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Sap18 is required for the maternal gene *bicoid* to direct anterior patterning in *Drosophila melanogaster*

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Abstract

Development of the insect head is a complex process that in *Drosophila* requires the anterior determinant, Bicoid. Bicoid is present in an anterior-to-posterior concentration gradient, and binds DNA and stimulates transcription of head-specific genes. Many of these genes, including the gap-gene *hunchback*, are initially activated in a broad domain across the head primordium, but later retract so that their expression is cleared from the anterior-most segmented regions. Here, we show that retraction requires a Bicoid-interacting protein, Sap18, which is part of the Sin3/Rpd3 histone deacetylase complex. In sensitized-mutant backgrounds (e.g., bcd^{E1}/+), removal of maternal *sap18* results in embryos that are missing labrally derived parts of the cephalopharyngeal skeleton. These *sap18* mutant embryos fail to repress *hb* expression, and show reduced anterior cap expression of the labral determinant *cap* '*n*' *collar*. These phenotypes are enhanced by lowering the dose of *rpd3*, which encodes the catalytic subunit of the deacetylase complex. The results suggest a model where, in labral regions of the head, Bicoid is converted from an activator into a repressor by recruitment of a co-repressor to Bicoid-dependent promoters. Bicoid's activity, therefore, depends not only on its concentration gradient, but also on its interactions with modifier proteins within spatially restricted domains.

Keywords: Transcription repression; Histone deacetylase; Pattern formation

Introduction

Anterior–posterior (A–P) patterning in the *Drosophila* embryo depends upon members of three maternal polarity systems encoded by the anterior, posterior, and terminal group genes. The head and thorax are specified by maternally deposited products of anterior group genes, including *bicoid* (*bcd*), which encodes a morphogen that stimulates expression of zygotic genes along the A–P axis in a concentration-dependent manner (Driever and Nüsslein-Volhard, 1988a,b; Frohnhöfer and Nüsslein-Volhard, 1986). Among its targets are the gap genes *hunchback* (*hb*), *giant* (*gt*), *krüppel* (*Kr*), and *knirps* (*kni*) and head-specific genes *orthodenticle* (*otd*), *buttonhead* (*btd*), and *empty spiracles* (*ems*) (Driever and Nüsslein-Volhard, 1989; Finkelstein and Perrimon, 1990; Struhl et al., 1989; Wieschaus et al., 1984).

Proper development of the head also requires a contribution from terminal group genes that encode components of a receptor-mediated MAPK signal-transduction pathway (Perrimon, 1993; St. Johnston and Nüsslein-Volhard, 1992). This pathway is activated at the two poles of the embryo by the somatic follicle cells, which generate a localized ligand that binds Torso, a receptor tyrosine-kinase (Perrimon et al., 1995). This pathway activates *tailless* (*tll*) and *huckebein* (*hkb*) at both ends of the embryo (Duffy and Perrimon, 1994; Pignoni et al., 1992; Weigel et al., 1990).

Interactions between the anterior and terminal systems that are required for head specification are not well

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understood. However, it is clear that both systems are required for a phenomenon known as "retraction," in which Bicoid-dependent genes are down-regulated at the anterior tip of the embryo during cellularization stages. This region has the highest concentration of Bicoid, and yet localized expression of genes such as *hb* and *otd* is cleared from this part of the embryo (Driever and Nüsslein-Volhard, 1989; Finkelstein and Perrimon, 1990; Gao et al., 1996; Tautz, 1988). In terminal-system mutants, such as the *torso* (*tor*) loss-of-function alleles, retraction is lost and head development is impaired (Ronchi et al., 1993). It had been hypothesized that phosphorylation of Bicoid by the terminal system kinases directly inhibits its activity thereby causing retraction, but this could not be demonstrated (Bellaïche et al., 1996; Janody et al., 2000a). An alternative possibility is that, under control of the terminal system, Bicoid interacts with other proteins that may inhibit its activity.

One such protein might be *Drosophila* Sap18, first identified as a Bicoid-interacting protein in a modified two-hybrid screen (Zhu et al., 2001). Sap18 is the fly ortholog of human SAP18, a Sin3A-associated protein, which is a component of the Sin3-Rpd3 histone deacetylase co-repression (HDAC) complex (Mannervik and Levine, 1999; Pennetta and Pauli, 1998; Zhang et al., 1997). In *Drosophila* S2 cells, Sap18 represses Bicoid-dependent gene expression, suggesting that interaction of Bicoid with Sap18 recruits the *Drosophila* Sin3-Rpd3 HDAC1 complex to DNA, turning Bicoid from an activator into a repressor (Zhu et al., 2001). This interaction might be necessary for the retraction phenomenon, which is critical for proper head development.

Although SAP18 is conserved among humans, *Caenorhabditis elegans*, and flies, little is known about its function. No clear ortholog exists in yeast, and there are no compelling data on its exact role in any organism. No functional motifs are revealed by its sequence. It was discovered by biochemical fractionation of human cell extracts as an 18-kDa peptide that co-purifies with the Sin3A/Rpd3 HDAC complex, and enhances Sin3-mediated repression (Zhang et al., 1997). *Drosophila* Sap18 also interacts with GAGA and E(z) proteins, which are implicated in Trithorax- and Polycomb-mediated regulation of homeotic genes, respectively (Espinás et al., 2000; Wang et al., 2002). Mammalian SAP18 interacts with Su(Fu), which is a repressor of the Gli transcription factor in the Hedgehog signaling pathway (Cheng and Bishop, 2002). In each study, SAP18 was proposed to be an adaptor protein that bridges the interaction between a DNA-binding protein and a Sin3-HDAC co-repression complex. SAP18 was also found to be part of a complex known as ASAP, which contains both an RNA-binding protein (RNPS1) and a caspase (Acinus), suggesting that it plays a role in RNA processing and apoptosis (Schwerk et al., 2003).

In this paper, we sought to test whether the interaction of Bicoid with Sap18 is important for embryonic head development, and whether Sap18 is required for retraction. To this end, we used P-element excision to generate a series of chromosomal deletions that removed the gene encoding Sap18 (*sap18*), and we examined the consequences on both germline and embryonic development. We found that *sap18* is required maternally and zygotically, and that embryos from *bcd sap18* double mutant mothers fail to undergo normal retraction of the gap gene *hb* at the anterior tip of the embryo. Failure to down-regulate *hb* leads to loss of expression of the labral determinant *cap* '*n*' *collar*, resulting in severe head defects. This phenotype is further enhanced by reducing the dosage of *rpd3*, indicating that repression by histone deacetylases is likely to be involved. Thus, Bicoid's activity is spatially regulated in the embryo, not only by its concentration gradient along the A–P axis, but also by its interaction with a modifier (co-repressor) protein that alters its activity. The results also reveal roles for Sap18 in oogenesis, and in larval and pupal development, that are independent of Bicoid.

Materials and methods

Fly stocks

The EP(3)3462 insertion line (Rorth, 1996) used to generate *sap18* alleles was w^{1118}; $P\{w^{+mC} = EP\}EP3462/TM6B\ Tb\ Hu$, the bcd^{E1} null stock was $st^1\ kni^{ri-1}\ bcd^6 roe^1\ p^p\ nos^{l7} e^1/TM3\ Sb^1\ Ser^1$, and a stock containing a strong hypomorphic rpd^- allele was $P\{ry^{+t7.2} = PZ\}l(3)04556^{04556} ry^{506}/TM3\ ry^{RK}\ Sb^1\ Ser^1$. The *torso* loss-of-function (tor^4) and gain-of-function (tor^{4021}) stocks were tor^4 *cn bw/CyO I(2)DTS513* and *T(1;2)Bld/ T(1;2)064/ tor4021*, respectively. The *lacZ* and *GFP* balancer lines were *P{ry[+t7.2] = ftz-lacZ ry[+]} TM3 Sb[1] ry[*]/Dr[Mio]* and w^*; $Sb^1/TM3\ P\{w^{+m} = Act\ GFP\}\ JMR2\ Ser^1$, *respectively.* Oregon R was used as a wild-type control.

Excision screen

The EP(3)3462 insertion is located 259 bp from the *sap18* start codon and was mobilized by crossing to females of the transposase line, w^{1118}; $P\{ry^{+t7.2} = \Delta 2-3\}99B,\ Sb/ TMb,Ubx$. Dysgenic *EP(3)3462/ P[Δ2−3]* F1 males were crossed to w^{1118}; *H[2]/TM3,* Sb^1 females. F2 non-Sb males (lacking the transposase *P[Δ2-3]*), were screened for loss of w^{+mC} marker caused by excision of the EP element. 500 white-eyed males were identified and were individually backcrossed to w^{1118}; *H[2]/TM3,* Sb^1 females, and the progeny were analyzed. Using PCR, we identified excisions in which the P3 end of the insertion (proximal to *sap18*) was intact, and these were discarded. For the remaining excisions (397), we carried out Southern hybridization (using Bgl2) to distinguish between precise and imprecise excisions, or deletions internal to the EP element. Balanced lines were made for 56 imprecise excision lines, and PCR and DNA