STUDIES OF CELL COMMUNICATION BY USING GAP JUNCTION CHANNELS RECONSTITUTED IN UNILAMELLAR LIPID VESICLES

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Gap junction channels were reconstituted into unilamellar liposomes using immunoaffinity purified connexin 32 gap junction protein from rat liver. Vesicles containing open channels and close channels were separated by means of iso-osmolar sucrose density gradient sedimentation. The open channels formed in lipid vesicles were permeable to a fluorescent dve molecule, lucifer vellow of which the hydrodynamic size is similar to pore size of gap junctions in vivo. However, the large fluorescent dye molecule, rhodamine dextran was not transmitted through the channels. Phosphorylation of reconstituted gap junctions by purified protein kinase C closed the gap junction channels and this gating was monitored in iso-osmolar sucrose density gradient sedimentation. Evidence for the transport of second messengers. IP3 and cAMP through gap junction channels was first presented by measuring the labelled second messengers incorporated into the lipid vesicles through the reconstituted gap junction channels.