Response to Comment on “Structural basis of histone H3K27 trimethylation by an active polycomb repressive complex 2”

Lianying Jiao and Xin Liu*

Zhang et al. suggested that in the crystal structure of a polycomb repressive complex 2 from Chaetomium thermophilum (ctPRC2), a flexible linker region, but not the H3K27M cancer mutant peptide, better fits the electron density. Based on our new data, we agree with this alternative interpretation and provide the crystal structure of ctPRC2 bound to a bona fide H3K27M sequence.

Although displaying obvious sequence diversity, Chaetomium thermophilum polycomb repressive complex 2 (ctPRC2) is functionally and structurally similar to human PRC2 in both basal and stimulated states (1–3). In our initial crystallization efforts, the H3K27M cancer mutant peptide, which also inhibited the enzymatic activity of ctPRC2 in solution, was strictly required to generate ctPRC2 crystals of sufficient quality for structural determination (4). In addition, electron density that clearly corresponded to a short peptide was observed within the substrate-binding groove of the Ezh2 active site. We assigned the H3K27M peptide to the electron density primarily based on the arginine residue in the replaced H3K27M sequence from a neighboring asymmetric unit was inserted into the active site, contacting SAM directly (Fig. 1).

Only the aliphatic portion of residue R26 side chain is ordered under the current condition. The remainder of the structure remained essentially identical to the published ctPRC2 structure in the stimulated state. H3K27M thus appears to use the same structural mechanism to inhibit ctPRC2 catalysis as previously suggested for human PRC2 (2, 5).

The new crystal structure of SAM and H3K27M-bound ctPRC2 in the stimulated state was deposited in the Protein Data Bank under PDB accession code 5KJL. We also updated the previously deposited PDB entries 5CH1 and 5CH2 to 5KJH and 5KJL, respectively. All other results and analyses of our original publication remain unaffected. We apologize for any confusion that this misinterpretation may have caused.

REFERENCES AND NOTES

ACKNOWLEDGMENTS
Preparation of this response was supported by Welch Foundation research grant I-1790, Rita Allen Foundation research grant R1119, Rita Allen Foundation.
research grant, University of Texas Southwestern Medical Center
Endowed Scholar fund, and NIH grant GM114576 to X.L. L.J. was
supported by American Heart Association postdoctoral fellowship
16POST30700004. X.L. is a W. W. Caruth, Jr. Scholar in Biomedical
Research. This research also received support from the Cecil H.
and Ida Green Center Training Program in Reproductive Biology
Sciences Research. This research used resources of the Advanced
Photon Source, a U.S. Department of Energy (DOE) Office of Science
User Facility operated for the DOE Office of Science by Argonne
National Laboratory under contract no. DE-AC02-06CH11357. The
Advanced Light Source is supported by the Director, Office of
Science, Office of Basic Energy Sciences, of the U.S. DOE
under contract no. DE-AC02-05CH11231. Use of the Stanford
Synchrotron Radiation Lightsource (SSRL). Stanford Linear
Accelerator Center (SLAC) National Accelerator Laboratory, is
supported by the U.S. Department of Energy, Office of Science,
Office of Basic Energy Sciences under contract no. DE-AC02-
76SF00515. The SSRL Structural Molecular Biology Program is
supported by the DOE Office of Biological and Environmental
Research and by the National Institutes of Health, National
Institute of General Medical Sciences (NIGMS) (including
P41GM103393). The contents of this publication are solely the
responsibility of the authors and do not necessarily represent the
official views of NIGMS or NIH.
19 September 2016; accepted 9 November 2016
10.1126/science.aaj2335
Response to Comment on "Structural basis of histone H3K27 trimethylation by an active polycomb repressive complex 2"
Lianying Jiao and Xin Liu

*Science* **354** (6319), 1543.
DOI: 10.1126/science.aaj2335