Selective Gap Junctional Communication within the V79-4 Chinese Hamster Cell Line

(gap junctions / selective communication / ethylene glycol / dye coupling / connexin43)

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Abstract. The probability of cell-to-cell coupling between directly adjacent cells (communication capability) in the V79-4 Chinese hamster cell line was evaluated under standard conditions or after 18-h treatment with EG. The cell monolayer did not form a continuous network of cells interconnected via gap junctions, but an average cell was coupled to only one half of its directly adjacent neighbours under standard conditions, or to one third of its directly neighbouring cells after 18-h exposure to EG. The rest of the directly adjacent neighbours did not establish functional gap junctions with an injected cell, although they were competent to couple to other cells with a probability similar to that of the coupling between the injected cell and its direct neighbours. Moreover, all the cells possessed the identical connexin – cx43, present on all cell membranes. The results indicated that the choice of a cell to which neighbour be coupled was rather random in the standard cell population as a whole, although the population contained some clones whose capability to couple was more or less different from that of the original cell population. Ethylene glycol reduced the gap junctional communication by increasing the frequency of cells not coupled to any of their direct neighbours from 1% for untreated cells to 23.3% for cells exposed to EG, and consequently by reducing the number of directly adjacent cells coupled to communicating injected cells. The communication capability of the cell population appeared to be unstable. It varied slightly in time and so did the response of the cells to EG. The results indicate that a cell can control its coupling to different directly adjacent neighbours independently, being able to control the gap junctional communication not only *in time* but *in space* as well. All control mechanisms of GJIC, known so far, affect a cell as a whole, while our results indicate that another regulatory mechanism may exist, controlling the gap junctional communication to different adjacent neighbouring cells independently.

Small molecules (up to 1000 daltons) such as various metabolites, cell signal molecules, including secondary messengers, diffuse between adjacent cells via membranous channels – gap junctions – which consist of a highly conserved family of proteins – connexins. This gap junctional intercellular communication (GJIC) mediates transport of molecules within and even between tissues, and it is considered as essential for the maintenance of homeostasis and the differentiation state with a tissue, transmission of various biological signals, synchronization of response to extracellular signals within a tissue, and for the nourishment of some highly specialized cells (Dermietzel, 1993; Banoub et al., 1996; Bruzzone et al., 1996; Munari-Silem and Rousset, 1996; Yamasaki and Naus, 1996; Brink and Barr, 2000; Perracchia, 2000). Impairments of GJIC are associated with the development of various pathological disorders including carcinogenesis, teratogenesis, Charcot-Marie-Tooth disease, Chagas' disease, nonsyndromic inherited deafness, various cardiomyopathies, etc. (Krutovskikh and Yamasaki, 2000; Peracchia, 2000). GJIC is inhibited by various agents of intra- or extracellular origin. In our experimental system we use ethylene glycol (EG) – a potent inhibitor of GJIC in cell cultures (Vítek, 1993; 1997).

While the gap junctional communication has been extensively studied at the level of cell populations, few data are available regarding the transport of biological molecules from a single cell to its directly adjacent neighbours. It is generally assumed that the molecules diffuse via gap junctions from a cell to all directly neighbouring cells, providing they are GJIC-competent and their gap junctions consist of identical or similar connexins. However, it has been shown that some cells do not communicate directly with all of their adjacent neighbours. For example, Banoub (1996) reported that the non-transformed mouse lung C10 cells were coupled to 75–85% of their neighbours and the neoplastic lung epithelial cells to only 10–35% of their directly adjacent cells. For this phenomenon that a cell is coupled to only a portion of its directly adjacent cells, the

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Abbreviations: cx43 – connexin43, DC – dye coupling, EG – ethylene glycol, GJIC – gap junctional intercellular communication.

term *selective communication* will be used in the present report. The subject of this paper is to deal with this phenomenon in detail.

We observed that V79-4 Chinese hamster cells were coupled to only a portion of their directly adjacent neighbours in spite of the fact that the non-coupled neighbours were also coupling-competent and contained the identical connexin. The treatment with the inhibitor of GJIC – ethylene glycol – increased the selectivity of communication between adjacent cells, i.e. a cell, under such conditions, chose a neighbouring cell to couple more strictly. The results indicate that a cell can control its gap junctional communication not only in time, but also in space.

Material and Methods

Terms

The following terms were used in this paper (see Fig. 1):

primary neighbours: cells directly adjacent to an injected cell

communicating primary neighbours: primary neighbours dye-coupled to an injected cell

"non-communicating" primary neighbours: primary neighbours not dye-coupled to an injected cell

secondary neighbours: cells directly adjacent to a primary neighbour but not to an injected cell

communication capability: probability of dye coupling between an injected cell and its primary neighbour expressed as the average percentage of primary neighbours to which the dye from an injected cell spread

non-communicating cell: an injected cell not dye-coupled to any of its primary neighbours

Cells and culture conditions

The V79-4 Chinese hamster cell line and its six independent clones were used. Four of them (II, III, IV, and VI) were isolated independently by cloning in 96-well plates (Corning, Corning, NY), while the other two (XIV and XV) were originally derived as independent clones resistant to 6-thioguanine. The cells were maintained in Eagle's minimum essential medium supplemented with non-essential amino acids (Bio Whittaker, Walkersville, MD), 5% foetal bovine serum (Bioclot, Aidenbach, Germany), and gentamicin 50 μg/ml (Lek, Ljubljana, Slovenia).

Analysis of GJIC by fluorescent dye microinjection

6.5×10^5 cells were plated into 35-mm Petri dishes (Corning, Corning, NY). Ethylene glycol (EG) produced by Serva (Heidelberg, Germany) was applied in the medium, if required, 4 h after the seeding to the final concentration of 40 μl/ml. At the same point of time standard medium in control dishes was exchanged. After 18 h there followed the assessment of GJIC by the dye-coupling method (DC). We used a TMD Nikon Diaphot inverted microscope (Nikon, Tokyo, Japan) equipped with an MMW-23 water hydraulic micromanipulator (Narishige, Tokyo, Japan) and epifluorescence illumination. The cells were injected with borosilicate glass capillary micropipets pulled with a P-97 puller

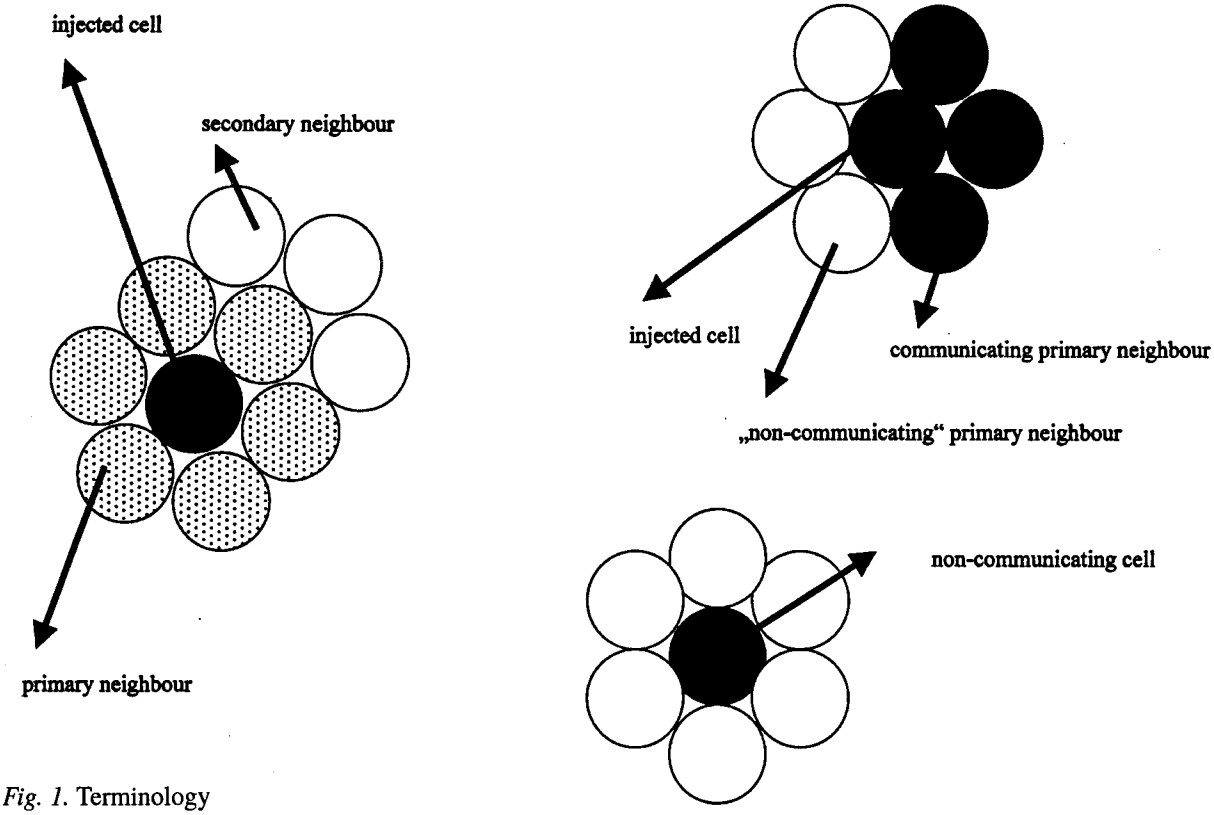

Fig. 1. Terminology

(Sutter Instrument Company, Leveroni Court Novato, CA). The micropipets were backfilled with Lucifer yellow CH (dipotassium or dilithium salt, Sigma, St. Louis, MO, 0.625% in 0.9% NaCl). The dye was introduced into the cells by a dual microiontophoresis current generator (2–10 nA, WPI, Sarasota, FL). The spread of dye to directly adjacent neighbouring cells (dye coupling) was assessed 1 min later. Fifteen cells per dish were injected. The dye coupling was evaluated as a total number of directly adjacent neighbours coupled to injected cells (A1) in relation to the total number of directly adjacent neighbours not coupled to injected cells (A2). The results of all dishes of one treatment group were evaluated together. Statistical significance was calculated by the χ^2 test or Student's t-test (Medcalc, Mariakerke, Belgium).

Immunocytochemical detection of connexins

Cells grown on microscopic coverslips were fixed by methanol : acetone (1 : 1), and non-specific binding sites were blocked by 0.2% bovine serum albumin (Vector, Burlingame, CA). Then followed incubation with the primary anti-connexin antibody (anti-cx43, 5 μg/ml, Zymed, San Francisco, CA), with the secondary antibody (biotinylated anti-mouse/anti-rabbit IGG, 20 μg/ml, Vector, Burlingame, CA), and with streptavidin labelled with fluorescein (30 μg/ml, Vector, Burlingame, CA), 1 h each. After mounting in glycerol the fluorescence was evaluated using an Eclipse E600 microscope (Nikon, Tokyo, Japan). In control coverslips the primary antibody was omitted.

Results

The initial task of this study was to evaluate the communication capability of the original cell population. The results are shown in Table 1. We injected 100 cells and assessed the total number of communicating primary neighbours (A1), total number of "non-communicating" primary neighbours (A2), and calculated the communication capability (%). It appeared that an injected cell communicated approximately with a half of its primary neighbours (communication capability = 52.6%). Only 1% of the injected cells did not communicate with any of their primary neighbours (non-communicating cells).

In order to find whether the "non-communicating" primary neighbours were GJIC-competent we injected their directly adjacent secondary neighbours and evaluated the dye coupling between the secondary neighbours and the "non-communicating" primary neighbours (the secondary neighbours not coupled to any other secondary neighbour were not included). The observed communication capability was 54.8% (Table 2). Consequently, the results clearly showed that the injected cells did not communicate with approximately 50% of their primary neighbours, although the "non-communicating" primary neighbours were dye-coupling competent as well, and were coupled to the secondary neighbours with a similar probability.

When the cells were exposed to EG for 18 h, the communication capability decreased to 25.1% (Table 1). At the same time the frequency of non-communicating cells increased from 1% in the control population to 23.3% in treated cells. However, when the non-communicating one fourth of the treated population was not taken into account, the communication capability increased to 33.3%. This value corresponded to that found for the probability of coupling between the "non-communicating" primary neighbours and the secondary neighbours under similar experimental conditions (Table 2). Thus, the treated "non-communicating" primary neighbours were coupled to secondary neighbours with the same probability as an injected cell to its primary neighbours, just as it was determined in the untreated cell population.

Table 1. Communication capability of the V79-4 cell line under standard conditions (control) or after treatment with ethylene glycol (EG, 18 h).

	DCO/ No. of injected cells	DCO (%)	A1/A2 – DCO	A1*100/ (A1+A2), (%)	A1/A2 +DCO	A1*100/ (A1+A2), (%)
control	1/100	1.0	331/293	53.0	331/298	52.6
chi (th.)			1 : 1		1 : 1	
P			0.3094		0.3846	
EG (18 h)	14/60	23.3	97/194	33.3	97/290	25.1
chi (th.)			1 : 2		1 : 3	
P			0.9340		0.9505	

A1 – No. of communicating primary neighbours
A2 – No. of "non-communicating" primary neighbours
DCO – No. of non-communicating cells
–DCO – non-communicating cells were omitted
+DCO – including non-communicating cells
chi (th.) – theoretical values of χ^2