proliferative or antiviral activity, IFN is apparently not the direct effector molecule responsible for inhibiting proliferation in the recipient cells. The evidence is as follows: (i) antiproliferative activity can be effectively transferred between xenogeneic cells that do not respond to heterologous IFN, (ii) transfer will occur if IFN is removed before the cells are cocultured, and (iii) supernatants from IFN-induced cells do not induce antiproliferative activity in recipient cells. Thus the transfer appears to be mediated by a contact-dependent mechanism and the results support the hypothesis that antiviral activity is transferred by means of a secondary messenger, possibly through low-resistance membrane channels such as gap junctions (*7*).

Our results suggest a novel mechanism by which IFN may indirectly regulate cell growth. These cellular interactions could be an important host defense mechanism against neoplasias in tissues, especially where IFN diffusion is poor. Gresser *et al.* reported that a strain of L1210 cells cloned for IFN resistance was still responsive to IFN antitumor therapy in mice (*17*) and attributed these results to the effect of IFN on the host's immune response. The present findings, together with data indicating the probable transfer of antiviral activity in vivo (*18*), suggest that the antitumor effects on IFN-resistant cells may have been due, in part, to the transfer of antiproliferative activity from responding normal mouse cells. Similarly, the human IFN–induced inhibition of human tumor cells in nude mice, which was attributed to a direct effect of IFN only on the human tumor cells, could also have been due to the transfer of activity from mouse effector leukocytes (*19*). Such a defense mechanism suggests that cells other than those of the immune system may play a role in controlling tumor growth where cell-to-cell contact occurs. This mechanism also provides a new system in which basic cell-to-cell communication may be investigated.

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Human Fetal Muscle and Cultured Myotubes Derived from It Contain a Fetal-Specific Myosin Light Chain

Abstract. *Human fetal muscles at ages 110, 125, and 132 days contain a fetal-specific myosin light chain. This light chain is absent in adult human muscle, copurifies with myosin, and is identified as a slow light chain because it reacts with purified antibody to chicken slow muscle light chains and does not react strongly with antibody to fast myosin light chains. This light chain is synthesized in cultures of fetal muscle along with normal myosin light chains. The presence of a fetal light chain in culture provides a marker for studies of human muscle disease in which it is important to know when or if the muscle makes a transition from embryonic or fetal expression to true adult phenotype.*

One-dimensional sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis of myosin from human muscle has shown conflicting results concerning the number and distribution of light chains (*1–3*) probably because most human muscles contain mixtures of slow and fast fibers (*4*). Two-dimensional polyacrylamide gel electrophoresis of adult human myosin (*2, 5*) has shown the presence of a slow light chain (SLC_1) with an isoelectric point (PI) and molecular weight closer to that of fast light chain (FLC_1) than in other mammalian species (*6*), although the exact relationship varies possibly because of slightly different analytical conditions. Fetal-specific light chains have been demonstrated in rat skeletal and cardiac muscle (*7, 8*), bovine cardiac muscle (*9*), and in human ventricle (*10*). Volpe *et al.* (*2*) were unable to demonstrate the presence of slow light chains in fetal or neonatal human skeletal muscle and attributed this to the predominance of fast type 2 fibers in these muscles. We now show that a fetal-specific slow light chain (fetal LC_1) is present in human fetal muscle and that it is synthesized in cultures of fetal muscle cells.

Since most human adult muscles contain a mixture of slow and fast fibers (*4*)

Table 1. Synthesis of myosin light chains in cultures of human fetal cells

Light chain fraction	Percent of total light chains*	
	Experiment 1	Experiment 2
Fetal LC_1	27.7	30.9
Slow LC_1	0.9	0.1
Fast LC_1	10.7	13.7
Slow LC_2	5.4	1.0
Fast LC_2	41.1	37.4
Fast LC_3	14.9	16.9

*After electrophoresis and fluorography of [^{35}S]-methionine-labeled myosin from 4- to 5-day-old cultures, stained light chains were cut from the dried gel and extracted with NCS and radioactivity was determined in Omnifluor-toluene scintillation fluid. Corrections for background were made.

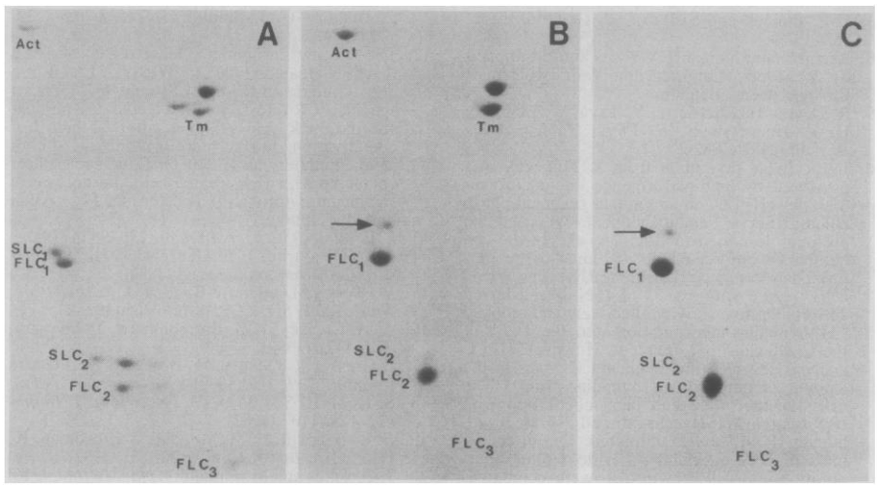

Fig. 1. Two-dimensional polyacrylamide gel electrophoresis of human myosin from adult and fetal tissues (*24*). Muscle was minced, homogenized, and washed in low salt buffer (20 m*M* potassium chloride, 2 m*M* potassium phosphate, and 2 m*M* EGTA, *p*H 6.8). Myosin was extracted with high salt buffer (40 m*M* sodium pyrophosphate, 2 m*M* EGTA, and 1 m*M* magnesium chloride, *p*H 9.5) for 1 hour, dialyzed against low salt buffer and then suspended in high salt buffer containing 50 percent glycerol. All procedures were carried out on ice, and myosin was stored at −20°C. Isoelectric focusing gels (*25*) contained a mixture of Ampholines (LKB, Gaithersburg, Maryland) (1.8 percent, *p*H 5 to 8; 0.2 percent, *p*H 3.5 to 10) and exposed for 16 hours to 400 V and for 2 hours to 800 V. The second-dimension gels were 12.5 percent SDS polyacrylamide. Only the acidic part of the gel stained with Coomassie blue containing contractile proteins is shown. (A) Adult human rectus abdominus muscle shows both slow and fast myosin light chain isozymes, SLC_1, FLC_1, SLC_2, FLC_2, and FLC_3. Actin (*Act*) and tropomyosin (*Tm*) are present; 30 to 40 μg of protein was placed on the gel. (B) Fetal human leg muscle (125 days old) shows predominantly FLC_1 and FLC_2 with a small amount of FLC_3 and SLC_2. The position of a fetal specific light chain is indicated (→); 50 μg of protein was placed on the gel. (C) Fetal myosin was further purified by DE-52 chromatography (*26*) to show that the fetal-specific light chain copurifies with other myosin light chains while actin and tropomyosin are removed; 50 to 60 μg of protein was placed on the gel.

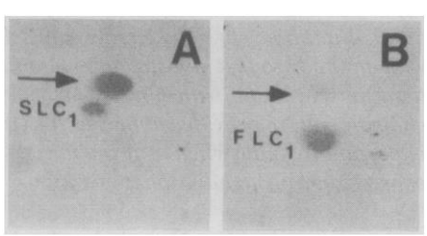

Fig. 2. Reaction of the light chains from fetal muscle with antibodies to SLC_1 and FLC_1 from the chicken. Myosin was prepared from adult chicken pectoralis and anterior latissimus dorsi (*ALD*) muscles (see legend to Fig. 1). Heavy and light chains were separated in urea (*27*) and dialyzed against low salt buffer to selectively precipitate the heavy chains. Light chains in the supernatant were separated on SDS polyacrylamide (12.5 percent) gels (*28*). The SLC_1 and FLC_1 bands were cut out, lyophilized, and ground in a mortar and pestle. The ground powder was mixed with Freund's adjuvant for immunization of rabbits. The ammonium sulfate precipitated immunoglobulin G's from immune serums were passed through Sepharose 4B columns bound with myosin from adult pectoralis (for the antibody to SLC_1) or ALD (for the antibody to FLC_1) (*29*) to remove cross-reacting antibodies. Fetal light chains, separated by two-dimensional polyacrylamide gel electrophoresis as in Fig. 1 were transferred to nitrocellulose (*30*) and reacted with antibodies. Horseradish peroxidase-conjugated swine antiserum to rabbit immunoglobulin G was used to stain bound antibodies (*31*). Only the LC_1 region is shown. The fetal-specific LC_1 is indicated by an arrow (→). (A) Reaction with antibody to SLC_1. (B) Reaction with antibody to FLC_1.

both slow and fast light chains are present (Fig. 1A). This pattern is similar to that shown by Volpe *et al.* (*2*) and exists with slight variations in the ratios of slow to fast isozymes in various adult human muscles. Multiple forms of SLC_2 and FLC_2 with the same molecular weights but different isoelectric points may be due to partial phosphorylation as reported in other species (*11*). A different pattern of light chains is obtained from myosin extracted from human fetal muscle (Fig. 1B). Very little SLC_1 can be seen, but another spot, here termed fetal LC_1, is present in myosin extracted from fetal samples taken at 110, 125, and 132 days) and copurifies with myosin (Fig. 1C). This spot was identified as a myosin slow light chain (Fig. 2). Fetal muscle myosin light chains, transferred from a polyacrylamide gel to nitrocellulose, were reacted with antibodies to chicken SLC_1 and FLC_1. The fetal LC_1 stains strongly with antibody to SLC_1 (Fig. 2A) and only slightly with antibody to FLC_1 (Fig. 2B). In addition, the presence of adult type SLC_1, not apparent in the original Coomassie-stained gel, can be shown with this more sensitive technique (Fig. 2A). The fetal light chain is missing in adult human rectus abdominus (Fig. 1A) and is also absent in adult human vastus lateralis, latissimus dorsi, and pectoralis minor (*2*).

Muscle cells from fetal tissue were cultured and labeled with [^{35}S]methionine before myosin extraction (Fig. 3A and Table 1). A large amount of fetal LC_1 (27 to 30 percent) was synthesized while only 10 to 14 percent of the labeled total light chain was in FLC_1 and very little adult-type SLC_1 (0.1 to 0.9 percent) was synthesized. These cells were obtained from younger fetuses (62 days) and were subcultured three times before labeling; one or both of these factors may contribute to the enhanced synthesis of an earlier stage light chain pattern than that from older fetuses (110 to 132 days) as shown by staining of myosin. The absence of a

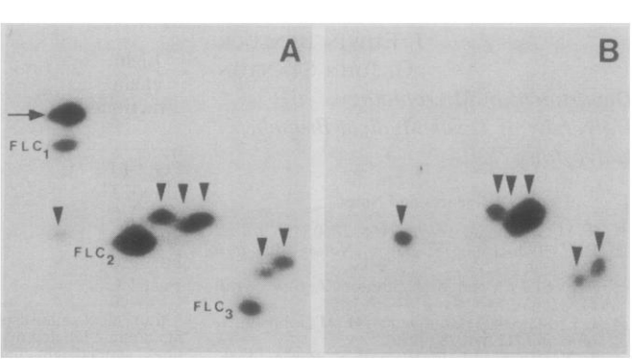

Fig. 3. Fluorographs (*32*) of [^{35}S]methionine-labeled myosin prepared from cultures and separated by two-dimensional polyacrylamide gel electrophoresis. (A) Third passage cells from 62-day-old fetal human leg muscle were cultured in minimum essential medium containing 10 percent horse serum and 2 percent chick embryo extract. Cells were labeled with [^{35}S]methionine (50 μCi/ml) for 2 hours on day 4. Myosin was extracted (described in Fig. 1), and the extract (200,000 count/min) was loaded on the gel. Light chains were identified by comigration with stained adult and fetal markers in the same gel. Fetal LC_1 is indicated by an arrow (→). (B) Cells were subcultured several more times by trypsinization and replating. Cultures were incubated and refed for long periods to reduce the muscle cell population relative to fibroblasts. When no further fusion was visually detected one more passage was performed and 60-mm plates were prepared. At 24 hours four plates of cells were labeled with [^{35}S]methionine (100 μCi/ml) for 2 hours. Cells were scraped into low salt buffer and myosin was extracted in the presence of added unlabeled fetal myosin. The extract (400,000 count/min) was then loaded onto the gel. The positions of fibroblast-specific proteins are marked (▼).

spot comigrating with the fetal LC_1 in labeled human fibroblast cultures (Fig. 3B) shows that it is not synthesized by fibroblasts.

Finally, a significant amount of FLC_3 is synthesized in cultures of fetal human tissue (Fig. 3A and Table 1). Of the total light chains 15 to 17 percent can be attributed to FLC_3 synthesis. In contrast, Whalen *et al.* (*9*) show very little FLC_3 in cultures of rat thigh muscle and L6 cells and Keller and Emerson (*12*) show from 1 to 4 percent in primary cultures of chicken and quail pectoralis muscle and quail clonal cultures. Blau and Webster (*13*) report only four light chains in clonal cultures of adult human muscle but do not clearly identify them, and it is unclear whether FLC_3 or a fetal light chain is present. Synthesis and accumulation of FLC_3 are normally associated with the early adult stage in both avian and mammalian muscle (*14–18*). Its relatively high concentration in our human cultures suggests that these culture conditions for human muscle will support, at least, a partial expression of adult myosin phenotype. Complete adult expression in cultured fibers originating from myogenic cells of normal and diseased muscle has not yet been obtained.

The presence of a fetal-specific myosin light chain in human muscle provides a marker with which to detect shifts from fetal to adult myosin gene expression. This is especially important for work in cell culture where it is highly desirable to define conditions that will allow the expression of adult gene patterns as opposed to embryonic or fetal patterns. In the search for a gene product associated with inherited, X-linked, Duchenne muscular dystrophy, for example, human muscle culture could be extremely useful. Usefulness is now limited because cultured muscle expresses a phenotype that is predominantly fetal (*19*). Where there are muscle-specific isoform shifts involving an embryonic (or fetal) to adult or a neonatal to adult transition in vivo, these shifts do not occur in vitro (*20*). For example, even when cultured chicken muscle fibers are maintained for 30 to 50 days, they express an embryonic pattern of myosin heavy and light chains and apparently completely repress the activation of the adult configuration (*16*). This is also true for patterns of tropomyosin and myosin expression in cultured muscle fibers derived from satellite cells of adult chicken muscle (*21*). Cultured rat muscle fibers synthesize a fetal light chain and fetal myosin heavy chains and, like cultured chicken muscle, apparently do not make the transition to expression of adult isoforms of myosin and perhaps other muscle-specific proteins (*22*). If genes responsible for diseases like Duchenne dystrophy are associated with the modification of adult gene expression as suggested by Fitzsimmons and Hoh (*23*), then cell culture studies should be valuable provided that we can devise conditions supportive of a full range of gene expression. The presence of a fetal-specific myosin light chain in human muscle and its synthesis in human muscle cultures provides a reliable marker for determining whether variations in culture conditions can induce shifts from fetal to adult modes of muscle-specific protein synthesis.

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Identification of Pro-Opiomelanocortin–Derived Peptides in the Human Adrenal Medulla

Abstract. *Extracts from adult human adrenals contained high concentrations of immunoreactive β-endorphin and α-melanotropin. Lower quantities of immunoreactive adrenocorticotropic hormone could also be detected. Distribution studies showed the presence of pro-opiomelanocortin fragments in the adrenal medulla. No α-melanotropin, β-endorphin, or adrenocorticotropic hormone could be found in adrenal extracts from several other mammalian species. Analysis of the β-endorphin-like immunoreactivity using region specific radioimmunoassays interfacing with gel filtration and reverse-phase high-performance liquid chromatography showed the majority of the β-endorphin-like material to exist as nonacetylated β-endorphin-(1–31) with a small percentage of lipotropin-sized molecules. The α-melanotropin-like immunoreactivity cochromatographed on gel filtration and reverse-phase high-performance liquid chromatography with desacetyl α-melanotropin. The data suggest that pro-opiomelanocortin is expressed in the adrenal medulla of humans but is not detectable in the adrenal glands of many other mammalian species.*

Since the characterization of Met- and Leu-enkephalin by Hughes *et al.* (*1*) in 1975, numerous other endogenous opiate active peptides have been detected (*2*). All endogenous opioid peptides thus far isolated from mammalian tissue are derived from three separate precursors, namely, pro-opiomelanocortin (*3*), pro-enkephalin (*4, 5*), and pro-neoendorphin/dynorphin (*6*). One of the richest sources