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ELECTRICAL CHARACTERIZATION OF GAP JUNCTION FORMATION

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It is generally accepted that single gap junction pores are formed by integral membrane proteins (connexons) which are arranged to a hexamer /3,7,9/. This hemichannel is impermeable to ions, dyes or metabolic active molecules. An intercellular passage for these substances may be enabled, however, when one hemichannel of a cell membrane is linked with another one of an attached cell membrane. Since gap junction channels are always arranged in a certain pattern within the membrane, an additional cooperative mechanism for opening (or closing) of the laterally organized channels might be possible. A single channel cannot be resolved morphologically, the well known gap junction pattern, however, is easily identified on freeze fracture replicas, even if only a few channels are arranged. An electrical characterization of single channels should preferentially be performed at the beginning of junction formation, because only then the ratio of nascent to open channels is optimal for a high electrical resolution. These measurements can help to answer two questions:

1. Does one channel open continuously or stepwise?
2. Is the pore size of all gap junction channels constant?

The cellular and temporal specificity of gap junctions during embryogenesis /1,5,8/ and polar permselective properties of gap junctions in cell culture /2/ point to different pore sizes. In Xenopus laevis embryos quantal steps of conductance increase have been described during formation of gap junction pores /6/, however, closing of the channels by Ca^{++} injection led to not integer multiples of the opening quantal steps.

As already shown /4/, homocaryons of cultured mammalian cells enable the investigation of membrane properties of those cultured cells which normally resist long lasting intracellular recordings. Using an electrophysiological high resolution method /6/ the formation of gap junction pores was followed between homocaryons of cultured mammalian tumor cell lines (BICR/M1R-K, BT5C2). Two homocaryons of similar size (about 5 to 8 nuclei) were manipulated into close contact by glass microelectrodes. Membrane potentials were recorded continuously and a sinusoidal electrical signal was applied to one of them.

As soon as electrical contact developed between these two cells, this signal can be traced in the second homocaryon. This very weak signal was detected by its amplitude and phase with a Lock-in amplifier (Ithaco, Mod. 393) which was automatically tuned to the signal frequency. No quantal steps of the signal amplitude in the coupling cell were resolved, however, different increases of the signal amplitude (increment of coupling) for BICR/M1R-K- and BT5C2-homocaryons were found after the electrical coupling started (see Table 1). The time between mechanical contact and electrical coupling varied with temperature between 1 and 16 min. The increment of coupling showed no correlation with temperature, however, below 25°C no onset of electrical coupling was observed. Within 15 min after first cell contact and incubation at 37°C gap junction areas have been detected on freeze fracture replicas of attached homocaryons.

Table 1: Increment of coupling amplitude within the first and the third minute of gap junction channel formation between homocaryons. (μV, mean ± standard error; time after initial coupling)

Cell line	1. min	3. min
BICR/M1R-K	1.17 ± 0.07	3.61 ± 0.30
BT5C2	1.59 ± 0.14	5.19 ± 1.10

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