Published in final edited form as:

Sci Signal. ; 13(616): . doi:10.1126/scisignal.aaz8130.

Fragile epitopes—antibody's guess is as good as yours

Kevin A. Janes^{1,*}

¹Departments of Biomedical Engineering and Biochemistry & Molecular Genetics, University of Virginia, Charlottesville, VA 22908, USA

Abstract

Monoclonal antibodies recognize epitopes so specifically that altering a single residue can disrupt binding. In this issue of *Science Signaling*, Schüchner *et al.* and Frohner *et al.* report that flanking amino acids and an underappreciated posttranslational modification perturb epitope affinity for two groups of widely used monoclonal antibodies.

One-Sentence Summary

Critical research antibodies need systematic validation (Frohner *et al.* and Schüchner *et al.*, in 28 January 2020 issue).

Few reagents in cellular signaling work are more pervasive and angst-inducing than the research antibody. The fickleness of antibody preparation and distribution is well documented—in the United States, an estimated \$350 million is wasted each year on poorly performing antibodies (1). To avoid costly trial and error, most researchers seek out the scientific consensus, assuming that the best antibody-producing hybridoma clone is the one most widely cited or most widely sold commercially. In two papers from the Ogris laboratory, Schüchner *et al.* (2) and Frohner *et al.* (3) give compelling examples where this conventional wisdom is incorrect.

The work of Schüchner *et al.* (2) focuses on monoclonals recognizing a peptide epitope derived from the transcription factor Myc. The minimal peptide sequence of the Myc epitope is commonly grafted onto the amino (N) or carboxy (C) terminus of ectopically expressed proteins as a “universal” affinity tag for immunodetection or immunopurification. However, for the most popular hybridoma clone (9E10) that recognizes Myc (4), the authors found serendipitously that immunodetection was substantially altered when the Myc sequence was situated in two slightly different N-terminal contexts of the same ectopic protein (Fig. 1, A and B). More broadly, 9E10 exhibited 100- to 1000-fold differences in target affinity according to custom peptide arrays synthesized with random amino acids flanking the minimal Myc epitope. The pronounced affinity dependence persisted with tandem Myc tags and was not unique to 9E10—a polyclonal antibody directed against Myc (9106) also exhibited affinity dependence but with context preference opposite that of 9E10. Encouragingly, context dependence was not endemic to the Myc tag itself, as Schüchner *et*

*Corresponding author. kjanes@virginia.edu.

Competing interests: The author declares that he has no competing financial interests.

al. (2) show by using a shorter Myc tag immunogen that harbors a C terminus comprised of unnatural amino acids. The resulting hybridoma clone (4A6) binds much more uniformly on peptide arrays (<10-fold affinity difference between peptides that differ in their flanking residues) and in immunoblots of various Myc-tagged fusion proteins (Fig. 1, A and B). Toward long-term reproducibility (1), the 4A6 light and heavy chains were sequenced and cloned together as a short-chain variable fragment for permanent distribution. 4A6 is a dedicated Myc-tag monoclonal and should become the antibody of choice for applications such as absolute quantitation (5).

The paper by Frohner *et al.* (3) is also a forensic study of epitopes, but here directed at an endogenous peptide antigen of protein phosphatase 2A catalytic subunit (PP2A C). The C terminus of PP2A C extends far away from the catalytic domain (6), making it a favorite target for monoclonal antibody producers (Fig. 1C). As enticing as those terminal amino acids may be for immunization, Frohner *et al.* (3) report that the very C terminus of PP2A C is problematic for making interpretable immunoreagents. In cells, multiple residues are phosphorylated within this C-terminal region, and the C-terminus is almost completely carboxymethylated en route to proper assembly of native heterotrimeric PP2A holoenzymes (Fig. 1D) (7). Accordingly, the authors found that most monoclonals directed against the C terminus of PP2A C had considerable preference for the rare, nonmethylated fraction of PP2A C. Antigen binding of these antibodies was also perturbed or eliminated when the C terminus was phosphorylated. The results should be eye-opening to casual users of a commercial PP2A C activity assay—published in dozens of studies—that deploys one of these monoclonals (1D6) for the first-step immunoprecipitation. More robust clones were verified for immunoblotting—notably, clone 52F8 raised with a peptide slightly upstream of the C-terminus (Fig. 1, C and D)—but none were suitable for PP2A holoenzyme immunoprecipitation. Global PP2A C activity assays of endogenous complexes await better affinity reagents; in the meantime, bulk assays against specific PP2A substrates may be an acceptable substitute for some applications (8).

A highly-appreciated quality of these latest papers (2, 3) is the systematic, comparative assessment of commercial and in-house antibodies in the same category. Side-by-side comparisons are the norm for other types of research reagents, such as fluorescent proteins, optogenetic constructs, and tissue-clearing solutions. By contrast, some commercial antibody producers are more inclined to validate and advertise than to vet their products against the competition, making the evaluation incumbent on investigators. One hopes that the publications here will emphasize how crucial such work is to the broader scientific community.

The studies are also refreshingly forthright. In one instance, the authors' new monoclonal is superior to competing alternatives (2). In the other, an “Ogris-grade” monoclonal suffers from the same epitope fragility as those commercially available (3). The results emphasize the frustrating combination of best practices and luck that goes into obtaining a good monoclonal.

Together, the two publications of Schüchner *et al.* (2) and Frohner *et al.* (3) remind that the definition of “epitope” is usually nebulous. Without detailed structural information about

how a monoclonal antibody recognizes its target (9), we cannot know which features of an antigen are critical for the epitope and which are not. A case in point is the 9E10 monoclonal, which binds to the extended Myc sequence (Fig. 1A) in an asymmetric 2:1 stoichiometry (10). Hybridoma clones that produce research-grade antibodies are stochastic “winners” in a process of recombination, hypermutation, and selection that we try to control but do not fully understand. Thus, insights can only arise from accidental discoveries (2) and educated guesses (3) about epitope fragility. The information in these papers should be circulated widely to avoid perpetuating unintended errors of the past.

Acknowledgments

I thank Cheryl Borgman for reviewing this manuscript.

Funding: K.A.J. is supported by the NIH (R01-CA214718, U01-CA215794, R01-CA194470) and the David and Lucile Packard Foundation (2009-34710).

References and Notes

1. Bradbury A, Pluckthun A, Reproducibility: Standardize antibodies used in research. *Nature* 518, 27–29 (2015); published online EpubFeb 5 (10.1038/518027a). [PubMed: 25652980]
2. Schüchner S, Behm C, Mudrak I, Ogris E, The 9E10 Myc tag monoclonal antibody displays highly variable epitope recognition dependent on neighboring sequence context. *Sci Signal* 13, eaax9730 (2019).
3. Frohner IE, Mudrak I, Kronlachner S, Schüchner S, Ogris E, Antibodies recognizing the carboxy-terminus of PP2A catalytic subunit are unsuitable to study PP2A activity and holoenzyme composition. *Sci Signal* 13, eaax6490 (2019).
4. Evan GI, Lewis GK, Ramsay G, Bishop JM, Isolation of monoclonal antibodies specific for human c-myc proto-oncogene product. *Mol Cell Biol* 5, 3610–3616 (1985); published online EpubDec (10.1128/mcb.5.12.3610). [PubMed: 3915782]
5. Janes KA, An analysis of critical factors for quantitative immunoblotting. *Sci Signal* 8, rs2 (2015)10.1126/scisignal.2005966. [PubMed: 25852189]
6. Cho US, Xu W, Crystal structure of a protein phosphatase 2A heterotrimeric holoenzyme. *Nature* 445, 53–57 (2007); published online EpubJan 4 (10.1038/nature05351). [PubMed: 17086192]
7. Yu XX, Du X, Moreno CS, Green RE, Ogris E, Feng Q, Chou L, McQuoid MJ, Pallas DC, Methylation of the protein phosphatase 2A catalytic subunit is essential for association of Balph regulatory subunit but not SG2NA, striatin, or polyomavirus middle tumor antigen. *Mol. Biol. Cell* 12, 185–199 (2001); published online EpubJan (10.1091/mbc.12.1.185). [PubMed: 11160832]
8. Shah M, Smolko CM, Kinicki S, Chapman ZD, Brautigan DL, Janes KA, Profiling Subcellular Protein Phosphatase Responses to Cocksackievirus B3 Infection of Cardiomyocytes. *Mol Cell Proteomics* 16, S244–S262 (2017); published online EpubApr (10.1074/mcp.O116.063487). [PubMed: 28174228]
9. Sheriff S, Silverton EW, Padlan EA, Cohen GH, Smith-Gill SJ, Finzel BC, Davies DR, Three-dimensional structure of an antibody-antigen complex. *Proc. Natl. Acad. Sci. U. S. A* 84, 8075–8079 (1987); published online EpubNov (10.1073/pnas.84.22.8075). [PubMed: 2446316]
10. Krauss N, Wessner H, Welfle K, Welfle H, Scholz C, Seifert M, Zubow K, Ay J, Hahn M, Scheerer P, Skerra A, Hohne W, The structure of the anti-c-myc antibody 9E10 Fab fragment/epitope peptide complex reveals a novel binding mode dominated by the heavy chain hypervariable loops. *Proteins* 73, 552–565 (2008); published online EpubNov 15 (10.1002/prot.22080). [PubMed: 18473392]

Fig. 1. Contextual fragility of antibody epitopes.

(A, B) Multiple antibodies directed against Myc (the monoclonal 9E10 and polyclonal 9106) depend on characteristics of the residues immediately C-terminal to the epitope. (C, D) Monoclonal antibodies raised against the C terminus (CT) of the PP2A catalytic subunit are disrupted by phosphorylation and carboxymethylation. Hybridoma clones 4A6 and 52F8 are robust in the contexts described in Schüchner *et al.* (2) and Frohner *et al.* (3), respectively.