**OCT PROTEINS AND OCT GENES: DNA RECOGNITION AND TRANSCRIPTIONALREGULATION.**

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Keywords: POU domain, oct proteins, oct genes, octamer site, homeospecific site, oct-1 promoter

Oct proteins belong to the POU domain transcription factorfamily (for review see, 1). POU domain is a bipartite modul containing POU homeo and POU specific subdomains, identified in more than 30 nuclear proteins which recognize relative cis-elementsin promoters, enhancers and silencers of many genes. Oct-1 and Oct-2 proteins recognize in regulatory gene elements octamersequence ATGCAAAT and with lower affinity oct-relative sequences. The aim of the first step of this work was to range "a menu" of the targets, recognized by Oct proteins. The random modification approach allowed to screen two sets ofnucleotide sequences: 'ocamer' and octamer-relative sequences and TAAT-core sites which contact with Oct proteins with lower affinity (2, 3). Methylation interference analysis allowed to establish nucleotides essential for contact with POU domain. These data increased the number of potential targets for Oct proteins on DNA and changed our view on the regulation of expression by these transcription factors.

The goal of the further experiments was to establish the role of the concrete amino acid residues responsible for both 'octamer' and homeo-site recognition. To this end the role of two amino acidresidues Val47 and Cys50, located in the third recognition helix of POU homeo domain was elaborated (4, 5). Conservative Val47 wasalternately substituted with other 19 amino acids. Affinity andspecificity of interaction with oct-site ATGCAAANGA and with homeo-specific site ATAANGA were determined for all mutants. The wild type protein (with Val47) has maximal affinity and specificity in POU domain interaction with octamer sequence. However, V47I mutant showed stonger interaction with homeo-specific site. Thehigest specificity of interaction with homeo-site was recorded for V47S mutant. We conclude that only Val47 provides sequence specifichigh-affinity binding of POU proteins with octamer targets otherthan homeospecific site. It is shown also that damages caused by point mutations may be at least partially compensated by participation in the oct-site recognition of both POUh and POUssubdomains.

Cys50 of POU homeodomain is absolutely conserved (in contrastto that in homeoproteins). It contacts in coincidence with X-raydata (6) with two nucleotides 3'-flanking oct-sequence. To asses the importance of this residue in determining the binding specificity of Oct proteins all amino acids were sustituted for Cys at position 50, and the resulting mutants were tested with probes containing octamer motive ATGCAAATNN or homeospecific sites TAATNN. Only the wild-type POU was shown to adequately discriminate between the octamer and homeospecific sites, and the protein affinity was only slightly affected by the nucleotides flanking the octamer at the 3'-end. Moreover any amino acid substitutions at position 50 reduce the discrimination in bindingaffinity to octamer and TAAT-like sequences. It seems to be, the evolutionary selection adapts not only the POU proteins to theirtargets, but concurrently the targets to POU proteins.

Recently the 5'-upstream region of Oct-1 transcription factor was cloned and sequenced (7). CAT reporter gene analysis of this region has detected a functionally active promoter. This region contains 24 TAAT-core sites, arranged in five A/T enriched clusters (four to six in one cluster). Two 'octamer' sites are located in the first and second clusters proximal to transcription initiation. As shown by elecrophoretic mobilityshift assay, Oct-1, Oct-2, and some unknown proteins from myeloma cell line NS/0 interact with the TAAT-core sites of this clusters. The results suggest autoregulation of Oct-1 gene expression that may also be controlled by other POU proteins.

From our data follows that there are two types of POU domain interaction with DNA: POU-specific (with 'octamer' site) and homeo type interaction (with TAAT-core sites). At least two amino acid residues are essential for discrimination between these two types of targets. Any mutants decrease the affinity and specificity of interaction. It is possible that evolutionary selection of transcription factors has been aimed both at increasing the affinity to their 'own' targets and decreasing that to 'foreign' DNA binding sites. Both, 'octamer' and homeospecific sites are presented in oct-promoter region. It is sugested that they participate in autoregulation of oct-1 gene expression.

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