Evolution of a derived protein–protein interaction between HoxA11 and Foxo1a in mammals caused by changes in intramolecular regulation

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AUTHOR SUMMARY

The evolution of gene regulation is a well-established mechanism for both developmental evolution and the origin of phenotypic novelties. To date, many studies have demonstrated that evolutionary changes in transcription factor binding sites, or cis-regulatory regions, can affect gene expression and drive phenotypic novelty. It has been suggested that changes to transcription factor proteins affect multiple phenotypic traits, and are therefore mostly deleterious (1). However, there are well-documented examples of transcription factor evolution affecting gene expression, indicating that this kind of change is minimally deleterious (2). Although it is increasingly clear that the evolution of interactions among transcription factors directly contributes to gene regulatory change, the mechanisms underlying the evolution of these protein-protein interactions among transcription factors are not well understood. A recent study suggested that the binding surfaces between interacting proteins are frequently nonadaptive, suggesting that small changes to protein structures can be introduced by chance, leading to small and local defects in the protein’s affinity for water (3). In this model, protein-protein interactions arise that stabilize these weakly deleterious mutations (3).

Here, we examine the evolutionary origin of a protein–protein interaction between two transcription factors, Homeobox A11 (HoxA11) and Forkhead box 01A (Foxo1a), which interact physically and cooperatively transactivate target gene expression, to identify when their physical interaction arose (4). Using communoprecipitation (CoIP), we analyzed the interaction capability of extant HoxA11 and Foxo1a proteins from humans, opossums, and chickens as well as “resurrected” proteins from ancestral eutherian, therian, mammalian, and amniote lineages. We found that the physical interaction between HoxA11 and Foxo1a originated before the most recent common ancestor of mammals and Foxo1a is located in the homeodomain of HoxA11, which is 100% conserved among the vertebrates, including mammals and birds. Thus, the only amino acid changes that occurred as the protein-protein interaction evolved are located in the N-terminal region of the HoxA11 protein. Because HoxA11 from chickens and the reconstructed ancestral amniote protein failed to interact with the Foxo1a protein, we conclude that amino acid substitutions outside of HoxA11’s homeodomain altered, directly or indirectly, Foxo1a’s access to the conserved binding interface in HoxA11’s homeodomain (Fig. P1).

Our results suggest that HoxA11 contains an unknown intramolecular regulatory region, located outside of the homeodomain, that controls Foxo1a’s access to the protein-protein interaction interface in the homeodomain (Fig. P1). It is likely that the intramolecular regulation serves to provide context-specific access to HoxA11’s interaction interface(s). The evolution of the protein–protein interaction was then attributable to amino acid changes in the regulatory domain. This mechanism of evolution for protein–protein interactions differs from the recently proposed model by Fernández and Lynch (3) in that the interaction surface itself is not changing. Evolution of intramolecular regulatory sequences also provides a possible explanation of how transcription factor changes can be adaptive.

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Evolutionary changes to these regulatory sequences may have more limited pleiotropic effects than alternations to the functional domains themselves.


