

TECHNICAL RESPONSE

CHROMATIN

Response to Comment on “A histone acetylation switch regulates H2A.Z deposition by the SWR-C remodeling enzyme”

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Wang *et al.* report a failure to reproduce our biochemical observation that the INO80C and SWR1C/SWR1/SWR-C chromatin remodeling enzymes catalyze replacement of nucleosomal H2A.Z with H2A when the substrate contains H3-K56Q. They point to technical problems with our dimer exchange assay. In response, we have recapitulated our findings using a mobility shift assay that was developed and employed by Wang and colleagues.

SWR1C and INO80C are two yeast members of the INO80 subfamily of remodelers (1). Whereas SWR1C promotes replacement of nucleosomal H2A/H2B dimers with H2A.Z/H2B in an adenosine triphosphate (ATP)-dependent reaction (2), we reported that INO80C catalyzes the reverse dimer exchange reaction, replacing H2A.Z/H2B dimers with H2A/H2B (3–5). A role for INO80C in removal of H2A.Z is consistent with global disruption of H2A.Z localization in yeast strains lacking INO80C (3). Furthermore, we found that this reverse reaction was stimulated when nucleosomes contained acetylated lysine 56 of histone H3 (H3-K56Ac) or a substitution of H3-K56 for glutamine (H3-K56Q) (5). Strikingly, H3-K56Q or H3-K56Ac switched the substrate specificity of SWR1C, inhibiting H2A.Z deposition while promoting exchange of H2A.Z with H2A (5). Furthermore, H3-K56Q altered the adenosine triphosphatase (ATPase) properties of SWR1C. Normally, the ATPase activity of SWR1C is activated by H2A nucleosomes and further stimulated by free H2A.Z/H2B dimers. However, we found that H3-K56Q allowed both H2A and H2A.Z nucleosomes to fully stimulate ATPase activity, and the enzyme was insensitive to histone dimers (5). These ATPase data support the observation of altered substrate specificity for dimer exchange activity.

In their Comment, Wu and colleagues used three gel-based assays to monitor dimer exchange by SWR1C and INO80C [Wang *et al.* (6)]. Although their assays detected H2A.Z deposition by SWR1C, they did not observe the reverse reaction by either SWR1C or INO80C. Interestingly, they did observe inhibition of H2A.Z deposition by H3-K56Q, similar to our previous results (5). They suggest that interpretation of our published results may be confounded by technical problems with our dimer

exchange assay. Our assay involves incubation of remodelers with recombinant yeast mononucleosomes and recombinant yeast H2A/H2B or H2A.Z/H2B dimers (3). Reaction products are electrophoresed on polyacrylamide gel electrophoresis (PAGE) to separate mononucleosome products from free histones or other non-nucleosomal products. Nucleosome integrity is monitored by Western blot for H3 or by visualizing DNA with ethidium bromide, and dimer exchange is assayed by Western blot. To avoid aggregation by free histone dimers, the concentration of free dimers is titrated for each experiment. With this assay, we

found that all dimer exchange activities are strongly ATP- and enzyme concentration-dependent (3, 4, 5, 7). Furthermore, in side-by-side comparisons, INO80C catalyzed the reverse reaction (replacement of H2A.Z with H2A), but the SWI/SNF remodeler was inactive (3). Likewise, SWR1C catalyzed the reverse reaction with H2A.Z/H3-K56Ac or H2A.Z/H3-K56Q nucleosomes but not with an unmodified substrate (5). All results have been confirmed numerous times with independent enzyme, histone, and nucleosome preparations. Wu and colleagues suggest that our results may be influenced by a failure to stop reactions with excess DNA, although they acknowledge that this cannot explain the ATP dependence (or enzyme specificity) of our results. The altered ATPase parameters of SWR1C with H3-K56Q nucleosomes cannot be explained by the specifics of the exchange assay.

To eliminate the possibility that our particular assay might have influenced results, we performed reactions using the direct electrophoretic mobility shift assay (EMSA) assay developed and employed by Wu and colleagues (8). Given the time frame allowed for our response, we have focused on INO80C. In their EMSA assay, the substrate is a fluorescently labeled mononucleosome, and dimer exchange is monitored by incorporation of a 3xFlag-tagged histone dimer, which causes a shift in mobility on PAGE. Using this assay, and an H2A.Z/H3-K56Q nucleosome, we confirmed that INO80C catalyzes incorporation of 3xFlag-tagged H2A (Fig. 1). Importantly, this dimer exchange reaction was ATP-dependent, and the amount of product increased with increasing enzyme concentration and time of incubation (Fig. 1). Interestingly, the reaction appears to favor replacement of both H2A.Z/H2B dimers, because the slower migrating species

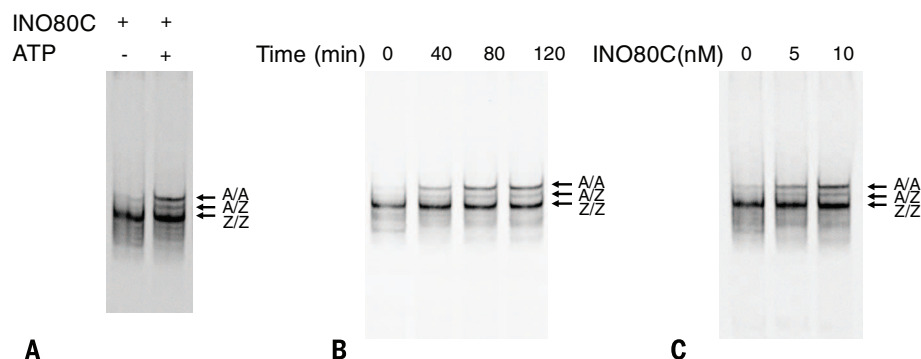


Fig. 1. Histone exchange by INO80C. EMSA shows Cy5-DNA (200 base pairs) of yeast H2A.Z/H3-K56Q mononucleosomes reconstituted on centrally located 601 nucleosome positioning sequence. Arrows indicate nucleosomes containing two H2A.Z/H2B dimers (Z/Z), one 3xFlag-H2A/H2B dimer (A/Z), or two 3xFlag-H2A/H2B dimers (A/A). Migration of each species was confirmed by assembly of “marker” nucleosomes created by reconstitution with H3/H4 tetramers and mixtures of H2A.Z/H2B and 3xFlag-H2A/H2B dimers. **(A)** INO80C catalyzes “reverse” dimer exchange. Reactions contained 10 nM INO80C, 100 nM H2A.Z/H3-K56Q nucleosomes, and 100 nM 3xFlag-H2A/H2B dimers. ATP (2 mM) was added as indicated. Reactions incubated 2 hours at 30°C in buffer [70 mM NaCl/10 mM Tris-HCl (pH8.0)/5 mM MgCl₂/0.1 mg/ml bovine serum albumin/1 mM dithiothreitol] ± 2 mM ATP as indicated. Stop solution (1 mg/ml salmon sperm DNA/50% glycerol) was added before loading on 6% native PAGE gel in 0.5X tris-borate EDTA, followed by Cy5 scanning on GE Typhoon imager. **(B)** Reactions as in (A) but samples incubated with 2 mM ATP for indicated times. **(C)** Reactions as in (A) and samples contained indicated amounts of INO80C with 2 mM ATP.

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accumulates at low enzyme concentrations and at early time points. Thus, using either our Western blot assay or the direct EMSA assay, INO80C catalyzes replacement of H2A.Z/H2B with H2A/H2B on an H2A.Z/H3-K56Q substrate.

At present it is not clear why the Wu group is unable to detect the reverse dimer exchange reaction, even when using the same assay. As they note, the differing results do not appear to be due to different methods of purifying enzymes, nor does the problem appear to be due to differences with recombinant histone dimers, as H2A/H2B dimers provided by the Wu group were functional in our hands. The only remaining variables are histone octamers and nucleosome reconstitutions. We find that yeast H2A.Z and H2A.Z/K56Q octamers do not reconstitute efficiently and that great care has to be taken to ensure that octamers do not contain free histone tetramers or dimers. The success of these dimer exchange reactions is highly sensitive to the quality and concentrations of both the reconstituted nucleosome and free dimers.

The only clear solution is to continue to share reagents until the source of the technical problem is identified.

The differing results obtained with gel-based assays for histone dimer exchange reactions reinforce the need for more sophisticated, solution-based assays that monitor each of the steps in the exchange reaction. Indeed, a previous fluorescence resonance energy transfer-based study suggested that the ATP-dependent steps of dimer exchange may occur quite rapidly and that formation of a product that is detectable by PAGE occurs on a much slower time scale (9). We anticipate that application of such methods will not only define the detailed mechanism of dimer exchange by INO80 subfamily members but that these more quantitative tools will enhance the reproducibility of data obtained among groups.

Experimental procedures were as follows: Purification of recombinant histones, reconstitution of mononucleosomes, preparation of yeast histone dimers, and purification of INO80C-TAP were as

published (3, 5, 10). Exchange assays were performed as published (3, 5).

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