# Intercellular communication through gap junctions is reduced in senescent cells

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## INTRODUCTION

Gap junctions are thought to play a major role in the control of cellular growth and differentiation (1). This idea received support from the demonstrations that intercellular communication mediated by gap junctions was highly responsive to a variety of growth signals, including epidermal growth factor, tumor promoters as well as viral and cellular src proteins (2). Because of the apparently intimate relationship between cell growth and gap junction permeability and the complete cessation of cell growth in cultured senescent cell populations (3), we sought to investigate the status of gap junction-mediated cell-to-cell communication as a function of cell age in vitro.

## RESULTS AND DISCUSSION

Intercellular communication was investigated using a technique known as GAP-FRAP which involves labeling live anchored cells with a fluorescent dye and monitoring the redistribution of the dye through gap junctions connecting adjacent cells after photobleaching the dye in one of the contacting cells (4). The cell model used was human umbilical vein endothelial cells (HUVEC) which has a finite lifetime in culture of ~60 population doublings (5). HUVEC at different population doubling levels (PDL) were labeled with 6-carboxyfluorescein diacetate (Molecular Probes, Eugene, OR) and examined by GAP-FRAP methods using a Meridian ACAS 570 Interactive Laser Cytometer (Meridian Instruments, Okemos, MI). Data was analyzed using Meridian software for cell–cell communication. The data in Fig. 1 show that older cells ($\geq$ 40 population doublings) exhibit a shift in the mean rate of fluorescence recovery in comparison to younger cells. To investigate whether this decrease in rate among older cells might be due to a constriction in channel size, cells were labeled with the diacetate derivatives of either calcein (mol wt = 622) or carboxyfluorescein (mol wt = 376). Results presented in Table 1 indicate that there is no significant difference in the recovery rates obtained using these two markers, suggesting no difference in channel size between young and old cells, at least up to a molecular weight cutoff of 622 D.

Because senescent cells have been reported to become arrested at a specific phase of the cell cycle (6), it was necessary to perform a cell cycle analysis of senescent HUVEC as well as to evaluate the cell cycle dependence of intercellular communication in these cells. To determine the population distribution of senescent cells among the cell cycle compartments, unsynchronized nonproliferating cells were fixed and stained with propidium iodide (PI) (Sigma Chemical Co., St. Louis, MO) to quantitate DNA content and the fluorescence distribution profile of this population was obtained using the ACAS 570 cytometer (Meridian Instruments). The PI fluorescence (DNA) profile of nondividing senescent cells at PDL 61 were compared to those of synchronized, growing populations of younger cells (PDL 24) at various times after release of a double thymidine (Sigma Chemical Co.) block which synchronizes cells at the G1/S interphase (7). The data indicated that much of the senescent population was characterized by lower PI fluorescence, suggesting predominance of the G1 or G0 phase (data not shown). This result was confirmed by flow cytometric analyses of parallel preparations of senescent and synchronized young cells which indicated a distribution of 70, 5, and 25% in G1/G0, S, and G2/M phases, respectively, for the PDL 61 cells (data not shown).

Given that the majority of the senescent cells were in the G1/G0 phase, GAP-FRAP studies were performed on synchronized PDL 24 cells to determine if intercellular communication was affected by cell cycle progression. Fig. 2 shows mean recovery rates as a function of time after release from synchrony. There is a decrease in the recovery rates after release from the G1/S block, with the lowest mean value corresponding to the population with the highest fraction of G2/M phase cells

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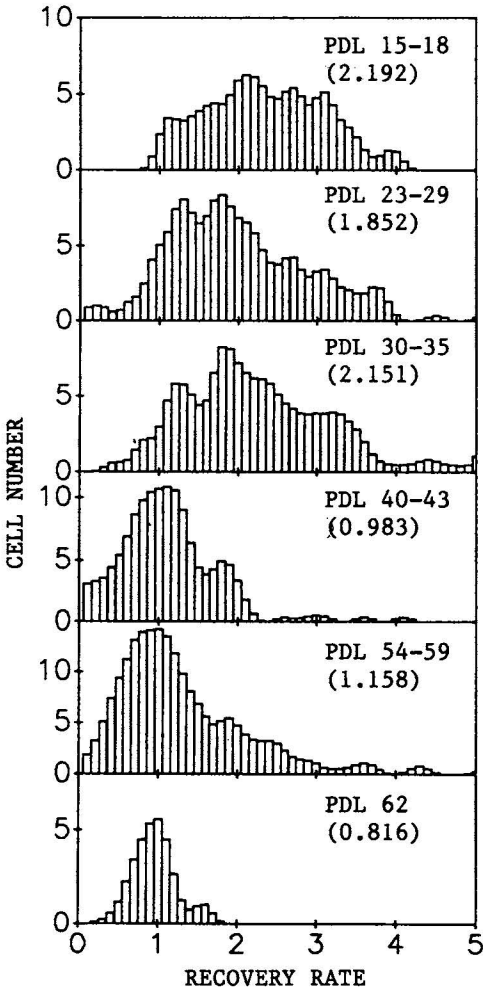


FIGURE 1 Change in cell–cell communication level as exemplified by fluorescence recovery rates (percent recovery/min evaluated over the first 3 min) as a function of in vitro senescence. HUVEC at different PDL were labeled with 6-carboxyfluorescein diacetate and analyzed by GAP-FRAP according to procedures described in reference 4. The histograms were smoothed according to standard methods. Mean recovery rates are given in parentheses.

**TABLE 1 Recovery rates as a function of marker size in young and old cell populations**

| | Recovery rate (percent/minutes) | |
|---|---|---|
| PDL | CF (mol wt = 376) | Calcein (mol wt = 622) |
| 20/21 | 1.844 ± 1.203 (39)* | ND‡ |
| 22 | ND | 1.813 ± 0.965 (17) |
| 29 | 2.022 ± 0.948 (34) | 2.056 ± 1.178 (36) |
| 61 | ND | 0.793 ± 0.662 (28) |
| 62 | 0.816 ± 0.263 (35) | ND |

*Number of cells analyzed; ‡not determined.

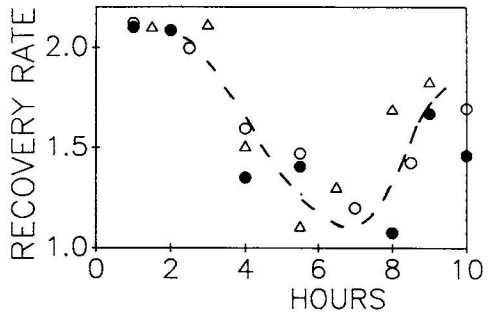


FIGURE 2 Cell cycle dependence of gap junction-mediated intercellular communication. Cells synchronized by a double thymidine block were labeled with 6-carboxyfluorescein diacetate and analyzed by GAP-FRAP methods at various times after thymidine washout. Data from three separate experiments are shown with each data point representing the average recovery rate of ~20 cells.

(~6–8 h after release). The mean recovery rate also shifts back to higher values as the cells progress through G2/M and back to G1. These data, together with the cell cycle compartment analysis of senescent cells, suggest that the lower communication level found in PDL 61 cells (mean recovery rate = 0.79) is not a direct result of cell cycle arrest. G1 and G0 cells (PDL 24) obtained in separate experiments by mitotic selection and serum deprivation, respectively, both exhibited mean recovery rates of 2.23. These results therefore leave open the question of why gap junction activity declines with age in culture. Also intriguing is the mechanism for the cell cycle dependence of gap junction activity, which has been noted previously for a few other cell types (8–10). We are examining both of these questions at the molecular level by studying the expression as well as post-translational modification (phosphorylation) of gap junction proteins.

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