

11. K. K. Tomczak *et al.*, *FASEB J.* **18**, 403 (2004).  
 12. P. K. Rao, M. Farkhondeh, S. Baskerville, H. F. Lodish, data not shown.  
 13. M. W. Rhoades *et al.*, *Cell* **110**, 513 (2002).  
 14. D. P. Bartel, C. Z. Chen, *Nat. Rev. Genet.* **5**, 396 (2004).  
 15. L. P. Lim *et al.*, *Nature* **433**, 769 (2005).  
 16. B. P. Lewis, I. H. Shih, M. W. Jones-Rhoades, D. P. Bartel, C. B. Burge, *Cell* **115**, 787 (2003).  
 17. J. G. Doench, P. A. Sharp, *Genes Dev.* **18**, 504 (2004).  
 18. E. C. Lai, B. Tam, G. M. Rubin, *Genes Dev.* **19**, 1067 (2005).  
 19. E. Eisenberg, E. Y. Levanon, *Trends Genet.* **19**, 362 (2003).  
 20. A. Ota *et al.*, *Cancer Res.* **64**, 3087 (2004).  
 21. L. He *et al.*, *Nature* **435**, 828 (2005).  
 22. H. B. Houbaviy, M. F. Murray, P. A. Sharp, *Dev. Cell* **5**, 351 (2003).  
 23. M. R. Suh *et al.*, *Dev. Biol.* **270**, 488 (2004).  
 24. X. Xie *et al.*, *Nature* **434**, 338 (2005).  
 25. We thank G. Ruby, M. Axtell, and H. Chang for helpful discussions. Supported by predoctoral fellowships from the Department of Energy (B.P.L.) and NSF (C.J.) and a postdoctoral fellowship and grants from the NIH (A.G., C.B.B., D.P.B.). D.P.B. is a Howard Hughes Medical Institute Investigator.

**Supporting Online Material**  
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11 October 2005; accepted 10 November 2005  
 Published online 24 November 2005;  
 10.1126/science.1121158  
 Include this information when citing this paper.

# Ubiquitin-Binding Domains in Y-Family Polymerases Regulate Translesion Synthesis

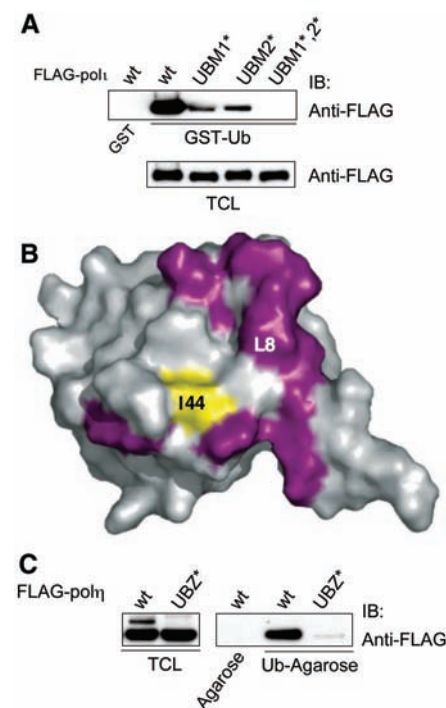
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Translesion synthesis (TLS) is the major pathway by which mammalian cells replicate across DNA lesions. Upon DNA damage, ubiquitination of proliferating cell nuclear antigen (PCNA) induces bypass of the lesion by directing the replication machinery into the TLS pathway. Yet, how this modification is recognized and interpreted in the cell remains unclear. Here we describe the identification of two ubiquitin (Ub)-binding domains (UBM and UBZ), which are evolutionarily conserved in all Y-family TLS polymerases (pols). These domains are required for binding of pol $\eta$  and pol $\iota$  to ubiquitin, their accumulation in replication factories, and their interaction with monoubiquitinated PCNA. Moreover, the UBZ domain of pol $\eta$  is essential to efficiently restore a normal response to ultraviolet irradiation in xeroderma pigmentosum variant (XP-V) fibroblasts. Our results indicate that Ub-binding domains of Y-family polymerases play crucial regulatory roles in TLS.

Signaling through ubiquitin (Ub) is generally thought to occur by low-affinity noncovalent interactions between Ub and a variety of specialized Ub-binding domains (UBDs) (1, 2). To analyze the Ub-interaction map, we performed yeast two-hybrid screens using wild-type Ub and Ub in which isoleucine 44 (I44) was mutated to alanine (Ub\*). To date, all known characterized UBDs require the conserved I44 in the hydrophobic patch on Ub for their binding (2), and proteins interacting with Ub\* might therefore contain previously un-

known Ub-interacting modules. Among the clones that interacted with Ub\* are two that encode the C terminus of TLS polymerase  $\iota$  (pol $\iota$ ) (fig. S1A). Moreover, full-length mouse pol $\iota$  expressed in human embryonic kidney (HEK) 293T cells bound to both glutathione *S*-transferase (GST)-Ub and GST-Ub\*, but not to GST alone (fig. S1A). Thus, pol $\iota$  contains a Ub-binding module in the C terminus that does not require I44 for its binding to Ub. Bioinformatic analysis of the C-terminal part of pol $\iota$  identified two copies of a previously unknown sequence motif termed UBM (Ub-binding motif). These repeats span ~30 residues and consist of two predicted helical segments, separated by an invariant "Leu-Pro" motif, which is conserved in all pol $\iota$  versions examined, as well as in Rev1, another Y-polymerase (fig. S1B). Missense mutations of the conserved residues with a presumptive crucial role in Ub binding (L508A, P509A in UBM1\*, L693A, P694A in UBM2\*) in either pol $\iota$  UBM substantially impaired pol $\iota$  binding to GST-Ub, whereas the inactivation of both domains by point mutations completely blocked the interaction (Fig. 1A). Similar results were obtained using pol $\eta$  de-

letion (pol $\iota$ - $\Delta$ 496-524 and pol $\iota$ - $\Delta$ 681-709) mutants (fig. S1C). We purified isolated GST-UBM1 and GST-UBM2 of pol $\iota$  and analyzed their binding to Ub and the Ub-I44A mutant by nuclear magnetic resonance (NMR) spectroscopy (fig. S1D). The estimated dissociation constant ( $K_d$ ) values for binding of UBM1 and UBM2 to both Ub and Ub-I44A were in the range of 180  $\mu$ M. Mapping of the

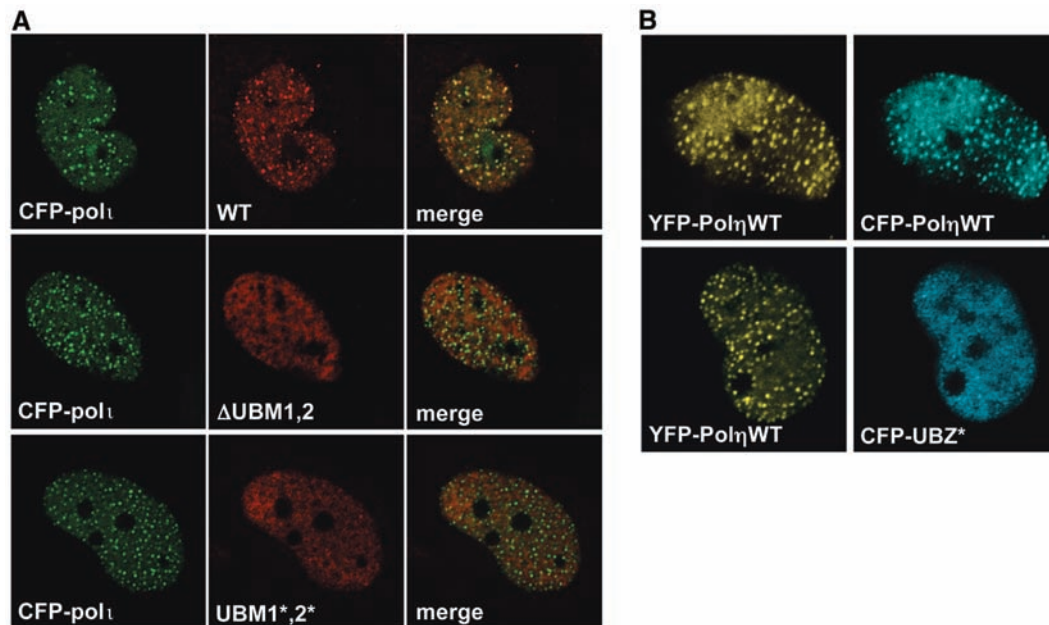


**Fig. 1.** (A) Identification of the UBDs in Y-polymerases. Point mutations of either UBM1 (L508A, P509A in UBM1\*) or UBM2 (L693A, P694A in UBM2\*) of mouse pol $\iota$  reduce its binding to Ub as compared with wild-type pol $\iota$  (wt). Mutating both UBMs (UBM1\*2\*) abolishes binding of pol $\iota$  to Ub in GST pull-down assays. (B) Surface representation of Ub interaction with UBM determined by NMR spectroscopy. The binding interface of GST-UBM2 on Ub defined by residues K6, L8, T9, G10, I13, T14, R42, K48, G53, and R72 (see supporting online material) is indicated in purple. Residue I44 (yellow) is indicated for orientation. (C) Pol $\eta$  UBZ mediates binding to ubiquitin. HEK293T lysates (TCL) containing FLAG-pol $\eta$  wild type or its UBZ mutant (D652A) (UBZ\*) were subjected to Ub-agarose pull-down assays. The shift in mobility of pol $\eta$  visible in lane 1 represents its monoubiquitinated form. IB, immunoblot.

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**Fig. 2.** UBMs and UBZ are essential for the accumulation of pol $\iota$  and pol $\eta$  in replication foci. (A) MRC5 fibroblasts were cotransfected with pECFP-pol $\iota$  wild type (left panels) and pCMV-FLAG pol $\iota$ , either wild type (WT) or mutants, as indicated (middle panels). The cells were UV irradiated with 15 J m $^{-2}$  and fixed 16 hours later. (B) MRC5 fibroblasts were cotransfected with YFP-pol $\eta$  wild type and CFP-pol $\eta$ , either wt or D652A, and treated as in (A).



UBM2 binding surface on Ub revealed binding around the previously defined hydrophobic patch, but the binding surface is displaced toward L8 and away from I44 (Fig. 1B).

Apart from the UBMs, we also identified several yeast two-hybrid clones containing mononucleate Zn fingers, which were required for their binding to Ub in yeast and mammalian cells. Using profile-based sequence comparisons (3, 4), we grouped these Ub-binding Zn fingers into a separate family, which we named UBZ (Ub-binding Zn finger). These sequence profile searches showed that the UBZ-family Zn fingers can be clearly separated from the presumed DNA-binding variety and are completely unrelated to PAZ (polyUb-associated Zn finger) (5) and NZF (Npl4 Zn finger) (6). UBZ-type fingers were also found in the remaining two Y-family polymerases pol $\eta$  and pol $\kappa$  (fig. S1E). Indeed, human pol $\eta$  is a Ub-binding protein because it interacted specifically with monoUb coupled to agarose (Fig. 1C), and point mutation of the conserved aspartate 652 to alanine in the UBZ (UBZ\*) prevented the interaction of pol $\eta$  with monoUb. Thus, all members of the Y-family polymerases contain UBMs in their C termini (fig. S1F).

Pol $\iota$  and pol $\eta$  colocalize with each other and with proliferating cell nuclear antigen (PCNA) in replication factories. These appear as bright foci in S-phase cells, which accumulate upon ultraviolet (UV) irradiation (7, 8). To examine whether this localization requires their UBMs, we cotransfected MRC5 fibroblasts with wild-type cyan fluorescent protein (CFP)-pol $\iota$  and wild-type or mutated FLAG-pol $\iota$ . The fraction of cells with foci increased in UV-irradiated cells to ~60%, and as expected, both wild-type constructs colocalized in foci. However, neither  $\Delta$ UBM1,2

nor UBM1\*,2\* mutants of pol $\iota$  localized in replication foci (Fig. 2A). Furthermore, the *Xenopus tropicalis* pol $\iota$ , which contains two UBMs but few other conserved amino acids in the C-terminal part (fig. S2A), bound to Ub (fig. S2B) and localized in replication factories in human cells (fig. S2C).

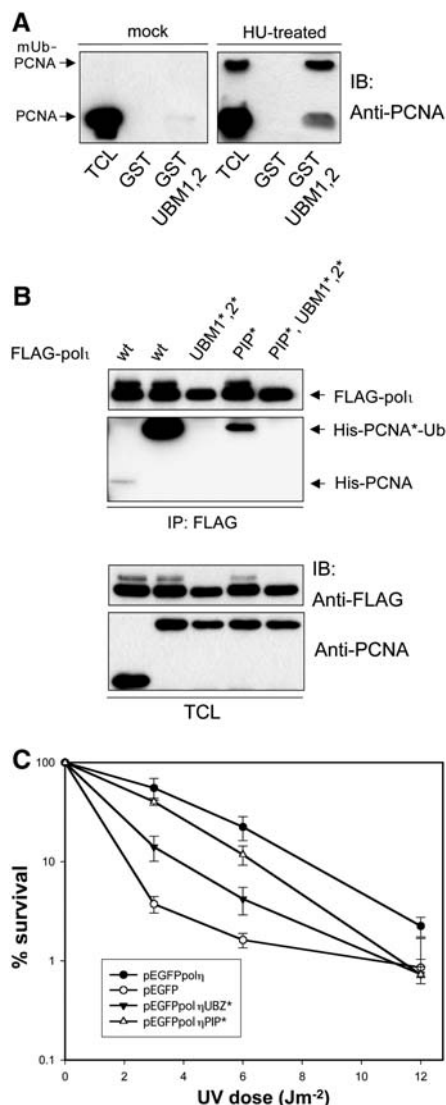
In similar cotransfection experiments, yellow fluorescent protein (YFP)- or CFP-tagged wild-type pol $\eta$  formed bright foci in S-phase cells accumulated upon UV irradiation, whereas CFP-pol $\eta$ -D652A mutant (UBZ\*) formed very faint or no foci (Fig. 2B). We conclude that foci localization of both pol $\eta$  and pol $\iota$  depends on their ability to interact with Ub, indicating a common mechanism for the accumulation of Y-polymerases in replication foci. Notably, the polymerase activity was identical in wild-type and mutant proteins used (fig. S3A).

Ubiquitination of the polymerase processivity factor PCNA controls switching of polymerases and replication of damaged DNA (9–13). TLS polymerases pol $\eta$  and pol $\iota$  bind directly to PCNA via their PCNA-interacting peptide (PIP box) (11, 14–16). In addition, DNA damage-induced ubiquitination of PCNA on K164 increases its interaction with pol $\eta$  in vivo (11, 13). We therefore investigated whether the UBM domains of pol $\iota$  directly bind to monoubiquitinated PCNA upon DNA damage. Isolated UBMs of pol $\iota$  readily precipitated monoubiquitinated PCNA generated in HEK293T cells by hydroxyurea treatment (Fig. 3A). UBMs also interacted directly with monoubiquitinated His-PCNA and with a permanently ubiquitinated PCNA, engineered by fusing the cDNA for Ub in frame with that for His-PCNA-K164R mutant (PCNA\*-Ub chimera) (fig. S3B). We next analyzed whether simultaneous binding of PIP and UBM do-

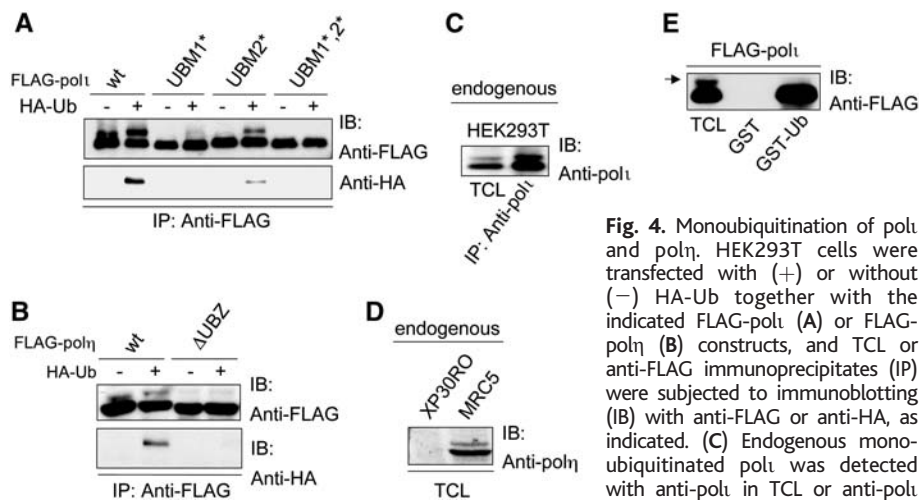
mains of pol $\iota$  to ubiquitinated PCNA enhanced this interaction in vivo. Coprecipitation of pol $\iota$  and unmodified PCNA from cells was hardly detectable, whereas the PCNA\*-Ub chimera strongly bound to pol $\iota$  in transiently transfected HEK293T cells (Fig. 3B). Coprecipitation was substantially reduced when the PIP box was mutated in pol $\iota$  and completely abolished if UBM domains or both PIP box and UBM domains of pol $\iota$  were mutated.

We next examined the importance of the UBMs in pol $\eta$  for its functions in vivo. Xeroderma pigmentosum variant (XP-V) patients are defective in pol $\eta$ , resulting in elevated levels of UV mutagenesis and skin cancers (17). XP30RO is an XP-V fibroblast line that is sensitive to UV irradiation in the presence of caffeine (17). Transfection of green fluorescent protein (GFP)-pol $\eta$  into XP30RO cells restores the UV plus caffeine sensitivity to that of normal human fibroblasts (Fig. 3C). However, when we transfected a UBZ mutant of pol $\eta$  (C638A) into XP30RO cells, the ability to confer resistance to UV plus caffeine treatment was substantially impaired. A reduction in this ability, though less marked, was also obtained with the PIP box mutation. Thus, both Ub- and PCNA-binding abilities of pol $\eta$  are required for efficient TLS. In previous work, we identified a motif in the little finger domain of pol $\eta$  that was required for its interaction with ubiquitinated PCNA; we thought this motif was similar to a CUE domain (11). Current bioinformatic and biochemical studies indicate that this motif is not a bona fide UBD. It is likely therefore that it is involved in the pol $\eta$ -PCNA interaction, irrespective of ubiquitination status.

UBMs can mediate monoubiquitination of the proteins containing them (1, 2). When FLAG-pol $\iota$  was transfected into HEK293T



**Fig. 3.** Dual mode of polt-monoUb-PCNA interaction. (A) The UBM domains of polt mediate interaction with monoubiquitinated PCNA (mUb PCNA) generated in HEK293T cells by hydroxyurea (HU) treatment. Total cell lysates or proteins bound to GST or GST-UBMs were analyzed by immunoblotting (IB) with antibodies to PCNA (anti-PCNA). A small amount of nonubiquitinated PCNA precipitated with GST-UBM domains, presumably owing to heterotrimerization of ubiquitinated monomers with nonubiquitinated ones. (B) Both the UBM and PIP box motifs of polt mediate its binding to PCNA\*Ub chimera. HEK293T cells were transfected with His-PCNA\*Ub and FLAG-polt (wt) or its PIP\* mutant (FLAG-polt-D424A/C425A/Y426A), UBM1\*2\* mutant, or PIP\*UBM1\*2\* mutant. The TCL shows the expression level of the corresponding proteins that were subsequently subjected to anti-FLAG immunoprecipitation (IP). Immunoprecipitated polt and PCNA\*Ub were detected with anti-FLAG and anti-PCNA, respectively. (C) XP30RO XP-V cells were transfected with pEGFP-polt constructs, and stable clones were isolated. The survival of these clones was measured after exposure to different doses of UV irradiation and plating in the presence of caffeine (75 μg/ml). UBZ\* represents the C638A mutation.



**Fig. 4.** Monoubiquitination of polt and polη. HEK293T cells were transfected with (+) or without (-) HA-Ub together with the indicated FLAG-polt (A) or FLAG-polη (B) constructs, and TCL or anti-FLAG immunoprecipitates (IP) were subjected to immunoblotting (IB) with anti-FLAG or anti-HA, as indicated. (C) Endogenous monoubiquitinated polt was detected with anti-polt in TCL or anti-polt immunoprecipitate. (D) Endogenous monoubiquitinated polη was detected with anti-polη in TCL from MRC5 but not in XP30RO cells (E) HEK293T lysates containing FLAG-polt were subjected to pull-down assays with GST-Ub, and bound proteins were detected by immunoblotting with anti-FLAG. The arrow indicates the monoubiquitinated form of polt.

cells, a band of polt of reduced mobility was detected by immunoblotting with antibodies to FLAG (Fig. 4A). Analysis of cells additionally transfected with hemagglutinin (HA)-Ub showed that the protein of reduced mobility was monoubiquitinated polt (Fig. 4A). Under similar conditions, we found a species of polη with reduced mobility (Fig. 4B; see also Fig. 1C), which we showed was monoubiquitinated polη (Fig. 4B). Mutational inactivation of UBMs in polt or UBZ in polη abolished their monoubiquitination (Fig. 4, A and B). We also detected endogenous levels of monoubiquitinated forms of both polymerases (Fig. 4, C and D). The monoubiquitinated species of polt appeared to be no longer capable of binding to Ub. In GST-Ub pull-down assays, only the unmodified form of polt was detected (Fig. 4E; compare lane 3 with lane 1). Similarly, only the unmodified form of polη bound to Ub-agarose beads (Fig. 1C). In accordance with these observations, creating a permanently ubiquitinated form of polη by fusing Ub to the C terminus of polη (polη-Ub chimera) strongly reduced its ability to bind to Ub-agarose beads (fig. S3C). These results indicate that monoubiquitinated polymerases might be blocked in binding to Ub because of autoinhibitory interactions with their own UBMs (18).

In conclusion, we have identified two previously unknown UBMs in the Y-family TLS polymerases that enable them to interact with monoubiquitinated targets and undergo monoubiquitination in vivo. UBMs are critical for accumulation of polt and polη in replication foci in human cells and are required for efficient restoration of normal TLS in XP-V cells. Both polt (Fig. 3, A and B) and polη (11, 13) preferentially interact with monoubiquitinated PCNA, which is generated at stalled replication forks (11, 13). The PIP provides the

specificity for the interaction, and the DNA damage-induced conjugation of a Ub moiety to PCNA increases the avidity of this binding by providing an interaction surface for the UBMs. We have also shown that polt and polη are themselves monoubiquitinated in vivo. Although the precise role of monoubiquitination of the polymerases remains to be established, it is tempting to speculate that a cycling between their nonubiquitinated and monoubiquitinated forms may contribute to regulation of their compartmentalization in or out of replication factories. Taken together, our data show that Ub binding of the Y-family polymerases plays an important role in translesion DNA synthesis and provide a long-sought clue to how these polymerases can gain preferential access to the stalled replication machinery at the sites of DNA damage.

**References and Notes**

1. K. Haglund, I. Dikic, *EMBO J.* **24**, 3353 (2005).
2. L. Hicke, H. L. Schubert, C. P. Hill, *Nat. Rev. Mol. Cell Biol.* **6**, 610 (2005).
3. K. Hofmann, P. Bucher, *Trends Biochem. Sci.* **21**, 172 (1996).
4. K. Hofmann, L. Falquet, *Trends Biochem. Sci.* **26**, 347 (2001).
5. D. Seigneurin-Berny *et al.*, *Mol. Cell. Biol.* **21**, 8035 (2001).
6. H. H. Meyer, Y. Wang, G. Warren, *EMBO J.* **21**, 5645 (2002).
7. P. Kannouche *et al.*, *EMBO J.* **22**, 1223 (2003).
8. P. Kannouche *et al.*, *Genes Dev.* **15**, 158 (2001).
9. E. C. Friedberg, A. R. Lehmann, R. P. Fuchs, *Mol. Cell* **18**, 499 (2005).
10. C. Hoegge, B. Pfander, G. L. Moldovan, G. Pyrowolakis, S. Jentsch, *Nature* **419**, 135 (2002).
11. P. L. Kannouche, J. Wing, A. R. Lehmann, *Mol. Cell* **14**, 491 (2004).
12. P. Stelter, H. D. Ulrich, *Nature* **425**, 188 (2003).
13. K. Watanabe *et al.*, *EMBO J.* **23**, 3886 (2004).
14. D. Y. Burnouf *et al.*, *J. Mol. Biol.* **335**, 1187 (2004).
15. A. E. Vidal *et al.*, *J. Biol. Chem.* **279**, 48360 (2004).
16. G. Maga, U. Hubscher, *J. Cell Sci.* **116**, 3051 (2003).
17. A. Lehmann, *Curr. Biol.* **13**, R585 (2003).
18. D. Hoeller *et al.*, *Nat. Cell Biol.*, in press.

19. We thank E. Friedberg and S. Tateishi for help with reagents and W. Mueller-Esterl, A. Carr, K. Caldecott, and members of the Dikic laboratory for critical reading of the manuscript. We also thank J. Wing and I. Konrad for technical assistance. This work was supported by a scholarship from the Ernst Schering Foundation (to M.B.), an MRC Programme Grant and EC Contract MRTN-CT-2003-503618 (to A.R.L.), grants from the

Swiss National Science Foundation and Eidgenössische Technische Hochschule Zürich (to M.P. and G.W.), and grants from the Deutsche Forschungsgemeinschaft (DI 931/1-1) and Boehringer Ingelheim Fonds (to I.D.).

**Supporting Online Material**  
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Materials and Methods  
Figs. S1 to S3  
References

27 September 2005; accepted 9 November 2005  
10.1126/science.1120615

# Chitin Induces Natural Competence in *Vibrio cholerae*

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The mosaic-structured *Vibrio cholerae* genome points to the importance of horizontal gene transfer (HGT) in the evolution of this human pathogen. We showed that *V. cholerae* can acquire new genetic material by natural transformation during growth on chitin, a biopolymer that is abundant in aquatic habitats (e.g., from crustacean exoskeletons), where it lives as an autochthonous microbe. Transformation competence was found to require a type IV pilus assembly complex, a putative DNA binding protein, and three convergent regulatory cascades, which are activated by chitin, increasing cell density, and nutrient limitation, a decline in growth rate, or stress.

Rivers, estuaries, and coastal waters are the principal reservoir for *Vibrio cholerae* in nature. In habitats of this kind, *V. cholerae* is found as a planktonic organism in the water column, in

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the mucilaginous sheaths of blue-green algae, and on the chitinous exoskeletons and molts of copepods (1). Population structure studies of aquatic habitats typically disclose ecosystems containing multiple microbial strains and species and high concentrations of phage and free DNA (2, 3). These features, when combined with mechanisms for HGT, likely explain why the *Vibrionaceae* have developed high levels of genomic diversity (4, 5).

One microenvironment where HGT could occur between *V. cholerae* and other strains

and species is within microbial assemblages on natural chitin surfaces. *V. cholerae* readily attaches to and degrades chitin, a polymer of  $\beta$ -1,4-linked *N*-acetylglucosamine (GlcNAc). Chitin induces the expression of a 41-gene regulon involved in chitin colonization, digestion, transport, and assimilation, including genes predicted to encode a type IV pilus assembly complex (6).

*V. cholerae* has never been shown to be competent for natural transformation, and thus, with respect to HGT events, its genome is presumed to have evolved by transduction (responsible for the acquisition of the *ctx* genes encoding cholera toxin) and conjugation (7, 8). However, the induction of type IV pilin by chitin and the association of type IV pili and competence in several other species led us to test if chitin might induce natural competence in *V. cholerae* (9). We grew *V. cholerae* O1 El Tor, strain A1552, in a liquid minimal medium containing 2.5 mM (GlcNAc)<sub>6</sub>, a soluble chitin oligosaccharide that induces the chitin regulon (6). Then, genomic DNA from the *V. cholerae* O1 El Tor strain VCXB21, which harbors a gene for kanamycin resistance on the chromosome, was added to the culture. After 18 hours of growth, the culture was plated onto antibiotic-free and kanamycin-containing LB agar; this yielded a transformation frequency [kanamycin-resistant (Kn<sup>r</sup>) colony-forming units (CFU)/

**Table 1.** Transformation of *V. cholerae*; data are the average of at least three experiments. Transformation frequency is Kn<sup>r</sup> or Str<sup>r</sup> CFU/total CFU; <DL, below detection limit (for values in (A),  $\sim 4.0 \times 10^{-8}$ ; for (B),  $\sim 3.0 \times 10^{-9}$ ; for (C),  $\sim 1.0 \times 10^{-7}$ ; for (D),  $\sim 4.0 \times 10^{-8}$ ).

Donor DNA	Recipient strain	Medium	Transformation frequency	Range
<b>A. Transformation in liquid medium</b>				
VCXB21	A1552	+ (GlcNAc) <sub>6</sub>	$2.7 \times 10^{-5}$	1.4 to $6.8 \times 10^{-5}$
VCXB21	A1552	+ (GlcNAc) <sub>6</sub> + DNase	<DL	
None	A1552	+ (GlcNAc) <sub>6</sub>	<DL	
VCXB21	A1552	+ (GlcNAc) <sub>6</sub> + Glucose	<DL	
VCXB21	A1552	+ Glucose	<DL	
VCXB21	A1552	+ GlcNAc	<DL	
<b>B. Transformation in liquid medium of His and Pro auxotrophs</b>				
VCXB21	A1552proC	+ (GlcNAc) <sub>6</sub>	$2.7 \times 10^{-6}$	$4.0 \times 10^{-7}$ to $1.1 \times 10^{-5}$
None	A1552proC	+ (GlcNAc) <sub>6</sub>	<DL	
VCXB21	A1552hisD	+ (GlcNAc) <sub>6</sub>	$6.8 \times 10^{-6}$	$4.0 \times 10^{-8}$ to $1.7 \times 10^{-5}$
None	A1552hisD	+ (GlcNAc) <sub>6</sub>	<DL	
<b>C. Transformation of chitin surface-attached bacteria</b>				
VCXB21	A1552	Crab shell	$3.5 \times 10^{-5}$	$5 \times 10^{-6}$ to $6.9 \times 10^{-5}$
None	A1552	Crab shell	<DL	
N16961	A1552	Crab shell	$1.8 \times 10^{-5}$	$5 \times 10^{-6}$ to $4.4 \times 10^{-5}$
VCXB21	A1552	Crab shell + DNase	$3.7 \times 10^{-7}$	<DL to $8.0 \times 10^{-7}$
VCXB21	N16961	Crab shell	<DL	
VCXB21	C6706	Crab shell	$2.8 \times 10^{-6}$	$8.0 \times 10^{-7}$ to $5 \times 10^{-6}$
VCXB21	0395 (classical)	Crab shell	<DL	
<b>D. Transformation in biofilm communities without exogenous DNA</b>				
None	A1552 -Kn/-Str	Crab shell	$4.4 \times 10^{-5}$	1.4 to $8 \times 10^{-5}$
None	A1552 -Kn/-Str	Crab shell + DNase	$<1.2 \times 10^{-7}$	<DL to $1.2 \times 10^{-7}$