Molecular Biology of Polyomaviruses and Herpesviruses

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PREFACE

Tremendous strides have been made in the past 10 years in understanding the molecular structures involved in the multiplicative cycles and processes of malignant transformation elicited by both DNA and RNA tumor viruses. We have selected members of two groups of DNA tumor viruses for presentation in this book: *Polyomavirus* and *Herpesvirus*. These viruses are at the same time among the simplest and the most complex of the animal and human viruses. With the development of modern biochemical and genetic engineering techniques for the elucidation of gene structure and function as well as protein identification and characterization, it has been possible to elucidate nearly completely the molecular biology of the polyomaviruses and to make great strides in the related work on such complex viruses as the herpesviruses. We hope that a presentation of the two different groups of viruses in this book will emphasize that particular point while providing updated information on the processes of gene organization and expression from these viruses. Let us keep in mind that much of our knowledge of the molecular mechanisms of gene expression in higher cells has come from studies of simpler genetic systems such as those represented in these viruses.

> The principle cannot be enunciated too often: that anything a cell is seen to do in culture must be counted among its potentialities.
>
> MARGARET R. MURRAY, 1977 (1)

It has been nearly a century since Wilhelm Rous first used explants of the chick embryo medullary plate to study *in vitro* the mechanism underlying closure of the neural tube (2). In the early days of tissue culture, workers necessarily confined themselves to asking biochemical questions of a very general nature. The modern era began in the 1950s, with the exploitation (particularly by virologists and molecular biologists) of cell and tissue-culture techniques to answer specific problems (3). Tissue culture not only released the virologist from an unsavory dependence on developing chick and mouse embryos; it also facilitated the discovery of new pathogenic viruses as well as viruses with no known association or implication in disease processes.

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CONTENTS

CHAPTER I

POLYOMAVIRUSES

I. THE PAPOVAVIRIDAE

A. IDENTIFICATION AND GENERAL DESCRIPTION

In 1971, two new viruses were isolated from human tissues. For want of more descriptive terms, they were labeled with the initials of the patients from which they had been isolated, BK and JC. Both viruses proved to be related to the two already well-studied mammalian viruses: simian virus 40 (SV40) and polyomavirus (Py) (1,2). These four viruses now are considered to be individual species of the genus *Polyomavirus,* which belongs to the family Papoviridae along with the genus *Papillomavirus.* Besides their structural similarities, the genera share the Greek suffix *-oma,* used to form nouns denoting tumorous (3). All papovaviruses have been shown to cause tumors in either their natural host (papillomaviruses) or in different species from the species of origin of the virus (polyomaviruses) (4).

Although papillomaviruses were the first DNA viruses demonstrated to cause tumors (5), understanding of the molecular biology of these organisms has been severely impeded by their inability to grow under current tissue culture systems. The polyomaviruses provide a much more accessible experimental target. While on the trail of a leukemogenic RNA virus in 1953, Gross discovered a separate filterable agent that produced parotid tumors in mice (6). Because the parotid agent was shown to transform many different cell types *in vivo,* the term "polyoma virus" became universally accepted (4), eventually as one word.

SV40 was also discovered as a contaminant in another virus preparation. In nature, SV40 is found persistently associated with kidney cells of the rhesus monkey. These cells were used routinely to propagate the human adenovirus and poliovirus used in vaccines. Before this discovery thousands of patients were inoculated with live SV40 (7). The subsequent discovery by Eddy and her colleagues that SV40 causes tumors (8) inspired intensive in

vestigations of both the virus and the vaccine recipients (4). Fortunately, no human disease has been linked unequivocally to an SV40 infection (although variants of SV40 have been isolated from certain human tissue). The original inspiration has led to innumerable contributions to our understanding of normal and abnormal cellular function (9).

All members of the *Polyomavirus* genus share many traits, not only in their gross physical properties (including specific regions of DNA sequence homology) but in their dynamics of growth as well.* They all contain approximately 3×10^6 daltons of double-strandard, circular DNA enclosed in a small (~45 nm diameter) icosohedral protein capsid (12). The DNA encodes five to seven known proteins. Three of these proteins are involved in capsid structure: VP1, VP2, and VP3 (4). In addition, at least four host-cell-encoded histones; H2A, H2B, H3, and H4, are associated with virion DNA and are responsible for a tertiary structure of the DNA similar to the cellular nucleosome (13,14) (also called minichromosomes).

B. BIOLOGICAL ACTIVITIES AND HOST RANGE

> The polyoma virus—a DNA-containing virus—is characterized by a duality of actions: it produces neoplasias of various types in different species of rodents and causes cell degeneration in mouse embryo tissue cultures. . . . the results so far . . . suggest the existence of host-virus interaction with characteristics reminiscent of temperate bacteriophage.
>
> Marguerite Vogt, 1960 (15)

* In addition to the four major *Polyomavirus* members there are the more recently discovered and poorly examined HD virus of the stump-tailed macaque (10) and the K virus of mice (11), which appear to be quite similar to BKV and JCV from initial superficial studies. They will not be discussed here.

In their classic report in 1960, Vogt and Dulbecco examined the two polar effects of Py virus infection. On one hand, an infection can cause cytocidal interaction leading to extensive viral synthesis and cell degeneration. On the other hand, a moderate interaction leads to the transformation of normal cells into neoplastic cells (15). These observations reflect both tissue culture and whole animal observations. Subsequently, it was observed that all polyomaviruses retain this duality of action to some degree in experimental tissue culture models. The full extent to which this holds true in the whole animal (particularly the human host) has yet to be determined. Cytocidal interaction is now most frequently referred to as lytic infection. The conversion to neoplastic characteristics is arrogantly described by those in the field simply as cellular transformation.

Each of the polyomaviruses has a separate and distinct host range—especially for lytic infection. SV40 grows well only in primary cells of African green monkey kidneys (AGMK), certain other monkey cells, and cell lines derived therefrom. By definition, it does not grow well in its persistently infected host–rhesus monkey kidney cells. It grew poorly, if at all, in human cells tested. SV40 has been shown to transform a variety of rodent cell cultures as well as certain human cells and readily causes the induction of tumors in newborn hamsters (especially gliomas and subcutaneous fibrosarcomas). When especially high titers of SV40 are used for intravenous inoculations, leukemias, lymphomas, osteosarcomas, and reticulum cell sarcomas develop. It does not induce tumors in the natural host nor, apparently, in humans (4,16,17).

Py virus multiplies in several cultures of mouse and other rodent cells, although it is endemic in most mouse populations. It has a broader range of oncogenicity than does SV40, transforming not only mouse cells but also cells of rats, rabbits, guinea pigs, dogs, cattle, monkeys, and man. Inoculations into animals (hamsters and mice are the most popular hosts) result in tumors

in virtually all organs and tissues except the brain. Tumor formation in rabbits has also been reported (16,17).

BK virus, in contrast, has a very limited host range. Highest virus titers are obtained only when primary human cultures of brain, kidney, or endothelial origin (18–21) are used. Vero cells (monkey origin), while adequate for the original isolation of the virus, replicate BKV poorly after as few as three passages. In general, the BKV lytic cycle is slower than that of either SV40 or Py. Other monkey cultures such as primary AGMK, CV-1, or BSC-1, do not produce significant amounts of virus (18) and are frequently abortively transformed (22).* In culture, BKV has been shown to transform hamster kidney cells (23–25), rat embryo cells (26), rabbit cells (27,28), and several primary human cells including brain (29), kidney (20), and vascular endothelial cells (21). *In vivo,* BKV has been shown to be weakly oncogenic only in the newborn hamster (23,30–32), where it demonstrates a propensity toward cerebral tumors (33), and mastomy (9). It has been recovered, however, from the brain tumor of an immunosuppressed human (34). Reports of BKV DNA sequences in human tumors are conflicting and will be dealt with more fully below (35–37).

Finally, JCV appears to have the most limited host range of all. Its lytic cycle is also the slowest. Although an enormous number of cell lines have been investigated, productive infection occurs only in primary human fetal glial cells and in secondary human amnion cells (38), while human lung and kidney cells function only as very poor hosts (39). *In vitro,* it has been shown to transform only hamster (40) or human (41) glial cells, human endothelial cells (21), or human amnion cells (42). In contrast to BKV, JCV is highly oncogenic in newborn hamsters, primarily producing tumors associated with the brain (43,44). It has also been shown to induce tumors of the brain when inoculated into owl monkeys (45). In humans, JCV has been found repeatedly

* Abortive transformation implies partial advancement towards the transformed state but not maintenance.

associated with the degenerative disease, progressive multifocal leukoencephalopathy (PML) (46–48).

C. VIRUS REPLICATION AND EXPRESSION

1. Temporal Considerations

The *Polyomavirus* lytic infection cycle is initiated by adsorption and viropexis (pinocytosis) of virions into permissive cells. The virions lose their capsids, and the DNA may associate with host proteins and enter the nucleus (16). Because the virion DNA has a coding capacity for only a few proteins, it must depend on the host cell to supply most of the functions of viral replication, transcription, and translation. Unlike many other viral infections, infection with one of the polyomaviruses does not inhibit normal cellular functions. In fact, infection forces resting cells to reenter the cell cycle, and the entire complement of host DNA is subsequently replicated. Histone synthesis is also enhanced (following DNA replication). The synthesis of several known enzymes, the overall rate of protein synthesis, host RNA synthesis, and the transport of hexose sugars across the cell membrane are also enhanced prior to DNA synthesis (4).

The lytic cycle is divided temporally. Early functions are those that appear prior to the onset of viral DNA replication, beginning 12 to 15 hours after infection. Late functions are those expressed thereafter. In general, early expressions are nonstructural viral proteins required for induction of the host-cell functions mentioned above and the initiation of viral DNA replication; while late functions are primarily the virion structural proteins (4).

2. Functional Subdivision of the Viral Genome

Correspondingly, the viral genome is also divided into early and late components. Studies on temperature-sensitive (ts) and dele-

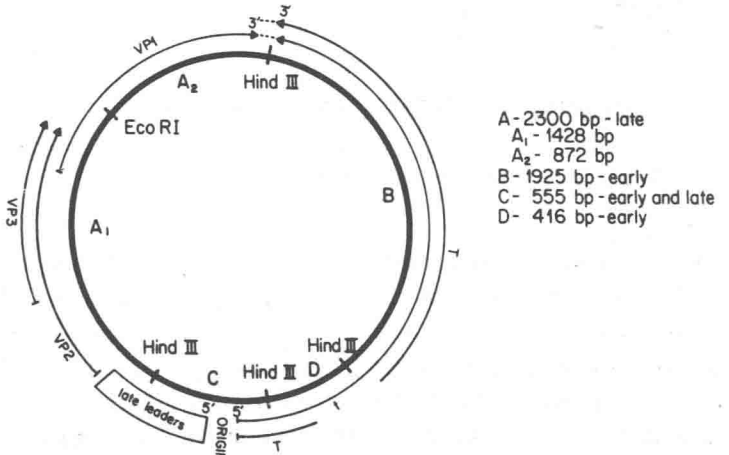

FIGURE 1. Schematic representation of a prototype *Polyomavirus* genome. Solid lines represent polypeptide coding regions; dashed lines equal full extent of 5′-capped and 3′-polyadenylated mature mRNA. Removal of RNA sequences by splicing events is indicated by *A*. General regions to which early mutations have been mapped are so noted.

tion (dl) mutants as well as analysis of mature messenger RNA (mRNA) species (4) and, ultimately, analysis of DNA sequences (49–55) have demonstrated that the *Polyomavirus* genome is virtually divided into functional halves. As shown in Fig. 1, all functions required during the early phase of infection are encoded by the right half of this composite genome, while the late (structural) functions are encoded by the left half.

Also indentified in Fig. 1 is the viral origin of DNA replication. Sequence analysis of SV40, Py, and BKV DNAs (49–55) indicates a high level of nucleotide conservation between viral origins which maintains the potential for extensive secondary structure. Early work which examined replicating structures by electron microscopy (56,57) or pulse-labeling (58) indicated that DNA replication proceeds at an equal rate in both directions from the sin-

gle origin. Subsequently, a small portion of replicating Py molecules were also observed as rolling-circle replicative intermediates (59). Bidirectional replication terminates when the growing forks meet and the two daughter molecules are separated by nick and repair procedures (4).

3. Early Gene Expression

Production of virus-encoded RNA is the first gene expression observed following infection (60,61). It is the harbinger of the early viral proteins known at T antigens. Following the lead of those studying adenovirus-induced tumors SV40 (and Py), workers indentified a soluble antigen in both lytically infected and virus-transformed cells. It reacted with serum from virally induced tumor-bearing animals in a standard complement fixation assay (C'F) (62–65). The reactive property was distinct from virion capsid antigens and appeared much sooner after infection than did virion antigens. Because the C'F activity was dependent upon sera from tumor-bearing animals, it was referred to as tumor, or T antigens. These sera reacted specifically with the nuclei of both infected and virally transformed cells in an indirect immunofluorescence assay (66,67).

By 1977, a total of three distinct T antigen polypeptides encoded by the early region of Py was discovered, two of which were also noted in SV40 (these two are also encoded by early BKV and JCV DNA). The first detected was the large T antigen whose molecular weight has been estimated as ranging from 81,000 to 100,000 (68–70,236). It is located primarily in the nucleus of the cell (68), but some forms have also been reported to be associated with the plasma membrane (71,72).

The second tumor antigen found in all polyomaviruses has a molecular weight of 17,000 to 22,000 and has been referred to as small t antigen, or t antigen (70,73,74). Although it shares significant amino acid homology with the large T antigen, several experiments (including extensive genetic studies) have shown it to

be a distinct protein, encoded by its own mRNA (49–55,70,73–86).

A third virally encoded tumor antigen has been identified only in Py-infected cells. Its molecular weight is estimated at 55,000 to 60,000 and accordingly it has been designated "middle T" (87). It also shares some, but not all, of its amino acid sequence with large T antigen and is encoded by its own mRNA (81–83,86). Searches for an analogous protein in other polyomavirus have not been successful, and examination of analogous DNA sequences suggests that no such protein can be encoded (49–55).

Various genetic evidence has indicated that one or another of the early viral antigens are required for specific steps in lytic infection and transformation (4,88–93). The early region of the DNA is divided into two complementation groups. The A group includes mutations that involve primarily the carboxyl half of the large T antigen, while the hr-t (Py) or dl 0.54-0.59 (SV40) groups involve mutations primarily in the region that encodes the unique portion of t antigen. (For Py, this also includes the region encoding the middle T antigen. The Py t and T genes have only recently been genetically separated (91). "A" mutations were first identified in ts mutants of SV40 and Py (4,88,92). The chemically induced mutants were isolated by their ability to form plaques at "permissive" temperatures (31–33°C) but not at "nonpermissive" temperatures (~40°C). Multiple analyses of these viruses demonstrated that a functional A gene was required to initiate (but not to complete) each new round of viral DNA replication (92,94). It is also required, probably only transiently, to induce transcription of late viral RNAs, and it appears to regulate the synthesis of its own mRNA (95,96). For our purposes, we consider the A gene synonymous with large T antigen.

The role of large T antigen in cellular transformation is not as consistent among the polyomaviruses as it appears to be for lytic infection. While it is clear that T is an absolute requirement for the initiation of SV40 transformation (88–93), Py transformation has been achieved with Py DNA molecules in which the coding

region for large T has been interrupted by cleavage or insertion of foreign DNA (97–99). Functional T may also be required for the maintenance of the transformed state in all but the Py system (100).

Other properties associated with large T antigen include:

(a) It is phosphorylated (70,73) and poly-ADP ribosylated (101).

(b) It is primarily sequestered in the nucleus (66,67), although it has been reported in the plasma membrane (71,72).

(c) It can induce synthesis of cellular enzymes (4) and is responsible for increased host RNA synthesis (102).

(d) It is required for the integration of tandem repeats of Py DNA into the host genome, and it facilitates excision of viral genomes (103–106).

(e) It binds to DNA in general and has high affinity for a discrete region of the viral origin of DNA replication (107–109).

(f) It copurifies initially with both an ATPase activity and a protein kinase activity, although rigorous assignment of these activities to the T molecule has not been proved. In fact, only the ATPase activity is maintained when an adenovirus-SV40 T antigen chimeric protein is highly purified (110–112).

(g) It is most "active" as a tetramer, perhaps in complexes with host-encoded proteins (108–108a).

(h) Preparation of T antigen can induce tumor immunity, so-called TSTA activity (see below) (113–114).

(i) During both lytic infection and transformation it can be observed to bind to discrete RNA molecules (114a–114b).

Less well established are the properties associated with small t antigen: