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Research Article

Nuclear actin and myosins in adenovirus infection

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ABSTRACT

Adenovirus serotypes have been shown to cause drastic changes in nuclear organization, including the transcription machinery, during infection. This ability of adenovirus to subvert transcription in the host cell facilitates viral replication. Because nuclear actin and nuclear myosin I, myosin V and myosin VI have been implicated as direct regulators of transcription and important factors in the replication of other viruses, we sought to determine how nuclear actin and myosins are involved in adenovirus infection. We first confirmed reorganization of the host's transcription machinery to viral replication centers. We found that nuclear actin also reorganizes to sites of transcription through the intermediate but not the advanced late phase of viral infection. Furthermore, nuclear myosin I localized with nuclear actin and sites of transcription in viral replication centers. Intriguingly, nuclear myosins V and VI, which also reorganized to viral replication centers, exhibited different localization patterns, suggesting specialized roles for these nuclear myosins. Finally, we assessed the role of actin in adenovirus infection and found both cytoplasmic and nuclear actin likely play roles in adenovirus infection and replication. Together our data suggest the involvement of actin and multiple myosins in the nuclear replication and late viral gene expression of adenovirus.

1. Introduction

Diverse bacterial and viral pathogens induce actin polymerization of host cells to facilitate infection. In the nuclei of host cells, a pathogenic mechanism for promoting dynamic actin assembly has been described to enable replication in a growing number of viruses. Baculovirus, a large double-stranded DNA virus that replicates inside the host nucleus, has been shown to manipulate nuclear actin for virus gene expression and progeny production [1,2]. Nuclear actin has also been implicated in herpes viral infection [3,4]. Replication compartments formed by herpes simplex virus in infected nuclei were shown to move by directed motion and require nuclear actin and myosins [5], and myosin Va shows nuclear enrichment upon herpes simplex virus infection [6]. Furthermore, nuclear actin has been implicated in the nuclear transport of unspliced mRNA from human immunodeficiency virus type 1 (HIV-1) and Mason-Pfizer monkey virus [7,8]. Although adenovirus infection has been shown to result in loss of nuclear actin from Cajal bodies [9], sites of RNA metabolism that disassemble in the late phase of adenovirus infection [10–12], no studies have directly investigated the role of nuclear actin and myosins in adenovirus infection.

In uninfected cells, nuclear forms of actin and myosins are involved in multiple steps required to produce mature transcripts [13]. Actin and myosins have been shown to interact with several chromatin remodeling complexes [14] and have been implicated in mediating long-range directed movement of chromatin [15,16]. Nuclear actin has been found to regulate transcription by all three RNA polymerases and is important for pre-initiation complex formation [13]. Nuclear actin is also associated with hnRNP A proteins [17], which along with Cajal bodies are reorganized during adenoviral infection [9,10]. Moreover, nuclear actin has been linked to the nuclear matrix through its interactions with lamins, emerin, and nuclear scaffolding proteins [18–21]. Recent work on nuclear actin binding proteins has implicated nuclear actin as a potential regulator of nuclear shape and organization [22,23]. Although the functions of actin and myosins in the nucleus are becoming clearer, the mechanisms by which they operate are largely unknown.

Human adenovirus type 5 (Ad5, family Adenoviridae, genus Mastadenovirus) is a non-enveloped icosahedral virus containing a linear double-stranded DNA molecule that replicates in the cell nucleus [24]. In a productive adenovirus infection there is a dramatic reorganization of the cell nucleus during the intermediate and late phase of lytic infection, whereas early viral gene expression does not alter the nuclear organization of mRNA biogenesis

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[24–29]. The transition to the late phase of infection is conventionally defined by the onset of viral replication and coincides with the accumulation of viral 72 kD DNA binding protein into spot, ring and crescent-like structures [24,25,30,31]. These virus-induced structures demarcate areas of active viral DNA replication. They also represent nuclear sites where newly replicated adenovirus DNA is heavily transcribed by the host cell's RNA polymerase II (pol II) and where the resulting pre-mRNAs are processed [24–26,29,32]. Adenovirus DNA replication occurs at the surface of these compact ring structures and transcription and splicing are predominantly detected around these structures [24].

Given the dynamic nuclear reorganization and transcriptional

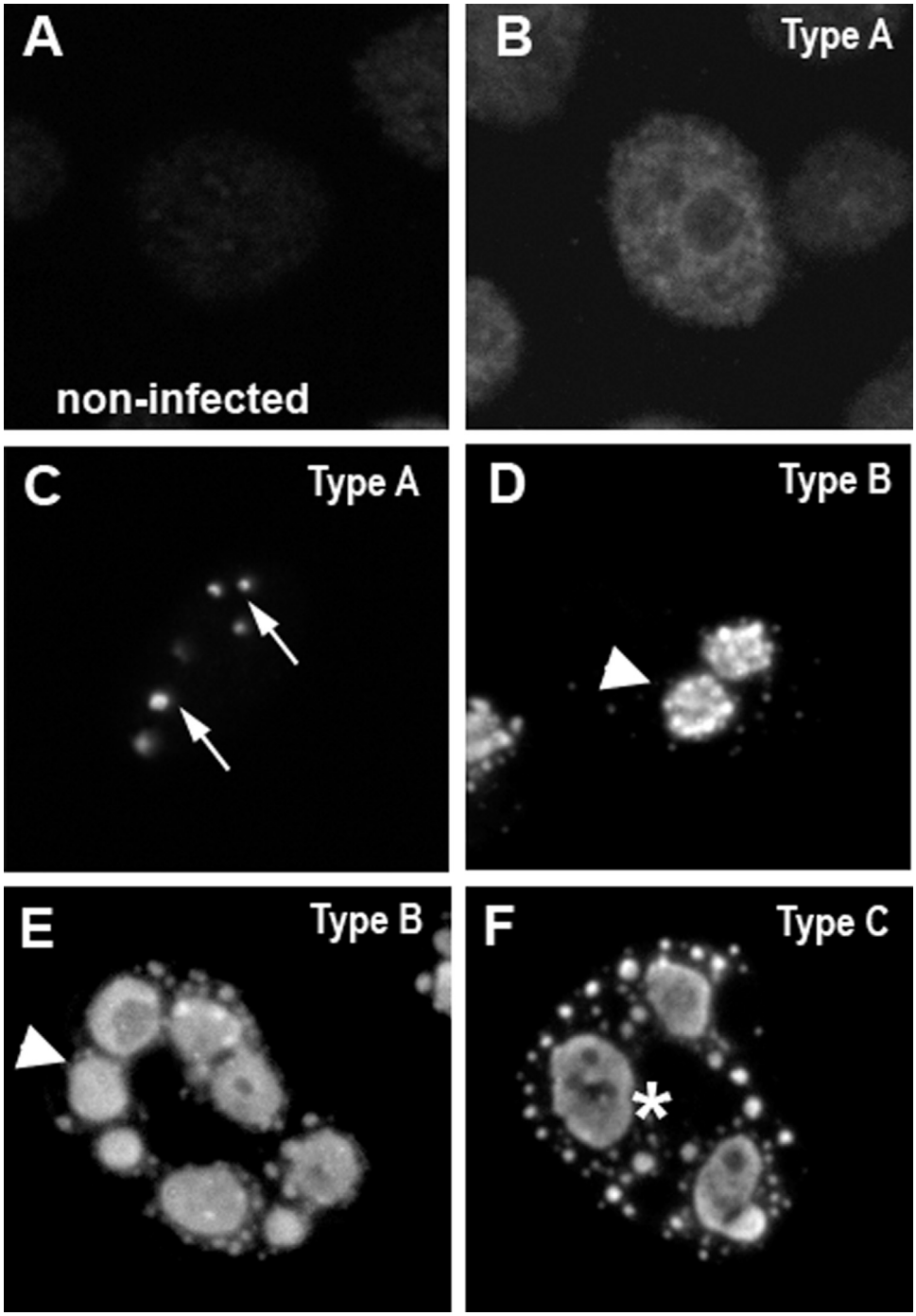

Fig. 1. Nuclear morphology of viral DNA centers. Confocal micrographs of a control (non-infected) HeLa cell (Panel A**;** no 72 kD staining) and HeLa cells infected with Ad5 wt (1000 VP/cell) and imaged at 21 hpi. Cells were labeled with an antibody against the viral 72 kD DNA binding protein. The homogenous distribution of 72 kD (Type A, Panel B) is consistent with the early stages of viral infection. The onset of viral DNA synthesis marks the transition from the early to the late phase of infection and is characterized by the compaction of viral centers labeled by the anti-72 kD antibody into dots (Type A, Panel C; arrows). The initial part of the late phase is termed the intermediate stage. As the intermediate stage progresses, viral centers labeled by the anti-72 kD antibody grow and are detected as rings (Type B, Panel D and E**;** arrowheads). Panels D and E also indicate the morphological heterogeneity of the ring structures. As the late phase of adenovirus infection continues, enlarged pools of 72 kD protein become present (Type C, Panel F; asterisk), which correspond to the advanced late phase. Therefore, Type A cells were classified as expressing 72 kD protein in a homogenous nuclear distribution or in discrete dots, indicating the transition from the early into the intermediate phase (i.e. the initial part of the late phase) (Panels B and C). Cells expressing the viral 72 kD DNA binding protein in rings were classified as Type B, indicating the progression of the intermediate phase (Panels D and E) and cells expressing enlarged pools of 72 kD were identified as Type C, corresponding to the advanced stage of the late phase (Panel F).