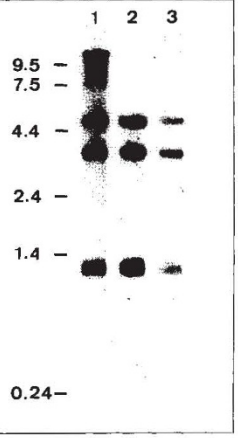
Ubiquitin in a togavirus

SIR—Khatchikian *et al.*[1] have shown that insertion of a 28S ribosomal sequence into the haemagglutinin gene of an influenza virus leads to increased viral pathogenicity. We now report the identification in another RNA virus of inserted cellular sequences, which may also be linked to the pathogenesis of a viral disease.

Pestiviruses, currently classified in the family Togaviridae, are positive-stranded RNA viruses that cause severe animal epidemics including bovine viral diarrhoea and hog cholera. Propagation of the bovine viral diarrhoea virus (BVDV) in tissue culture allows one to discriminate between cytopathic (cp-BVDV) and non-cytopathic (noncp-BVDV) strains, both of which can be pathogenic in cattle. One remarkable difference between the two strains is that a non-structural virus-encoded protein (p120) of unknown function is processed to a small product of M_r about 80,000 only in cp-BVDV-infected cells. Interestingly, the coexistence of both biotypes in persistently infected animals is linked to pathogenesis of fatal mucosal disease[2].

On comparing the genomic sequences of two cp-BVDV strains Osloss[3] and NADL[4] and one hog cholera virus strain[5] we found insertions in the BVDV gene



Northern blot analysis of total RNA from MDBK cells infected with BVDV strains Osloss (lane 1), NADL (lane 2) and Oregon (lane 3). The blots were hybridized with pCL208, kindly provided by Dr K.H. Scheit.

coding for p120, which may affect its processing. The Osloss insertion comprises 228 nucleotides, the NADL insertion 270 nucleotides. The two inserted sequences are not similar in either nucleotide or deduced amino-acid sequence. Whereas the NADL insertion is not similar to any sequence in the current data bank, the amino-acid sequence deduced from the Osloss insertion is almost identical to animal ubiquitin: compared to the ubiquitin sequence conserved in all animals[6], only 2 of the 76 amino acids differ.

To examine whether the ubiquitin coding sequence is specific for the Osloss strain, a *Bgl*II fragment derived from a porcine polyubiquitin complementary DNA clone (pCL208, ref. 7) was hybridized to total RNA of cells infected with different strains of cp-BVDV. The ubiquitin probe recognized genomic RNA from the Osloss strain only (see figure; the other three bands visible in all lanes obviously represent bovine ubiquitin messenger RNAs).

Based on these observations, we would like to suggest a novel model for the pathogenesis of mucosal disease. In persistently infected animals a noncp-BVDV mutates to a cp-BVDV biotype by taking up cellular sequences during a recombination event. This would explain the isolation of serologically closely related noncp-BVDV and cp-BVDV from one animal suffering from the disease[8,9]. Once this recombination has occurred, horizontal transmission of the resulting cp-BVDV strain follows. Further efforts will be directed towards a comparison of cp-BVDV and noncp-BVDV strains, including the detailed investigation of their p120 and its processing.

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Particle migration on cells

SIR—In their recent article entitled 'Nanometre-level analysis demonstrates that lipid flow does not drive membrane glycoprotein movements'[1], Sheetz and colleagues claim to have eliminated the membrane flow hypothesis of cell locomotion[2]. Their conclusion arises from experiments in which they add 40-nm gold particles coated with concanavalin A (Con A) to migrating macrophages. Using an ingenious optical technique involving image processing, they follow the movements of individual gold particles on the dorsal surfaces of these cells. They observe that the particles alternate between two modes of behaviour: either they diffuse by brownian motion, or they move (more or less directly) away from the leading edge of the cell. As analysis of particles in the diffusive mode shows that there is no superimposed directional drift on a particle, they believe that there can be no bulk (lipid) flow in the cells' plasma membranes. They suggest that the alternative mode — direct motion away from the leading edge — is driven by the actin cytoskeleton.

There appears, however, to be a conceptual error in the article. In measuring the diffusion of particles on the dorsal surface (finding there is no superimposed drift), the authors use a stationary marker on the glass slide as a reference point (see legend to Fig. 2 in ref. 1). But if the cells are actually moving (which is assumed, but not shown), then any particle on their dorsal surface that is stationary with respect to the substratum must be moving rearwards with respect to the cell. In other words, the particles, even when in a diffusive mode, must be moving away from the cell's leading edge. This is what is expected on the membrane flow scheme (see ref. 3, where this point has been discussed): the observations of Sheetz *et al.* could therefore be used to argue that membrane flow does exist in these cells.

There is an additional problem in the interpretation of the observations. Sheetz *et al.* assume the particles are on the cells' dorsal surfaces, but do not establish this. They could be inside the cells, or between the cells and substrate (under the cells). The reason for this is that the thickness of the leading lamella of a cell is usually about 250 nm (for example, ref. 4 shows the lamella varies in thickness between about 150 nm to 700 nm in one fibroblast), and the depth of focus of the microscope used is 250 nm (M. Sheetz, personal communication). It seems likely that some of the particles observed are inside the cells because macrophages are very active in endocytosis and phagocytosis. Con A actually stimulates membrane internalization in these cells[5], about half the surface-bound Con A being internalized in a few minutes[6]. Alternatively, it is conceivable that the particles are semi-trapped under the cells. In either case, if the particles are not on the dorsal surface, conclusions derived from the assumption that they are, would not be valid.

Finally, Sheetz *et al.* resurrect one of the old arguments against membrane flow: the intracellular vesicles that, on the membrane flow scheme, are supposed to move forward inside the cell and exocytose at the cell's leading edge have

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