

Determinative properties of muscle lineages in ascidian embryos

THOMAS H. MEEDEL, ROBERT J. CROWTHER and J. R. WHITTAKER

Laboratory of Developmental Genetics, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA

Summary

Blastomeres removed from early cleavage stage ascidian embryos and reared to 'maturity' as partial embryos often elaborate tissue-specific features typical of their constituent cell lineages. We used this property to study recent corrections of the ascidian larval muscle lineage and to compare the ways in which different lineages give rise to muscle. Our evaluation of muscle differentiation was based on histochemical localization and quantitative radiometric measurement of a muscle-specific acetylcholinesterase activity, and the development of myofilaments and myofibrils as observed by electron microscopy. Although the posterior-vegetal blastomeres (B4.1 pair) of the 8-cell embryo have long been believed to be the sole precursors of larval muscle, recent studies using horseradish peroxidase to mark cell lineages have shown that small numbers of muscle cells originate from the anterior-vegetal (A4.1) and posterior-animal (b4.2) blastomeres of this stage. Fully differentiated muscle expression in isolated partial embryos of A4.1-derived cells requires an association with cells from other lineages whereas muscle from B4.1 blastomeres develops autonomously. Clear

differences also occurred in the time acetylcholinesterase activity was first detected in partial embryos from these two sources. Isolated b4.2 cells failed to show any muscle development even in combination with anterior-animal cells (a4.2) and are presumably even more dependent on normal cell interactions and associations. Others have noted an additional distinction between the different sources of muscle: muscle cells from non-B4.1 lineages occur exclusively in the distal part of the tail, while the B4.1 descendants contribute those cells in the proximal and middle regions. During the course of ascidian larval evolution tail muscle probably had two origins: the primary lineage (B4.1) whose fate was set rigidly at early cleavage stages and secondarily evolved lineages which arose later by recruitment of cells from other tissues resulting in increased tail length. In contrast to the B4.1 lineage, muscle development in the secondary lineages is controlled less rigidly by processes that depend on cell interactions.

Key words: ascidian, muscle, cell lineage, *Ciona intestinalis*, *Ascidia ceratodes*.

Introduction

In the past decade there has been a resurgence of research interest in the cell lineages of several animal species in an attempt to define the various mechanisms that operate in establishing cell commitments during embryogenesis. Considerable differences occur in the time at which embryonic cells of the particular model organisms chosen first establish 'clones' of invariant cell fate; these studies have been the subject of a number of recent reviews (e.g. Davidson, 1986; Slack, 1983; Stent, 1985). Development of larval tail muscle in ascidian embryos remains the classic example of very early and seemingly complete cell lineage determination. Conklin (1905) described in detail the occurrence of a crescent-

shaped region of yellow cytoplasm in the fertilized egg of *Styela partita* and noted the segregation of this yellow cytoplasm into the presumptive tail muscle cells of the embryo. The developmental autonomy implied by his findings was later confirmed in histological investigations, most notably by Reverberi & Minganti (1946, 1947): isolation of the two B4.1 blastomeres from the 8-cell stage, into which most of the mitochondria-enriched yellow crescent material becomes segregated, resulted in partial embryos containing differentiated muscle cells. Other blastomere isolates did not make obvious muscle. This 'self-differentiation' has also been demonstrated by the occurrence of acetylcholinesterase and myofilaments/myofibrils in B4.1 partial embryos (Whittaker, Ortolani & Farinella-Ferruzza, 1977; Whittaker, 1982;