Zeitschrift: Verhandlungen der Schweizerischen Naturforschenden Gesellschaft. Wissenschaftlicher und administrativer Teil = Actes de la Société Helvétique des Sciences Naturelles. Partie scientifique et administrative = Atti della Società Elvetica di Scienze Naturali

Herausgeber: Schweizerische Naturforschende Gesellschaft

Band: 149 (1969)

Artikel: The use of polyoma virus for studies on genetic regulation in mammalian cells

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DOI: https://doi.org/10.5169/seals-90681

The use of polyoma virus for studies on genetic regulation in mammalian cells

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1. *General properties of polyoma virus*

Polyoma virus, which is endemic in mice, induces in its natural host and in related rodents a broad spectrum of histologically different types of tumors and thus exhibits an action which is similar to that of certain chemical carcinogens [5]. Several other tumor- or wart-producing viruses, present in a number of mammalian species *(Tab. 1)*, are similar to polyoma virus in structure and biological properties, and like polyoma virus contain circular DNA as their genetic material.

Infection of cultured mouse cells leads to the production of relatively large amounts of polyoma virus, followed by cell death. The resulting crude viral preparation can be purified and concentrated by treatment with deoxyribonuclease and ribonuclease followed by differential and CsCl equilibrium density gradient centrifugation.

According to the model proposed by KLUG and FINCH [14] the polyoma viral capsid, with an outer diameter of 450 Å, is made up of 72 capsomeres (subunits) arranged in icosahedral symmetry and is composed of one type of polypeptide [6, 20] *(Fig. 1a)*. Infective polyoma virions consist of the capsid which encloses one molecule of circular polyoma DNA [25, 31] *(Fig. 1c)*. From newer experimental results it follows, however, that 10 to 90% of the viral particles in purified preparations of polyoma virus are actually non-infective *pseudovirions (Fig. 1b, d)*, i.e. polyoma capsids that contain, instead of circular polyoma DNA, linear fragments of mouse chromosomal DNA (see below) [18, 19].

2. *Physico-chemical and biological properties of polyoma DNA*

From purified viral preparations total DNA can easily be extracted with phenol or with sodium dodecyl sulfate. Sedimentation velocity analyses in the analytical ultracentrifuge of the DNA extracted from highly purified viral preparations regularly reveal three discrete components which we designated, in order of decreasing sedimentation coefficients, as polyoma DNA I (20 S), II (16 S) and III (~ 14–7 S). The properties of the three types of polyoma DNA [18, 25, 28, 31] are summarized in *Figure 2*. Polyoma DNA I and II are circular molecules with the same

molecular weight (3×10^6), length (1.5 μ) and buoyant density in CsCl solutions (1.709 g cm^{-3}) and thus the same base composition (49% cytosine + guanosine). Both I and II are infective, i.e. they contain the genetic information for the production of progeny virus and for the induction of tumors. Polyoma DNA I is a twisted circular helix which consists of two separately continuous strands which are unable to unwind and to separate. After thermal denaturation it exhibits spontaneous, monomolecular renaturation. The unusual thermal stability of the infective titer of polyoma DNA preparations (it remains essentially unchanged

Table 1

POLYOMA AND RELATED VIRUSES
common denominator : circular DNA

	endemic in:
Polyoma virus	mice
Simian virus SV-40	monkeys
Shope papilloma virus	rabbits
Canine " "	dogs
Bovine " "	cattle
Human * " "	man

* induces warts (*verruca vulgaris*)

during boiling for 20–30 min) is explained by the ring structure of polyoma DNA I [28, 31]. The introduction of one single interruption in either of the two complementary strands converts polyoma DNA I into a "relaxed" ring corresponding to polyoma DNA II which exhibits a denaturation behavior which is comparable to that of linear double-stranded DNA [25]. As shown by HIRT [10, 11], circular polyoma DNA undergoes semiconservative replication, a process which requires the introduction of at least one discontinuity into one of the strands of the parental DNA molecule. Following duplication, the continuity of the strands of the daughter molecules is restored *(Fig. 3)*.

Surprisingly, component III consists essentially of linear fragments of mouse chromosomal DNA which are excised at random during the lytic infection of mouse cells and which are enclosed in polyoma viral capsids [18, 19]. Polyoma viral capsids that contain mouse cellular DNA instead of the viral genome (or fragments thereof) are not infective and are referred to as *polyoma pseudovirions*. The possibility is considered that they may transduce mammalian genetic markers.

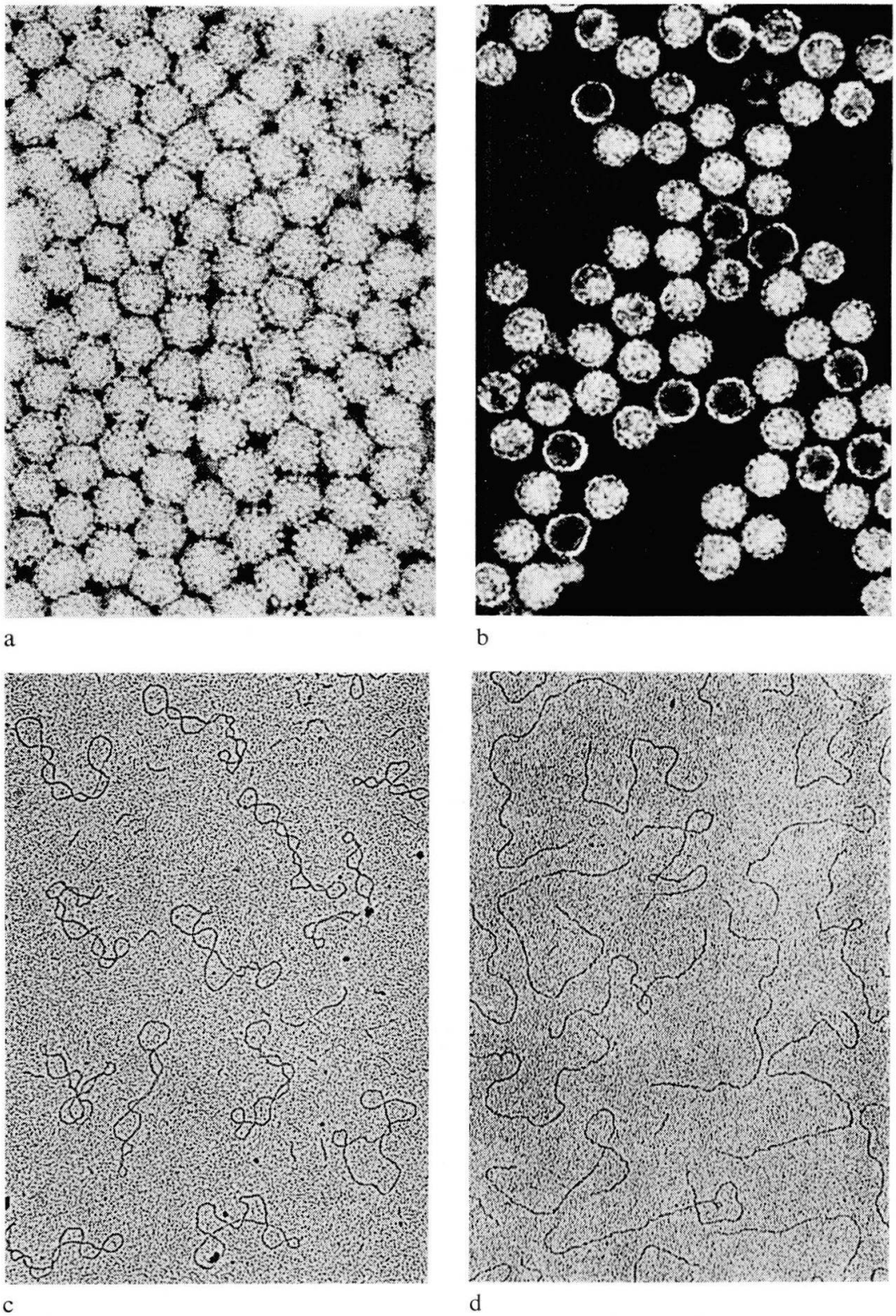


Figure 1

a) Polyoma virions (magnification 150000×)—b) Polyoma pseudovirions (magnification 150000×)—c) Polyoma DNA I (magnification 38000×)—d) Polyoma DNA III (magnification 38000×)

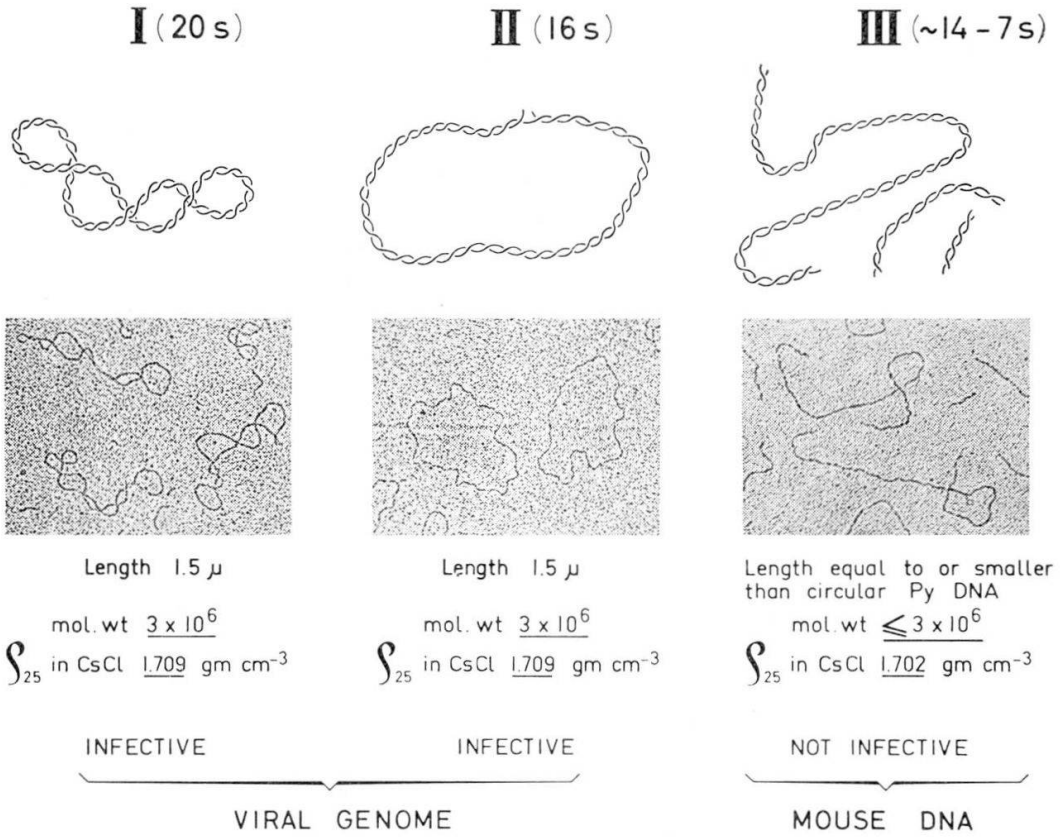


Figure 2
Polyoma DNA

3. *The interaction of polyoma virus with cultured mammalian cells*

3.1 *Lytic infection*

a) This type of interaction leads to the production of polyoma viral particles and is followed by cell death [26, 32]. It can conveniently be studied in primary, confluent mouse kidney cultures, made up mainly of "contact-inhibited", epithelial-like cells which have stopped cellular DNA synthesis and mitotic division prior to infection. Combined biological, cytochemical and chemical studies have led to the conclusion that, in individual cells, the lytic cycle proceeds in two distinct phases [29, 30] which are summarized in *Figure 4:* phase 1 corresponds to a sequence of early events that precede and initiate phase 2, i.e. the activation of the cellular DNA-synthesizing apparatus [13, 22, 30] and the actual production of progeny virus. A few hours after infection parental polyoma DNA molecules are found in the nuclei of most cells, in close association with the chromosomes [9]. Newer experimental results point to the possibility that in all infected cells at least one viral DNA molecule may become integrated into the chromosomal DNA and that a minor fraction of total viral messenger RNA, designated as "early" polyoma RNA, might be transcribed from the integrated polyoma DNA [12]. During phase 1 the infected cells start synthesizing a new, virus-specific antigen, referred to

as T- or neo-antigen [23] which can be visualized intranuclearly by immunofluorescence. At present neither the chemical nature nor the function of this antigen are known. As a working hypothesis we consider the possibility that it may be linked to the activation of the cellular DNA-synthesizing apparatus. This activation comprises the coordinate increase in the activities of the enzymes involved in the synthesis of pyrimidine-deoxyribonucleotides and of DNA *(Fig. 5)* and, possibly, also the conversion of viral and cellular DNA from a "non-priming" into a "priming state" for DNA polymerase [13, 22, 30]. Synthesis of polyoma viral DNA is regularly accompanied by the replication of the mouse chromosomal DNA [4, 29] and, as was shown lately [8], of the chromosome-associated histones and non-histone proteins. Immediately after the onset of viral DNA synthesis, increasing amounts of "late" polyoma messenger RNA are synthesized in the form of large, polycistronic RNA molecules which carry most or all of the genetic information contained in one strand of circular polyoma DNA [12]. The viral messenger RNA is then transferred from the nucleus into the cytoplasm. There it forms polysomes and (presumably) directs the synthesis of viral capsid protein which is rapidly transported into the nucleus where progeny virus is assembled.

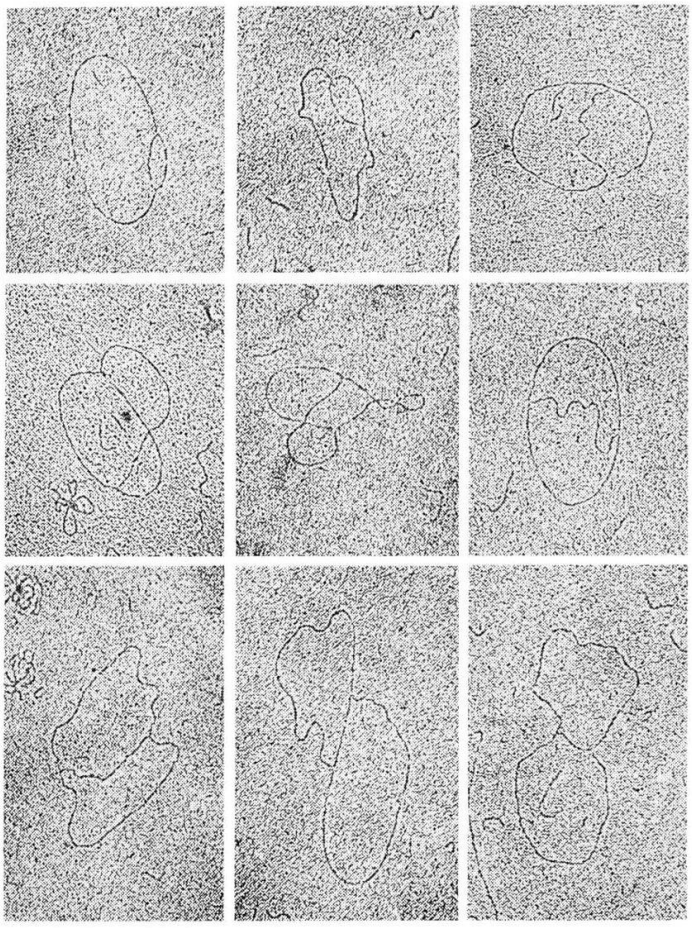


Figure 3
Replicating polyoma DNA (magnification 38000×)—Preparation: Dr. B. HIRT

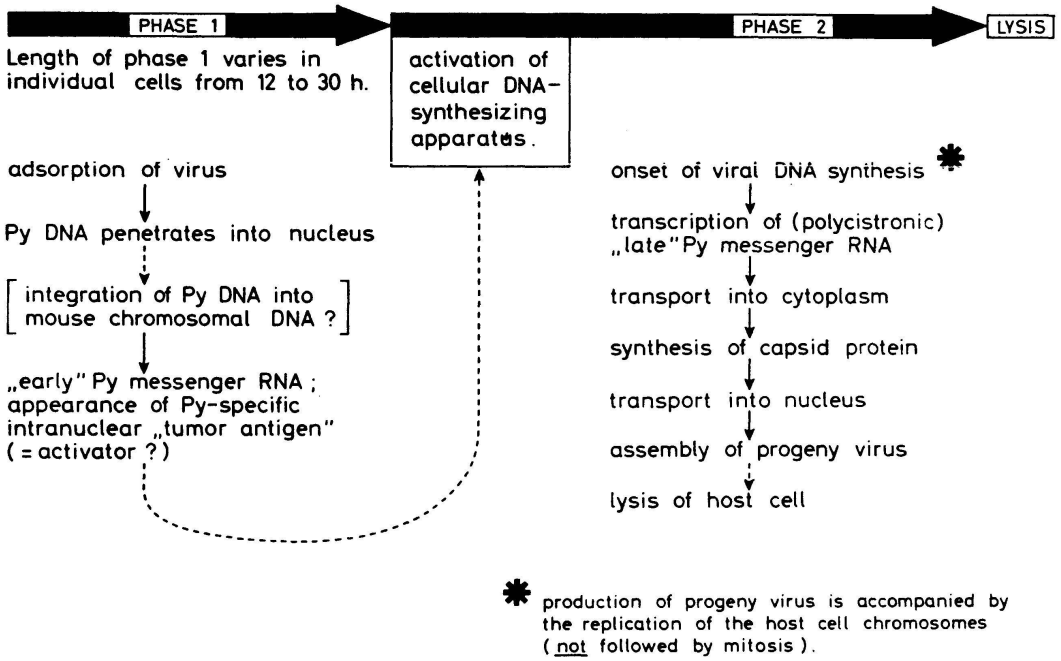
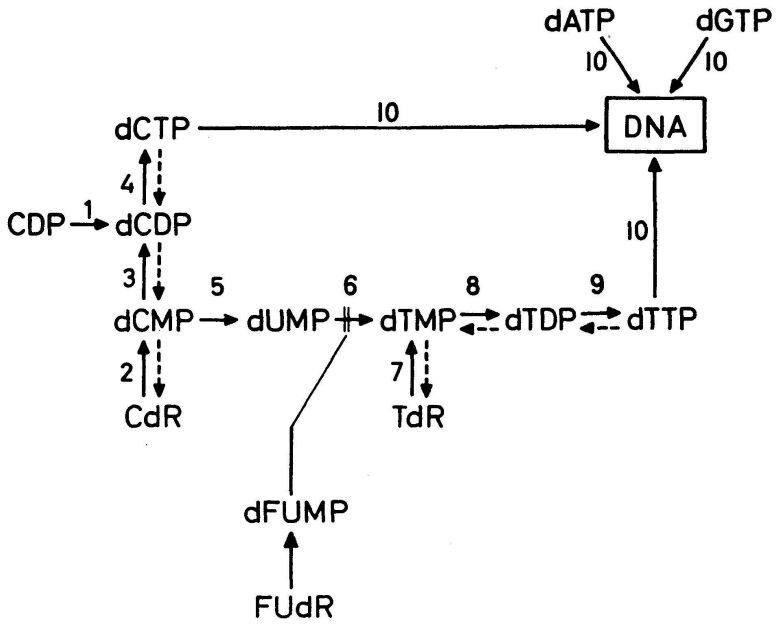


Figure 4
Polyoma virus: the infective cycle in "contact-inhibited" mouse kidney cells

b) *Synchronization of polyoma-induced DNA synthesis.* Due to the markedly varying length of phase 1 (12–30 h) in individual cells, the time course of infection in mouse kidney cultures is very asynchronous *(Fig. 6)*: the first cells are activated, i.e. start polyoma-induced DNA synthesis, around 12 h after infection. Thereafter, the number of activated cells rapidly increases and reaches a plateau around 30 h after infection [22, 30]. In order to study in detail the sequential steps of the infective cycle, particularly the transcription of polyoma messenger RNA, we synchronized the onset of polyoma-induced DNA synthesis by the use of 5-fluorodeoxyuridine (FUdR; *Fig. 5 and 7)* [22]: if FUdR is added to the culture medium immediately after the adsorption of the virus, cellular and viral DNA synthesis are inhibited and little if any "late" viral messenger RNA or viral capsid protein are synthesized [12, 22]. However, the early events of the infective cycle such as the production of T-antigen [23] and of "early" polyoma RNA [12] and also the activation of the cellular DNA-synthesizing apparatus [13, 22, 30] take place in the presence of FUdR just as they do in parallel cultures infected in the absence of the inhibitor *(Fig. 7)*. Addition of thymidine to the culture medium releases immediately and specifically the block imposed by FUdR. Therefore, if thymidime is added around 30 h after infection, i.e. when most cells are activated, polyoma-induced DNA synthesis starts synchronously in all cells.

c) *Working hypothesis.* Certain stimuli such as phytohemagglutinin, partial hepatectomy and others trigger the mitotic machinery of differen-



1 = CDP-reductase
2 = CdR-kinase
3 = dCMP-kinase
4 = dCDP-kinase
5 = dCMP-deaminase
6 = dTMP-synthetase
7 = TdR-kinase
8 = dTMP-kinase
9 = dTDP-kinase
10 = DNA-polymerase

CDP = cytidine-5'-diphosphate
CdR = deoxycytidine
TdR = deoxythymidine
dCMP, dCDP, dCTP = deoxycytidine-5'-mono-, di- and triphosphate
dTMP, dTDP, dTTP = deoxythymidine-5'-mono-, di- and triphosphate
dUMP = deoxyuridine-5'-monophosphate
dATP = deoxyadenosine-5'-triphosphate
dGTP = deoxyguanosine-5'-triphosphate
FUdR = 5-fluorodeoxyuridine
dFUMP = 5-fluorodeoxyuridine-5'-monophosphate
DNA = deoxyribonucleic acid

Figure 5
Schema of the biosynthesis of pyrimidine-deoxyribonucleotides and of DNA

tiated cells [3, 16, 17]: in these systems induced cellular DNA synthesis is preceded by a markedly increased production of cellular RNA and is followed by mitotic division. In contrast, polyoma-induced cellular DNA synthesis is neither preceded nor accompanied by an overall stimulation of cellular RNA synthesis nor is it followed by mitosis. Our experimental results are compatible with the hypothesis that *in "contact-inhibited" mouse kidney cells polyoma virus activates (derepresses?) a specific genetic regulatory element of the host cell which controls initiation of chromosome replication.*

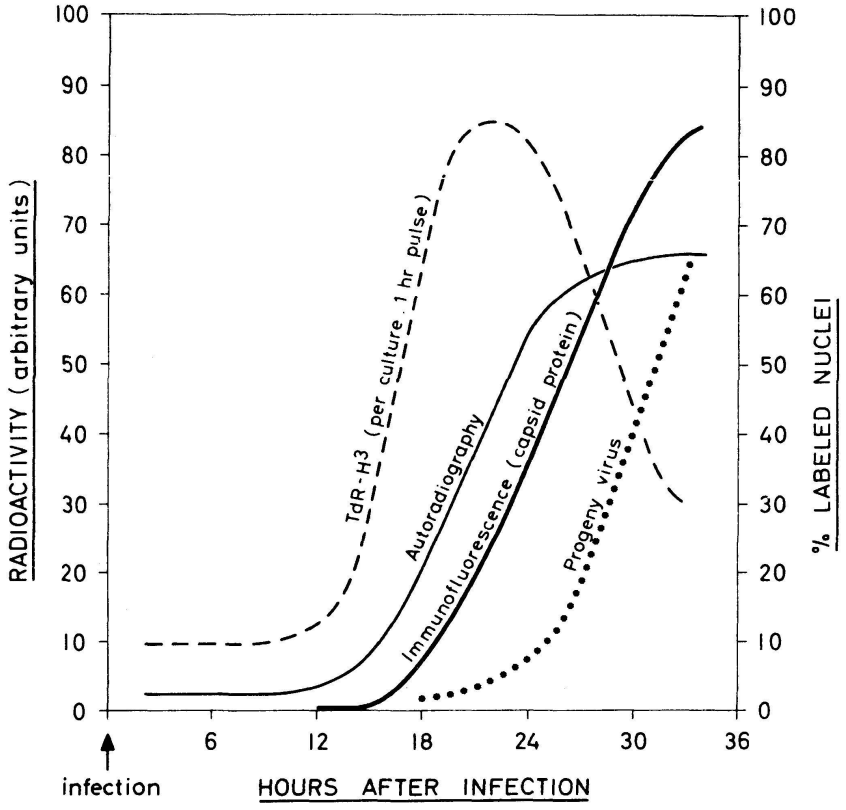


Figure 6
Scheme of the time course of infection with polyoma virus in confluent mouse kidney cultures (Autoradiography: 1 hour pulse with thymidine —H^3)

3.2 *The non-lytic infection ("transformation")*

Infection of cultured hamster cells does not lead to the production of progeny virus [26] since the infective cycle is blocked at an early stage, prior to virus-induced DNA synthesis. Intact polyoma DNA rapidly penetrates into the nuclei of most cells present in the infected cultures and is found closely associated with the chromosomes [9]. One to two days after infection, intranuclear T-antigen can be detected in a small number of cells [23]. Many of these "transformed" cells, furthermore, contain new virus-induced surface antigens which, however, may actually be of cellular origin [21]. Since the virus-induced antigens represent inheritable and, in general, fairly stable imprints of the transforming virus, they are regarded as the first solid, though indirect, experimental evidence in support of the continued presence of viral DNA in transformed cells, an assumption which was strengthened by the finding that polyoma-transformed cells continuously produce small amounts of polyoma viral RNA [1]. Several laboratories [2, 7, 15, 24, 27] have reported direct evidence for the continued presence of the viral genome in the (chromosomal?) DNA of cells "transformed" by virus SV40: replication of the viral DNA and production of infective SV40 virus can be induced by fusion of the "transformed" cells with uninfected, cultured monkey

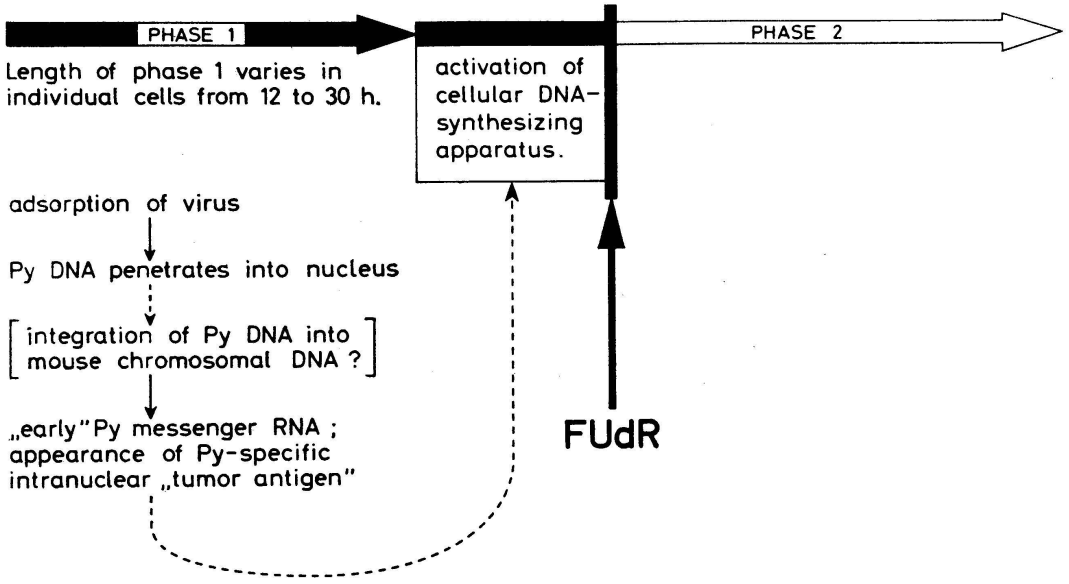


Figure 7
The block imposed by 5-fluorodeoxyuridine (FUdR) on the infective cycle of polyoma virus

kidney cells. In view of the similarities between virus SV40 and polyoma virus, an analogous situation may exist for polyoma-transformed cells.

The synonymous use of the terms "transformed", "neoplastic" and "malignant" has created considerable conceptual confusion. Therefore, it should be pointed out that neither specific morphological properties nor loss of contact-inhibition nor malignancy (as judged by transplantability) are regularly associated with polyoma- (or SV40-) transformed cells, and, furthermore, that the mechanism of the oncogenic effect of polyoma and related viruses remains unknown.

Summary

Judging from molecular weights, circular polyoma DNA contains 5 to 7 genes as compared to an estimated 5×10^6 genes present in mammalian cells. Because of its small size and its remarkable physical and biological stability circular polyoma DNA is a useful tool to study genetic regulation in mammalian cells.

Zusammenfassung

Aus dem Molekulargewicht zu schliessen, enthält die ringförmige Polyoma DNS 5 bis 7 Gene, im Vergleich zu etwa 5×10^6 Genen in der Säugetierzelle.

Infolge ihres kleinen Molekulargewichts und ihrer ausserordentlichen biologischen und strukturellen Stabilität eignet sich die Polyoma DNS als Mittel zum Studium genetischer Regulationsmechanismen der Säugetierzelle.

Résumé

La comparaison des poids moléculaires permet d'estimer que l'ADN polyome circulaire contient de 5 à 7 gènes alors que celui des cellules de mammifères en contiendrait environ 5×10^6.

Grâce à sa petite taille et à sa remarquable stabilité physique et biologique, l'ADN circulaire polyome est un moyen utile pour l'étude de la régulation génétique des cellules mammifères.

Riassunto

Il confronto dei pesi molecolari permette di calcolare che l'ADN circolare del Polyoma contiene da 5 a 7 geni mentre quello delle cellule dei mammiferi ne conterrebbe circa 5×10^6.

Grazie alla sua piccola taglia e alla sua considerevole stabilità fisica e biologica, l'ADN circolare del polyoma rappresenta un utile mezzo di studio sulla regolazione genetica delle cellule dei mammiferi.

This work was supported by grants from the Swiss National Foundation for Scientific Research.—The electron microscopy of polyoma virus and polyoma DNA was performed by Dr. G. PÉTURSSON and Dr. B. HIRT respectively at the Centre de Microscopie Electronique of the University of Lausanne.

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