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Previews

# Conservation and Variation of Structural Flexibility in Protein Families

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**In this issue, Raimondi et al. (2010) obtained interesting insights concerning structural flexibilities in the Ras superfamily that are essential to both function retention and specialization by analyzing the deformation patterns from physical models of protein structure and from crystal structures of homologous proteins.**

Enabled by impressive advances in processing speed and low-latency intercomputer communications, computational biophysics has trended toward studies at longer and longer timescales. Although still relatively rare, the massive computing power now available to investigators has allowed for the brute force calculation of the $10^{12}$-molecular dynamics steps necessary to reach the millisecond-time regime (Klepeis et al., 2009). As this ability becomes more widespread, it promises to probe deeper into the nature and functional consequences of biomolecular motions, especially in conformational changes that have large kinetic barriers and occur relatively slowly. While long timescale investigations of large systems are clearly an exciting and powerful prospect, the field of biomolecular simulation is not limited to leveraging the horsepower of immense computational resources. Clever analyses using established techniques with well-chosen approximations have continually proven to be of great value in investigating interesting biophysical and biochemical problems. The article by Raimondi, Orozco, and Fanelli (2010) published in this issue of *Structure* is an excellent example of this mode of investigation. The authors use the familiar techniques of principal component analysis (PCA) and normal mode analysis (NMA) (Cui and Bahar, 2006) to identify the important structural flexibilities that enable proteins in the Ras superfamily to switch between their active and inactive states. In addition, this analysis leads to an interesting hypothesis regarding the evolutionary adaptation of structural deformations by the individual members of the superfamily to fulfill their specialized function.

The Ras superfamily comprises many guanine nucleotide-binding proteins that are essential to intracellular signal transduction. These proteins adopt an active conformation when bound to guanine triphosphate (GTP); the subsequent hydrolysis of GTP to guanine diphosphate and the concomitant conformational changes switches the protein to its inactive form and reduces its downstream signaling effects. The structural transition between the different conformational states is striking in scale and crucial to the function of the Ras superfamily, referred to as "molecular switches" in the literature. Activating mutations in Ras are found in 20%–25% of human tumors, and up to 90% in specific tumor types (Downward, 2003), which highlights the importance of understanding the mechanism of structural transitions in Ras, such as the intrinsic structural flexibilities that facilitate functional transitions.

Using principal component analysis on a set of Ras superfamily structures from five separate subfamilies and various functional states, the authors were able to identify deformation patterns ("motions") that are conserved across different families. Such transfamily flexibility is made most clear by further investigations using NMA with a physical model (elastic network) for protein structural deformation; the comparison of physical and evolutionary deformability patterns (Figure 1) makes this study an interesting extension to a previous investigation that compared elastic network NMA results to PCA of a collection of crystal structures for a single protein (HIV protease) (Yang et al., 2008). An important observation from this work is that the inherent flexibility coded in the protein structure that is revealed by the NMA overlaps well with those deformations sampled in the database-driven PCA that examined changes among functional states. Moreover, the doubly-detected deformability involves residues in lobe 1 of the Ras-like domain; they are important to the nucleotide-binding region and the interswitch region implicated in the active/inactive nucleotide switch functionality. The phenomenon that functional motions are coded into the structure and represent the most energetically accessible deformations has been observed frequently in biomolecules (Tama and Brooks, 2006), and seems to be a logical stratagem for accomplishing conformational change. One dramatic example of this has been illustrated in the DNA and RNA polymerases (Van Wynsberghe et al., 2004; Delarue and Sanejouand, 2002), in which the crab claw nature and the relative mobility of both pincers are easily deformable and likely to play important functional roles. Additional evidence that the Ras superfamily members share a set of switching dynamics is given by the authors' identification of hinge points throughout all subfamilies. Rather than being present at the nucleotide binding loops, the hinges are found at the lobe 1/lobe 2 domain interface and are likely to be involved in relative motion of the two domains.

Another interesting observation that applies to all family members is that the $S^{GEF}$ state has normal modes that lead to the $S^{GTP}$ (active) or $S^{GDP}$ (inactive) states, but that neither of these latter two states' normal modes indicate easy

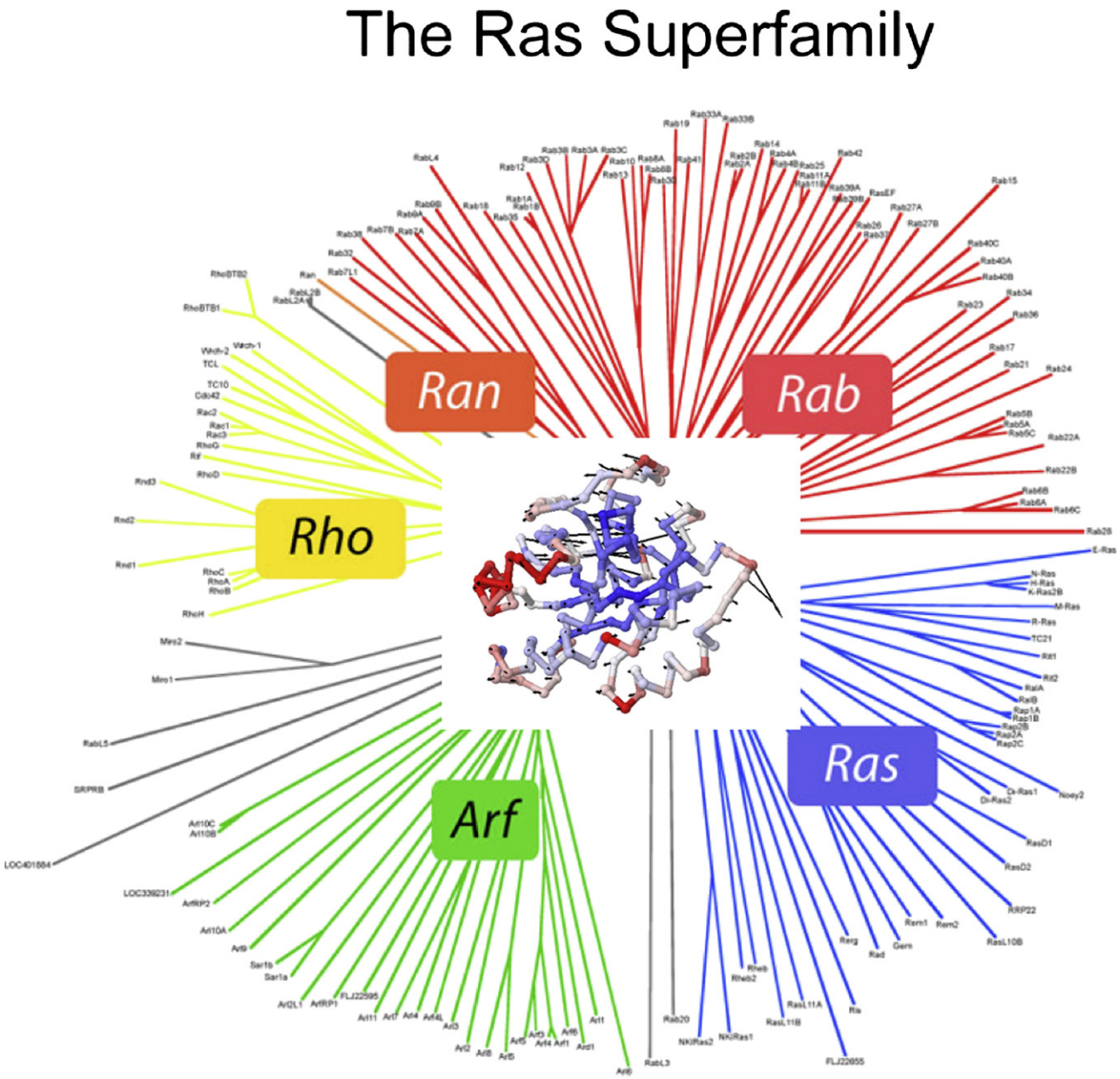


**Figure 1. Comparison of Protein Flexibility across the Ras Family**
Using structural information for homologous proteins and elastic network models, Raimondi et al. (2010) explore the structural flexibilities of proteins in the Ras superfamily that are essential to function retention and specialization. The illustration of normal modes based an elastic network model is generated using the ANM webserver (http://ignmtest.ccbb.pitt.edu/cgi-bin/anm/anm1.cgi).

transitions to the other functional states. This suggests that $S^{GEF}$ is the most flexible of the three states, a reasonable conclusion given that $S^{GEF}$ is an intermediate state whose role is the transfer of the nucleotide. In this aspect, the Ras system is similar to the aforementioned polymerase systems, in that the polymerases were seen to be more flexible in their "open" configuration than their "closed" configuration (Van Wynsberghe et al., 2004; Delarue and Sanejouand, 2002).

In addition to the identification of conserved structural flexibility among the Ras superfamily members, the PCA also allowed the authors to distinctly cluster the evolutionarily-sampled deformations and therefore hint at the variation of structural flexibility among different families. Most notably, the PCA on the conserved core of the entire Ras superfamily revealed a distinction between the Ras, Rab, and Rho subfamilies, and the Arf and Gα subfamilies along the first principal component, which involves deformation in both the lobe 1 and lobe 2 regions, the latter being distal to the nucleotide-binding site. Interestingly, the same motion component is observed for the principal components that

characterize the transition between different functional forms of Arf when PCA is applied to the Arf family members. In other words, it appears that the deformations dictated by evolution (between Ras/Rab/Rho and Arf/Gα subfamilies) also serve for specialization in selected family members. Taken as a whole, the PCA and NMA results suggest that the Ras superfamily utilizes a hierarchical organization of its structural flexibilities; the lobe 1 motions associated with its switching function must be retained in order to accomplish the primary G protein function of changing its affinity to effector proteins with different bound nucleotides, but additional motions across both lobes of the protein are family specific and play a role in determining the unique functional characteristics of specific members.

In summary, by taking advantage of structural information across a superfamily and adopting relatively simple physical models of proteins, the authors have been able to gain interesting insights concerning structural flexibilities in the Ras superfamily that are essential to both function retention and specialization. This line of research touches upon the emerging topic of protein dynamics, or "dynamism" (Tokuriki and Tawfik, 2009), and evolvability. Indeed, the idea that functional specialization results from the mixture between the same deformation patterns essential to function retention and those instrumental in selective intermolecular interactions (Raimondi et al., 2010) is sensible and likely applicable to many protein families. Given the intimate linkage between protein structure and functional flexibilities that has emerged in many recent studies, an emerging challenge is to better define the connection between the sequence and the dynamical properties of proteins that dictate their function (Smock and Gierasch, 2009), not only to better understand protein function evolution but to also rationally design proteins with new functions.

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