Establishment of cell fate during early *Drosophila* embryogenesis requires transcriptional Mediator subunit dMED31

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**Abstract**

During early *Drosophila* embryogenesis, formation of the anterior–posterior (A/P) axis depends on spatial gradients of maternal morphogens. It is well recognized that positional information is transmitted from these morphogens to the gap genes. However, how this information is being transmitted is largely unknown. The transcriptional Mediator complex is involved in the fine tuning of the signaling between chromatin status, transcription factors and the RNA polymerase II transcription machinery. We found that a mutation in the conserved subunit of the Mediator complex, dMED31, hampers embryogenesis prior to gastrulation and leads to aberrant expression of the gap genes *knirp* and *Krüppel* and the pair-rule genes *fusarityazu* and *even-skipped* along the A/P axis. Expression of the maternal morphogens *dorsal* and *hunchback* was not affected in *dMED31* mutants. mRNA expression of *dMED31* exactly peaks between the highest expression levels of the maternal genes and the gap genes. Together, our results point to a role for dMED31 in guiding maternal morphogen directed zygotic gap gene expression and provide the first *in vivo* evidence for a role of the Mediator complex in the establishment of cell fate during the cellular blastoderm stage of *Drosophila melanogaster*. © 2007 Elsevier Inc. All rights reserved.

**Keywords:** A/P polarity; Mediator complex; Cell fate; dMED31

**Introduction**

Proper fine tuning of the eukaryotic transcriptome depends on numerous *cis* and *trans* acting factors that modulate the chromatin environment of genes and influence the RNA polymerase II (RNAPII) transcription machinery. The Mediator complex is a core processor in the signaling between RNAPII and transcription factors. This complex is an evolutionary conserved protein assembly of 25–30 subunits (Boube et al., 2002; Bourbon et al., 2004; Guglielmi et al., 2004; Linder and Gustafsson, 2004; Blazek et al., 2005) which was first identified in budding yeast (Flanagan et al., 1991) and consists of four large modules; head, middle, tail and an Srb8–11 module (Asturias et al., 1999; Borggrefe et al., 2002; Samuelsen et al., 2003; Guglielmi et al., 2004).

Support for a specific role during development of Mediator subunits is provided by several studies in *Drosophila melanogaster* (Boube et al., 2000; Gim et al., 2001; Treisman, 2001; Garrett-Engele et al., 2002; Janody et al., 2003; Loncle et al., 2007) and *Caenorhabditis elegans* (Kwon et al., 1999; Zhang and Emmons, 2001; Howard and Sundaram, 2002; Yoda et al., 2005). These studies describe mostly functions of Mediator subunits during late developmental stages, but a role of the subunits during early embryonic development is largely unknown. The Mediator consists of more than 25 subunits, pointing to a multifaceted role of this complex during metazoan development. Understanding this complexity starts with the identification of the function of each subunit.

*D. melanogaster* MED31 was identified by bioinformatics analysis (Boube et al., 2000) and its presence in the Mediator complex was confirmed in purified complexes from embryos and cells (Park et al., 2001; Gu et al., 2002). In a pull down assay, the Mediator (containing dMED31) complex binds to the transcription factors bicoid (bcd), Krüppel (Kr), fushi tarazu (ftz), dorsal (dl) and HSF, but not twist (twi), hunchback (hb) and even-skipped (eve) (Park et al., 2001). Moreover, the Mediator complex is required for *in vitro* transcription from...
developmentally important promoters regulated by these transcription factors. Despite these in silico and in vitro results, to date the functional role of MED31 in eumetazoan remains elusive. Here we report the identification of the highly conserved Drosophila Mediator subunit dMED31 as a novel maternal-effect gene necessary for proper segment specification during early embryogenesis. dMED31 mutant females have fecundity defects and embryos deposited by homozygous mothers display severe defects along the anterior–posterior (A/P) axis when gastrulation is initiated. Whereas expression of maternal morphogens is not affected, alterations in gap and pair-rule gene expression during the proceeding blastoderm stage correlate with these defects observed in dMED31 mutant embryos. Remarkably, a small percentage of the progeny of homozygous mutant females escape from embryonic death and develop into adults. These escapers have defects in their abdominal segmentation pattern, a phenotype enhanced by mutations in dMED13. Our findings provide the first in vivo evidence for a specific role of dMED31 in establishing cell fate in the cellular blastoderm and point to a role for the Mediator in guiding maternal morphogen directed zygotic gap gene expression.

Material and methods

Drosophila stocks and genetics

Fly stocks were maintained at 22 °C according to standard protocols. The E709 stock was a generous gift of A. Ephrussi (Heidelberg, Germany). The dMED17Δ and dMED31Δ P element insertion lines are previously described (Bougie et al., 2000) and were obtained from the Bloomington stock center (Indiana University, USA). P element excision lines were generated with the ∆2–3 transposase (Robertson et al., 1988). From a collection of approximately 75 individual excision lines, a back to wt allele (dMED13) was isolated. Genomic DNA surrounding the P element insertion site was sequenced to confirm precise excision.

dMED31 mapping and transgene construction

Plasmid rescue analysis was performed to recover the 3′ and 5′ flanking genomic sequences of the pLaCw insertion site (Guo et al., 1996). Subsequent DNA sequencing and database searches revealed the exact genomic position of the P element. PCR analysis combined with complementation tests by means of standard crosses to deficient chromosomes encompassing the identified cytological position was used to confirm the localization of the P element and to confirm a link between the P element insertion and the female fertility phenotype of the E709 mutant. A genomic fragment containing the dMED31 locus (CG1057), its preceding intergenic region together with approximately 250 bp of genomic DNA flanking the polyadenylation site was PCR amplified with primer pair 5′-GAAGGATCCCGCCATACATAGGTTAG-3′ and 5′-AGGCGAATTCTGTGGGTCTGTGAATTCAC-3′, cut with BamHI and EcoRI and cloned in an identically digested pCasperd-Pmel provided by L.G. Fradkin (Leiden, The Netherlands). A transgenic fly carrying the P(dMED31) transgene on the second chromosome was created by Genetic Services Inc. (Sudbury, USA) and was crossed into the E709 strain. Single fly PCR analysis was used to confirm its presence (Gloor et al., 1993).

Assessment of embryonic viability and abdominal defects

To assay embryonic viability, embryos were collected (0–6 h) on apple juice plates and counted, and the hatch rate was determined by visual inspection of the egg cases 2 days after egg laying. Female fecundity was assayed by crossing 10 1-week-old females with 5 males in standard food vials containing yeast paste. After 3 days, flies were transferred to vials without yeast granules/paste and the amount of embryos deposited after 20 h was determined. Groups were compared by Student’s t-test. Inspection of abdominal defects was carried out by visual inspection of 2–5 day-old flies. Images were captured with a Olympus BX50 light microscope.

Immunofluorescence

For these studies, embryos deposited by dMED311/w mothers crossed with wt males or embryos deposited by wt females crossed with wt males were used. Isolation, fixation and immunolabeling of embryos were performed as described (Theurkauf, 1994). Blocking of embryos and labelings were performed in PBS + 0.3% TritonX-100 + 5% BSA. Primary antibodies included mouse anti-pH3S10 (1:200, Cell Signaling), rabbit anti-tailless (1:1000), rabbit anti-Krüppel (1:500) and rabbit anti-ABX-2 (1:50) (generously provided by H. Jäckle and P. Carrera, Göttingen, Germany) and concentrated supernatants of mouse anti-even-skipped (1:5) (3C10 developed by C. Goodman) and mouse anti-dorsal (1:5) (7A4 developed by R. Steward) obtained from the Developmental Studies Hybridoma Bank (Iowa, USA). Secondary antibodies included FITC-conjugated goat anti-mouse or goat anti-rabbit IgGs (Jackson ImmunoResearch). Embryos were incubated in a 0.2 μg/ml DAPI solution in PBS to visualize the DNA, mounted in citifluor (Agar scientific) and analyzed by FM, LM (Carl Zeiss Axioskop2) or CLSM (Leica TCS SP2). Captured images were processed using Paint Shop Pro.

In situ hybridization

For in situ hybridization experiments, embryos deposited by dMED311/w mothers crossed with either dMED311/w or dMED311/w males were pooled and compared to embryos deposited by wt mothers crossed with wt males. Hybridizations were essentially carried out as described (Tautz and Pfeifle, 1989). Plasmids for probe production against Krl (pT:Krl) (Knipple et al., 1985), kni (pcJ5) (Pankratz et al., 1990), eve (p48-X1.4) (Macdonald et al., 1986), fit (pT-fit) (Ish-Horowicz and Pinchin, 1987) and en (pen) were generously provided by P. Gergen (Stony Brook, USA). Digoxigenin (DIG) incorporated RNA probes were generated from linearized plasmids by in vitro transcription in the presence of a DIG RNA labeling mixture (Roche). Hybridizations were carried out at 55–60 °C and the probes were detected with anti-digoxigenin-AP Fab fragments (Roche). Chi square tests were used to compare the percentages of abnormal mRNA expression in control versus dMED31 mutant stage 3–8 embryos. The quantified data represent the total amount from 3 independent hybridization experiments.

Results

Mutations in dMED31 cause defects in fecundity and embryogenesis

We analyzed libraries of existing single P element insertions in D. melanogaster for novel genes involved in embryogenesis. From a collection of mutants (kindly provided by A. Ephrussi) that affect female fertility, we recovered a single P element insertion line E709 (dMED311, see below) as a candidate for further investigation. Embryos deposited by homozygous females crossed with wild-type (wt) males (further referred to as mutant embryos) rarely hatched (2.1%) compared to embryos deposited by wt females (88.2%) further referred to as wt embryos (Table 1). Unless otherwise specified, all studies in this manuscript were performed with embryos derived from females that were crossed with wt males. In the mutant, the P element was mapped within the 5′-UTR of CG1057 (Fig. 1A) and its
location was confirmed by PCR mapping (not shown). This gene encodes the fly homologue of the \textit{S. cerevisiae} suppressor of HPR1 (SOH1) and is part of the transcriptional Mediator complex (Boube et al., 2000; Park et al., 2001). SOH1 is also known as TRAP18, but recently unified nomenclature designated the protein MED31 (Bourbon et al., 2004), therefore the mutant will be referred to as \textit{dMED311}. The \textit{dMED31} locus encodes two alternative transcripts as evident from the expressed-sequence-tagged database (www.fruitfly.org), which both encode the full length \textit{Drosophila} \textit{dMED31} protein (Fig. 1C). PCR analysis and rapid amplification of cDNA ends (RACE) analysis of cDNA isolated from \textit{dMED311/1} females revealed the presence of P-lacW specific sequences in the 5′-UTR of the \textit{dMED31} transcript, indicating that transcription originates from the P element (not shown). As the transposon landed in the first intron of CG1057-RA, exactly after the first splice donor site of CG1057-RB, it is likely that appropriate splicing and production of CG1057-RB do not occur (Fig. 1B). Therefore, we conclude that the P element insertion in the \textit{dMED31} mutant alters \textit{dMED31} mRNA structure and production.

In addition to poor hatching rates of embryos deposited by \textit{dMED311/1} females, mutant females also displayed fecundity defects and cohorts of 10 \textit{dMED311/1} females produced only 7.4±1.5 eggs/20 h (\(p<0.0001\)) compared to 46.0±3.3 eggs deposited by wt females (Table 1). In order to confirm linkage between the observed mutant phenotype and the P element insertion, \textit{dMED311} was placed over the deficiency Df(3R)Z1,ry*, in which at least 82 genes surrounding the \textit{dMED31} locus are deleted. Transheterozygous \textit{dMED31 1/Df(3R)Z1,ry*} females showed more severe fecundity defects (\(p<0.05\))

<table>
<thead>
<tr>
<th>Genotype female</th>
<th>Hatching rate (%)</th>
<th>Fecundity (eggs/20 h±SEM)</th>
</tr>
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<tbody>
<tr>
<td>(y^{1/1}w^{118})</td>
<td>88.2 ((n=1005))</td>
<td>46.0±3.3 ((n=45) cohorts)</td>
</tr>
<tr>
<td>(dMED311/1)</td>
<td>2.1 ((n=574))</td>
<td>7.4±1.5 ((n=42) cohorts)</td>
</tr>
<tr>
<td>(dMED311/Df(3R)Z1,ry*)</td>
<td>3.2 ((n=632))</td>
<td>3.2±0.4 ((n=51) cohorts)</td>
</tr>
<tr>
<td>P[dMED31],dMED311/2</td>
<td>91.6 ((n=1287))</td>
<td>ND</td>
</tr>
<tr>
<td>P[dMED31],dMED311/1</td>
<td>57.9 ((n=401))</td>
<td>ND</td>
</tr>
</tbody>
</table>

Flies of indicated genotype were crossed with wt males. Hatching of \(n\) embryos was monitored and the fecundity of \(n\) cohorts of 10 females was determined.
compared to \(dMED31^{1/1}\) females (Table 1). A small percentage of embryos derived from \(dMED31^{1/2}\) mothers is able to hatch and a small percentage of these larvae is able to reach the adult stage (see also below). A small percentage (3.2%) of embryos derived from transheterozygous \(dMED31^{1/2}/\text{Df}(3R)Z1,ry^*\) mothers was also able to hatch; however, none of these larvae reached the adult stage. Based on these observations, we conclude that the \(dMED31^{1/2}\) allele is hypomorphic. Next, we restored the \(dMED31\) insertion allele by remobilizing the P element (Robertson et al., 1988). When we placed \(dMED31\) over a precise excision allele \(dMED31^{21}\), the hatching rate of embryos deposited by \(dMED31^{1/2}\) mothers was restored to 91.6% (Table 1). Finally, when we introduced a genomic fragment that harbors the entire \(dMED31\) locus including its flanking intergenic regions \(P[dMED31]\), the hatching rate was restored to 57.9% (Table 1). Together these results show that an intact \(dMED31\) allele is required for female fecundity and intact function of maternal \(dMED31\) is required for embryonic viability.

**Establishment of embryonic A/P polarity requires Mediator subunit \(dMED31\)**

In order to determine at which stage embryogenesis is disrupted in eggs deposited by \(dMED31^{1/1}\) females, embryos derived from \(dMED31^{1/1}\) females were labeled with an antibody directed against serine 10 phosphorylation of histone H3 (pH3Ser10), which enabled us to visualize mitotic domains within the developing embryo. Close inspection of these mitotic domains in combination with morphological analysis revealed that mutant embryos reach the cellular blastoderm stage (Figs. 2F, G), but begin to display severe defects upon gastrulation (Figs. 2H, I). During wt gastrulation and germ band elongation, cells associated with the migrating polar plate and stomodeum are highly proliferating (Figs. 2C, D). In mutant embryos, these mitotic domains were missing or completely mislocalized and coincided with altered embryonic morphology. The formation of the posterior located pole cells appeared unaffected in mutants (Fig. 2L) but, quickly after their formation cells at the anterior and posterior pole started to delaminate toward the interior of the embryo (Fig. 2N). Normally, pole cells migrate during gastrulation; first over the dorsal surface, then the pole cells migrate inwards the embryo to the posterior end, where they form the future reproductive system. Upon gastrulation, the formation of the major invaginations (proctodeal invagination, transverse furrow, stomodeal invagination and the cephalic furrow) was observed in \(dMED31\) mutant embryos (Fig. 2G). Nevertheless, cells associated with the anterior and posterior pole of mutant embryos frequently displayed abnormal migratory behavior. During subsequent embryonic stages, abnormal cell migration and degeneration at both poles eventually resulted in severe polarity problems within the developing embryo. Especially the cells that accompany the migrating polar plate behaved abnormal (Fig. 2). Moreover, cell loss was frequently more profound at the dorsal-posterior region of mutant embryos. Similar defects were found in embryos deposited by \(dMED31^{1/2}/\text{Df}(3R)Z1,ry^*\) or \(dMED31^{1/2}\) females crossed with \(dMED31^{1/2}\) males. The majority of the embryos deposited by \(dMED31^{1/2}/\text{Df}(3R)Z1,ry^*\) or \(dMED31^{1/2}\) mothers (independent of the paternal genotype) displayed severe morphological defects beyond st.8 (estimated >90%). Thus, a mutation in the \(dMED31\) locus causes a maternal effect phenotype in over 90% of the embryos and leads to aberrant cell migration and impaired anterior–posterior (A/P) axis formation in the embryo, suggesting that \(dMED31\) is an essential factor required for establishing cell fate in the cellular blastoderm.

**Mediator subunit \(dMED31\) is necessary for zygotic gap and pair-rule gene transcription**

Previously, *in vitro* experiments demonstrated that the *Drosophila* Mediator complex is able to bind developmental transcription factors and is essential for the *in vitro* transcription from promoters regulated by these transcription factors (Park et al., 2001). Moreover, immunoprecipitation of embryonic nuclear extracts with an antibody directed against \(dMED31\) abolished Mediator composition and affected *in vitro* RNA transcription, suggesting that the Mediator complex is required to control gene transcription during early development (Park et al., 2001). To test this suggestion, we first analyzed expression profiles of all Mediator genes using available data sets (www.fruitfly.org; Tomancak et al., 2002). These data show that the entire Mediator complex is maternally supplied to the embryo and is highly expressed prior to gastrulation (see Supplementary Fig. 1), indicating that this complex is required at this specific stage during early embryonic development. Comparing the expression profile of the Mediator with expression profiles of maternal genes and gap genes revealed that expression levels of Mediator genes exactly peak between highest expression levels of maternal genes and gap genes during development (see Supplementary Fig. 1), suggesting a specific function of the Mediator complex at this specific moment.

Upon egg deposition, maternally contributed morphogen gradients of genes such as; \(dl\), \(cad\), \(nos\), \(bcd\) or \(hb\) define the embryo’s polarity, the dorsal–ventral (D/V) axis and the A/P axis (Rivera-Pomar and Jackle, 1996). Because expression of \(dMED31\) peaks at a time point later than the maternally supplied morphogens, we hypothesized that localization and expression levels of these morphogens in \(dMED31\) mutants are not affected. In agreement with this hypothesis, the localization and the expression of the maternal morphogens \(dl\) and \(hb\) were unaffected in embryos deposited by \(dMED31^{1/1}\) mothers (Fig. 3E).

During wt embryogenesis, \(cad\), \(nos\), \(bcd\) and \(hb\) regulate expression of the gap genes \(tailless\) (\(tl\)), \(giant\) (\(gt\)), \(knirps\) (\(kni\)) and \(Kr\) (Hulskamp et al., 1990; Kraut and Levine, 1991). \(dMED31\) expression peaks before high expression levels of the gap genes (see Supplementary Fig. 1) which coincided with the timing of the morphological abnormalities observed in \(dMED31\) mutant embryos (Fig. 2). These data suggest that gap gene expression may be affected in \(dMED31\) mutant embryos. To investigate whether \(dMED31\) indeed affects embryonic gap gene expression, we analyzed mRNA and
protein distribution of the gap genes *kni* and *Kr* (Fig. 3A). Early mRNA expression of these genes can be observed at the anterior tip and as a band in the center of wt embryos. Furthermore, *Kr* expression is also strong at the posterior end of wt embryos during gastrulation and germband elongation. These patterns of mRNA expression change during subsequent embryonic stages. The protein expression of both genes globally parallels their mRNA expression pattern. In mutant embryos deposited by
deposited by dMED31/+ females, kni and Kr mRNA expression during st.4–5 is much lower compared to wt embryos (Fig. 3A). Moreover, abnormal mRNA and protein expression patterns of these two genes were observed during early stages of embryogenesis. Interestingly, the polar expression of kni and Kr is hardly detectable or even absent. dMED31/+ embryos also displayed abnormal patches of kni expression in cells that reside within the ventral region (Fig. 3A), suggesting that these cells received incorrect information from the maternal morphogens. The central band of the Kr protein is sometimes smaller in mutant embryos compared to wt Kr expression and high Kr expression could also be found in cells outside the central band. Overall 44.6% of dMED31/+ embryos deposited by homozygous mutant females displayed abnormal Kr mRNA expression and 18.1% of the embryos displayed abnormal kni expression (Fig. 3D).

The maternal terminal system controls the restricted expression of the terminal gap genes tll and huckebein (hkb) in the embryonic termini via the localized activation of the Torso (Tor) receptor tyrosine kinase signaling pathway (Klingler et al., 1988; Weigel et al., 1990; Bronner and Jackle, 1991). Because dMED31 mutant embryos show terminal defects, we analyzed protein expression of tll, whose activity is required to specify cell fates at the embryonic poles (Strecker et al., 1986). Protein expression of tll was unaffected in embryos deposited by dMED31/+ females compared to wt. However, as previously noted, we frequently found gaps in the cellular blastoderm at both termini and we quantified the amount of st.5 embryos in which posterior located tll expressing cells were missing (Fig. 3C). In 28.0% (n=82) of st.5 embryos produced by dMED31/+ mothers, posterior located tll expressing cells were missing (arrows), which was 3.1% in wt embryos (n=96). These data suggest that dMED31 is not required for the activation of the terminal gap genes in response to Tor signaling at the embryonic termini, but dMED31 is required to maintain cell viability at the termini.

In wt embryos, gap gene expression proceeds pair-rule gene expression. Pair-rule gene patterning occurs in 7 distinct stripes. Eve, ftz and other pair-rule genes provide cell identity to cells in the blastoderm stage. In 25% of the embryos deposited by dMED31/+ mothers, the mRNA expression of ftz is lower and abnormal compared to wt embryos (Figs. 3B, D). It should be noted that abnormalities in ftz expression are mainly observed during stages 6–8 and ftz expression seems not severely altered in earlier stages. Similar results were obtained when expression patterns of eve were analyzed in mutant embryos and compared to wt embryos. Wt mRNA expression of eve is initially blurred, but rapidly resolve into sharply defined stripes. In the cellular blastoderm of mutant embryos, only minor alterations in mRNA expression of eve could be observed (Figs. 3B, D) during early stages. The availability of an antibody against Eve allows the analysis of protein expression patterns and this analysis sometimes revealed less than the normal 7 stripes (Fig. 3B). Comparable to abnormalities in ftz expression, abnormal expression patterns of eve mRNA were most obvious in later stages (stages 6–8) (Fig. 3C, data not shown). Based on these observations, we conclude that in dMED31/+ mutant embryos, expression of eve and ftz is affected. Although at this point, we cannot distinguish whether the observed abnormalities are due to primary defects in dMED31 mediated eve and ftz expression or due to embryonic morphological alterations induced by a mutation in dMED31.

Expression of the segment polarity and Hox genes is activated by the pair-rule genes and a subset of the gap genes during late stage embryogenesis (>st.10). We also analyzed the expression of the segment polarity gene en whose expression is restricted to 14 stripes along the A/P axis. Only a limited number of mutant embryos could be scored positive for en expression and did not allow quantification (Fig. 3B). Likely late stage en expression could not be detected in dMED31/+ mutant embryos due to severe defects during gastrulation. Thus, although the morphological abnormalities (Fig. 2) are uniform and observed in over 90% of mutant embryos, it is more complicated to define precisely the abnormalities in zygotically gene expression because of variability (Fig. 3). Despite this, our results demonstrate that the dMED31 subunit is required to establish domains of gap and pair-rule gene expression along the A/P axis during embryogenesis.

Mutations in Mediator subunits dMED31 and dMED13 abrogate abdominal segmentation

During our analysis of the embryonic hatching rate of eggs deposited by dMED31/+ females, we found that 2.1% (n=574) of the embryos was able to hatch. Moreover, we noticed that some of these larvae could also progress through subsequent developmental stages and produced viable adults. Interestingly, morphological analysis of the adult survivors revealed the presence of deformations in abdominal segmentation (Figs. 4A–G, Table 2) and we used these abdominal defects as a quantifiable marker for further analysis of dMED31 function during development. When dMED31/+ females were crossed with wt males 33.7% of the cohort of rare escapers that reached the adult stage developed segmentation defects, while this percentage was 45.6% when dMED31/+ females were crossed with dMED31/+ males. Mutant flies of both sexes displayed features of incomplete tergite separation, showed the formation of additional tergites, developed distinctive patches of pigment in the abdominal epidermis, had abnormally positioned sternites or lacked halteres (Figs. 4B, C, E, G). These defects were not present in flies that carried either one or two copies of the P[dMED31] transgene. Transheterozygous dMED31/+;Df(3R)Z1, ry* or dMED31/+ adults derived from heterozygous parents did not develop significant abdominal deformations (Table 2). Together, this indicates that abnormal maternally supplied dMED31 results in segmentation defects and this effect is enhanced due to aberrant zygotically expression of dMED31. Furthermore, since adults produced by dMED31/+ mothers did not develop segmentation defects (Table 2), the dMED31/+ allele is recessive.

We wondered whether mutations in other Mediator subunits also caused segmentation defects during early embryogenesis. Previously it was shown that the dMED13 and dMED17 Mediator subunits are required for segment identity
specification controlled by the Hox genes proboscipedia and Sex combs reduced during larval development (Boube et al., 2000). Mutant adults develop abnormal labial palps, while dMED13 mutants alone also formed ectopic sex comb teeth on the second tarsal segment of the prothoracic (T1) leg (Boube et al., 2000). Since both mutations are lethal, we investigated abdominal segmentation in heterozygous mutants. Heterozygous dMED17 mutants displayed normal segmentation (not shown); however, when dMED13+/− females were crossed with wt males, 22.7% of the dMED133/+ adult progeny had segmentation defects, while 9.8% of the +/- offspring had defects (Table 2). This indicates that dMED13 is a dominant modifier of segmentation in Drosophila. Most likely abnormal maternal deposition of dMED13 causes segmentation defects in

![Diagram](image_url)
Table 2
Mutations in Mediator subunits dMED13 and dMED31 disrupt abdominal segmentation

<table>
<thead>
<tr>
<th>Crossings (genotype female × male)</th>
<th>Adult offspring</th>
<th>% abnormal</th>
<th>n</th>
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<tr>
<td>y1w1118 × y1w1118</td>
<td>dMED311/+</td>
<td>2.5</td>
<td>315</td>
</tr>
<tr>
<td>dMED311/+ × y1w1118</td>
<td>dMED311/+</td>
<td>3.0</td>
<td>291</td>
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<td>147</td>
</tr>
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<td>dMED311/TM3 × Df(3R)Z1,ry*/TM3</td>
<td>dMED311/1</td>
<td>0.0</td>
<td>139</td>
</tr>
<tr>
<td>dMED311/+ × y1w1118</td>
<td>dMED311/1</td>
<td>1.9</td>
<td>265</td>
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<tr>
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<td>33.7</td>
<td>196</td>
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<td>dMED311/+ × y1w1118</td>
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<td>53</td>
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<td>dMED311/TM3 × Df(3R)Z1,ry*/TM3</td>
<td>dMED311/1</td>
<td>15.1</td>
<td>205</td>
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</table>

The total percentage of abdominal defects of n flies in the progeny derived from flies of indicated genotype is shown.

The +/− offspring and these defects are enhanced by aberrant zygotic expression of dMED13 in the dMED13+/− progeny. Next we analyzed whether the dMED31+/− allele could enhance the dominant dMED31+/− phenotype. When dMED31+/− females were crossed with dMED13+/− males, 54.7% of the dMED31+/−/dMED13+/− adults had abdominal defects (Table 2), demonstrating that zygotic expression of the dMED13 allele enhanced the dMED31 allele phenotype (χ² p<0.006 compared to the progeny from dMED31+/− females crossed to wt males). However, no increase in segmentation defects was observed in the progeny from dMED13+/−/dMED13+/− mothers compared to defects observed in progeny from dMED13+/−/+ mothers (Table 2), demonstrating that dMED31+/− is not an enhancer of dMED13+/− when maternally supplied. This indicates that the dMED31 and dMED13 subunits of the Mediator are both required for proper cell fate specification along the embryonic A/P axis.

Discussion

Cell fate specification requires maternally supplied components of the transcriptional Mediator complex

Our findings identify a component of the conserved eukaryotic transcriptional Mediator complex, dMED31, that is required for normal initiation of zygotic gene expression during the blastoderm stage of Drosophila embryogenesis. Female flies that carry a mutation in the dMED31 gene suffer from fecundity defects and the embryos deposited by these females display abnormal embryogenesis due to aberrant cell migration events upon gastrulation. Impaired embryogenesis coincided with changes in kni, Kr, ftz and eve expression along the A/P axis. Furthermore, adult flies derived from embryos that escaped from embryonic death displayed severe defects in their abdominal segmentation. Because mRNA production was hampered in dMED31+/−, these abdominal defects were likely the result of abnormal maternal and zygotic dMED31 mRNA production. A mutation in the Mediator subunit dMED13 also caused segmentation defects and this mutant enhanced the dMED31 mutant maternal effect phenotype. Therefore, our data indicate that the Mediator complex directs zygotic gene expression upon egg deposition to establish cell fate in the embryonic blastoderm.

In order to accomplish cell fate determination, cells gain a transcriptional poised state during early embryogenesis that is maintained throughout development and requires many cis and trans acting factors that modulate the chromatin environment of the genes involved. In D. melanogaster, cell identity along the A/P is established in the blastoderm stage when the pair-rule genes are expressed. A/P polarity is controlled by the maternal morphogenes cad, nos, bcd and hb whose activity results in the spatio-temporal expression of the gap genes gt, kni, tll and Kr (Hulskamp et al., 1990; Kraut and Levine, 1991; Rivera-Pomar and Jackle, 1996). These gap genes are the first genes expressed along the A/P axis and encode transcription factors that in turn govern patterned expression of the pair-rule genes. Pair-rule gene expression occurs in distinct stripes and is accompanied by cellularization. Thus when cellularization takes place, large clusters of cells gain an imprint that defines the primordial segments. Cell identity is fine tuned when expression of the segment polarity and Hox genes is activated (Sanson, 2001). Although this cascade of maternal, gap, pair-rule and segment polarity genes is well studied, much remains unknown how the maternal morphogens regulate RNAPII activity at their cognate promoters in order to establish regional domains of gap gene expression.

Because segmentation defects in escaper flies derived from dMED31+/− mothers were restricted to the abdomen, it is possible that the bithorax complex (BX-C) is abnormally expressed. This complex contains the homeotic genes Ultra-bithorax, abdominal A and Abdominal B, which control the identity of the posterior two-thirds of the fly (Maeda and Karch, 2006). Mutations in hb, Kr, tll and kni affect expression of BX-C and result in homeotic transformation (Nauber et al., 1988; Shimell et al., 1994; Casares and Sanchez-Herrero, 1995). We did not observe complete homeotic transformations of entire
parasegments, suggesting an indirect effect of \textit{dMED31} on Hox activation. Since segment identity is established during early embryogenesis, this implies that only groups of cells and not whole primordial segments gained abnormal imprinting. Regional errors in cellular imprinting are supported by the variety of the abdominal defects we observed in adult flies. Moreover, defects in embryogenesis were accompanied by cell loss at the embryonic poles and aberrant migratory behavior of cells upon gastrulation, processes which occur prior to the activation of the segment polarity and the Hox genes. Finally, early developmental defects coincided with abnormal expression of the gap genes \textit{kni} and \textit{Kr} and subsequently the pair-rule genes \textit{ftz} and \textit{eve}. These genes are expressed prior to Hox gene expression and are required for activation/repression of the Hox cluster. Although it is possible that the abdominal region is preferentially sensitive for a mutation in \textit{dMED31}, it is more likely that random defects during formation of the abdomen are tolerated, whereas defects in other regions of the embryo are incompatible with adult viability and these adults never eclose.

\textbf{\textit{dMED31} and Mediator functioning}

Several intriguing questions remain: why is the embryonic phenotype so variable (>90% of the mutant embryos die, while a small percentage of embryos is able to reach the adult stage), why are mainly embryos affected by a mutation in \textit{dMED31} and what is the primary embryonic defect caused by a mutation in \textit{dMED31}? Answers to these questions can be derived from studies of the Mediator in yeast in combination with our data. The budding yeast MED31 protein is part of the Mediator transcription initiation complex (Bourbon et al., 2004; Guglielmi et al., 2004; Linder and Gustafsson, 2004). Although a mutation in yeast \textit{MED31} affects gene expression (van de Peppel et al., 2005), mutants displayed no sensitivity to transcriptional inhibition by 6-azauracil and \textit{MED31} was not essential for growth (Fan et al., 1996; Malagon et al., 2004). However, yeast \textit{MED31} mutants have a synthetic growth defective phenotype when combined with mutations in genes encoding for the two largest subunits of RNAPII (RPB1, RPB2) and the transcription initiation factors TFIIB and TFIIS (Fan et al., 1996; Malagon et al., 2004, 2006). Like in yeast, depletion of \textit{dMED31} in \textit{Drosophila} SL2 cells by RNAi did not interfere with the Mediator composition and no growth alterations were reported (Gu et al., 2002). Thus, \textit{Drosophila} \textit{MED31}, like yeast \textit{MED31}, might not be essential for RNAPII activity per se, but could be an auxiliary factor involved in the signaling between specific transcription factors and the RNAPII machinery (Park et al., 2001). Together, these findings and our data suggest that \textit{dMED31} is not required for transcription in general, but is merely required for the fine tuning of transcription of specific genes.

Largely, based on studies in yeast, it was proposed that the Mediator functions as a platform that allows rapid regulation of transcription at (re)initiation (Asturias, 2004). Fast regulation and (re)initiation of transcription might be key during the interphase periods of the final syncytial cell cycles when zygotic transcription is initiated, while such large scale, strict and “fast” control over transcription would not be essential during subsequent stages of development and thus may explain why \textit{dMED31} function is essential during early embryogenesis. The observation that a small percentage of embryos derived from \textit{dMED31}\textsuperscript{-/-} mothers was able to develop into an adult, while the majority of the embryos displayed severe defects during embryogenesis, might also be attributed to such auxiliary function(s) of the \textit{dMED31} protein. Minor differences in \textit{dMED31} protein levels, due to the hypomorphic \textit{dMED31}\textsuperscript{+} allele, may result in subtle changes in the expression of the gap and pair-rule genes and allow embryos to progress throughout embryogenesis, but with the formation of segmentation defects. On the other hand, in the majority of embryos, more severe changes in gap and pair-rule patterning occur, which results in embryonic death.

In summary, we demonstrate that \textit{dMED31} is essential to establish regional domains of expression of cell fate determinants \textit{kni}, \textit{Kr}, \textit{ftz} and \textit{eve}. mRNA expression of \textit{dMED31} peaks exactly between maternal morphogen and gap gene expression and it was demonstrated that the Mediator complex is able to bind to several maternal transcription factors (Park et al., 2001). Together this indicates that the Mediator complex constitutes an interface between the maternal morphogens and the RNAPII machinery to guide zygotic gene expression of cell fate determinants that specify primordial segment identity. These findings provide the first \textit{in vivo} evidence for a role of the Mediator complex in establishing cell fate during early embryogenesis and since \textit{MED31} resembles one of the most conserved subunits within the Mediator complex (Linder and Gustafsson, 2004; Blazek et al., 2005) this protein could serve a crucial role in the control of RNAPII activity during early developmental processes in all higher eukaryotes.

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\section*{Appendix A. Supplementary data}

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2007.11.019.

\section*{References}


