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# Nuclear myosin 1 associates with papillomavirus E2 regulatory protein and influences viral replication

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ABSTRACT

Nuclear myosin 1c (NM1) associates with RNA polymerases and is a partner in the chromatin remodeling complex B-WICH. This complex, which also contains WSTF and SNF2h proteins, is involved in transcriptional regulation. We report herein that papillomavirus protein E2 binds to NM1 and co-precipitates with the WSTF and SNF2h proteins. Our data suggest that E2 associates with the cellular B-WICH complex through binding to NM1. E2 and NM1 associate via their N-terminal domains and this interaction is ATP dependent. The cellular multifunctional protein Brd4 and beta-actin are also present in the NM1-E2 complex. NM1 downregulation by siRNA increases the replication of the BPV1 and HPV5 genomes but does not affect HPV18 genome replication. These results suggest that the B-WICH complex may play a role in the papillomavirus life cycle through NM1 and E2 protein interaction.

## 1. Introduction

Myosins are actin activated ATP hydrolases, and actomyosin complexes function as molecular motors to power muscle contraction, cell motility and cell division (Sellers, 2000). During the last two decades, various studies have demonstrated that myosin and actin are present in both the cytoplasm and the cell nucleus, where myosins perform vital functions in rRNA biogenesis and processing, chromatin dynamics and modulating the export of preribosomal subunits.

Nuclear myosin 1c (NM1) was the first molecular motor described in the cell nucleus (Nowak et al., 1997). NM1 is an isoform b of myosin 1c (Myo1c) that contains a unique N-terminal 16 amino acid sequence. Myo1c was previously thought to reside only in the cell cytoplasm, but later studies revealed that both Myo1c and NM1 are present in the nucleus and in the cytoplasm (Dzijak et al., 2012), showing also that Myo1c can functionally substitute for NM1 in Pol I and Pol II transcription (Venit et al., 2013). Since NM1 was discovered in 1997, five other myosins have been found in the cell nucleus (myosin Va, Vb, VI, XVIb and XVIIIb), whose exact functions are still unknown (Simon and Wilson, 2011). NM1 is a 1079 amino acid protein and similar to other myosin I family members, NM1 has a N-terminal head domain, a linker neck domain with the IQ calmodulin binding motifs and a tail, that is too short to form filaments. The head domain contains motifs for ATP and actin binding, the nuclear localization signal is located in the IQ domain, and nuclear import of this protein is regulated by calmodulin (Dzijak et al., 2012; Sarshad et al., 2013). In the nucleus, NM1 is found in ribonucleoprotein particles together with polymeric actin in association with RNA polymerases I and II, and it is required for nuclear transport and chromatin relocation (Philimonenko et al., 2004; Hofmann et al., 2004, 2006; Kukalev et al., 2005; Visa, 2005; de Lanerolle and Serebryannyy, 2011).

The interaction of the regulatory protein TIF-1 with NM1 promotes the initiation of Pol I transcription (Philimonenko et al., 2004), but it also has a primary role in promoting PCAF-mediated H3K9 acetylation at the gene promoter, which allows transcription activation and cell cycle progression (Sarshad et al., 2013). In addition to transcription initiation, NM1 is also required during the elongation phase. Together with WSTF and SNF2h, NM1 belongs to the multiprotein complex B-WICH that is involved in the RNA pol I and pol III transcription through a chromatin-based mechanism (Percipalle et al., 2006), remodeling the chromatin of active rRNA genes and allowing histone acetyl transferases to associate with histones (Vintermist et al., 2011; Cavellan et al., 2006; Percipalle and Farrants, 2006).

However, until now no information has been available about the NM1 interactions with non-cellular viral proteins that are often involved as regulators in analogous processes such as viral DNA

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replication and maintenance, viral gene expression and mRNA transport in the nuclei of infected cells.

The papillomaviruses (PV) comprise a large family of DNA viruses infecting basal skin or mucosal epithelium in a wide range of hosts. The papillomavirus genome contains extrachromosomal circular dsDNA that is maintained in the nuclei of infected cells, where viral replication and transcription occur. The viral early protein encoded by the E2 ORF is a multifunctional master regulator throughout the viral life cycle. E2 has a structure common to many DNA-bound regulatory proteins. Its N-terminal domain is the transcription activation domain (TAD), and through the C-terminal DNA binding/dimerization domain (DBD) E2 binds to its binding sites present in the PV genomes (Giri and Yaniv, 1988). The BPV1 E2 TAD is important for transcriptional regulation, replication and interaction with the E1 protein and with multiple cellular proteins, including Brd4, EP300and NAP1L1. Both E2 (Muller and Demeret, 2012) and NM1 (Dzijak et al., 2012; Sarshad et al., 2013) interact with PCAF protein complexes and mediate PCAF recruitment to chromatin, thereby influencing the H3K9 acetylation supporting transcription activation. The conserved DBD interaction partners include BRCA1, TAF1, TBP and TP53 (Muller and Demeret, 2012). The TAD and DBD domains are linked by a hinge region, which is variable between PV genera. E2 is a replication factor that together with the viral protein E1 initiates PV genome replication by binding to the replication origin in the upstream regulatory region (URR) that contains at least one E2 binding site, E1 binding site and A/T rich region (Ustav et al., 1991, 1993). The number and position of E2 binding sites are well conserved among viruses of the same PV genus. For example, the HPV18 URR contains four consensus binding sites, the HPV5 URR has four consensus and one non-canonical binding site, and the BPV1 URR has twelve binding sites. In addition, full-length E2 and the shorter products of the E2 ORF are also transcription regulators controlling the expression of viral genes by recruiting cellular factors to the viral URR regions. In BPV1 two repressor proteins have been identified in infected cells, designated E8/E2, which is potentially expressed by all PV and E2C (E2-TR), that is transcribed from a promoter P3080 and initiated from an internal ATG in the E2 ORF (Lambert et al., 1987; Choe et al., 1989). The E2 proteins can activate or repress transcription depending on the binding site context, associated cellular factors and E2 protein levels (Bernard et al., 1989; Bouvard et al., 1994; Dong et al., 1994; Stubenrauch et al., 1996; Hou et al., 2000; Fujii et al., 2001).

E2 also mediates the association of viral genomes with host mitotic chromosomes, which ensures the proper segregation and maintenance of viral genomes during mitosis (Ilves et al., 1999). The main tethering factor on the mitotic chromosomes is the chromatin adapter protein Brd4 (You et al., 2004; Wu et al., 2006), which is one of the most studied E2 cellular interaction partners. Brd4 is essential for the transcriptional activation of E2 (McPhillips et al., 2006; Schweiger et al., 2006) and is also involved in E2-mediated transcriptional repression (Wu et al., 2006; Schweiger et al., 2007) and replication (Ilves et al., 2006; Wang et al., 2013). Brd4 is also required for the initiation of E2-dependent replication in HPV16 (Gauson et al., 2015). In addition to Brd4, more than 200 cellular proteins have been identified as nuclear interaction partners of PV E2 proteins (Muller and Demeret, 2012; McBride, 2013; Jang et al., 2015). These interaction partners include histone modifiers such as acetyl transferases (CBP/p300), deacetyl transferases, methylases, splicing factors (SRPK1), replication factors (RFC), transcription factors (TBP) and chromatin remodelers (SMARC family, INO80) (Jang et al., 2015). Because the double-stranded viral DNA of the papillomaviruses is also packed into nucleosomes, viral interactions with chromatin remodeling factors change the nucleosome structure to enable or facilitate viral transcription and replication (Euskirchen et al., 2012; Cha and Seo, 2011). There are many works showing that HPV E2 interacts with SWI/SNF chromatin remodeling complex proteins: HPV18 E2 interacts with SMARCB1 (SNF5) protein (Lee et al., 1999), and SMARCB1 and SMARCA4 (BRG1) enhance the E2-dependent transcription activation and replication of HPV18 (Cha and Seo, 2011). HPV16 E2 interacts with the SWI/SNF component SMARCA2 (Brm) through its N-terminal domain (Kumar et al., 2007), and HPV16 E7 binds to Brg1 to control the cell cycle (Lee et al., 2002). The HPV E1 protein directly interacts with the nucleosomal protein H1 (Swindle and Engler, 1998) and with SMARCB1 (hSNF5) (Lee et al., 1999), suggesting that E1 engages these enzymes during S phase to drive viral episome replication (Kumar et al., 2007). A recent proteomics study revealed that the E2 protein of the beta-papillomavirus group HPV8 binds to several chromatin remodeling complex proteins including SMARCA4 (BRG1), SMARCB1 (SNF5), WSTF and INO80D (Jang et al., 2015). In the same study BPV1 E2 was also shown to interact with chromatin remodelers.

In this study, we demonstrate that different PV E2 proteins interact with NM1 and co-immunoprecipitate with the cellular proteins WSTF and SNF2h, all belonging to the multiprotein assembly complex B-WICH, which is involved in chromatin remodeling. These interactions can directly influence the PV life cycle, as downregulation of NM1 elevated PV genome replication, supporting the idea that reducing the expression of NM1 releases E2 protein from the chromatin remodeling complex.

## 2. Materials and methods

### 2.1. Plasmids and antibodies

The expression vector of full-length human NM1, pCMV-NM1-FLAG, was a kind gift from Pavel Hozak and has been described previously (Pestic-Dragovich et al., 2000). The expression constructs for the NM1 N-terminus (aa 1–709) and C-terminus (aa 704–1028) were cloned into the *Eco*RI site of the pCMV-FLAG vector (Invitrogen, US). The heterologous SV40 LT NLS signal CCCAAGAAGAAACGCAAAGTT was inserted to the C-terminus of both the NM1 N- and C-terminal sequences. The BPV1 E2 protein expression vectors included: (i) pCGE2, which expressed full-length BPV1 E2 protein, (ii) pCGE2-TAD, which expressed the E2 TAD domain (aa 1–218), (iii) pCGE2C, which expressed the E2 C-terminal part (aa 162–410) (Ustav and Stenlund, 1991), and (iv) pCGE2 37/73, which expressed full-length E2 with alanine substitutions in residues R37 and I73 (Baxter et al., 2005). The plasmid containing the C-terminal domain of Brd4, pcDNA4C-SV40NLS-hBrd4-CTD (p4948), has been described (You et al., 2004). The pET plasmid series (Novagen, US) for protein production, including pET41a-NM1, pET41a-NM1-N, pET41a-Brd4CTD and pET24d-E2, was generated following the manufacturer's instructions.

Plasmid pQMNE2-18 (HPV18 E2) was described previously (Kadaja et al., 2007), plasmid pQMNE2-5 (HPV5) was prepared by cloning E2 ORFs from HPV5 into pQMNE2-18, replacing HPV18E2 region and codon optimized to human expression.

Antibodies against FLAG epitope M2 (F 7425), NM1 (M 3567), myosin 9 (M 8064) and beta-actin (A 2228) were purchased from Sigma-Aldrich, US. Anti-WSTF (ab50850) and anti-SNF2h (ab3749) were purchased from Abcam, UK. The monoclonal anti-E2 antibodies 5E11-HRP, 1E4, 3E8 and 5H4 (Kurg et al., 1999) and the secondary anti-mouse and anti-rabbit antibodies conjugated to horseradish peroxidase were purchased from LabAS, Estonia. Human NM1 siRNA (Silencer Select s9200) with sequence CGUGCGGACAAUAAGCAAAtt and its mouse homolog were obtained from Ambion (Thermo Fisher Scientific, Waltham, MA).

### 2.2. Cell culture

Chinese hamster ovary (CHO) cells and the derivative CHO4.15.5 cells (expressing BPV1 E1 and E2 (Piirsoo et al., 1996)) were maintained in Ham's F12 medium supplemented with 10% fetal calf serum (GE Healthcare Life Sciences, Pittsburgh, PA). COS-7 and U2OS were grown in IMDM medium containing 10% fetal calf serum. The cells were transfected via electroporation using a Bio-Rad Gene Pulser II