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Intracellular Trafficking of Lipid Gene Vectors Investigated by Three-Dimensional Single Particle Tracking

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Three-dimensional single particle tracking (SPT) was applied to investigate the intracellular trafficking of multicomponent (MC) lipoplexes in CHO-K1 cells. In untreated (NT) cells, we have found that: (i) intracellular lipoplex motion was either directed or Brownian; (ii) the occurrence of directed motion was more frequent (more than 70%) than the Brownian one;(iii) within experimental error, the Brownian motion (D~0.7 10-3 umt2/s) was faster than the directional movement (D~0.35 10-3 umt2/s);(iv) the directed motion mean velocity was about v=0.032 um/s;(v) the calculated three-dimensional asphericity, A3, was close to unity denoting the privileged occurrence of movement along a direction. To elucidate the role of the cytoskeleton structure in the lipoplex trafficking, cells were treated with cytoskeleton (actin microfilaments and microtubules) polymerization inhibitors (Latrunculin B and Nocodazole, respectively). In inhibitor-treated cells, we have found that: (i) the percentage of directional movement decreased balanced by the simultaneous increase in the occurrence of Brownian motion; (ii) reduction of directional movement was large but never complete. Such observation might reflect either an incomplete disruption of cytoskeleton network by drug treatment and/or its recovery due to the kinetic profile of the drugs employed;(iii) the effect of Nocodazole on the reduction of directional movement was definitely stronger than that of Latrunculin B;(iv) lipoplex mobility increased. Indeed, within each motion category (i.e. directed or Brownian), the diffusion coefficients were, in general, higher than the corresponding values obtained in NT cells. However, a very precise trend could not be found probably due to the low accuracy of experimental data;(v) within experimental error, the mean velocities were in the same range of those obtained in NT cells;(vi) the calculated asphericities were lower than that calculated in NT cells and were found to be close to the theoretical random walk value.