Muscle development: **Reversal of the differentiated state**

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Cell fate selection and cell cycle exit are fundamental features of differentiation during animal development. Accumulating data suggest that these processes are more readily reversible than previously supposed and are beginning to point at the underlying molecular mechanisms.

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Dolly the sheep and other cloned animals have taught us that, in an appropriate cytoplasmic environment, nuclei of differentiated cells can be reprogrammed to follow any fate [1,2]. However, the molecules responsible for such reprogramming are unknown. A recent study [3] has shown that Msx1, a transcription factor which has a homeobox DNA-binding motif, has the ability to reverse both terminal differentiation and commitment to the muscle cell fate.

Vertebrate muscle development has yielded a paradigm for thinking about the relationship of growth and differentiation (Figure 1). First, a population of committed, yet dividing, muscle precursor cells, called myoblasts, arises from the somitic mesoderm. A separate process then triggers the myoblasts to stop dividing, start expressing muscle-specific genes and terminally differentiate into myocytes. Many other cell types, notably neurons, undergo analogous steps of commitment and terminal differentiation. Muscle is a particularly dramatic example of terminal differentiation because myocytes subsequently fuse with one another to form multinucleate fibres containing thousands of terminally differentiated muscle nuclei.

Twenty years ago, experimental fusions between various cell types, including neurons, and muscle cells showed that cytoplasmic components can reprogramme gene expression in terminally differentiated cells [4,5]. At a molecular level, members of the MyoD family of basic helix–loop–helix myogenic transcription factors (MRFs) are likely to account for the reprogramming of non-muscle cell nuclei, because forced expression of an MRF switches cell fate to myogenesis [6]. MRFs are a key to normal myogenic commitment, as expression of an MRF is required for the generation of myoblasts *in vivo* [7]. Moreover, the activity of an MRF is also required for terminal differentiation [8]. Consistent with this, growth factors, which normally prevent terminal differentiation of cultured myoblasts, inhibit the function of MRF proteins [9]. Thus, there appear to be intimate molecular links between commitment and terminal differentiation of muscle cells bound up in the activity of MRFs. Strikingly, families of basic helix–loop–helix proteins also control neurogenesis and haematopoiesis [10,11]. However, until recently, commitment and terminal differentiation were

Figure 1

The steps in muscle differentiation and their reversal. Schematic summary of normal myogenesis from uncommitted mesoderm to myotube. A generalised indication of some genes expressed at each stage is given below each cell type in bold, but is far from precise. Factors in blue promote myogenesis, those in red prevent or induce the reversal of myogenesis, and those in black promote differentiation towards other lineages. Uncommitted mesoderm, as well as myoblasts, can give rise to fat cells and osteoblasts with appropriate extracellular signals. Hh, hedgehog proteins; WNT, wingless/WNT family proteins; BMP, bone morphogenetic proteins; FFA, nonesterified (free) fatty acids.

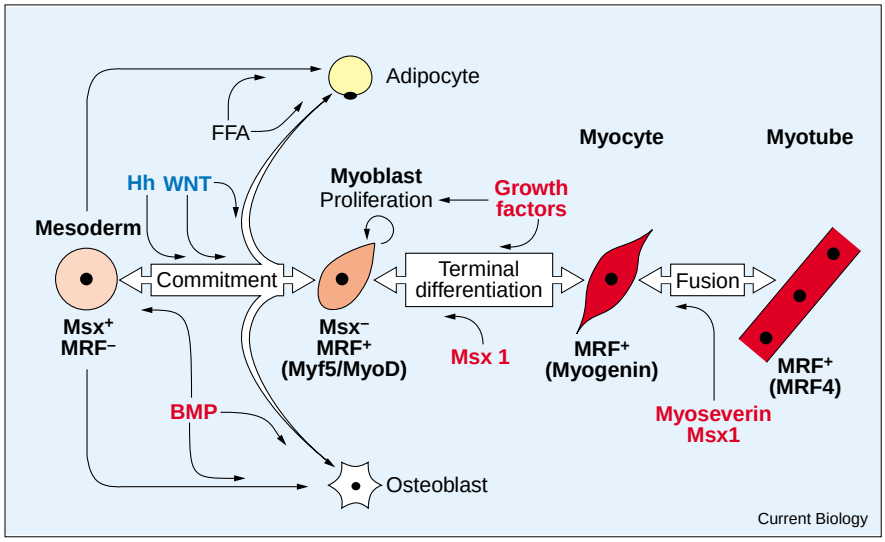
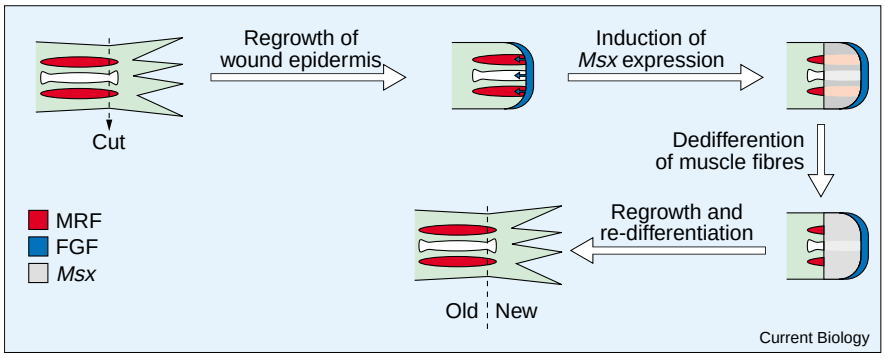


Figure 2



Hypothetical scheme for control of limb blastema dedifferentiation. FGF from wound epidermis induces *Msx* gene expression in the blastema. This could lead to suppression of MRF expression in adjacent muscle, and possibly of other differentiation-promoting genes in tissues such as bone. After regrowth, these dedifferentiated cells may re-differentiate into a variety of new cell types.

thought to be irreversible in the absence of experimental interference with intracellular components.

This view was changed by the seminal work of Brockes and collaborators [12,13] showing that terminally differentiated multinucleate urodele muscle fibres can dedifferentiate to yield dividing mononucleate cells that contribute to various cell lineages in a regenerating limb stump. Further work, however, highlighted a fundamental difference between urodele and mammalian muscle fibres, which parallels the inability of mammalian limbs to regenerate. The nuclei of urodele muscle fibres can be triggered to re-enter the cell cycle by a serum-derived extracellular factor, whereas those of mouse origin cannot [14]. On the other hand, mammalian myotube nuclei are driven into S phase by overexpression of the viral oncoproteins E1A or SV40 large T antigen, showing that myotubes remain capable of re-entering the cell cycle and suggesting that mammalian muscle fibres simply lack the signal transduction machinery found in urodele fibres [14]. What is the difference in the machinery, and could an extracellular signal induce it, thereby permitting mammalian muscle fibres to dedifferentiate *in vivo*? Hope that this will be possible is stimulated by the recent report [15] that myoseverin, a small microtubule-binding purine analogue, can induce myotube cytokinesis and help trigger reversal of terminal differentiation of the murine C2C12 myogenic cell line.

Turning attention from reversal of terminal differentiation to reversal of commitment, C2C12 cells, and even primary myoblasts, have also been shown to give rise to fat cells or osteoblasts when extracellular signals are modified, suggesting that commitment of myogenic cells to myogenesis is reversible [16–18]. The mechanism involves suppression of MRF expression or activity. MRF suppression can be achieved in C2C12 cells and primary myoblasts by a variety of intracellular manipulations, such as forced expression of activated E1A, ras or Msx1 [19–21]. Thus, reversal of the key step of MRF activation seems to be a prerequisite for reversal of commitment.

In the new work, these various strands have been combined into a single elegant series of experiments. Using C2C12 cells, Odelberg *et al.* [3] introduce a retroviral vector capable of expressing *Msx1* under control of tetracycline. They confirm that *Msx1* expression in myoblasts inhibits terminal differentiation. But they go further and provide convincing timelapse evidence that induction of *Msx1* in terminally differentiated multinucleate myotubes leads to cytokinesis, which is accompanied by loss of expression of three out of the four known MRFs (Myf5 was not analysed). The newly derived mononucleate cells have lost the cell cycle inhibitor p21, replicate and are capable of differentiating along osteogenic, chondrogenic, adipogenic or myogenic pathways, depending on culture conditions.

The importance of this work is not so much in the demonstration of multiple lineages derived from C2C12 cells, as this was known previously. Rather it is the reversal of terminal differentiation and the focusing of attention on the molecular mechanism by which Msx1 reverses the entire process of myogenesis. Msx1 is a member of a family of known transcriptional inhibitors and probably targets at least some MRF regulatory elements directly [22]. Strikingly, *Msx* gene expression is induced in outgrowing limb or fin bud tips by fibroblast growth factor (FGF) signals from the adjacent ectoderm [23,24]. Such FGF signals are capable of suppressing MyoD accumulation in myoblasts and this correlates with the inhibition of terminal differentiation *in vivo* [25]. There is an excellent correlation between the ability of an appendage to regenerate and the activation of *Msx* gene expression [24,26,27]. Taken together, these data suggest the attractive hypothesis that *Msx* gene induction in differentiated tissues, elicited by growth factors such as FGF, leads to reversal of the differentiated state (Figure 2).

As Odelberg *et al.* [3] themselves point out, the work leaves a number of issues still to be resolved. One is to determine whether *Msx1* expression can be induced in muscle fibres *in vivo*. Equally important is whether Msx1 can cause real muscle fibres to dedifferentiate, since they are substantially

different from cultured myotubes. Affirmative answers to these questions would increase the attraction of skeletal muscle tissue as a substrate for tissue engineering — the technology of generating spare body parts.

A fundamentally interesting question for biologists, however, is whether *Msx* genes ever cause muscle dedifferentiation in real life. Since the discovery of the satellite cell — the quiescent myoblast present in adult muscle — and the demonstration that muscle fibres are formed by fusion of myocytes, rather than replication of myoblast nuclei without cytokinesis, it has been supposed that all regenerated muscle tissue arises from the satellite cell pool. Recent data have questioned this in two ways. First came demonstrations that haematopoietic cells can contribute to muscle fibres (see [28] for example), suggesting that non-muscle cells may aid muscle regeneration. The new results [3] resurrect the old question of whether some muscle, and indeed other tissues, may arise from terminally differentiated muscle fibres. Does activation of *Msx* gene expression mediate the dedifferentiation of urodele myotubes? Is this process ever elicited in amniote muscle? Given the widespread expression of *Msx* genes in situations where cells are kept undifferentiated such as cranial sutures and early neural tube, how common is this role of *Msx* genes? Could they, or other similar genes, return a nucleus to the basal state required for the creation of Dolly?

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