Muscle development: **A transcriptional pathway in myogenesis**

Michael V. Taylor

Recent studies have substantially advanced our understanding of the transcriptional program regulating development of the different muscle types in *Drosophila*. For body wall muscle, a pathway can now be drawn that links the transcription factor Dorsal, inherited from the egg, with the differentiated-muscle protein tropomyosin.

Address: Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, UK.

Current Biology 1998, 8:R356–R358
http://biomednet.com/elecref/09609822008R0356

 ISSN 0960-9822

During *Drosophila* embryogenesis, the mesoderm is defined on the ventral side of the embryo in response to the maternal transcription factor Dorsal. At gastrulation, these cells migrate into the embryo and form the mesoderm from which many cell types develop, including the muscle of the body wall, the gut and the heart. The gene *twist*, which is activated by Dorsal, plays a central role in these events [1,2]. It is required firstly for proper gastrulation and formation of the mesoderm, and subsequently for somatic (or body wall) muscle development. Twist is a basic-helix–loop–helix protein that is known to be an activator of transcription, and the suggestion has been that its profound effect on mesoderm development is mediated by the activation of a battery of genes [3].

A central question in this field has therefore been that of the identity of the direct transcriptional targets of Twist. In a recent paper from Cripps *et al.* [4], this question has come together with another in the field of muscle development: how are the regulators of muscle differentiation themselves regulated? The regulators in question here are the *MEF2* genes, which encode transcription factors of the MADS family and have important functions in the regulation of muscle gene transcription and differentiation in both flies and vertebrates [5].

Cripps *et al.* [4] explored the relationship between Twist and the *Drosophila MEF2* gene *Dmef2*, which is required for the differentiation of the various muscle types of the embryo and thoracic somatic muscles of the adult [5]. They focused on the so-called adepithelial cells, from which these muscles of the adult thorax develop and which are associated with the imaginal discs and put aside during embryogenesis. They showed that Twist and Dmef2 are co-expressed in these cells, and identified an enhancer from the *Dmef2* gene that drives expression in them. They also showed that Twist binds to a specific site in this enhancer, and that it activates the enhancer in both cell culture and embryos. Furthermore, the enhancer activity is dependent on both *twist* function and the Twist-binding site. Together, these results provide strong evidence that Twist is a direct regulator of *Dmef2* in the adepithelial cells.

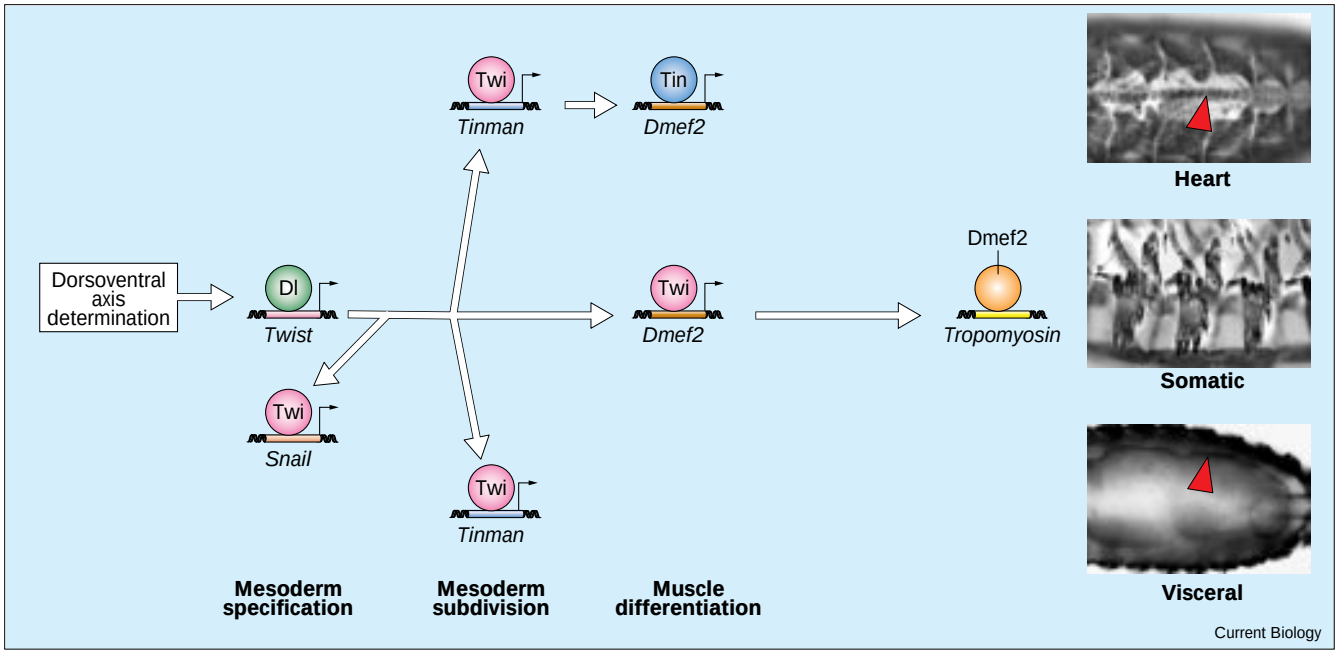
Does the same relationship exist in the somatic muscle of the embryo? This has been indicated previously, but not demonstrated. *Dmef2* and *twist* are co-expressed at certain times; at gastrulation, *Dmef2* expression is dependent on *twist*; and ectopic expression of Twist induces ectopic *Dmef2* expression with a time-course that suggests direct activation [6]. The recent study of Cripps *et al.* [4] shows that the adepithelial cell enhancer from the *Dmef2* gene is also active in the embryo, where it is dependent on the Twist-binding site, and that reducing *twist* function after gastrulation severely reduces Dmef2 protein expression. Taken together with the earlier work, these findings are strong evidence that Twist is indeed also a direct regulator of *Dmef2* in the embryo. It appears, however, that activation of this *Dmef2* enhancer by Twist is only a partial explanation for the regulation of *Dmef2* expression in the somatic muscle during embryogenesis; for example, the enhancer is not active early (although another might be), and Twist and Dmef2 are not co-expressed late.

The direct regulation of *Dmef2* by Twist is significant, because *twist* is specifically required for somatic muscle development. A temperature-sensitive allelic combination previously revealed this in the embryo [7] and Cripps *et al.* [4] have now shown this for thoracic indirect flight muscles, using the same temperature-sensitive condition. This regulation of *Dmef2* by Twist can now be fitted into a broader picture of transcriptional regulation in muscle differentiation, where there has also been substantial recent progress in defining direct regulation of one gene by another using similar criteria to those used by Cripps *et al.*

Dmef2 has a relatively complex pattern of expression — it is expressed in all muscle types at many stages of development — and it is probable that there are a number of regulators of its expression. An example of such a regulator is Tinman, a homeodomain-containing transcription factor related to the vertebrate Nkx family, which has an essential role in the development of the heart, gut (or visceral) muscle and some somatic muscle. Tinman can directly activate *Dmef2* through a distinct enhancer that drives expression in the heart lineage [8].

There are, therefore, defined regulators of *Dmef2* expression in addition to Twist. Are there other defined targets

Figure 1



A simplified summary of transcriptional activation steps in the developing mesoderm and differentiating muscle-types of *Drosophila*. These interactions have been established using both genetic and biochemical tests. A pathway of direct gene activation can be traced from the egg right through to the fully differentiated somatic muscle. This pathway begins after the maternal 'dorsal group' of genes acts to produce a dorsal–ventral nuclear concentration gradient of the Dorsal (Dl) transcription factor. There is also substantial evidence for additional aspects to transcriptional regulation during muscle differentiation (co-regulation, autoregulation, other genes – see text for details). The three muscle types – heart (arrowhead), somatic and visceral (arrowhead) – are revealed with an anti-Myosin antiserum.

of Twist? The answer is yes. Firstly, Twist is an activator of *snail*, a second gene with a crucial early role in mesoderm development [9]. Twist and Dorsal collaborate in this activation. Secondly, in two papers [10,11] reporting results that parallel those for Twist on *Dmef2*, it was found that another direct target of Twist is *tinman*, which has separate enhancers that drive expression in the differentiating heart and visceral muscle. Twist can specifically bind to and activate each of these enhancers. The next step in assembling a framework for understanding transcriptional regulation in muscle development is to determine target genes for Dmef2. *Dmef2* can activate a number of terminal muscle differentiation genes [12]. To date, however, only one direct target gene has been clearly defined: this is tropomyosin in the somatic muscle [13].

The regulatory interactions I have described are summarised in Figure 1. There is good evidence for each interaction illustrated, and although it is possible to argue that certain steps, including the action of Twist on *Dmef2*, are not the major ones that operate directly *in vivo*, this is a time to wield Occam's razor to produce a satisfactory model. Figure 1 is centred on a pathway of direct transcriptional regulation for the somatic muscle, which links the egg with terminally differentiated muscle. The pathway starts with Dorsal, the key maternal transcription factor in the establishment of the dorsal–ventral axis. It proceeds through *twist*, with roles in the early mesoderm and in promoting somatic muscle development, and then Dmef2, which is a direct transcriptional regulator of muscle differentiation. Finally, it ends with tropomyosin, which is a functional feature of the fully differentiated muscle cell.

Although a number of targets of Twist and regulators of *Dmef2* have already been described, the picture of transcriptional regulation in the different muscle types is certain to become more complex. There are probably many other targets of both Twist and Dmef2. Candidate genes activated by Twist include those that are expressed early in the mesoderm in a *twist*-dependent manner (references in [11]). Another likely target for Dmef2 is *PS2α* in the visceral muscle, and other possibilities include a set of myosin genes in the heart [14]. One also anticipates that other activators of the *Dmef2* gene will be discovered.

Furthermore, there is likely to be distinct regulation at different times and in different muscle types. An example is tropomyosin expression in the heart and visceral muscle, which, in contrast to that in somatic muscle, is not dependent on *Dmef2* [13]. Lastly, transcription factors and binding sites in enhancers do not function in isolation, but

rather in combinations. For example, the Twist-binding sites in the *tinman* gene cooperate with Tinman-binding sites [10], the Dmef2-binding sites in the Tropomyosin gene cooperate with another site [13], and the 30 base pair *Dmef2* enhancer fragment that contains the necessary Twist-binding site and an adjacent 'E-box' is not sufficient to drive expression in muscle [4].

The regulatory pathway shown in Figure 1 is of wider significance for two reasons. Firstly, this is the first demonstration of such a direct pathway linking patterning events initiated in the egg with a fully differentiated muscle cell-type. This is relevant to the entire field of cell differentiation, not least because over the past decade muscle differentiation has become a paradigm for differentiation in general. Secondly, specific aspects of these pathways are likely to be important in other animals because, as in other processes, many of the players and molecular mechanisms of muscle differentiation are conserved between flies and vertebrates.

In the case of *twist*, however, functional comparisons between flies and vertebrates remain difficult. It has been suggested that mouse *twist* (*Mtwist*) is actually an inhibitor of myogenesis: it can inhibit muscle gene transcription and muscle cell differentiation in cell culture [2]. However, the phenotype of *Mtwist* knockout mice, and the distribution of Mtwist protein in embryogenesis, are not easily reconciled with any early function in myogenic differentiation [15]. Additional studies on *twist* in both flies and vertebrates are now needed to reveal all facets of its function and the extent to which these have been conserved.

Notwithstanding this issue, parallels can be drawn between the scheme shown in Figure 1 and the world of vertebrate skeletal muscle. Although analyses of vertebrate *MEF2* gene enhancers have not been reported, the myogenic regulatory factors MyoD and myogenin induce MEF2 activity in cell culture [5]. It is therefore possible that, in this case, the basic-helix–loop–helix protein that regulates *MEF2* expression is the relevant myogenic regulatory factor, rather than Twist. Some genes for myogenic regulatory factors have essential MEF2-binding sites in their control regions, so this is not a simple linear pathway [5]. What flanks this possible step in the transcription pathway? Downstream of MEF2 is the gene for muscle creatine kinase [5], regulated by the enhancer where the whole MEF2 story began, and upstream of MyoD lie all the events of patterning the developing somites, which is an area of intense research [16].

Much of the complexity of transcriptional regulation during muscle development remains to be unravelled, and one can anticipate new findings, many of which will undoubtedly reflect fundamental similarities in the ways that different animals develop.

References

1. Thisse C, Perrin-Schmitt F, Stoetzel C, Thisse B: Sequence-specific transactivation of the Drosophila ***twist*** gene by the dorsal gene product. *Cell* 1991, 65:1191-1201.
2. Taylor MV: A myogenic switch. *Curr Biol* 1996, 6:924-926.
3. Leptin M: ***twist*** and ***snail*** as positive and negative regulators during ***Drosophila*** mesoderm development. *Genes Dev* 1991, 5:1568-1576.
4. Cripps RM, Black BL, Zhao B, Lien C-L, Schulz RA, Olson EN: The myogenic regulatory gene Mef2 is a direct target for transcriptional activation by Twist during Drosophila myogenesis. *Genes Dev* 1998, 12:422-434.
5. Olson EN, Perry M, Schulz RA: Regulation of muscle differentiation by the MEF2 family of MADS box transcription factors. *Dev Biol* 1995, 172:2-14.
6. Taylor MV, Beatty K, Hunter K, Baylies MK: ***Drosophila*** MEF2 is regulated by twist and is expressed in both the primordia and differentiated cells of the embryonic somatic, visceral and heart musculature. *Mech Dev* 1995, 50:29-41.
7. Baylies M, Bate M: Twist: a myogenic switch in ***Drosophila***. *Science* 1996, 272:1481-1484.
8. Gajewski K, Kim Y, Lee YM, Olson EN, Schulz RA: ***D-mef2*** is a target for ***Tinman*** activation during ***Drosophila*** heart development. *EMBO J* 1997, 16:515-522.
9. Ip YT, Park RE, Kosman D, Yazdanbakhsh K, Levine M: Dorsal–twist interactions establish ***snail*** expression in the presumptive mesoderm of the ***Drosophila*** embryo. *Genes Dev* 1992, 6:1518-1530.
10. Lee YM, Park T, Schulz RA, Kim Y: Twist-mediated activation of the ***NK-4*** homeobox gene in the visceral mesoderm of ***Drosophila*** requires two distinct clusters of E-box regulatory elements. *J Biol Chem* 1997, 272:17531-17541.
11. Yin Z, Xu X-L, Frasch M: Regulation of the Twist target gene ***tinman*** by modular ***cis***-regulatory elements during early mesoderm development. *Development* 1997, 124:4971-4982.
12. Lin M-H, Bour BA, Abmayr SM, Storti RV: Ectopic expression of MEF2 in the epidermis induces epidermal expression of muscle genes and abnormal muscle development in ***Drosophila***. *Dev Biol* 1997, 182:240-255.
13. Lin M-H, Nguyen HT, Dybala C, Storti RV: Myocyte-specific enhancer factor 2 acts cooperatively with a muscle activator region to regulate ***Drosophila*** tropomyosin gene muscle expression. *Proc Natl Acad Sci USA* 1996, 93:4623-4628.
14. Ranganayakulu G, Zhao B, Dokidis A, Molkentin JD, Olson EN, Schulz RA: A series of mutations in the D-MEF2 transcription factor reveal multiple functions in larval and adult myogenesis in ***Drosophila***. *Dev Biol* 1995, 171:169-181.
15. Gitelman I: Twist protein in mouse embryogenesis. *Dev Biol* 1997, 189:205-214.
16. Tajbakhsh S, Sporle R: Somite development: constructing the vertebrate body. *Cell* 1998, 92:9-16.