ABSTRACT

Papillomaviruses (PV) infect the basal cells of the skin or mucosal epithelium thereby inducing benign tumors which may undergo malignant transformation dependent on the infecting PV type. The viral E2 protein plays an essential role within the regulation of the viral life cycle. It is able to activate or repress viral gene expression, and together with the PV E1 protein E2 fulfills functions in the activation of viral replication. In this study the role of the interaction of E2 with cellular proteins which are important for transcriptional activation were investigated. In previous experiments the human Nucleosome Assembly Protein 1 (hNAP-1), a histone chaperone, was identified as a protein interacting with the activation domain of the transcriptional activator BPV1 E2 (Bovine PV1 E2) by a yeast two-hybrid screen. This interaction was confirmed in vitro by GST pull-down experiments with the E2 proteins of BPV1, HPV8 (Human PV8) and HPV18. The aim of this study was to characterize the importance of the interaction of E2 and hNAP-1 for E2-specific functions. The binding of hNAP-1 to these three E2 proteins was confirmed in vivo by coimmunoprecipitations. In addition, it was shown that the interaction is direct and not merely mediated by the cellular coactivator p300, which is bound by E2 and hNAP-1. E2-mediated activation of transcription was strongly stimulated by coexpression of hNAP-1, indicating a role of this interaction for the activator function of E2. Two separable domains of hNAP-1 are bound by E2, one within the C-terminus and an internal domain. By using hNAP-1 deletion mutants it turned out, that the binding of hNAP-1 to E2 is necessary for the cooperativity between both factors but in addition, the N-terminal 91 amino acids of hNAP-1 also are crucial for its coactivator function. Moreover, for the first time evidence for the existence of a ternary complex consisting of hNAP-1, E2 and p300 in vitro was provided by a competition experiment and a glycerol gradient sedimentation. This ternary complex seems to be very efficient in activation of transcription since E2, hNAP-1 and p300 cooperated in activating HPV8 gene expression. Hence, p300 and hNAP-1 as well may be recruited to the DNA by E2 to efficiently activate PV gene expression. Furthermore, p53 which also uses p300 as a coactivator, directly interacts with hNAP-1. This interaction was also shown to be functional since hNAP-1 could enhance p53-mediated activation of gene expression. In contrast, TEF-1 which does not use p300 as a coactivator did not bind to hNAP-1 in vitro and in correlation hNAP-1 was not able to stimulate TEF-1-mediated activation of transcription.