

Molecular recognition in helix-loop-helix and helix-loop-helix-leucine zipper domains: Design of repertoires and selection of high affinity ligands for natural proteins - (session: Structural Genomics)

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Helix-loop-helix (HLH) and helix-loop-helix-leucine zipper (HLHZip) are dimerization domains, which mediate selective pairing among members of a large transcription factor family involved in cell fate determination. To investigate the molecular rules underlying recognition specificity and to isolate molecules interfering with cell proliferation and differentiation control, we assembled two molecular repertoires obtained by directed randomization of the binding surface in these two domains. For this strategy we have selected the Heb HLH and Max Zip regions as molecular scaffolds for the randomization process and we have displayed the two resulting molecular repertoires on lambda phage capsids. By affinity selection, many domains were isolated that bound to the proteins Mad, Rox, MyoD and Id2 with different levels of affinities. Although several residues along an extended surface within each domain appeared to contribute to dimerization, some key residues critically involved in molecular recognition could be identified. Furthermore, a number of charged residues appeared to act as switch points that facilitate partner exchange. By successfully selecting ligands for four out of four HLH or HLHZip proteins, we have shown that the repertoires that we have assembled are rather general and possibly contain elements that bind with sufficient affinity to any natural HLH or HLHZip molecule. Thus they represent a valuable source of ligands that could be used as reagents for molecular dissection of functional regulatory pathways.