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Zinc Finger-DNA Recognition: Transcriptional Repression via Zinc Finger Design and Selection

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Zinc fingers of Cys_2His_2 class constitute one of the most common DNA binding motifs in eukaryotes. Unlike other DNA binding motifs, zinc finger proteins recognize very diverse DNA sites, and their sequence specificities can be systematically changed by phage display. A series of studies were performed to determine whether zinc finger peptides could efficiently repress transcription from RNA polymerase II promoters in vivo and to determine how such repression might depend on the position of the zinc finger binding site with respect to those of the TATA box or the initiator element. We found that the peptide containing the three zinc fingers of Zif268 could efficiently repress activated transcription when bound to a site near the TATA box or near the initiator element. Novel zinc finger peptides that had been selected via phage display also served as repressors of activated transcription in vivo. We then used structure-based design methods to link zinc finger peptides and make poly-finger peptides that have dramatically enhanced affinity and specificity. Our studies focused on a fusion in which the three-finger Zif268 peptide was linked to a designed three-finger peptide (designated "NRE") that specifically recognizes a nuclear hormone response element. Gel shift assays indicated that this six-finger peptide, 268//NRE, binds to a composite 18-bp DNA site with a dissociation constant in the femtomolar range. Transfection assays in human cell culture also showed that the 268//NRE peptide is an extremely effective repressor, giving 72-fold repression when targeted to a binding site close to the initiator element. Linking peptides selected via phage display should allow the design of novel DNA-binding proteins with extraordinary affinity and specificity for use in biological research and gene therapy.
(This work has been performed in the laboratory of Carl O. Pabo at the Department of Biology, Massachusetts Institute of Technology)