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## A human skeletal muscle cell line obtained from an adult donor

Raul Caviedes<sup>a</sup>, Jose L. Liberona<sup>a</sup>, Jorge Hidalgo<sup>a</sup>, Soledad Tascon<sup>a</sup>, Karime Salas<sup>a</sup>  
and Enrique Jaimovich<sup>a,b</sup>

<sup>a</sup> Departamento de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile, Santiago (Chile)  
and <sup>b</sup> Centro de Estudios Científicos de Santiago, Santiago (Chile)

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A cell line (RCMH) in permanent culture was established from surgically removed adult normal human skeletal muscle by exposure to conditioned media obtained from thyroid cells. Cells proliferated indefinitely but displayed density inhibition of growth while maintaining some differentiated markers. Under certain incubation conditions, cells fused into myotube-like structures, with a concomitant increase in muscle specific proteins, such as human myoglobin, skeletal muscle myosin, desmin and dystrophin, as identified using immunocytochemical procedures. In addition, RCMH cells displayed high affinity receptors for  $\alpha$ -bungarotoxin ( $B_{\max} = 0.7$  pmol/mg protein,  $K_d = 1.5$  nM) and dihydropyridines ( $B_{\max} = 0.3$  pmol/mg protein,  $K_d = 0.5$  nM for [<sup>3</sup>H]PN200-110); these values are comparable to those reported for muscle cells in primary culture. Patch-clamp studies showed the presence of 42 pS carbachol gated channels and of 5 pS calcium channels (current carried by barium); chloride and potassium channels were also seen. This new cell line appears to be a convenient model system to study skeletal muscle function.

### Introduction

Cell cultures are a useful tool for understanding the physiology and pharmacology of biological systems. Human skeletal muscle cells in culture are likely to be a good model for the study of both basic muscle physiology and human muscle disorders, particularly those of genetic origin [1-6].

A major obstacle to the use of muscle cell cultures is their heterogeneity; they contain a variety of cells, namely fibroblasts, endothelial cells, myoblasts and myotubes in several stages of differentiation (for review see Ref. 1). This problem can be partially circumvented by preparing clonal cultures, where cells can be assessed for their ability to fuse and form myotubes; cultures of 'pure' myogenic and non myogenic cells have been obtained in this way [1]. Clonal cultures have been used extensively in studies of human fetal muscle [7] but only recently experiments have been extended to postnatal human muscle [1].

One of the problems of studying human muscle in vitro arises from the difficulty of having a regular tissue supply. The relatively short life of human cells in

culture imposes a limit on the time in which a particular cell strain can be studied, and in the quantities of cells that can be grown. A solution to these problems would be to transform human muscle cells to obtain 'immortalized' cells, as done by Yaffe [8] with the L6 cell line, produced by transformation of rat myoblasts with methyl cholanthracene. Miranda et al. [9] have used SV-40 to produce two lines of transformed human myoblasts. These cells have some characteristics of SV-40 transformed cells; however, one consequence of transformation is that the myoblasts lose the ability to differentiate, as judged by a total lack of fusion and the presence of only the B-isoenzyme form of creatine kinase.

We have produced a stable cell line in permanent culture, starting from adult human skeletal muscle. We extended a previously used procedure for cell transformation and the transformed cells so obtained were capable of fusion and exhibited several markers of differentiated muscle. Preliminary data on this cell line have been presented elsewhere [10].

### Materials and Methods

#### Conditioned media to immortalize muscle cells

Cell free human thyroid conditioned media (HTCM) were prepared by harvesting the complete media used

Correspondence: E. Jaimovich, Centro de Estudios Científicos de Santiago, Casilla 16443, Santiago 9, Chile.

to grow normal human thyroid monolayer cultures. These thyroid primary cultures were obtained from normal-looking tissue removed after lobectomy from a thyroid gland bearing a benign adenoma. These tissue samples were routinely obtained from the Clinical Hospital of the Universidad de Chile, with previous patient consent. Histopathology studies of the tissue used were also routinely performed. Cells were cultured in growth medium (GM) composed of equal parts of Ham F12 nutrient mixture, Dulbecco's modified Eagle's medium, 10% bovine serum, 2.5% fetal bovine serum, 15 mM HEPES (pH 7.2), plus penicillin (100 mg/l) and streptomycin (50 mg/l). These epithelial-like thyroid cultures proliferated until the 30th passage in 1:2 duplications, to later decline and die. HTCMT was collected during the first ten passages, frozen at  $-20^{\circ}\text{C}$ , thawed and filtered through 0.2 micron Millipore membranes.

#### *Development of a new skeletal muscle cell line*

Normal muscle (a 2 by 0.5 cm piece of quadriceps) was obtained from trauma repairing surgery carried out at the Clinical Hospital of the Universidad de Chile, with previous patient consent. Finely, chopped muscle tissue was digested with collagenase (Sigma, St. Louis, MO, USA, Type V 0.2 g%) in  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  free Hanks' balanced salt solution, under sterile conditions. Washed pellets were suspended in GM 10% HTCMT (see above) and incubated in glass dishes with no matrix added, in 10%  $\text{CO}_2$ -air atmosphere, 100% humidity at  $36^{\circ}\text{C}$ .

After the first passage, proliferating cells were enriched in myocytes using the 'selective serial passage' method described by Yaffe [8]. Two successive subcultures were used; each one was followed by the corresponding 24 h preplating period, to select the slowest attaching cells, i.e., myoblasts. 10% of HTCMT added to GM was used until sustained cell growth was attained or multi transformed cell foci were seen. Established cultures went through several passages (20 to 30 subcultures) and several clones were obtained. At this stage of culture, HTCMT was removed from the cultures without affecting significantly cell growth parameters; control cultures did not proliferate in the absence of HTCMT.

Cell fusion was induced using the method described by Olson [11]; cultures were transferred to GM with 1% bovine serum and 10  $\mu\text{g}/\text{ml}$  of both insulin and transferrin.

All cultures were mycoplasma free as judged by standard bacteriological procedures.

#### *Immunocytochemical procedures*

Cultures grown on glass coverslips were either frozen in liquid nitrogen or fixed for 15 min at  $-20^{\circ}\text{C}$ , first with methanol and then with acetone. After rinsing in PBS, a drop of the diluted primary antibody was placed

over the monolayer and biotinylated or unconjugated linking antibodies were added. Peroxidase anti-peroxidase (PAP) or streptavidin were used to visualize the antigen-antibody reaction. The expression of muscle proteins, such as myosin, myoglobin or dystrophin, was analyzed using polyclonal antibodies. Most antibodies and PAP complexes were obtained from Sigma, St. Louis, MO, USA. Anti human skeletal myosin antibodies, developed using myosin heavy and light chains, were reported to specifically stain the A band of human and animal skeletal muscle and do not stain smooth muscle. Antisera reported to be specific to human myoglobin was obtained from Miles Laboratories, Elkhart, IN, USA. Anti dystrophin antibodies (mouse DMD homologs [12]) were kindly provided by Dr. E.P. Hoffman; in these experiments, frozen cells were sequentially incubated with anti DMD in sheep, biotinylated anti-sheep IgG and streptavidin-fluorescein [12]. Control experiments in which the first antibody was replaced by normal serum from the corresponding animals were routinely performed. In some cases (myosin, myoglobin), additional control experiments were done. To test the specificity of the antibody used, the reaction was performed in the presence of an excess of externally added antigen.

#### *Binding experiments*

Receptors for ion channels markers were measured using techniques previously described [13]. [ $^3\text{H}$ ]PN 200-110 was used as a marker for voltage dependent calcium channels and [ $^{125}\text{I}$ ]iodo- $\alpha$ -bungarotoxin as a marker for nicotinic acetylcholine channels.

Cells were washed three times with 5 ml PBS buffer and detached using a rubber policeman in ice-cold buffer (mM: 140 KCl, 5.4 NaCl, 1.8  $\text{CaCl}_2$ , 1.7  $\text{MgCl}_2$ , 25 HEPES-Na, pH 7.4). Homogenization was performed in a Ultrasonic-Homogenizer for 10-15 s in the presence of saponin (1 mg/ml). To measure equilibrium binding of  $\alpha$ -bungarotoxin, cell homogenates (0.1-0.2 mg/ml) were incubated in the same buffer with the addition of [ $^{125}\text{I}$ ]iodo- $\alpha$ -bungarotoxin (0.5-15 nM) for 30 min at room temperature, in the presence or absence of an excess of cold ligand (0.1  $\mu\text{M}$ ) to measure non-specific binding.

[ $^3\text{H}$ ]PN200-110 binding was measured in cell homogenates following the same protocol as above; the incubation medium contained (mM) 140 choline chloride, 50 Tris-HCl (pH 7.4) and [ $^3\text{H}$ ]PN200-110 (0.5-12 nM). Samples were incubated for 60 min at  $10^{\circ}\text{C}$ , in the presence or in the absence of nifedipine (2  $\mu\text{M}$ ) to measure non-specific binding.

#### *Single channel recording*

Patch clamp experiments were carried out using a commercial patch clamp amplifier (Axopatch 1-A, Axon Instruments, Foster City, CA). Patch pipettes were

obtained from 100  $\mu$ l glass capillaries (Drummond Microcaps), using a two stage puller (BBCH, Geneva, Switzerland). Sylgard coated pipettes were fire polished in a microforge. Successful recordings were obtained when the electrode's resistance in solution ranged from 3 to 8 M $\Omega$ .

The patch clamp amplifier outputs for current and command voltage were recorded on video tape using a PCM/VCR set (Dagan DAS900, Dagan, Minneapolis, MN, USA). Computer controlled on-line data acquisition and pulse generation, were performed through an Axolab (Axon Instruments, Foster City, CA) interface controlled with pClamp software.

Previous to recording, the cells were rinsed with 10 ml of saline with the following composition (mM): 135 NaCl, 5 KCl, 1 MgCl<sub>2</sub>, 1 CaCl<sub>2</sub>, 10 HEPES-Na and 1 mg/ml glucose; the solution was adjusted with NaOH to pH 7.4. The final volume left in the dish was 1.5 ml. Patch clamp recordings were difficult to obtain due to a low rate of successful seals. This difficulty seemed to arise from the large amount of extracellular matrix present in the cells, which interfered with seal formation. Cleaning of the cell membrane by incubation with  $1 \cdot 10^{-6}$  M colchicine for 2 h previous to use improved the gigaseal rate formation slightly. Unfortunately, this treatment left the cell membrane very fragile and susceptible to rupture.

## Results

### *Establishment of the RCMH cell line*

A procedure previously shown to transform rat adult cells (nerve and kidney) in culture [15,16] was applied in this work to human muscle. In our previous work, rat cells were transformed after incubation (for variable periods of 15 to 90 days) with conditioned media obtained from thyroid tumor cell monolayers, or by exposure *in vivo* to thyroid tumor implants [15,16].

When we applied a similar procedure, using conditioned media obtained from human primary cultures derived from normal thyroid glandular material, the same immortalization event obtained in adult rat cultures incubated with UCHT1 conditioned media was observed in adult human skeletal muscle. After approx. 3 months, the presence of HTCM in the culture media was no longer needed to sustain indefinite growth of cells.

The resulting cell line showed short doubling times (ca. 24 h), cell density-dependent inhibition of growth (46 000/cm<sup>2</sup>), and plating efficiencies of around 10%, which allowed clonal analysis. The mother cell line and two clones, used in this work, exhibited similar markers and morphological characteristics. Cells also exhibited chromosomal rearrangements (data not shown) but maintained an acceptable repertoire of differentiated markers, even after several cycles of freezing and thaw-

ing. Muscle cultures not treated with HTCM displayed poor cell proliferation, subcultures stopped dividing and ended in cell lysis.

RCMH cells proliferated in complete media containing 10% bovine serum and 2.5% fetal calf serum (Fig. 1A). Transfer to media containing 1% bovine serum, insulin and transferrin (fusion-promoting medium) caused aggregate formation within 48 h (Fig. 1B) followed by fusion. Ratio of fused over non fused cells was variable but up to 85% of the cells were involved in fusion in a given plate. Multinucleated myotube-like structures were also obtained after transfer of RCMH cells to media containing 5% horse serum for a period of 48 h (Fig. 1C and D). Fused cells do not exhibit spontaneous contractions at this level of differentiation; nor do they contract when electrically stimulated, indicating that the maturation process that leads to a functional contractile machinery is not complete at this stage.

### *Immunocytochemical markers*

Fig. 1E, illustrates cells cultured in fusion-promoting media for 5 days; Fig. 1F shows the fluorescent signal from the same cells labelled with anti dystrophin antibodies conjugated with streptavidin-fluorescein. Fluorescent granular bodies were detected through all the area of the cell cytoplasm both in fused and in non fused cells, while nuclei remained unstained.

RCMH cells maintained the genetic expression of specific cytoskeletal proteins as striated muscle myosin and desmin, a marker observed in many muscle cells (and certain non muscle cells) grown in culture [17]. Fig. 2 illustrates the specific immunostaining against myosin (panel B) and desmin (panel D); in Fig. 2A and C, control experiments using normal rabbit serum instead of the respective first antibody are shown for comparison. Myosin staining is visible through all the cytoplasm and appears to concentrate in filamentous regions in some cells. In practically all the cells of RCMH monolayers, there was a strong immunoreaction of the intermediate size filament systems with anti-desmin sera (Fig. 2D), with a preferential accumulation of PAP complexes around the nuclei.

Myoglobin, a muscle phenotype marker, was also retained under continuous proliferation conditions (Fig. 2E and F). Again, the specific anti-myoglobin stain was seen labeling the entire cytoplasm in almost all cells attached to coverslips (Fig. 2F). In all cases, replacement of primary antisera by non immune sera gave negative results (Fig. 2A, C and E). The specificity of anti-myosin and anti-myoglobin antibodies was tested in experiments where the reaction took place in the presence of an excess of externally added antigen and negative results, similar to those depicted in Fig. 2A and E, were obtained. The presence of creatine kinase, another muscle phenotype marker, was detected in

these cells by studying the MM isozyme. In the RCMH cell line the amount of this isozyme increased with cell differentiation, parallel to cell fusion (not shown).

#### *Receptors for ion channels*

A study of the binding of drugs and toxins indicated the presence of specific membrane receptor sites for  $\alpha$ -bungarotoxin (Fig. 3) and PN-200-110 (Fig. 4), blockers of the cholinergic nicotinic ion channels and

of voltage dependent calcium channels, respectively. In both cases, the maximal binding capacity is on the order of 2-5 pmol/mg protein, similar to that described for primary cultures and is high enough to allow reproducible cell based binding studies. Interestingly, some differences were apparent when we compared binding to RCMH cells with binding to purified membranes from skeletal muscle. The data for binding of  $\alpha$ -bungarotoxin (Fig. 3) and that of dihydropyridines

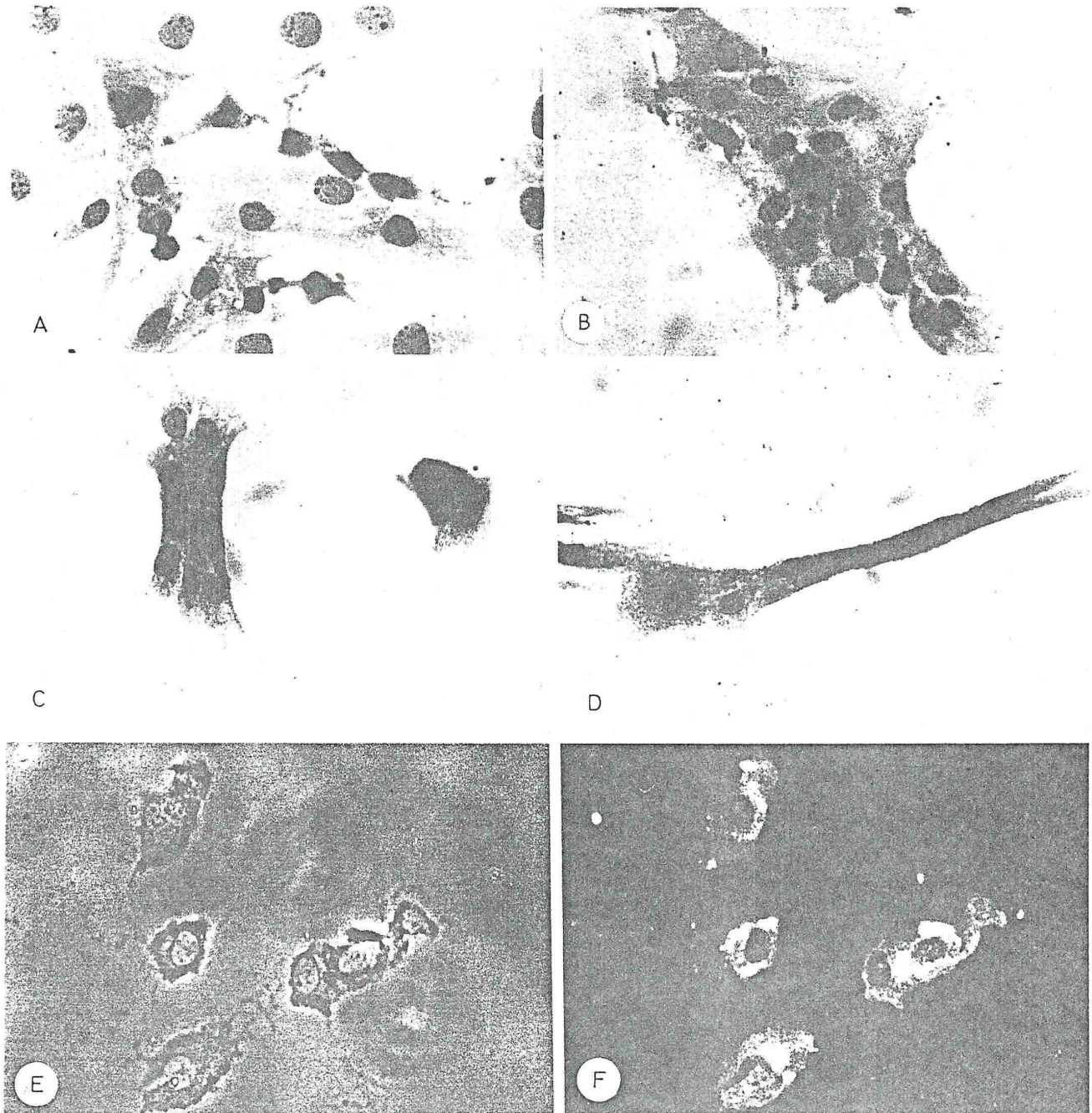


Fig. 1. (A) Confluent monolayer of RCMH cells growing in complete serum containing medium for 2 days ( $\times 200$ ); note the absence of fusion and the presence of several mitosis. (B) ( $\times 200$ ) Sister RCMH cultures growing in fusion promoting media for 2 days. (C and D) Sister RCMH cultures incubated for 2 days in growing medium containing 5% horse serum ( $\times 200$ ). RCMH cells cultured for 5 days in fusion promoting media were fluorescence immunolabeled for dystrophin; (E) phase contrast and (F) epifluorescence optics ( $\times 200$ ).

(Fig. 4) can be fitted assuming two families of receptors for RCMH cells, whereas only one (high affinity) receptor site is seen in muscle membranes isolated from frog or mammalian skeletal muscle [13,18].

Cells placed in a culture medium that favors differentiation displayed an increase in specific binding of  $\alpha$ -bungarotoxin with time of exposure (Fig. 5). This increase correlates well with the amount of myotube-like structures seen in the culture plates. The increase

occurred at all the toxin concentrations tested but more data are needed in order to ascertain whether both binding components or only the high affinity binding increases.

#### *Single channels*

Acetyl choline-gated ion channels were studied in the presence of carbachol in the patch pipette. Single ion channel recordings obtained under these condi-

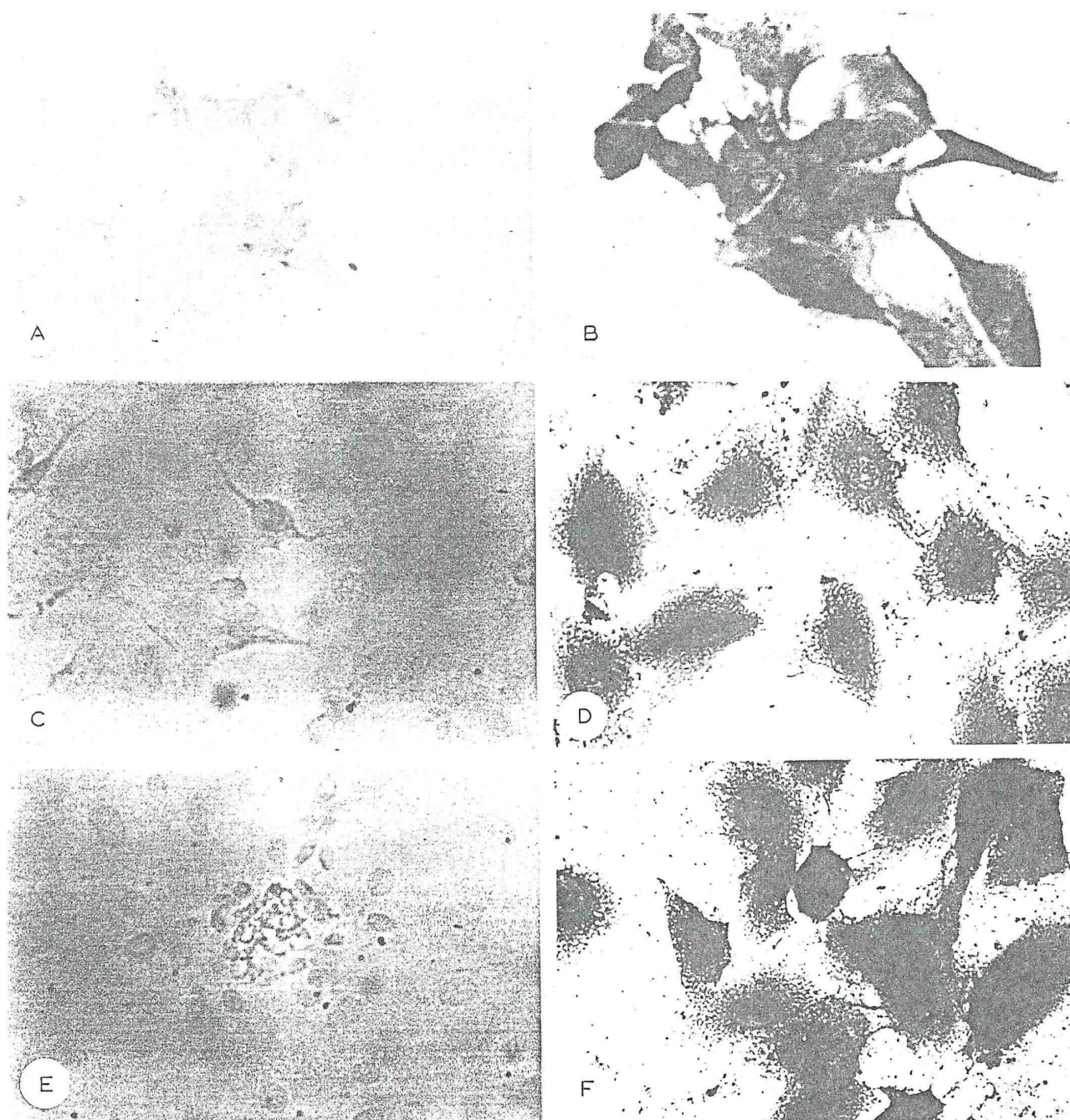


Fig. 2. (B,D and F) Positive immunostained material in RCMH cells as seen after decoration with specific rabbit antibodies against human skeletal myosin (B,  $\times 200$ ), desmin (D,  $\times 200$ ) and human myoglobin (F,  $\times 200$ ). Stain was developed by PAP complexes. In all cases close to 100% of the cells reacted with the antibody. (A ( $\times 200$ ), C and E ( $\times 100$ )) Control cultures using normal rabbit serum instead the first antibody.

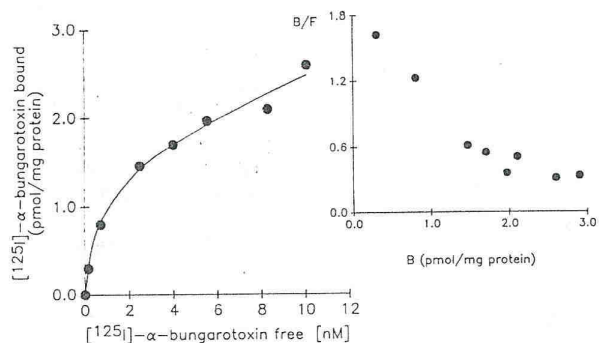


Fig. 3. Specific binding at equilibrium of [ $^{125}$ I]iodo- $\alpha$ -bungarotoxin to cell homogenates of RCMH. The Eadie-Scatchard analysis shows two populations of receptors. Curve fitting to two sites gave the following values for  $K_d$  and  $B_{max}$ : High affinity site; 0.7 nM and 1.48 pmol/mg protein. Low affinity site; 1.62  $\mu$ M and 178.8 pmol/mg protein, respectively.

tions are illustrated in Fig. 6. The results shown were obtained from two separate patches under identical applied voltage (+100 mV) and ionic composition in the pipette, except for the carbachol concentration which was 200 nM in panel A and 1  $\mu$ M in panel B. The calculated conductance of 42.5 pS was obtained by fitting a straight line to the channel amplitudes obtained at several hyperpolarizing voltages (data not shown). The bursting pattern is clearly different at the two concentrations of agonist. Curve fitting of the histograms for closed and open durations was obtained using two exponentials for the open dwell times and three exponentials for the closed dwell times; the time constants obtained in this way are summarized in Table I. The shorter open duration is dose dependent and probably represents the intra burst opening rate. The longer open lifetime may reflect burst duration.

Fig. 7 shows data from a cell-attached patch with the pipette filled with 100 mM BaCl<sub>2</sub> and 10 mM Hepes-K at pH 7.4. The pipette potential was 0 mV, thus the potential across the patch membrane was essentially equal to the cell resting potential. Small events are distinguishable at a low bandwidth (digital gaussian filter at 500 Hz). Although we measured current carried by barium ions, the observed activity is

TABLE I

Time constants for carbachol gated channels

[Carbachol]	$\tau_S$	$\tau_M$	$\tau_L$
	on times (ms)		
200 nM	0.38 $\pm$ 0.04	—	9.3 $\pm$ 0.74
1 $\mu$ M	0.10 $\pm$ 0.04	—	8.67 $\pm$ 0.43
	off times (ms)		
200 nM	1.26 $\pm$ 0.13	20.0 $\pm$ 3.0	49.0 $\pm$ 4.0
1 $\mu$ M	0.29 $\pm$ 0.01	1.9 $\pm$ 0.4	308 $\pm$ 7.0

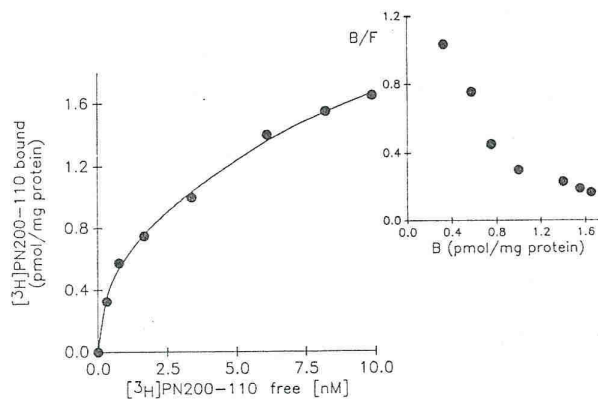


Fig. 4. Specific binding at equilibrium of [ $^3$ H]PN200-110 to cells homogenates of RCMH. The Eadie-Scatchard analysis shows similarly to what we find for  $\alpha$ -BTX binding, the curve fit to two sites gave the following values for  $K_d$  and  $B_{max}$ : high affinity site; 0.28 nM and 0.54 pmol/mg protein. Low affinity site; 12.8 nM and 2.62 pmol/mg protein, respectively.

consistent with a Ca<sup>2+</sup> selective channel of ca. 5 pS. Activity consistent with the presence of more than one type of K<sup>+</sup> channels was evident when K<sup>+</sup> ions were placed in the pipette (not shown).

Fig. 8 shows a steady state recording of the same patch shown in Fig. 7, after it was excised from the cell. The bandwidth for the analysis was in this case 2 kHz; the patch pipette potential was held at -10 mV. Large unitary currents with a slope conductance close to 350 pS were seen, as well as events of smaller amplitude which may be interpreted as subconductance states of the same channel. The amplitude distribution histogram presents two peaks above baseline, clearly describing the sublevel. Given the ionic and voltage conditions used, these events are suggestive of a Cl<sup>-</sup> selective channel.

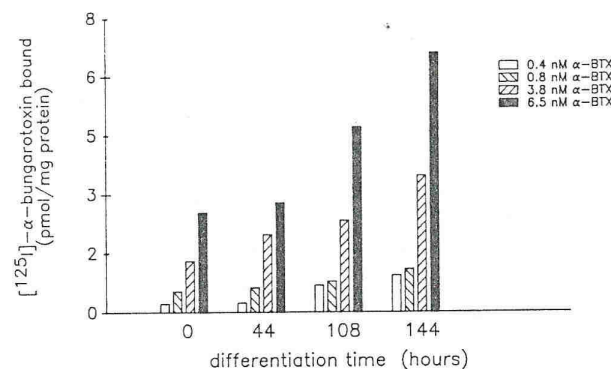


Fig. 5. Increase of the specific binding of [ $^{125}$ I]iodo- $\alpha$ -bungarotoxin to RCMH cells homogenates after differentiation. Cells were incubated for a variable period in a medium containing 1% bovine serum, 10  $\mu$ g/ml insulin and 10  $\mu$ g/ml transferrin.  $\alpha$ -Bungarotoxin binding was measured at different toxin concentrations as described in Materials and Methods.

## Discussion

A non traditional procedure was used to obtain a cell line, RCMH, from human skeletal muscle. These cells, grown in contact with a thyroid cell line conditioned medium, represent a new type of transformed cells; they continuously divided in the presence of this medium and proliferated indefinitely following its removal. After this process, we produced a cell line that shares with transformed cells the capacity to grow permanently in culture (it continues to grow after more than 400 generations) but, that, unlike other transformed cells, exhibits contact inhibition of growth, does not grow in soft agar, and maintains differentiated markers while dividing.

Human cells are less prone to transform than rodent cells in culture, maybe because of their relatively higher karyotypic stability [19]. Traditional methods used to immortalize human cells include fusion with already

transformed cells and exposure of primary cultures to carcinogens, tumor promoters, radiation, oncogenic virus or tumorigenic nucleic acids [19]. RCMH cells are a consequence of an irreversible transforming effect caused by a product of an apparently normal cell; the fact that sister cultures without HTCM did not survive beyond the first passage, rules out a 'spontaneous' transformation. In a rather similar manner, conditioned media from murine pokeweed mitogen-stimulated splenocytes has been reported to transform mouse bone marrow mast cells [20].

The actual factor that causes immortalization remain to be identified. This factor (or factors) will probably differ from the many transforming growth factors (TGFs) that have been purified from media conditioned by several normal or neoplastic cells, because their common characteristic is that the anchorage independent growth they induce is reversible upon their removal [21] whereas with HTCM, the effect is

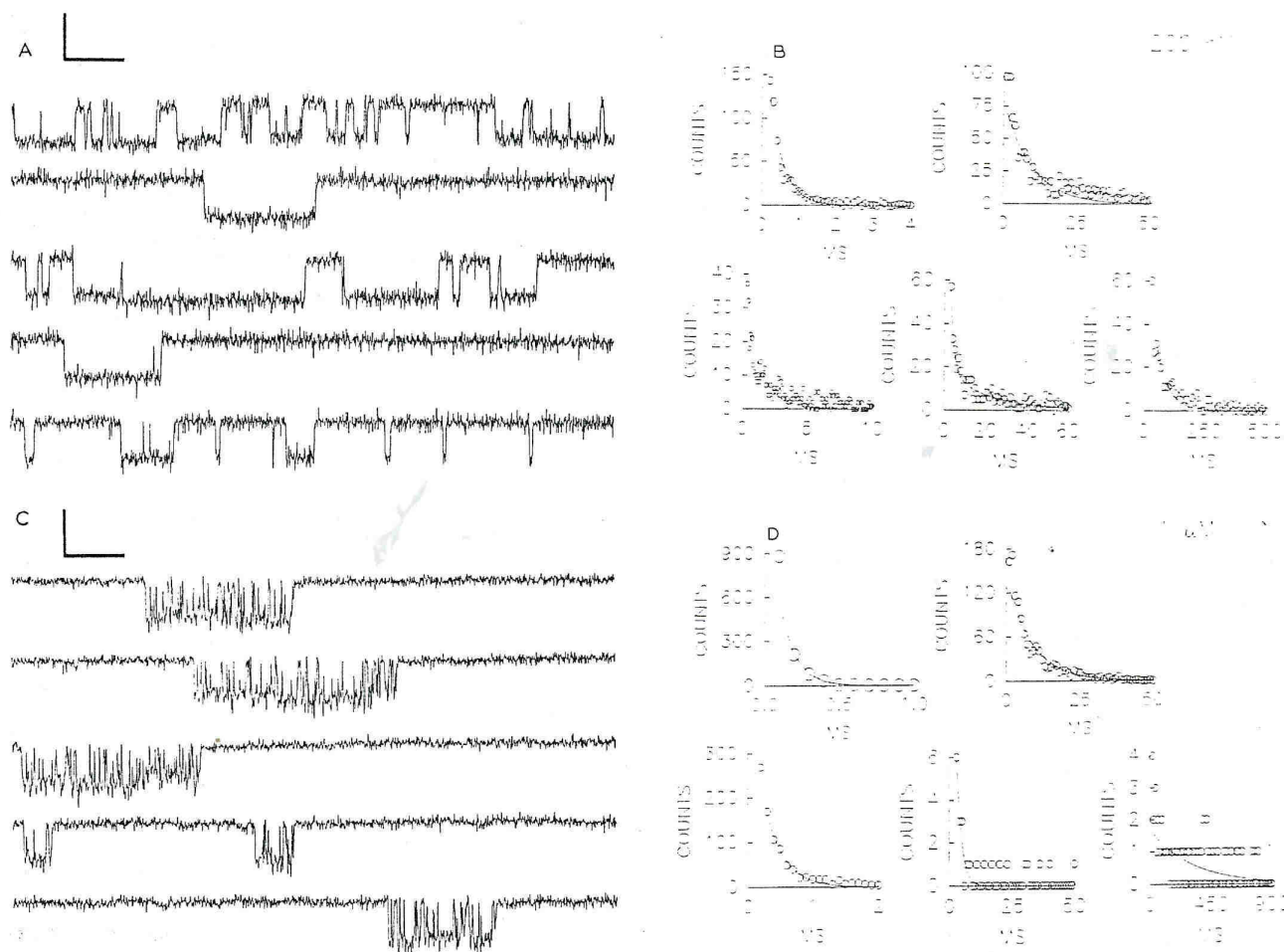


Fig. 6. Selected non-contiguous traces of single channel recordings from two different experiments obtained in cell-attached conditions using 200 nM (A) and 1  $\mu$ M (C) carbachol in the patch pipette. The potential inside the pipette is in both cases 100 mV hyperpolarized with respect to the membrane potential. Channel openings are shown as downward deflections representing cationic inward current. The mean amplitude of the events calculated from the amplitude histograms is 3.0 (B) and 3.4 (D). The calibration bar of 20 ms and 2 pA is the same for both panels.

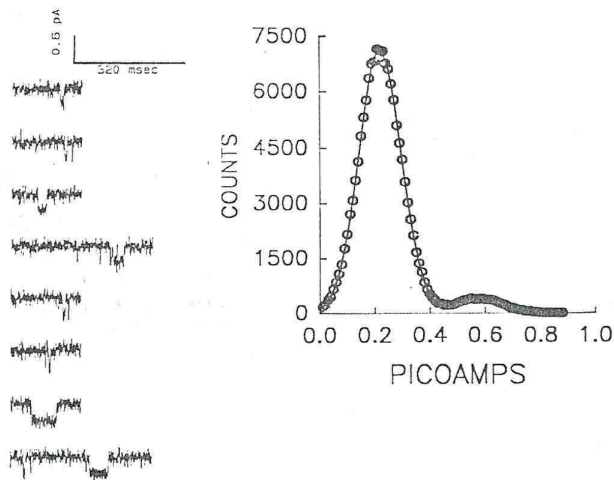


Fig. 7. Selected traces of a cell-attached record obtained with a pipette filled with 100 mM  $\text{BaCl}_2$  with no applied potential, thus corresponding to the cell's resting membrane potential. Small inward currents of ca. 360 fA are detected when the data is filtered to 500 Hz using a low pass filter. The amplitude histogram is well fitted by two Gaussian distributions.

irreversible. Studies in laboratory animals clearly demonstrate the influence of thyroid hormones on induction and growth of several types of experimental tumors, i.e., lymphoma, mammary tumors, primary and transplanted hepatoma (see Ref. 22 for review). Triiodothyronine plays also a role in neoplastic transformation of cultured cells by X-rays, chemical carcinogens and both RNA and DNA virus [23–25]. The fact that the cellular counterpart of a viral oncogene (*v-erbA*) encodes a thyroid hormone receptor [26,27], suggests that this hormone has either a direct or a non-direct effect of the hormone on tumor growth; nevertheless, the presence of the hormone alone seems not to be sufficient to induce transformation since no cell transformation nor tumor promotion has ever been documented as an effect of triiodothyronine alone and the simultaneous presence of either known growth factors or of other unknown factors may be needed [22].

The skeletal muscle markers that are present in the permanent cell line include the capacity of cells to fuse spontaneously and the expression of a variety of proteins characteristic of skeletal muscle. Consistent with the finding that desmin and vimentin can co-exist in muscle and certain non muscle cells [17], both gene products persist in our human muscle lineage under continuous culture. Immunocytochemical localization of myoglobin and human myosin together with the presence of the MM isoform of creatine kinase and of dystrophin, define a set of markers for gene expression that make this cell line worthy of further study. We have not been able to monitor contractions in these cells, possibly meaning that some key molecules that

participate in the process leading to contraction are not being expressed in our culture conditions and further differentiation may be needed.

Functional ion channels were detected directly in patch clamp records and indirectly through receptors for ion channel blockers; the nicotinic acetylcholine receptor channel, expressed as an  $\alpha$ -bungarotoxin receptor, is present in non fused cells at levels comparable to those described for differentiated skeletal muscle in primary culture [28,29] making feasible to study the location of receptors within the cell membrane by using autoradiography. Serum deprivation plus addition of insulin and transferrin to the incubation media induced cell fusion as well as a time dependent increase in  $\alpha$ -bungarotoxin receptors; a process that mimics myoblast differentiation [29] and suggests that this cell line behaves in this respect like adult muscle satellite cells in primary culture [2,3].

The data gathered with patch-clamp recordings add evidence to the binding data, reinforcing the skeletal muscle cell lineage of this line. The conductance for the carbachol gated channel is in the same range than the 40–45 pS channel reported by Adams and Bevan [28]. Kinetically, the longer open lifetime found is

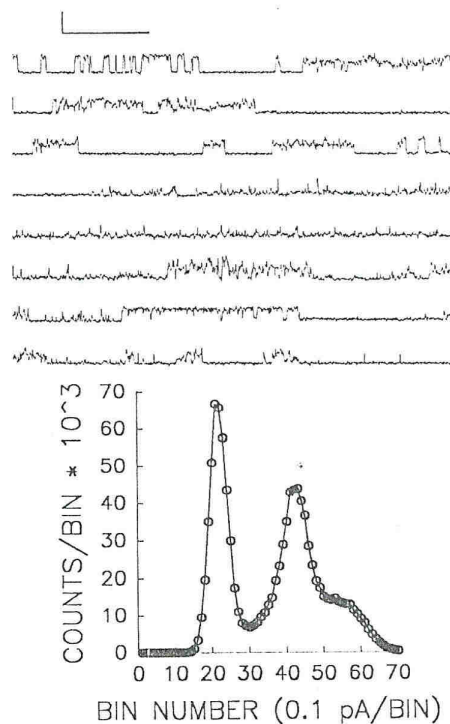


Fig. 8. Data extracted from the same experiment of Fig. 7 after the electrode was excised from the cell carrying with it a small patch of membrane. In this case the intracellular face of the membrane is exposed to the extracellular saline bathing the dish (normal Ringer). The pipette potential is  $-10$  mV. The large amplitude events, with a mean amplitude of 3.5 pA correspond to a channel with a conductance close to 350 pS. The baseline-corrected total amplitude histogram shows activity of a not fully open channel which is also clearly seen in the current traces.



similar to the 6.9 ms open lifetime reported by Mancinelli et al. [30] for acetylcholine gated currents in primary culture of human skeletal muscle. A full characterization of these ion channels is under way.

The density of high affinity dihydropyridine receptors in these cells is also comparable to that described for differentiated skeletal muscle in culture [31,32]. These receptors represent voltage dependent calcium channels found in skeletal muscle [18,31-34], although a role for them as voltage sensors in excitation-contraction coupling has also been proposed [31,35].

We found more than one family of dihydropyridine receptors in these cells, in contrast to what has been found in both frog and mammalian transverse tubule membranes [13,18]. Two types of calcium channels were reported in cultured rat skeletal muscle as well as in rat ventricular muscle, where a difference in sensitivity for dihydropyridines was evident [36]; the possible role of the different families of receptors in these cells deserves further study.

Human skeletal muscle cell lines may be especially important if we consider the possibilities they offer to research in human muscle pathologies. Conversely, cell lines tend not to express many of the features characteristic of differentiated cells from adult tissues; therefore, the value of the cell line we describe here as a research tool will depend on the markers of differentiated skeletal muscle that it is able to express. The characteristics of RCMH cell line suggest that it may be useful as a tool both for the study of muscle physiology and in the search of new cell based therapies for human genetic diseases affecting skeletal muscle [4-6,37], where myogenic properties and dystrophin production are necessary (though not sufficient) conditions.

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