JBC Papers in Press. Published on May 23, 2017 as Manuscript M117.790386
The latest version is at http://www.jbc.org/cgi/doi/10.1074/jbc.M117.790386

Functional role of the three conserved cysteines in the N-domain of visual arrestin-1

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Running title: The role of native cysteines in visual arrestin activation

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Keywords: Arrestin, activation, rhodopsin, cysteines, conformational change

Arrestins specifically bind active and phosphorylated forms of their cognate G protein-coupled receptors, blocking G protein coupling and often redirecting the signaling to alternative pathways. High-affinity receptor binding is accompanied by the two major structural changes in arrestin: the release of the C-tail and rotation of the two domains relative to each other. The first requires the detachment of the arrestin C-tail from the body of the molecule, whereas the second requires the disruption of the network of charge-charge interactions at the interdomain interface, termed the polar core. These events can be facilitated by mutations destabilizing the polar core or the anchoring of the C-tail, which yield "pre-activated" arrestins that bind phosphorylated and unphosphorylated receptors with high affinity. Here we explored the functional role in arrestin activation of the three native cysteines in the N-domain, which are conserved in all arrestin subtypes. Using visual arrestin-1 and rhodopsin as a model we found that substitution of these cysteines with serine, alanine or valine virtually eliminates the effects of the activating polar core mutations on the binding to unphosphorylated rhodopsin, while only slightly reducing the effects of the C-tail mutations. Thus, these three conserved cysteines play a role in the domain rotation, but not in the C-tail release.

Visual arrestin-1[1] has three cysteines in the N-domain (Cys63, Cys128, and Cys143 in bovine arrestin-1), which are conserved in all vertebrate species, even in Ciona intestinalis, an ascidian belonging to a sister clade of vertebrates (1). Interestingly, the same cysteines are present in the other three vertebrate arrestins, although in non-visual subtypes there are numerous additional cysteines in the C-domain (1). This conservation suggests a high functional importance of these residues. Indeed, the substitution of the last cysteine (Cys 147 in human arrestin-1) with phenylalanine is the only dominant arrestin-1 mutation causing retinal degeneration in humans discovered so far (2). However, in most invertebrate species only this last of

the three cysteines is conserved (1). The crystal structures of arrestin-1 (3,4), and the other three subtypes of vertebrate arrestins (5-8), show that the homologue of Cys128 is the most exposed of the three, whereas the other two are buried between β-strands, interacting with numerous hydrophobic partners (Fig. 1). To generate cysteine-less bovine arrestin-1 as a base mutant for site-directed spin labeling/EPR studies we replaced buried Cys63 and Cys143 with smaller alanines or slightly larger valines, whereas more exposed Cys128 was replaced with a serine. Both cysless forms, termed ASA and VSV, respectively, appeared to be competent to bind phosphorylated light-activated rhodopsin (P-Rh*) (9-11) and to form physiologically relevant oligomers (12,13). However, we found that the replacement of cysteines in arrestin-1 virtually eliminates the activating effects of some mutations that render arrestin-1 phosphorylation-independent, while only slightly reducing the effect of other mutations that yield similar "pre-activated" phenotype. Therefore, we tested the effect of replacing individual cysteines with different residues arrestin-1. The data suggest that cysteines in specific positions in the arrestin-1 molecule help it maintain optimal flexibility for receptor binding.

RESULTS

Previously, we substituted both Cys63 and Cys143 with either alanines (ASA base mutant) or valines (VSV). Both base mutants performed like WT arrestin-1 (9,10,12,13). As both residues appeared to substitute successfully, we also generated ASV and VSA mutants. Since the most critical biological function of arrestin-1 is the ability to discriminate between light-activated phosphorylated rhodopsin (P-Rh*) and unphosphorylated light-activated rhodopsin (Rh*), we tested the binding of WT and four cys-less mutants to these two forms. We found that all mutants demonstrate essentially the same selectivity, with ASA mutant even binding P-Rh* slightly better than WT (Fig. 2A). Arrestins are elongated two-domain molecules (4) (Fig. 1A,D). WT arrestin-1 is held in the basal conformation by two key interactions: the polar core between the two domains, where the critical salt bridge between Arg175 and Asp296 plays a key role (14), and hydrophobic three-element interaction of the C-tail with the α-helix and β-strand I in the N-domain (15) (Fig. 1B,C). Both interactions are destabilized by the receptor-attached phosphates (14,15). Destabilization of either of these "clasps" greatly enhances arrestin-1 binding to Rh*, yielding pre-activated phosphorylation-independent mutants (4,14,16-20) that appear to have a reduced energy barrier for activation (21), in contrast to the fairly high activation energy of WT arrestin-1 (22). Mutations on the receptor-binding surface K257Q and E346H in bovine arrestin-1 (23), as well as homologous K258Q and E347H mutations in a mouse protein (20), also enhance Rh* binding. Therefore we introduced these activating mutations on the background of the four sets of substitutions that remove native cysteines. To our surprise, ASA, ASV, VSA, and VSV mutations dramatically reduced Rh* binding of R175E and D296R mutants with a destabilized polar core (Fig. 2B,C), but only marginally affected Rh* binding of mutants activated by destabilization of the three-element interaction: truncated arrestin-1-(1-378), arrestin-1-3A (triple alanine substitution of bulky hydrophobic residues in the C-tail, F375, V376, and F377), as well as combinations of these mutations with K257Q and E346H (Fig. 2D,E,F). Some cysteine substitutions even reduced P-Rh* binding of polar core mutants R175E and D296R (Fig. 2B,C).

As this result was rather unexpected, we tested whether this is a common feature of mammalian arrestin-1 or a peculiarity of the bovine protein. To this end, we made cysteine-less mouse arrestin-1 by introducing C64A, C129S, C144A mutations, and showed that this protein retains high selectivity for P-Rh* over Rh* (Fig. 3). Next we used homologous mutations in the polar core (R176E and D297R) and in the C-terminus (3A and

truncation) that were shown to pre-activate mouse arrestin-1 just like the bovine protein (16,20) and compared the effect of these mutations on the WT and ASA background (Fig. 3). The results were the same as in the bovine arrestin-1: ASA essentially eliminated Rh* binding of R176E and D297R mutants, while only somewhat reducing Rh* binding of the Tr mutant and not affecting 3A mutant at all (compare Figs. 2 and 3). Thus, elimination of native cysteines blocks Rh* binding of polar core mutants, but does not demonstrate a similar devastating effect in the case of 3A mutation and the deletion of the C-tail (Tr).

To determine whether the substitution of a particular cysteine is responsible for Rh* binding reduction of R175E and D296R mutants, we combined these mutations with individual cysteine replacements, C63A, C128S, and C143A, and compared these mutants to triple substitution ASA (Fig. 4). We found that in case of activating polar core mutations, R175E and D296R, the substitution of each individual cysteine somewhat reduces Rh* binding, although only one and two reached statistical significance with R175E and D296R, respectively. Thus, the effect of the triple substitution ASA appears to be the result of additive effects of the individual mutations, suggesting a role of each of the three conserved cysteines in arrestin-1 activation.

In many cases the reduction of activation barrier reflected in enhanced Rh* binding is associated with overall loss of arrestin-1 stability (11,16,20,21,24). Thus, reduced Rh* binding might reflect a higher activation barrier, which would be expected to increase thermal stability of the basal conformation. To test whether this is the case, we compared the thermal stability of WT arrestin-1, its cysteine-less ASA mutant, pre-activated R175E and D296R mutants, as well as combinations of these activating mutations with the substitution of individual cysteines and triple ASA mutation. We found that cysteine-less arrestin-1-ASA is as stable as WT arrestin-1, fully retaining the ability to bind P-Rh* after 1 h incubation at 39^{o}C (Fig. 5). As we found earlier in less comprehensive experiments (16), polar core mutations R175E and D296R significantly reduced thermal stability of arrestin-1 (Fig. 5). Contrary to our expectations, the addition of triple substitution ASA further reduced thermal stability of both R175E and D296R mutants (Fig. 5A,B). Proteins where R175E or D296R mutations were combined with the substitutions of individual cysteines, demonstrated intermediate stability between these mutants on WT and cysteine-less background (Fig. 5A,B). Thus, it appears that each cysteine substitution reduces stability in the context of pre-activated polar core mutants, with Cys143Ala being the least detrimental. The effect of triple substitution ASA on stability (Fig. 5), similar to its effect on binding (Fig. 4), appears to be the result of additive effects of individual substitutions.

Arrestin-1 activation energy (the energy barrier for P-Rh* binding) is unusually high (22). Both types of activating mutations, R175E and D296R in the polar core, as well as 3A and truncation of the C-tail, reduce the energy barrier of arrestin-1 activation, which is reflected in elevated Rh* binding at 37^{o}C and much higher than WT P-Rh* binding at low temperatures, including 0^{o}C (21). Thus, one possible mechanism of disparate effects of cysteine substitutions on the background of activating polar core and C-terminal mutations could be their differential action on the energy barrier. If cysteine substitutions increase the activation energy of polar core mutants more than that of the C-terminal mutants, they would reduce Rh* binding of R175E and D296R to a much greater extent, than that of 3A or Tr mutants, as observed (Figs. 2,3). To test this idea, we compared P-Rh* binding of R175E and Tr mutants on the background of WT and two cysteine-less variants, ASA and VSV at 37^{o}C and 0^{o}C (Fig. 6). We found that even at physiological temperature (37^{o}C) ASA and VSV reduced P-Rh* binding of R175E and Tr mutants. VSV substitutions suppressed P-Rh* binding to a greater extent than ASA in all cases (Fig. 6). Importantly, the binding at 0^{o}C revealed striking difference between the

effects of cysteine substitutions on different pre-activated mutants. While ASA-Tr and VSV-Tr combination mutants demonstrated virtually the same binding level at 0°C, P-Rh* binding of ASA-R175E and VSV-R175E demonstrated much greater sensitivity to temperature, being reduced by 50% or more at 0°C relative to 37°C (Fig. 6). Thus, it appears that cysteine substitutions counteract the lowering of the activation energy barrier by polar core mutations to a much greater extent than by the C-terminal mutations.

DISCUSSION

High conservation of the three cysteines in the N-domain of all vertebrate arrestins (1) suggests their functional importance. Our mutagenesis data (Figs. 2-4) indicate that these cysteines likely play a role in receptor binding. However, the replacement of cysteines on the WT background does not significantly change arrestin-1 selectivity for the active phosphorylated rhodopsin (9), whereas the substitutions that are well tolerated in that case produce unexpected effects on the background of enhanced pre-activated arrestins: it completely suppresses the activating effect of polar core mutations, but only slightly reduces Rh* binding of mutants with detached (3A) or deleted (Tr) C-terminus (Figs. 2-4). The effect of triple substitution appears to be the sum of the effects of single substitutions (Fig. 4), each of which also greatly reduces the stability of polar core mutants (Fig. 5). Thus, the key question is why the effects of cysteine replacements on polar core and C-terminus mutations, which yield very similar phosphorylation-independent phenotypes (14,16,17,19), are dramatically different. Careful inspection of the position and interactions of these cysteines in the basal arestin-1 (here the highest resolution structure is available for the bovine protein, PDB 1CF1 (4)) and in the rhodopsin-bound arrestin-1 (the only available structure includes mouse arrestin-1, PDB 4ZWJ (25)) reveals that all three conserved cysteines are at or near the inter-domain interface, and all shift upon receptor binding relative to their basal positions (Fig. 7). One of the two key interactions that hold the arrestin molecule in the basal state, the polar core (Fig. 1B) (4), is localized on the inter-domain interface (Figs. 1,7). The global conformational change triggered by the receptor binding (11,22) includes the rotation of the two arrestin domains relative to each other (25-27). Thus, the localization of the three cysteines is consistent with their role in arrestin activation upon receptor binding. As polar core mutations directly destabilize the inter-domain interface (28), the mutations that counteract this destabilization would be expected to suppress their effect, as appears to be the case of cysteine substitutions (Figs. 2-4, 6).

However, the binding of the C-terminus mutants to Rh* also likely involves arrestin transition into the active conformation, which includes domain rotation. Thus, a significantly more modest effect of cysteine substitutions on Rh* binding of 3A and Tr mutants calls for an explanation. These mutations mimic the other conformational rearrangement associated with receptor binding – the release of the arrestin C-terminus (9,29,30), which is in the basal state anchored to the N-domain via the three-element interaction of hydrophobic residues in the C-terminus, β-strand I, and the α-helix (4) (Fig. 1C). This interaction is also destabilized by the receptor-attached phosphates (15) that bind via numerous positive charges in the arrestin N-domain (23,31). The original model of multi-site arrestin-1 binding to P-Rh* suggested that it can first engage either phosphorylated receptor elements or the parts of the receptor that change conformation upon activation, and then the other element (32), responding to the simultaneous activation and phosphorylation of the target receptor like a coincidence detector (33). However, recent data suggest that arrestins can engage the receptor partially, particularly by binding only to the phosphorylated receptor C-terminus (34), which apparently displaces the negatively charged arrestin C-terminus (9,10), an effect that can be mimicked by polyanionic heparin (29,35). A complex,

where arrestin is interacting only with the receptor C-terminus, while the G protein engages the cavity between the trans-membrane helices that opens upon receptor activation (36), as in the receptor-G protein complex (37,38), was recently visualized (34). The binding of the phosphorylated receptor C-terminus displaces the arrestin C-terminus from its basal location in the cavity of the N-domain (9,10,25,27,30), and both truncation and 3A mutation mimic this C-terminus displacement. Our data show that cysteine substitutions do not dramatically affect Rh* binding of mutants pre-activated by truncation and 3A mutation (Figs. 2,3), and do not reduce P-Rh* binding of truncated mutant at very low temperature of 0^{o}C (Fig. 6). Cysteine substitutions also have minimal effect on the P-Rh* binding of WT and mutant arrestin (Figs. 2-4), when the receptor C-terminus is phosphorylated, and therefore structurally equipped to engage the cavity of the N-domain and displace the arrestin C-terminus. In contrast, cysteine substitutions significantly reduce P-Rh* binding of R175E polar core mutant at 0^{o}C (Fig. 6), suggesting that these mutations increase the activation energy of R175E mutant (although not to the WT level), but not the activation energy of the truncated form of arrestin-1.

Our data are consistent with the following model. Receptor binding involves two structural rearrangements in the arrestin molecule: the release of the C-terminus and the rotation of the two domains relative to each other. Activating mutations differentially affect these two changes: 3A mutation and truncation forcibly detach or eliminate the arrestin C-terminus, respectively, leaving the N-domain binding site fully accessible for phosphorylated, as well as unphosphorylated rhodopsin. After this initial interaction the incoming receptor induces the rotation of the two domains to the position where they fit the active receptor, as revealed in the structure of the complex (25). The replacement of the cysteines, which apparently makes domain rotation less likely, reduces, but does not prevent the binding of these mutants to P-Rh* or Rh*. In contrast, the polar core mutations make the domain rotation easier, but do not induce the release of the C-terminus. Therefore, their effect is counteracted by cysteine substitutions, restoring high selectivity of cysteine-less polar core mutants for P-Rh* essentially to WT levels. Obviously, in case of WT arrestin-1, where activation-associated conformational change has a high energy barrier (22), the displacement of the arrestin C-terminus by the phosphorylated rhodopsin C-terminus is sufficient to allow the rest of the active receptor to "force" the arrestin molecule into active conformation. Thus, despite similar phenotype, i.e., greatly increased Rh* binding, different activating mutations act via distinct molecular mechanisms, and this difference is revealed by cysteine substitutions, which selectively impede domain rotation.

EXPERIMENTAL PROCEDURES

Materials. [g-^{32}P]ATP, [^{14}C]leucine, and [^{3}H]leucine were from Perkin-Elmer (Waltham, MA). All restriction and DNA modifying enzymes were from New England Biolabs (Ipswich, MA). Rabbit reticulocyte lysate was from Ambion (Austin, TX), SP6 RNA polymerase was prepared as described (39).

Mutagenesis and plasmid construction. For *in vitro* transcription bovine arrestin-1 was subcloned into pGEM2 (Promega; Madison, WI) with "idealized" 5-UTR (39) between Nco I and Hind III sites, as described (32,40). All mutations were introduced in transcription construct by PCR, using the strategy previously described (14). All constructs were confirmed by dideoxy sequencing.

In vitro transcription, translation, preparation of phosphorylated and unphosphorylated rhodopsin were performed as described (14).

Direct binding assay was performed, as described (14). Briefly, 1 nM arrestin-1 (50 fmol) was incubated with 0.3 mg of P-Rh* in 50 ml of 50 mM Tris-HCL, pH 7.4, 100 mM potassium acetate, 1 mM EDTA, 1 mM DTT for 5 min at 37^{o}C or at 0^{o}C (in ice-water

bath) under room light. Samples incubated at 37°C were cooled on ice, and bound and free arrestin-1 was separated at 4°C by gel-filtration on 2-ml column of Sepahrose 2B-CL. Arrestin-1 eluting with rhodopsin-containing membranes was quantified by liquid scintillation counting. Non-specific "binding" was determined in samples where rhodopsin was omitted and subtracted.

In vitro arrestin stability assay. Translated radiolabeled arrestin-1 was incubated for indicated time at 39°C and cooled on ice. The binding of arrestin-1 in these samples to P-Rh* was compared to that of control sample kept on ice, as described above; 1 nM arrestin-1 (50 fmol per sample) was used, as in the direct binding assay.

Statistical analysis. The data were statistically analyzed using, where appropriate, one-way ANOVA with protein as a factor, followed by post-hoc Bonferroni-Dunn test with correction for multiple comparisons, Student's t-test (mouse arrestin-1 data), comparing each cys-less mutant to its WT counterpart, or repeated measure ANOVA (stability data). In all cases P-Rh* and Rh* binding was analyzed separately and $p<0.05$ was considered significant (actual significance levels are indicated in figures).

Acknowledgements
This work was supported by NIH R01 grants to VVG (EY011500, GM077561, GM109955; the latter two were replaced with R35 GM122491) and HEX (DK071662).

Conflict of Interest
The authors declare no conflict of interest.

Author Contributions
SAV, RJL, AF, and QX generated mutants and performed binding experiments; SAV, VVG, XEZ, and HEX designed the study and analyzed the data. VVG, XEZ, HEX, and SAV wrote the manuscript with input from the other authors.

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FOOTNOTES

[1] We use the systematic names of the arrestin proteins: arrestin-1 (historic names S-antigen, 48 kDa protein, visual or rod arrestin), arrestin-2 (β-arrestin or β-arrestin1), arrestin-3 (β-arrestin2 or hTHY-ARRX), and arrestin-4 (cone or X-arrestin; for unclear reasons its gene is called "arrestin 3" in the HUGO database).

FIGURES AND LEGENDS

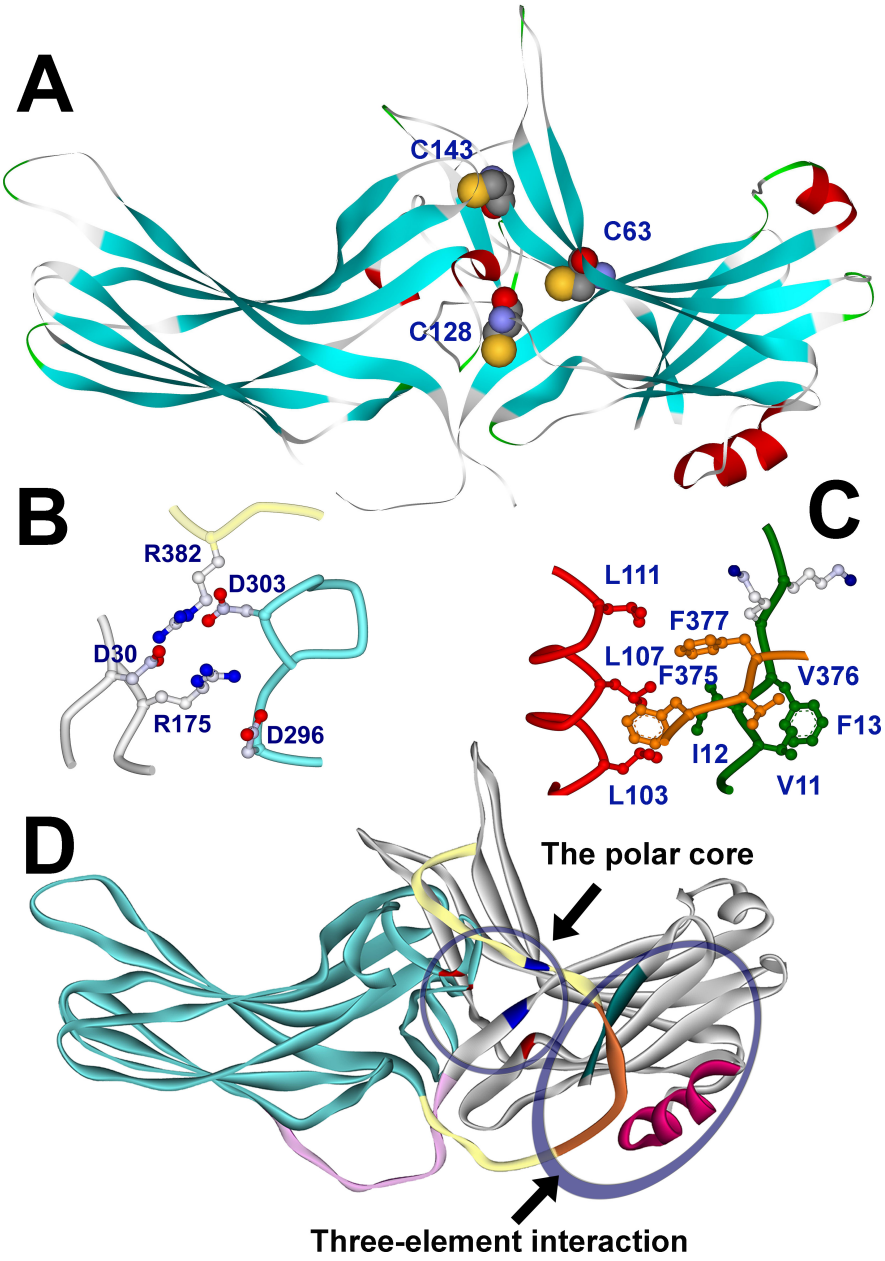

Fig. 1. The three cysteines in arrestin-1 are located near the polar core. A. Crystal structure of bovine arrestin-1 (PDB 1CF1 (4)) with β-strands shown in blue, α-helices in red, β-turns in green, and elements with no secondary structure in gray. Cys63, Cys128, and Cys 143 are shown as CPK models colored by atom (gray, carbon; blue, nitrogen; red, oxygen; yellow, sulfur). **B.** The polar core, consisting of Arg175 forming a salt bridge with Asp296, Arg382 of the C-tail, Asp 30 of the N-domain, and Asp303, which is located on the lariat loop along with Asp296. **C.** Three-element interaction mediated by hydrophobic residues Val11, Ile12, and Phe13 on the β-strand I, Leu103, Leu107, and Leu111 on the α-helix I, and Phe375, Val376, and Phe377 of the C-tail. **D.** The location of the polar core between the two arrestin domains and the three-element interaction in the arrestin-1 molecule is shown by circles. In **B, C,** and **D** the elements of the arrestin molecule are colored, as follows: gray, the N-domain; light blue, the C-domain; red, α-helix I; green, β-strand I.

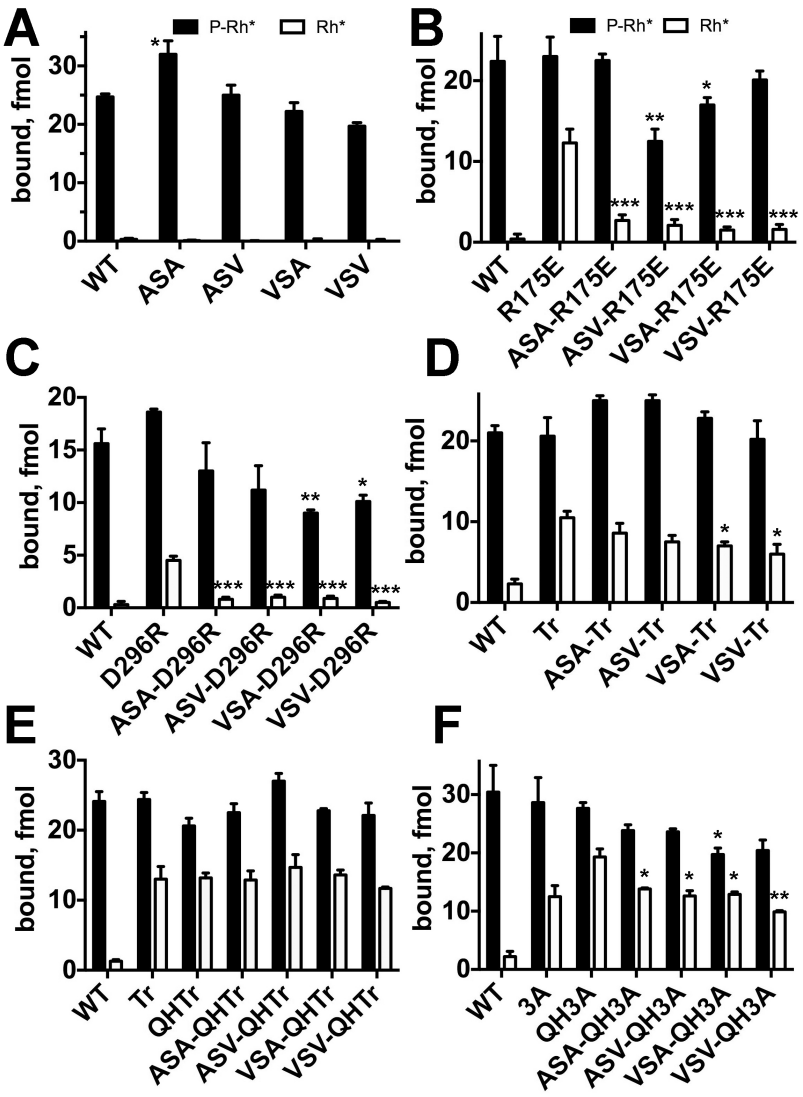

Fig. 2. Cysteine substitution obliterates increased Rh* binding of pre-activated polar core mutants. A. The binding of WT bovine arrestin-1 and indicated cysteine-less mutants. Here and in other panels ASA stands for Cys63Ala, Cys128Ser, Cys 143Ala; ASV – Cys63Ala, Cys128Ser, Cys143Val; VSA – Cys63Val, Cys128Ser, Cys143Ala; VSV – Cys63Val, Cys128Ser, Cys143Val. **B.** The binding of WT arrestin-1, pre-activated R175E mutant, and R175E mutation on indicated cysteine-less backgrounds. **C.** The binding of WT arrestin-1, pre-activated D296R mutant, and D296R mutation on indicated cysteine-less background. **D.** The binding of WT arrestin-1, pre-activated truncated (Tr) arrestin-1-(1-378), and truncated mutants on indicated cysteine-less backgrounds. **E.** The binding of WT arrestin-1, pre-activated truncated (Tr) arrestin-1-(1-378), truncated arrestin-1 carrying additional K257Q and E346H mutations (QHTr), and QHTr mutants on indicated cysteine-less backgrounds. **F.** The binding of WT arrestin-1, pre-activated arrestin-1 with triple substitution F375A, V376A, F377A (3A), arrestin-1-3A carrying additional K257Q and E346H mutations (QH3A), and QH3A mutants on indicated cysteine-less backgrounds. In all panels the bars are colored, as follows: black, P-Rh*;

white, Rh*. The means ± SD of two experiments each performed in duplicate are shown. The data were analyzed by one-way ANOVA (separately for P-Rh* and Rh* binding) with protein as a main factor, followed by Bonferroni-Dunn test with corrections for multiple comparisons. Statistical significance of the differences (as compared to respective base mutant with WT cysteines) is shown, as follows: *, p<0.05; **, p<0.01; ***, p<0.001.

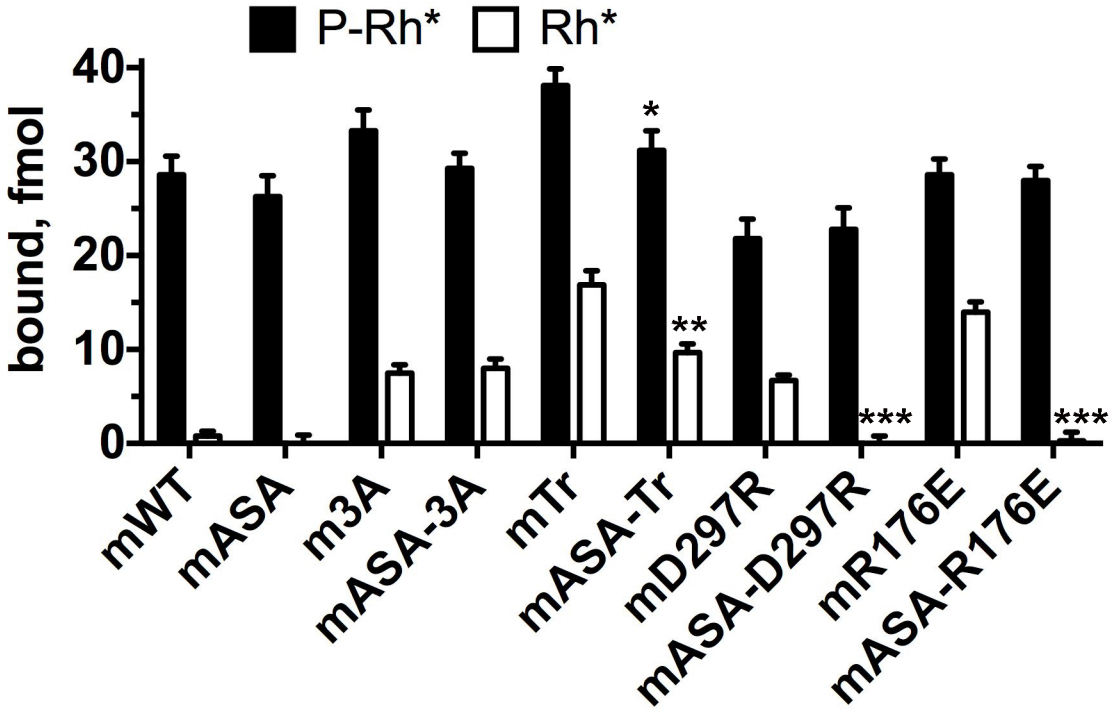

Fig. 3. Cysteine substitutions affect mouse arrestin-1 exactly like the bovine homologue. The binding of WT mouse arrestin-1 (mWT), mASA cysteine-less mutant (Cys64Ala, Cys129Ser, Cys 144Ala), as well as mutants with activating mutations, 3A (Leu374Ala, Val375Ala, Phe376Ala), Tr (truncated 1-377), D297R, and R176E, on mWT and mASA background. The bars are colored, as follows: black, P-Rh*; white, Rh*. The means ± SD of two experiments each performed in duplicate are shown. The data (separately for P-Rh* and Rh* binding) were analyzed by unpaired Student's t-test, where each cys-less mutant was compared to corresponding mutant with WT cysteines. Statistical significance of the differences is shown, as follows: *, p<0.05; **, p<0.01; ***, p<0.001.

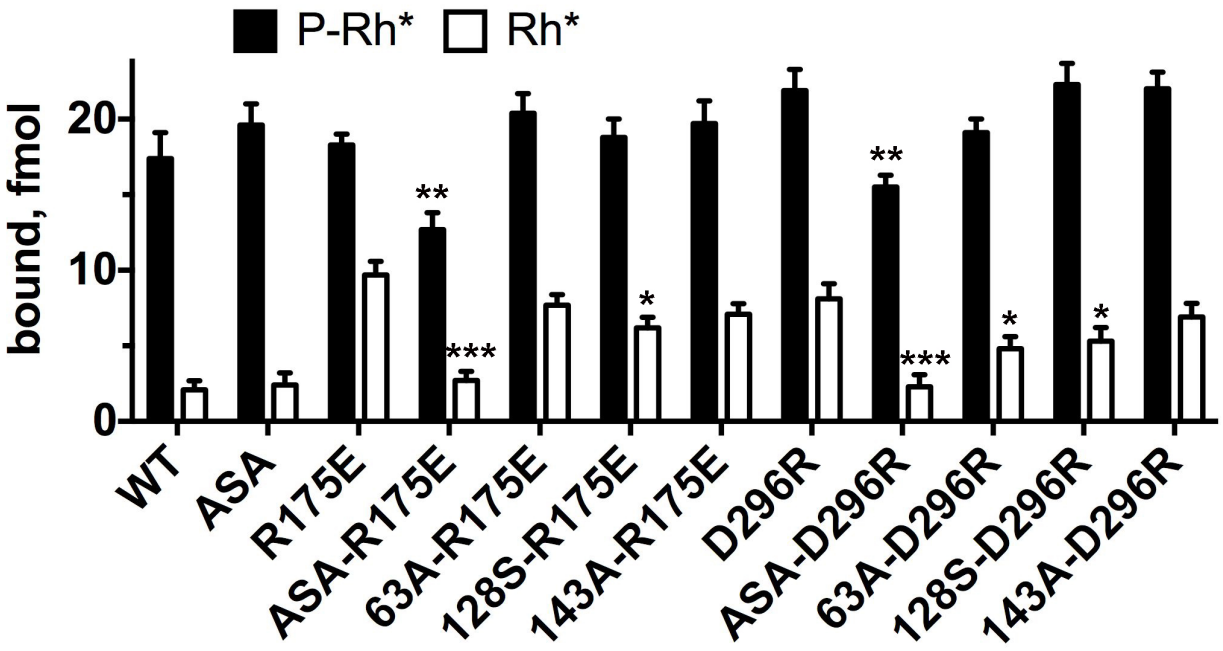

Fig. 4. All three cysteines contribute to the reduction of Rh* binding of pre-activated polar core mutants. The binding of WT bovine arrestin-1 and indicated mutants. ASA stands for Cys63Ala, Cys128Ser, Cys143Ala; individual mutations are indicated. The bars are colored, as in Fig.2. The means ± SD of two experiments each performed in duplicate are shown. The data (separately for P-Rh* and Rh* binding) were analyzed by one-way ANOVA with protein as main factor, followed by Bonferroni-Dunn test with correction for multiple comparisons. The R175E and D296R mutants with cysteine substitutions were compared to the corresponding mutants with native cysteines. Statistical significance of the differences is shown, as follows: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

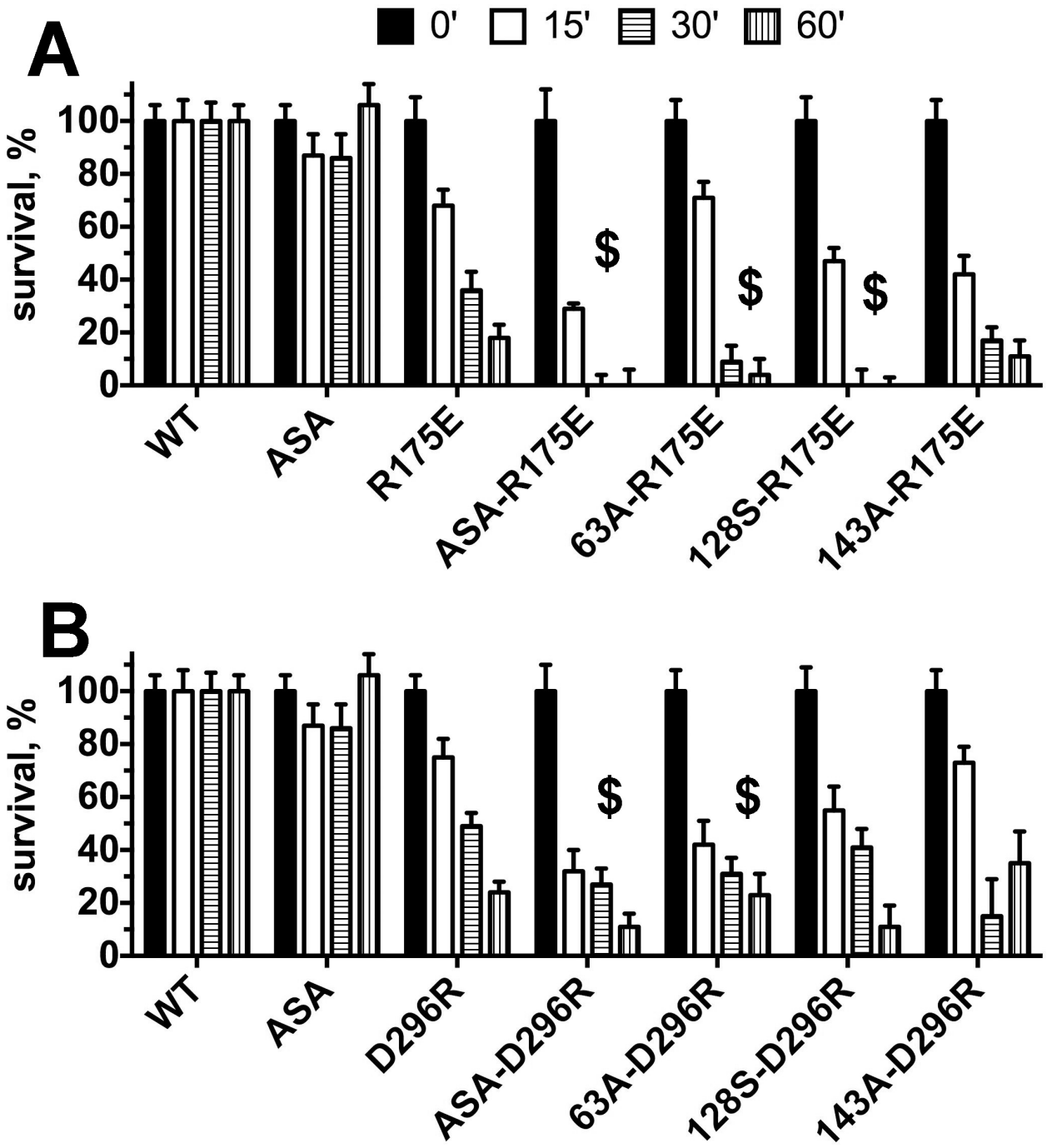

Fig. 5. All three cysteines contribute to reduced stability of pre-activated polar core mutants. A. Comparison of WT arrestin-1, cys-less ASA mutant, and R175E mutant. **B.** Comparison of WT arrestin-1, cys-less ASA mutant, and D296R mutant. Both panels show the binding of WT bovine arrestin-1 and indicated mutants to P-Rh* after incubation for 15, 30, and 60 min at 39^{o}C; controls (0') were kept on ice. The means ± SD of two independent experiments performed in duplicate are shown in both panels. The data were analyzed by repeated measure ANOVA with protein as main factor and time as repeated measure factor. Statistical significance of the differences in the survival time course, as compared to base R175E (panel **A**) or D296R (panel **B**) mutant is indicated by the $ sign.

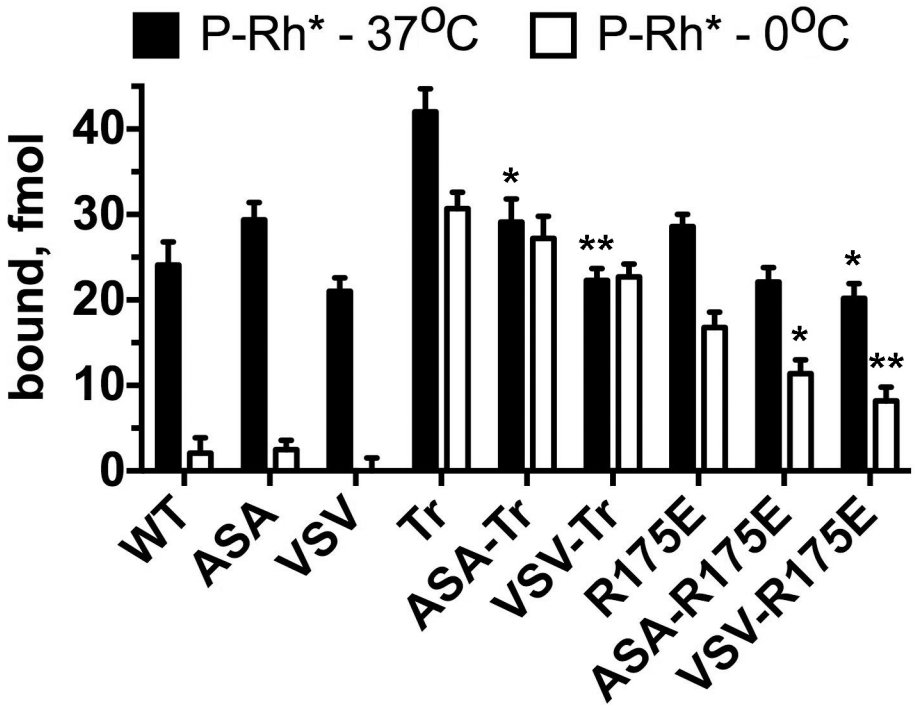

Fig. 6. Cysteine substitutions increase the activation energy of the polar core mutant to a greater extent than the C-terminal mutant. The binding of WT bovine arrestin-1 and indicated mutants to P-Rh* at physiological (37°C, black bars) and very low (0°C, white bars) temperature. The means ± SD of two independent experiments performed in duplicate are shown. The data for each group (WT, Tr, and R175E) and each temperature were analyzed separately by ANOVA with protein as main factor, as compared to the corresponding protein with WT cysteines at the same temperature. Statistical significance of the differences is shown, as follows: *, $p<0.05$; **, $p<0.01$.

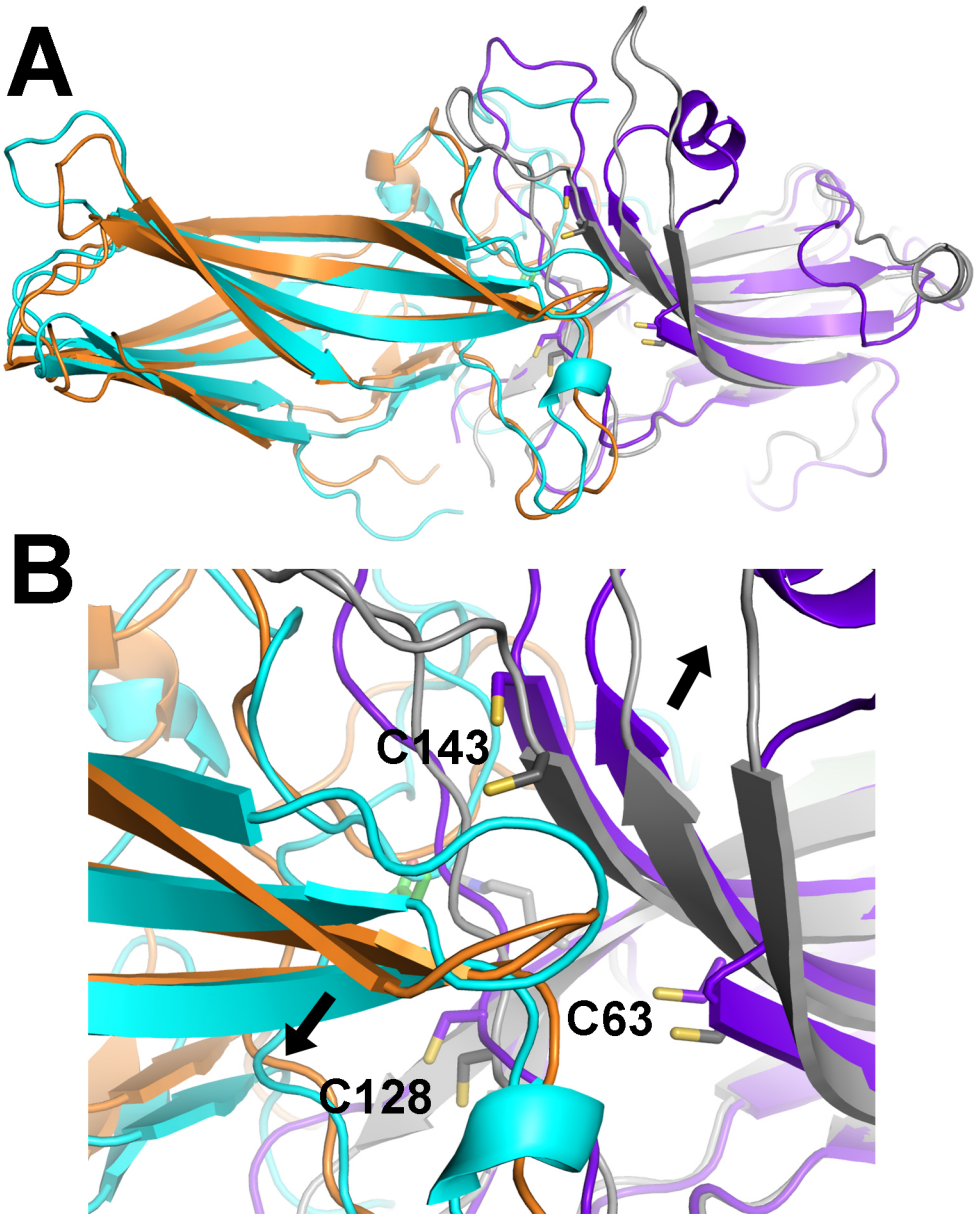

Fig. 7. Structural basis of the function of native arrestin-1 cysteines. A. Superposition of the structures of basal bovine arrestin-1 (PDB 1CF1, molecule C (4)) and rhodopsin-bound mouse arrestin-1 (PDB 4ZWJ (25)), with the three cysteines shown as stick models. Here and in panel **B** the molecules are colored, as follows: basal state N-domain, gray; C-domain, cyan; rhodopsin-bound state N-domain, purple; C-domain, brown. **B.** Inter-domain interface in arrestin-1 (blow-up of the middle part of of panel **A**). Bovine cysteine residue numbers are shown (mouse arrestin-1 numbers are n+1). Arrows indicate the shift (reflecting binding-induced rotation of the N- and C-domains relative to each other) that opens up the cleft between the loops in the two domains for the interaction with the intracellular part of the receptor.

Functional role of the three conserved cysteines in the N-domain of visual arrestin-1

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J. Biol. Chem. published online May 23, 2017
