Finding MyoD with a little help from my friends

Andrew Lassar

One of the mysteries that fascinated me when I was finishing my graduate work in 1983 was how a cell becomes specified to a particular cell fate. Papers published by Helen Blau and Woody Wright's groups in 1983 and 1984 pointed to the existence of a factor (or several factors) in skeletal muscle cells that could activate muscle gene expression in non-muscle cell types. A logical path to identify such myogenic regulators would have been to study the transcription factors that bind the regulatory regions of muscle structural genes. Given the technology of the early 1980s, this would have entailed fractionating muscle cell nuclear extracts in search of DNA-binding activities that recognized these regulatory regions. During my graduate career, I developed an aversion to working in the cold room and knowing that this approach would involve months, if not years, of column chromatography at 4°C, I wondered if there was another road I could take to identify muscle regulatory molecules.

The key to unravelling this problem came from a series of fortuitous events, starting with my recollection of two papers published by Peter Jones and Shirley Taylor in 1979 and 1982. These studies reported the striking finding that brief treatment of 10T1/2 cells (a mouse fibroblast line) with the DNA-demethylating agent 5-azacytidine could stably convert them into adipogenic, chondrogenic or myogenic cells. This suggested that demethylation of distinct regulatory loci was inducing different cellular phenotypes. If myogenic conversion of 10T1/2 cells was caused by demethylation of a single regulatory locus, I reasoned that transfection of this unmethylated locus into 10T1/2 cells would convert them into myocytes,

even in the absence of 5-azacytidine treatment. I remember being pretty excited about this approach, as it would both keep me out of the cold room and potentially identify a regulator of cell fate.

I had already arranged my postdoctoral position with Hal Weintraub, whose lab was at the Fred Hutchinson Cancer Research Center in Seattle. Hal was a wonderful mentor who became a great friend. After arriving in Seattle in November 1983, I outlined my scheme to Hal at 'The Surrogate Hostess', a restaurant that sold terrific cinnamon rolls and served as Hal's early morning office. Although he initially wanted me to work on transcription factors that regulated globin gene expression, Hal was enthusiastic about new ideas to identify cell-fate-determining factors and gave me the go-ahead to work on the 10T1/2 project. I spent my first three years in Hal's lab deriving myogenic cell lines from 10T1/2 cells and transfecting DNA from these myogenic derivatives back into the parental cells. These experiments were massive, each typically involving 100 15-cm tissue culture dishes that I had to analyse for the presence of muscle cells before mould ruined them. The hard work paid off when I eventually demonstrated that muscle DNA transfection could induce myogenic conversion of 10T1/2 cells at a rate consistent with the notion that a single regulatory locus had activated the muscle cell program. I soon learned at a Keystone Conference on muscle biology that Charlie Emerson from the University of Virginia had arrived at the same conclusion through similar experiments.

Despite these early successes, however, the regulatory locus conferring muscle fate remained elusive. The approach that eventually led me to its identification was a dividend of attending a Gordon Conference. After listening to Dan Littman describe how he had identified CD4 by employing the recently developed subtractive hybridization technique, I realized

that myogenic cell lines derived from 10T1/2 cells treated with 5-azacytidine would contain RNA transcripts that were absent from the parental 10T1/2 cells, and that one of these transcripts would be the myogenic regulator that I sought. The problem was to somehow identify the RNA encoding the muscle regulatory factor in a sea of transcripts giving rise to more prosaic muscle structural proteins. Although myogenic cell lines only activate their differentiation program following mitogen withdrawal, the 5-azacytidine-derived myogenic cells lines had apparently undergone an epigenetic change that was stably maintained in proliferating myoblasts before their differentiation. Thus, I conjectured that 5-azacytidine may have induced the expression of a myogenic regulator in proliferating myoblasts, which allowed them to activate the muscle differentiation program upon mitogen withdrawal.

At that point, I was very fortunate to be joined by Robert Davis, a 'laboratory dynamo' who had recently joined Hal's lab for his graduate work. Armed with the hypothesis that proliferating myoblasts express a 'determination gene' that maintains their cellular phenotype, we screened a skeletal muscle cDNA library with a proliferating-myoblast-specific probe and identified three myoblast-specific genes whose expression coincided with muscle cell commitment. When ectopically expressed in other cell types, one of these cDNAs displayed myogenic potency and was accordingly named myogenic determination gene number 1, also known as MyoD. Subsequent work established that MyoD activates the expression of other muscle regulators such as MEF2 and myogenin, which are necessary for induction of the skeletal muscle differentiation program. This hierarchical positioning of MyoD as a regulatory node upstream of other essential myogenic regulators was no doubt also key to its discovery — luckily for us!

Andrew Lassar is at the Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, Massachusetts 02115, USA.
e-mail: andrew_lassar@hms.harvard.edu