GAP JUNCTION FORMATION BETWEEN MAMMALIAN CELLS IN CULTURE

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The formation of low resistance junctions between mammalian cells - both normal and malignant - is difficult to follow, because most cells do not survive manipulation with impaled electrodes for a sufficient time. Fusion of the cells by polyethylene glycol increases the cell size, resulting in a decrease of the input resistance without changing specific membrane properties. These giant cells allow long lasting intracellular recordings, which are impossible with the respective parental cells.

BICR/M1R-K cells (rat mammary tumour) were grown in plastic petri dishes to confluency and treated with 40 % (w/w) polyethylene glycol (MW 1540, Koch-Light) for 1 to 4 min to form homocaryons of more than 10 nuclei (1). When isolated homocaryons were manipulated into close contact, they formed gap junctions - as has been investigated by high resolution electrophysiology and electronmicroscopy. The time between first contact and the detection of the formation of the first gap junction pore is independent of the applied signal current (2-10 nA) but shows a temperature dependence (30° C : 8 min; 27° C : 16 min). The formation rate of the following pores seems to be temperature independent.

Homocaryons often display hyperpolarizing (up to 50 mV) oscillations of their membrane potentials. In non coupled cells lines (HeLa, L, Cl 1D) the frequencies of these endogenous signals are 3 oscillations per minute. Trypsinated homocaryons of coupled cell lines (BICR/M1R-K, 3T3, BT5C2) have frequencies of 0.3 oscillations per minute. By recording the membrane potential oscillations of two contacting homocaryons, the onset of coupling was followed by a superposition of the individual oscillations.

(1) Krähling,H., U.Schinkewitz, A.Barker and D.F. Hülser
Cytobiologie, European Journal of Cell Biology 17 (1978) 51-61