Supplementary Material

STRUCTURAL ANALYSIS OF THE CONSERVED UBIQUITIN-BINDING MOTIFS (UBMS) OF THE TRANSLESION POLYMERASE IOTA IN COMPLEX WITH UBIQUITIN

Burschowsky, Daniel1‡, Rudolf, Fabian2.3.§, Rabut, Gwénaël2.§, Herrmann, Torsten4, Peter Matthias2.* and Wider, Gerhard1.*

1 Institute of Molecular Biology and Biophysics, ETH Zurich, 8093 Zurich, Switzerland; 2 Institute of Biochemistry, ETH Zurich, 8093 Zurich, Switzerland; 3 Competence Center for Systems Physiology and Metabolic Diseases, 8093 Zurich, Switzerland; 4 Université de Lyon, CNRS/ENS Lyon/UCB Lyon-1, Centre Européen de RMN à très hauts champs, 5 Rue de la Doua, 69100 Villeurbanne, France

‡ these authors contributed equally
§ present address: BSSE, ETH-Zurich, Mattenstr. 26, 4058Basel, Switzerland
§ present address: CNRS, UMR6061, Université Rennes 1, Institute of Genetics and Development, 35043 Rennes, France
* to whom correspondence should be addressed:
email: matthias.peter@bc.biol.ethz.ch
gsw@mol.biol.ethz.ch
Tel: MP: +41-44-63 36 586; GW: +41-44-63 33 455
Fax: MP: +41-44-63 21 298; GW: +41-44-63 31 484
Supplementary Figures

**Supplementary Figure 1.** Domain organization (to scale) of murine Pol ι. The N-terminal domains are structured; the PIP (PCNA interaction peptide) just after the PAD domain and the UBMs are located in the flexible C-terminal tail.

**Supplementary Figure 2.** \([^{1}H,^{15}N]\)-HSQC spectra with the assignment of the amide resonances of (A) UBM1 and (B) UBM2 bound to ubiquitin. Peaks arising from secondary conformations due to Pro cis/trans isomerism in the flexible regions are marked with asterisks; for UBM1 these peaks could be assigned. NHε resonances of arginines are aliased into the spectrum from their real chemical shifts of approx. 82-84 ppm.
Supplementary Figure 3. Amino acid sequences of the UBM constructs that were used for NMR and fluorescence measurements. The grey N-terminal residues represent residues that were remaining from cloning/cleaving and which are not part of the original sequence of Pol ι. The central LP motif is marked with a box.

| Seq.№ | Seq.№ |
|-------|-------|
| UBM1* 480 | GSPEFEKDTSDLPLQALPEGVDQEVFKQLP 509 |
| UBM1 485 | GS DTSDLPLQALPEGVDQEVFKQLP 509 |
| UBM2 668 | GSPEFDSAEKLPFPIDPQVYELP 694 |
| UBM1* 510 | ADIQEILSGKSRENLKGSLSCPPLLTR 538 |
| UBM1 510 | ADIQEILSGKSRENLKGKGSLS 532 |
| UBM2 695 | EEVQKELMAEWERAGAARPSAHR 717 |

Supplementary Figure 4. Distance difference mapping of UBM1 (sequence on top of figure) and UBM2 (sequence below figure). The per-residue backbone RMSD values of the UBM structure bundles are shown (UBM1 in orange; UBM2 in blue) in comparison to the distances between the backbones of the two mean structures (black line). The mean structures were fitted on the helix-turn-helix motif of UBM1 (16 residues), where the fold is very similar.
Supplementary Figure 5. Superposition of UBM1 (orange) and UBM2 (blue) structures (ribbon representation) bound to ubiquitin (surface representation); the lowest energy structures are shown.
Supplementary Figure 6A. Superposition of a series of $[^{1}H,^{15}N]$-HSQC spectra measured upon titration of UBM1* with ubiquitin. The concentration ratios of [Ub]:[UBM] are indicated by different colors of the peaks: red – 0.0, magenta – 0.5, violet – 1.1, cyan – 5.0.
Supplementary Figure 6B. Superposition of a series of $[^1\text{H}, ^{15}\text{N}]$-HSQC spectra measured upon titration of UBM2 with ubiquitin. The concentration ratios of [Ub]:[UBM] are indicated by different colors of the peaks: red – 0.0, magenta – 0.5, violet – 1.1, cyan – 5.0.
Supplementary Figure 7. Thermal denaturation of free UBM1 (left) and UBM2 (right) monitored by a series of $^{13}$C-HSQC spectra. Resonances with relatively large chemical shift changes ($\Delta \delta$) upon temperature changes were selected; a resonance from a less structured region is included for comparison (green stars). Combined chemical shift changes for $^1$H and $^{13}$C were calculated as described in the NMR analysis (see main text).

Supplementary Figure 8. [$^1$H,$^1$H]-Strips from an aliphatic $^{13}$C-resolved [$^1$H-$^1$H]-NOESY spectrum that show long-range NOEs between methyl groups of the five residues, which mainly make up the hydrophobic pocket in UBM1 (left; V500, V504, L508, I512, I516) and UBM2 (right; I685, V689, L693, V697, L701). Intraresidual and interresidual NOEs between the five residues forming the pocket NOEs are marked with a “×” and a “+”, respectively, all other NOEs are not marked.
Supplementary Figure 9. Combined chemical shift changes of amide groups for the ratio Ub:UBM of 5:1 for all residues in UBM1 (top) and UBM2 (bottom) versus the sequence.
Supplementary Figure 10. Sequence alignment of UBMs of Pol ι and Rev1 of different species (Hs: Homo sapiens; Mm: Mus musculus; Dm: Drosophila melanogaster; Sp: Schizosaccharomyces pombe; Sc: Saccharomyces cerevisiae); the numbers indicate the position of the first residue in the sequence of the polymerase, with the UBMs being numbered according to their position relative to the N-terminus. Residues in structural features that were found in this study are highlighted by colored boxes (red: hydrophobic interface – green: ionic interaction – cyan: hydrophobic core), residues that have earlier been found to be conserved (1) are shown in grey and black. Residues that are involved in the hydrophobic core are only marked for Pol ι UBMs. The sequence differences between UBM domains of Rev1 and of Pol ι and the fact that the hydrophobic core structures of UBM1 and UBM2 of Pol ι differ significantly (length of helices and/or fold of terminal regions) do not warrant an inclusion of Rev1. For the UBMs of Pol ι the residues that build the hydrophobic cores are framed in cyan. Interestingly, the sequence of the single UBM of Pol ι in Drosophila combines features of both mammalian UBMs, especially concerning the hydrophobic core (e.g. Trp at the end of the domain as in UBM2, but Tyr is substituted by Lys as in UBM1). The residues that constitute the interface (framed red and green) are well conserved in all UBMs (including Rev1) and we therefore speculate that they also support similar structural features in all functional UBMs.
Supplementary Figure 11. (A) Superposition of the 20 lowest-energy NMR structures of the UBM1-ubiquitin complex, in contrast to Figure 2 A and B the RMSD is minimized only for the residues 1 to 73 of ubiquitin. (B) Same for UBM2-ubiquitin complex.
**Supplementary Figure 12.** Superposition of the 20 lowest-energy NMR structures of UBM1 (left) and UBM2 (right) in complex with ubiquitin. The residues that are involved in electrostatic interactions are represented as stick models. R42 on ubiquitin interacts with D511/E515 of UBM1 and with E700 of UBM2. No restraints for the interactions were used during the structure calculations, but more than 60% of the structures show the ion pairs that were also verified by mutational analysis (see main text).
Supplementary Figure 13. 2D Strips from a 3D HNCACB spectrum of UBM1 bound to ubiquitin. The sequential assignment path for the residues 502 to 512 is indicated by a black line for the $^{13}$C$^\beta$ (upper line) and for the $^{13}$C$^\alpha$ resonances (lower line); residue 509 is Pro.
## Supplementary Tables

### Supplementary Table 1 – List of yeast strains used in this study

| Name       | Genotype                                                                 | Source                      |
|------------|---------------------------------------------------------------------------|-----------------------------|
| LHY461     | MATa lys2-801, leu2-3,112, his3-Δ200, trp1-1(am) ubi1-Δ1::TRP1, ubi2-Δ2::ura3, ubi3-ΔUb-2, ubi4-Δ2::LEU2 (pUB100) (YPE pCUP1 Ub(K6R) LYS2) | (2)                         |
| scGR837    | MATa lys2-801, leu2-3,112, his3-Δ200, trp1-1(am) ubi1-Δ1::TRP1, ubi2-Δ2::ura3, ubi3-ΔUb-2, ubi4-Δ2::LEU2 (pUB100) (YPE pCUP1 Ub URA3) | This study                  |
| scGR838    | MATa lys2-801, leu2-3,112, his3-Δ200, trp1-1(am) ubi1-Δ1::TRP1, ubi2-Δ2::ura3, ubi3-ΔUb-2, ubi4-Δ2::LEU2 (pUB100) (YPE pCUP1 Ub(U68A) URA3) | This study                  |
| scGR840    | MATa lys2-801, leu2-3,112, his3-Δ200, trp1-1(am) ubi1-Δ1::TRP1, ubi2-Δ2::ura3, ubi3-ΔUb-2, ubi4-Δ2::LEU2 (pUB100) (YPE pCUP1 Ub(T66A) URA3) | This study                  |
| scGR842    | MATa lys2-801, leu2-3,112, his3-Δ200, trp1-1(am) ubi1-Δ1::TRP1, ubi2-Δ2::ura3, ubi3-ΔUb-2, ubi4-Δ2::LEU2 (pUB100) (YPE pCUP1 Ub(T9A) URA3) | This study                  |
| scGR937    | MATa lys2-801, leu2-3,112, his3-Δ200, trp1-1(am) ubi1-Δ1::TRP1, ubi2-Δ2::ura3, ubi3-ΔUb-2, ubi4-Δ2::LEU2 rev1::KAN (pUB100) (YPE pCUP1 Ub(K6R) LYS2) | This study                  |
| scGR938    | MATa lys2-801, leu2-3,112, his3-Δ200, trp1-1(am) ubi1-Δ1::TRP1, ubi2-Δ2::ura3, ubi3-ΔUb-2, ubi4-Δ2::LEU2 rev1::KAN (pUB100) (YPE pCUP1 Ub(H68A) URA3) | This study                  |
| scGR939    | MATa lys2-801, leu2-3,112, his3-Δ200, trp1-1(am) ubi1-Δ1::TRP1, ubi2-Δ2::ura3, ubi3-ΔUb-2, ubi4-Δ2::LEU2 rev1::KAN (pUB100) (YPE pCUP1 Ub(H68A) URA3) | This study                  |
| scGR940    | MATa lys2-801, leu2-3,112, his3-Δ200, trp1-1(am) ubi1-Δ1::TRP1, ubi2-Δ2::ura3, ubi3-ΔUb-2, ubi4-Δ2::LEU2 rev1::KAN (pUB100) (YPE pCUP1 Ub(T66A) URA3) | This study                  |
| scGR942    | MATa lys2-801, leu2-3,112, his3-Δ200, trp1-1(am) ubi1-Δ1::TRP1, ubi2-Δ2::ura3, ubi3-ΔUb-2, ubi4-Δ2::LEU2 rev1::KAN (pUB100) (YPE pCUP1 Ub(T9A) URA3) | This study                  |
**Supplementary Table 2** – Comparison of the ubiquitin-complex structures of UBM2 of human polymerase iota (3) and UBM2 of murine polymerase iota.

| Regions                  | $Mm$ vs. $Hs_{\text{mean}}$ (a) | $Hs$ vs. $Mm_{\text{mean}}$ (a) |
|--------------------------|---------------------------------|---------------------------------|
| UBM2-HTH$^{(b)}$         | 1.00 ± 0.09                     | 0.98 ± 0.02                     |
| UBM2-domain              | 2.22 ± 0.08                     | 2.20 ± 0.06                     |
| UBM2-HTH$^{(b)}$ + ubiquitin | 1.26 ± 0.14                     | 1.19 ± 0.03                     |
| UBM2-domain + ubiquitin  | 1.91 ± 0.18                     | 1.92 ± 0.06                     |

(a) RMSD to the mean coordinates, Å

(b) HTH: helix-turn-helix motif (residues 687-707 for $Mm$ and 685-705 for $Hs$.

Supplementary Table 2: The RMSD values were calculated for the backbone atoms N, C$^{\alpha}$ and C'. $Mm$ refers to UBM2 of *Mus musculus*, $Hs$ to UBM2 of *Homo sapiens*. For the comparisons of UBM2-HTH and UBM2-domain the structures were fitted on the 21 residues of the central helix-turn-helix motif; for the comparisons of the complex the structures were fitted simultaneously on ubiquitin (1-73) and the HTH motif. The entire domain was compared for the residues 678-709 for $Mm$ and 767-707 for $Hs$, respectively.
References

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