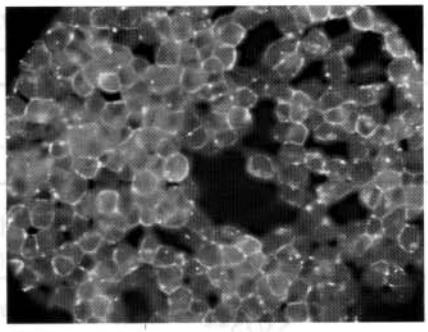

*Fig. 4.* Immunocytochemical detection of cx43 in the original cell line. Connexin43 is present, in most cells, on cell membranes shared with all neighbouring cells, in spite of the fact that an average cell is coupled only to a half of its adjacent neighbours. Magnification 400x.

XIV was tested 8 times while clone XV even 15 times at intervals of one or two days. The results depicted in Fig. 5 demonstrate the variability of the values studied in time both under standard conditions and after treatment with EG.

## Discussion

The V79-4 Chinese hamster cell population does not form a network of cells continuously coupled via gap junctions, as is shown in this report. An average cell is coupled to only a half of its primary neighbours. The principal question of this study is: why do the cells not communicate to the second half of their primary neighbours? In this report we tested whether the "non-communicating" primary neighbours were dye-coupling incompetent, or in a stage of the cell cycle with a diminished communication capability. The experimental answer was negative. We found that the „non-communicating" primary cells were in fact dye-coupling competent and, moreover, at the time of the test they communicated to the secondary neighbours with a probability similar to that found for the communication between an injected cell and its primary neighbours. Similar results were found even for cells exposed to EG. Consequently, a cell communicates selectively under both, i.e. standard conditions as well as during the treatment with EG, choosing only a portion of its neighbours to communicate with. The probability of communication between two directly adjacent cells found for the original cell population (P = 0.5) offers the explanation that a standard cell chooses its neighbour to couple randomly.

The population distributions of cells coupled to different portions of their primary neighbours resembled, under standard conditions, the *normal distribution*, at least for the original cell population and some of the clones (see Fig. 3). The portions of non-communicating cells among the clones were also very variable and always higher than the value found for the original cell line (Table 3). All these findings clearly indicate that the

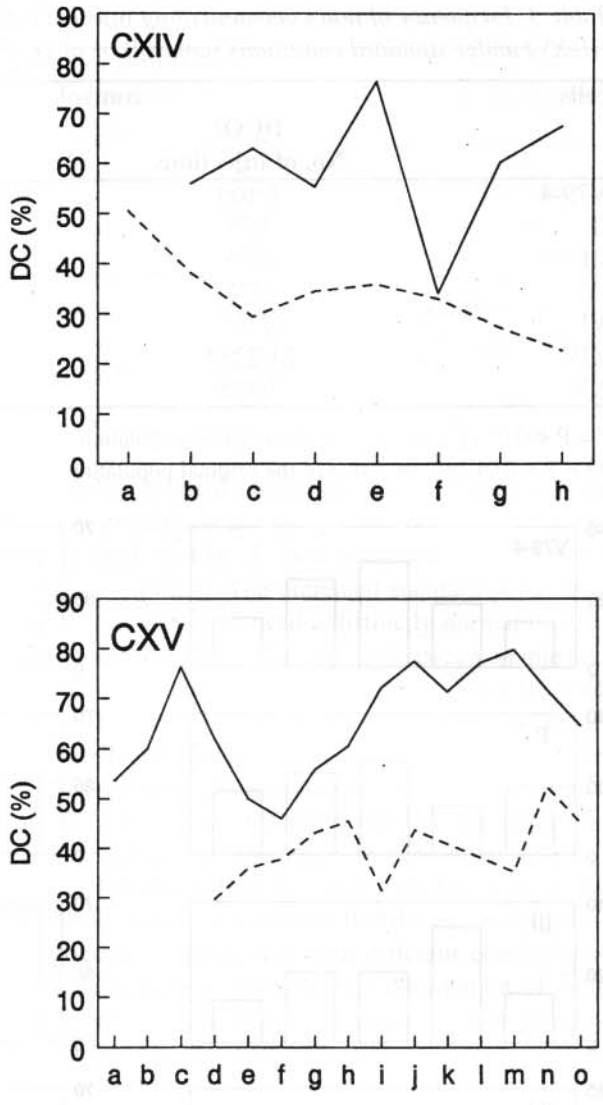


*Fig. 5.* Variations in the communication capability in time under standard conditions or after treatment with EG (intermittent line) in two clones derived from the original cell line (each value represents the result of 15 injections/Petri dish)
X-axis - time intervals of the assessment ( always 1 or 2 days)
Y-axis – average percentage of primary neighbours coupled to an injected cell (%) = communication capability

V79-4 cell population is not entirely homogeneous but contains some clones whose capabilities to communicate with their directly adjacent neighbours decline more or less from that of the original cell population as a whole.

It has to be noted that the results concerning the percentage of communicating or "non-communicating" primary neighbours as obtained by our technique were not always fully true. We observed repeatedly that a cell that we classified as a communicating neighbour because it contained the dye, in fact had got the dye not directly from an injected cell but rather via another

directly adjacent primary neighbour. However, the observations of such false classifications of the cells were very rare and probably could not distinctly affect the results in this experimental system. On the other hand, some of the "non-communicating" primary neighbours actually did not communicate only to an injected cell but also to one or two of the directly adjacent primary neighbours, or otherwise they would have got the dye from them and, consequently, they would not be examined as "non-communicating" primary neighbours.

The exposure to EG leads to a decrease in the communication capability of cells (Table 1). The source of the reduction in the communication capability is mainly the increase in the portion of non-communicating cells from 1 to 23.3% in the original population and even higher than that in its clones, with a maximum of 70.7% (clone VI, Table 3). Thus, the sensitivity of the cells to the treatment with EG varies within the cell population studied, and while a great deal of the cells respond to EG exposure with a total loss of the communication capability, the others are affected in a lesser extent (Fig. 3 – EG). The fact that we succeeded in the isolation of clones with a remarkably different responses to the treatment with EG suggests the idea that the sensitivity of cells to the treatment with this drug might somehow be genetically controlled.

The communication capability of the V79-4 cell line and its clones changes in time, i.e. between independent experiments, as illustrated in Fig. 5 for clones XIV and XV. The results for the other clones and the original cell line are not given, but they showed similar variations of this value. Both the characteristics tested, i.e. communication capability and response to EG, seem to behave more or less independently.

In another series of experiments we compared the direction of the changes of communication capability values between control dishes and experimental dishes treated with EG within experiments (above versus below the average value). A similar direction of the change in the communication capability was found in five cases out of twelve experiments (data not given), indicating that both events were rather independent. Consequently, these results again support the explanation that the communication capability in the untreated population and the response of the same population to EG are two different phenomena, which seem to change in time independently of each other.

On the other hand, the variability of this parameter within one day, i.e. within an experiment, has been found very low (data not given), thus indicating that the observed variability between the experiments found in our experimental system is not produced by a failure of the technique used, but that it reflects the really existing changes in the communication capability of these cells in time.

The changes of the communication capability in time may be very rapid, as was demonstrated in Fig. 5, but their source remains unclear. The variability of the dye coupling in V79 cells has been observed previously, when the suitability of this cell line for detection of tumour-promoting agents was tested (Zeilmaker and Yamasaki, 1986). The authors proposed three possible sources of the variability: (1) heterogeneity of the cell line, which they excluded by cloning the cells and finding that the variability in clones was similar to that of the original cell line (in contrast to our results), (2) lower reliability of the technique of detection. This source was improbable, because they argued that the technique had given sufficiently stable and homogeneous results with a different cell line. (3) Effect of the cell cycle stage. We suppose that none of these interpretations could be applied for the explanation of variations in the communication capabilities found by ourselves. We believe that the changes of the communication capability in time reflect the standard variations of various parameters of cell populations that could be observed during the microevolution of cell populations in culture.

Selective communication as a phenomenon is supposed to exist in living organisms on borders between various compartments of cells, i.e. between tissues or organs. The cells on such borders do communicate with cells within their own compartment, but not with "foreign" cells of the other compartment. This selectivity seems to be achieved by the inability of gap junctions containing some different connexins to form functional intercellular channels, and it results in preventing the escape of specific biological signals from one tissue to the other. However, the principle of the selective communication in our cell line seems to be different, because all the cells in our cell line do contain the identical connexin.

The most important finding of this report is that a cell in our cell line communicates with only a half of its neighbouring cells, although the other half, i.e. the "non-communicating" neighbours, are (1) cell-coupling competent, (2) do contain the same connexin, and (3) this connexin is present on cell membranes adjacent to all neighbouring cells, whether they do communicate with the injected cell or not, as we showed by the immunocytochemistry. The mechanisms of control of GJIC known so far affect the communication of a cell as a whole (Perrachia, 2000), but our results indicate that a cell may also possess a mechanism that can control the gap junctional communication to different neighbouring cells independently, even if they contain similar connexins. That means a cell may control its communication not only *in time*, as is fairly known (e.g. Peracchia, 2000), but *in space* as well. But such regulatory mechanism was not identified yet.

We have observed the selective communication not only in the V79-4 Chinese hamster cell line, but also in

the VUPT cell line derived from a human malignant melanoma of the chorioid as well as in the 3T3 mouse cell line (results not published). All of these cell lines are transformed, and hence one may postulate that the selective communication observed may represent one form of impairments of the gap junctional communication, which seem to be characteristic of neoplastic cells (Yamasaki, 1990a, 1990b). However, by contrast, Banoub (1996) reported the selective communication not only in neoplastically transformed cells, but also in non-transformed mouse lung C10 cells, indicating that the selective communication might not be limited only to transformed cells. One could speculate that the selective communication may exist within a non-transformed cell population or even a tissue, but it is not revealed because the "non-communicating" primary neighbours obtain the dye from other primary or secondary neighbours.

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