsal, but not for repression by the dedicated repressor Hairy (Jimenez et al. 2000).

Like Dorsal, Runt-family proteins are Groucho-dependent repressors that can also function as activators (Aronson et al. 1997). Recruitment of Groucho by these factors is at least partially dependent on a C-terminal WRPY motif that can be viewed as a degenerate version of the WRPW motif found in Hairy family proteins. It will therefore be interesting to determine if repression by Runt family proteins also requires the formation of a repressosome and if this repression is Capicua-dependent.

Although the repressosome appears to explain how a single factor can function as both an activator and a repressor, it does not by itself explain the mechanism of long-range repression. An understanding of how Groucho silences transcription requires a consideration of what is known about the interactions between the repressosome and other nuclear components, such as chromatin and the general transcriptional machinery.

Histone deacetylases in long-range repression

One way that repressors may mediate long-range repression is by organizing chromosomal domains into a transcriptionally silent state. Multiple clues suggest that Groucho/Tup1 superfamily proteins may function in this way. This includes evidence suggesting functional interactions between Groucho/Tup1 and histone deacetylases (Chen et al. 1999; Choi et al. 1999; Watson et al. 2000; Brantjes et al. 2001; Wu et al. 2001).

It is well established that the acetylation state of histones has a major influence on transcriptional activity (Struhl 1998). The eight histone subunits found in the nucleosome core particle each contains a globular C-terminal domain and an extended N-terminal tail. The globular domains coalesce to form the interior of the particle, whereas the N-terminal tails project outward and may be involved in the internucleosomal interactions that stabilize higher order chromatin structure (Kornberg and Lorch 1999). The controlled acetylation and deacetylation of highly conserved lysine side chains in the tails appear to play roles in the regulation of gene expression. Transcriptionally silent genes tend to be associated with hypoacetylated histones, whereas transcriptionally active genes tend to be associated with hyperacetylated histones. Although the mechanism by

which histone acetylation controls gene activity is unclear, one plausible idea is that acetylation disrupts internucleosomal contacts, resulting in a less compact form of chromatin that is more accessible to the transcriptional machinery. Alternatively or in addition, covalently modified histone tails could function to recruit chromatin-remodeling factors to the template (Jenuwein and Allis 2001).

In accord with the correlation between histone acetylation state and gene activity, a number of coactivators have been found to function as histone acetyl transferases (HATs), whereas a number of corepressors have been found to function as histone deacetylases (HDACs; Struhl 1998). One of the most widely studied corepressors with histone deacetylase activity is HDAC1 (encoded in *Drosophila* and yeast by the *rpd3* gene). This factor is usually found in one of several multisubunit complexes including the Sin3 and NuRD complexes (Knoepfler and Eisenman 1999).

In studies using Groucho as an affinity reagent to purify proteins from crude embryo extracts, HDAC1 was identified as a Groucho-interacting protein (Chen et al. 1999). Further analyses revealed the interaction to be direct and showed that a glycine/proline-rich region (the GP domain) in Groucho probably provides the interaction surface (Chen et al. 1999; Brantjes et al. 2001). Experiments in which Groucho was expressed in human cells showed that in addition to HDAC1, at least two additional components of the Sin3 complex, namely, Sin3 and RbAp48, coimmunoprecipitate with Groucho (Choi et al. 1999). Groucho may therefore serve as an adaptor between the Sin3 complex and DNA-bound transcriptional repressors. In this respect, Groucho is analogous to the corepressor SMRT, which mediates repression by nuclear hormone receptors (Knoepfler and Eisenman 1999), except that SMRT is believed to bind Sin3, but Groucho appears to bind directly to HDAC1.

Tissue culture studies provide evidence that the Groucho/HDAC1 interaction is functional. For example, in transfection assays, HDAC1 was found to enhance repression of reporter genes containing Gal4-binding sites by Gal4–Groucho fusion proteins. The function of HDAC1 in this assay was abrogated by a point mutation in the histone deacetylase active site, suggesting that deacetylase activity is required for function (Chen et al. 1999).

The genetic evidence for a functional interaction between *Drosophila* Groucho and HDAC1 is mixed. Simultaneously cutting the maternal dosage of *groucho* and *rpd3* in half results in a high level of lethality in *Drosophila* embryos and a high incidence of a bicaudal defect in which the anterior half of the embryo is replaced with a mirror image duplication of the posterior half (Chen et al. 1999). Although the molecular basis for this phenotype is unclear, it hints at a role for Groucho and HDAC1 in the localization of the components of the posterior maternal pattern-forming system (St. Johnston and Nusslein-Volhard 1992). On the other hand, the recessive maternal effect phenotype associated with a P-insertion allele of *rpd3* is much milder than the recessive maternal effect phenotype associated with strong *groucho* alleles (Mannervik and Levine 1999). A careful analysis of the *rpd3* phenotype suggests that it may be attributable to an inability of the pair-rule transcription factor Even skipped (Eve) to repress *odd* expression in the absence of HDAC1. For a number of reasons, however, this analysis does not rule out the possibility that HDAC1 plays a central role in Groucho-mediated repression. First, the available P-allele of *rpd3* is a hypomorphic allele and not a null. Owing to a requirement for HDAC1 in oogenesis, it is not readily possible to determine the null *rpd3* maternal effect phenotype (A.J. Courey and C. Winkler, unpubl.). Second, although Eve was originally thought to be a Groucho-independent repressor (Jimenez et al. 1997), more recent evidence suggests that Groucho may indeed be a corepressor for Eve (Kobayashi et al. 2001). Finally, the *Drosophila* genome encodes multiple histone deacetylases (Johnson et al. 1998; Barlow et al. 2001). These include class I histone deaceyltases such as HDAC1, which are defined by their similarity to yeast Rpd3, and class II histone deacetylases, which are defined by their similarity to yeast Hda1. These multiple enzymes may function redundantly with one another, thereby partially masking the *rpd3* mutant phenotype.

Additional evidence in favor of a role for histone deacetylases in the function of Groucho/Tup1 superfamily corepressors comes from studies suggesting that a variety of histone deacetylases may contribute to repression by yeast Tup1. For example, yeast cells that are triply mutant for the three class I deacetylases Rpd3, Hos1, and Hos2 exhibit increased acetylation of histones H3 and H4 at genes normally repressed by Tup1, as well as a severe defect in Tup1-mediated repression (Watson et al. 2000). Furthermore, an *rpd3* mutation severely compromises repression by a LexA–Tup1 fusion protein of a reporter containing LexA-binding sites (Wu et al. 2001). Finally, Hos2 and Rpd3 were found to interact physically with Tup1, although it is not clear if the interaction is direct or is mediated by the Tup1-partner protein Ssn6 (Watson et al. 2000).

Although the above findings strongly suggest a role for class I histone deacetylases in Tup1 function, other studies suggest a role for the class II enzyme Hda1 (Wu et al. 2001). Mutations in this enzyme result in hyperacetylation of histones H2B and H3, and a defect in LexA–Tup1-mediated repression similar to that observed in an *rpd3*-mutant background. In this study, the changes in histone acetylation pattern caused by a *tup1* mutation were found to more closely parallel the changes resulting from an *hda1* mutation than the changes resulting from an *rpd3* mutation. This suggests that class II enzymes like Hda1 may have more important roles in Tup1-mediated repression than do class I enzymes like Rpd3. However, it is important to note that the different studies cited above (Watson et al. 2000; Wu et al. 2001) focused on different Tup1 target genes. It is entirely possible that Tup1 represses different genes using different classes of histone deacetylases.

The finding that Groucho/Tup1 superfamily proteins

may function by recruiting histone deacetylases resulting in the production of a large transcriptionally silent chromosomal domain appears to provide an explanation for how such corepressors may function to repress transcription at long range. However, a detailed analysis of the effects of *rpd3* mutations in yeast on the acetylation state of histones may be at odds with this model (Kadosh and Struhl 1998; Rundlett et al. 1998). The *INO1* gene contains a single binding site for the Rpd3-dependent transcription factor Ume6 located a short distance upstream of the transcriptional start site. In an *rpd3* mutant background, the chromatin near the Ume6-binding site exhibits increased acetylation, but the increase only extends over a small region of, perhaps, two nucleosomes. If these findings are general, they suggest that the targeted recruitment of a histone deacetylase by a sequence-specific transcriptional repressor is not, by itself, sufficient to result in the production of a large transcriptionally silent chromosomal domain. A possible way to resolve this apparent paradox is provided by a consideration of Sir-dependent silencing in yeast.

Sir-dependent silencing as a model for long-range repression

The phenomenon of long-range repression was first characterized in studies of the yeast silent mating type loci *HMR* and *HML* (Loo and Rine 1995). The *HM* loci contain extra cassettes of mating type information. Pairs of silencers that flank each *HM* locus maintain these cassettes in a silent state. These silencers can function over large distances in an orientation-independent manner to prevent transcription of heterologous genes. A large number of proteins are required for optimal silencer function. These include DNA-binding proteins such as Rap1, Abf1, and ORC, and the silent information regulators Sir1, Sir2, Sir3, and Sir4. The Sir proteins do not bind the silencers directly but are apparently recruited by protein–protein interactions with sequence-specific factors to form a repressosome.

A great deal of evidence suggests that the Sir repressosome blocks transcription by remodeling a large domain of chromatin into a repressed conformation (Loo and Rine 1995). For example, histones associated with the silenced loci have hypoacetylated N-terminal tails, and mutations that alter or delete the N-terminal tails of histones H3 and H4 result in a loss of silencing. In addition, physical analyses suggest that the silenced loci are organized into a compact conformation that may be inaccessible to the transcriptional machinery or that may reduce the processivity of transcription. Furthermore, in addition to being required for HM silencing, most Sir proteins are required for the formation of telomeric heterochromatin and for the silencing of genes by telomeres.

One of the most remarkable components of the Sir repressosome is Sir2. Not only is this protein required for *HM* and telomeric silencing, but it is also required for transcriptional and recombinational silencing of the rDNA repeats in the nucleolus (Lustig 1998). Through its function in the nucleolus, it suppresses nucleolar fragmentation and thereby serves as a longevity factor. Recent biochemical studies of Sir2, which is the only Sir protein that is conserved in multicellular eukaryotes, have revealed that it is the prototype for a novel family of histone deacetylases (Imai et al. 2000; Landry et al. 2000; Smith et al. 2000). A surprising aspect of Sir2 enzymology is that NAD is required as a cofactor. In the deacetylation reaction, the acetyl group is apparently transferred to the nicotinamide-linked ribose residue in NAD, displacing nicotinamide and producing acetyl-ADP-ribose (Tanner et al. 2000).

The finding that Sir2 is a histone deacetylase provides the first known mechanistic link between the Sir repressosome and the hypoacetylated state of the loci silenced by the Sir repressosome. Unlike the local deacetylation brought about by the Ume6-recruited Sin3 complex, deacetylation by the Sir repressosome is apparently a long-range phenomenon. Some of the other Sir proteins, particularly Sir3 and Sir4, may provide the explanation for this difference. Sir3 and Sir4 are able to bind the hypoacetylated N-terminal tails of histones H3 and H4, perhaps allowing Sir3 and Sir4 to spread along the chromatin fiber from the silencer (Grunstein 1998). The recruitment of Sir2 by Sir3 and Sir4 may then result in the spread of the deacetylated domain. By strengthening the Sir-protein/histone interaction, deacetylation could also serve to stabilize the transcriptionally repressed state.

The spreading of a repressed chromosomal state is not by any means limited to Sir-dependent repression in yeast. A classic example of this kind of spreading is provided by the phenomenon of position effect variegation (PEV) in *Drosophila* (Reuter and Spierer 1992). In this process, genes that become mislocalized to regions close to centromeric heterochromatin are silenced by the spreading of heterochromatin. Extensive genetic analysis of PEV has shown that the likelihood of spreading can be altered by changes in the concentration of chromatin components or in the concentration of enzymes that covalently modify histones (Wallrath 1998).

The possible spreading of a Groucho-induced chromosomal state

Just as the Sir repressosome generates a transcriptionally silent chromatin structure that is able to spread along the chromatin fiber, it is possible that the Groucho repressosome nucleates a silent chromosomal state. A number of studies indicate that like Sir3 and Sir4, Groucho/Tup1 superfamily proteins can bind hypoacetylated histone tails (Edmondson et al. 1996; Flores-Saaib and Courey 2000). This may allow these corepressors to spread along the chromatin fiber. By recruiting HDAC1 and/or other histone deacetylases, they may then generate a large deacetylated transcriptionally silent chromosomal domain (Fig. 2).

Although studies that directly address the possibility of Groucho spreading have not yet been reported, studies looking at the possibility of Tup1 spreading at the yeast *STE6* locus have recently been carried out by two labs,

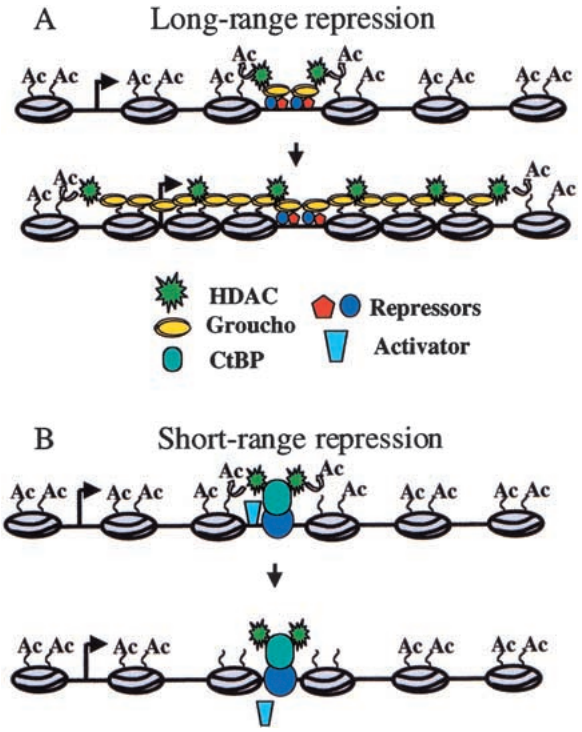

Figure 2. Spreading of corepressors along chromatin may be a key to long-range repression. (*A*) After recruitment to DNA by sequence-specific repressors, long-range corepressors such as Groucho or Sir3/Sir4 may recruit histone deacetylases to nearby histone tails, resulting in an altered chromatin structure. The corepressors may then spread along chromatin by virtue of their ability to bind hypoacetylated histones. The corepressor polymer may then recruit additional histone deacetylase (HDAC). Through such a repetitive process, a large chromosomal locus may be organized into a repressed state. (*B*) Short-range corepressors such as CtBP can also recruit histone deacetylase. This may result in the local deacetylation of nucleosomes, forming an altered chromatin structure that may displace neighboring activators. As a result of the hypothesized inability of short-range repressors to polymerize, the effect may be strictly local.

and the results are contradictory. Like all **a** cell-specific genes, *STE6* is repressed by the homeodomain protein α2, which is a Tup1-dependent repressor. Repression of *STE6* by Tup1 results in the precise positioning of nucleosomes around the α2-binding site and an increased nucleosomal density (Shimizu et al. 1991; Cooper et al. 1994). Furthermore, nucleosome positioning and repression are both dependent on the histone tails (Roth et al. 1992; Edmondson et al. 1996). In one report examining the question of Tup1 spreading, chromatin immunoprecipitation (ChIP) assays with Tup1 antibodies suggest a high density of Tup1 along the entire *STE6* locus under repressive conditions (Ducker and Simpson 2000). Furthermore, these experiments showed a sharp drop-off in Tup1 density upstream and downstream of the gene, suggesting the existence of boundary elements that somehow limit the spread of Tup1. However, similar ChIP experiments from another laboratory failed to reproduce these findings (Wu et al. 2001). This second study suggests instead that Tup1 is localized almost exclusively to the region containing the α2-binding site in *STE6*. The reason for the discrepancy is unclear and therefore the question of Tup1 spreading remains unresolved.

Even if Groucho/Tup1 superfamily proteins do not themselves spread along chromatin in a manner analogous to Sir3/Sir4 spreading, it is possible that these factors nucleate an altered chromatin structure that is able to spread along the template. For example, by recruiting histone deacetylases, Tup1 might generate a change in the local histone acetylation pattern. This altered acetylation state could serve as a signal for the recruitment of factors that are able to cooperatively spread along the template, organizing a repressed chromosomal domain. Indeed, the idea that the covalent modification state of histone tails might serve as a code that is read by various effector proteins to generate changes in gene expression has been the subject of much recent interest (Jenuwein and Allis 2001).

Long-range repression via basal machinery interactions

Although histone deacetylation probably accounts for part of the ability of Groucho to repress transcription, it is unlikely to represent the whole story. For example, the histone deacetylase inhibitor TSA only partially blocks Gal4–Groucho-mediated repression (Chen et al. 1999). Furthermore, additional regions of Groucho outside of the HDAC1-interacting GP domain function as repression domains (Fisher et al. 1996).

Just as activation by enhancers has long been thought to involve stimulatory interactions between enhancer-bound activators and the basal machinery, it is likely that repression involves inhibitory interactions between silencer-bound repressors and the basal machinery. Long-range repression could therefore require the formation of a DNA loop that brings a silencer, with its interacting repressors and corepressors, into the vicinity of the core promoter, with its interacting basal transcriptional machinery.

Evidence that basal machinery interactions might mediate repression by Groucho family repressors comes from studies of the androgen receptor (AR; Yu et al. 2001). In these studies, N-terminal enhancer of split (AES), a TLE family protein, was shown to bind the N-terminal region of AR. Furthermore, in an in vitro transcription system reconstituted from highly purified components presumably devoid of histones, AES abolished transcriptional activation by AR, although basal transcription was unaffected. Finally, AES was found to interact specifically with TFIIE, a component of the basal machinery. A possible interpretation of these findings is that, after recruitment of AES by a regulatory factor, loop formation allows an interaction between AES and TFIIE that serves to block preinitiation complex function. Although this is an attractive model, it does not account for the inability of AES to interfere with basal transcription. If AES truly inhibits the basal machinery, one might expect to observe repression of basal transcription.

A number of studies have suggested that Tup1 might function by basal machinery interactions, and, in particular, by interactions with the mediator complex (Gromoller and Lehming 2000; Papamichos-Chronakis et al. 2000; Zaman et al. 2001). This large polypeptide complex associates with the C-terminal domain of the large subunit of RNA polymerase II (Berk 1999). It was first characterized in yeast, and analogous complexes have been identified in metazoans. In both yeast and metazoans, the mediator complex is believed to interact functionally with a wide variety of activators.

In an elegant study showing a functional interaction between Tup1 and the basal machinery, the Srb7 subunit of the mediator complex was found to bind Tup1 (Gromoller and Lehming 2000). A mutant allele of *srb7* was created encoding a protein that was unable to bind Tup1, but that was able to rescue the lethality caused by an *srb7* deletion. This *srb7* allele was found to result in phenotypes reminiscent of those associated with *tup1* mutations, such as cell clumping and decreased mating efficiency. In addition, several Tup1 target promoters displayed severely compromised Tup1-mediated repression. These experiments strongly suggest that an interaction between Tup1 and the holoenzyme interferes with the function of the basal machinery.

Further experiments included in the study described above suggest that the same region of Srb7 that contacts Tup1 also contacts Med6, a component of the mediator complex that is required for the stimulation of transcription by a number of activator proteins. It was therefore proposed that the interaction between Med6 and Srb7 is required for activation by factors that work through Med6, and that Tup1 might prevent activation by blocking the Med6/Srb7 interaction (Fig. 3). This intriguing model has a very interesting implication. It suggests that factors that work through the basal machinery to mediate long-range repression may work in an activator-selective manner. For example, if the model is correct, it would suggest that Tup1 should preferentially interfere with activation by factors that work through Med6, whereas factors that work by other mechanisms should be relatively resistant to repression by Tup1.

Short-range repression

In some cases of gene regulation, mechanisms are required that will allow repressors to block activation of a given promoter by activators bound close to the repressor binding site, while still allowing activation by more distantly bound activators. Although, as discussed above, long-range repression may sometimes allow for activator-specific repression, short-range repression may be a more flexible way to achieve this kind of control. For example, the distance over which a short-range repressor is able to work appears to be dependent on repressor concentration. Thus, short-range repressors may provide a sensitive means of responding to a transcription factor concentration gradient (Hewitt et al. 1999).

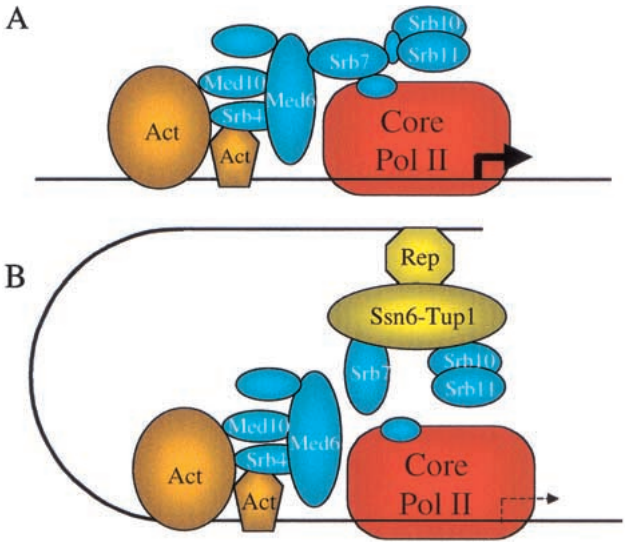

Figure 3. Interactions between Tup1 and the mediator. (*A*) The RNA polymerase II holoenzyme consists of core Pol II and a mediator, which contains multiple subunits, only a few of which are illustrated here (blue ellipses). For simplicity, the general transcription factors have been omitted. A number of activators (Act) require Med6 to activate transcription. These activators may stimulate an interaction between Med6 and Srb7, leading to activation. (*B*) After recruitment by a repressor (Rep), Tup1 (as a component of the Ssn6–Tup1 complex) may block activation by competing with Med6 for binding to Srb7. Tup1 has also been proposed to engage in an inhibitory interaction with Srb10/Srb11.

Short-range repression and enhancer autonomy in pair-rule gene expression

Repressors that regulate the expression of the *Drosophila* pair-rule genes such as *eve* and *hairy* provide an excellent example of short-range repression. Pair-rule genes are generally expressed in seven transverse stripes along the anteroposterior axis of the early embryo (Ingham 1988). The spatial control of pair-rule gene expression is largely dependent on the transcription factors encoded by the gap genes (e.g., *giant*, *hunchback*, *Krüppel*, *knirps*) and by the maternal polarity genes (e.g., *bicoid*). These factors work via multiple autonomous enhancers in the pair-rule genes (Akam 1989). An individual enhancer often directs a single stripe of expression. Because the enhancers function independently of one another to direct stripes of expression at different positions along the anteroposterior axis, the characteristic seven-stripe expression pattern can be generated by an appropriate combination of enhancers within a single locus.

The ability of these multiple enhancers to function autonomously is critically dependent on the ability of the repressor proteins that interact with these enhancers to function in a short-range manner. For example, in a textbook example of how combinatorial control can direct stripe formation, the two short-range repressors Giant and Krüppel bind to the *eve* stripe 2 enhancer to block activation by Hunchback and Bicoid, thereby setting the borders of stripe 2 (Gray and Levine 1996b). Gi-

ant and Krüppel are able to block activation by Hunchback and Bicoid because the activator and repressor binding sites in the stripe 2 enhancer are spaced by less than ~100 bp, which seems to be the limit for this type of short-range repression. At the same time, these repressors are unable to interfere with activation by other stripe enhancers in the *eve* locus because the activator binding sites within the other enhancers are hundreds to thousands of base pairs away, thus ensuring enhancer autonomy.

A possible clue to the mechanism of short-range repression comes from the observation that many short-range repressors found in the early embryo, including Giant, Krüppel, Knirps, and Snail, are at least partially dependent for their function on a common corepressor named CtBP (Mannervik et al. 1999; Nibu and Levine 2001). Therefore, an understanding of how CtBP represses transcription may go a long way toward explaining short-range repression. It should be noted, however, that *Drosophila* CtBP was first isolated in a yeast two-hybrid screen for proteins that interact with Hairy, which, as discussed above, is a Groucho-interacting long-range repressor (Poortinga et al. 1998). Recent results suggest that this may reflect a role for CtBP in down-regulating Hairy function (Zhang and Levine 1999; Phippen et al. 2000). It should also be noted that the short-range repressors at work in the early embryo can, at least in some cases, function by CtBP-independent mechanisms (La Rosee-Borggreve et al. 1999; Keller et al. 2000).

Whereas the *Drosophila* version of CtBP interacts with the short-range repressors mentioned above, mammalian CtBP has been found to interact with a number of mammalian factors, including E1A (Boyd et al. 1993; Schaeper et al. 1995), TCF (Criqui-Filipe et al. 1999), and Ikaros (Koipally and Georgopoulos 2000). Many of these mammalian and *Drosophila* CtBP-interacting proteins contain a motif with similarity to a PxDLS consensus sequence, and this motif is apparently required for CtBP recruitment. Therefore, the theme of employing short peptide motifs to recruit corepressors seems to extend to CtBP as well as Groucho.

Histone deacetylases in CtBP function

A number of studies suggest that CtBP may function, at least in part, by recruiting histone deacetylases (Sundqvist et al. 1998; Criqui-Filipe et al. 1999; Zhang et al. 2001). For example, the C-terminal domain of E1A was found to bind a CtBP/HDAC1 complex (Sundqvist et al. 1998). In addition, repression by a Gal4–CtBP fusion protein was found, at least in some cases, to be sensitive to TSA, a specific inhibitor of histone deacetylases (Criqui-Filipe et al. 1999).

How do we explain the apparent contradiction that arises from the possibility that both long- and short-range corepressors may function through histone deacetylation? There are a number of possibilities. First, as mentioned previously, long-range corepressors may have the ability to spread along the template recruiting histone deacetylases and/or other chromatin modifying activities to a large domain, but short-range repressors may lack the capacity to spread. Alternatively, the differences between long- and short-range corepressors could relate to the different properties of different histone deacetylases. Groucho has thus far only been found to bind class I histone deacetylases, whereas CtBP appears to bind both class I and class II histone deacetylases (Bertos et al. 2001). Perhaps the different repertoires of histone deacetylases recruited by different corepressors result in different histone acetylation patterns in the surrounding chromatin. As discussed previously, certain histone acetylation patterns could result in the recruitment of chromatin-remodeling enzymes that organize large, transcriptionally repressed domains. In contrast, other histone acetylation patterns might only generate short-range changes in chromatin structure that result in the ejection of activators from nearby binding sites (Fig. 2).

Other mechanisms for short-range repression

As with Groucho, it is likely that histone deacetylase interactions do not fully account for the ability of CtBP to repress transcription. For example, although TSA largely blocks repression by a Gal4–CtBP fusion protein in CHO cells (Criqui-Filipe et al. 1999), it is apparently unable to do so in 293 cells (Koipally and Georgopoulos 2000).

Another possible mechanism for short-range repression, which is sometimes referred to as quenching (Gray and Levine 1996b), involves interactions of repressors (and the corepressors they recruit) with activators bound to nearby sites. Thus, once a short-range corepressor is recruited to a gene by an interaction with a repressor, it could be transferred to a nearby DNA-bound activator protein. The transferred corepressor could then serve to block activation, perhaps by obstructing an interaction between the activation domain and the general machinery.

One aspect of CtBP-mediated repression would seem to make the quenching model unattractive. In particular, available evidence suggests that the CtBP-dependent short-range repressors in the *Drosophila* embryo will quench any activator as long as the repressor and activator binding sites are within ~100 bp of one another (Gray and Levine 1996a). Given the great diversity in activation domains, the interactions between CtBP and the activators would therefore need to be quite promiscuous. Such promiscuity might be expected to result in a myriad of nonproductive interactions with irrelevant nuclear components. A possible solution to this problem comes from the idea that, in the absence of highly evolved complementary interaction surfaces, it may nonetheless be possible to impose specificity by localization (Ptashne and Gann 1998). In the particular case of quenching, the closeness of the repressor and activator binding sites may result in a high enough effective concentration of the corepressor in the vicinity of the activator to favor relevant corepressor/activator interactions over irrelevant interactions.

Flexibility in transcriptional repression

The ability of repressors and corepressors to function by multiple mechanisms, including chromatin interactions and transcriptional machinery interactions, appears to be widespread in eukaryotic gene regulation. This multifunctionality may allow corepressors to shut off gene expression in ways that are tailored to the goal of the repression. For example, if the goal is to transiently repress transcription in response to a temporary change in the environment, then repression via transcriptional machinery interactions, which should be rapidly reversible, might be the best option. In contrast, if the goal is to generate a repressed epigenetic state, then repression via covalent changes in histone structure might be the preferred option. This is because the semiconservative redistribution of histones during S phase might allow such changes to be maintained from one cell generation to the next. Indeed, a number of repressed states that have been linked to changes in chromatin including HM silencing and PEV are known to be heritable (Jenuwein and Allis 2001).

The availability of both short- and long-range repressors adds yet another layer of flexibility to gene regulation. Long-range repression provides the possibility of shutting down an entire locus regardless of how many separate regulatory modules control the activity of that locus. On the other hand, short-range repression provides a way to control the activity of one enhancer without interfering with the activity of others. This enhancer autonomy appears to be especially important at complex loci containing multiple enhancers, each required for a distinct portion of an intricate pattern of expression.

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Transcriptional repression: the long and the short of it

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