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E1B 55k-independent dissociation of the DNA ligase IV/XRCC4 complex by E4 34k during adenovirus infection

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ABSTRACT

The ligase IV/XRCC4 complex plays a central role in DNA double-strand break repair by non-homologous end joining (NHEJ). During adenovirus infection, NHEJ is inhibited by viral proteins E4 34k and E1B 55k, which redirect the Cul5/Rbx1/Elongin BC ubiquitin E3 ligase to polyubiquitinate and promote degradation of ligase IV. In cells infected with E1B 55k-deficient adenovirus, ligase IV could not be found in XRCC4-containing complexes and was observed in a novel ligase IV/E4 34k/Cul5/Elongin BC complex. These observations suggest that dissociation of the ligase IV/XRCC4 complex occurs at an early stage in E4 34k-mediated degradation of ligase IV and indicate a role for E4 34k in dissociation of the ligase IV/XRCCC4 complex. Expression of E4 34k alone was not sufficient to dissociate the ligase IV/XRCC4 complex, which indicates a requirement for an additional, as yet unidentified, factor in E1B 55k-independent dissociation of the ligase IV/XRCC4 complex.

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Introduction

DNA damage occurs in all living cells either as a result of environmental insults or normal metabolic processes and presents a major threat to the integrity of the genome. In particular, DNA double-strand breaks (DSBs) can lead to genetic rearrangements that promote tumor formation. Eukaryotic cells repair DSBs by two mechanisms: homologous recombination, which is used for repair of DSBs that occur during late-S or G2 phases of the cell cycle, and non-homologous end joining (NHEJ), which is used during G0 and G1 phases of the cell cycle (Lieber et al. 2004, Sonoda et al. 2006, Weterings and Chen 2008). In addition to spontaneous DSBs, somatic cell recombination processes required for immunoglobulin (Ig) production and diversification are initiated by DSBs that are repaired by NHEJ (Lieber et al. 2004, Weterings and Chen 2008). Because of the large proportion of non-cycling cells in multicellular organisms and the added role of DSBs in Ig production, the majority of DSBs in humans are repaired by NHEJ (Lieber et al. 2004, Sonoda et al. 2006).

The repair of the DSBs by NHEJ involves recognition of DSBs, localization of repair enzymes to the DSB, processing of the DNA ends to yield ligatable termini, and ligation to reseal the break. In mammalian cells, DSB recognition requires phosphorylation of histone H2AX and processing of DNA ends may involve one of several factors, which include aprataxin and polynucleotide kinase (Chappell et al. 2002, Koch et al. 2004, Ahel et al. 2006). Mammalian NHEJ also requires the heterotrimeric DNA-dependent protein kinase (DNA-PK), a nuclear serine/threonine protein kinase belonging to the phosphatidylinositol 3-related protein kinase (PI3K) family (Smith and Jackson 1999, Weterings and Chen 2008). This heterotrimeric kinase consists of a 460 kDa catalytic subunit (DNA-PKcs) and a heterodimeric DNA binding subunit (Ku) (Smith and Jackson 1999). Ku is thought to be involved in the recognition of DSBs based on its abundance in nuclei and its affinity for DNA ends (Smith and Jackson 1999, Weterings and Chen 2008). In mice, disruption of genes encoding DNA-PKcs or either of the Ku subunits results in a spectrum of DSB repair and Ig-production defects (Smith and Jackson 1999, Weterings and Chen 2008).

In mammalian NHEJ, the ligation event is catalyzed by DNA ligase IV, which forms a complex with the DNA-binding proteins XRCC4 and XLF (Bryans et al. 1999, Ahnesorg et al. 2006, Buck et al. 2006, Sonoda et al. 2006, Weterings and Chen 2008). XRCC4 makes direct contact with ligase IV and XLF, stimulates ligase IV activity and is essential for ligase IV stability (Critchlow et al. 1997, Grawunder et al. 1998, Ahnesorg et al. 2006, Buck et al. 2006, Sonoda et al. 2006, Weterings and Chen 2008). Structural studies have shown that both XRCC4 and XLF form homodimers with remarkable similarity in overall fold (Junop et al. 2000, Andres et al. 2007, Li et al. 2007). Direct-binding and structural studies paint a picture of a complex composed of 1 ligase IV: 2 XRCC4: 2 XLF that is held together through strong, direct ligase IV/XRCC4 and XRCC4/XLF interactions with weaker, possibly indirect, ligase IV/XLF contacts (Deshpande and Wilson 2007).

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Cells lacking ligase IV, XRCC4 or XLF cannot carryout NHEJ and are therefore radiation-sensitive and unable to carry out Ig-gene rearrangement by V(D)J recombination (Barnes et al. 1998, Frank et al. 1998, Lieber et al. 2004, Buck et al. 2006, Weterings and Chen 2008). In mice, disruption of ligase IV or XRCC4 results in embryonic lethality (Barnes et al. 1998, Frank et al. 1998, Weterings and Chen 2008). The severe phenotype associated with ligase IV or XRCC4 gene ablation likely reflects the central role of the ligase IV complex in mammalian NHEJ. Interaction between the DNA-PK and ligase IV/XRCC4/XLF complexes has been observed (McElhinny et al. 2000, Hsu et al. 2002, Costantini et al. 2007, Yano et al. 2008). Previous reports have described ligase IV/Ku and XRCC4/DNA-PKcs interactions, which are believed to be important in coordinating the efforts of these two large multi-protein complexes (Hsu et al. 2002, Costantini et al. 2007).

During infection of human cells with adenovirus, replication of the linear dsDNA adenovirus genome and the presence of adenovirus-genome termini can activate the host cell DSB repair response (Weitzman and Ornelles 2005). Unperturbed, this response can result in adenovirus genome concatamer formation and inhibits productive lytic infection. Adenovirus genome concatenation is an NHEJ-dependent process that requires DNA-PK, ligase IV and the Mre11 protein (Boyer et al. 1999, Stracker et al. 2002, Weitzman and Ornelles 2005). To prevent genome concatenation, human adenovirus type 5 (Ad5) encodes early proteins that specifically target ligase IV for polyubiquitination and proteasome-mediated degradation (Baker et al. 2007). This process requires viral proteins encoded by early region 1B (E1B 55k) and early region 4 (E4) open reading frame 6 gene product (E4orf6, E4 34k), which also target Mre11 and p53 to effectively disable DNA damage sensing, DSB repair and apoptosis (Carson et al. 2003, Berk 2005). To further prevent genome concatenation, the E4 open reading frame 3 gene product (E4orf3, E4 11k) causes mis-localization of the nuclear Mre11 protein to cytoplasmic aggresomes, which prevents Mre11 from acting on the newly replicated Ad5 genomes (Araujo et al. 2005).

Study of the molecular mechanism that underlies adenovirus-directed protein degradation has shown that the Ad5 E1B 55k and E4 34k proteins form a complex that re-directs a host ubiquitin E3 ligase to polyubiquitinate host factors (Querido et al. 2001, Blanchette et al. 2004, Cheng et al. 2007). This system has been most thoroughly studied in the context of p53 degradation during Ad5 infection. The Cul5/Rbx1/Elongin BC ubiquitin E3 ligase is bound by E4 34k through direct contact with Cul5 and the Elongin BC complex to form an Ad5-specific E4 34k ubiquitin E3 ligase (Blanchette et al. 2004, Cheng et al. 2007). It is thought that efficient selection and delivery of p53 to the E4 34k ubiquitin E3 ligase is mediated by E1B 55k, which binds to E4 34k and delivers p53 only after assembly of the Ad5-specific E4 34k/Cul5/Rbx1/Elongin BC ubiquitin E3 ligase (Blanchette et al. 2004). Cul5-dependent polyubiquitination of p53 has been observed, and because polyubiquitination acts as a general signal for proteasome-mediated degradation, viral redirection of the Cul5/Rbx1/Elongin BC ubiquitin E3 ligase results in effective elimination of targeted host proteins (Querido et al. 2001, Blanchette et al. 2004, Cheng et al. 2007).

Ad5-directed degradation of ligase IV requires the same factors necessary for degradation of p53 and is thought to proceed by the same mechanism. Interestingly, while ligase IV is known to form a stable complex with the XRCC4 and XLF proteins, Ad5-directed degradation of ligase IV does not appear to affect XRCC4 or XLF protein levels (Baker et al. 2007). This observation suggests that the ligase IV/XRCC4/XLF complex is dissociated at some stage during the virus-specific pathway that targets ligase IV for degradation. Previous observation of an E1B 55k/ligase IV complex suggests that ligase IV, like p53, is specifically recognized by E1B 55k and then delivered to the E4 34k/Cul5/Rbx1/Elongin BC ubiquitin E3 ligase.

In this communication, we show that in the absence of E1B 55k, ligase IV is bound by E4 34k to form a complex that contains Elongins B and C, but does not contain XRCC4. Our findings show that ligase IV, like p53, is recognized by both E1B 55k and E4 34k and indicate an important role for E4 34k in dissociation of the ligase IV/XRCC4 complex. Because expression of E4 34k alone was not sufficient to cause dissociation of the ligase IV/XRCC4 complex, our observations suggest the possibility that an additional factor is required for E4 34k-dependent, E1B 55k-independent dissociation of the ligase IV/XRCC4 complex.

Results

Infection with Ad5 deletion mutants reveals that viral expression of E4 34k compromises the DNA-PKcs/ligase IV interaction in an E1B 55k-independent fashion

While DNA-PK has been shown to functionally and physically interact with the ligase IV/XRCC4 complex (Hsu et al. 2002), during Ad5 infection, DNA-PK and XRCC4 appear to be unaffected, while ligase IV is degraded (Baker et al. 2007). Because E4 34k participates in ligase IV degradation and co-immunoprecipitates (co-IP) with DNA-PKcs (Boyer et al. 1999, Baker et al. 2007), we wanted to examine the effect of Ad5 infection on the interactions between the DNA-PK and ligase IV/XRCC4 complexes. To address this question we prepared lysates from cells infected with wild type or deletion mutants of Ad5, used immunoprecipitation (IP) to isolate DNA-PKcs-containing protein complexes and then used Western blot analysis to determine if XRCC4 and ligase IV were associated with DNA-PKcs. In extracts prepared from mock-infected cells, we were able to detect both ligase IV and XRCC4 in the input lysate and in the DNA-PKcs co-IP (Fig. 1, top). We interpret these data as the result of direct protein–protein interactions between XRCC4 and DNA-PKcs (Hsu et al. 2002) and between XRCC4 and ligase IV (Critchlow et al. 1997, Grawunder et al. 1998), which result in an indirect (i.e. through XRCC4) interaction between ligase IV and DNA-PKcs (Fig. 1). In extracts prepared from cells infected with wild type Ad5, it is worth noting that ligase IV was absent and the co-IP between XRCC4 and DNA-PKcs was still observable (Fig. 1, top). Similar results were obtained with extracts prepared from cells infected with H5*dl*1013 and H5*pm*1020, which

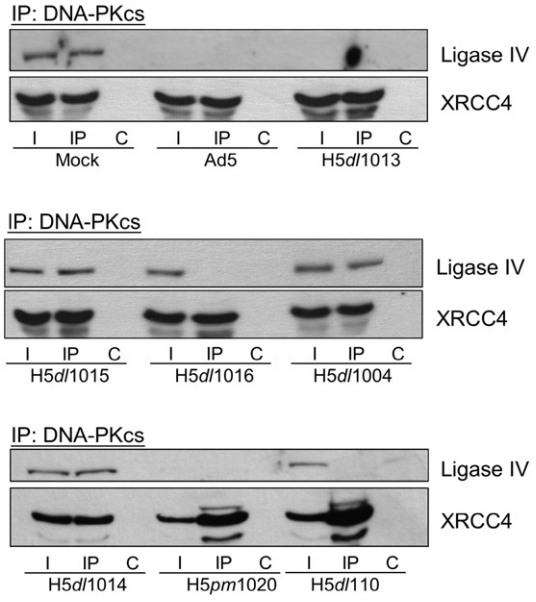

Fig. 1. E1B 55k-independent loss of ligase IV interaction with DNA-PKcs. (A) HeLa cells were Mock, Ad5 or Ad5-mutant (H5*dl*1013, H5*dl*1015, H5*dl*1016, H5*dl*1004, H5*dl*1014, H5*pm*1020 or H5*dl*110) infected and lysed in NP40 lysis buffer 20 h post-infection. 200 μg of the total extracts were subjected to immunoprecipitation (IP) with anti-DNA-PKcs antibodies. DNA-PKcs-containing complexes were recovered, resolved by SDS-PAGE, Western transferred and probed for Ligase IV and XRCC4. I, 15% of input lysate; IP, immunoprecipitation using anti-DNA-PKcs antibodies; C, control IP.

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