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Connexin43 and connexin45 form gap junctions with different molecular permeabilities in osteoblastic cells

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We examined the expression and function of gap junctions in two rat osteoblastic cell lines, ROS 17/2.8 and UMR 106-01. The pattern of expression of gap junction proteins in these two cell lines was distinct: ROS cells expressed only connexin43 on their cell surface, while UMR expressed predominantly connexin45. Immunoprecipitation and RNA blot analysis confirmed the relative quantitation of these connexins. Microinjected ROS cells passed Lucifer yellow to many neighboring cells, but UMR cells were poorly coupled by this criterion. Nevertheless, both UMR and ROS cells were electrically coupled, as characterized by the double whole cell patch-clamp technique. These studies suggested that Cx43 in ROS cells mediated cell-cell coupling for both small ions and larger molecules, but Cx45 in UMR cells allowed passage only of small ions. To demonstrate that the expression of different connexins alone accounted for the lack of dye coupling in UMR cells, we assessed dye coupling in UMR cells transfected with either Cx43 or Cx45. The UMR/Cx43 transfectants were highly dye coupled compared with the untransfected UMR cells, but the UMR/Cx45 transfectants demonstrated no increase in dye transfer. These data demonstrate that different gap junction proteins create channels with different molecular permeabilities; they suggest that different connexins permit different types of signalling between cells.

Key words: cell-cell communication/connexin/gap junction/osteoblast

Introduction

Gap junctions contain aqueous channels connecting the cytoplasmic matrices of adjacent cells. Studies in several cell types have demonstrated that gap junction channels allow molecules as large as 1000 daltons to pass between coupled cells, and that these channels exhibit little charge selectivity (Flagg-Newton *et al.*, 1979; Neyton and Trautmann, 1985; Imanaga *et al.*, 1987). However, these studies were performed before any gap junction proteins had been identified. Over the last several years, ~12 related proteins, called connexins (Cx), have been identified as molecules that comprise gap junctions (Beyer *et al.*, 1990). Although electrophysiologic studies have revealed that different connexins form junctional channels with different unitary conductances and regulation (Veenstra *et al.*, 1992), the size selectivities of channels formed by different connexins have not been measured.

We examined connexin expression and cell coupling in two osteoblastic cell lines, ROS 17/2.8 (ROS) and UMR 106-01 (UMR). While both of these cell lines are derived from rat osteogenic sarcomas, ROS cells produce bone matrix proteins and may therefore represent a more highly differentiated state than UMR cells. We found that these two osteoblastic cell lines express different connexins on their cell surface and also differ in the molecular permeabilities of the gap junctions they form. These data suggested that the gap junctions expressed by these cells, Cx43 in ROS cells and Cx45 in UMR cells, have different molecular permeabilities. To examine this hypothesis, we overexpressed Cx43 and Cx45 in UMR cells and examined the ability of these transfectants to pass dye between cells.

Results

Expression of connexin mRNA in ROS and UMR cells

We performed RNA blots to determine whether ROS and UMR cells express members of the connexin family of gap junction proteins. Total cellular RNA was isolated from ROS and UMR cells and hybridized with ^{32}P-labelled cDNA probes for Cx26, Cx32, Cx37, Cx40, Cx43, Cx45 and Cx46. RNA from both cell lines hybridized to the Cx43 probe (Figure 1, left panel), although ROS cells appeared to express more Cx43 mRNA than did UMR cells. We

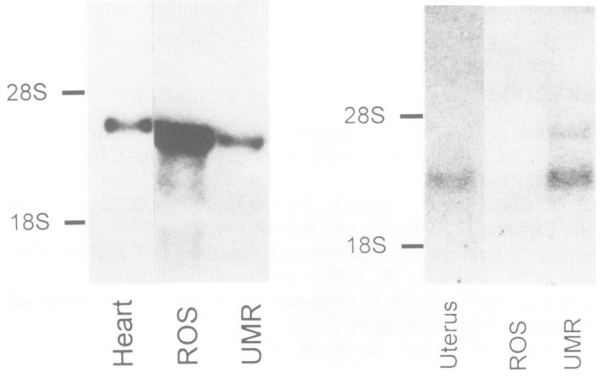

Fig. 1. Expression of Cx43 and Cx45 mRNA in rat osteoblastic cell lines. RNA blots of total RNA from ROS and UMR cells were hybridized with radiolabelled DNA probes for Cx43 (left panel, rat heart RNA as control) or Cx45 (right panel, rat uterus RNA as control).

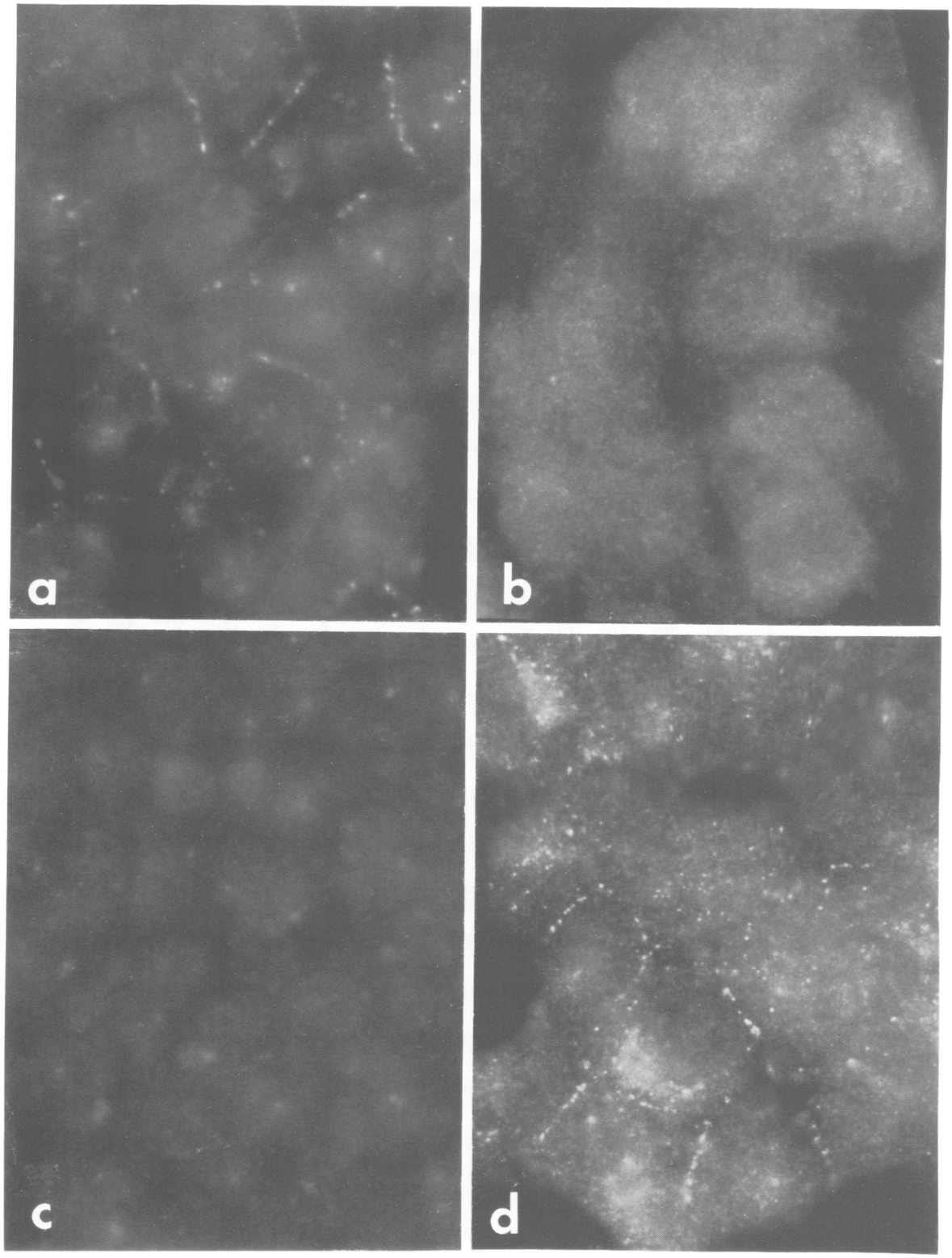

Fig. 2. Immunofluorescence staining of ROS and UMR cells for Cx43 and Cx45. ROS cells (**a** and **b**) and UMR cells (**c** and **d**) were fixed in methanol–acetone and immunostained for Cx43 (a and c) or Cx45 (b and d).

confirmed this observation by reprobing the RNA blots with an actin probe, quantitating the connexin and actin bands using densitometry, and expressing the relative abundance of RNA as the ratio of connexin to actin band density. We found that the ROS cells consistently expressed 3-fold more Cx43 mRNA than did UMR cells. UMR cells also expressed mRNA that hybridized to the probe for Cx45; the major band co-migrated with the band in rat uterus RNA (Figure 1, right panel). No Cx45 band was identified in ROS cell RNA. Both cells also expressed mRNA for Cx46 (data not shown). Neither cell line expressed mRNA for Cx26, Cx32, Cx37 nor Cx40 (data not shown).

Expression of connexin protein in ROS and UMR cells

The expression of Cx43 and Cx45 proteins was assessed by immunofluorescence and immunoprecipitation. These studies demonstrated a greater difference in the expression of Cx43 between ROS and UMR cells than did the RNA blots. By immunofluorescence, ROS cells demonstrated abundant specific staining for Cx43 in a linear punctate pattern at the plasma membrane between adjacent cells, characteristic of gap junctions (Figure 2a). In contrast, UMR cells displayed little, if any, specific staining for Cx43 (Figure 2c). Conversely, UMR cells displayed specific cell surface immunofluorescent staining for Cx45 (Figure 2d). ROS cells showed no reactivity (Figure 2b), consistent with their lack of Cx45 mRNA (Figure 1). In all four instances, immunostaining performed with preimmune serum did not show the punctate fluorescence pattern characteristic of specific gap junction staining (not shown). Staining of both the UMR and ROS cells with either a polyclonal or monoclonal antibody for Cx46 revealed a punctate intracellular staining pattern; no plasma membrane staining for Cx46 was identified (not shown).

To confirm the relative expression of the gap junction proteins in the rat osteoblastic cell lines, we metabolically labelled cells with [^{35}S]methionine and immunoprecipitated with anti-Cx43 or anti-Cx45 antibodies, preimmune serum or immune serum plus 100 μg/ml of the immunizing peptide. In both ROS and UMR cells, labelled protein bands of ~40 and 42 kDa were precipitated with anti-Cx43 immune serum but not with preimmune serum or in the presence of peptide (Figure 3, top panel). These bands correspond, respectively, to non-phosphorylated and phosphorylated forms of Cx43, as demonstrated by the presence of only the lower molecular mass form of the protein in phosphatase-treated samples (data not shown; Musil *et al.*, 1990). Both forms of Cx43 were much more abundant in ROS cells than in UMR cells; however, the relative amounts of the high and low molecular mass forms of the protein were similar in both cell types. Affinity-purified anti-Cx45 precipitated a single 48 kDa protein from UMR lysates, but precipitated no protein from ROS cells. Precipitation of this polypeptide from UMR was specifically inhibited by the immunizing peptide (Figure 3, bottom panel). This confirmed that UMR cells expressed Cx45 protein whereas ROS cells did not. Thus the two osteoblastic cell lines ROS and UMR differ in the gap junction proteins they express: ROS cells expressed only Cx43 on the cell surface, while UMR cells expressed predominantly Cx45.

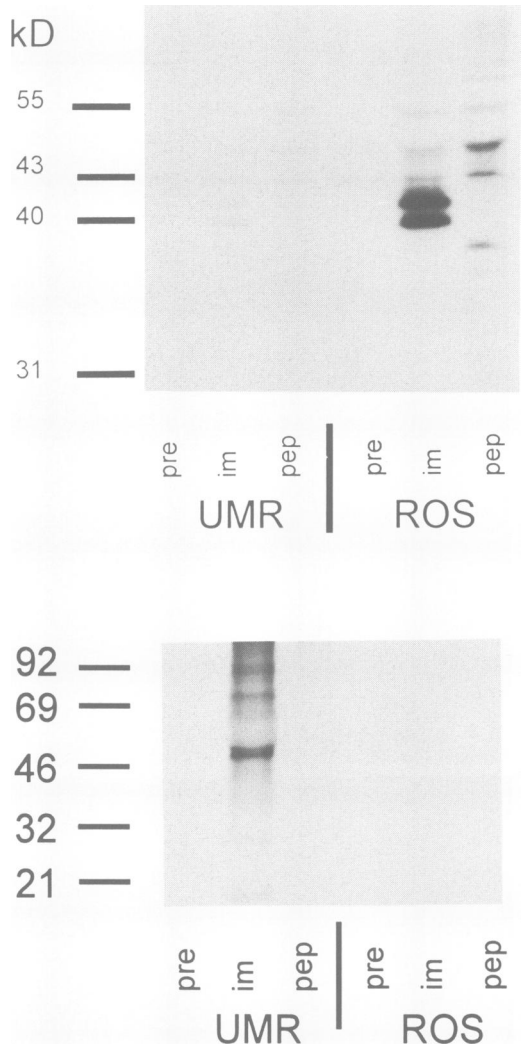

Fig. 3. Immunoprecipitation of Cx43 (**top**) and Cx45 (**bottom**) in rat osteoblastic cell lines. ROS and UMR cells were metabolically labelled with [^{35}S]methionine and immunoprecipitated with preimmune serum (pre), immune serum (im) or immune serum and 100 μg/ml of the immunizing peptide used to generate the antibody (pep). Top panel: anti-Cx43 serum; bottom panel: anti-Cx45 antibodies.

ROS cells are highly dye-coupled but UMR cells transfer dye poorly

We assessed the presence of functional gap junctions in ROS and UMR cells using dye transfer and electrophysiologic studies. Transfer of the fluorescent dye Lucifer yellow from a single microinjected cell to its neighbors has been used widely as a measure of gap junction patency, usually in cells expressing Cx32 or Cx43. We found that ROS cells were highly coupled by this assay (Figure 4). Up to 25 cells were coupled to each microinjected cell and only 18% of injected cells showed no dye passage. The average number of cells coupled to microinjected ROS cells was 9.1 ± 7.2 (mean ± SEM, n = 82). Frequently Lucifer yellow diffused to cells that were three or four cells removed from the microinjected cell. Dye transfer in ROS cells was abolished by addition of 2 mM octanol or 1 mM heptanol, inhibitors of gap junctional permeability. On the contrary, the UMR cells were poorly coupled as assessed by dye transfer. In the majority of these cells, the fluorescent dye did not diffuse to any other cell, with an average coupling of 1.4 ± 1.8 (mean ± SEM, n = 100). Dye passage did not increase appreciably when observation periods were extended beyond 5 min. Similar results were obtained when the dye carboxyfluorescein was microinjected into cells (not shown).

Both ROS and UMR cells are electrically coupled

To determine whether UMR cells expressed functional gap junctions, we examined electrical coupling between these cells using the double whole cell patch–clamp technique (Figure 5). Electrical coupling was detected in 50% of the UMR cell pairs examined with junctional conductances of 1–2 or 8–11 nS (5.5 ± 4.8 nS, mean ± SD, n = 4). Instantaneous junctional currents increased linearly with transjunctional voltage over ± 100 mV range. A pronounced time- and voltage-dependent relaxation of junctional current

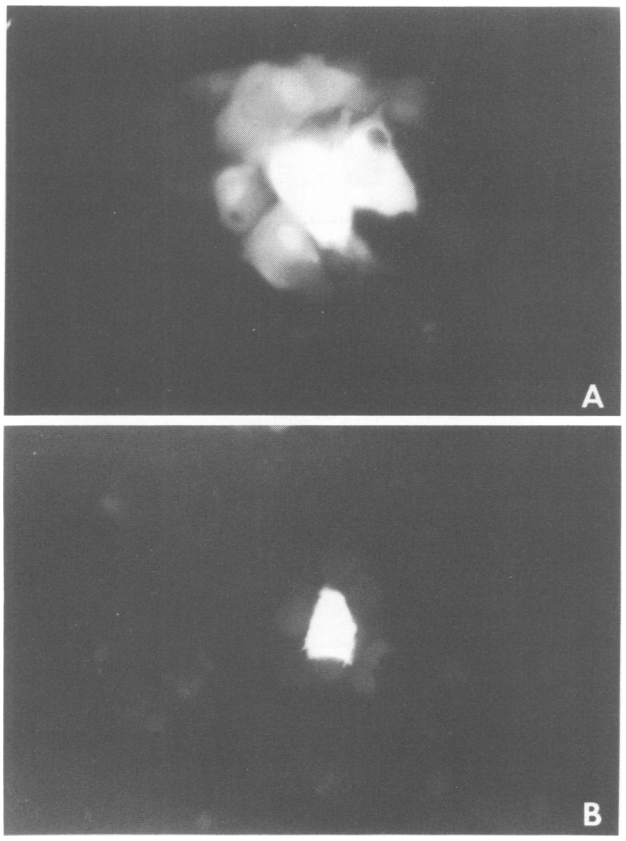

Fig. 4. Dye coupling in ROS and UMR cells. Cell adherent to glass coverslips were microinjected with 10 mM Lucifer yellow. Transfer of dye to neighboring cells was assessed by epifluorescence microscopy 3–5 min later. **A**, ROS cells; **B**, UMR cells.

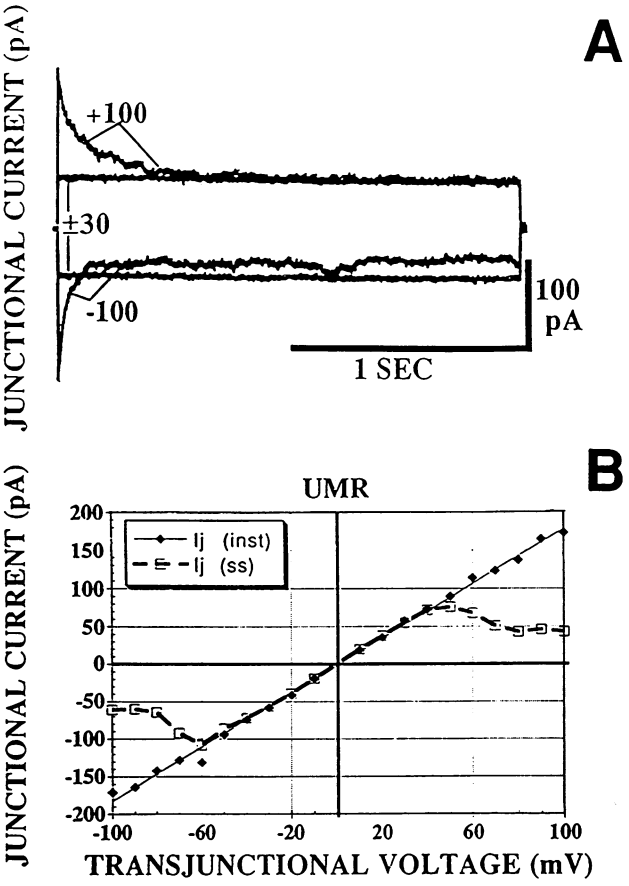

Fig. 5. Double whole cell patch–clamp studies of UMR cells. (**A**) Whole cell junctional current traces obtained from the unpulsed cell of a UMR cell pair. Both cells were voltage clamped to −40 mV, a 2 s duration voltage pulse was applied once every 7 s to cell 1 and junctional currents were recorded from cell 2. (**B**) Instantaneous (filled diamonds) and steady-state (open boxes) junctional current–voltage relationship for the same experiment illustrated in panel A over the entire ±100 mV transjunctional voltage range. Instantaneous and steady-state junctional currents were measured from the first and last 10 ms of each current pulse, respectively.

was observed in the 1.2 and 1.8 nS UMR cell pairs when transjunctional voltage exceeded ±50 mV. Steady-state junctional currents were reduced by a maximum of 75% at transjunctional voltages of ±100 mV, consistent with the presence of a strongly voltage-dependent gap junctional channel. Electrical coupling was also evident in 75% of the ROS cell pairs examined (data not shown). Total junctional conductance ranged from 4 to 31 nS (16.5 ± 10.0 nS, n = 6). No time- or voltage-dependent decline in junctional current was seen in ROS cell pairs. In both UMR and ROS cells, the gap junction inhibitor heptanol blocked the observed junctional conductances.

These electrophysiologic studies demonstrate that although UMR cells are not 'chemically coupled' as assessed by transfer of the dye Lucifer yellow among cells, they are 'electrically coupled' and by inference express gap junctions that allow small ions to pass between cells.

Dye coupling in UMR transfectants

The above experiments suggested that UMR cells were poorly dye-coupled because they expressed little Cx43. However, we could not exclude the possibility that some other difference between UMR and ROS cells accounted for the lack of dye transfer in UMR cells. To compare dye permeability of Cx43 and Cx45 channels in the same cell type, we overexpressed either Cx43 or Cx45 by stable transfection of UMR cells. Single cell clones were selected in geneticin, harvested and analyzed by RNA blots and immunofluorescence for expression of Cx43 and Cx45. RNA blots of the transfected cells revealed hybridizing bands both for the endogenous connexins and for mRNA corresponding to the transfected protein (not shown). By immunofluorescence, the UMR cells transfected with Cx43 (UMR/Cx43) demonstrated a high level of cell surface staining for Cx43 (Figure 6a). Similarly, the cells transfected with Cx45 (UMR/Cx45), displayed markedly increased cell surface staining for Cx45 (Figure 6b). In both transfectants the staining was similar to the pattern seen in ROS cells stained for Cx43. UMR/Cx45 transfectants did not show increased staining with anti-Cx43 sera (not shown); their appearance was similar to that of the parental cells.

We next microinjected Lucifer yellow into single cells from monolayers of UMR, UMR/Cx43 and UMR/Cx45 (Figure 7). The UMR cells (n = 144) again demonstrated little dye transfer, with only 21% of cells passing dye to more than one neighbor. The cells transfected with Cx43 showed a marked increase in dye coupling. On average, each microinjected UMR/Cx43 cell (n = 55) transferred Lucifer yellow to 5.4 neighboring cells and 78% of microinjected cells passed dye to more than one neighboring cell. In contrast, there was no increase in dye coupling in the UMR/Cx45 transfectants (n = 110). Only 21% of these cells

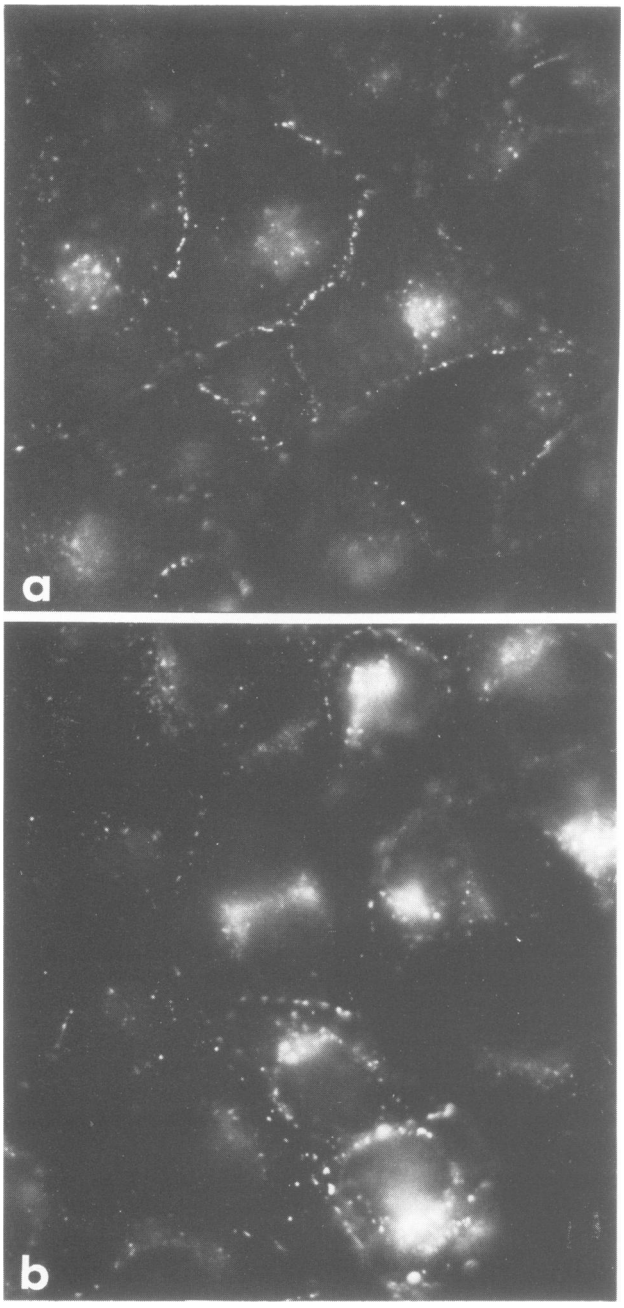

Fig. 6. Immunofluorescence studies of UMR cell transfectants. (**a**) UMR cells transfected with Cx43 and stained with anti-Cx43; (**b**) UMR cells transfected with Cx45 and stained with anti-Cx45.

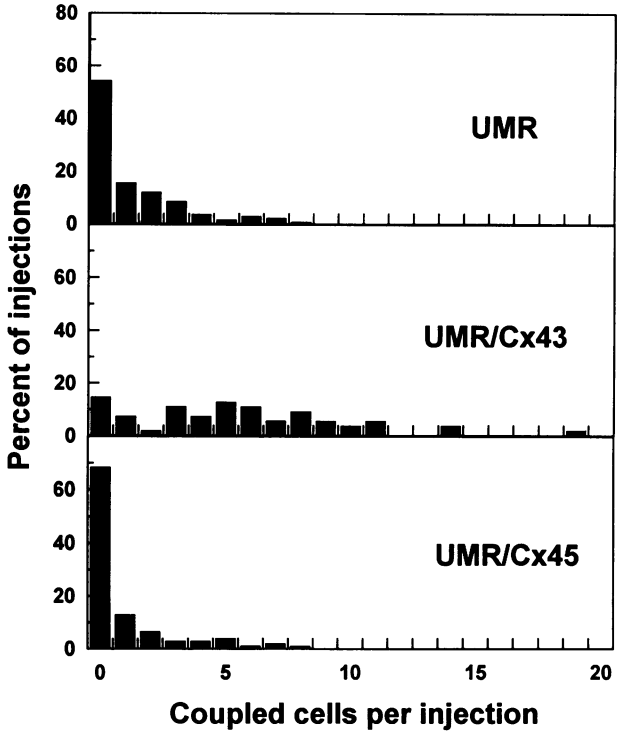

Fig. 7. Dye coupling in UMR cells and Cx43 or Cx45 transfectants. UMR (n = 144), UMR/Cx43 (n = 55) and UMR/Cx45 (n = 110) were microinjected with Lucifer yellow and the number of neighboring cells that contained dye at 2 min was assessed.

passed dye to more than one neighbor and the dye transfer histograms of these transfectants and the parent UMR cells were similar.

To determine whether differences in whole cell conductance could account for the differences in dye transfer, we also measured junctional conductances in the UMR cell transfectants and compared these with additional measurements in the parent UMR cell line. The mean conductances were similar in all cells: 4.95 ± 0.91 nS for parent UMR (n = 11), 5.58 ± 1.19 nS for UMR/Cx43 (n = 15) and 3.18 ± 1.22 nS for UMR/Cx45 (n = 12). One cell pair from each transfectant was found to have a junctional conductance of >15 nS, significantly higher than the rest of the population. These studies confirm that gross alterations in whole cell conductance could not account for the different abilities of UMR/Cx43 and UMR/Cx45 to transfer dye. We further analyzed the voltage dependence of the transjunctional currents in the three different cells. Analysis of the junctional current–voltage (I_j-V_j) relationships for the additional pairs from the parent UMR cell line confirmed the pronounced voltage-dependent decreases in junctional current reported above (Figure 5). In contrast, the I_j-V_j relationships for the UMR/Cx43 and UMR/Cx45 clones were somewhat more complicated; the majority of pairs transfected with either connexin yielded I_j-V_j curves demonstrating asymmetric steady-state voltage dependence (data not shown). While the analysis of these data is complicated, the results confirmed that overexpression of the transfected connexin altered the normal behavior of these cells.

Discussion

These studies demonstrate that two different gap junction proteins, Cx43 and Cx45, form channels with different molecular permeabilities in osteoblastic cells. Two lines of evidence support this conclusion. First, gap junctional communication in ROS cells, which express only Cx43 on their cell surface, allowed both intercellular transfer of the dye Lucifer yellow and electrical coupling of cells. UMR cells, which express predominantly Cx45 on the plasma membrane, were electrically coupled but failed to pass Lucifer yellow between cells. Second, UMR cells transfected with Cx43 allowed extensive intercellular transfer of Lucifer yellow whereas the parent UMR cells and UMR/Cx45 transfectants were poorly coupled. Electrophysiological studies on these transfectants demonstrated that their whole

cell conductances were not dramatically altered, indicating that differences in dye transfer could not be attributed to alterations in total conductance through coupled cells. Nevertheless, in both transfectants immunofluorescence studies revealed characteristic junctional immunofluorescence and in both transfectants current−voltage relationships were different from those of the parent cell line, suggesting that the transfected connexins were functional in both instances.

Our data argue that Cx43 is responsible for coupling in ROS cells and that Cx45 mediates cell−cell communication in UMR cells. The double whole cell patch−clamp studies of UMR cells demonstrated a strongly voltage-dependent conductance, characteristic of Cx45 but not Cx43. Thus the electrophysiologic data are consistent with the protein and RNA data that Cx45 mediates cell−cell communication in UMR cells and suggest that Cx45 channels are smaller than Cx43 channels. In transfected cells, Cx43 forms channels with a unitary conductance of 45−50 pS (Fishman *et al.*, 1990; Veenstra *et al.*, 1992) while Cx45 forms channels of 22−30 pS (Veenstra *et al.*, 1992). Thus the relative size of the single channel conductance in junctions formed by these two connexins is consistent with the reduced molecular permeability of Cx45 channels.

Cx45 gap junctions in UMR cells were not permeable to dyes such as Lucifer yellow (mol. wt 457). This was also true in UMR/Cx45 transfectants that overexpressed Cx45 protein. Lucifer yellow is well below the gap junction permeability limit of ~1000 daltons defined in previous studies. However, most studies of gap junctional molecular permeability antedated the identification of the specific connexins involved and many of these studies were performed on fibroblastic cells that express Cx43. Clearly, the size selectivity of gap junctions formed by individual connexins will need to be investigated in carefully defined systems. An ideal system would be a cell devoid of endogenous connexins, but which supported gap junctional communication after cloned connexins are introduced. Unfortunately, currently available models, such as SK Hep1 which expresses endogenous connexins and N2A which supports only limited dye coupling when transfected (Beyer *et al.*, 1992), do not fulfill these criteria. It is also evident from the current work that dye transfer experiments cannot be used as a sole criterion for cell coupling.

One implication of this work is that gap junctions formed by different connexins may be capable of transmitting different types of signals. It is probable that all junction channels mediate electrical coupling of cells by allowing passage of small ions. This type of electrical coupling is important in coordinating contractility in cardiac and smooth muscle. At least some gap junctions also allow transfer of molecules as large as 1000 daltons, including intracellular second messengers such as cyclic nucleotides and inositol phosphates (Saez *et al.*, 1989). Our studies suggest that these intracellular messengers can diffuse between cells coupled by Cx43, but not cells coupled by Cx45. It is possible that cells control the type of gap junctional intercellular communication that they employ by altering the connexins they express. Different connexins are expressed preferentially by certain cells and at certain stages of development (Gimlich *et al.*, 1990; Nishi *et al.*, 1991); this might regulate intercellular diffusion of molecular second messengers and in some instances allow only ionic coupling of cells.

Thus, our data demonstrate that gap junction channels formed by different connexins can have different molecular permeabilities, and specifically that Cx45 forms channels that have a smaller size exclusion than do channels formed by Cx43. These differences may dictate the nature of the intercellular communication supported by different connexins.

Materials and methods

Reagents

Lucifer yellow CH was purchased from Molecular Probes (Eugene, OR). The polyclonal antibodies detecting Cx43 and Cx45 have been previously reported. They were generated by immunizing rabbits with synthetic peptides corresponding to amino acids 252−271 from rat Cx43 (Beyer and Steinberg, 1991) and amino acids 285−298 from dog Cx45 (Kanter *et al.*, 1992). The anti-Cx45 serum was affinity-purified on peptide−agarose and has been characterized previously (Kanter *et al.*, 1993). Rhodamine-conjugated goat anti-rabbit IgG was from Boehringer Mannheim (Indianapolis, IN). Antibodies specific for Cx46 were kind gifts of L.J.Takemoto (Paul *et al.*, 1991) and C.Louis (Tenbroek *et al.*, 1992).

Cell culture

The cell line UMR 106-01 was a gift of Dr Nicola C.Partridge (St Louis University). These cells are derived from the rat osteogenic sarcoma cell line UMR 106, which has been characterized as having an osteoblastic phenotype (Shupnik and Tashjian, 1982; Boland *et al.*, 1986). These cells fail to produce the bone matrix protein osteocalcin (Fraser *et al.*, 1988); they may therefore represent osteoblasts in an early stage of differentiation. They were maintained in Eagle's MEM, supplemented with 10% FBS. The ROS 17/2.8 cells are a subclone of ROS 17/2 cell line, originally derived from a transplantable rat osteosarcoma. They express several osteoblastic features, including production of matrix protein, which suggests they are relatively more differentiated than UMR (Majeska *et al.*, 1980, 1985). They were provided by Dr Gideon Rodan (Merck, Sharp & Dohme, West Point, PA) and were maintained in DMEM/Ham's F-12 (1:1), supplemented with 10% FBS.

RNA blots

RNA blots were performed as previously described (Beyer and Steinberg, 1991). Total cellular RNA was isolated using guanidinium isothiocyanate (Chomczynski and Sacchi, 1987). Samples (10 μg/lane) were separated on formaldehyde−agarose gels and transferred to nylon membranes. The membranes were hybridized (0.75 M sodium phosphate, 1.0% SDS, 100 μg/ml salmon sperm DNA, 65°C) and washed under high stringency conditions (30 mM sodium phosphate, 1.0% SDS, 65°C). The following ^{32}P-labelled cDNA probes were used: rat Cx26 (Zhang and Nicholson, 1989), Cx32 (Paul, 1986), Cx37 (Reed *et al.*, 1993), Cx40 (Beyer *et al.*, 1992), Cx43 (Beyer *et al.*, 1987), Cx45 (Kanter *et al.*, 1992) and Cx46 (Paul *et al.*, 1991).

To confirm the integrity of the mRNA present on these blots, we reprobed the membranes with a human fibroblast γ actin cDNA probe (Gunning *et al.*, 1983) and found similar actin bands on all samples. To quantitate Cx43 mRNA, we performed densitometry on the RNA blots and normalized the density value of Cx43 mRNA for that of actin mRNA present.

Immunoprecipitation

Cells were cultured on 100 mm tissue culture plates to 75−85% confluence, incubated in methionine-free medium for 30 min and labelled with [^{35}S]Trans-S (300 μCi/plate) for 6 h. Cells were then washed and solubilized in 150 mM NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 0.5% BSA, 50 mM Tris, pH 8 containing a cocktail of protease and phosphatase inhibitors. After cell solubilization radioactivity was measured in a 5 μl aliquot from each sample and equivalent c.p.m. were used for each precipitation. Samples were clarified and supernatants were divided into aliquots, incubated overnight in 5 μl of preimmune serum, immune serum or immune serum with 100 μg/ml of the immunizing peptide at 4°C, precipitated with protein A−Sepharose and boiled in Laemmli sample buffer for 10 min. Samples were electrophoresed on polyacrylamide gels and autoradiograms were performed.

Immunofluorescence

Cells adherent to glass coverslips were fixed in 50% methanol−50% acetone for 2 min at room temperature and incubated in primary (rabbit polyclonal) and secondary (goat anti-rabbit IgG, rhodamine conjugated) antibodies at

room temperature for 45 min with intervening washes. Coverslips were viewed on a Zeiss axioscope with epifluorescence.

Dye coupling

Cell–cell dye coupling was assessed by intercellular transfer of microinjected Lucifer yellow as described (Civitelli *et al.*, 1993). For the initial ROS and UMR cell experiments, cells adherent to 31 mm glass coverslips were grown to 80% confluence. The coverslips were mounted in a tissue chamber (Biophysica Technology, Baltimore, MD) and placed on the stage of an inverted epifluorescence microscope. Cells from confluent areas of the culture were microinjected with 10 mM Lucifer yellow (1100–1200 p.s.i. applied for 0.2–0.3 s). Lucifer yellow fluorescence was monitored using a low light silicon intensified target camera (SIT-66, Dage MTI, Michigan City, IN) and the number of adjacent cells containing dye 3–5 min after the injection was recorded. For the UMR transfection experiments, cells were plated on plastic tissue culture dishes and cell coupling was assessed 2 min after injection.

Electrophysiological studies

The junctional conductances of paired ROS, UMR and UMR transfectants were directly determined using the double whole cell patch–clamp recording technique as previously described (Veenstra, 1990; Rup *et al.*, 1993). Patch electrodes were filled with 100 mM potassium glutamate, 15 mM NaCl, 1 mM KH_2PO_4, 4.6 mM $MgCl_2$, 0.68 mM $CaCl_2$, 5 mM EGTA, 3 mM Na_2ATP, 3 mM sodium phosphocreatine, 25 mM HEPES, pH 7.1. Recordings were performed on cells incubated at room temperature in buffered saline solution (142 mM NaCl, 1.3 mM KCl, 0.8 mM $MgSO_4$, 0.9 mM NaH_2PO_4, 1.8 mM $CaCl_2$, 5.5 mM dextrose, 10 mM HEPES, pH 7.2). Transjunctional voltage differences (5 s pulses, four per minute) were applied to calculate transjunctional conductance.

Transfection of UMR cells

UMR cells expressing high levels of Cx43 or Cx45 were generated by transfecting these cells with a construct containing the pSFFVneo vector (Carel *et al.*, 1989) and a cDNA encoding either rat Cx43 (Beyer *et al.*, 1987) or chick Cx45 (Beyer, 1990). These constructs have previously been shown to produce significant electrical coupling in transfected N2A cells (Veenstra *et al.*, 1992). Cells were transfected using lipofectin and selected in 0.50 mg/ml geneticin. Several single cell colonies were selected and screened by RNA blot and immunofluorescence. All successful transfectants expressed mRNA for the transfected protein and higher levels of protein than the parent UMR cells.

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