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Minireview

SV40 DNA replication: From the *A* gene to a nanomachine

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ABSTRACT

Duplication of the simian virus 40 (SV40) genome is the best understood eukaryotic DNA replication process to date. Like most prokaryotic genomes, the SV40 genome is a circular duplex DNA organized in a single replicon. This small viral genome, its association with host histones in nucleosomes, and its dependence on the host cell milieu for replication factors and precursors led to its adoption as a simple and powerful model. The steps in replication, the viral initiator, the host proteins, and their mechanisms of action were initially defined using a cell-free SV40 replication reaction. Although our understanding of the vastly more complex host replication fork is advancing, no eukaryotic replisome has yet been reconstituted and the SV40 paradigm remains a point of reference. This article reviews some of the milestones in the development of this paradigm and speculates on its potential utility to address unsolved questions in eukaryotic genome maintenance.

Introduction

The study of bacteriophage and viruses over the past 50 years laid the foundations of modern molecular biology. The physicists, chemists, biologists, and physicians pioneered this frontier with the hope that the relative simplicity of these agents might allow them to serve as tools to understand their vastly more complex infected host cells. The discovery of simple DNA viruses that propagated in mammalian cell nuclei and caused tumors in experimental animals led the way to an explosion of eukaryotic molecular biology and its applications to understanding, treating, and preventing human disease. For pioneering studies of the DNA tumor viruses polyomavirus and SV40 in the 1969's, Renato Dulbecco was awarded the 1975 Nobel Prize in Physiology or Medicine. Equally importantly, the many young scientists who trained in his laboratory were inspired to pursue and expand on this fruitful approach in ever more exciting new directions. The development of the field and collegial interactions among members of the DNA tumor virus community were greatly fostered by annual meetings sponsored by Cold Spring Harbor Laboratory and Imperial Cancer Research Fund, as well as by review volumes edited by John Tooze beginning in 1973 (Tooze, 1973).

This article reviews some of the fundamental lessons on genome structure, DNA replication, and genome maintenance that these deceptively simple viruses have revealed over the past 4 decades. The utility of these viral paradigms in guiding the investigation of mammalian DNA replication is considered. The article concludes with reflections on how the rapidly growing understanding of host genome maintenance is leading to a re-consideration of how these viruses exploit their host cells.

The SV40 minichromosome: genetic and physical maps linked through DNA sequence

The SV40 genome is a covalently closed circular duplex DNA molecule of 3.6×10^6 Da (Crawford and Black, 1964; Dulbecco and Vogt, 1963; Weil and Vinograd, 1963). Biophysical characterization of superhelical SV40 and polyomavirus DNA provided the first insight into the initially puzzling ability of supercoiled DNA to renature rapidly after exposure to alkali (Vinograd et al., 1965; Weil, 1963; Weil and Vinograd, 1963), its limited uptake of intercalating dyes, e.g. ethidium bromide, and other properties typical of supercoiled DNA. The SV40 genome exists in the virus particle and in infected cells as a mini-chromosome packaged with host cell histones into nucleosomes that closely resemble those of the host chromatin (Bonner et al., 1968; Germond et al., 1975; Griffith, 1975; White and Eason, 1971) (Fig. 1). Purification of SV40 DNA from minichromosomes reveals its negatively supercoiled topology. As we now know, this topology endows chromatin with the capacity to readily denature for initiation of DNA replication or transcription.

Genetic studies of replication in prokaryotes had led to a potentially general model for control of replication: the replicon model of Jacob, Brenner, and Cuzin (Jacob and Brenner, 1963). The model postulated a cis-acting element, the replicator, recognized by a trans-acting factor, the initiator. This interaction would lead to locally denatured duplex DNA in or near the replicator element and initiation

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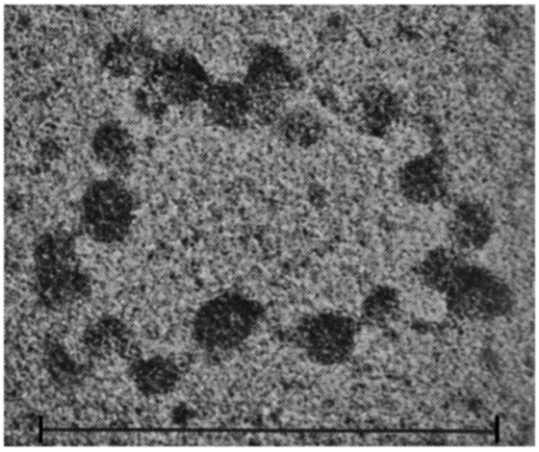

Fig. 1. Electron micrograph of an SV40 minichromosome isolated from productively infected cells. (Scale bar 100 nm). (Reprinted from Griffith, 1975 with permission from AAAS.)

of replication. Each replicator with its initiator would thus govern the replication of the flanking regions of DNA, the replicon. If this model were general, one might expect eukaryotic DNA to be organized into replicons in a similar manner. If SV40 DNA represents a eukaryotic replicon, one would predict a genetically definable viral replicator element and an initiator that recognized it.

In the mind of Daniel Nathans at Johns Hopkins University, the appeal of SV40 as an object for genetic analysis converged with the discovery of the first sequence-specific restriction endonucleases by his colleague Hamilton Smith (Fig. 2). Nathans and colleagues generated the first restriction cleavage map of SV40 DNA, by determining the physical order of the Hind II/III and Hpa I/II sites around the SV40 DNA genome (Danna and Nathans, 1971; Danna et al., 1973). A unique restriction cleavage site by Eco RI (Morrow and Berg, 1972) provided a point of reference in the viral genome. By 1972, Danna and Nathans had combined a radiolabeled thymidine pulse-chase approach with their restriction map to determine the physical start site for SV40 DNA replication, the origin, and show that replication proceeded bidirectionally to terminate on the opposite side of the DNA molecule (Danna and Nathans, 1972; Nathans and Danna, 1972). [For a fascinating overview of these discoveries, see Brownlee, 2005; Roberts, 2005] This physical map greatly facilitated determination of the 5243 bp sequence of SV40 DNA, the first eukaryotic genome to be completely sequenced (Fiers et al., 1978; Reddy et al., 1978). Moreover, the map and the sequence enabled the classical mutational analysis of the SV40 genome (Chou and Martin, 1974; Tegtmeyer, 1972; Tegtmeyer and Ozer, 1971) to be correlated with nucleotide sequence changes that affected viral DNA replication (temperature-sensitive complementation group A (tsA)), cell transformation (tsA), and virion production (tsB, C, BC, D) [for a personal account, see Nathans, 1978].

With the viral DNA sequence in hand and new restriction endonucleases rapidly emerging in several laboratories, the Nathans lab moved quickly to test the function of the SV40 origin of DNA replication by mutational analysis. They devised site-directed mutagenesis protocols for deletions and base substitutions followed by selection for resistance to cleavage by Bgl I, which has a single recognition site in SV40 DNA at the origin (DiMaio and Nathans, 1980; DiMaio and Nathans, 1982; Shortle and Nathans, 1978). These mutations were mapped by DNA sequencing and shown to render SV40 replication defective when the genome was introduced into host cells, satisfying one criterion for a replicator element. To examine the relationship between the putative replicator element and the *tsA* gene that was also involved in replication, the Nathans lab carried out a mutational screen for second site revertants of the replication-defective mutant origins. These pseudorevertant mutations were then mapped and shown to reside at positions outside of the origin region and to alter the coding sequence of the *tsA* gene (Margolskee and Nathans, 1984; Shortle et al., 1979). The *A* gene encodes the SV40 large tumor (T) antigen (Tag), a multifunctional protein whose structure and roles in viral DNA replication are reviewed below. Thus, the origin element interacted genetically with a viral gene that regulated the rate of viral DNA replication, providing strong evidence for a replicon model in controlling replication of SV40 DNA. Biochemical investigation of Tag promptly confirmed the interaction, paving the way for new experiments to elucidate the mechanism of SV40 DNA replication.

Further dissection of the viral replicator in multiple laboratories revealed a 64 bp core composed of three elements. A central element contains a palindromic array of four GAGGC pentanucleotides that, as we now know, serve as binding sites for Tag. The binding sites are flanked by an easily denatured imperfect palindrome (EP) on one side and by an AT-rich sequence on the other side. The two flanking elements undergo local distortion or melting during initiation of replication (Borowiec et al., 1990). The early and late promoter elements flank the viral core origin and stimulate its activity in infected cells, as does the viral enhancer element. These auxiliary elements may stimulate initiation of replication from the viral core origin at multiple levels, some of which may reflect the close relationship between origins of replication and transcription. The first level may be by modulating the structure of the core origin DNA to facilitate distortion by Tag, e.g. through intrinsically bent AT-rich DNA sequences, or local modulation of supercoiling. These physical properties of origin DNA are also found in eukaryotic chromosomal origins of replication and are thought to be important for binding of the origin recognition complex ORC (Remus et al., 2004). Proteins bound to the auxiliary elements may also facilitate Tag assembly on the core origin DNA, Tag remodeling into an active helicase, or recruitment of host replication proteins. For example, chromatin remodeling to generate a nucleosome-free origin region and histone modifications are likely to be important for initiation at the SV40 origin and at chromosomal origins (Saragosti et al., 1980). Lastly, replication of the viral minichromosome appears to take place in specific subnuclear domains (Ishov and Maul, 1996; Staufenbiel and Deppert, 1983; Tang et al., 2000), but how the minichromosome is targeted to these sites remains poorly defined.

Given the sequence specificity of the SV40 core origin and the dependence of the virus on host cell proteins for DNA replication, it was tempting to imagine that chromosomal origins of replication might also be composed of modules with defined sequences that

Fig. 2. Daniel Nathans (left) and Hamilton Smith in the laboratory at Johns Hopkins University. (Reprinted from Roberts, 2005 with permission from Copyright 2005 National Academy of Sciences, U.S.A.)