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PATTERNS OF PROLIFERATION AND DIFFERENTIATION OF ADULT MYOBLASTS DEFINE A UNIQUE MYOGENIC POPULATION

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In here we summarize some of our recent studies on adult chicken myoblasts. We discuss a) myosin isoform expression by the differentiated myoblasts, and b) the role of PDGF during proliferation of the cells. These studies demonstrate that adult myoblasts represent a unique myogenic population (or lineage) which emerges in late development. Furthermore, we discuss possible lineal relationship between adult and somitic/limb bud cells. Unless otherwise noted, the source of myoblasts was chicken pectoralis muscle, and all procedures and antibodies were as described in previous publications (Yablonka-Reuveni, et al., 1987; Yablonka-Reuveni and Seifert, submitted; Hartley, et al., 1991; 1992).

INTRODUCTION

Throughout development of skeletal muscle, myogenic precursor cells (known as myoblasts), may replicate, withdraw from the cell cycle and give rise to terminally differentiated, fusion-capable myoblasts. These differentiated cells can then undergo fusion with each other to form multinucleated myofibers. As development progresses, de novo muscle fiber formation no longer occurs and instead, myogenic cells fuse into pre-existing muscle fibers, adding new nuclei. During late embryogenesis myotubes become encased by a basement membrane and it is generally accepted that the myogenic progenitors at this stage are closely associated with the muscle fiber, situated between the basement membrane and the plasma membrane of the fiber in a position 'satellite' to the muscle fiber. It is because of this position that myogenic precursors of post-natal and adult muscles are often referred to as satellite cells

(Mauro, 1979; Campion, 1984; Grounds and Yablonka-Reuveni, 1992). In the neonate, these satellite cells are proliferative, adding nuclei to the myofibers (Moss and Leblond, 1970). In the adult, satellite cells are mitotically quiescent but can reinitiate proliferative activity in response to injury (Mauro, 1979; Campion, 1984; Grounds and Yablonka-Reuveni, 1992), as well as in response to more subtle stresses such as stretch, exercise, denervation and compression (reviewed in White and Esser, 1989; Bischoff, 1989). The resulting progeny (adult myoblasts) either fuse into pre-existing fibers or form new myofibers (Snow, 1977a and 1977b; Mauro, 1979; Grounds and Yablonka-Reuveni, 1992).

Data from different laboratories has indicated that the myogenic precursor cells can be further sub-divided into unique populations with specific characteristics. These finding have been further put forward in support of the hypothesis that there are multiple myogenic lineages in the developing and mature muscle (Feldman and Stockdale, 1991). We summarize below some of the findings which show that there are multiple myogenic populations. We then continue by discussing some of our recent studies on adult myoblasts.

EVIDENCE FOR MULTIPLE MYOGENIC POPULATIONS DURING DEVELOPMENT OF SKELETAL MUSCLE

It is generally accepted that all of the skeletal muscle (except for some head muscles) arises from a common somitic progenitor pool, but there are many studies which indicate that there are multiple myogenic populations (or lineages) in the developing embryo. The notion of multiple myogenic lineages during development is based on in vivo and cell culture studies where myoblasts with different characteristics have been identified when somitic and limb bud myoblasts were compared (Christ, et al., 1977; Chevallier, et al., 1977; Sassoon, et al., 1989; Ordahl and Le Douarin, 1992), and when myoblasts from early and late stages of limb and wing development were compared (White, et al., 1975; Seed and Hauschka, 1984; 1988; Mouly, et al., 1987; Vivareli, et al., 1988; Miller and Stockdale, 1989; Smith and Miller, 1992; and reviewed in Cossu and Molinaro, 1987; Stockdale and Miller, 1987; Hartley and Yablonka-Reuveni, 1992). Stockdale and colleagues have further introduced the terms 'embryonic' and 'fetal' myoblasts for early and late myoblasts which are present during the embryonic and fetal phases of embryogenesis, respectively (Stockdale and Miller,1987; Miller and Stockdale 1989). In the chicken, the embryonic phase lasts from embryonic day 4 through 7, followed by the fetal phase. Embryonic myoblasts

are most abundant on embryonic day 5, while fetal myoblasts are most abundant between embryonic days 8-12 (Stockdale and Miller, 1987). Collectively, the above studies have indicated that there are several distinct myogenic populations (or lineages) in the developing muscle. These populations include the initial precursors present in the somites and limb buds, and as development proceeds, those present during the embryonic and fetal periods. Early (or embryonic) myoblasts, and late (or fetal) myoblasts can be further subdivided based on characteristics of their progeny in culture such as medium requirements or myosin expression.

ADULT MYOBLASTS REPRESENT A UNIQUE MYOGENIC POPULATION

Chick-quail chimera studies suggest that, like myoblasts found during development, satellite cells originate in the somites (Armand, et al., 1983). However, the hypothesis that satellite cells are identical to myoblasts found in the developing muscle but were simply trapped underneath the fiber basement membrane has been challanged in recent years by many studies which identified differences between myoblasts from mature and developing muscle. Studies on mammalian myoblasts (reviewed by Cossu and Molinaro, 1987) have shown differences between fetal and adult myoblasts in their sensitivity to a tumor promoter (Cossu, et al., 1983), in expression of acetylcholine receptors (Cossu, et al., 1987) and in the form of acetylcholine esterase expressed (Senni, et al., 1987). Studies on avian myoblasts have demonstrated that compared to fetal myoblasts, cultured satellite cells fuse into myotubes later (Yablonka-Reuveni, et al., 1987), express desmin as cycling cells more frequently (Yablonka-Reuveni and Nameroff, 1990), have a different response to merocyanine 540 (Nameroff and Rhodes, 1989), have specific patterns of myosin heavy chain isoform expression (Hartley, et al., 1991; 1992; Feldman and Stockdale 1992), and express more binding sites for platelet-derived growth factor (Yablonka-Reuveni, et al., 1990; Yablonka-Reuveni and Seifert, submitted). Furthermore, many of these studies suggest that myogenic precursors with the characteristics of adult myoblasts become dominant in late stages of embryonic development.

MYOSIN ISOFORM EXPRESSION DIFFERS IN FETAL AND ADULT MYOGENIC CULTURES

Terminal differentiation of myoblasts results in the expression of muscle specific proteins. One of the most

prominent muscle specific proteins is sarcomeric myosin, which consists of two heavy chain subunits and several light chain subunits. It is now well established that in both avian and mammalian systems, myosin heavy chain (MHC) consists of multiple isoforms which are differentially expressed during development and regeneration (reviewed in Cerny and Bandman, 1987; Bourke, et al., 1991). It is because of this distinct developmental pattern that we examined MHC isoform expression by cultured myoblasts in search of a traceable marker of myogenic cells from the adult chicken.

When we used a general monoclonal antibody against all known forms of sarcomeric MHC (mAb MF20, Bader, et al., 1982) we observed that adult myogenic cultures differentiate and fuse later than myogenic cultures prepared from embryonic day 10 (E10 or fetal cultures). These results are consistent with the delayed fusion in adult compared to embryonic cultures reported previously by us (Yablonka-Reuveni, et al., 1987). Surprisingly, most myocytes (differentiated myoblasts) and myotubes in 3 to 5 day-old cultures from adult muscle were not reactive with a monoclonal antibody against fast embryonic MHC (mAb EB165, Cerny and Bandman, 1987) while myocytes and myotubes in E10 cultures reacted with this antibody (Hartley, et al., 1991). This was surprising because fast embryonic MHC was thought to be the major isoform of MHC expressed in short-term myogenic cultures from chicken pectoralis muscle (Cerny and Bandman, 1986; Hartley and Yablonka-Reuveni, 1990). Yet, the reactivity of satellite cell cultures with mAb MF20 but not with mAb EB165 suggested that the MHC isoform expressed by adult cells is different from embryonic MHC. (It should be noted that mAb EB165 reacts with both embryonic and adult isoforms of chicken MHC, but with the current experiments it is used as a marker for embryonic MHC since adult MHC is not expressed, as shown with a monoclonal antibody specific for adult MHC (Hartley and Yablonka-Reuveni, 1990)). Recently, it became evident that a ventricular isoform of MHC is expressed transiently in both developing and regenerating chicken muscle, concomitantly with embryonic fast MHC (Kennedy, et al., 1989; Sweeney, et al., 1989; reviewed in Hartley et al., 1991; Bourke et al., 1991), so we set out to determine if the MHC isoform present in adult myogenic cultures was ventricular MHC. This was done employing a monoclonal antibody specific for the major form of chicken ventricular MHC (mAb HV11, Bourke, et al., 1991; Hartley, et al., 1991; 1992). Most myocytes and myotubes in E10 myogenic cultures co-expressed both ventricular and embryonic MHCs (a low percentage of myocytes expressed ventricular myosin only; see discussion below). In contrast, myocytes and newly formed myotubes in adult cultures expressed ventricular but

not embryonic MHC. More advanced myotubes eventually co-expressed embryonic MHC along with ventricular MHC but adult myocytes never expressed embryonic MHC (Hartley, et al., 1991).

Thus, there are two patterns of ventricular/embryonic MHC expression which are specific for fetal and adult myoblasts. The 'fetal pattern' is the co-expression of embryonic MHC along with ventricular MHC in both myocytes and myotubes, while the 'adult pattern' is the expression of ventricular MHC only in myocytes and newly-formed myotubes, and subsequent co-expression of embryonic MHC in more advanced myotubes. These adult and fetal patterns are also demonstrated by clonal cultures derived from adult and E10 myoblasts (Hartley, et al., 1991). Adult chicken myoblasts isolated from injured muscle (where they were induced to proliferate prior to isolation) as well as passaged adult and fetal myoblasts, maintain in culture their appropriate adult or fetal MHC expression pattern (Harltey, et al., 1991). The adult and fetal patterns of MHC expression are also seen in cultures from other adult and fetal skeletal muscles of the chicken regardless whether the cells are isolated from a fast or a slow muscle (Hartley, et al., 1992). Hence, these adult and fetal patterns of MHC expression are not peculiar to the pectoralis muscle, but are present in a developmentally specific manner in different muscles.

MYOBLASTS WITH THE ADULT PATTERN OF MHC EXPRESSION BECOME PREDOMINANT DURING LATE CHICKEN DEVELOPMENT

We used the appearance of myocytes and newly formed myotubes expressing ventricular but not embryonic MHC as a marker to determine when adult myoblasts first appear during chick development. The expression of ventricular and embryonic MHCs was examined in primary mass cultures and clones established from progressive stages of chicken embryogenesis and post-natal growth (E14, E18, 1-day post-hatch, 3-week post-hatch) and compared with expression patterns of E10 and adult cultures (Hartley, et al., 1991; 1992). E14 myogenic cultures, like those from E10, contained a small percentage of myocytes expressing only ventricular MHC, while the majority of myocytes and myotubes co-express both ventricular and embryonic MHCs. In contrast, most of the myocytes and the newly formed myotubes in E18 cultures expressed ventricular MHC only. the percentages of myocytes expressing only ventricular MHC were 16% for E10, 24% for E14, and 86% for E18 on the days that the highest number of myocytes are present in the mass cultures. Cultures from 1-day and 3-week post-hatch chicks uniformly

exhibited a pattern of ventricular and embryonic MHC expression identical to that of adult cultures. Collectively, these studies show that adult myoblasts are present in small numbers at E10 and begin to predominate during late (or mid-fetal) development.

THE FREQUENCY OF CHICKEN MYOBLASTS RESPONSIVE TO PDGF IS HIGHER IN THE ADULT THAN IN THE FETAL MYOGENIC POPULATIONS

In studies examining the effect of the three PDGF isoforms on the mouse myogenic cell line C2 (derived from adult mouse muscle, presumably from a satellite cell) we showed that PDGF-BB enhances proliferation and suppresses differentiation of the cells, PDGF-AB has only a modest effect on proliferation and PDGF-AA has no effect on either proliferation or differentiation of the cells (Yablonka-Reuveni, et al., 1990a). In earlier studies on the role of PDGF during myogenesis we detected low level binding of PDGF-AB to myogenic cultures from adult chickens whereas binding of PDGF-AB to myogenic cultures derived from E10 was barely detectable (Yablonka-Reuveni et al., 1988; 1990b). These studies have suggested a possible role of PDGF during myogenesis in adult organisms but perhaps not during development. With this possibility in mind we have examined PDGF receptor expression by chicken myoblasts taken at different developmental stages. We focused on cells from mid and late chicken development (E10 and E19, respectively) and analyzed to a lesser extent cells from post-hatch chickens (3 week-old) (Yablonka-Reuveni and Seifert, submitted). We selected the E10 and E19 stages as the myoblasts from these sources represent two fetal and adult myogenic populations (see discussion above). To ensure the purity of the myoblast preparation and to study potential heterogeneity between myogenic precursors within the same developmental stage, the study was performed with clonally-derived myoblasts as well as with primary cultures. We showed that myogenic progeny of muscle colony forming cells from the E10 and E19 stages as well as from the post-hatch stage express binding sites for PDGF-AB and PDGF-BB and both PDGF isoforms promote proliferation of these clonally-derived myoblasts. However, when analyzing binding of PDGF isoforms to primary cultures from E10 and E19 we noted that E19 cultures have high levels of receptors for PDGF-AB and PDGF-BB compared to E10 cultures (Yablonka-Reuveni and Seifert, submitted). These results are consistent with our earlier studies discussed above where we found that primary mass cultures of E10 myoblasts express very few binding sites for PDGF-AB whereas cultures of adult myoblasts expressed a greater number of receptors for PDGF-AB). The fact that most E10 clones do

express PDGF receptors at levels comparable to E19 clones is consistent with the possibility that most, if not all, the clonable myoblasts from E10 breast muscle are a sub-population which, unlike the majority of E10 myoblasts, express PDGF receptors at levels comparable to adult myoblasts.

We conclude that PDGF is a potent mitogen for chicken myoblasts and that myoblasts responsive to PDGF are more frequent in late stages of chicken development as well as in post-hatch stages. The timing of appearance of myoblasts responsive to PDGF (i.e., late development) seems to overlap the time of emergence of myoblasts with the adult pattern of MHC expression.

RELATIONSHIP BETWEEN ADULT MYOBLASTS AND SOMITIC/LIMB BUD MYOBLASTS

We have demonstrated differences between adult and fetal myoblasts yet it is generally accepted that all myoblasts originate in the somites. The questions remain: where does divergence of adult myoblasts begin? Do adult myoblasts become committed to this population very early in development (i.e., in the somite) or does the divergence occur late in development? Does a precursor of fetal myoblast gives rise to adult myoblast during late development, or the fetal myoblast itself change into adult myoblast during late development? To begin addressing such issues we cultured chicken somitic and limb bud cells and analyzed the co-reactivity with the antibodies against embryonic and ventricular MHC. We were able to detect, at about equal frequencies, myocytes expressing ventricular MHC only and myocytes co-expressing ventricular and embryonic MHC (Hartley and Yablonka-Reuveni, unpublished). It is attractive to suggest that these two different myocyte phenotypes represent the fetal and adult myoblasts, thus the potential to give rise to these two populations already exists during early development. However, myoblasts in these cultures did not fuse into myotubes, and their relationship to the fetal and adult myoblasts present at later stages of development has yet to be resolved. Furthermore, it is also possible that during the time the smoitic/limb bud cells spend in culture, the myogenic precursor cells have sufficient time to give rise to the fetal and adult phenotype, but in vivo this divergence may only happen later during development. Our clonal studies suggest that even at E14 and E18 stages there are myogenic precursor cells with the potential of giving rise to both fetal and adult type myoblasts. This was determined by identifying myocytes expressing ventricular MHC only and myocytes co-expressing

ventricular and embryonic MHC within individual clones employing double immunofluorescence. Progenitors with the dual potentialities were not detected following hatching and all myocytes within individual clones were of the adult type only (i.e., expressing ventricular but not embryonic MHC)(Harltey, et al., 1992).

CONCLUDING REMARKS

In this paper we reviewed some of our recent results indicating that adult myoblasts represent a unique myogenic population (or lineage) which become dominant during late chicken development. Upon differentiation, these adult myoblasts have a specific pattern of MHC expression compared to fetal myoblasts and their proliferation in culture is regulated by specific isoforms of PDGF. A more complete analysis is needed to address lineal relationship and divergence between adult, fetal, limb bud and somitic myoblasts.

Some of our current studies are focused on the question whether adult and fetal myoblasts differ in expression patterns of myogenic determination genes. Studies following the timing and sequence of expression of myogenic determination genes during development suggest heterogeneity in the myogenic lineage, even before formation of the muscle primordial (Sassoon, et al., 1989; Lyons, et al., 1991). Similarly, distinct lineages or phenotypes of fetal and adult myoblasts can be correlated with differences in expression of such genes.

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