**Drosophila** Brakeless Interacts with Atrophin and Is Required for Tailless-Mediated Transcriptional Repression in Early Embryos

Achim Haecker¹, Dai Qi¹, Tobias Lilja¹, Bernard Moussian², Luiz Paulo Andrioli³, Stefan Luschnig²*, Mattias Mannervik¹*

1 Developmental Biology, Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, 2 Abteilung Genetik, Max-Planck Institut für Entwicklungsbiologie, Tübingen, Germany, 3 Department of Genetics and Evolution, University of Sao Paulo, Sao Paulo, Brazil

Complex gene expression patterns in animal development are generated by the interplay of transcriptional activators and repressors at cis-regulatory DNA modules (CRMs). How repressors work is not well understood, but often involves interactions with co-repressors. We isolated mutations in the Brakeless gene in a screen for maternal factors affecting segmentation of the *Drosophila* embryo. Brakeless, also known as Scribbler, or Master of thickveins, is a nuclear protein of unknown function. In brakeless embryos, we noted an expanded expression pattern of the Krüppel (Kr) and knirps (kni) genes. We found that Tailless-mediated repression of kni expression is impaired in brakeless mutants. Tailless and Brakeless bind each other in vitro and interact genetically. Brakeless is recruited to the Kr and kni CRMs, and represses transcription when tethered to DNA. This suggests that Brakeless is a novel co-repressor. Orphan nuclear receptors of the Tailless type also interact with Atrophin co-repressors. We show that both *Drosophila* and human Brakeless and Atrophin interact in vitro, and propose that they act together as a co-repressor complex in many developmental contexts. We discuss the possibility that human Brakeless homologs may influence the toxicity of polyglutamine-expanded Atrophin-1, which causes the human neurodegenerative disease dentatorubral-pallidoluysian atrophy (DRPLA).

**Introduction**

The generation of complex spatial and temporal gene expression patterns during embryo development is achieved through gene regulatory networks in which broadly distributed transcriptional activators act in combination with repressors with a more restricted distribution (reviewed in [1]). Repressors have an essential role in establishing gene expression boundaries. The mechanisms by which repressors act are not well understood, but may involve competition for DNA binding sites, inhibition of activator function (quenching), and direct repression (reviewed in [2–5]). Many activators and repressors require co-regulators for activity (reviewed in [6,7]). One way that co-regulators work is to modulate the chromatin structure in order to facilitate or restrict transcription initiation complex assembly. However, co-regulators may have other functions as well, such as mediating an association between transcription factors and the basal transcription machinery. It is possible that the type of co-regulator that is recruited determines the mechanism of transcriptional control at use. For example, during *Drosophila* embryo development, repressors acting over a short range recruit the CtBP co-repressor, whereas several long-range repressors interact with the co-repressor Groucho (reviewed in [8]). Yet, the mechanism by which several important transcription factors in the embryo work remains unknown. We therefore set out to isolate novel transcriptional regulators that are required for *Drosophila* embryo segmentation, and identified the Brakeless protein as a co-repressor that is required for function of the transcription factor Tailless.

Segmentation of the *Drosophila* embryo is achieved through a hierarchy of transcriptional control (reviewed in [9,10]). The maternal mRNAs *bicoid* (bcd) and *nanos* (nos) localize to the anterior and posterior poles of the embryo, respectively, from where they give rise to protein gradients in the syncytial embryo. Bcd activates transcription of the *hunchback* (hb) gene and represses translation of maternal *caudal* (cad) mRNA, whereas Nanos represses translation of maternal hb message (reviewed in [11]). The resulting Bcd, Hb, and Cad protein gradients act in combination to turn on expression of the first zygotic patterning genes, the gap genes, in restricted domains in the embryo. The gap gene products in turn are transcriptional repressors that regulate the next level in the hierarchy, pair-rule gene expression. Pair-rule proteins are transcription factors that control the segment-polarity genes, which in turn specify the positions of the 14 segments of the animal. The positioning of gap gene expression domains relies on interpretation of the Bcd, Hb, and Cad activator

**Academic Editor:** Matthew P Scott, Stanford University, United States of America

**Received** May 24, 2006; **Accepted** March 26, 2007; **Published** May 15, 2007

**Copyright:** © 2007 Haecker et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Abbreviations:** aa, amino acid; bp, base pair; CD, central domain; ChIP, chromatin immunoprecipitation; CRM, cis-regulatory DNA module; EL, egg length; GFP, green fluorescent protein; kb, kilobase; NEE, neuroectoderm enhancer; RT-PCR, reverse transcription PCR; wt, wild-type

* To whom correspondence should be addressed. E-mail: mannervik@devbio.su.se

* Current address: Bayreuth Center for Molecular Biosciences, Department of Genetics, University of Bayreuth, Bayreuth, Germany
Nuclear receptors play important roles in embryonic development and cellular differentiation by regulating gene expression at the level of transcription. The functions of transcriptional repressors, including nuclear receptors, are often mediated by other proteins, so-called co-repressors. We performed a genetic screen in the fruit fly *Drosophila melanogaster* to search for novel co-repressor proteins. We isolated mutations in the brakeless gene that alter normal transcriptional repression in early fly embryos. Brakeless was already known to regulate axon guidance in the eye, larval behavior, and gene expression in wing imaginal discs. However, the molecular function of this protein was unknown. Here we show that Brakeless is a co-repressor required for function of the Tailless nuclear receptor. Tailless was previously shown to interact with another co-repressor, Atrophin. Here, we demonstrate that Brakeless and Atrophin can bind to one another and that this interaction is conserved between a human Brakeless homolog, ZNF608, and human Atrophin-1. A polyglutamine expansion in Atrophin-1 is the cause of the neurodegenerative disease dentatorubral-pallidoluysian atrophy (DRPLA). It is possible that the interaction with ZNF608 could contribute to the pathogenesis of polyglutamine-expanded Atrophin-1.

**Results**

**Severe Segmentation Defects in Embryos Derived from brakeless (bks) Germline Clones**

From a screen for new maternal genes involved in embryonic pattern formation [13], we searched for mutant phenotypes that reflect defects in the transcriptional regulation of segmentation. We found a mutant, 2R-14, that displayed severe segmentation defects in embryonic cuticle preparations (Figure 1). 2R-14 germline clone larvae show deletions of denticles belts to a variable extent. All of the abdominal segments, as well as terminal structures, can be affected, but there is no effect on dorsal-ventral patterning. Two additional alleles (2R-278 and 2R-339) were isolated that also show this phenotypic variability, and differ only in the frequency of the phenotypic classes. In contrast to the maternal phenotypes, patterning of 2R-14 zygotic mutant larvae is fully normal, but they die at later stages of development.

We mapped the 2R-14 locus to an approximately 600-kilobase (kb) interval between 55B and 55E, uncovered by the deficiency *Df(2R)PC4*. We performed complementation tests with all available lethal mutants in this interval and found that the 2R-14, 2R-278, and 2R-339 alleles fail to complement the *brakeless* (*bks*) alleles *l(2)04440, bks*1 and *bks*2 (described in [15,17]). Thus the lethality of the 2R-14 locus maps to the *bks* gene. We cleaned the 2R-14 chromosome by recombination and did complementation tests with the *bks* alleles, as well as generated germline clone embryos from the resulting recombinants. The phenotype of these embryos is essentially identical to embryos derived from the original 2R-14...

---

**Author Summary**

Nuclear receptors play important roles in embryonic development and cellular differentiation by regulating gene expression at the level of transcription. The functions of transcriptional repressors, including nuclear receptors, are often mediated by other proteins, so-called co-repressors. We performed a genetic screen in the fruit fly *Drosophila melanogaster* to search for novel co-repressor proteins. We isolated mutations in the brakeless gene that alter normal transcriptional repression in early fly embryos. Brakeless was already known to regulate axon guidance in the eye, larval behavior, and gene expression in wing imaginal discs. However, the molecular function of this protein was unknown. Here we show that Brakeless is a co-repressor required for function of the Tailless nuclear receptor. Tailless was previously shown to interact with another co-repressor, Atrophin. Here, we demonstrate that Brakeless and Atrophin can bind to one another and that this interaction is conserved between a human Brakeless homolog, ZNF608, and human Atrophin-1. A polyglutamine expansion in Atrophin-1 is the cause of the neurodegenerative disease dentatorubral-pallidoluysian atrophy (DRPLA). It is possible that the interaction with ZNF608 could contribute to the pathogenesis of polyglutamine-expanded Atrophin-1.
chromosome (Figure 1C). In order to confirm that mutations in the bks gene indeed cause a maternal segmentation defect, we recombined the independently isolated bks1 and bks2 alleles onto FRT G13 (42B) chromosomes. bks1 and bks2 germ-line clone–derived larvae and early embryos (Figure 1D and unpublished data) are phenotypically indistinguishable from 2R-14 mutants. Thus, we named our alleles bks14, bks278, and bks339.

The bks locus encodes at least two proteins, Bks-A and Bks-B (Figure 2A). Bks-A is a 929–amino acid (aa)-long protein, whereas Bks-B consists of 2,302 aa and has the first 929 aa in common with Bks-A [14,15,17,18]. The only sequence similarity to known functional domains is a single C2H2 zinc finger located in the unique region of Bks-B. One additional domain (D2) is highly conserved and present also in sequences from deuterostome species (Figure S1). In vertebrates, a duplication has resulted in two genes with sequence similarity to Bks, encoding zinc-finger protein 608 (ZNF608) and ZNF609. In addition, we identified three domains (D1, D3, and D4) that are highly conserved in insects and that contain limited similarity to vertebrate sequences (Figure 2A).

We sequenced our three bks alleles in order to identify the molecular lesions associated with the mutations (see Figure 2A). For bks14, we were unable to amplify the first exon using various primer combinations. No mutation was detected in the rest of the gene. Sequencing of bks278 revealed a 345–base pair (bp) large deletion after nucleotide 2,365 of the Bks-B cDNA. This deletion together with a 8-bp insertion causes a frame shift at aa 741 that results in addition of 79 novel amino acids. The weaker bks339 allele is due to a C to T transition at position 5,485 that converts Q1758 into a stop codon. This truncates the protein before the conserved D3 domain and shows that the maternal function of the Bks-B subtype is necessary for embryo development.

Bks Is Ubiquitously Expressed in Early Embryos

We examined the bks expression pattern during embryogenesis by whole-mount in situ hybridization with a probe that recognizes both bks isoforms. Due to the maternal contribution, bks transcripts are present ubiquitously in the embryo. Anterior is to the left, and dorsal is up.

We performed reverse transcription PCR (RT-PCR) on mRNA from early embryos (0–3 h) using primers specific for bks-A or for bks-B and primers specific for bks-A or for bks-B were used in the PCR reaction. Products of the expected size were obtained after oligo-dT–primed reverse transcription, but not in the absence of reverse transcriptase.

We examined the maternal Bks protein expression pattern during embryogenesis by whole-mount in situ hybridization with a probe that recognizes both bks isoforms. Due to the maternal contribution, bks transcripts are present ubiquitously in the embryo. Anterior is to the left, and dorsal is up. We performed reverse transcription PCR (RT-PCR) on mRNA from early embryos (0–3 h) using primers specific for bks-A or for bks-B. We found bks mRNA to be expressed in the egg and throughout all stages of embryogenesis. At the blastoderm stage, ubiquitous expression is caused by the maternal contribution of the Bks-B subtype, which is necessary for embryo development.

We sequenced our three bks alleles in order to identify the molecular lesions associated with the mutations (see Figure 2A). For bks14, we were unable to amplify the first exon using various primer combinations. No mutation was detected in the rest of the gene. Sequencing of bks278 revealed a 345–base pair (bp) large deletion after nucleotide 2,365 of the Bks-B cDNA. This deletion together with a 8-bp insertion causes a frame shift at aa 741 that results in addition of 79 novel amino acids. The weaker bks339 allele is due to a C to T transition at position 5,485 that converts Q1758 into a stop codon. This truncates the protein before the conserved D3 domain and shows that the maternal function of the Bks-B subtype is necessary for embryo development.

We sequenced our three bks alleles in order to identify the molecular lesions associated with the mutations (see Figure 2A). For bks14, we were unable to amplify the first exon using various primer combinations. No mutation was detected in the rest of the gene. Sequencing of bks278 revealed a 345–base pair (bp) large deletion after nucleotide 2,365 of the Bks-B cDNA. This deletion together with a 8-bp insertion causes a frame shift at aa 741 that results in addition of 79 novel amino acids. The weaker bks339 allele is due to a C to T transition at position 5,485 that converts Q1758 into a stop codon. This truncates the protein before the conserved D3 domain and shows that the maternal function of the Bks-B subtype is necessary for embryo development.
expression phenotypes in bks embryos. In wild-type (wt) pre-cellular embryos, the gap gene kni is expressed in two domains, one in the anterior-ventral end of the embryo, the other one as a stripe in the posterior half (Figure 3A). In bks mutants, this posterior domain is broadly expanded (Figure 3B). Whereas in wt, the posterior kni domain extends from 27% to 43% egg length (EL, where 0% is the posterior pole and 100% the anterior pole), in bks14 embryos, it extends from 15% to 41% EL. In cellularizing bks embryos, the posterior domain remains expanded, and additional ectopic expression is found in the posterior-ventral end of the embryo (arrowhead in Figure 3D). To determine whether the effect on kni expression is transcriptional or post-transcriptional, we introduced a kni 4.4-kb CRM-lacZ transgene [23] into bks germline clone embryos. As shown in Figure 3F, lacZ expression expands towards the posterior as compared to wt embryos (Figure 3E). The kni-lacZ pattern extends from 31% to 43% EL in wt embryos, and expands to 22%–41% EL in bks278 mutant embryos. We conclude that expansion of the kni pattern in bks mutant embryos is due to transcriptional deregulation.

Kr is first expressed in a central domain (CD) of the embryo (Figure 3G). Later, additional anterior and posterior domains are detectable (unpublished data). In bks embryos, the CD is broadly expanded both in an anterior and a posterior direction (Figure 3H). We measured the CD to 51%–62% EL in bks14 embryos, compared to 40%–57% EL in wt. Intensity of expression also appears enhanced and persists into later stages of embryogenesis. In addition, expression of the anterior domain is enhanced, expanded, and expressed earlier in bks embryos as compared to wt (arrowhead in Figure 3H). Thus, Bks is necessary to restrict Kr expression.

The gt expression pattern develops from a broad domain in the anterior half and one narrower domain in the posterior half in the early blastula embryo, to three anterior stripes and a posterior stripe in cellularizing embryos at mid-cycle 14. In bks embryos, gt expression is variable, but in a majority of embryos, resolution of the anterior domain into stripes is delayed (compare Figure 3J with 3I). The posterior domain is less affected, but in about 25% of the embryos, its expression is reduced (unpublished data).

The gap genes are activated by the maternal factors Bcd, Cad, and Hb. The terminal gene products Tll and Huckebein (Hkb) act as repressors that restrict gap gene expression together with mutual inhibition by gap gene products. Expression of these upstream regulators is mostly normal in bks mutants (Figure S2), and cannot be responsible for the gap gene phenotypes observed.

In conclusion, three gap genes are de-repressed in bks mutants. Similar to previous findings [16,18], absence of Bks leads to de-repression of transcription, indicating that Bks may normally be involved in transcriptional repression.

**Tll Function Is Impaired in bks Embryos**

Gap gene expression boundaries are set by repressor proteins. For example, Kr and Gt restrict each other’s expression [24–27], and Tll represses Kr and kni [23,28–30]. We therefore tested whether Bks is a co-repressor required for the activity of regulators of Kr and kni expression.

We first investigated whether the activities of Tll and Hb are affected in bks embryos by misexpressing them in wt and bks mutant backgrounds, and compared their ability to
A hb transgene driven by the snail promoter was introduced into wt embryos or bks14 germline clone embryos. Lateral views of late cellularizing embryos show that ectopic Hb can repress kni expression ventrally in both wt (C) and bks (D) mutants (arrows). Note that the posterior patch of kni expression that occurs in bks mutants is unaffected (star in [D]), presumably because the snail expression pattern does not extend all the way to the posterior.

E–J Assay of endogenous Knirps (Kni), Krüppel (Kr), and Giant (Gt) function on reporter transgenes containing synthetic repressor binding sites (schematic drawings of the transgenes are presented below the embryo images). (E and F) Males harboring a lacZ reporter transgene driven by a modified rhomboid NEE enhancer with synthetic Kni binding sites were crossed with wt females or females containing bks14 germline clones. Embryos were collected and hybridized with a lacZ probe. Ventro-lateral views of cellularized wt (E) and bks (F) embryos demonstrate that endogenous Kni protein represses reporter gene expression in both genotypes (arrows). (G and H) Introduction of a modified NEE reporter gene with synthetic Kr binding sites into wt embryos (G) and embryos derived from bks14 germline clones (H). Ventral views of cellularized embryos hybridized with a lacZ antiense probe show that endogenous Kr protein can repress reporter gene expression in both genotypes (arrows). (I and J) Lateral views of a cellularized wt embryo (I) and a cellularized embryo derived from a bks14 germline clone (J) containing a reporter gene with synthetic Gt binding sites, activated by a twist PE enhancer and the rhomboid NEE enhancer, stained with a lacZ probe. Endogenous Gt protein can repress the reporter in both wt and bks mutant embryos (arrows).

Dorsal (dl) and Twist (twi) activators bind the rhomboid and twist enhancers.
doi:10.1371/journal.pbio.0050145.g004

repress transcription. We used a snail promoter construct that directs ectopic Tll or Hb expression in the ventral domain of the embryo (described in Protocol S1 and in [31]). When Tll is misexpressed in wt embryos, kni expression becomes repressed in ventral cells (Figure 4A, arrow). By contrast, in a majority of bks mutant embryos, the kni expression pattern is unaffected by misexpressed Tll (Figure 4B, arrow; and Table S1), suggesting that full Tll activity depends on wt Bks function.

On the other hand, misexpression of Hb from the snail promoter causes repression of kni in the ventral half of the embryo in both wt (Figure 4C) and in a bks mutant background (Figure 4D). Despite the enhanced and expanded levels of kni expression in bks embryos, ectopic Hb is sufficient to repress kni ventrally. The ectopic patch of kni expression in the posterior-ventral part of the embryo remains unaffected (star in Figure 4D), presumably because snail expression does not extend to the very posterior of the embryo [32].

To examine the repressor activities of Kni, Kr, and Gt proteins, we introduced lacZ reporter gene constructs into bks mutant embryos. LacZ expression is driven by a modified rhomboid neuroectoderm enhancer (NEE) that is activated on the ventral side of the embryo by the Dorsal and Twist proteins. In addition, the enhancer constructs contain either Kni, Kr, or Gt binding sites (described in [33–35]). In wt embryos, binding of the corresponding gap protein leads to repression of the reporter gene in the domain of gap gene expression (Figure 4E, 4G, and 4I). Similarly, lacZ expression is repressed in the gap gene expression domains in a bks background (Figure 4F, 4H, and 4J). Thus, in bks mutants, the three gap proteins Kni, Kr, and Gt are able to perform repression at least on the artificial enhancer constructs used, indicating that Bks is not required for the repressor activities of these proteins. We conclude that Tll-mediated repression is impaired in a bks mutant background, whereas the Hb, Kni,
Kr, and Gt repressors are not affected under these conditions.

Interactions among Bks, Tll, and Atrophin

We tested whether the dependence of Tll repressor function on Bks might be due to a molecular interaction between these proteins. Tll and Bks-A were expressed as GST-fusion proteins in bacteria, and mixed with radiolabeled in vitro–translated proteins. As shown in Figure 5A, in vitro–translated Bks-B binds weakly to a GST-Tll fusion protein lacking the DNA binding domain (GST-Tll 101–452), and more strongly with GST-full-length Tll.

(B–E) Genetic interaction of bks with tll mutants. Cellularizing embryos hybridized with a kni probe are oriented with anterior to the left and dorsal up. The kni pattern in wild-type (wt) embryos (B) and embryos from bks homozygous mothers (C) are indistinguishable. In tll homozygous embryos (D), the posterior kni domain expands slightly towards the posterior. In tll homozygous embryos derived from bks heterozygous females (E), there is a further expansion of the kni pattern (see arrow).

(F) Bks interacts with the C-terminus of Atrophin. Amino acids (aa) 1,324–1,966 of Atrophin binds the ligand binding domain of Tll, as well as GST-BksA. Truncation of the conserved Bks D2 region (GST-Bks 1–780) does not disrupt binding, but a weaker, independent interaction is found with the D2 domain together with the zinc finger (GST-Bks 834–1,151).

(G) Bks and Tll can be co-immunoprecipitated with Atrophin from Drosophila S2 cells. A stable cell line expressing V5-tagged Bks-B was generated and transiently transfected with FLAG-tagged Tll. Immunoprecipitations with V5, Atrophin, and FLAG antibodies were performed from these cells and compared to normal S2 cells lacking tagged Bks and Tll. The leftmost panel shows a short exposure of a membrane immunoblotted with the V5 antibody, demonstrating the presence of Bks-V5 in transfected cells. The middle panel shows a longer exposure of the same membrane, where Bks-V5 is co-immunoprecipitated with endogenous Atrophin. In the right panel, FLAG-Tll is detected both in the Atrophin and FLAG immunoprecipitates. Arrowheads point to Bks-V5 and Tll-FLAG.

(H) The human Bks homolog ZNF608 (aa 1–600) interacts with aa 600–1191 from human Atrophin-1, showing that the Bks-Atrophin interaction is evolutionarily conserved.

doi:10.1371/journal.pbio.0050145.g005

This shows that Bks and Tll interact in vitro, and that the Tll DNA binding domain is important for the interaction.

A functional interaction between Tll and Bks was demonstrated in vivo by genetic means. We found that lowering the dose of bks in a tll mutant background causes enhanced derepression of kni expression. In embryos derived from a tll hypomorph, kni expression expands towards the posterior (compare Figure 5D with Figure 5B). By contrast, embryos receiving half the dose of maternal bks have an essentially wt kni expression pattern (Figure 5C). However, in tll mutant embryos with reduced amounts of maternal bks product, the kni expression pattern expands even further to the posterior.

Figure 5. Bks Interacts with Tll and Atrophin

(A) Binding of Bks to Tll in vitro. Left panel shows that in vitro–translated Tll interacts with bacterially produced GST-BksA, but not with GST alone. In the right panel, in vitro–translated Bks-B binds weakly to a GST-Tll fusion protein lacking the DNA binding domain (GST-Tll 101–452), and more strongly with GST-full-length Tll.

(B) Genetic interaction of bks with tll mutants. Cellularizing embryos hybridized with a kni probe are oriented with anterior to the left and dorsal up. The kni pattern in wild-type (wt) embryos (B) and embryos from bks heterozygous mothers (C) are indistinguishable. In tll homozygous embryos (D), the posterior kni domain expands slightly towards the posterior. In tll homozygous embryos derived from bks heterozygous females (E), there is a further expansion of the kni pattern (see arrow).

(F) Bks interacts with the C-terminus of Atrophin. Amino acids (aa) 1,324–1,966 of Atrophin binds the ligand binding domain of Tll, as well as GST-BksA. Truncation of the conserved Bks D2 region (GST-Bks 1–780) does not disrupt binding, but a weaker, independent interaction is found with the D2 domain together with the zinc finger (GST-Bks 834–1,151).

(G) Bks and Tll can be co-immunoprecipitated with Atrophin from Drosophila S2 cells. A stable cell line expressing V5-tagged Bks-B was generated and transiently transfected with FLAG-tagged Tll. Immunoprecipitations with V5, Atrophin, and FLAG antibodies were performed from these cells and compared to normal S2 cells lacking tagged Bks and Tll. The leftmost panel shows a short exposure of a membrane immunoblotted with the V5 antibody, demonstrating the presence of Bks-V5 in transfected cells. The middle panel shows a longer exposure of the same membrane, where Bks-V5 is co-immunoprecipitated with endogenous Atrophin. In the right panel, FLAG-Tll is detected both in the Atrophin and FLAG immunoprecipitates. Arrowheads point to Bks-V5 and Tll-FLAG.

(H) The human Bks homolog ZNF608 (aa 1–600) interacts with aa 600–1191 from human Atrophin-1, showing that the Bks-Atrophin interaction is evolutionarily conserved.

doi:10.1371/journal.pbio.0050145.g005
These results suggest that Bks and Tll cooperate to set the normal posterior boundary of \( \text{kni} \) expression. It was recently demonstrated that Tll also interacts with the Atrophin protein, and that Atrophin and Bks genetically interact in adult flies [21,36]. We therefore performed a GST pulldown assay to investigate whether Bks and Atrophin can interact in vitro. As previously published [21], the C-terminus of Atrophin interacts with the ligand binding domain of Tll (Figure 5F). We found that the Atrophin C-terminus interacts with GST-BksA as well. A truncated Bks protein (Bks 1–780) lacking the evolutionarily conserved D2 region still binds to Atrophin, but a weaker, independent interaction was also found with a Bks portion consisting of the conserved D2 region and the zinc finger (Bks 834–1,151, Figure 5F). Thus, Atrophin can bind to at least two separate parts of the Bks protein. These results show that Tll can interact with both Bks and Atrophin, and that Bks and Atrophin can bind to one another as well. This suggests that a tripartite complex consisting of Tll, Bks, and Atrophin might form. We confirmed the interactions among Bks, Atrophin, and Tll in S2 cells expressing V5-tagged Bks-B and FLAG-tagged Tll proteins. Using an Atrophin antibody, we could co-immunoprecipitate V5-tagged Bks and FLAG-tagged Tll with endogenous Atrophin (Figure 5G).

We then tested whether this interaction is evolutionarily conserved. We made a GST-fusion protein consisting of the first 600 aa of the human Bks homolog ZNF608 (including the conserved D2 domain and the zinc finger), and mixed it with radiolabeled C-terminus of human Atrophin-1. A strong interaction between these proteins was observed (Figure 5H). We conclude that the interaction between Bks and Atrophin has been conserved during evolution.

**Bks Associates with \( \text{kni} \) and \( \text{Kr} \) CRMs**

An interaction with Tll is expected to bring Bks to the \( \text{kni} \) and \( \text{Kr} \) CRMs to directly regulate their expression. To determine if Bks is associated with the \( \text{Kr} \) and \( \text{kni} \) CRMs, we performed chromatin immunoprecipitations (ChIP) from S2 cells expressing V5-tagged Bks-B protein. We found a 23-fold and 4.7-fold enrichment at the \( \text{kni} \) and \( \text{Kr} \) CRMs, respectively, with the V5 antibody compared to a control green fluorescent protein (GFP) antibody (Figure 6A and 6B). As a control, we performed ChIP from normal S2 cells lacking the tagged Bks protein. From these cells, the V5 antibody precipitated less \( \text{kni} \) and \( \text{Kr} \) CRM DNA than the control GFP antibody (Figure 6A and 6B). A comparable amount of \( \text{kni} \) 5' UTR DNA was precipitated with the V5 antibody from V5-tagged Bks-B–expressing cells as from normal S2 cells (2.8-fold and 2.7-fold compared to GFP antibody; Figure 6C). A locus on Chromosome 4 was precipitated at a similar efficiency with V5 and GFP antibodies (1.7-fold enrichment; Figure 6D). From these results, we conclude that Bks specifically associates with \( \text{kni} \) and \( \text{Kr} \) CRM sequences when expressed in S2 cells.

We extended these results to Drosophila embryos using an affinity-purified antibody raised against Bks amino acids 450–620. We found an enrichment of \( \text{kni} \) CRM sequences with Bks,
Bks Proteins Can Repress Transcription When Tethered to DNA

When tethered to a promoter. We fused bks coding regions to the DNA binding domain of the tetracycline repressor (TetR-DBD) and expressed the fusion constructs in Drosophila tissue-culture cells. We co-transfected a luciferase reporter construct driven by the actin5C enhancer that also contains tet operators, binding sites for the TetR-DBD (described in [37]). We compared luciferase activity of cells that expressed TetR-Bks fusion proteins with those that expressed the TetR-DBD protein alone. We found that both Bks-A and Bks-B are able to repress transcription when tethered to DNA in mbn-2 as well as in S2 cells (Figure 7A and unpublished data).

We also investigated Bks repressor activity in a transgenic embryo assay. Bks-A coding sequence was fused to the Gal4 DNA binding domain and placed under control of the Kr CD enhancer, which directs expression in the CD of the early embryo. A lacZ reporter gene containing a modified rhomboid NEE lacking Snail repressor sites and containing three upstream activation sequence (UAS) sites was used to monitor Gal4-Bks repressor activity (described in [34]). In a wt background, the reporter gene is expressed in ventral regions of the embryo (Figure 7B). However, when crossed into transgenic embryos expressing the Gal4-BksA fusion protein, the reporter is repressed in central regions (Figure 7C).

In conclusion, our data show that Bks proteins are capable of repressing transcription when bound to a promoter. Taken together with our other results, we conclude that Bks acts as a transcriptional co-repressor.

Discussion

Repression plays a pivotal role in establishing correct gene expression patterns that is necessary for cell fate specification during embryogenesis. For example, in the early Drosophila embryo, repression by gap and pair-rule proteins is essential for specifying the positions of the 14 segments of the animal. The mechanisms by which transcriptional repressors delimit gene expression borders are not well understood. However, many repressors require co-repressors for function. In the Drosophila embryo, the CtBP and Groucho co-repressors are required for activity of many repressors (reviewed in [8,38]). More recently, Atrophin has been identified as a co-repressor for Even-skipped and Tll [21,39]. Still, co-regulators for several important transcription factors in the early embryo have not yet been identified. We therefore performed a screen for novel maternal factors that are required for establishing correct gene expression patterns in the early embryo.

From this screen, we identified mutations in the bks gene that cause severe phenotypes on gap gene expression and embryo segmentation. The Bks protein is evolutionarily conserved between insects and deuterostomes, but has not been characterized in any species except Drosophila, in which it has been shown to repress runt expression in photoreceptor cells and thickveins expression in wing imaginal disks [16,18]. However, the molecular function of Bks was unknown. We show here that Bks interacts with the transcriptional repressor Tll, is recruited to target gene CRMs, and will repress transcription when targeted to DNA.

Tll was recently shown to utilize Atrophin as a co-repressor [21]. Atrophin genetically interacts with Tll and physically interacts with its ligand binding domain. Atrophin binding is conserved in nuclear receptors within the same subfamily,
such as Seven-Up in *Drosophila* as well as Tlx and COUP-TF in mammals [21,40]. When expressed in mammalian cells, *Drosophila* Atrophin and mouse Atrophin-2 interact with the histone deacetylases HDAC1 and HDAC2 [21,41]. Histone deacetylation may therefore be part of the mechanism by which Atrophin functions as a co-repressor. Another recent report described genetic interactions among *bks* and *atrophin* mutants in the formation of intercellular bristles in adult flies [36]. Furthermore, it was shown that *atrophin* mutants have virtually identical phenotypes as *bks* mutants, including derepression of *runt* expression in the eye, *thickveins* expression in the wing, and *Kr* and *kni* expression in the embryo [21,36,42].

We now show that both proteins are recruited to the *kni* CRM, a Tll-regulated target gene, in the embryo. Importantly, we further demonstrate that Atrophin and Bks interact in vitro and that they can be co-immunoprecipitated from S2 cells. We propose that Bks and Atrophin function together as a co-repressor complex, and based on the similar *bks* and *atrophin* mutant phenotypes at several developmental stages, the complex may function throughout development. Our results are compatible with the existence of a tripartite complex consisting of Tll, Bks, and Atrophin. Bks binding to Tll is enhanced by the Tll DNA binding domain, whereas the interaction of Tll with Atrophin is mediated through the C-terminal ligand binding domain. Tll may therefore simultaneously interact with Bks and Atrophin. Alternatively, Tll interacts separately with Bks and Atrophin on the *kni* CRM. In either case, both Bks and Atrophin are required for full Tll activity. However, at high enough Tll concentration, Bks activity is dispensable. Some *bks* embryos misexpressing Tll still repress *kni* expression (Table S1), and overexpressing Tll from a heat-shock promoter can repress the posterior *kni* stripe in both wt and *bks* mutant embryos (unpublished data).

For this reason, we believe that Bks and Atrophin are cooperating as Tll co-repressors, so that Tll function is only partially impaired by the absence of either one. We found that Tet-Bks-mediated repression in cells is insensitive to the zinc finger inhibitor trichostatin A (TSA; unpublished data). It is possible, therefore, that whereas Atrophin-mediated repression may involve histone deacetylation, Bks could repress transcription through a separate mechanism.

Our results have not revealed any differences between the molecular functions of the two Bks isoforms. Both Bks-A and Bks-B repress transcription when tethered to DNA, and the sequences that mediated binding to Tll and Atrophin are shared between the two isoforms. However, the *bks*339 allele that selectively affects the Bks-B isoform causes a weaker, but comparable phenotype to the stronger *bks* alleles that disrupt both isoforms. Therefore, the C-terminus of Bks-B provides a function that is indispensable for embryo development and regulation of *kni* expression. This part of Bks-B contains two regions (D3 and D4) that are highly conserved in insects and loosely conserved in deuterostome Bks sequences, but does not resemble any sequence with known function. The only sequence similarity to domains found in other proteins is a single zinc-finger motif in Bks-B. Preliminary results indicate that the zinc finger in isolation or together with the conserved D2 domain does not exhibit sequence-specific DNA binding activity (unpublished data). Indeed, multiple zinc fingers are generally required to achieve DNA binding specificity (reviewed in [43]). Instead, Bks is likely brought to DNA through interactions with Tll and other transcription factors.

Atrophins are required for embryonic development in *Caenorhabditis elegans, Drosophila*, zebrafish, and mice [39,41,42,44–47]. In vertebrates, two atrophin genes are present. Atrophin-1 is dispensable for embryonic development in mice, and lacks the N-terminal MTA-2 homologous domain that interacts with histone deacetylases [48]. However, the homologous C-termini of Atrophin-1 and Atrophin-2 can interact, and we found that this domain can also bind to the human Bks homolog ZNF608 (Figure 5H). Atrophin-1 interacts with another co-repressor–associated protein as well, ETO/MTG8, and can repress transcription when tethered to DNA [49]. These data are consistent with the emerging view that deregulated transcription may be an important mechanism for the pathogenesis of polyglutamine diseases (reviewed in [50,51]). Recent evidence indicates that interactions with the normal binding partners may cause toxicity of polyglutamine-expanded proteins such as Ataxin-1 [52]. It will be interesting to investigate whether the interaction between human Bks homologs and Atrophin-1 is important for the neuronal toxicity of polyglutamine-expanded Atrophin-1.

**Materials and Methods**

*Generation of germline clones, cuticle preparations, in situ hybridization and immunohistochemistry, molecular cloning, P element transformation, GST pulldowns, RT-PCR, cell culture and transient transfections, immunoprecipitation, and chromatin immunoprecipitation* are described in Protocol S1.

**Bks alleles.** The *bks* alleles *bks*14, *bks*278, and *bks*339 were generated on an FRT2R-G13-containing chromosome in germline clone ethyl-methane sulfonate (EMS) screens performed in Tübingen ([13] and N. Vogt, unpublished data). Recombination mapping placed the 2R-14 locus on chromosome arm 2R between the markers curved (52D) and flexes (58E). Complementation tests with deficiencies covering this area narrowed the 2R-14 locus down to approximately 600 kb between 55B and 55E, uncovered by the deficiency Df(2R)PC4. We performed complementation tests with all available lethal mutants in this interval and found that the 2R-14, 2R-278, and 2R-339 alleles fail to complement the *bks* alleles *l(2)04440*, *bks*14, and *bks*339 (2R04444 is a P element insertion described in [17]. The *bks*14 and *bks*339 EMS-induced alleles, kindly provided by Barry Dickson (described in [15]), were recombined to an FRT2R-G13-containing chromosome (using stock #19058 in [53]). The *bks*14 and *bks*339 alleles were outcrossed against an FRT2R-G13 c px spCyO hs-hid chromosome to clean the stock from additional mutations. Four different recombinants (two from both sides of the bks locus) were tested and showed no significant phenotypic differences from the parental chromosomes.

The *bks*14, *bks*278, and *bks*339 alleles were balanced over CyO tubulin-GFP to enable isolation of homozygous mutant larvae. Genomic DNA was prepared and *bks* exonic sequences amplified by PCR, sequenced, and compared to an FRT2R-G13 chromosome derived from another mutant from the screen, 2R-91.

**Genetics.** Females harboring *bks* germline clones were crossed with transgenic males to introduce various transgenes into *bks* mutant embryos. To determine if *kni* expansion in *bks* germline clone embryos is due to transcriptional control, we crossed males containing a lacZ reporter regulated by a 4.4-kb *kni* enhancer (GO125 kni-lacZ; [23]) to *bks*339 germline clone females and analyzed the resulting embryos for *lacZ* expression using in situ hybridization.

To analyze the activity of Tll and Hb in a *bks* mutant background, males containing transgenes misexpressing *tll* or *hb* were crossed to *bks*278 or *bks*14 germline clone females or to wt females. Expression in a ventral domain of the embryo was achieved by use of the snail promoter (*snail* stocks s196 and s197, described in Protocol S1, and *snail* stock s227, described in [31]). The constructs contain transcriptional stop signals flanked by FRT sites downstream of the snail promoter to allow maintenance of transgenic lines. Ventral expression was activated by crossing in a B2-tubulin-FLP transgene [54]. Male progeny containing both FLP and snail promoter transgenes (in whose
spermatocytes recombination occurred) were crossed to virgins with bks germline clones or to wt virgins; embryos were then collected and processed for in situ hybridization with a kini probe.

To test the repressor activities of Kni, Kr, and Gt proteins in a bks germline clone background, we crossed wt females or females with bks germline clones with males containing modified rhomboid NEE enhancer. The NEE-kni-lacZ transgene (lab stock A45) is described in [33] and contains synthetic Kni binding sites, but lacks Snail sites. NEE-Kr-lacZ (lab stock G5.3) contains synthetic Kr sites, but lacks Snail sites, and is described in [34]. The 2xgt-55 lacZ reporter gene is described in [35]. It is activated by the rhomboid NEE as well as the 2xFPE. twist enhancer and contains two Gt sites situated 55 bp upstream of the transcription start site. Embryos were collected and fixed 2–4 h after egg laying, and lacZ expression patterns were analyzed by in situ hybridization.

Flies containing a modified rhomboid NEE-lacZ reporter gene with three UAS sites (described in [34]) were crossed to wt or Kreggy-BksA transgenic flies (see Protocol S1), embryos collected, and lacZ reporter gene expression analyzed by in situ hybridization.

Genetic interactions between bks and tll were tested by crossing bks;+/; tll females with tll/TM3 Sb males. Embryos from this cross were compared to embryos derived from bks;+/; females crossed to wt males, and with embryos derived from the tll; stock.

Chromatin immunoprecipitation and real-time PCR. A detailed description of this procedure can be found in Protocol S1. In brief, we established a stable S2 cell line expressing V5-tagged Bks-B, prepared sheared chromatin from this and a control S2 cell line, as we established a stable S2 cell line expressing V5-tagged Bks-B, and performed ChIP essentially as from 2–4-h wt embryos, and performed ChIP essentially as described in [33]. ChIP protocol (Upstate Biotechnology, http://www.upstate.com). Real-time PCR was performed on an ABI prism 7900 machine using Power SYBR Green reagent (Applied Biosystems). PCR was performed on 1 μl (cells) or 3 μl (embryos) template DNA in triplicate samples, and immunoprecipitated DNA was compared against standard curves from serial dilutions of input DNA. The values are plotted as percent input DNA from the corresponding extract, and the standard deviation within the triplicate samples indicated. Similar results were obtained in independent ChIP experiments.

Supporting Information

Figure S1. Protein Sequence Alignment of Brakeless Homologs

The sequences spanning the D2 domain and the C2-H2 zinc finger were aligned with ClustalW (DNASTAR Lasergene, http://www.dnastar.com). Species included in the analysis are the fruit flies Drosophila melanogaster (Dm) and D. pseudoobscura (Dp), the mosquito Anopheles gambiae (Ag) and Aedes aegypti (Aae), honeybee Apis mellifera (Am), flour beetle Tribolium castaneum (Tc), sea urchin Strongylocentrotus purpuratus (Sp), pufferfish Tetraodon nigroviridis (Tn), zebrafish Danio rerio (Dr), mouse Mus musculus (Mm), and human Homo sapiens (Hs).

References

1. Levine M, Davidson EH (2005) Gene regulatory networks for development. Proc Natl Acad Sci U S A 102: 4936–4942.
2. Hanna-Rose W, Hansen U (1996) Active repression mechanisms of embryonic gene expression in Drosophila melanogaster. Adv Dev Biol Biochem 12: 155–204.
3. Gray S, Levine M (1996) Transcriptional repression in development.Curr Opin Cell Biol 8: 358–364.
4. Ip YT, Hemavathy K (1997) Drosophila development. Delimiting patterns by repression. Curr Biol 7: R216–R218.
5. Nibu Y, Senger K, Levine M (2003) CtBP-independent repression in the Drosophila embryo. Mol Cell Biol 23: 3990–3999.
6. Mannervik M, Nibu Y, Zhang H, Levine M (1999) Transcriptional repression: The long and the short of it. Trends Genet 15: 478–483.
7. Rosenfeld MG, Lunyak VV, Glass CK (2006) Sensors and signals: A coregulators in development. Science 284: 606–609.
8. Courjey R, Jia S (2001) Transcriptional repression: The long and the short of it. Genes Dev 15: 2786–2789.
9. Niewiadomska A, Rivera-Pomar R, La Rosee A, Hader T, Schock F, et al. (1997) A cascade of transcriptional control leading to axis determination in Drosophila. J Cell Physiol 173: 162–167.
10. Rivera-Pomar R, Jacek H (1996) From gradients to stripes in Drosophila embryogenesis. Filling in the gaps. Trends Genet 12: 478–483.
11. Rivera-Pomar R, Lu X, Perrimon N, Taubert H, Jackle H (1995) Activation of posterior gap gene expression in the Drosophila blastoderm. Nature 376: 252–256.
12. Luschnig S, Mousian S, Krauss J, Desjeux I, Perkovic J, et al. (2004) An F1 genetic screen for maternal-effect mutations affecting embryonic pattern formation in Drosophila melanogaster. Genetics 167: 323–342.
13. Yao R, Pang P, Ruan W, Gunning D, Zupursky SL (2000) Brakeless is required for photoreceptor growth-cone targeting in Drosophila. Proc Natl Acad Sci U S A 97: 5966–5971.
14. Senti K, Kelemen K, Eisenhaber F, Dickson BJ (2000) Brakeless is required for lamina targeting of R1-R6 axons in the Drosophila visual system. Development 127: 2291–2301.
15. Kaminker JS, Canon J, Salecker I, Banerjee U (2002) Control of photoreceptor axon target choice by transcriptional repression of Runt. Nat Neurosci 5: 746–750.
16. Yang P, Shaver SA, Hilliker AJ, Sokolowski MB (2000) Abnormal turning behavior in Drosophila larvae. Identification and molecular analysis of an sbbl (sb) allele. Genetics 155: 1161–1174.
17. Funakoshi Y, Minami M, Tabata T (2001) mts shapes the activity gradient of the Dpp morphogen through regulation of thickveins. Development 128: 67–74.
18. Moran E, Jimenez G (2006) The tailless nuclear receptor acts as a dedicated repressor in the early Drosophila embryo. Mol Cell Biol 26: 3446–3454.
19. Nuclear Receptors Nomenclature Committee (1999) A unified nomenclature system for the nuclear receptor superfamily. Cell 97: 161–163.
20. Wang L, Rajan H, Pitman JL, McKeown M, Tsai CC (2006) Histone
deacetylase-associating Atrophin proteins are nuclear receptor corepressors. Genes Dev 20: 525–530.

22. Kanazawa I (1999) Molecular pathology of dentatorubral-pallidolysian atrophy. Philos Trans R Soc Lond B Biol Sci 354: 1069–1074.

23. Pankraz MJ, Busch M, Hoch M, Seifert E, Jackle H (1992) Spatial control of the gap gene knirps in the Drosophila embryo by posterior morphogen system. Science 255: 986–989.

24. Kraut R, Levine M (1991) Mutually repressive interactions between the gap genes giant and Kruppel define middle body regions of the Drosophila embryo. Development 111: 611–621.

25. Kraut R, Levine M (1991) Spatial regulation of the gap gene giant during Drosophila development. Development 111: 601–609.

26. Eldon ED, Pirrotta V (1991) Interactions of the Drosophila gap gene giant with maternal and zygotic pattern-forming genes. Development 111: 367–378.

27. Capovilla M, Eldon ED, Pirrotta V (1992) The giant gene of Drosophila encodes a b-ZIP DNA-binding protein that regulates the expression of other segmentation gap genes. Development 114: 99–112.

28. Steingrimsson E, Pignoni F, Liaw GJ, Lengyel JA (1991) Dual role of the Drosophila pattern gene tailless in embryonic termini. Science 254: 418–421.

29. Pankraz MJ, Hoch M, Seifert E, Jackle H (1989) Knüppel requirement for knirps enhancement reflects overlapping gap gene activities in the Drosophila embryo. Nature 341: 337–340.

30. Hoch M, Gerwin N, Taubert H, Jackle H (1992) Competition for overlapping sites in the regulatory region of the Drosophila gene Knüppel. Science 256: 94–97.

31. Clyde DE, Corado MS, Wu X, Pare A, Papatsenko D, et al. (2003) A self-enhancing network of repressor gradients establishes segmental complexity in Drosophila. Nature 426: 849–853.

32. Alberga A, Boulay JL, Kempe E, Dennefeld C, Haenlin M (1991) The snail gene encodes a bZIP DNA-binding protein that regulates the expression of other segmentation gap genes. Development 109: 493–502.

33. Arnosti DN, Gray S, Barolo S, Zhou J, Levine M (1996) The gap protein knirps mediates both quenching and direct repression in the Drosophila embryo. EMBO J 15: 3659–3666.

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

35. Hewitt GF, Strunk BS, Margalis C, Pripinut T, Wang XD, et al. (1999) Transcriptional repression by the Drosophila giant protein: cis element positioning provides an alternative means of interpreting an effector gradient. Development 126: 1201–1206.

36. Wehn A, Campbell G (2006) Genetic interactions among scribbler, Atrophin and groucho in Drosophila uncover links in transcriptional repression. Genetics 173: 849–861.

37. Ryu JR, Arnosti DN (2003) Functional similarity of Knirps CtBP-dependent and CtBP-independent transcriptional repressor activities. Nucleic Acids Res 31: 4654–4662.

38. Mannervik M (2001) Corepressor proteins in Drosophila development. Curr Top Microbiol Immunol 254: 79–100.

39. Zhang S, Xu L, Lee J, Xu T (2002) Drosophila atrophin homolog functions as a transcriptional corepressor in multiple developmental processes. Cell 108: 45–56.

40. Zhang CL, Zou Y, Yu RT, Gage FH, Evans RM (2006) Nuclear receptor TLX prevents retinal dystrophy and recruits the corepressor atrophin1. Genes Dev 20: 1308–1320.

41. Zoltewicz JS, Stewart NJ, Leung R, Peterson AS (2004) Atrophin 2 recruits histone deacetylase and is required for the function of multiple signaling centers during mouse embryogenesis. Development 131: 3–14.

42. Erkner A, Roure A, Charroux B, Delage M, HoÌławy N, et al. (2002) Grunge, a novel zinc finger protein, is related to human Atrophin-like proteins, has multiple functions in Drosophila development. Development 129: 1119–1129.

43. Iuchi S (2001) Three classes of C2H2 zinc finger proteins. Cell Mol Life Sci 58: 625–635.

44. Ch'ng Q, Kenyon C (1999) egl-27 generates anteroposterior patterns of cell fusion in C. elegans by regulating Hox gene expression and Hox protein function. Development 126: 3303–3312.

45. Herman MA, Ch'ng Q, Lettahh SM, Ratliff TM, Kenyon C, et al. (1999) EGL-27 is similar to a metastasis-associated factor and controls cell polarity and cell migration in C. elegans. Development 126: 1055–1064.

46. Solari F, Bateman A, Ahringer J (1999) The Caenorhabditis elegans gene egl-27 and egr-1 are similar to MFA1, a member of a chromatin regulatory complex, and are redundantly required for embryonic patterning. Development 126: 2483–2494.

47. Asai Y, Chan DK, Starr CJ, Kappler JA, Kollmar R, et al. (2006) Mutation of the atrophin2 gene in the zebrafish disrupts signaling by fibroblast growth factor during development of the inner ear. Proc Natl Acad Sci U S A 103: 9069–9074.

48. Shen Y, Lee G, Choe Y, Zoltewicz JS, Peterson AS (2007) Functional architecture of atrophins. J Biol Chem 282: 5037–5044.

49. Wood JD, Nucifora FC Jr, Duan K, Zhang C, Wang J, et al. (2000) Atrophin-1, the dentato-rubral and pallido-lysian atrophy gene product, interacts with ETOMT8 in the nuclear matrix and represses transcription. J Cell Biol 150: 939–948.

50. Helminger D, Zora I, Devys D (2006) Transcriptional alterations and chromatin remodeling in polyglutamine diseases. Trends Genet 22: 562–570.

51. Butler R, Bates GP (2006) Histone deacetylase inhibitors as therapeutics for polyglutamine disorders. Nat Rev Neurosci 7: 784–796.

52. Lam YC, Bowman AB, Jafar-Nejad P, Lim J, Richman R, et al. (2006) ATAXIN-1 interacts with the repressor Capicua in its native complex to cause SCA1 neuropathology. Cell 127: 1335–1347.

53. Chou TB, Ferrimon N (1996) The autosomal FLP-DFS technique for generating germline mosaics in C. elegans. Development 126: 3303–3312.

54. Struhl G, Fitzgerald K, Greenwald I (1993) Transcriptional repression by the Lin-12 and Notch intracellular domains in vivo. Cell 74: 331–345.