The Insertion of DNA into Vaccinia Virus

Abstract. *Cells infected with vaccinia virus in the presence of hydroxyurea (HU), which blocks DNA replication, were examined in thin sections by electron microscopy at intervals after removal of HU. Dense, fibrillar material was observed at the orifice formed just before closure of the membrane constituting the envelope of the immature form of the virus. It is concluded that synchrony of assembly enabled stages in the condensation and insertion of viral deoxyribonucleoprotein to be observed. The mechanism appears to be similar to that encountered in morphologic studies of herpes simplex virus and in biochemical studies of poliovirus, adenovirus, and several bacteriophages.*

Investigation of bacteriophages indicates that after assembly of the head, insertion of the nucleoprotein results in the formation of a "provirus," which subsequently matures into the complete or infectious virion (*1, 2*). Biochemical studies suggest that in the case of poliovirus (*3*) and adenovirus (*4*), at least, the nucleic acid is inserted into a preformed "procapsid." A period of transient synchrony of assembly follows the release of infected cells from blockade with hydroxyurea (HU) and, by taking advantage of this, it has been possible to demonstrate by electron microscopy the stages in the entry of herpes simplex virus nucleoprotein into preformed capsids (*5*). In view of these observations, I examined cells infected with vaccinia virus and found that the process of entry of nucleoprotein could be observed.

In the normal course of events after infection with vaccinia virus, large, relatively homogeneous aggregates of fine granules and filaments form in the cytoplasm and constitute the sites at which viral assembly occurs (*6*). Biochemical studies indicate that, although DNA is replicated at these sites, the proteins are synthesized elsewhere and transported to the foci of assembly (*7*). The next structural event observed is the formation of membranes coated with spicules (*8*). These grow in an extraordinarily controlled manner to enclose uniform portions of matrix (viroplasm). With the appearance of the electron-dense, eccentrically placed bodies (nucleoids) the immature form of the virus is completed. Subsequently, the surface spicules disappear; the nucleoid moves to the center of the particle (*7, 9*) and becomes a biconcave disk or core; the viroplasm condenses to form lateral bodies and the infectious virion is formed. In the presence of HU, the synthesis of early proteins takes place but DNA replication is prevented and infectious virus is not found (*10, 11*). Upon release from blocking with HU, there is rapid synthesis of viral DNA, characteristic immature virus makes its appearance, and the production of infectious virions results (*9, 12*).

Specimens were prepared for electron microscopic examination as follows. Cells were infected with vaccinia virus at a viral multiplicity of 10 : 1 in the presence of $5 \times 10^{-2}M$ HU. After incubation for 18 hours, the cells were washed with balanced salt solution and incubation was continued for 3, 5, 7, and 9 hours. The preparations were fixed in glutaraldehyde and osmium tetroxide, embedded, cut into thin sections, and stained. Although the aforementioned cytoplasmic foci were readily visible at 3 hours, it was not until 5 hours after the HU was removed that viral membranes in process of differentiation were encountered. These membranes with characteristic surface spicules progressively enfolded the fine fibrils and granules termed viroplasm. Electron-dense material, which frequently had the appearance of closely packed filaments, was seen at the orifice before the membranes closed to complete the immature form of the virus (Fig. 1). The location of these filaments, their opacity, and close morphologic similarity to the nucleoid left little doubt that they were, in fact, the substance of the nucleoid encountered in the process of condensation and entry. This event was first observed 5 hours after the removal of HU. Upon completion of the nucleoid the membranes appeared to finish the process of closure (Figs. 2 and 3). Several lines of evidence suggest that the nucleoid of the immature form of the virus is, in fact, the viral deoxyribonucleoprotein. First, its density to the electron beam is in keeping with the appearance of the nucleoprotein cores of all viruses so far examined. Second, electron microscopic studies (*7, 9*) have shown that the nucleoid migrates centrally and appears to become transformed into the biconcave disk of the mature virion. This central structure contains the DNA (*13*). Third, in the presence of agents blocking synthesis of DNA and the formation of infectious virus, the immature forms differentiate but lack a nucleoid (*10, 12, 14*). In the normal course of infection, the incorporation of DNA into the virus begins relatively late—that is, at 5 hours, by which time a pool of DNA has already formed (*7*). This pool of DNA is not structurally recognizable at the foci of assembly. Only when condensation or close packing of

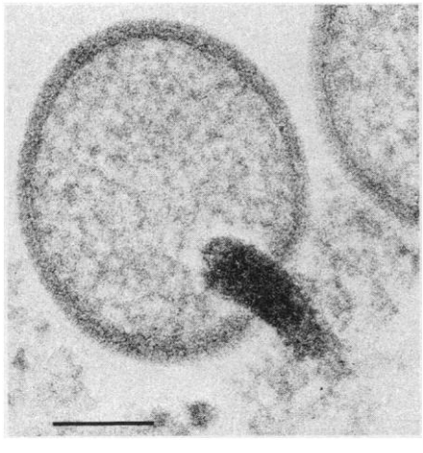

Fig. 1. Deoxyribonucleoprotein apparently at midstage of condensation and insertion into the virus. Scale bar, 0.1 μm.

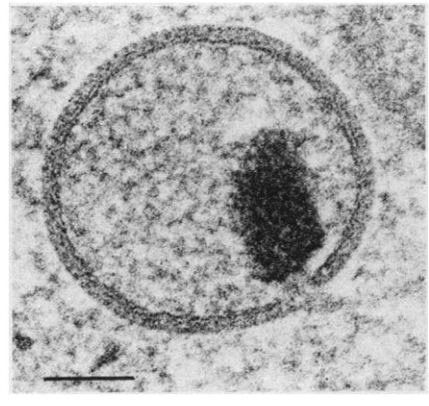

Fig. 2. The process of insertion nearly finished. Fibrinous material still extends from the cytoplasm to the nucleoid. Surface spicules are evident at the upper left. Scale bar, 0.1 μm.

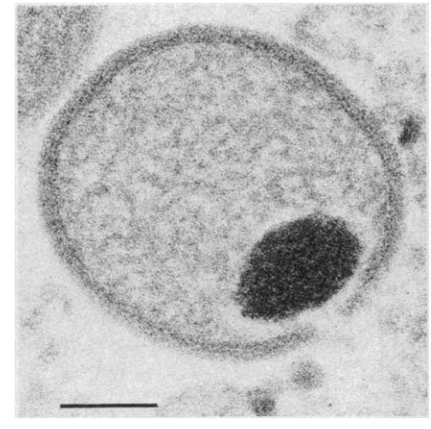

Fig. 3. A fully developed nucleoid. The small, remaining orifice would seem about to close, thus completing envelopment of the virus. Scale bar, 0.1 μm.

the nucleoprotein occurs at the instant of entry does it become visible in the electron microscope.

The immature form of the virus appears to be unstable, because it is never encountered extracellularly. It thus resembles the "fragile precursor heads" described in the case of T4 bacteriophage (*2*). Finally, the point should be made that maturation of vaccinia virus requires protein synthesis (*7, 15*) in a manner analogous to that encountered in recent studies of the conversion of precursor particles to mature T4 phage (*2*). It is suggested that in the future the "immature" form of the pox viruses be called provirus in keeping with bacteriophage nomenclature.

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Benzo[*a*]pyrene Diol Epoxides as Intermediates in Nucleic Acid Binding in vitro and in vivo

Abstract. *Evidence has been obtained that a specific isomer of a diol epoxide derivative of benzo[a]pyrene, (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, is an intermediate in the binding of benzo[a]pyrene to RNA in cultured bovine bronchial mucosa. An adduct is formed between position 10 of this derivative and the 2-amino group of guanine.*

Two hundred years have elapsed since the British surgeon Sir Percival Pott made the observation that chimney sweeps developed skin cancer because of their exposure to soot (*1*). This astute observation was the first causal association between an environmental (in this case, occupational) agent and human cancer. We now know that some of the carcinogenic principles in soot and coal tars are polycyclic aromatic hydrocarbons (PAH) (*1, 2*). These compounds are widely distributed in the human environment and thus represent major potential human hazards. Perhaps the most extensively studied PAH is benzo[*a*]pyrene (BP) which is emitted into the air of the United States at an estimated rate of about 1300 tons per year (*3*).

It has become an axiom in cancer research that many chemical carcinogens, including BP, are metabolized in vivo to reactive intermediates that become covalently bound to cellular macromolecules (*4, 5*). There is also evidence that this binding is a critical event in the process of carcinogenesis. Some studies have suggested that the major reactive intermediate in vivo in the case of BP is a 7,8-diol-9,10-epoxide metabolite (*6*). Two isomers, **1** and **2** (Fig. 1), of this compound have been synthesized (*7, 8*). In this study we examine the binding in vitro of these two isomers to various nucleic acids, compare the products to adducts of BP and nucleic acids formed intracellularly when bovine bronchial explants were incubated with ^{3}H-labeled BP, and report the structure of one of these products.

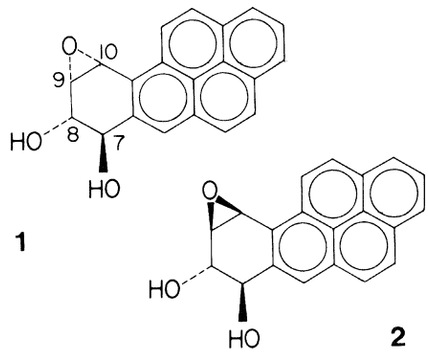

Fig. 1. Structures of compound **1**, (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, and compound **2**, (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene.

Table 1 indicates the binding in vitro of either isomer **1** or isomer **2** to various natural and synthetic nucleic acids. The 7,8,9,10-tetrahydro-BP derivatives have a pyrenelike absorption spectrum with a strong maximum between 340 and 350 nm. The unmodified nucleic acids did not show absorption at 350 nm, whereas the modified polymers showed a distinct absorption peak in this region. Thus, the absorption at 350 nm could be used to estimate the extent of covalent binding of the BP residue to nucleic acids (legend to Table 1). With respect to the synthetic ribonucleotide homopolymers, at *p*H 5 isomer **1** reacted considerably with polyguanylic acid [poly(G)], polyadenylic acid [poly(A)], and polycytidylic acid [poly(C)]. Isomer **2** also reacted with poly(G) and poly(A), and to a lesser extent with poly(C). A somewhat similar pattern of base specificity was seen with synthetic deoxyribonucleotide homopolymers. Little or no reaction was detected with polyinosinic acid [poly(I)], polyuridylic acid [poly(U)], or polydeoxythymidylic acid [poly(dT)]. Extensive reactions were seen with RNA and DNA, which provide all three residues—guanine (G), adenine (A), and cytosine (C)—as substrates. When reactions were performed at *p*H 8.0 with either isomer **1** or **2**, the total extent of modification of nucleic acid was less than at *p*H 5.0 and significant reaction was obtained only with poly(G), RNA, and DNA (Table 1). Separate time course studies indicated that at *p*H 5 isomer **1** or **2** reacted most rapidly with poly(G) and reached a plateau at about 1 hour. Poly(A) reacted somewhat more slowly and reached a plateau at about 3 hours. Poly(C) reacted very slowly. At 3 hours the modification was less than 15 percent that obtained with poly(G) or poly(A), and only after a 26-hour incubation period (Table 1) was there extensive modification of poly(C). Differences in the stabilities of the BP-diol epoxides, as well as the reactivities of the nucleophiles, may account for these results.

Because of the preferential reaction