Evidence for the presence of virus in clonal insulin-producing RINm5F cells[1]

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Summary. The ultrastructural morphology of the clonal insulin-producing cell line, RINm5F, was investigated. Virus-like particles, probably C type viruses, were identified both intra- and extracellularly. Because these particles could not be found in the original transplantable tumor, it is probable that viruses were induced at some later stage in the development of the RINm5F cell line. All investigators using the RINm5F cells should be aware of the fact that these cells may contain one or several types of viruses, and of the possibility that these particles may interfere with a variety of cellular functions.
Key words. Clonal insulin-producing cells; RINm5F cells; virus-like particles; C type viruses.

The clonal insulin-producing cell line, RINm5F, has been used extensively in studies of the mechanisms regulating insulin release[2-6]. Although this cell line has been subjected to thorough biochemical and biophysical characterization[2-11], no detailed ultrastructural studies have been performed. During an investigation of the ultrastructural morphology of RINm5F cells during the activation of the release mechanism, virus-like particles were identified within the cells. Future studies utilizing this cell line should therefore take into consideration the fact that RINm5F cells may contain one or several types of viruses.

Materials and methods. Various passages of RINm5F cell cultures, originally obtained from the Swedish laboratory, were grown in 50-ml flasks as previously described[9] in the USA laboratory. Cultures of RINm5F cells obtained from the Department of Molecular Biology and Biochemistry, University of Chicago, Chicago, IL (through the courtesy of M. Welsh) were also examined.

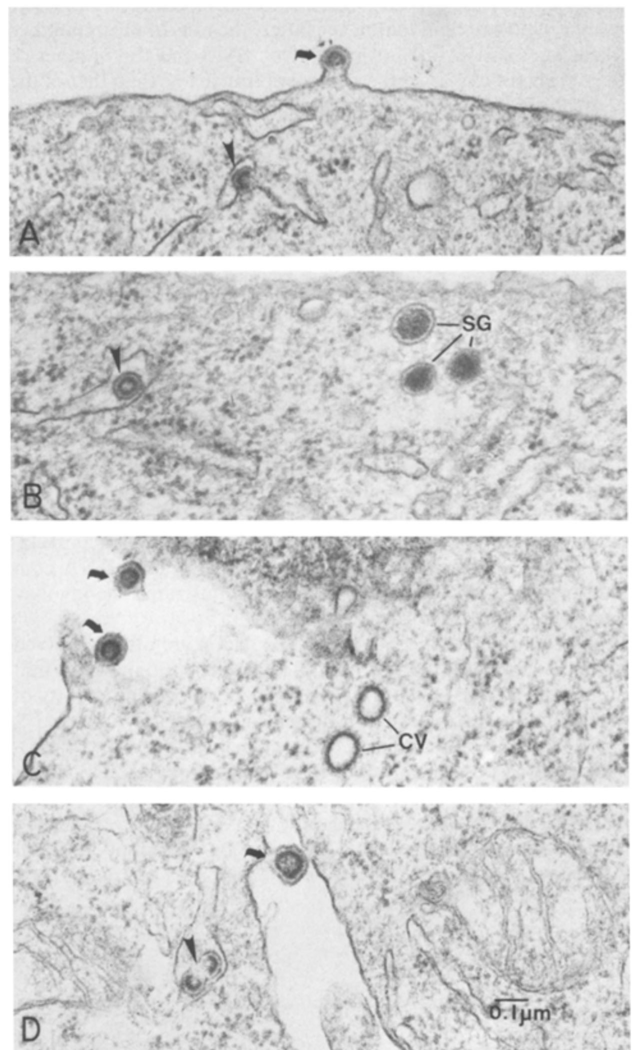
Solid tumor samples, obtained as fresh-fixed tissue (courtesy of P. Flatt, Department of Biochemistry, University of Surrey, England and M. C. Appel, Department of Pathology, University of Massachusetts Medical School, Worcester, MA, USA) or as frozen fresh tissue maintained at $-70\,^{\circ}C$ (courtesy of M. Welsh) were also viewed.

Monolayer cultures of RINm5F cells were fixed in 3% glutaraldehyde buffered in 0.1 M sodium phosphate buffer, pH 7.2 at 4 °C for 2 h. While still attached to the bottom of the culture flasks, the cells were washed in buffer and post-fixed with 2% osmium tetroxide (OsO_4) in veronal acetate buffer at 4 °C. Following a brief rinse in distilled H_2O, the cells were treated with 2% aqueous uranyl acetate for 2 h and dehydrated in alcohols. The cells were detached from the culture flask with a rinse of propylene oxide and transferred to vials and capsules for final embedding in Polybed 812 (Polysciences).

Solid tumors were treated in a similar manner, either fixed by immersion of 1 mm^3 pieces of the fresh tissue or by thawing and dicing the frozen tumor in fixative. Final processing of the tissue was the same as described above.

Sections, stained with uranyl acetate and lead citrate, were viewed in a JOEL 100 CX transmission electron microscope.

Results. As demonstrated in the figure, virus-like particles (indicated by arrows and arrowheads) can be identified both intra- and extracellularly. The particles have a core of intermediate density, a surrounding portion of higher density and a halo of lower density with a surrounding bilaminar membrane. Panel B allows the direct comparison of the ultrastructure of the virus-like particles with that of secretion granules (SG). The particles have a smaller total diameter ($\sim$ 1000 Å) as well as a smaller core diameter ($\sim$ 700 Å) than do the secretion granules ($\sim$ 1600 Å and $\sim$ 1250 Å, respectively). As can be seen in panels A, B and D, the virus-like particles bud from both the apical surface of the cells as well as into the cisternae of the endoplasmic reticulum (ER). Another commonly observed morphological feature of the virus-like particles is the double profile demonstrated in panel D. These profiles are associated with the cisternae of the ER and on the basis of their frequent occurrence, it is unlikely that they represent only a random association of two adjacent particles. Despite extensive viewing, none of these types of profiles were seen in any of the three solid tumors.



Ultrastructural evidence for the existence of virus-like particles in cultured RINm5F cells. Panel A shows the presumptive virus either budding from the cell surface (arrow) or into the cisternae of rough endoplasmic reticulum (RER) (arrowhead). Panel B illustrates the difference in size of the virus-like particles (arrowhead) in a RER cistern and the secretion granules (SG) typical of these cells. Panel C has both extracellular virus-like particles and intracellular coated vesicles (CV) evident, with very different morphological features. Panel D shows both an extracellular virus-like particle and a double profile of virus-like particles frequently seen budding into a RER cistern.

Discussion. The clonal insulin-producing cell line, RINm5F, does not display a normal secretory response to glucose, but responds with a significant release of insulin after stimulation by other secretagogues including high concentrations of K^+[2,4]. In view of the defective secretory response to glucose this tumor cell line might be used as a model for the pancreatic β-cells in non-insulin dependent diabetics.

In attempts to further characterize possible changes in the ultrastructural morphology associated with secretion, virus-like particles were identified in the RINm5F cells. When investigating RINm5F cells also from other laboratories similar virus-like particles were identified. These latter findings suggest that the virus-like particles identified in our RINm5F cells are not simply due to an infective contamination.

The virus-like particles could not be identified in samples of the original transplantable tumor, obtained from three different sources. The fact that it is not possible, at least by morphological means, to identify virus-like particles in the original tumor may indicate that these were induced in the RINm5F cell line at some later stage. Whether the virus-like particles were induced by processing the original tumor cells[13] through the athymic nude mouse or by the cloning procedure[12] will be the subject of further investigations.

Previous studies have shown that multiple injections of streptozotocin into mice resulted in a syndrome characterized by diabetes mellitus, insulitis and the induction of endogenous C type virus in pancreatic β-cells[14]. Although more detailed investigations are needed, preliminary characterization strongly indicates that the virus-like particles expressed in RINm5F cells are C type viruses.

We have no evidence, so far, that the functions of the RINm5F cells are affected by the presence of the viruses but all investigators using this line should be aware of their possible existence. Furthermore, from the present study it is obvious that this cell line is not a suitable candidate for use in investigations of the cellular effects of viral infections[15].

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Multiplied nucleolus organizer regions (NORs) in polyploid nuclei of *Vicia faba* revealed by ammoniacal silver staining

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Summary. The number of chromatids with transcriptionally active NORs in 4-, 8-, and 16-ploid restitution nuclei, as well as in endopolyploid nuclei with ploidy degrees of 4 and 8, were investigated by Ag-staining. In the genomes studied, the frequency of Ag-dots was equal to the number of chromatid NORs.

Key words. Vicia faba; endopolyploidy; nucleolus organizer region; silver staining; restitution nucleus.

The technique of ammoniacal silver staining is a standard method for staining chromosomal sites of rDNA (nucleolus organizer regions)[1,2], which are actively involved in rRNA transcription during the preceding interphase[3]. Chromosomal sites of rRNA activity can be recognized by dark brown silver precipitates. In late metaphase, when both chromatids of a nucleolar chromosome are separated, both are silver-dotted in the NOR. These double dots give evidence for rRNA gene activity of the parental codogen DNA-strand in one chromatid and of the just-replicated filial codogen DNA-strand in the other chromatid. Thus, the method offers the means of measuring the number of chromatids with transcriptionally active NORs in individual cells. This possibility complemented the investigation in the genome of *Vicia faba* to study proportional multiplication and transcriptional activity of chromatid NORs during somatic polyploidization. We first examined 4-, 8- and 16-ploid restitution nuclei of root tip meristems after colchicine treatment, and then endopolyploid nuclei with ploidy degrees of 4 and 8 of differentiated epidermis cells in the shoot axis. From the frequency and size of multiple chromatid Ag-dots in all types of nuclei investigated we conclude that all of the genomic NORs are proportionally multiplied and transcriptionally active during the course of somatic polyploidization.

Material and methods. Plants: Seeds of *Vicia faba* L. var minuta 'Kleine Thüringer', 2n = 12, were purchased locally.

Root tip meristem: Seeds were germinated in a desiccator at 19 ± 1 °C for 80 h. To increase the number of metaphases roots were immersed in a well-aerated colchicine bath, of concentration 0.05%, for 2 h. To induce restitution nuclei, roots were immersed in a colchicine bath, concentration 0.005%, to grow for 20, 40, 80 and 100 h respectively, as described by Deka and Sen[4]. Subsequently, primary root tips of 5 mm in length were harvested.