Molecular and Cell Isoforms During Development

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Summary. Development proceeds by way of a discrete yet overlapping series of biosynthetic and restructuring events that result in the continued molding of tissues and organs into highly restricted and specialized states required for adult function. Individual molecules and cells are replaced by molecular and cellular variants, called isoforms; these arise and function during embryonic development or later life. Isoforms, whether molecular or cellular, have been identified by their structural differences, which allow separation and characterization of each variant. These isoforms play a central and controlling role in the continued and dynamic remodeling that takes place during development. Descriptions of the individual phases of the orderly replacement of one isoform for another provides an experimental context in which the process of development can be better understood.

Embryonic development proceeds through a series of replacement events wherein one molecule or cell state is replaced by the next more complex state. The replacement does not occur instantaneously, nor does it involve a completely different moiety. The molecules or cells taking part in these replacement events are both similar and distinctly different; they are called isoforms. Examples of molecular isoforms are myosin heavy chain (MHC) or muscle actins, which have slightly different primary structures from those found in embryonic or neonatal, compared to adult, muscle; these isoforms arise from different genes in the case of actins (*1*). An example of a cellular isoform is the replacement of a chondrocyte by an osteoblast; both cells synthesize and secrete an unusual extracellular matrix that provides rigid structural support as an embryonic cartilage model, as for the tibia and its bony replacement (*2, 3*). The replacement of like molecules or cells for like is the basis for the isoformic replacement mechanism for continued embryonic development extending into adulthood and later maturation events.

In this article, we discuss the principle of isoformic replacement with emphasis on muscle and cartilage. However, other examples (*4*) also illustrate the general principle and strengthen our proposition that isoform transitions are necessary during development.

Cell Isoforms and Muscle Development

Isoforms arise during discrete developmental stages in which molecular and cell demands are changing or are a result of the genetic program of the organism. In the case of muscle, for example, a newly formed and emerging fetal musculature of the mouse functions in random movements that are essential in the forming of joint structures (*5*) and for space-filling characteristics that enable major myogenic expression to take place shortly after birth (*6*). This fetal muscle does not experience highly stringent demands with regard to load-bearing capacity, yet it is essential for subsequent complex developmental events. Shortly after birth, for example, the mouse requires muscle with slightly different properties in order to move around and eventually to nurse for nourishment. Within several days after birth, the newly born mouse slowly gains in stature and strength, and within a matter of a few weeks, develops a fully innervated complex musculature that can function with regard to load-bearing.

Thus, as shown in mouse muscle, three distinct developmental periods can be defined:

1) The fetal or late embryonic period in which the tissue architecture, individual functioning muscle units [such as the extensor digitorus longus (EDL)], and temporary innervation patterns are established.

2) The neonatal period in which limited movement becomes possible.

3) The growing mouse or adult where full mobility and full load-bearing properties are acquired. Each of these individual periods of development is overlapping (*7*), yet is distinctive at the molecular and the cellular level.

The fetal period of muscle development is more complex in regard to cell isoforms than any of the other developmental time periods. Here, the embryo converts relatively uniform mesenchymal cells into distinctive myoblasts, which eventually fuse to form multinucleated myotubes. These myotubes further develop by synthesizing complex arrays of macromolecules that are organized into the contractile apparatus. Three distinct isoformic phases of myoblast development have been described (*8*). The initial phase involves the conversion of mesenchymal cells into myoblast I. This phase is preceded by a differential vascularization of premyogenic as compared to prechondrogenic mesenchyme (*9*). This differentiation of the vasculature provides a separated and rich nutrient space in which initial myogenesis can take place. In both tissue culture and in vivo, early myogenesis is marked by large separations between individual cells and the replacement of the extracellular matrix from an environment rich in hyaluronic acid to one structured by complex proteins and polysaccharides (*10*). Myoblasts I undergo limited fusion to form small multinucleated myotubes that seem to function in a highly dispersed manner and to set up broad limits for major myogenesis and the musculature to follow (Fig. 1).

This initial phase of mesenchymal cell conversion into myogenic tissue is followed by the emergence of myoblast II cells which are capable of forming larger multinucleated cells that predominate over the tissue originated from the myoblast I population (*8*). Coincident with the appearance of the myoblast II population is the distal elaboration of major motor axons in the limb (*5*). These axons enter as large bundles containing many axons that then sprout and diverge through the length of the innervated appendage. Coincident with the appearance of nerve is the last subgroup of myoblast, myoblast III, whose appearance has been shown to be nerve-dependent (*11*). In the absence of such neural involvement, this group of myogenic cells is absent. All through this process,

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myoblasts fuse and form large and complicated multinucleated structures that synthesize a complex contractile apparatus containing muscle-specific isoforms within a single cell membrane.

In contrast to the myoblast I, which forms only small myotubes with four to six nuclei per cell, myoblast II and III form myotubes with 20 to 100 or more nuclei per cell. Major muscle development and the replacement of soft tissue with complex muscle units occur during the stage of development predominated by myoblast III units. When the embryo emerges, at birth, very few new cells are added to the muscle tissue; growth is accomplished by myotubes becoming larger and the deposition of larger amounts of contractile proteins. The myoblast III thus becomes the primary embryonic muscle unit accounting for a significant proportion of muscle found in this and subsequent stages of development. Muscle-associated connective tissue (*12*), produced by both the muscle and fibrogenic connective tissue cells, is responsible for segregating individual groups of myotubes into discrete muscles such as EDL. The transition from myoblast I to II to III exemplifies cell isoforms and replacement phenomena and is a general embryonic mechanism for building complex tissues capable of providing complex functions.

Nerve-muscle interaction follows a number of discrete and complex development steps in which the muscle is first innervated by a multiplicity of "bouton" type innervations with no discrete nerve-muscle junctions (*13*). Eventually, in fast muscles, all but one of these foci of innervation are lost; this surviving focus differentiates into a highly structured, nerve-muscle junction with distinctive morphological and functional characteristics (*14*). The final architecture of this synapse with its single myelinated nerve fiber and its characteristic electrical and chemical properties eventually becomes an intransient structure, which will serve as a guide for its own reinnervation after injury (*15*). The general trend in all these developmental processes, but especially the nerve-muscle process, is that both cytological and chemical reactivities undergo isoformic transitions, from those which are highly plastic and capable of modification to one which has highly characteristic and restrictive reactivities. Hence, we would conclude that these isoformic transitions involve the orderly replacements of distinctive cell types and reactivities by the next generation of functioning units, either of cellular or molecular type.

Developmental Compared to Physiological Transitions

With the emergence of myoblast III and major myogenesis, the soft tissue of a limb is replaced, to a major extent, by fetal muscle. Discrete molecules, such as fetal muscle myosin, fetal muscle actin, and muscle-type creatine kinase, are packaged into these muscles along with organizational molecules never before produced, such as myomesin (*16*) and other M- and Z-line proteins (*17*). Interestingly, in chicken and in mouse, the H-type isoform of lactate dehydrogenase (LDH) is produced during these stages of development (*18*). The H-type LDH is usually found in highly aerobic tissues and functions in a manner that is kinetically very different from that of the M-type LDH, which is associated with more anaerobic metabolism (*19*). However, with the transition from fetal to neonatal functioning, the newly synthesized LDH is almost exclusively of the M type. Correlated with major load-bearing responsibilities, the more anaerobically functioning LDH isozyme is produced. Thus, the metabolic and physiologic demands on muscle tissue are communicated, and somehow one isoform of LDH is selectively accumulated over the other (*20*).

Great caution, however, should be applied to analyzing this last example because there are distinct mechanisms involved in the control processes that govern the synthesis of developmentally programmed macromolecules as opposed to those that arise through physiological changes, that is, those due to some change in the cell's external environment and that are reversible. Thus, the presence of a hormone can cause a shift in the biosynthetic patterns of particular cells yet, when this hormone is removed, the cells will usually shift back to the original pattern. In the case of a developmental process, stimuli in the environment are eventually sensed by the cell's genome and result in the synthesis of a particular macromolecule or macromolecules. As this stimulus is withdrawn, the synthesis of any of these particular macromolecules may or may not fluctuate, but the cell does not revert to its original state since other developmental changes have also occurred. In this context, developmental changes usually seem to be nonreversible while physiological changes are reversible. Such differences between physiologically and developmentally regulated synthesis are not separate functions but obviously interact in a complex manner. For example, although major myogenic events take place in cell culture (myoblast proliferation, myoblast fusion, and

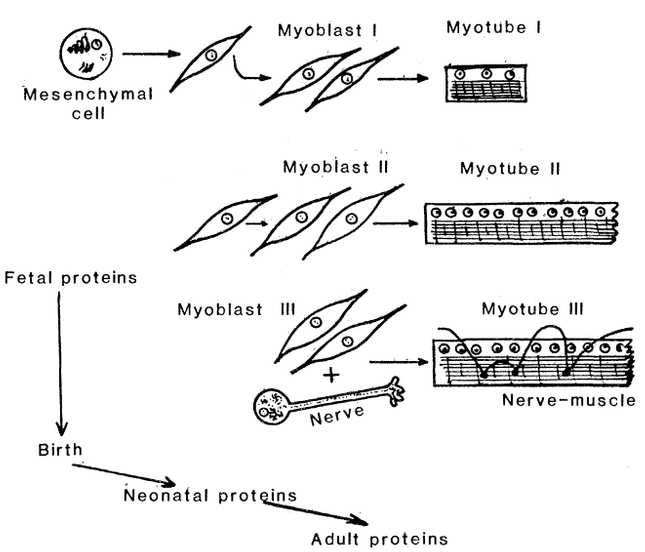

Fig. 1. Isoformic transitions during muscle development are schematically depicted. Mesenchymal cells become committed to a myogenic lineage and form myoblasts. The myoblasts multiply and eventually fuse into myotubes where the contractile apparatus is synthesized and assembled. The first myoblasts (I) fuse to form only small myotubes (I), while myoblasts found at later stages of development form very large myotubes with many tens of nuclei per myotube (II and III). The myoblast III cells seem to arise in a nerve-dependent series of events (*11*). After fusion of myoblasts into myotubes, motor nerves initially make numerous "bouton" contacts with a single muscle fiber. However, only one such tenuous contact ultimately matures into the nerve-muscle junction controlling that entire fiber. This maturation includes the myelination of the nerve, the concentration of acetylcholine receptors from a uniform dispersion on the myotube into the nerve-muscle junction, and the formation of the characteristic ultrastructure of nerve-muscle junction. While these complex cellular and nerve-related transitions are occurring, isoformic transitions of muscle-specific proteins occur with the appearance and replacement of fetal proteins, then neonatal proteins and, finally, adult proteins.

multinucleated myotube maturation to provide a spontaneously contractile cellular element), many of the major contractile macromolecules synthesized by the myotubes in these cultures remain of the fetal type (*21*). It is possible that load-bearing is a physiological stimulus for the initiation of the developmental remodeling of muscle isoforms between the adult, neonatal, and embryonic or fetal types.

Muscle Proteins: Molecular Isoforms

Analyses of muscle proteins, especially those associated with the contractile apparatus, add a molecular dimension to the isoform mechanism. Proteins similar to those of the contractile apparatus are also present in nonmuscle cells where they contribute to cell mobility (*22*). However, these molecules in nonmuscle cells are of different primary structure compared to the contractile proteins specific for muscle (*23*). Furthermore, muscle-specific contractile proteins are different for the muscle of heart, smooth muscle, fast muscle, and slow muscle as well as for nonmuscle tissues (*24*). Two important observations arise from the detailed characterization of these contractile proteins: first, three distinct transitions within the same muscle cell occur, with certain contractile proteins being replaced by their isoforms; these isoforms provide similar function but they have different primary structure. These developmental transitions occur during an embryonic, a neonatal, and an adult stage of development (*7*). And second, a contractile protein isoform found in the embryonic stage of one tissue may serve as another tissue's adult isoform. In particular, one of the myosin light chains of the adult heart atria appears in early skeletal muscle and in the embryonic ventricular heart muscle; in the adult organism, however, this heart-specific light chain is absent in skeletal muscle and ventricular heart but present in heart atria and Purkinje fiber muscle (*25*). Also in this context creatine kinase plays a dual role in muscle: as a component of the structure of the contractile apparatus (M-line protein) and as an enzyme involved in muscle energy metabolism.

Myosins

Myosin is a multi-subunit protein that consists of two heavy chains with a molecular size of 200,000 daltons and a series of light chains that can be divided into phosphorylated light chains (LC_2) and nonphosphorylated light chains (LC_1 and LC_3) (*26*). In fast skeletal muscle, there are two LC_2 chains of 18,000 daltons and an LC_1 and an LC_3 of 25,000 and 16,000 daltons, respectively (*26*). The amino acid sequence of LC_3 is found in LC_1; and data obtained with molecular probes from cloned segments of the genome suggest that these two light chains arise from the same gene but through a different splicing mechanism in the processing of the primary transcript (*27*). When analyzed under native conditions, the fast muscle myosins can be resolved into three bands, which correspond to homopolymers (2 HC, 2 LC_1, 2 LC_2 or 2 HC, 2 LC_3, 2 LC_2) or heteropolymers (2 HC, 1 LC_1, 1 LC_3, 2 LC_2) (*27*). In slow skeletal muscle, the myosins consist of two heavy chains (HC) associated with two LC_1 and two LC_2 of 27,000 and 20,000 daltons, respectively (*26*). These slow and fast myosins differ in their activated adenosinetriphosphatase activity, a property that may contribute to the physiological differences between the two types of fibers (*28*).

Table 1. Myosin light and heavy chain isoforms.

Fetal tissue or first culture	7 to 11 days after birth	Adult
MHC_{emb}	MHC_{neo}	MHC_F
$LC_{1(emb)} \rightarrow LC_{1F}$	LC_{1F}	LC_{1F}
LC_{2F}	LC_{2F}	LC_{2F}
	LC_{3F}	LC_{3F}

In studies of muscle development in mammals, Whalen *et al.* (*29*) demonstrated the existence of a new myosin light chain which they called $LC_{1(emb)}$. This light chain is present in embryonic muscles from skeletal and cardiac tissue; it is expressed by muscle cells that differentiate in vitro. This embryonic light chain is replaced by an adult form of LC_1 that is muscle-type specific. This isoformic transition takes place in the embryo in situ as well as during myogenesis in culture (*29*).

In addition, the myosin heavy chain that is present in fetal muscle differs from that found in adult muscle (*1*). Results of polypeptide mapping indicated that fetal myosin differs from adult myosin heavy chain and, in addition, that a new form of myosin transiently appears between the time of the disappearance of embryonic heavy chain (MHC_{emb}) and emergence of the adult heavy chain (MHC_a). This myosin has been called myosin heavy chain neonatal (MHC_{neo}).

These transitions in rat fast muscle development are outlined in Table 1. A similar situation seems to exist during the development of avian muscles as evidenced by studies with polypeptide mapping and monoclonal antibodies (*30, 31*). Several important considerations support our proposed sequence of events (Table 1). First, $LC_{1(emb)}$ is replaced by $LC_{1(F)}$ (emb, embryo; F, fast); the transition is not seen in muscle cell lines, such as the L_6 or L_8, that differentiate in culture. This transition is not seen in the rhabdomyosarcoma cells which also differentiate in culture. In contrast, $LC_{1(emb)}$ continues to be expressed in adult heart muscle where it is localized in the atrium (*25*).

A second aspect of $LC_{1(emb)}$ is that it has never been observed in avian muscles. When developing muscles or myotubes formed in vitro are analyzed, they synthesize and accumulate myosin light chains which by two-dimensional gel analysis cannot be distinguished from authentic adult myosin light chains. However, birds may have evolved another type of "embryonic" myosin. When newly formed embryonic muscles, whether future fast or slow, are isolated from an 8- or 9-day-old chick embryo, this tissue accumulates a myosin whose light chains are essentially LC_{1F}-associated with LC_{2S} and LC_{2F}. In this case, the stoichiometry is such that the amount of LC_{1F} is equal to the amount of LC_{2S} plus LC_{2F}, with LC_{2S} being slightly more abundant than LC_{2F} (*32*). The nature of the MHC chain still remains to be elucidated but such a myosin whose light chains are a mixture of fast and slow isoforms could represent the first type of myosin to be synthesized by early avian embryonic muscles. Later during development, the myosin that is synthesized consists of an embryonic heavy chain associated with the adult light chains corresponding to the type of the muscle (*21*).

A third consideration raises the question as to how many MHC_{emb} exist in the embryo? Is there an MHC that is specific for each type of skeletal muscle? Is there only one single embryonic MHC? From histochemical observations of adenosinetriphosphatase activity, it appears possible to distinguish histochemical differences between presumptive slow and fast muscles in embryos. If these differences in any way reflect the nature of the myosin heavy chains, they would indicate the presence of at least two skeletal MHC_{emb}. The tentative suggestion, therefore, is that each skeletal muscle type synthesizes its own specific MHC_{emb}. In cultures of myogenic cells,

it appears that differentiated myotubes only synthesize MHC_{emb} that corresponds to the fast skeletal muscle type and that the cues necessary for the next isoform are not present (*21*).

Actins

There has been no description of embryonic actin. There is, however, a transition involving the replacement of nonmuscle β- and γ-actins, which are present in proliferating myoblasts by the skeletal α-actin which is synthesized by muscle fibers (*33*). Two observations, however, may indicate that this is not a simple transition. First, in the mouse L_6 line, before a skeletal muscle α-actin appears, there is the transient synthesis of a smooth muscle α-actin (*34*). This same observation has been made with normal mouse myogenic cells in culture by peptide analysis (*35, 36*). And second, with molecular probes from cloned segments of the genome, evidence has been obtained that, during skeletal muscle development, one can detect messenger RNA (mRNA) sequences which correspond to cardiac α-actin sequences (*37*). Taken together, these two observations indicate that both cardiac and smooth muscle α-actins could play the role of embryonic or fetal forms during the development of skeletal muscle, although this suggestion requires further analytical documentation.

Creatine Kinase

It remains an important question whether newly appearing isoforms have a functional role in differentiated cells that cannot be fulfilled by the isoforms found in the precursor cells. At present there are only very few cases known that show such a difference; among such cases is the one for creatine kinase (CK). A transition during development from a ubiquitous or embryonic isoform (BB-CK) to a muscle-specific form (MM-CK) has been demonstrated for CK in a number of species (*38*). A complete transition from the two-subunit form BB-CK to exclusively MM-CK in adult skeletal muscle occurs in vivo, whereas in cell cultures the transition has not been demonstrated to go to completion (*39*). During this transition period, MB-CK is also observed, an indication that, at some time, both CK subunits were synthesized and had randomly assembled into the hetero dimer of the MB type within the same cell. With the use of the DNA probe, $pMCK_1$, evidence has been obtained that in early embryonic skeletal muscle as well as in proliferating myoblast culture, no mRNA sequences are present that can hybridize to the M-CK DNA (*38, 40*). Therefore, the transcription of the M-CK gene is an integral part of the synthetic program for terminally differentiating muscle cells.

The central question is whether there is a special feature of MM-CK as compared to the nonmuscle BB-CK in less-differentiated muscle cells. In this regard, a significant difference has been observed between MM-CK and BB-CK with respect to their ability to bind to the myofibrils. One component of the electron-opaque M line, which traverses the center of the sarcomere of myofibrils (Fig. 2) and consists mostly of the so-called M bridges, has been identified as MM-CK (*17*). M-bridge material can be removed concomitantly with the removal of all the CK activity (*16*). On the other hand, BB-CK has never been shown to be localized within the M line; in chicken heart myocytes, the B to M transition does not take place nor can the electron-opaque M line be demonstrated (*39*). Recent data suggest that the M line–bound MM-CK may be an intramyofibrillar adenosine triphosphate–regenerating system besides being a structural part of the contractile apparatus (*38, 39*). For isolated chicken skeletal muscle myofibrils, the bound CK (5 percent of total) is sufficient for rephosphorylating all the adenosine diphosphate which is formed by the myofibrillar actin-activated Mg^{2+} adenosinetriphosphatase (*38*).

Cell culture myotubes contain all three CK isoforms as demonstrated cytologically with fluorescently labeled antibody to M-CK and antibody to B-CK, but only MM-CK is localized in the M line. Thus, even though both M- and B-CK subunits are present in these cells, only the MM-CK isoform is able to associate with the M region of the myofibril even in culture conditions where the CK transition does not proceed to completion. M line–attached MM-CK represents, on the one hand, an enzyme involved in adenosine triphosphate regeneration and, on the other hand, a structural reinforcement of myofibrils by means of the M bridges in such muscle types where a higher degree of maintenance of filament order is required. The isoform-specific incorporation into the myofibrillar M-line structure during terminal differentiation seems to represent a functional advantage only available in cells that can express the M-CK gene.

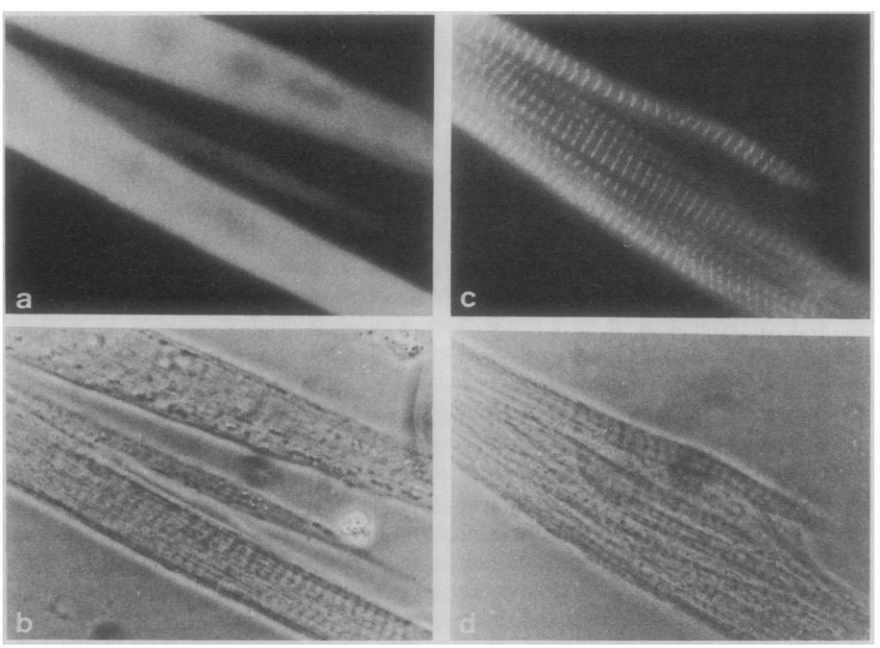

Fig. 2. Presence of MM-CK in myotubes of a 6-day-old cell culture. Stained by indirect immunofluorescence using antichicken M-CK antibodies and a second antibody labeled with rhodamine (*38, 39*); (a) total cytoplasmic MM-CK after treatment of the cells with Triton X-100; (c) M-line bound MM-CK after permeabilization and subsequent extraction of cytoplasmic MM-CK; (b and d) phase contrast images corresponding to (a) and (c), respectively.

Coordinate Expression

Another important point to stress (*41, 42*) indicates that the program for the biosynthesis of muscle macromolecules is coordinately regulated so that, after fusion, when major biosynthesis of MM-CK, myosin, actin, light chains, and the like are called for, these are all synthesized at compatible rates in compatible

quantities. On the basis of these studies, the hypothesis put forth is that there is an organizational unit in the genome that calls for these various gene products as a coordinate group, especially since these muscle-specific genes are not linked. When there is a transition within the same cell from fetal to, eventually, adult-type myosin, for example, the question thus arises whether these replacement isoforms are called forth as multicomponent units or whether these are synthesized in a less coordinated fashion during the transition from neonatal to adult. This latter situation seems to be the case since (Table 1) the transition from $LC_{1(emb)}$ to LC_{1F} is not accompanied by a transition at the level of the myosin heavy chains.

Transitions Within One Cell

The above discussions are oversimplified in the sense that fast muscles are made up not only of "fast" individual myotubes. This is clear from the work (*43*) in which monoclonal antibodies to various myosin light chains were used to show that developing and adult muscles are made of a mixture of fibers. Some fibers in the same muscle mass show positive staining for fast light chains, while next to them are fibers staining for slow light chains. Thus, when analyzing a specific muscle for its molecular composition by two-dimensional gel analysis, a mixture of fast and slow or embryonic and adult isoforms may reflect fiber heterogeneity as opposed to a functional mixture of these molecules.

In addition, there are two levels of isoformic transitions: First, during embryonic life, isoformic transitions usually involve cell replacement such as myoblast II for myoblast I or erythrocytes which make adult hemoglobin replacing those making fetal hemoglobin; and second, such as in muscle during later development where molecular isoformic transition can take place within the same cell.

Extracellular Matrix, Cartilage, and Bone

The general thesis described above in the developing limb musculature holds for another set of complicated macromolecules that are continually changing during the developmental process. This group of macromolecules is summarily referred to as extracellular matrix and consists of a complex mixture of proteins and polysaccharide-containing macromolecules. During the transition from mesenchymal cells to muscle or cartilage phenotypes, the extracellular environment is characterized by the presence of small amounts of type I collagen and a relatively large amount of hyaluronic acid (*44*). Hyaluronic acid seems to be present in areas of accelerated rates of cell division and is inhibitory toward the differentiation of mesenchymal cells into discrete phenotypes. Prior to the transition of mesenchymal cells to discrete phenotypes, increased hyaluronidase activity is observed which theoretically destroys much of the extracellular hyaluronic acid present and is coordinated with the transition of mesenchymal cells into their first embryonic phenotypes (*10, 45*). In the case of cartilage, this environment rich in hyaluronic acid is replaced by an environment high in new isoforms of collagen, type II collagen (*46*), and cartilage-specific proteoglycan (*47*).

The proteoglycan itself is synthesized in a slightly different isoform with each succeeding time interval or stage of development both in vitro and in situ (*48, 55, 56*). This proteoglycan seems to have the same core protein, but has a different arrangement and chemical properties of its large and complex polysaccharide side chains. Embryonic chondrocytes synthesize proteoglycans that have larger chondroitin sulfate chains and smaller keratan sulfate chains than those synthesized by more adult cells (Fig. 3). Ultimately, the senescent chondrocyte synthesizes cartilage-specific proteoglycans having age-dependent size and chemical characteristics. Since the chondroitin sulfate chains are the predominant component for structuring large amounts of water (*49*), which gives cartilage its properties of resiliency, the programmed change in the biosynthesis of this extracellular matrix component changes the physiological functioning of the tissue. It has been thought that senescent chondrocytes synthesize a proteoglycan that is less able to structure water and, thus, does not support the force transmitted through bone joints (*50, 51*). This inability to function as a cushion at joints results in tissue damage, inflammation, and eventually, symptoms of osteoarthritis. Studies indicate that this ever-changing biosynthetic pattern is part of the normal developmental program of chondrocytes. Thus, the extracellular matrix of this phenotype changes primarily due to the biosynthesis of an ever-changing isoform which, in this case, involves primarily posttranscriptional,

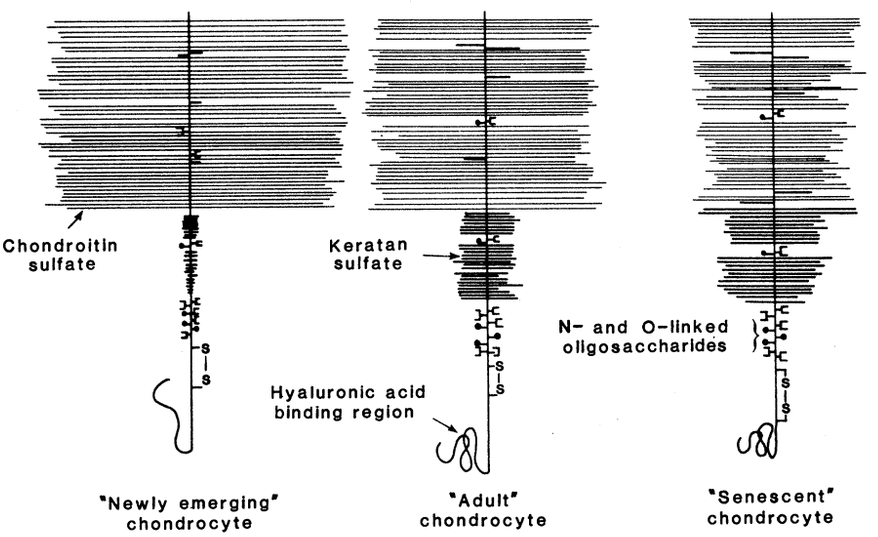

Fig. 3. Schematic representation of the comparative structure of proteoglycans from cartilage during early development, maturity, and advanced age of chondrocytes. The schematic is based on data from chick (*48*), bovine (*55*), and human (*56*). It is assumed that the core protein for these progressive, developmental states has the same primary structure. The chondroitin sulfate chains are larger as synthesized by young chondrocytes and eventually are about one-half the size comparing those of proteoglycans from senescent chondrocytes with newly emerging chondrocytes. The glycosaminoglycan chains from older chondrocytes are synthesized shorter as opposed to being degraded extracellularly since the keratan sulfate chains are found to be shorter from young chondrocytes and longer in older cells. For most cases, the ratio of chondroitin sulfate to keratan sulfate chain size is indicative of chondrocyte age with this ratio larger for young cells. Experimentation supports the view that these synthetic changes represent a natural developmental progression which is programmatic and irreversible (*49, 51*). The cartilage tissue derives its resiliency from the interaction of these proteoglycans with other molecules, namely, collagen and water (*47*). Thus, the loss of resiliency of joint cartilage is probably a direct result of these programmatically controlled biosynthetic changes throughout the life of the organism.

posttranslational modifications and the age-dependent control of that complex biosynthetic pathway.

This programmatic changing of the biosynthetic pattern is also seen in the development of other tissues, for example, cornea (*52*) or muscle (*12*). In the case of muscle development, myogenic cells synthesize a proteoglycan found only during early embryogenesis of muscle and not found in later stages of muscle development and maturation. The fibrous connective tissue associated with muscle then becomes the primary biosynthetic center for extracellular matrix components during periods when there are major changes in muscle morphology and the generation of various tissue and molecular isoforms. The proteoglycan molecules in the extracellular matrix have been proposed to be special categories of isoforms: common core proteins with different polysaccharide compositions and constituents.

In the developing limb, the transition of mesenchymal cell to cartilage is followed by another and more dramatic transition. The developing cartilage in the limb forms a model of the central skeletal appendage, and this model serves as the morphogenetic scaffolding on which future bone is built (*2*). The chondrocytes found in the cartilage core eventually go through hypertrophy, which is followed by vascular invasion, subsequent remodeling of the cartilage core, and the introduction of osteogenic cells into this remodeling matrix. The form and dimensions of the cartilage model serve to structure the future bone formation after vascular invasion. This invasion first occurs in the hypertrophying mid-diaphyseal region of the cartilage model, and then progressively proceeds in both proximal and distal directions until the two ends of the cartilage model are reached. At these ends, there is a distinct directional transition in which a growth plate forms and is involved in the endochondral lengthening of the skeletal appendages. The transition between cartilage and bone is marked by osteoblast-directed biosynthesis of bone-specific type I collagen (*53*) and other macromolecules that form the general framework on which calcium apatite of bone is organized and deposited. The replacement of the polysaccharide-rich cartilage matrix with its type II collagen by type I collagen and calcium apatite provides the major structural changes found in the transition between cartilage and bone (*54*). This replacement is the direct result of the replacement of chondrocytes by osteoblasts, both of which take part in synthesizing and assembling a specific extracellular matrix. Thus, the limb core region goes through three discrete phases: (i) mesenchymal cell phase, (ii) the production of a chondrogenic model, and (iii) bone formation, which in itself is a complex tissue necessary for skeletal support and for the location of complex hemopoietic events. This process is not completed during embryonic development since endochondral bone formation is a dynamic process that continues throughout most of the growth phase of the organism and is involved with the gradual lengthening of the long bones in a developmental process that continues into adulthood.

Although the cartilage model serves a space-filling role during this early and active period of development, it also provides rigidity, yet flexibility, for the developing appendage. The development of rigid and inflexible bone at this stage of development would not be supportive of all of the other complex events going on in the soft tissue areas, including the changes in myoblast type and segregation of individual muscle units. The transition from cartilage into bone occurs simultaneously with the emergence and domination of myoblast III and major myogenesis. The coordination of these two events is essential in the final formation and architecture of the developing limb. This shift of various tissue isoforms from mesenchymal through cartilage types and eventually to various forms of bone also involves a complex shifting of various isoformic macromolecules such as proteoglycans and collagens. Thus, isoformic transitions of both molecular and cell types are occurring in a very complex and regulated fashion culminating in the structural and functional specification of an appendage. This appendage is fabricated for specific functions of movement and support as integrated into the overall survival patterns of each animal. Other isoformic transitions occur during embryology and later development (*3*).

Summary and Conclusions

Sequential replacement of discrete cellular and molecular units is a normal, general, and important part of ontogeny. Information has been presented which details that within the same cell or within the same tissue, isoforms arise in a programmatic manner during development. Perhaps the single most intriguing observation is that entire subcellular organizations can be replaced by a particular isoformic configuration. This is clearly the case in muscle where the transitions between neonatal and adult isoforms of myosin or CK seem to take place within the same myotube. The fact that myogenic cells in culture undergo substantial differentiation and development, but do not demonstrate a transition to the adult isoform of MHC, suggests that cues stimulating such transitions are absent in tissue culture.

There are at least three avenues for interpreting the isoform model that are amenable to experimental inquiry. First, the emergence of isoforms represents a response to a change in functional demand on a molecule or cell. For example, fetal myosin is produced during a developmental period when essentially no load is presented to the muscle while adult myosin forms during a load-bearing period. This predicts that adult myosin has different functional characteristics. Second, isoforms may arise as a result of a change in the control sequences governing transcription or RNA processing as opposed to a change in function of the translation product. In this case, isoforms of the same or different structures may be produced as a result of a developmental program which coordinately dictates the production of different genomic sequences. And last, the isoforms represent evolutionary dictated specialization for which hierarchical sequences were generated. These three considerations can be applied to molecular (myosins), cellular (chondrocyte rather than osteocyte), or tissue (tooth replacement) considerations. The recognition of isoforms also stimulates the question of which isoform is the progenitor of its evolutionary offspring. For example, is adult myosin evolutionarily younger or older than fetal myosin? With the articulation of the isoform model, the challenge remains to provide experimental evidence to help choose between these various considerations.

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