J. Membrane Biol. 113, 169–175 (1990)

The Journal of
Membrane Biology

Inhibition of Voltage-Dependent Na^+ Current in Cell-Fusion Hybrids Containing Activated c-Ha-*ras*

Mark Estacion
Department of Physiology and Biophysics, University of California, Irvine, Irvine, California 92717

Summary. The electrophysiological properties of EJ (human bladder carcinoma), GM2291 (human fetal lung fibroblast), and of three hybrid cell lines obtained from their cell fusion were investigated using the patch-clamp technique. GM2291 cells, which are nontumorigenic, express voltage-dependent Na^+ channels. The pharmacology and gating properties of the Na^+ channels in GM2291 cells are distinct from neuronal and cardiac Na^+ channels. EJ cells, which are tumorigenic and contain activated c-Ha-*ras*, express inward rectifier K^+ channels. The three cell-fusion hybrid lines, named 145 (nontumorigenic), 145L (nontumorigenic but morphologically altered), and 147TR2 (fully tumorigenic segregant), have been previously shown to express levels of activated c-Ha-*ras* similar to those of the EJ parental line. Voltage-dependent Na^+ channels were observed in none of the hybrid cell lines, while inward rectifier K^+ channels were observed in each of the hybrid cell lines. The possibility that c-Ha-*ras* inhibits expression of a voltage-dependent Na^+ channel is discussed.

Key words patch-clamp · Na^+ channel · inward rectifier · *ras* oncogene · fibroblasts · cell-cell coupling

Introduction

The *ras* protein is well characterized biochemically and its oncogenic form is commonly found in human tumors (Barbacid, 1987). Oncogenic forms of *ras* are formed by point mutations in the cellular proto-oncogene, which result in single amino acid changes in one of a few key positions of the resultant protein p21. Functional domains for the *ras* protein have been identified from sequence analysis and from the three-dimensional structure (De Vos et al., 1988). The Val-12 mutation, which is the form found in EJ cells, changes the binding site for guanine nucleotides (Tong et al., 1989); this could account for the reduced GTPase activity of oncogenic *ras*. The localization of *ras* to the plasma membrane and the moderate similarity of *ras* to G-proteins support the hypothesis that *ras* functions as part of a signal transduction mechanism.

In addition to oncogenes, tumor suppressor genes play a role in tumorigenesis. Tumor suppressor genes are hypothesized to account for the nontumorigenic phenotype of hybrid cells resulting from the fusion of a tumor cell expressing an activated oncogene and a nontumorigenic cell. Such a series of cells (Geiser et al., 1986) is the focus of this study. The EJ cell line, a human bladder carcinoma, contains activated c-Ha-*ras* and is highly tumorigenic. GM2291 cells are normal human fibroblasts isolated from fetal lung and are not tumorigenic. The resultant hybrid cells, named 145, were found to be nontumorigenic but expressed levels of activated *ras* similar to those found in EJ cells. A number of revertants that displayed a spectrum of phenotypes were isolated from the hybrid cells in culture. One isolate, named 145L, showed reduced anchorage-dependent growth and altered cell morphology, yet remained nontumorigenic. A full revertant to the tumorigenic phenotype, named 147TR2, was also characterized. No difference in the amount of *ras* expression was found in any of these hybrid cell lines. Thus tumor suppressor genes are hypothesized to account for the nontumorigenic phenotype of these hybrid cells expressing the *ras* oncogene.

This paper characterizes the ion channels of EJ, GM2291, and three EJ *x* GM2291 hybrid cell lines. Fibroblasts and tumor cell lines are often used in the study of the mechanisms of cell growth control but are, for the most part, uncharacterized electrophysiologically. Since ion channels play a role in signal transduction in the nervous system, ion channels may play an analogous role in signal transduction for mitogenesis. This study will attempt to correlate differences in ion channel expression, or of the properties of the ion channels expressed, with

the phenotype of the cells. It is probable that expression of ion channels is regulated at the level of the nucleus. On the other hand, changes in the properties of a given ion channel in the different cell lines may reflect differences in post-translational covalent modifications or perhaps differences in splicing and processing of ion channel genes.

Materials and Methods

The cells are the generous gift of Dr. Eric Stanbridge, Department of Microbiology and Molecular Genetics, University of California, Irvine. Serial cultures are maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (JR Scientific, Woodland, CA), 1% glutamine and no antibiotics. The cells are incubated at 37°C in humidified air with 5% carbon dioxide. Electrophysiological experiments are performed on cells subcultured onto sterile glass coverslips. The subconfluent cells are allowed at least 24 hours to attach and regain normal morphology before use. Immediately before recording, coverslips are transferred to a perfusable chamber mounted on an inverted microscope with Nomarski optics.

Patch-clamp experiments were performed as described by Hamill et al. (1981). An L/M-EPC-7 amplifier (LIST Electronics, Darmstadt, FRG) was used to measure the ionic current. The current signal was then passed through an eight-pole Bessel filter (Frequency Devices, Haverhill, MA) before being digitized and stored on computer hard disk. Hardware and software for PC-based stimulation and acquisition were built from a system developed by Dr. F. Bezanilla at UCLA. Upward deflections correspond to outward current through the membrane. The membrane area for normalizing the amount of channel expression was quantified electrically since fibroblast morphology makes a geometric calculation very difficult. The charging current for a small test pulse is integrated to determine membrane capacitance (C_m) which is proportional to membrane area. For pulse stimuli, the membrane charging current and linear leak currents were subtracted using a P/4 procedure. The ramp stimulus is a smooth and continuous sweep of the applied potential of the patch clamp from negative to positive. Corrections for liquid junction potentials were made during analysis.

The bath solution is standard mammalian Ringer containing (in mM): 160 NaCl, 4.5 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, and 5 HEPES adjusted to pH 7.4 with NaOH with a measured osmolarity of 290–320 mosmol. The pipette solution was composed of (in mM): 160 K-aspartate, 2.0 $MgCl_2$, 1.1 K_2EGTA, 0.1 $CaCl_2$, and 10 HEPES adjusted to pH 7.2 with KOH. The EGTA buffers give a calculated free calcium activity of 10^{-8} M. Tetrodotoxin was obtained from Calbiochem; all other chemicals used were obtained from Sigma. Experiments were performed at room temperature (22–25°C).

Results

GM2291 Normal Human Fibroblast

GM2291 cells express voltage-dependent Na^+ channels. Figure 1*A* shows typical currents elicited by a series of 20-msec depolarizing pulses. The pulse to

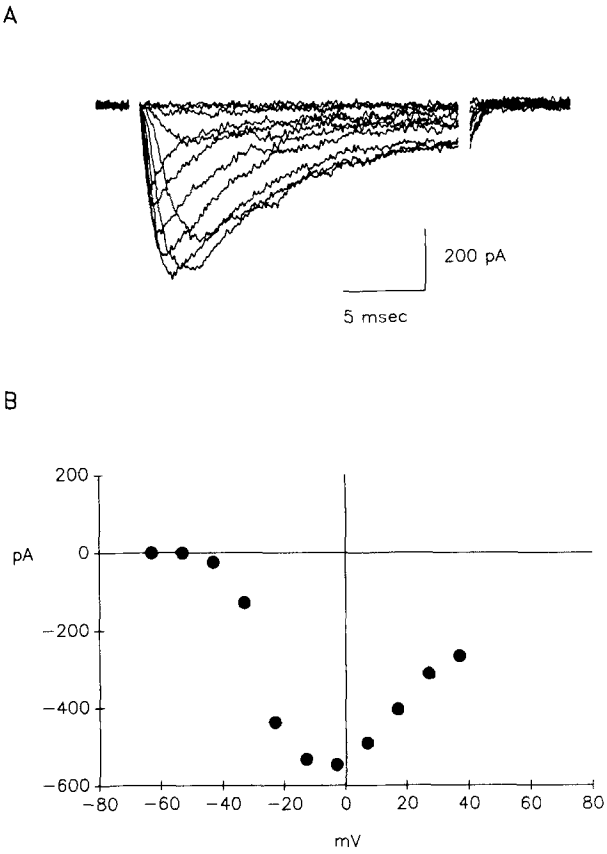

Fig. 1. (*A*) Whole-cell currents from GM2291 cells elicited by a series of depolarizing pulses of 20 msec duration. After correcting for junction potentials, the holding voltage was −93 mV and the pulses were in 10 mV increments from −63 to 37 mV. A 50-msec prepulse to −133 mV was applied to remove inactivation. Linear currents have been subtracted using a P/4 protocol in the range of −140 to −100 mV. This run was also series-resistance compensated to reduce the voltage error. Solutions are described in Materials and Methods. (*B*) Peak-current *vs.* voltage relation obtained from the data in *A*

−3 mV, which corresponds to the maximum of the *I-V* curve, shows that the current peaks in about 2 msec and then inactivates, but is not fully inactivated by the end of the 20-msec pulse. The current-voltage relation is plotted in Fig. 1*B*. The current is selective for Na^+ and is abolished when the Na^+ is replaced with potassium, tetramethylammonium, or choline. This current is also blocked with high affinity by tetrodotoxin (TTX) (Fig. 2), which is a specific blocker of voltage-dependent Na^+ channels. Dose-response curves to TTX were performed in five cells and each gave K_d's in the range of 30 to 60 nM. Thus by gating properties, ion selectivity, and pharmacology, this current is identified as due to voltage-dependent Na^+ channels.

GM2291 cells touching neighboring cells express electrical behavior consistent with coupling

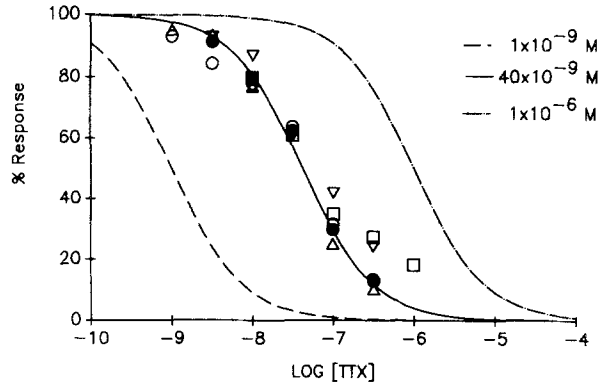

Fig. 2. Dose-response curve of the GM2291 Na^+ current to TTX. Data from five different experiments are plotted using different symbols. The solid line is a binding curve fit to the closed circles, which corresponds to the same cell as Fig. 1, and has an apparent K_d of 40 nM. The dashed line and the dotted line are curves corresponding to K_d's of 1 nM and 1 μM, respectively

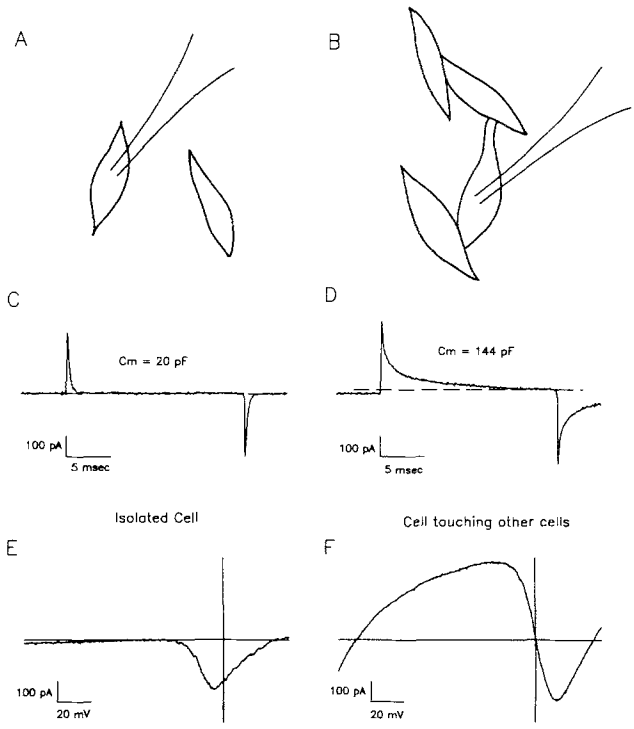

Fig. 3. Differences between isolated GM2291 cells and GM2291 cells in contact with neighbor cells. The left-hand side represents isolated cells. (*A*) A drawing illustrates a cell physically isolated from its neighbors. (*C*) Response to a 5-mV depolarizing pulse is shown illustrating the capacity transient typical of isolated cells. The capacitance value is obtained by integrating the current under the transient and dividing by the applied pulse voltage. (*E*) Response is illustrated of an isolated cell to a ramp from −120 to 40 mV. Note that the slope of the ramp is very slight in regions where the Na^+ channels are closed. The right-hand side represents behavior of cells that are electrically coupled. (*B*) A drawing illustrates cells as they make cell-cell contact. (*D*) The capacity transient to an identical pulse as in *C* is shown. The dashed line is fit to the end of the pulse to remove offsets due to linear leak and sets the baseline for the capacitance integration. Note the increase in area and the multiple decay rates. (*F*) Response is shown to an identical ramp as in *E*. The inward deflection indicating Na^+ current is shifted slightly to the right and is steeper, consistent with the poor voltage control of neighboring cells. The added slope is a relatively voltage-independent current that is selective for potassium. The curvature at the beginning of the ramp is due to poor capacity compensation (the clamp can only compensate a single time constant with a maximum value of 100 pF)

through gap junctions. Figure 3 illustrates currents in GM2291 cells that are isolated or are contacting neighboring cells. The measured capacitance of those cells that are in contact with neighboring cells is much higher than would be expected for a similarly-sized isolated cell. The multiple time constants of membrane charging (Fig. 3*D*) are consistent with the interpretation that the membranes of neighboring cells are being charged up by the voltage clamp through the gap junctions. The ramps still exhibit voltage-dependent Na^+ current in those cells (n = 7) that are electrically coupled and are nearing confluence (Fig. 3*F*). The extra current in Fig. 3*F* is potassium selective but attempts to pharmacologically block it have been unsuccessful. The appearance of this extra current and the multi-exponential capacity transient are absolutely correlated with cell-cell contact and are never seen in isolated cells. Because the assumptions of voltage clamp are not met for neighboring cells coupled by gap junctions, quantification of channels is not done when signs of coupling are present. The presence of Na^+ channels in confluent cells show that the expression of Na^+ channels is not limited to subconfluent GM2291 cells.

The voltage-dependent Na^+ channel is the predominant channel expressed in GM2291 cells. Figure 1 shows a lack of outward current, indicating low expression of voltage-dependent K^+ channels. Raising the external potassium did not reveal any current through inward rectifier K^+ channels nor through voltage-dependent K^+ channels. The possibility that calcium current may contribute to the observed net inward current is ruled out by the following observations: When extracellular sodium is replaced with isotonic calcium or barium, the current is completely abolished. In addition, the TTX block is complete and leaves no residual inward current. While Na^+ channels are expressed in nearly every cell, the amount of Na^+ channel expression was somewhat variable. In the isolated cells that allowed quantification (n = 14), the average conductance was 7.7 nS (SD = 4.7 nS) and the average capacitance was 26.8 pF (SD = 10 pF).

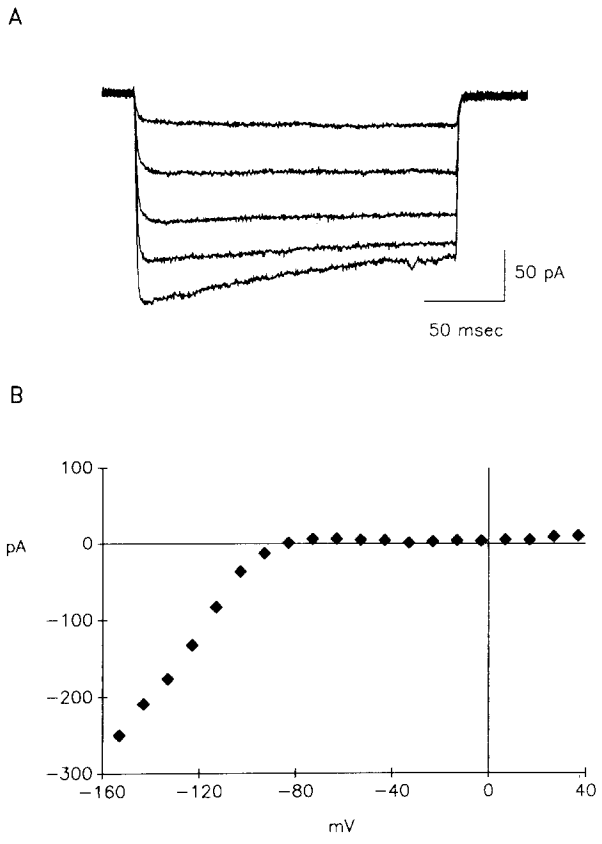

Fig. 4. (*A*) Whole-cell voltage-clamp currents elicited in EJ cells in response to 10-mV increment hyperpolarizing pulses. After correcting for junction potentials, the holding potential is −93 mV. Linear components of current have been subtracted using a P/4 procedure over the range of −50 to −20 mV. Solutions are described in Materials and Methods. (*B*) Peak-current *vs.* voltage relation derived from the currents elicited during pulses in 10-mV increments from −153 to 37 mV

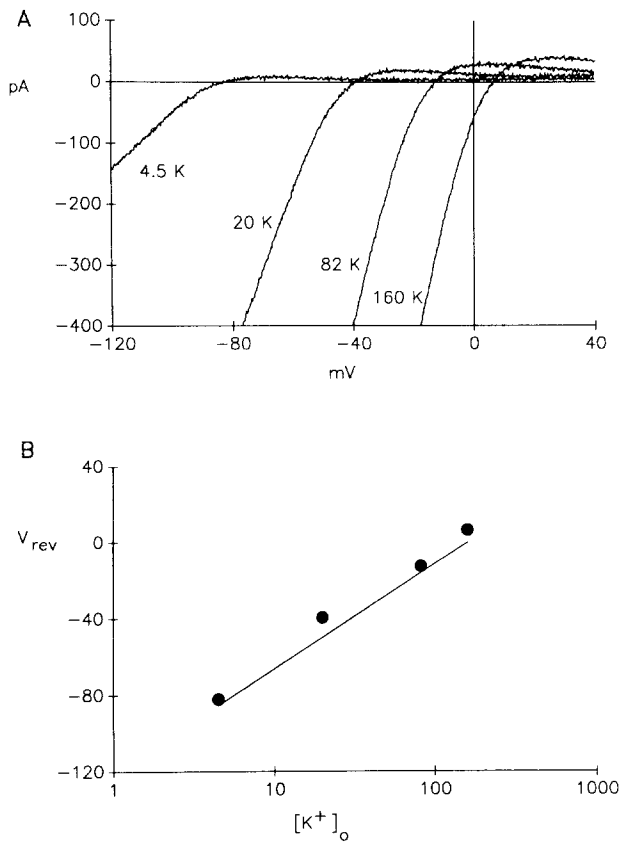

Fig. 5. (*A*) Whole-cell currents in EJ cells elicited by a ramp stimulus in the presence of external potassium concentrations. The extra K^+ was exchanged with Na^+ to keep total ionic strength constant. (*B*) The potentials at which the ramp traces cross the zero current axis are plotted *vs.* the logarithm of $[K^+]_o$. For comparison purposes, the solid line corresponds to the behavior of a potassium-selective electrode as predicted by the Nernst equation

EJ Human Bladder Carcinoma

EJ cells express classical inward rectifier K^+ channels. Figure 4 shows inward currents elicited by selected hyperpolarizing pulses and the current-voltage relation derived from an entire family of pulses. The shape of the *I-V* curve (Fig. 4*B*) is characteristic of the inward rectifier K^+ channel, showing increased current as the potential moves below the reversal potential for potassium. The ionic dependence of this current is illustrated in Fig. 5, where the external potassium concentration was raised in discrete steps. The traces show that the potential at which the current starts to flow inward shifts to more depolarized potentials with increasing external potassium. This shift is predicted by the Nernst equation assuming high selectivity for potassium (Fig. 5*B*). Another property of inward rectifier potassium channels is strong voltage-dependent block by Cs^+; the inward current is blocked more strongly as the potential moves below the reversal potential for potassium (*data not shown*). Taken together, these properties identify the predominant channels in EJ cells as inward rectifier potassium channels.

Inward rectifier K^+ channels are expressed in nearly every cell. The level of expression, however, is not tightly regulated and has an average slope conductance of 2.8 nS (SD = 2.4 nS). Evidence for other types of ion channels was seen in less than 10% of the cells. In contrast to GM2291 cells, EJ cells do not show evidence for electrical coupling, even for cells in highly confluent cultures. The morphology of the EJ cells differed from the GM2291 cells and showed a tighter distribution of sizes as measured by capacitance (average C_m = 25 pF, SD = 4 pF).

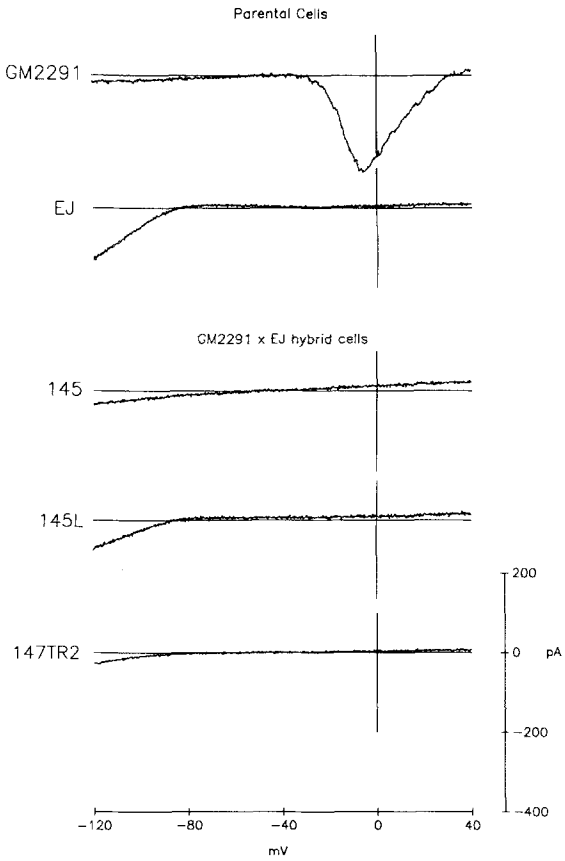

Fig. 6. Representative ramp current traces from each of the cell lines are plotted at identical scaling. The axes drawn are applicable to each of the records. Solutions are as described in Materials and Methods

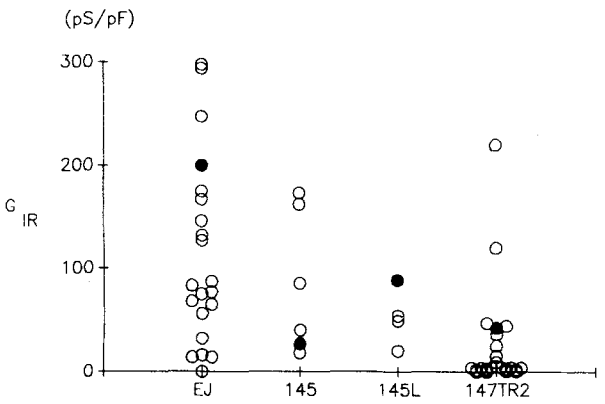

Fig. 7. Plot of inward rectifier potassium conductance normalized to measured capacitance for each cell, grouped by cell line for the *ras*-containing cell lines. EJ and 147TR2 are tumorigenic, while 145 and 145L are not tumorigenic. Filled symbol represents corresponding cell illustrated in Fig. 6

GM2291 *x* EJ Cell-Fusion Hybrids

Each of the hybrid cell lines was surveyed for expression of ion channels, and explicitly tested for the presence of voltage-dependent Na^+ channels and of inward rectifier K^+ channels. The results of surveying the nontumorigenic (145), morphologically altered (145L), and tumorigenic (147TR2) hybrid cell lines are seen in Fig. 6. Current responses to identical ramp stimulus protocols illustrating representative behavior for each cell line are shown. The major result is the complete absence of voltage-dependent Na^+ current in any of the cell fusion hybrids, using either ramp or pulse voltage stimuli. In contrast, inward rectifier K^+ channels were expressed in cells from each hybrid line. The gating and ion selectivity of the inward rectifier K^+ channels in each of the hybrid cell lines are similar to those described for the parental EJ cell line (*data not shown*). Inward rectifier K^+ channel expression in the hybrid cell lines is not tightly regulated. The inward rectifier expression is examined by calculating the conductance normalized to cell capacitance for each cell. Figure 7 shows that in addition to the wide distribution of levels of expression there are more cells with very low levels of expression. In the 147TR2 line, for example, a few cells expressed high levels of inward rectifier while the majority of cells showed no detectable expression of inward rectifier. Since GM2291 cells show electrical coupling while EJ cells do not, each of the hybrid cell lines was tested for evidence of electrical coupling through gap junctions (*data not shown*). The 145 line showed the increased capacitance response when cells were chosen that contacted neighboring cells. The 145L and the 147TR2 lines showed little evidence for electrical coupling, even when cells from a confluent dish were chosen.

Discussion

Ion Channels in Hybrid Cells

Interpretation of the patterns of ion channel expression in the GM2291 *x* EJ hybrid cells is facilitated by the dramatic differences in the ion channels expressed by the two parent cell lines. If channel expression in the hybrid cell represented the combination of the properties found in the parents, then both inward rectifier and Na^+ channels would be expressed. The lack of Na^+ channels in the hybrids, however, shows that ion channel expression is complex and suggests regulation of the GM2291 Na^+ channel. The possibility that all the GM2291 properties are suppressed in the hybrids is refuted by the

appearance of electrical coupling and the loss of tumorigenicity.

What could account for the lack of Na^+ channel expression in the GM2291 *x* EJ hybrids? One possibility is loss of chromosomes, which is common in somatic cell hybrids. One argument against this possibility is that the chromosomes from both human parent lines are the same and thus the hybrid cell would have to lose all copies of the chromosome that contained the Na^+ channel gene. The possibility that Na^+ channels are never expressed in hybrid cells formed with a tumor cell is refuted by the observation by Minna et al. (1971) that the action potential and, by inference, Na^+ channels are maintained in neuroblastoma *x* L-cell hybrids. A possibility which is more interesting to consider is that some factor from the EJ parent (e.g., the activated *ras* oncogene) suppresses Na^+ channel expression.

Does *ras* Modulate Na^+ Channels?

A number of observations support the speculation that *ras* affects the expression of the Na^+ channel found in GM2291 cells. The *ras* protein is thought to effect a signal transduction event in a manner analogous to the action of G-proteins. Among the actions of G-proteins (Stryer & Bourne, 1986; Neer & Clapham, 1988) is the modulation of ion channels. Perhaps *ras* modulates Na^+ channel proteins in the plasma membrane. Alternatively, *ras* might affect Na^+ channel activity through changes in gene expression. The H-*ras* oncogene is implicated in modulating the expression of voltage-dependent channels in other systems. In 3T3 fibroblasts, for example, transfecting H-*ras* was found to block the activity of T-type but not L-type Ca^{2+} channels (Chen et al., 1988). In BC_3H1 monocytes, Caffrey, Brown and Schneider (1987) showed that transfecting H-*ras* blocked the differentiation-induced expression of voltage-dependent Ca^{2+} channels and of voltage-dependent Na^+ channels. The results of this study showed a correlation between expression of H-*ras* and a lack of Na^+ current, consistent with the hypothesis that H-*ras* can modulate Na^+ channels. It would be of interest to explore the relationship between expression of GM2291 Na^+ channels and H-*ras* more directly either by microinjecting *ras* protein or by transfecting the GM2291 cell line with the H-*ras* gene.

Properties of GM2291 Na^+ Channels

The Na^+ channel expressed in GM2291 cells is similar but not identical to the "classical" Na^+ channel found in neurons. The pharmacology and gating kinetics of GM2291 Na^+ channels are measurably distinct from values previously reported in the literature. The differences in properties could arise either by post-translational modification of the "classical" Na^+ channel or by a unique Na^+ channel gene.

Tetrodotoxin is a specific blocker of Na^+ channels, but Na^+ channels from different tissues show a spectrum of sensitivity to TTX block. TTX binding curves for neuronal and cardiac Na^+ channels are included in Fig. 2. Neuronal Na^+ channels are blocked by 1–10 nM TTX (Catterall, 1980), while cardiac Na^+ channels require μM amounts of TTX to block (Cohen et al., 1981). The data obtained for GM2291 cells cannot be explained by postulating that GM2291 cells express a mixture of neuronal and cardiac Na^+ channels because an inflection of the TTX dose-response curve would be apparent. Normal muscle cells show TTX block with an apparent K_d of 8 nM, but express TTX-resistant Na^+ channels with an apparent K_d of 300 nM either when denervated or during development before formation of the synapse (Lombet et al., 1982). In muscle fibers there is electrophysiological evidence for a subpopulation of Na^+ channels with an apparent K_d of 40 nM (Jaimovich et al., 1983), a value similar to that found in GM2291 cells. The TTX dose response measured in this study suggests that the GM2291 Na^+ channel is neither a neuronal nor a cardiac Na^+ channel but may be a muscle Na^+ channel.

The inactivation kinetics of the GM2291 Na^+ channel are also distinct from the "classical" Na^+ channel. Most published records of Na^+ currents at similar ionic conditions and temperature show complete inactivation within 10 msec. GM2291 Na^+ channels, in contrast, have not fully inactivated by 20 msec. The Na^+ channels expressed in the muscle cell line BC_3Hl (Caffrey et al., 1987), however, shows kinetics similar to those of GM2291 cells. The inactivation kinetics and TTX block are most consistent with the view that the GM2291 Na^+ channel is related to a muscle-type Na^+ channel gene.

Inward Rectifier

The physiological roles for inward rectifier currents are not well established. Inward rectifier potassium channels are thought to contribute to maintaining the membrane potential near the Nernst potential for potassium. Oligodendrocytes are reported to express inward rectifier, and this may act to help maintain low extracellular potassium concentrations in regions of high neural activity (Barres,

Chun & Corey, 1988). The results of this study showed widely varying levels of inward rectifier expression for each *ras*-containing cell line. Since similar levels of activated *ras* are expressed in each of the hybrids and in the parent EJ line, changes in levels of expression of inward rectifier are probably due to other influences. In rat brain neurons, Nakajima, Nakajima and Inoue (1988) report an inhibition of an inwardly rectifying K^+ current by substance P acting via a pertussis toxin-insensitive G protein. In contrast, Fredrich et al. (1988) described a calcium-sensitive K^+ channel which was weakly inward rectifying in MDCK epitheloid cells. Thus changes in G-protein activity or changes in intracellular calcium activity might explain the varied levels of inward rectifier observed.

I would like to thank Dr. Michael Cahalan for his support and guidance while I was a member of his lab. I would like to thank Ruth Davis for help with cell culture. I am also grateful to Dr. Eric Stanbridge for supplying the cells and for critical reading of the manuscript. This work was supported by NIH grant NS14609.

References

Barbacid, M. 1987. *ras* genes. *Annu. Rev. Biochem. 56*:779–827

Barres, B.A., Chun, L.L.Y., Corey, D.P. 1988. Ion channel expression by white matter glia: I. Type 2 astrocytes and oligodendrocytes. *Glia* **1**:10–30

Caffrey, J.M., Brown, A.M., Schneider, M.D. 1987. Mitogens and oncogenes can block the induction of specific voltage-gated ion channels. *Science* **236**:570–573

Catterall, W.A. 1980. Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. *Annu. Rev. Pharmacol. Toxicol.* **20**:15–43

Chen, C., Corbley, M.J., Roberts, T.M., Hess P. 1988. Voltage-sensitive calcium channels in normal and transformed 3T3 fibroblasts. *Science* **239**:1024–1026

Cohen, C.J., Bean, B.P., Colatsky, T.J., Tsien, R.W. 1981. Tetrodotoxin block of sodium channels in rabbit purkinje fibers. *J. Gen. Physiol.* **78**:383–411

De Vos, A.M., Tong, L., Milburn, M.V., Matias, P.M., Jancarik, J., Noguchi, S., Nishimura, S., Miura, K., Ohtsuka, E., Kim, S. 1988. Three-dimensional structure of an oncogenic protein: Catalytic domain of human c-Ha-*ras* p21. *Science* **239**:888–893

Friedrich, F., Paulmichl, M., Kolb, H.A., Lang, F. 1988. Inward rectifier K channels in renal epithelioid cells (MDCK) activated by serotonin. *J. Membrane Biol.* **106**:149–155

Geiser, A.G., Der, C.J., Marshall, C.J., Stanbridge, E.J. 1986. Suppression of tumorigenicity with continued expression of the c-Ha-*ras* oncogene in EJ bladder carcinoma-human fibroblast hybrid cells. *Proc. Natl. Acad. Sci. USA* **83**:5209–5213

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391**:85–100

Jaimovich, E., Chicheportiche, R., Lombet, A., Lazdunski, M., Ildefonse, M., Rougier, O. 1983. Differences in the properties of Na^+ channels in muscle surface and T-tubular membranes revealed by tetrodotoxin derivatives. *Pfluegers Arch.* **397**: 1–5

Lombet, A., Frelin, C., Renaud, J., Lazdunski, M. 1982. Na^+ channels with binding sites of high and low affinity for tetrodotoxin in different excitable and non-excitable cells. *Eur. J. Biochem.* **124**:199–203

Minna, L., Nelson, P., Peacock, J., Glazer, D., Niremberg, M. 1971. Genes for neuronal properties expressed in neuroblastoma *x* L-cell hybrids. *Proc. Natl. Acad. Sci. USA* **68**:234–239

Nakajima, Y., Nakajima, S., Inoue, M. 1988. Pertussis toxin-insensitive G protein mediates substance P-induced inhibition of potassium channels in brain neurons. *Proc. Natl. Acad. Sci. USA* **85**:3643–3647

Neer, E.J., Clapham, D.E. 1988. Roles of G protein subunits in transmembrane signalling. *Nature (London)* **333**:129–134

Stryer, L., Bourne, H.R., 1986. G proteins: A family of signal transducers. *Annu. Rev. Cell Biol.* **2**:391–419

Tong, L., de Vos, A.M., Milburn, M.V., Jancarik, J., Noguchi, S., Nishimura, S., Miura, K., Ohtsuka, E., Kim, S. 1989. Structural differences between a *ras* oncogene protein and the normal protein. *Nature (London)* **337**:90–93

Received 20 July 1989