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Recent advances suggest increased influence of selective pressure in allostery

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Abstract

Allosteric regulation of protein functions is ubiquitous in organismal biology, but the principles governing its evolution are not well understood. Here we discuss recent studies supporting the large-scale existence of latent allostery in ancestor proteins of superfamilies. As suggested, the evolution of allostery could be driven by the need for specificity in paralogs of slow evolving protein complexes with conserved active sites. The same slow evolution is displayed by purifying selection exhibited in allosteric proteins with somatic mutations involved in cancer, where disease-associated mutations are enriched in both orthosteric and allosteric sites. Consequently, disease-associated variants can be used to identify druggable allosteric sites that are specific for paralogs in protein superfamilies with otherwise similar functions.

Introduction

Allostery is a protein regulatory mechanism where perturbations to a site distal from the active site induce conformational and/or functional change. Allostery is induced when information is transferred between a distal allosteric site and the orthosteric site [1••,2]. The perturbations at the allosteric site can be due to multiple factors [3], commonly in the form of either covalent or non-covalent binding of a molecule, or due to environmental factors such as light absorption or high-pressure [4–7]. Allosteric regulation has been extensively studied due to its role in a wide range of biological processes [8–17]. The concept of allostery was discovered five decades ago, after which several mechanisms have been proposed relating allosteric binding to conformational change of protein structure or protein dynamics [1••]. Recent ensemble-based techniques used to study allosteric mechanisms are well outlined in a recent review [4].

While these theories address the physical mechanisms of allostery, their relationship to molecular evolution remains less clear. Some suggest allosteric modulation of extant

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Conflict of interest statement

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proteins arose in ancestor proteins with a latent capacity for allosteric modulation [18,19]. Alternately, allosteric proteins could have arisen from non-allosteric predecessors that bind the same orthosteric ligand [20,21]. Importantly, the existence of allostery can impose epistatic (non-additive) effects on mutations, which were evaluated for potential allosteric networks in PDZ domains using statistical covariation of residues [22]. Such work suggests that conditional neutrality mediates evolutionary adaptation of PDZ domains and that this allosteric network has its origins in the capacity of proteins to adapt to binding alternate ligand classes [23]. While the presence of allosteric networks in PDZ domains has since been challenged (reviewed in Ref. [24]), the hypothesis that local changes in phase and structure can lead to long range negative epistasis in a model system of ligand binding supports this idea of conditional neutrality [25].

Allosteric sites are diverse, in contrast to active sites that are conserved [26]. Accordingly, allosteric modulators can achieve a high level of selectivity for individual proteins in large superfamilies [27]. Furthermore, allosteric modulators do not compete with substrate/ligands that bind to the active site, but instead, work together to induce allosteric activity [26]. These properties offer the potential for allosteric sites to serve as drug targets, allowing development of safer medicines [26]. Moreover, certain mutations at allosteric sites do not hinder catalytic activity of the protein [28], although they might profoundly influence evolution. As such, allosteric mutations have shown to be deleterious and disease-causing in many cases [29–31], supporting their roles in fitness. This review summarizes current views of allosteric evolution driven by the need for specificity in extant paralogs and the existence of latent allostery in ancestors of large superfamilies. Lastly, this work highlights how studying allosteric mutations in the context of evolution has proven useful for identifying both disease-associated genes and drug targets.

Large-scale existence of latent allostery in ancestors of protein superfamilies

It is unknown whether the acquisition of allostery results from natural selection of allosteric regulation over non-regulated paralogs, or whether it stems from existing co-evolving residues. Elastic network models (ENMs) can access much larger conformational ensembles than more intensive all-atom molecular dynamics methods [32]. Abrusan *et al.* investigate the hypothesis that allostery is an evolutionary mechanism to regulate slow-evolving homomeric protein complex specificity, where ligand binding sites are made up of residues from multiple chains [1••]. Expanding on previous analysis of homologous protein complexes with binding sites composed of residues from either multiple chains (MBS) or single chains (SBS) [33•], they analyze differences in residue correlated motion between MBS and SBS complexes from the Allosteric Database [34]. Using ENMs they find MBS complexes more likely to have residue communities (i.e. networks of residues with correlated motions) spanning multiple chains, whereas those in SBS complexes tend to be within single chains. *In silico* alanine mutation scanning of interface residues by FoldX [35] found that interface mutations in MSB communities weaken the binding energy significantly more than mutations from SBS communities. Taken together, these results suggest that the type of ligand-binding site is intimately linked to the strength of allosteric communication.

The authors suggest that for paralogous proteins, binding site similarity drives allosteric evolution.

As the ensemble model of allostery has gradually come to replace canonical symmetry model [36], more focus has turned to studying relationships between protein dynamics and allosteric signal transmission. The 'intrinsic dynamics' of a protein, or its native conformational ensemble under physiological conditions, can be used to probe residue motion types accompanying allostery. Zhang *et al.* modeled protein intrinsic dynamics on a large scale using SignDy [37••], which uses an ENM simulation pipeline designed to compare correlated motions between equivalent positions of homologs (from structural superposition and sequence alignment). The intrinsic dynamics of three structurally distinct superfamilies (LeuT, PBP-1, and Tim Barrel) shows that each retains conserved 'global' modes. Interestingly, these global modes have been shown in some cases to correspond to the structural rearrangements induced by ligand binding. Higher frequency modes do not show the same level of similarity between families but are distinctly conserved within sequence families from these CATH superfamily groups. This differentiation between conservation of higher frequency modes may provide some mechanism by which homologous proteins that share global modes may become subject to differing allosteric mechanisms.

Allosteric mutations contribute to evolutionary fitness and disease

Mutations contribute to genetic variation within a population, providing material for evolution. Advantageous mutations undergo positive selection and get fixed within a population and increase fitness [38,39]. Deleterious mutations decrease fitness and can lead to disease [40,41]. Furthermore, distal mutations of >15Å from the active site of a protein have been associated with networks of coupled residues that alter function [42,43•,44–47]. Because of this interconnection between mutations, disease, and allostery, current research has focused on studying disease-associated mutations in allosteric dysregulation. Shen *et al.* [48••] systematically investigated cancer-based regulation perturbed by somatic mutations at allosteric sites. Their dataset included 574 manually curated proteins from the Allosteric Database [49], out of which 74 proteins had experimentally validated allosteric sites and PDB structures. The evolutionary rate [50] and dN/dS ratio of human-mouse orthologous gene products were generally lower for allosteric proteins, suggesting a purifying selection.

To study the relationship between allosteric mutations and disease, the authors designed a pipeline to identify variants in allosteric sites among over 47 000 somatic missense mutations from 6958 pairwise tumor-normal matched pairs across 33 cancer types. They mapped 1990 predicted deleterious and 2461 known disease-causing variants on 74 allosteric structures and showed their enrichment at both allosteric and orthosteric sites, highlighting the potential role of allostery in disease pathology. The authors developed a statistical approach to predict cancer-associated allosteric genes, AlloDriver, using pan-cancer data. Known BRAF, HRAS, and AKT1, and four others, and unknown GCK and SERPINC1 cancer-associated proteins exhibited-enriched somatic missense mutations in their allosteric sites. Extending analysis to individual cancer types, they identify 20 known and 15 novel cancer proteins, suggesting allosteric dysregulation as an important factor

influencing tumorigenesis. These results not only provide allosteric examples for further experimental investigation of fitness but can also help with developing novel targeted cancer therapies.

Evolutionary selective pressure on allosteric sites is used for drug development

Selective pressures are factors that contribute to variant selection and adaptation within a population. Over time, evolution occurs as a result of these selective pressures. In relation to protein sequence, selective pressures provide the basis for conservation among homologs [51]. Allosteric sites tend to be less conserved than the active sites they regulate [26,52,53], which has increased the popularity of their use as drug targets [26,53,54•]. Selective pressure is also expressed through rare variants in the population, and studying these can identify uncharacterized regulatory areas that can be exploited for drug development [54•].

Agne *et al.* [55••] used rare variants to identify a putative allosteric site in Caspase-6 (Casp6) and developed inhibitors to target the site. Casp6 functions in cell-apoptosis and is activated by a unique self-cleavage of its L2 loop that stabilizes the substrate-binding pockets [56]. Casp6 is allosterically inhibited by phosphorylation at S257 that prevents the L2 loop from attaining an active conformation for self-cleavage [57,58]. Similarly, zinc binding at another allosteric site (via K36, E244 and H287) locks Casp6 in a non-canonical inactive conformation [59]. The authors investigated four rare natural variants in Casp6 that lead to missense mutations. Three of these (A34, E35 and A109) line up a well-defined pocket 30 Å from the catalytic site [55••,56]. In an active VEID-bound CASP6 dimer, the binding pocket is near a zinc binding site, a phosphorylation site from the adjacent subunit, and the L2 cleavage loops of both subunits (Figure 1a). Being unconserved and identified by a prediction tool as potentially druggable, the authors pursued *in silico* screening for allosteric inhibitors targeting the site (Figure 1b), which likely transmits a signal to the active site of the adjacent subunit through conformational change of L2, and disorder-to-order transition of the active site ‘loop bundle’ (Figure 1a and c).

In silico screening followed by fluorescence-based Casp6 enzyme assays found two inhibitory compounds (S10G and C13) with low micromolar K_i (inhibitor constant). Kinetic analysis showed that they non-competitively inhibited Casp6 activity, suggesting binding to an allosteric site. Although the predicted molecular binding model (C13) and binding evaluation using Hydrogen-Deuterium Exchange Mass Spectrometry (S10G) hinted at the allosteric pocket, future experimental structures are required for conformation. Despite this drawback, reports of known of allosteric regulation by residues around this binding pocket and the structural involvement of L2 loops suggests that this site could be allosteric, and the work provides a successful example of using rare variants to discover drug target sites.

Conclusion

Allosteric regulation could be an inherent property of biological polymer molecules, or could have evolved from non-allosteric proteins (Introduction and Figure 2). The degree to which ‘latent’ or potential allosteric sites exist in ancestor proteins is still a matter of

discussion. Recently developed methods to study protein dynamics are rein-forcing the notion of latent allostery in protein superfamilies [1••,32,37••]. Many known allosteric mechanisms involve protein oligomerization [20,60,61]. Molecular dynamics studies of protein complexes described by Abrusan *et al.* suggest that active site similarity in paralogous proteins could be the driving force behind the evolution of allostery [1••], which would allow selective control of similar active sites through divergent allosteric sites (Figure 2). Their observation of residue communities spanning across dimeric interfaces and their key role in interaction suggest that monomer to homodimer equilibrium represents the latent allosteric property of the ancestor. Similarly, the discovery by Zhang *et al.* of conserved global modes in various protein superfamilies suggests that their common ancestors possessed similar intrinsic dynamics [37••]. In these cases, conserved global modes likely transmit allosteric communication between active sites and allosteric sites displaying less conserved high frequency modes. The key roles of allosteric residues are also revealed in the investigation of somatic mutations by Shen *et al.* [48••]. They describe purifying selection for allosteric proteins and find enriched disease mutations at allosteric sites, suggesting that signal transmission between allosteric and orthosteric sites in extant proteins contributes to fitness. Such key roles of allosteric sites in disease have led to their use in developing novel drug therapies [48••,62], but also for discovering novel allosteric sites that can be exploited for drug development in the work by Agne *et al.* [55••]. Their use of rare variants to identify an allosteric pocket in Casp6 highlights the advantage of using allosteric site specificity in drug development.

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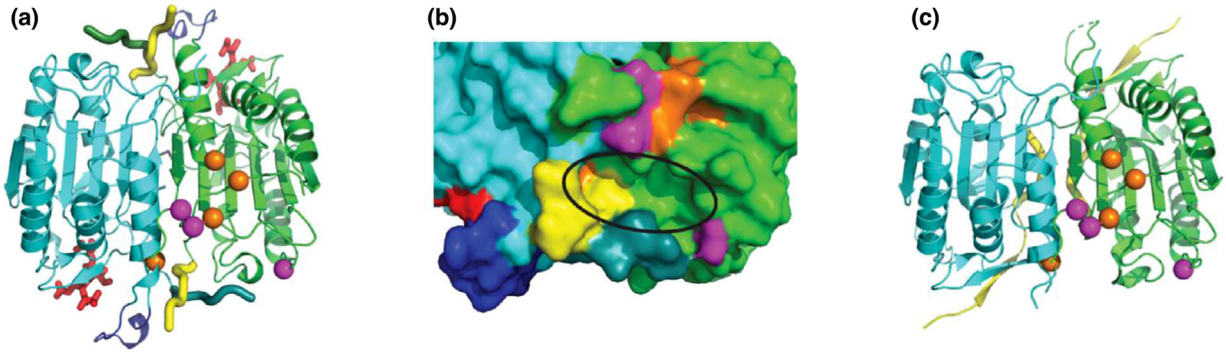

Figure 1.

Casp6 allostery and activation.

(a) Casp6 dimer (PDB 3OD5; green and cyan chains) in active conformation with VEID (red sticks) bound in the active site. Loop2′ C-terminus (yellow tube) interacts with Loop2 N-terminus (dark cyan/green tube) and stabilizes the active site 'loop bundle' (blue cartoon). Allosteric phosphorylation (S257) and Zinc binding residues (K36, E244, and H287; orange spheres) are near two of the rare variant residues (A34E and E35K; magenta spheres) depicted in the green subunit. **(b)** Zoom of allosteric pocket (black ellipse) surface in active conformation formed by L2 loops, parts of allosteric sites, and variant residue (A109T; magenta). **(c)** Conformation change of Loop2 (yellow) and disordered active site 'loop bundle' in inactive zymogen (PDB 3NR2).

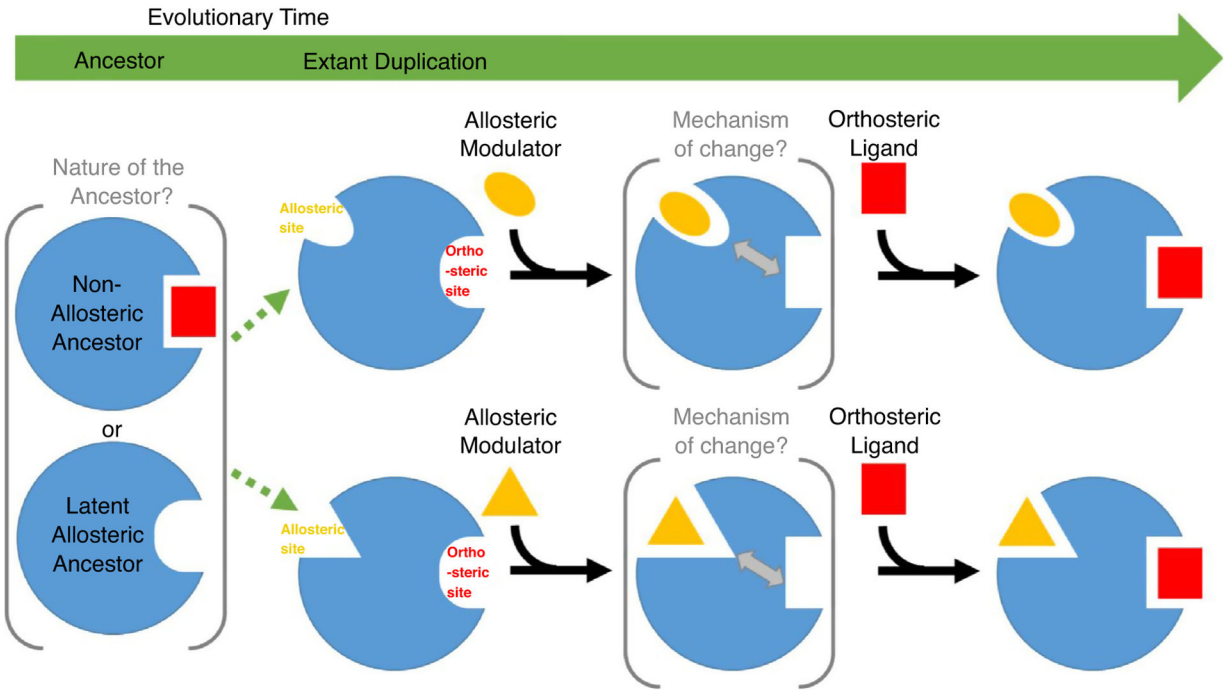

Figure 2.

Evolution of allostery.

Ancestral proteins that are either non-allosteric with similar active site ligands or have latent allosteric properties duplicate and diverge (green dotted arrows) to acquire divergent allosteric sites. Allosteric modulators induce change at the conserved active site through several possible mechanisms guiding communication between sites.