protein-synthesizing cell extract is made in which a single type of protein is synthesized from its messenger RNA in the presence of the molecular chaperones that occur naturally in that extract. The chemical denaturing system permits the use of much more sophisticated analytical techniques than the second because its chemical composition is both simple and defined, and it is thus favored by protein chemists. The second system is more similar to the in vivo situation because the polypeptide chain is synthesized vectorially in the extract as it is in the cell, and it is thus favored by cell biologists. In the chemical denaturing system, all parts of the polypeptide chain are simultaneously available for binding to the pure molecular chaperones, whereas in the second system, the polypeptide chain grows steadily in length as ribosomes traverse its messenger RNA and becomes progressively available for binding to whatever endogenous chaperones are present in the crude extract (see the figure).

Both systems have been used separately before, but Frydman and Hartl (*1*) have now directly compared the folding of the same proteins in the two systems. They also combined the two approaches—that is, chemically denatured full-length protein is diluted into a crude cell extract. Their results document that full-length, chemically denatured polypeptide chains and growing polypeptide chains interact differently with chaperones.

There are two contrasting models of chaperone-polypeptide interaction in the intact cell (*5*). One model suggests that chains that have folded incorrectly are unfolded by binding to chaperones after release from the ribosome; the proteins are then released in the unfolded state into the intracellular medium where they have another chance to fold correctly and, if they fail, may rebind the chaperones (*6*). This chaperone cycling model does not provide a means of avoiding aggregation of misfolded proteins; there is genetic evidence that, unless avoided, this is a hazard for some folding chains in vivo (*7*). The other model proposes that there is a selective and sequential binding of different kinds of chaperones to the polypeptide chain as it grows on the ribosome (*8*). Small chaperones of the heat shock protein 70–DnaJ–GrpE families bind first to the elongating chain and prevent it from folding prematurely, whereas large chaperones of the chaperonin family [called TRiC chaperones from the eukaryotic cytosol (*9*)] bind later to longer chains and provide them with a sequestered environment within which each complete chain can fold correctly without aggregating with other folding chains (*10*). In the latter model, the chain is released from the chaperones into the intracellular medium only after it has folded sufficiently for aggregation not to be a problem.

The results described in the new work (*1*) show that two polypeptides synthesized in a cell extract prepared from reticulocytes (actin and firefly luciferase) behave differently from full-length, chemically denatured chains of the same proteins refolding in the same extract, therefore supporting the second type of model—sequential binding to the growing chain. The inference is that chains of these proteins do not fold by cycling between chaperones and the free solution in the cell, but are released from chaperones into the intracellular medium only after they are committed to the correctly folded state.

Several questions remain to be resolved. If it occurs, the association of TRiC with growing chains should be demonstrable by pulse-chase immunoprecipitation experiments and visible in the electron microscope. What determines the sequential binding of different chaperones to particular polypeptide chains? The TRiC chaperone appears to be specific for a few proteins, especially actin and tubulin, so what about the folding of all the other chains in the eukaryotic cytosol? Perhaps chaperones bind in vivo only to polypeptides that are especially prone to aggregation. Establishing the relevance to the living cell of conclusions based on in vitro data is a continuing challenge to the ingenuity of biochemists.

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A New Turn (or Two) for Twist

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Like scientists progressing through their careers, the cells of the embryo become more and more specialized. In an early embryonic stage of the fruit fly *Drosophila*, cells are assigned to particular fates characteristic of the primordial germ layers—the ectoderm, the mesoderm, and the endoderm. To form the mesoderm (*1*), a complex hierarchy of signals activates transcription of the gene *twist* (*twi*), which encodes a basic helix-loop-helix (bHLH) transcription factor required for early mesoderm formation (*2*). But then how is mesoderm subsequently partitioned into its more specialized derivatives? In a surprising finding reported in this issue of *Science*, Twi itself is reported to have a second function: participating in the choice between alternative mesodermal cell fates (*3*), in addition to its well-known role in specifying the early mesoderm. In a separate report, a vertebrate homolog of Twi (Mtwist) is also shown to be an important regulator of muscle differentiation, although it functions quite differently than its fly counterpart (*4*).

In *Drosophila*, Twi is initially expressed in the entire mesoderm, but later its expression becomes quite restricted (*2*, *5*). As the mesoderm separates into somatic and visceral components, the amount of Twi protein remains high in the somatic regions but is markedly reduced in visceral regions (see the figure). Subsequently, Twi is rapidly down-regulated in differentiating embryonic muscle cells but persists in the progenitors of the adult myoblasts that are specified during embryogenesis (*6*). In the new work, Baylies and Bate have elegantly addressed the functional significance of this modulation (*3*). The simplest hypothesis was that high levels of Twi would inhibit embryonic somatic muscle development. However, even with continuously high levels of Twi, embryonic muscles formed normally. An alternative possibility was that the relative amounts of Twi might distinguish among different mesodermal cell fates. Indeed, a high level of Twi suppressed heart and visceral muscle formation but was essential for proper somatic myogenesis. Furthermore, when ectopically expressed in the ectoderm, Twi repressed epidermal and nervous system differentiation while activating a myogenic program in these cells. So Twi is both essential for the establishment of mesodermal cell fate before gastrulation and is involved in subdividing the mesoderm later in development.

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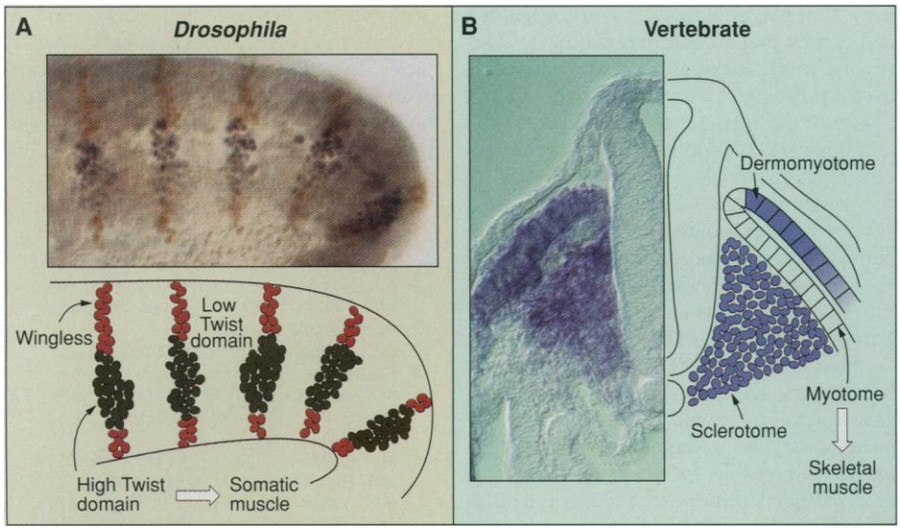

Vertebrate and invertebrate Twist expression. (**A**) In the *Drosophila* embryo, the regions of high Twist expression give rise to somatic muscles and are in register with Wingless expression in the overlying ectoderm (*5*). Visceral muscle derives from the low Twist region. (**B**) In vertebrates, Twist is initially expressed throughout the epithelial somite but then is excluded from the myotome, which gives rise to skeletal muscle cells (*7*). [Photo in (B) courtesy of D. Spicer]

A vertebrate Twist homolog also is expressed in a dynamic pattern (*7*) (see the figure). In mouse, Mtwist initially is found throughout the somitic mesoderm but subsequently is excluded from the myotome, persisting only in dermomyotomal and sclerotomal cells. This repression of Mtwist in the myotome coincides with the up-regulation of two classes of myogenic regulatory factors, the MyoD and MEF2 families (*8*). These reciprocal expression patterns suggested that Mtwist might influence the compartmentalization of the vertebrate mesoderm. However, in this instance, instead of promoting somatic myogenesis, as Twi does in flies, Mtwist would inhibit this process. Spicer and co-workers confirm this inhibition hypothesis in a murine tissue culture model of myogenesis (*4*). Mtwist inhibits muscle differentiation by two mechanisms: It prevents trans-activation of muscle target genes by MEF2 and it dimerizes with the bHLH binding partners of MyoD, termed E proteins, thereby blocking MyoD binding to DNA. Extrapolating from these and related findings in cultured muscle cells (9), the authors suggest that Mtwist may regulate myotome formation by restricting the spatial or temporal activation of the positive feedback loop linking the MyoD and MEF2 families of myogenic factors (*4*). The phenotype of Mtwist knockout mice partially supports this idea (*10*).

How are the dynamic patterns of Twist expression established in each species? Wingless (Wg), a signaling molecule whose expression coincides with the high Twi domain (see the figure), is a candidate positive regulator of *Drosophila twi*. Consistent with this possibility, Wg is essential for the specification of certain somatic muscle and cardiac cell fates (*11*). In vertebrates, repression by myogenic transcription factors might contribute to the exclusion of Twist from the myotome.

Spicer and co-workers also find that in their murine tissue culture system, *Drosophila* Twi can function as an inhibitor of either MyoD- or MEF2-dependent transcription (*4*). How can this result be reconciled with the observation that *Drosophila* Twi promotes rather than suppresses somatic muscle development in the intact fly embryo? There are several possible explanations for this apparent paradox. Inhibition of both MyoD and MEF2 by Mtwist is dependent on E protein association, and *Drosophila* Twi apparently functions in a similar manner in mammalian cells (*4*). Whether Twi acts in the *Drosophila* embryo as a homodimer or as a heterodimer with Daughterless (Da), the fly homolog of E proteins (*12*), remains to be determined. However, muscle development in *Drosophila* is not entirely dependent on Da (*12*), and the level of Da in the mesoderm may be insufficient to maintain Twi as a heterodimer.

One can pursue the latter idea by examining the effects of ectopic Twi expression in embryos in which Da levels are varied genetically. Alternatively, Twi-Da heterodimers may form but lack inhibitory activity because Da does not confer the same repressive function on Twi as do mammalian E proteins. Other dimer partners for Twi also might exist in the *Drosophila* embryo. Another interspecific difference may lie in the myogenic functions of the Mtwist targets, MyoD and MEF2. Whereas murine MyoD (together with another family member, Myf-5) is required globally for muscle development (*13*), *nautilus*, the *Drosophila* MyoD homolog (*14*), is expressed in only a subset of somatic muscles. Finally, *Drosophila* Twi may not inhibit DMEF2 directly because it acts at a different step in embryonic myogenesis—DMEF2 is required for muscle differentiation but not commitment (*15*), whereas Twi determines early muscle cell fates (*3*). Although numerous components of the myogenic pathway have been conserved, the actual mechanisms by which they act are distinct.

In comparing the function of Twist in *Drosophila* and mouse, we find both conserved and divergent features. Although the consequences and mechanisms of their actions may differ in detail, the one common element between vertebrate and *Drosophila* Twist is their contribution to the diversification of mesodermal cell subtypes. As future chapters in this story unfold, we can anticipate additional twists and turns for this important embryonic determinant.

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