of the embryo; and at the end of and after reversion still counterclockwise in relation to the original chorion axis, but clockwise in relation to the axis of the embryo (Figure 2). During reversion, the plane of ciliary movement in fact is not exactly perpendicular to the axis of the chorion (i.e. the axis of actual rotation), but slightly 'preceding' the plane of rotation, thus demonstrating a certain timelag due to the resistance of the bulky eggmass. The change of direction of the ciliary beat can also be followed directly on one and the same embryo by tracking particles at different times (Figure 1).

The pulsation of the outer yolk sac starting at stage IX does not interfere with the rotation, which works through all the stages of early and later organogenesis and ceases at about stage XVI when the ciliary surface in contact with the chorion becomes too small to move the then large embryo.

Discussion. All observations thus far reported in the literature perfectly match the 'model' described here. Embryos failing in undergoing the first reversion evidently start ciliary activity at a stage too late for reversion, the embryo having lost its high deformability; at stage X, however, when normal embryos have completed the first reversion, they also rotate counterclockwise thus demonstrating the autonomy of the reversal of ciliary beat. In species with very large eggs (e.g. *Octopus briareus*), the early embryo is very small compared to the egg mass and is – in the case of belated reversion – dragged forth by the large rotating yolk sac[13]. Embryos with only part of the yolk mass enclosed in the outer yolk sac can also undergo reversion. The partial reversion of aberrant, invaginated germs[9] can be explained by contraction only of the yolk mass that forces the uvula of embryonic tissue sooner or later into a curved, oblique position; its gradual deformation is about 10 times slower than normal reversion.

The fact that rotation does not cease after completion of the first reversion endorses the assumption that rotation also fulfills other physiological tasks such as acceleration of oxygen uptake by the yolk sac and maintainance – by revolving the perivitellin fluid – of a balanced microenvironment. This physiological viewpoint is meaningful for any attempt to evaluate the evolutionary pathway leading to the establishment of such a reversion, the significance of which is unknown. From a more biophysical point of view, however, the first reversion stands out against other functions of the ciliary activity. Whereas processes such as revolving the perivitellin fluid can be carried by cilia that never change the direction of their beat[1], the first reversion demands a more sophisticated ciliary apparatus. The present study coping only with the performance of this apparatus, the problems of the structure of the cilia envolved[14] and of the control and coordination system for this well-timed process are still open.

Zusammenfassung. Cilien des äusseren Dottersackes versetzen Octopoden-Embryonen in Rotation innerhalb ihrer Eihülle. Die erste Umdrehung oder «Blastokinese» ist die Folge einer einmaligen, innert Stunden ablaufenden Schwenkung der Cilienschlagrichtung um 180°.

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Influences of Cell Cycle on Uptake of SV40-DNA by Diploid Human Cells

Cells from a variety of animal species can be transformed in vitro by SV40. The transformed cells show loss of certain regulatory properties such as cellular sensitivity to contact inhibition and changes in morphology. Both structural and numerical aberrations of chromosomes occur in transformed cells[1,2]. Cells transformed by SV40 contain virus specific tumor antigens as well as messenger RNA which hybridizes specifically with SV40 DNA. These observations indicate that the viral genome interacts with that of the host cell and that all or part of the genetic information of the virus persists in the transformed cells. It has been demonstrated that cells transformed by DNA tumor viruses contain at least part of the virus genome in stable association with the host cell DNA[3–5]. The present study was undertaken in an attempt to utilize autoradiographic methods to determine the fate of tritium labeled SV40 DNA following infection of synchronized human cells by the virus and thus to get an insight into the influence of the physiological state of the cells on virus infection.

Materials and methods. SV40 clone 307L was grown in monolayers of CV-1 cells. For preparation of tritium labeled SV40, growth medium (Eagle's medium supplemented with 10% calf serum) was removed from 7-day-old CV-1 monolayer cultures and replaced with maintenance medium (Eagle's with 2% calf serum). In addition the maintenance medium contained tritiated-thymidine (TdR-H^3). 24 h later the cultures were infected with SV40 at 10 PFU per cell. After infection the culture were incubated at 37 °C in maintenance medium containing TdR-H^3 at 0.5 μc/ml. Cultures were harvested on the seventh day after infection when they were extensively cytopathic. Cells and maintenance medium from 10 cultures were pooled and the virus was purified and concentrated by a technique similar to that of LUBORSKY[6]. The infectivity titer of the final material was 6.5×10^6 PFU/ml and the specific activity was 1.5×10^{-3} counts/min per PFU. It is important to point out that the final virus suspension contained no free or DNAase-sensitive counts higher than background. This indicates that there was no free TdR-H^3 or cellular DNA in the preparation.

A human diploid cell line developed from skeletal muscle tissue at our laboratory was used in this study and grown in Ham's F-10 medium supplemented with

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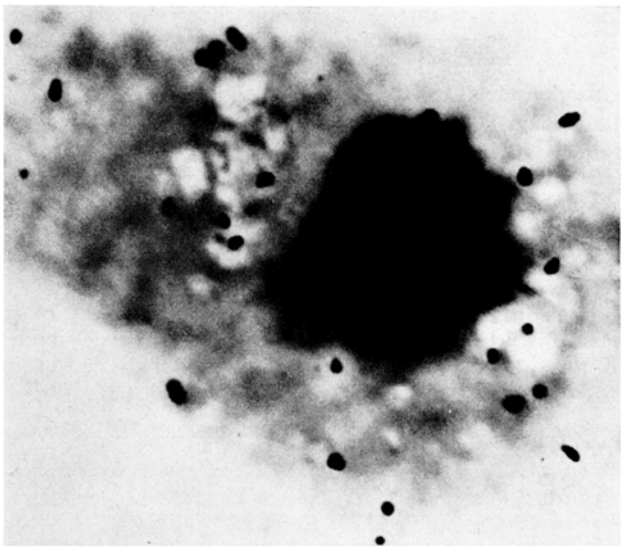

Fig. 1. Accumulation of H^3 labeled SV40 primarily in the cytoplasm 4 h post infection of cells synchronized at S phase of cell cycle.

highly sterile 20% fetal calf serum (Gray Industries, Inc., Fort Lauderdale, Florida). A moderate degree of synchrony of cells was achieved by single exposure of the Petri-dish cultures to excess of unlabeled thymidine. By this method cells were blocked at the beginning of S phase[7]. 12 h exposure of cultures to colcemide arrested a moderate number of cells at metaphase. Cultures with confluent growth of cells, arrested by contact inhibition, had cells mostly in the G_1 phase of the cell cycle[8]. All cultures were washed free of synchronizing agents and infected with the tritium labeled SV40 following the standard infection technique as described previously[9].

After infection the cultures were freed of nonabsorbed virus by repeated washing with saline. One group of cultures was harvested immediately after the completion of infection period and others were fixed after 1, 3, 5, 8, 10, 12, 14, 22, and 24 h from the completion of 3 h infection period. Coverslips with infected cells were coated with Eastman Nuclear Track Emulsion NTB-3 and stored in the dark for 2 weeks in a cold, dry atmosphere. The slides were developed in D-19 and stained with modified Giemsa.

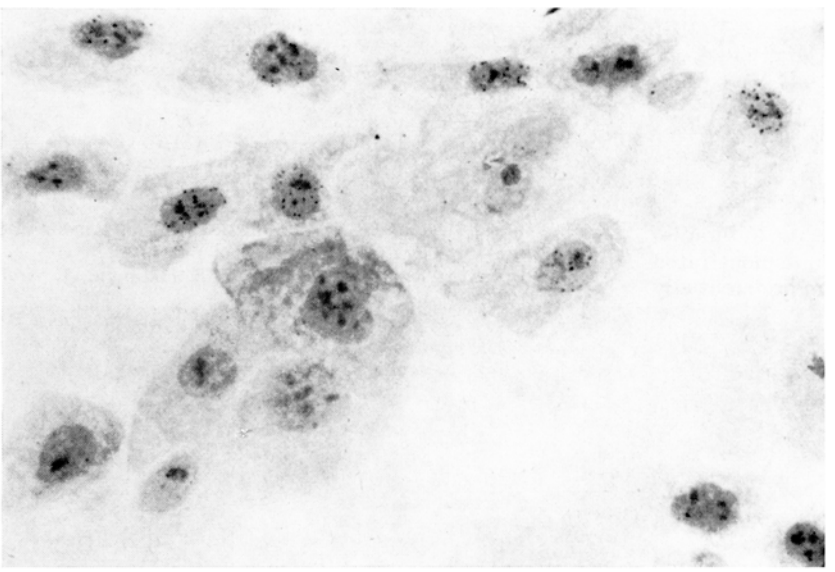

Fig. 2. Intranuclear accumulation of H^3 labeled SV40-DNA 8 h post infection of cells synchronized at S phase of cell cycle.

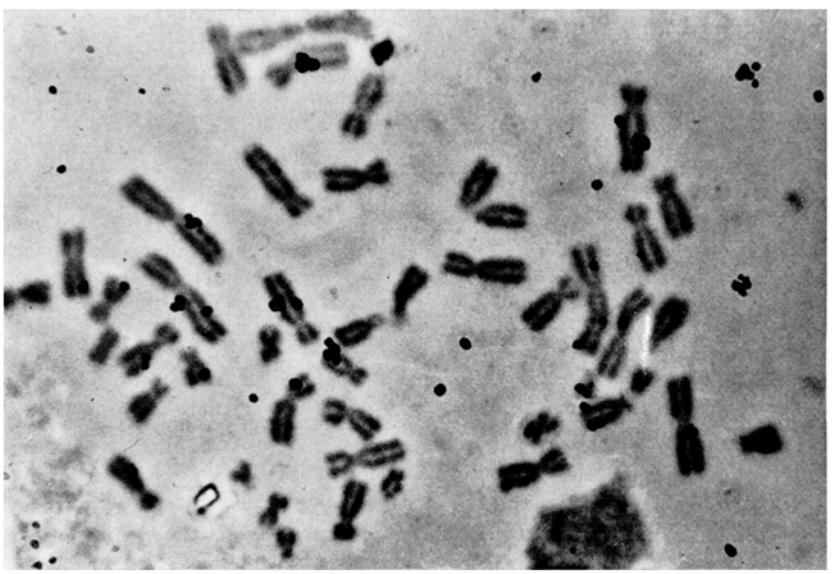

Fig. 3. Localization of H^3 labeled SV40-DNA on metaphase chromosomes 10 h post infection of cells synchronized at S phase of cell cycle.

Results. The objective was to determine whether cells infected with the labeled virus at the beginning of S, M and G_1 phases retained the labeled virus particles when allowed to proceed to enter in other phases of the cell cycle. Cells infected with labeled virus at M and G_1 did not contain any significant number of grains. Cells infected at S but harvested after 4 and 6 h from the onset of infection period retained grains primarily in the cytoplasm (Figure 1). Cells which were harvested 8 h or later showed intranuclear accumulation of labeled virus particles (Figure 2). It was also observed that cells infected by labeled SV40 at S phase and then arrested at metaphase retained grains primarily on the chromosomes (Figure 3).

Discussion. It has been shown that the anti-viral action of interferon could not protect cells from transformation by SV40 if a cycle of cellular DNA synthesis had occurred in the infected cells prior to the addition of interferon[10]. In addition it has been suggested that non-dividing cells are resistant to virus induced transformation[11]. Our results suggest that cells in the DNA synthetic phase of the cell cycle are the only ones which interact with viral DNA and that viral genomes retained by these cells as they proceed further in the cell cycle. The association of grains with the metaphase chromosomes, after infection of cells at S phase, indicates that viral DNA localizes on the chromosomes. It should be pointed out that retention of labeled viral DNA on the chromosomes may not mean that the entire genetic material of the virus is integrated into host DNA. However, it has been shown that a portion of viral DNA is integrated in the host genome in viral transformed cells[3–5]. Localization of tritium-labeled adenovirus 12 on metaphase chromosomes has been demonstrated using autoradiographic methods[12–14]. It has recently been demonstrated that rat embryo cells synthesizing DNA are preferentially infected in vitro by rat virus[15].

From these observations it has become evident that infection of cells by SV40 is influenced by the physiological state of the host cell and that cells engaged in DNA synthesis appear to be the only ones vulnerable to viral action. These observations further suggest that chromosomes are primary sites of viral action. It would be interesting to see whether selective distribution of virus DNA on the chromosomes synthesizing DNA can be achieved by infecting cells at different stages in the progression of DNA synthesis[16].

Zusammenfassung. Autoradiographische Untersuchungen an menschlichen, diploiden, synchronisierten Zellen, in der S-Phase mit Tritium markiertem SV40 infiziert, zeigten intranukleären Einbau von markierter DNS (in Metaphase vor allem Chromosomenmarkierung). Keinerlei Kernmarkierung wurde beobachtet, wenn die Zellen in anderen Phasen ihres Zyklus infiziert wurden.

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Two Types of Viruslike Particles in *Drosophila* Midgut

The midgut of the adult, wild type, Canton-S strain *Drosophila melanogaster* was the subject of electron microscopic investigations concerning the nature of submicroscopic alterations, studied at graded intervals of time, subsequent to ^{60}Co γ-radiation. During these investigations hexagonal, viruslike particles (VLP) of 2 different sizes were observed, 1 in the nucleus and the other in the cytoplasm of some cells (Figure 1).

Lightly etherized flies were dissected in cold, 5% glutaraldehyde, and the midgut was immediately transferred to a cold, one per cent solution of osmium tetroxide[1] buffered at a pH of 7.4. Subsequent to dehydration procedures in a graded series of alcohols and finally propylene oxide, the tissue was embedded in Epon 812, sectioned either on a Porter Blum hand ultramicrotome or on an LKB automatic ultramicrotome, and mounted on bare copper grids. The sections were stained with uranyl acetate[2] for 10 min, followed by lead citrate[3] for 6 min, and then viewed with an RCA EMU-3F electron microscope.

The presence of slightly elliptical[4,5] and spherical[6] viruslike particles of uniform size have already been reported in larval and in adult tissues, as well as in cell cultures of *Drosophila*. None of the previous reports indicated possible size differences between nuclear and cytoplasmic VLP, and all 3 groups of investigators showed similar electron micrographs, for the spherical VLP of one[6] were slightly elliptical in shape. Such variations in the morphology of these particles were also evident in this study, but were attributed to the plane of sectioning, for in some instances clearly hexagonal shapes were observable (Figures 2 and 3), each containing a dense, central core. The nuclear VLP were 565 ± 21 Å in dimension and those in the cytoplasm were 727 ± 23 Å ($P \ll 0.001$), when the distances between opposing sides were measured. The dimensions evident in the present material are considerably larger than those previously reported[4–6]. The VLP may be found

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