

Intrinsic and Extrinsic Regulation of the Development of Myogenic Precursors in the Chick Embryo

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Abstract

Embryonic somites give rise to a variety of tissues including skeletal muscle and cartilage. Determination of when, where, and how somite cells become specified to follow a particular pathway of differentiation has been investigated for over four decades. A synthesis of the results from *in vivo* and *in vitro* analyses suggest that the presomitic segmental plate mesoderm and newly formed somites contain cells that are stably committed to undergo either myogenesis or chondrogenesis and an uncommitted population of cells with the potential to differentiate along either of these pathways. A model is presented in which the neural tube and notochord promote differentiation, in part, by producing factors that overcome inhibitory cell-cell interactions within the somite. Stably committed cells then influence the pathway of differentiation of their uncommitted neighbours. Commitment of some cells to the myogenic and possibly chondrogenic lineages occurs prior to gastrulation, as cells reside in the epiblast layer of the embryo.

Key words: regulation, development, myogenic precursors.

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The formation of skeletal muscle requires *de novo* synthesis of cell surface, cytoplasmic, and nuclear proteins. While some proteins accumulate during myogenesis, others disappear. Molecular interactions occurring at the plasma membrane regulate the alterations in gene expression that accompany differentiation. In turn, the production of new gene products results in altered cell surface interactions. Stimulatory pathways promote the differentiation and maturation of muscle cells while inhibitory signals maintain muscle precursor cells in the undifferentiated state until they have migrated to their proper positions and replicated a sufficient number of times to create appropriately sized muscle masses. The following discussion focuses on intrinsic and extrinsic factors that regulate the initial appearance of myogenic precursors and their differentiation in the chick embryo.

Myogenic Potential within the Paraxial Mesoderm

Practically all skeletal muscle of the body arises from the paraxial mesoderm consisting of the segmental plate and somites [13, 16-18] (Fig. 1A). Somites form from the rostral end of the segmental plate every 1.5 hours [78-79].

Skeletal muscle differentiation begins in the myotome of the somite in the 48 hour chick embryo [55, 114]. Somite cells also form the dermamyotome and sclerotome that give rise to the dermis of the back and cartilages of the vertebrae and ribs, respectively [19]. Development of these different cell types is regulated, in part, by tissues lying in close proximity to the somite [2, 7, 9, 10, 20, 27, 31, 32, 38, 39, 41, 54, 63, 65, 72, 75, 84-86, 93, 94, 99, 111, 114, 117, 119, 120, 125]. Candidates for differentiation promoting molecules secreted by the notochord and neural tube are Sonic hedgehog and members of the Wnt family [31, 32, 63, 85, 111]. Sonic hedgehog is produced by the notochord and floor plate of the neural tube and influences sclerotome development [31, 32, 63]. Wnts 1, 3, and 4 are secreted by the dorsal neural tube, and in combination with Sonic hedgehog, promote myogenesis [85, 111].

Several possibilities exist for the nature of the interactions between the somite and adjacent structures. If somite cells have the potential to differentiate along multiple pathways, extrinsic factors would be required to instruct them to follow a specific pathway of development. Alternatively, if somite cells are irreversibly committed to de-

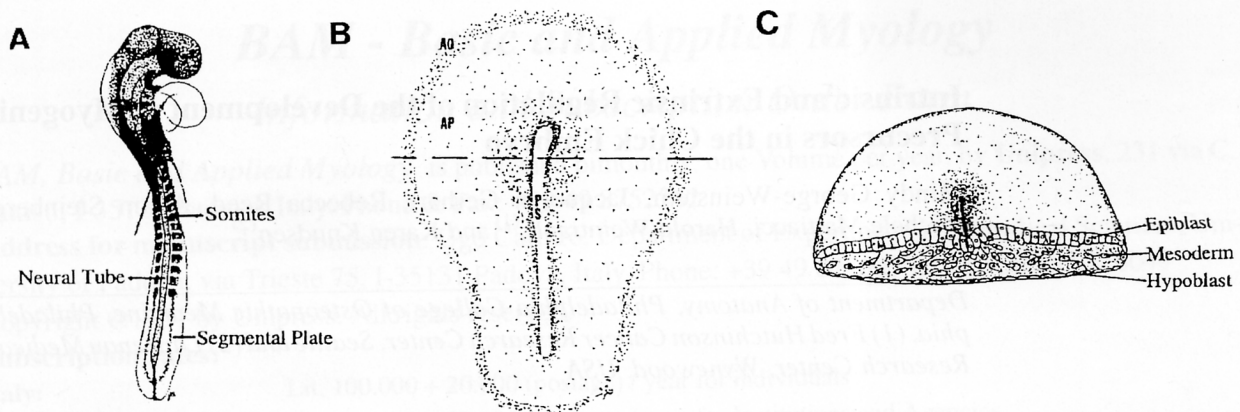


Figure 1. Stages 4 and 14 chick embryos. A. The stage 14 chick embryo (50 hours) contains approximately 22 pairs of somites. Somites arise from the cranial end of the segmental plate mesoderm. B. The stage 4 embryo (18 hours) contains a full-length primitive streak (PS). The embryo is viewed from its dorsal surface. Embryonic tissue is derived from the area pellucida (AP) while the area opaca (AO) gives rise to extraembryonic tissue. C. Transverse section through the stage 4 embryo at the level indicated by the hatched line in B. Cells of the dorsal, epiblast layer enter the primitive streak and then migrate laterally forming the mesoderm. The hypoblast is eventually replaced by the endoderm.

velop along a single pathway, signaling molecules may simply promote their differentiation. Support for the notion that cells of the newly formed somite are multipotential comes from experiments in which the position of the somite was changed relative to its surrounding tissues. These manipulations altered the cells' normal pathway of differentiation from myogenesis to chondrogenesis, and *vice versa* [1, 7, 9, 38, 93, 94, 125]. Additional support for the existence of multipotentiality comes from the observation that single cells labeled within the segmental plate mesoderm give rise to progeny found in both myogenic and chondrogenic regions of the somite [113]. Together these results suggest that the segmental plate mesoderm and newly formed somites contain cells whose fate is directed by the environment.

In contrast, transplantation studies support a model in which cells of the segmental plate and somites are inherently programmed to follow separate pathways of differentiation. Segmental plate tissue implanted into the limb bud forms both skeletal muscle and cartilage [122]. Cell culture studies also indicate that some segmental plate cells are inherently myogenic or chondrogenic. When segmental plates and somites are isolated from surrounding tissues, dissociated to produce a single cell suspension, and cultured at relatively high density, they differentiate into skeletal muscle and chondroblasts [36]. Additional evidence that at least some somite and segmental plate cells are inherently myogenic *in vivo* is that mRNA for MyoD is detected in these tissues by reverse transcription-polymerase chain reaction (RT-PCR) (Fig. 2). MyoD is a basic helix-loop-helix transcription factor that regulates the expression of some contractile protein genes [25]. Upregulation of MyoD expression (detection of MyoD mRNA by *in situ* hybridization) coincides with the apparent timing of stabilization of myogenic potential within the somite [95].

Different results were obtained when segmental plates and newly formed somites were placed in culture as an intact tissue instead of a single cell suspension. In this case, the cells did not differentiate into muscle unless co-cultured with the neural tube and/or notochord [2, 10, 65, 85, 111, 120]. The conflicting results of experiments in which cells were cultured as an intact tissue versus a single cell suspension may be resolved by assuming that some somite and segmental plate mesoderm cells are inherently myogenic or chondrogenic, but they are inhibited from differentiating by local cell-cell interactions.

Myogenic Potential of Primitive Streak Stage Epiblast Cells

Previous studies revealed that cells capable of forming skeletal muscle are present in the chick embryo prior to the formation of the first pair of somites [12, 14, 53, 56, 74, 121]. In an effort to determine when and how cells develop the potential to form skeletal muscle, we turned our attention to the tissue that gives rise to the segmental plate mesoderm, the epiblast. During gastrulation, some epiblast cells ingress through the primitive streak and then migrate laterally to form the mesoderm and endoderm layers (Fig. 1B and C). Other epiblast cells remain in the dorsal layer and develop into ectoderm and neur ectoderm.

Epiblasts were isolated from the mesoderm, hypoblast, and primitive streak, enzymatically dissociated, plated at high density, and grown in protein free medium [37]. Although *in vivo* the epiblast gives rise to all tissues of the embryo [5, 33, 100, 112], the dominant pathway of differentiation *in vitro* is skeletal myogenesis [37]. MyoD mRNA is present in the epiblast *in vivo*. MyoD gene expression is rapidly upregulated *in vitro* and within 2 hours of plating, 99% of the cells contain MyoD protein. Cultured epiblast cells also express other members of the basic helix-loop-helix family of muscle regulatory factors

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Table 1. Effect of cell density on muscle differentiation in epiblast cultures. Stages 3-4 epiblast cells were obtained as described previously [37]. Low density cultures were prepared by adding 2×10^4 cells in 1 ml 5% fetal calf serum, 5% horse serum, and 5% chick embryo extract (DES medium), to 35 mm tissue culture plates coated with gelatin and fibronectin. One hour after plating, the medium was replaced with protein free DMEM/F12 medium. Epiblast cells were cultured at high density by plating 2×10^4 cells in 10 μ l of DES medium on coated plates. One hour later, the dishes were flooded with 1.5 ml DMEM/F12 medium. Cells were fixed and labeled with the MF20 monoclonal antibody to myosin heavy chain [4]. Values are the mean standard deviation. The number of cultures scored is indicated in parenthesis. A minimum of 200 cells were scored per culture. The plating efficiency for low density cultures was 84 $\pm$ 8 (4) and 96 $\pm$ 1 (3) in high density cultures. Differentiation occurred to a greater extent when epiblast cells were plated at high density than low density ($p < .0005$).

	% MYOSIN POSITIVE CELLS					
	24 Hours		48 Hours		120 Hours	
Low Density	7	2 (6)	9	4 (6)	23	1 (4)
High Density	14	6 (7)	33	5 (4)	77	11 (6)

including myf5 and myogenin, and the MADS box transcription factor, MEF2 (Fig. 3). MEF2 binds DNA cooperatively with the muscle regulatory factors [88]. Within one week after plating, epiblast cells assemble sarcomeres containing fast embryonic myosin (Fig. 3), titin, α -actinin, the skeletal muscle specific molecules skeletal troponin T and 12101 antigen [37], and were capable of contracting.

Myogenesis was repressed when epiblasts were cultured either as an intact epithelium or in the presence of the mesoderm and hypoblast [37]. These studies indicate that cells have the potential to undergo myogenesis prior to entering the primitive streak, but are inhibited from differentiating in vivo by cell-cell and tissue interactions.

Releasing epiblast cells from the inhibitory influences present within the embryo is not by itself sufficient to trigger widespread differentiation in vitro. Cell-cell interactions also appear to be important to promote myogenesis. This was revealed when differentiation was compared in cultures plated at different cell densities. Myogenesis occurred to a significantly greater extent at high cell density than low density (Table 1).

An effect of cell-cell interactions on myogenesis also was observed in cells of the early *Xenopus laevis* embryo [45, 52, 103] and mouse somites [23] in vitro. This phenomenon, called the Community Effect [43], could result from secretion of locally acting factors and/or direct cell-cell contacts. Analyses of *Xenopus* [52] and chick embryos [George Weinstein M, Gerhart J, Blitz J, Simak E, Knud-

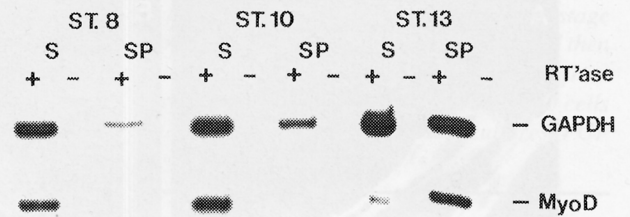


Figure 2. Analysis of MyoD expression by reverse transcription-polymerase chain reaction (RT-PCR). The most caudal 8 pairs of somites (S) and segmental plates (SP) were isolated from stages 8, 10, and 13 embryos (26, 35, and 48 hours) and immediately placed in 4.5 M guanidinium thiocyanate solution for extraction of RNA [15]. RT-PCR was carried out as described in George-Weinstein et al. [37]. Samples were incubated in the presence (+) or absence (-) of reverse transcriptase (RTase). The ubiquitously expressed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was included as an internal control for the amount of RNA tested. MyoD mRNA was detected in somites and segmental plate mesoderm at all stages tested.

sen K: N-cadherin promotes the commitment and differentiation of skeletal muscle precursor cells. Manuscript submitted] indicate that direct cell-cell contacts mediated by the calcium-dependent cell surface adhesion proteins, cadherins, are important for myogenesis. Injection of a dominant negative N-cadherin RNA into 2-4 cell *Xenopus* embryos suppressed the expression of MyoD in muscle forming cells of stages 16-18 embryos [52]. Experiments with chick embryo cells demonstrated that function-perturbing antibodies to N-cadherin, but not E-cadherin or NCAM [29, 48], inhibit the accumulation of myosin in epiblast, segmental plate, and somite cells in vitro [same authors and title as above. Manuscript submitted].

Development of Myogenic Potential within the Epiblast

Determination of the timing of development of myogenic potential was carried out by culturing epiblast cells from progressively younger chick embryos. A few cells from Hamburger and Hamilton's stages 1 and 2 embryos [47] differentiated into skeletal muscle (1% in 8 day cultures), however, they represented a very small percentage compared to what was observed in cultures of stages 3-4 embryos (approximately 83% in 5 day cultures). Thus a change occurs within the epiblast between stages 2 and 3 during primitive streak formation, that results in a dramatic increase in the number of cells with myogenic potential.

The factors responsible for the increase in the number of myogenic cells within the epiblast are unknown. One hypothesis that was tested was whether communication between the epiblast cells themselves would enhance muscle forming potential. Stage 1 epiblasts (Eyal-Giladi and Ko-

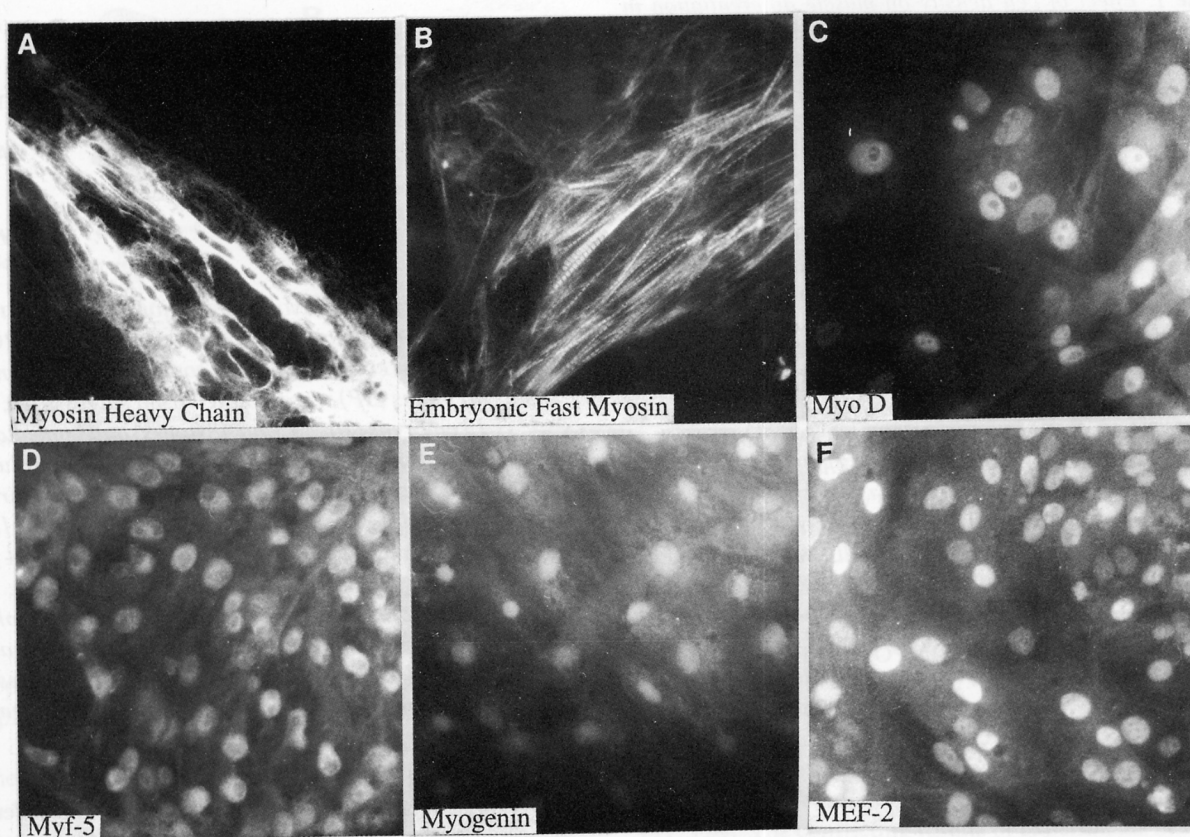


Figure 3. Expression of myogenic proteins in primitive streak stage epiblast cells *in vitro*. Stage 4 epiblast cells were cultured and labeled by the immunofluorescence localization method as described in George-Weinstein *et al.* [37]. Differentiated muscle cells were detected with the MF20 monoclonal antibody to myosin heavy chain [4] (Developmental Studies Hybridoma Bank) and the F59 monoclonal antibody to embryonic fast myosin [80], provided by Dr. Frank Stockdale. Epiblast cells also were labeled with polyclonal antisera to MyoD, Myf-5, and myogenin, provided by Dr. Bruce Paterson, and to MEF-2 (Santa Cruz Labs.).

chav's stages X-XII [30]) were isolated from the underlying hypoblast and cultured as intact epithelia for 20 hours, allowing them to age *in vitro* to the equivalent of stage 4. The tissue was then dissociated into a single cell suspension and replated under the same conditions that promoted myogenesis of stage 4 cells. Although the cells reattached to the dish, the percentage of cells differentiating into muscle remained low, similar to stage 1 cells initially plated as a single cell suspension. Although the mechanisms of cell-cell interactions within the epiblast may not be the same *in vitro* and *in vivo*, these results raise the possibility that intercellular communication within the epiblast itself is not sufficient to trigger the increase in myogenic potential that occurs between stages 2 and 3. Rather, interactions between the epiblast and surrounding tissues may mediate this change within the epiblast.

Two candidates for promoters of change within the epiblast are activin and fibroblast growth factor (FGF). Both molecules are present in the chick embryo at this stage of development [82, 83]. Activin can induce a secondary primitive streak and/or reorganize its position [21, 22, 66,

83, 115, 128]. Activin also can promote the differentiation of extraembryonic *area opaca* cells into smooth and striated muscle and notochord [115], and epiblast cells to undergo cardiac myogenesis *in vitro* [Ladd AN, Yatskievych TA, Antin PB: Regulation of avian heart muscle cell specification: The role of activin. Manuscript submitted]. Type-II receptors for activin are expressed in the primitive streak [115]. FGF has been implicated in the induction of nonaxial mesoderm such as blood cells, and together with other factors, in later stages of mesoderm development [82]. Both FGF and activin can induce mesoderm formation in the *Xenopus laevis* embryo [62, 108].

Activin and FGF were tested for an effect on epiblast myogenesis by adding them either alone or in combination, in various doses, for different amounts of time (1-3 days), to cultures containing intact epiblast tissue from stage 1 embryos (Eyal-Giladi and Kochav's stages X-XII) or cells initially plated as a single cell suspension. In no case was an increase in the number of skeletal muscle cells observed in these cultures (Table 2). Addition of activin and FGF also were without effect on myogenesis in cultures of

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Table 2. Effects of activin and fibroblast growth factor (FGF) on muscle differentiation and proliferation in epiblast cultures. Activin and/or FGF were added to stage 1 or 4 epiblast cells one hour after plating. The number of cells present in stage 1 cultures was determined 24 hours later. Stage 1 cells were fixed after 72 hours and stage 4 cells after 24 hours and then labeled with the MF20 monoclonal antibody to myosin heavy chain [4]. Values are the mean standard deviation from 4 cultures except that 7 untreated cultures were analyzed for the percent myosin positive cells. A minimum of 200 cells were scored per culture. Activin and/or FGF did not affect muscle differentiation in stage 1 or 4 epiblast cultures. FGF stimulated stage 1 epiblast cell proliferation ($p < .0005$).

Stage 1 Epiblast Cells		% Myosin Positive: 72 h	Cell Number: 24 h
Untreated		0	17000 1500
Activin:	0.1 ng/ml	0	
	1.0 ng/ml	0	16500 1200
	10 ng/ml	0	
	100 ng/ml	0	
FGF	0.1 ng/ml	0	26700 2600
	1.0 ng/ml	0	
	10.0 ng/ml	0	
Activin: 1 ng/ml and FGF: 0.5 ng/ml		0	
Activin: 10 ng/ml and FGF: 0.1 ng/ml		0	
Stage 4 Epiblast Cells		% Myosin Positive: 24 h	
Untreated		20	2
Activin: 1.0 ng/ml		20	1
FGF: 0.1 ng/ml		17	6

stages 3-4 epiblast cells (Table 2). Cultured epiblast cells do respond to these molecules as FGF stimulated their proliferation (Table 2) and activin induced the organization of cells into structures with different morphologies (not shown).

One treatment that was effective in promoting myogenesis of stage 1 cells was co-culturing them with stage 4 epiblast cells (Table 3). The percentage of differentiated stage 1 cells was greatest when they were combined in a 1:1 or 1:2.5 ratio with stage 4 cells and declined when the number of either stage 1 or 4 cells was increased. Most differentiated stage 1 cells were located in close proximity to stage 4 cells although a few myosin positive stage 1 cells (approximately 10% of the myosin positive population) were completely surrounded by myosin negative stage 1 cells. It is possible that the small numbers of stage 1 cells that differentiate presumably without direct contact with stage 4 cells was the same population that forms muscle in the absence of the older epiblast cells. Thus, close range cell-cell communication may be required for the transfer of information from stage 4 to stage 1 cells. This interpretation is consistent with the results of Salvatori et al. [102] who demonstrated that myoblasts from older tissues stimulated fibroblasts to undergo myogenesis and that close cell-cell interactions were required for this effect. Interac-

tions between the stage 1 cells themselves also appear to be important for their differentiation since an increase in the percentage of stage 4 cells resulted in a decrease in stage 1 myogenesis.

Heterogeneity of Epiblast Cells

Although most epiblast cells from primitive streak stage embryos differentiate into skeletal muscle *in vitro*, small populations of neurons and cells synthesizing type II collagen were present in these cultures [37]. Small numbers of nonmuscle cell types also emerged in cultures of stage 1 prestreak epiblasts (Fig. 4). The type II collagen positive cells may be chondroblasts, however, other cell types synthesize this molecule during early stages of development [73]. Previous studies demonstrated that cells from stages 1-3 embryos also can give rise to hematopoietic cells and smooth and cardiac muscle when cultured in the presence of serum or activin [14, 53, 81]. In addition, a subpopulation of cells destined to form the mesoderm and endoderm can be distinguished from those that remain in the ectoderm by their expression of the cell surface glycoprotein HNK-1 [11, 112]. These studies indicate that the epiblast is a heterogeneous tissue.

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Table 3. Effect of co-culturing stages 1 and 4 epiblast cells on myogenesis. Stage 1 epiblast cells were prelabeled with fluorescein isothiocyanate [37] then mixed with unlabeled stage 4 epiblast cells in the indicated ratios. A total of 2×10^4 cells were plated at high density as described in Table 1. Cells were stained with the MF20 monoclonal antibody to myosin heavy chain [4] and a secondary antibody conjugated with rhodamine. % Myosin positive stage 1 epiblast cells = rhodamine and fluorescein positive cells $\div$ total fluorescein positive cells $\times 100$. Results are from 2 cultures or the mean $\pm$ standard deviation of the number of cultures indicated in parenthesis. A minimum of 200 cells were scored per culture. Stage 4 cells promoted the differentiation of stage 1 cells.

	% Myosin Positive: 72 hr	
	Stage 1 Cells	Stage 4 Cells
Stage 4 cells alone		35 $\pm$ 7 (4)
Stage 1 cells alone	0 (7)	
Ratio of stages 1 to 4 cells:		
10:1	3 $\pm$ 1 (4)	29 $\pm$ 13 (4)
5:1	3 $\pm$ 2	36 $\pm$ 32
2.5:1	3 $\pm$ 2 (4)	25 $\pm$ 6 (4)
1:1	10 $\pm$ 2 (4)	32 $\pm$ 4 (4)
1:2.5	9 $\pm$ 4 (4)	30 $\pm$ 7 (4)
1:5	7 $\pm$ 3 (4)	31 $\pm$ 7 (4)
1:10	2 $\pm$ 2 (4)	44 $\pm$ 15 (4)

Regulation of Myogenesis in the Early Chick Embryo

Cells with the potential to differentiate into skeletal muscle are present in the chick embryo approximately 20 hours after fertilization (Hamburger and Hamilton's stage 1; Eyal-Giladi and Kochav's stage X), and possibly earlier. The fact that nonmuscle cell types also emerge in culture, even under conditions that clearly favor skeletal myogenesis [37], suggests that the epiblast contains small numbers of cells stably programmed to follow different pathways of development. Holtzer et al. [53] proposed that these cells were founder cells for different lineages. In invertebrate embryos, founder cells have been defined as those that are autonomously specified to follow a particular pathway of development and are responsible for establishing tissues by a direct transfer of information to neighboring cells [40, 44, 57, 96, 107, 116].

The majority of cells in cultures of stage 1 epiblasts grown in serum free medium do not differentiate into muscle, neurons, cells synthesizing type II collagen, or cells with the morphological appearance of hematopoietic cells [37, present study]. Although the use of other antibodies or nucleic acid probes may reveal additional differentiated cell types, it also is possible that most stages 1 and 2 epiblast cells are incapable of differentiating under these culture conditions. However, a dramatic change occurs in

the epiblast over the next 6 hours that results in practically all cells acquiring the potential to undergo skeletal myogenesis [37]. This must be a transient state *in vivo* since only a portion of the epiblast eventually differentiates into skeletal muscle within the embryo [33, 100, 112].

The mechanism that promotes the increase in the number of myogenic cells within the epiblast between stages 2 and 3 is unknown. Preliminary studies suggest a requirement for tissue interactions. It is likely that a molecule(s) secreted by the hypoblast, posterior marginal zone, and/or developing primitive streak brings about this change in the epiblast. Experiments that demonstrated the ability of stage 4 epiblast cells to promote the differentiation of stage 1 cells suggests that the stage 1 cells are competent to respond to a factor(s) that either directly or indirectly stimulates myogenesis. Whether the factor that is produced by the stage 4 cells is the same as that which causes the increase in myogenic potential within the epiblast *in vivo* between stages 2 and 3 remains to be determined. A question also remains as to whether the increase in myogenic potential within the epiblast results from a change in the ability of muscle founder cells to recruit their neighbours or in the responsiveness of the originally nonmyogenic cells.

Although activin and FGF are present in the early embryo [82, 83], they do not appear to be solely responsible for the transition that occurs within the embryonic epiblast since they do not promote muscle differentiation in cultures of stage 1 epiblast cells. In contrast, activin can induce myogenesis in cultures of the extraembryonic *area opaca* tissue (Fig. 1) [115]. These conflicting results may reflect differences in the source of the cells (epiblast from the *area pellucida* versus *area opaca*) and/or different culture conditions. Since the *area opaca* was cultured in medium containing fetal calf serum it is possible that activin promotes myogenesis in combination with other factors.

While myogenic potential is wide-spread within the primitive streak stage 4 epiblast, differentiation is prevented by cell-cell and tissue interactions [37]. It is likely that these inhibitory mechanisms allow some epiblast cells to proliferate and enter the mesoderm before differentiating into skeletal muscle, and other cells to become programmed to follow nonmuscle pathways of development. Inhibition of differentiation persists in the segmental plate mesoderm and newly formed somites. Again, tissue interactions are partially responsible for this effect. For example, the lateral plate mesoderm tissue appears to maintain those somite cells that migrate into the limb in the precursor state [94].

Inhibition of differentiation also may be regulated by direct cell-cell interactions mediated by the cell surface protein Notch. Notch is present in the segmental plate mesoderm of the mouse [34, 70, 71, 97] and chicken embryos [86]. Forced expression of Notch inhibits myogenesis in the *Xenopus* embryo and in cultured mouse cells by interfering with the activity of MyoD [87], possibly by associating with the suppressor of hairless protein and

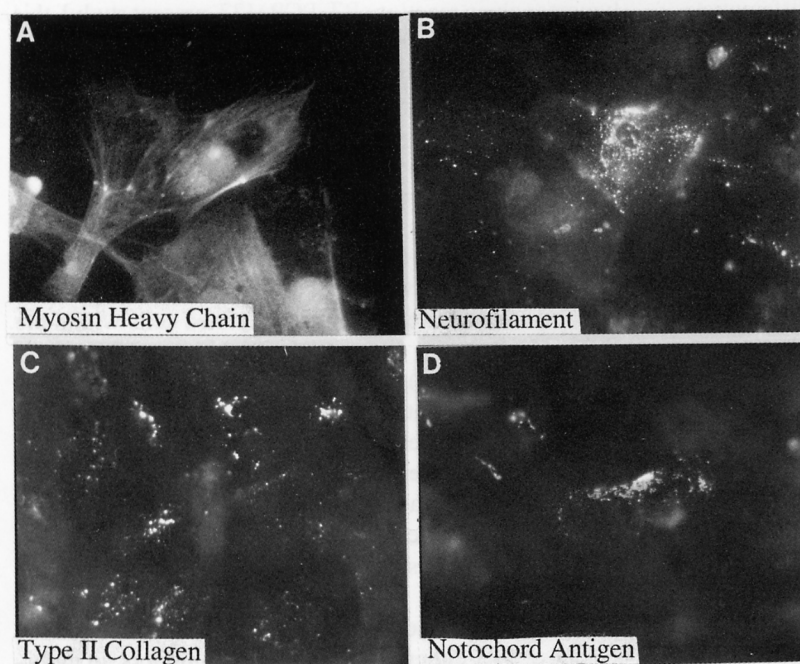


Figure 4. Expression of cell type specific proteins in stage I epiblast cells in vitro. Stage I epiblast cells (Eyal-Giladi and Kochav's stages X-XII) were cultured and labeled by the immunofluorescence localization method as described in George-Weinstein et al. [37]. After 5 days in culture, a few epiblast cells were labeled by the following monoclonal antibodies: MF20 to myosin heavy chain [4], 2H3 to neurofilament protein, CIIC1 to type II collagen [51], and NOT-1 to notochord [127], all obtained from the Developmental Studies Hybridoma Bank.

inducing the expression of the basic helix-loop-helix protein Enhancer of split [59, 61, 104].

A third potential mechanism for inhibiting muscle differentiation in these tissues is the accumulation of the basic helix-loop-helix protein Id. Id is present in the segmental plate of the mouse embryo [124] and prevents the binding of muscle regulatory factors and E-proteins to each other and to DNA [6, 60]. In addition, another basic helix-loop-helix protein Twist inhibits myogenesis by blocking the binding of MyoD to DNA and by affecting cooperative interactions between MyoD, MEF2, and E proteins [110]. Thus, multiple mechanisms may be operating to prevent premature differentiation within the embryo.

Differentiation promoting molecules produced by the neural tube and notochord act, at least in part, to override inhibitory signals in the somite. One intriguing possibility is that the Wnt secreted by the neural tube acts to interfere with the Notch signaling pathway. In cells of the *Drosophila* embryo, the Wnt homologue wingless appears to promote the association of the intracellular molecule Dishevelled with Notch resulting in a suppression in the transmission of the inhibitory Notch signal [3].

An additional effect of Wnt/wingless signalling is the accumulation of the cytoplasmic proteins armadillo/ β -catenin [50, 67, 91]. Armadillo is a *Drosophila* segment polarity gene [92] and its vertebrate homologue is β -catenin [77]. Each protein simultaneously binds transmembrane cadherins and cytoplasmic α -catenin. α -Catenin also interacts with actin [98] and α -actinin [68] thereby forming the linkage between the cadherin/catenin complex and the cytoskeleton that is essential for strong cell-cell adhesion. Wnt stabilizes cadherin-mediated cell-cell adhesion, most likely a result of its effect on β -catenin [8, 50]. Since cadherin-mediated adhesion promotes muscle differentia-

tion [52; manuscript in preparation], Wnt may promote differentiation by enhancing the adhesion between cells in the somites that express N-cadherin. The increased cell-cell adhesion that promotes compaction and epithelialization of the newly formed somite may be an obligatory step in skeletal muscle differentiation.

In addition to binding to cadherins at the plasma membrane, β -catenin and Armadillo associate with other proteins such as the tumor suppressor gene product APC [101, 118], and are found throughout the cytoplasm and sometimes in the nucleus. This pool of β -catenin appears to participate in regulating cell proliferation [58] and gene expression [35, 42, 46, 49, 90]. It has been suggested that β -catenin might directly or indirectly regulate MyoD expression in the *Xenopus* embryo [52]. In the chick embryo, β -catenin is present in epiblast cells and in the somite [George-Weinstein M, Gerhart J, Blitz J, Simak E, Knudsen K: N-Cadherin promotes the commitment and differentiation of skeletal muscle precursor cells. Manuscript submitted]. Thus the Wnt signaling pathway may promote myogenesis by interfering with the Notch pathway, promoting N-cadherin mediated cell-cell adhesion, and by altering transcription within the nucleus.

Once the cells of the somite receive signals from the neural tube and notochord and the influence of inhibitors such as Notch is neutralized, MyoD and other muscle regulatory factors could upregulate their own transcription. This would coincide with the detection of mRNAs for muscle regulatory factors in the somite by *in situ* hybridization [89, 95, 105, 106]. Downregulation of Id could result in heterodimer formation between MyoD and E-proteins, and along with MEF2, initiate the transcription of contractile protein genes [6, 60, 64, 88].

The assumption made in this model for the regulation of muscle differentiation in the somite is that this tissue

contains cells that are stably programmed to differentiate into muscle or cartilage. This premise can be reconciled with results that demonstrated the ability of somite cells to switch from a myogenic to chondrogenic fate after changing their environment [7, 9, 38, 63, 93, 94, 113, 125] by proposing that the somite contains both committed and uncommitted cells. Those cells that are stably committed to the myogenic or chondrogenic lineage appear to be present throughout the somite and may regulate the pathway of differentiation of their uncommitted neighbours. Evidence for the presence of stably committed cells throughout the somite comes from transplantation studies in which both the normally myogenic dorsal region of the somite and the chondrogenic ventral somite formed cartilage and muscle when implanted into the limb bud [122].

When the position of the newly formed somite is altered relative to its surrounding tissues, there is an apparent switch from a myogenic to chondrogenic fate and *vice versa* [7, 9, 38, 63, 93, 94, 113, 125]. This may reflect a reversal in the subpopulation of stably committed cells that is activated to recruit surrounding cells. Thus, committed muscle precursor cells located in close proximity to the myogenesis-promoting dorsal neural tube would influence their uncommitted neighbours to differentiate into muscle. In contrast, muscle precursors lying closer to the chondrogenesis-promoting notochord would remain inhibited while the chondrogenic precursors recruited their neighbours.

The population of uncommitted cells in the segmental plate mesoderm and newly formed somite is uncharacterized. Cell culture [36] and transplantation studies [122] suggest that these cells have a propensity for differentiating into skeletal muscle. Furthermore, the paired box genes Pax-3 and -7 initially are expressed throughout the segmental plate mesoderm and later become restricted to myogenic regions of the somite [7, 38, 39, 126]. The recruitment of presumably uncommitted cells to the chondrogenic lineage correlates with the downregulation of Pax-3 and -7 and upregulation of Pax-1 and -9 [9, 26-28, 31, 32, 39, 63, 72, 76, 109, 123, 126] and the basic helix-loop-helix protein scleraxis [24]. These results may be interpreted to mean that somite myogenesis is triggered by permissive cell and tissue interactions while the spread of chondrogenic potential to uncommitted cells depends more on instructional interactions. On the other hand, the apparent dominance of the myogenic pathway may reflect a more potent influence of the stably committed myogenic cells over the uncommitted population than from the committed chondrogenic cells. It also is possible that the uncommitted population expresses genes for both lineages, and therefore, are equally poised to follow either pathway of differentiation.

Testing various aspects of this model for the regulation of differentiation in the somite will be aided by the application of more sensitive methods of *in situ* analysis. Although MyoD mRNA is detectable in the epiblast and segmental plate mesoderm using reverse transcriptase-po-

lymerase chain reaction (RT-PCR) [37, present study], this technique does not provide information on the number and location of cells expressing MyoD. *In situ* RT-PCR [69] or conventional *in situ* hybridization coupled with a more sensitive detection system such as labeled DNA dendrimers (Polyprobe, Phila., PA) may reveal whether the uncommitted cells express MyoD and/or other positive and negative regulators of myogenesis or chondrogenesis. These techniques, along with identification of earlier markers for myogenic and chondrogenic precursors may reveal the location of stably programmed cells within the embryo.

The mechanism(s) whereby committed cells might promote the differentiation of their uncommitted neighbours is speculative; however, adhesion of cells via N-cadherin appears to play a role in the transfer of information between cells. Cell-cell interactions via N-cadherin, as well as those mediated by soluble factors secreted by surrounding tissues, ultimately must be linked with molecules that regulate transcription within the nucleus. Defining the intermediaries in these pathways may identify molecules that are active, either as stimulators or inhibitors, in multiple signaling pathways. The interplay between extrinsic and intrinsic factors, and between parallel signalling pathways, provides checks and balances on the rate of cell differentiation within the developing embryo.

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