Letter to the Editor

Insights into the regulation of human Rev1 for translesion synthesis polymerases revealed by the structural studies on its polymerase-interacting domain

Dear Editor,

Translesion synthesis (TLS) allows the DNA replication machinery to bypass an unrepaired DNA damage site using special polymerases called TLS polymerases (Fischhaber and Friedberg, 2005). When compared with the replicative polymerases, TLS polymerases have comparatively large active sites to incorporate the base opposite the damaged DNA and low fidelity to ensure progression of synthesis using the damaged template (McCulloch and Kunkel, 2008). Though TLS rescues the cells from the collapse of the replication fork, the bypass of the lesions can be a potential cause for the mutation generation (Wang, 2001). Therefore, tight regulation of the TLS polymerases is extremely important. Recent research shows that Rev1 can act as a regulator and defines distinct mechanism for TLS when compared with PCNA (Edmunds et al., 2008; Hendel et al., 2011). In comparison to other TLS polymerases, the catalytic function of Rev1 is not required for the mutagenic DNA damage tolerance (Lawrence, 2004; Prakash et al., 2005). Instead the C-terminus of Rev1, which interacts with TLS polymerases κ, η, i and ζ (consisting of Rev3 and Rev7) in eukaryotes (Murakumo et al., 2001; Guo et al., 2003; Ohashi et al., 2004), is reported to be required for the DNA damage tolerance (D’Souza et al., 2008). Therefore, the understanding of the molecular basis of the C-terminus of Rev1 and its related interactions with TLS polymerases is important in comprehending the mechanism of the TLS polymerases regulation. Here, we report the first structural studies on the TLS polymerase-interacting domain of human Rev11156–1251 [hRev1-polymerase-interacting domain (PID)] and its interactions with TLS polymerases κ, η, i and ζ.

In order to understand how Rev1 recruits the TLS polymerases, the complex structure of hRev1-PID with Pol κ 562–577 was first characterized using NMR spectroscopy (Supplementary Table S1). The overall structure of bound hRev1-PID consists of four α-helices oriented anti-parallel to each other and formed a four α-helix bundle. The two turned α-helix of Pol κ formed by residues from F567 to E574 contacts the surface formed by α1, α2 and the rigid N-terminus of hRev1-PID (Figure 1A). Further double titration experiments among the polymerase κ, η and i showed that at same concentration Pol κ can completely replace the Pol η and i for the Rev1 interaction (Supplementary Figure S1A and B), therefore, the competition binding observed here indicates a shared binding interface on Rev1 for Pol κ, η and i. In addition, similar heteronuclear single quantum coherence patterns (Supplementary Figure S1C) and dynamic properties (Supplementary Table S2) have been observed for 15N-hRev1-PID bound with polymerase κ, η and i, suggesting that hRev1-PID, when interacting with Pol η and Pol i adopts a structure similar to when it binds with Pol κ. To further elucidate the recruitment manner of Rev1 binding to Pol ζ, the complex structure between hRev1-PID and Pol ζ (Rev71–211 and Rev3846–908) was solved using crystallography at 1.9 Å resolution (Supplementary Table S3). The hRev1-PID in complex with Pol ζ remains the identical conformation compared with its bound form with Pol κ with R.M.S. deviation of 0.7 Å (Supplementary Figure S2A and B).

Based on our complex structure, the binding interface for Pol ζ locates in the N-terminal end of α3 and C-terminal end of α4 on hRev1-PID (Figure 1B), which is different from the binding sites for Pol κ. Taken together, Rev1 uses the same tertiary structure and two surfaces to recruit TLS polymerases, with one site for polymerase κ, η, i and the other site for polymerase ζ.

To shed light on the recognition mechanism between Rev1 and TLS polymerases, the Rev1-interacting motifs (RIM) of polymerase κ, η, i and ζ were investigated. Previous report showed that two highly conserved FF-motif is important for the Rev1 recognition (Ohashi et al., 2009). However, based on the sequence alignment on the Rev1-interacting region of polymerase κ, η and i, we found that K571 is also highly conserved (Figure 1C). Further mutation studies on the Pol κ K571G or hRev1-PID E1174K showed the significant decrease of binding affinity to hRev1-PID and Pol κ, respectively, indicating that K571 is indispensable for the Rev1 recognition through the electrostatic interaction with E1174 of Rev1 (Figure 1D). Therefore, we refer the ‘xxxFFxxK’ (where x represents any residue) as the RIM of Pol κ, η, i. On the basis of our complex structure, F568 of the Pol κ fits into the deep hydrophobic pocket formed by L1159, L1171, L1172, W1175 and V1190 of Rev1, while F567 of the Pol κ is involved in the hydrophobic interaction with W1175 of Rev1 and the side chain of K571 of Pol κ (Supplementary Figure S3). Further electrostatic surface analysis reveals that the RIM of polymerases κ, η and i is recognized by Rev1 through the unmarked hydrophobic pocket acting as the ‘lock’...
Figure 1 Structures of hRev1-PID in complex with TLS polymerases reveal the hub function of human Rev1 in TLS. (A) Solution structure of hRev1-PID (magenta) in complex with Pol κ (green) presented in ribbon (ensemble with 15 structures) and cartoon modes. (B) Complex structure of hRev1-PID (magenta) and Pol ζ (blue) shown in a cartoon mode. (C) Sequence alignment for Rev1-interacting regions of polymerase κ, η and ζ. (D) Fluorescence anisotropy for Pol κ titrated with hRev1-PID (solid circle), hRev1-PID E1174K (solid triangle) and Pol κ K571E titrated with hRev1-PID (empty circle). (E) Recognition between hRev1-PID (surface) and Pol κ (cartoon and stick). (F) Key residues for the interaction between hRev1-PID (hot pink) and Pol ζ (blue). (G) Surface representation of complex hRev1-PID (hot pink) and Pol ζ (blue) and the binding interfaces for hRev1-PID (bottom left) and Pol ζ (bottom right). (H) Confocal images of HEK293T cells co-transfected with...
and negative charge as ‘pin’ for the TLS polymerase location (Figure 1E). Intriguingly, the RIM presented by polymerase κ, η and ι is absent in the Rev7 subunit of polymerase ζ. Instead, the key residues responsible for the recognition are E101, L186, P188, K190, Q200, Y202, E205 of Rev7 subunit of Pol κ and K1201, L1203, E1204, Y1244a, L1248, K1249 of hRev1-PID (Figure 1F). Albeit distal in the primary sequence (Supplementary Figure S4A and B), these residues cluster into a surface in the tertiary structure of Rev1 and Rev7 subunit of Pol ζ, respectively (Figure 1G). Different from the polymerase κ, η and ι, the recognition between Rev1 and Rev7 subunit of Pol ζ is through the surfaces, which contains the center core for hydrophobic interaction and surrounding shell for electrostatic interaction (Figure 1G). Further mutation studies proved that both surfaces are required for the recognition of Rev7 subunit of Pol ζ by hRev1-PID (Supplementary Table S4).

Based on the catalytic functions of TLS polymerases, polymerase κ, η and ι are identified as inserters, while polymerase ζ is considered as extender for the TLS (Boiteux and Guillett, 2004). Subsequently two-polymerase mechanism: TLS requires both inserter polymerase for damage bypass and extender polymerase for further synthesis, has been proposed (Johnson et al., 2000; Shachar et al., 2009; Hicks et al., 2010). The recruitment and recognition mechanisms between TLS polymerases and Rev1 revealed by our studies exactly agree with the functions of TLS polymerases in TLS. Remarkably, our fluorescence anisotropy studies show that the RIM of inserter polymerase κ and extender polymerase ζ (Rev7/Rev3) can bind to the PID of Rev1 simultaneously (Supplementary Figure S5A and B), which is in accord with the parallel work published recently (Wojtaszek et al., 2012). Further confocal images of the HEK-293T cells prove that the triple complex of Rev1-PID/Polκ-RIM/Polζ (Rev7/Rev3) can present even in cells (Figure 1H). Taken together, our results suggest that Rev1 directly interacts with Pol κ and Pol ζ simultaneously forming a multi-polymerase inserter/extender complex. In addition, the presence of Pol κ does not significantly affect the binding affinity between hRev1-PID and Pol ζ (Rev7/Rev3) (Supplementary Table S4). Therefore, we propose that hRev1-PID functions as a ‘hub’, utilizing two preformed docking sites to recognize two different RIMs presented by inserter and extender translesion polymerases (Figure 1).

The results presented here provide the first structural insights into the regulation of human Rev1 for TLS polymerases. Detailed structural information on the recruitment and recognition might provide the basis for the future anti-cancer drug design. [Supplementary material is available at Journal of Molecular Cell Biology online. This work was supported by the National Research Foundation of Korea grants (2009-0092818, 2009-220-C00036, 2011-0020322 to B.S. C) and the high field NMR research program, Korea Basic Science Institute to K.S. Ryu. We are grateful to M.K. Yoon and S.H. Bae for the helpful discussions and comments.]

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References


PKCD-mRFP-Rev1-PID (bait), eGFP-Pol κ (560–615) (target), pTagBFP-Rev7 (full length 1–211) and pC3N1-Rev3 (1847–1898) (non-fluorescence). Top row: co-transfected HEK-293T without PMA treatment; Bottom row: co-transfected HEK-293T cells were treated with PMA (1 μM). The scale bar is 10 μm. (I) Triple complex model for hRev1-PID (magenta), Pol κ (green) and Pol ζ (blue) as shown in both cartoon and surface modes.