How many signals does it take?

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Summary

Although the genetics of dorsal-ventral polarity which leads to mesoderm formation in *Drosophila* are understood in considerable detail, subsequent molecular mechanisms involved in patterning the mesoderm primordium into individual mesodermal subtypes are poorly understood. Two papers published recently[1,2] suggest strongly that an inductive signal from dorsal ectoderm is involved in subdividing the underlying mesoderm, and present evidence that one of the signalling factors is Decapentaplegic (Dpp), a member of the bone morphogenetic protein subgroup of the Transforming Growth Factor-β (TGF-β) super family of proteins.

Introduction

Specification of cell fate during development is a multistep process. Cells often receive different signals from neighboring cells at different stages during their development, which play a significant role in determining their developmental fate. Members of the TGF-β superfamily, which are widely conserved in different organisms, constitute a major group of signalling molecules. They appear to mediate key events in normal growth and development and exhibit diverse activities like specification of body axis and induction of mesoderm in Xenopus, and control sexual development and creation of bones and cartilage in mammals (reviewed in ref. 4). In *Drosophila*, *decapentaplegic* (*dpp*) is required for formation of the embryonic dorsoventral axis of ectodermal cuticular structures, communication between tissue layers in gut development and correct proximal distal axis of adult appendages[5-9]. The two papers reviewed here demonstrate a new inductive role for *dpp* expressed in the dorsal ectoderm, to pattern the underlying mesoderm during early embryogenesis[1,2].

Mesoderm formation in *Drosophila*

Over the past two decades, enormous progress has been made in understanding the molecular mechanisms of cell fate specification in *Drosophila*. Most of these discoveries have concerned the specification of body axis, segmental patterning of the ectoderm and diversification in nervous system. Not much has been known about the molecular basis of mesodermal pattern formation and specification of mesodermal derivatives, with the exception of initial determination of the mesodermal anlagen. Mesoderm formation at blastoderm stage is controlled by a cascade of maternally active genes that generate dorso-ventral polarity. This process culminates in a nuclear gradient of the maternally expressed morphogen, *dorsal*, which determines the domains of two zygotic genes *twist (twi)* and *snail (sna)* in the ventral region of the early embryo (for a review, see ref. 3). During gastrulation this region of the embryo invaginates along the ventral midline into the interior of the embryo to form the mesoderm (Fig. 1A). The products of these two genes are likely to act as transcription factors, and the activities of both are required for mesoderm formation. It has been suggested that *twist*, which encodes a basic helix-loop-helix protein, functions as a positive activator of mesoderm differentiation whereas *snail*, encoding a zinc finger protein, represses non-mesodermal genes in the mesodermal primordium. Mesodermal cells do not appear to be committed to particular developmental fates during and shortly after gastrulation[10] and additional factors are obviously involved in further differentiation of the mesoderm into individual tissue types. The molecular basis of events after the formation of mesodermal primordium is beginning to be understood.

Mesodermal subdivision apparently occurs after the completion of gastrulation. Following invagination, the mesodermal cell mass flattens into a single layer which migrates below the ectoderm dorsally and extends to the border between dorsal ectoderm and amnioserosa[11] (Fig. 1B). Shortly thereafter the first morphological manifestation of mesodermal subdivision is the formation of two layers in the dorsal mesoderm: the inner layer, the visceral mesoderm, located dorsally and interiorly, contributes to the visceral gut muscles, while the outer layer, which remains in contact with the ectoderm (and extends to ventral midline), gives rise to somatic body wall muscles[12] (Fig. 1C). The presumptive cardiac mesoderm (heart precursors) derives from two rows of dorsal-most mesodermal cells on both sides of the embryo[13,14] (Fig. 1C). Recently several genes

with a function in the mesoderm have been isolated and their functional analysis has facilitated the study of the molecular basis of mesoderm differentiation (reviewed in refs 15 and 16). Two homeobox genes, *tinman (tin)* and *bagpipe (bap)* are required for determination of cell fates in the dorsal mesoderm[13,17,18]. *tin,* which becomes restricted to the dorsal mesoderm, specifies both visceral and cardiac mesoderm, whereas *bap* is involved in the formation of the visceral mesoderm only. *tin* specifies visceral mesoderm by controlling the expression of *bap* in the dorsal mesoderm[18].

Mesoderm patterning: signalling from ectoderm by *dpp*

dpp is expressed throughout the dorsal half of the early embryo and is required for the development of dorsal and lateral derivatives of the epidermis[5,6]. Midway through embryogenesis, *dpp* is also expressed in the visceral mesoderm and acts as an inductive signal across germlayers. *dpp* controls midgut morphogenesis by inducing the expression of the homeotic gene *labial* in the underlying midgut endoderm (reviewed in ref. 7). TGF-β proteins have also been shown to induce mesoderm in amphibian embryos (for a review, see ref. 4). Different members of the TGF-β family can induce different mesodermal tissues in animal cap assays (reviewed in ref. 19). Earlier transplantation experiments in other insects had indicated a role for ectoderm in patterning the mesoderm[20,21]. More recently an instructive role for the ectoderm in mesoderm patterning was suggested by experiments involving a block of gastrulation movements[22]. Experiments described by Staehling-Hampton *et al.*[1] and Frasch[2] in the two papers discussed here address the role of Dpp as an inductive signal secreted by the ectoderm in patterning the mesoderm. By ectopically expressing *dpp* in the ventral ectoderm or in the mesoderm itself, they show that Dpp acts as an inductive signal from the dorsal ectoderm to regulate *tin* and *bap* expression in the underlying dorsal mesoderm. As a consequence, the mesoderm subdivides into somatic, visceral and cardiac mesoderm.

Although *tin* is initially expressed uniformly in the mesoderm and requires the function of *twi*[13] (T. V. Venkatesh and R. Bodmer, unpublished), shortly after mesodermal migration *tin* expression is diminished in the ventral portions of the mesoderm but is maintained in the dorsal mesoderm[17,18]. Later *tin* expression splits into two domains, the visceral mesoderm (transient expression) and the precursor cells of the heart (persistent expression), but is excluded from the somatic mesoderm. *bap* is first expressed in the dorsal mesoderm in segmental patches of cells that migrate inside to give rise to visceral mesoderm[18].

By analyzing the expression of *tin* and *dpp* in the same embryo, Frasch[2] has shown that mesodermal cells expressing *tin* in the dorsal region of the mesoderm are located directly below the dorsal ectodermal cells expressing *dpp*. Mesodermal cells that do not 'contact' *dpp* lose *tin* mRNA expression shortly after dorsal migration of the mesoderm. Expression of *dpp* was monitored with a *LacZ* reporter gene driven by *dpp* regulatory sequences and *tin* was detected by mRNA expression. In contrast to wild-type, in *dpp* mutant embryos *tin* expression is not maintained in the dorsal mesodermal cells. However, the early *twi*-dependent expression of *tin* in all the mesoderm was not affected in *dpp* mutants. This loss of *tin* expression resulted in the absence of *bap* expression, and in turn no visceral mesoderm formed. Another consequence of loss of *tin* expression in the dorsal mesoderm was the failure to develop cardiac mesoderm. These results suggest that Dpp may act as a signalling molecule secreted from the ectoderm, required for maintaining *tin* expression in the dorsal mesoderm and for the formation of visceral and cardiac mesoderm. To test this hypothesis, *dpp* was expressed in the entire dorsoventral circumference of the ectoderm but not in the mesoderm. This was achieved by using the GAL4 targeting system[23]. This is a powerful system for specifically targeting the expression of cloned genes in desired tissues in *Drosophila*. In this method, transgenic flies carry-

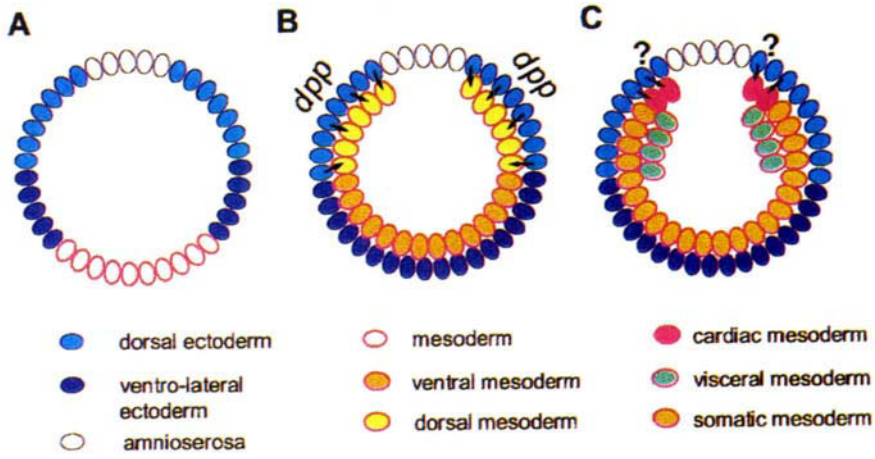

Fig. 1. Gastrulation and mesoderm formation in *Drosophila*. (A-C) Schematic representation of transverse sections of *Drosophila* embryos at different stages. Embryos are oriented dorsal surface up. (A) Blastoderm stage embryo (3 hours of development) showing the fate of cells along the dorsoventral axis. Ventral-most cells (red ovals) are the primordial mesodermal cells that during gastrulation (3.5 hours of development) invaginate (see text). (B) Early gastrula (4.5 hours of development). The invaginated mesoderm has spread dorsally along the basal surface of the ectoderm. Mesoderm cells juxtaposed to the dorsal ectoderm are induced by *dpp* to maintain *tin* expression (arrows), while ventral cells (not induced) lose *tin* expression. (C) Embryos after subdivision of the mesoderm: dorsal mesodermal cells differentiate into different tissue types. Additional signals (?) from ectoderm (arrows) are probably required for differentiation of the cardiac mesoderm.

ing GAL4, a potent transcriptional activator, from yeast driven by a tissue-specific enhancer of choice are crossed to another transgenic line, carrying the cDNA of the gene of interest (in this case *dpp*) under the control of GAL4 upstream activating sequences (UAS). To obtain ectopic expression of *dpp* in the ventral mesoderm, Frasch[2] used a synthetic enhancer which combined the enhancer elements from the gap gene *kruppel* and ventral repressor elements of *zen*[24] to drive GAL4 in the ectodermal primordium, but not in the mesodermal anlagen. As expected of a necessary and sufficient inductive signal for specifying dorsal mesoderm, ectopic expression of *dpp* in all ectodermal cells caused an expansion of *tin* expression towards the ventral midline. The ventrally maintained *tin* expression resulted in a similar expansion of the *bap* domain and the visceral mesoderm towards ventral parts of the mesoderm. These observations put *dpp* in a strong position as an inductive signal for mesodermal patterning. Surprisingly expansion of dorsal mesoderm did not cause a proportionate expansion of the cardiac mesoderm: the number and location of the heart progenitors appeared unaltered. Thus, additional patterning factors must contribute to the spatial specification of the heart.

Staehiling-Hampton *et al.*[1] used a similar approach to ectopically express *dpp* with the GAL4 system. In contrast to the other paper, they expressed *dpp* throughout the mesoderm, to test whether or not *dpp* can induce dorsal mesoderm when it is expressed in the ventral mesodermal primordia. This was achieved by using the enhancer elements of *twi* to drive GAL4 expression. In embryos carrying *twi*-GAL4 and UAS-*dpp*, *bap* expression appeared prematurely as mesodermal cells invaginated through the ventral furrow and its domain remained expanded to include the ventral mesoderm, similar to Frasch's observations[2]. Thus, supplying of Dpp directly by the mesoderm is sufficient to induce dorsal mesoderm. As *tin* is present uniformly throughout the mesoderm at this stage, this result suggests that Dpp does not simply act through *tin* for *bap* expression in the mesoderm, but in addition seems to be required in parallel to *tin.* Therefore, it is possible that Dpp-dependent factors may be able to directly activate or maintain *bap* expression along with *tin* in the dorsal mesoderm of the wild-type embryo. To address the question of whether or not induction of dorsal mesoderm suppresses ventral mesoderm, Staehling-Hampton *et al.*[1] also expressed *dpp* throughout the ectoderm by an ectoderm-specific GAL4 line, 69B[23]. In these embryos *bap* expression extended ventrally (see also Frasch[2]), while the expression of *poxmeso*, a transcription factor normally expressed in the ventral mesoderm, was absent. Therefore *dpp* is not only involved in the activation of dorsally restricted mesodermal genes, but may also direct the repression of genes whose expression is normally confined to the ventral mesoderm. Consistent with this conclusion, in embryos mutant for *dpp*, not only was a loss of *bap* expression in the dorsally located mesoderm observed, but also expanded expression of *poxmeso* throughout the circumference of the embryo. Interestingly, the results further show that expression of *dpp* in the mesoderm can generate completely dorsalized cuticle, implying that Dpp can act across germlayers in either direction.

Implications and future studies

These studies clearly demonstrate a role for Dpp in patterning the mesoderm: Dpp affects the differentiation of the dorsal mesoderm by controlling the expression of mesodermal genes. Considering that Dpp is a secretory signalling molecule, there should be a distinct signalling pathway involved in this cellular communication. Recently serine/threonine transmembrane kinase receptors for Dpp have been identified[25,26] (for a review, see ref. 27). One of the type I receptors encoded by *thickvein* is expressed in the mesoderm[26]. Other molecules involved in transducing the Dpp signals downstream of these receptors have not been identified, with the exception of *schnurri*, a recently identified zinc-finger-containing putative transcription factor gene. Apparently, the *schnurri* transcription factor mediates Dpp signalling from the visceral mesoderm to the adjacent endoderm[28,29] and presumably also in the early mesoderm[28]. It would be interesting to see if *schnurri* directly controls the expression of *tin* and *bap* by binding to their promoters.

It is not known what else contributes to the determination of the spatial domain of cardiac mesoderm. There should be additional factors which mediate the patterning of the cardiac mesoderm (see ?, Fig. 1C), since ectopic expression of the *dpp* in the ectoderm did not alter the domain of heart progenitor cells. Interestingly, very recently it has been reported that the secreted signalling molecule, Wingless (Wg), which is known for its function in determining the anterior-posterior segment-polarity of the ectoderm (reviewed in ref. 30), is involved in the formation of cardiac mesoderm in *Drosophila*[31]. By using a temperature-sensitive allele of *wg,* it has been shown that elimination of *wg* gene function for a short time period after gastrulation results in the selective elimination of the heart progenitor cells. Formation of the visceral mesoderm and segmentation was essentially normal in these embryos. Elimination of *wg* function resulted in the selective loss of *tin* expression in the presumptive cardiac precursor cells, without affecting the *tin* expression in the early mesoderm or its subsequent dorsal restriction. Further experiments show that Wg may be a direct signal for heart formation as overexpression of *wg* rescues heart formation in mutants of *hedgehog* (M. Park, X. Wu, K. Golden and R. Bodmer, unpublished). It thus appears that both dorso-ventral and anterior-posterior patterning signals from ectoderm are involved in patterning the mesoderm. Taken together these studies indicate that the mesoderm development involves complex molecular mech-

anisms that are mediated by signals from the ectoderm and factors endogenous to the mesoderm. Given the fact that many mesodermal genes including *tinman, Dmef2* and *nautilus* (myoD homologue) are conserved in vertebrates, it will be interesting to see how these recent discoveries in *Drosophila* lead to new discoveries in vertebrate mesoderm patterning.

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