

Differential activity by DNA-induced quarternary structures of POU transcription factors

Attila Reményi^a, Alexey Tomilin^b, Hans R. Schöler^b, Matthias Wilmanns^{a,*}

^aEMBL, Hamburg Outstation, c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany

^bDepartment of Animal Biology, Center for Animal Transgenesis and Germ Cells Research, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, 382 West Street Road, Kennett Square, PA 19348, USA

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Abstract

Recent structural studies on transcription factors from the POU family in complex with multiple cognate DNA enhancer elements have established a novel concept in DNA-mediated formation of distinct conformations of transcription regulator assemblies. Two crystal structures of the Oct-1 transcription factor in the presence of two different DNA sites have demonstrated how its POU DNA-binding segment is capable in forming two unrelated dimer arrangements, which is DNA motif dependent. While one arrangement allows binding of the Oct-1 specific coactivator OBF-1, binding of this coactivator is blocked in the second arrangement because the binding site is involved in its own dimer assembly. Conversely, two crystal structures of another POU transcription factor, Pit-1, have demonstrated how the same overall assembly is maintained in the presence of two different DNA response elements. However, since the distance of the two Pit-1 half-binding sites on these elements differ by two base pairs, the overall dimensions of the two complexes vary, allowing binding of a specific repressor (N-CoR) in one conformation but not in the other. Thus, despite the occurrence of different DNA-mediated molecular mechanisms, the net result, conformation-dependent binding of further regulators, is equivalent. These data introduce a concept where the DNA motif not only serves as binding site for specific transcription factors but also regulates their function by mediating specific transcription factor assemblies, which determine binding to conformation-dependent coregulators.

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One of the fundamental requirements for generating a complex organism from a single cell is the provision of a system in which genes are precisely expressed both temporally and spatially. Regulation of this process is mediated by a limited number of divergent transcription factors that specifically bind to DNA enhancer and promoter sequences of many genes. These proteins, apart from recognizing and binding specific short stretches of DNA, interact with different components of the basal transcription machinery, either directly or indirectly *via* transcriptional coregulators, leading to and/or facilitating transcription [1]. This simplistic model, however, is not sufficient to explain various experimental observations of eukaryotic transcriptional activation, such as the presence of architectural protein components that bend DNA and the relative positions and

orientations of protein-binding sites. According to the “enhanceosome” theorem, enhancer regions of eukaryotic genes mediate the assembly of stereo-specific, multiprotein-containing transcription factor complexes [2]. These assemblies, rather than single transcription factors, can be regarded as the basic unit of transcriptional regulation. Their protein components often function differently depending on the specific interacting partners and the nature of the DNA enhancer site. Therefore, it has become apparent that the architecture of the DNA-binding site is critical in determining whether and to what extent a certain transcription factor, as part of a multiprotein/DNA enhanceosomal complex, will activate or repress gene transcription. For example, non-steroid members of the nuclear receptor superfamily, which possess a zinc-finger DNA-binding domain, operate by binding to the hormone response elements. These elements consist of two minimal core hexad sequences, AGGTCA, which can be integrated into various functional motifs. The orientation and spacing between these two hexamers as well

* Corresponding author. Tel.: +49-40-89902-110; fax: +49-40-89902-149.
E-mail address: wilmanns@embl-hamburg.de (M. Wilmanns).

as subtle differences in their sequence dictate the identity and the mode (monomer, hetero- or homo-dimer) of nuclear receptor binding that results in diverse transcriptional outcomes [3].

Similarly, the operation of members of the POU transcription factor family, which is the focus of this review, is highly dependent on the nature of their cognate DNA elements. These factors all contain a central DNA-binding segment, referred as POU domain, while their flanking regions are generally unrelated. They are involved in a broad range of biological processes ranging from house-keeping gene functions (Oct-1) to programming of embryonic stem cells (Oct-4), the development of immune responses (Oct-1, Oct-2) and the pituitary gland (Pit-1) [4,5]. However, the low number [15] of POU transcriptional regulators in the human genome contrasts their versatile functions. Therefore, members of this transcription factor family are anticipated to rely on multilevel control mechanisms to perform these multiple tasks, such as posttranslational modification, interaction with heterologous transcription regulators, and flexible DNA-binding. The DNA-binding segment of POU family members consists of two canonical DNA-binding domains, the POU-specific (POU_S) and the POU-homeo (POU_H) domain, joined together by a unique and flexible linker region. This linker largely differs both in sequence and length (15–56 residues) among the members of the POU family. Since both DNA-binding domains are structurally and functionally autonomous, various arrangements on the DNA are possible [6].

POU factors were originally identified to function as monomeric transcriptional regulators. However, more recently, their capability to homo- and hetero-dimerize on specific DNA response motifs has received substantial attention [7–10]. Furthermore, new POU dimer crystal structures of Pit-1 and Oct-1 in complex with four different natural enhancer elements [11,12] have demonstrated the role of the cognate DNA sites in defining the structure and organization of the POU dimer/DNA interaction. These studies have established a role for DNA enhancer elements in mediating differential quaternary structural arrangements of the same transcription factor. Biochemical and *in vivo* studies in parallel then showed that different quaternary arrangements result in differential interaction with transcriptional coregulators, evoking diverse, sometimes even contrasting, transcriptional outcomes.

1. Differential Oct-1 dimer activities by selective recruitment of a coactivator

Two recently identified DNA response elements from promoter/enhancer regions of POU factor target genes have become critical tools to uncover the basis of the DNA-mediated structural plasticity of POU factors. The Palindromic Oct-factor Recognition Element (PORE),

ATTTGAAATGCAAAT, is localized within the first intron of the *osteopontin* (*OPN*) gene and was initially identified as an Oct-4 DNA responsive element. It mediates strong transcriptional activation in preimplantation mouse embryos and cell lines derived thereof [8]. The second POU factor recognition element, which bears the consensus sequence ATGCATATGCAT, was identified within immunoglobulin heavy chain enhancers, whose expression is also regulated by Oct-1 and/or Oct-2 [13]. This site was termed the MORE (More of PORE). Because members of the POU family tested thus far (Oct-1, Oct-2, Oct-4) bind to their consensus sequence in a cooperative fashion as homo- and hetero-dimers, both the PORE and MORE have been studied further as general Oct factor recognition elements.

Since transcription factors often interact with the basal transcriptional machinery *via* bridging factors or transcriptional coactivators, there is an ongoing search for such mediators. At present, a lymphoid-specific coactivator OBF-1 (OCA-B, BOB-1) has been identified to regulate the transcriptional activity of monomeric Oct-1 on the octamer motif in B-cells. OBF-1 clamps the POU_H and POU_S DNA-binding domains, leading to an enhancement of their cooperative DNA-binding affinity [14,15]. However, the Oct-1 dimer formed on MOREs within immunoglobulin heavy chain promoters (V_H), fails to interact with OBF-1. In contrast, the Oct-1/PORE dimeric complex can interact and synergize in transcriptional activation with this cofactor like the Oct-1/octamer monomer complex.

The rationale for these opposite binding properties has recently been provided by the two dimeric Oct-1 crystal structures: one bound to the MORE and the other to the PORE (Fig. 1). These structures revealed that the POU domain segment of same Oct-1 sequence is capable of interacting with the DNA in different quaternary arrangements while maintaining essentially identical protein–DNA interactions. The two Oct-1 protomers bind to the MORE in a compact fashion by almost entirely wrapping the DNA double helix, whereas in the Oct-1/PORE complex, the POU domains bind mainly to one side of the DNA. A key difference between the complexes is the strict spatial requirement between the two DNA half-sites in the PORE-mediated POU dimers, while additional base pairs may be inserted between DNA motif half-sites of the MORE without compromising its ability to mediate POU factor dimerization [10] (Fig. 2). The two recent Oct-1 dimer structures have provided the molecular rationale for this difference by revealing unrelated dimer interfaces in the PORE- and MORE-mediated POU–DNA complexes, ‘across half-sites’ and ‘within half-sites,’ respectively. As a structural consequence, the dimer interface of the Oct-1/MORE dimer is not affected by changes in the distance and orientations of the two half binding sites. In contrast, the flexible linker regions connecting the POU_S and the POU_H domains of each of the two protomers are opposite, ‘within half-sites’ in the PORE complex and ‘across half-sites’ in the MORE complex. This result, taken

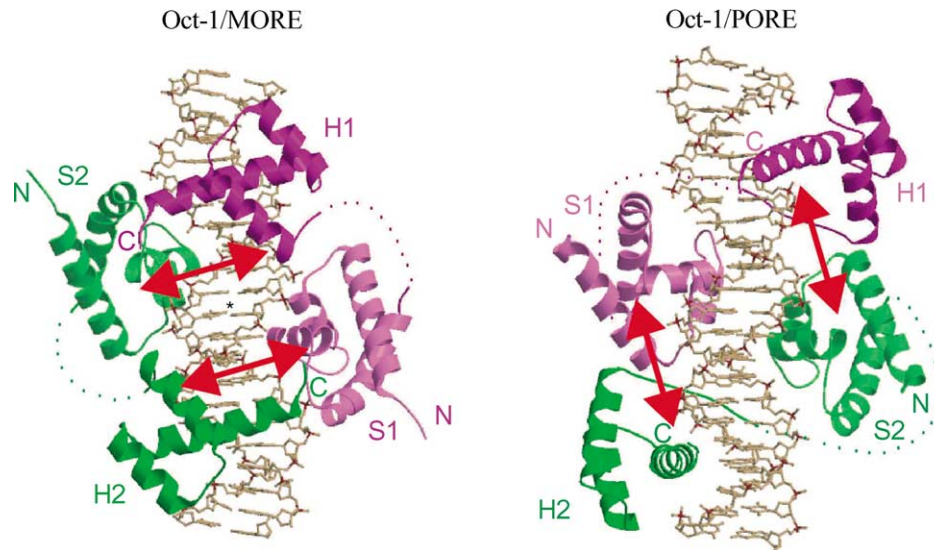


Fig. 1. The Oct-1 POU domain is capable of binding to DNA enhancer elements in different quaternary structural arrangements with different dimerization interfaces. The bipartite Oct-1 POU domain binds to both the MORE and PORE DNA responsive elements as a homo-dimer. In both structures, each POU-specific domain (POU_S , S) is folded as a four-helix bundle and each POU-homeo domain (POU_H , H) is comprised of three α -helices. The position of the MORE- and PORE-type interfaces are indicated with red arrows. The pseudo-palindromic symmetry of the MORE motif is indicated by an asterisk (*).

together with previous band shift data, implies that there may be other MORE-type enhancers with different spacings for the two half binding sites (Fig. 2). In contrast, as inferred from the structural model, the protein dimer interface of the Oct-1/PORE dimer is directly affected by changes in distance and orientation of the half binding sites either leading to sterical clashes (if the number of base pairs between the two half binding sites is diminished) or loss of specific dimer interactions (if the number of base pairs is increased).

The two recent dimeric Oct-1/DNA complex structures revealed not only how this member of the POU family uses its structural arsenal, two autonomously folded DNA-binding domains connected by a flexible linker, but also introduced the novel concept of the availability of a redundant set of two equivalent protein surfaces on each of the two DNA-binding POU_S and POU_H domains that have the capability to form protein–protein dimers. In both dimer types, the protein–protein interface is largely formed by the side chain of an exposed hydrophobic residue

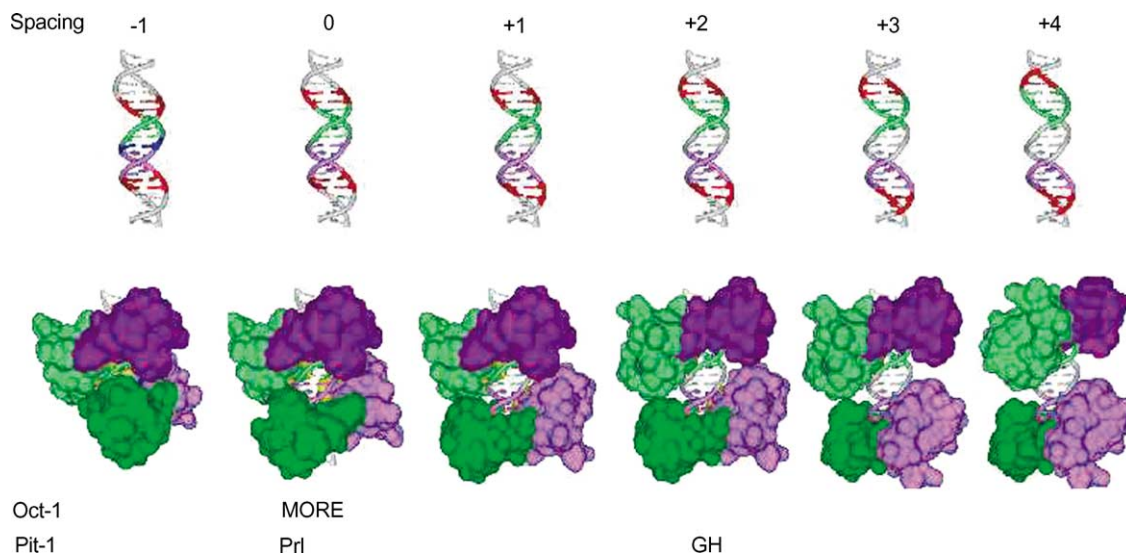


Fig. 2. Effects of spacing of POU binding sites using the structure of the Oct-1/MORE complex. Spacings of -1 , $+1$, $+2$, $+3$, $+4$ with respect to the MORE motif have been used for modeling. The colors of the POU_S and POU_H domains are as in Fig. 1. The distal DNA-binding sites for the POU_H domains are in red. For the two Pit-1/DNA complexes [11], the corresponding binding site spacings in relation to the MORE motif are indicated. The figure shows how the POU_S – POU_H dimer interface remains conserved at different DNA-binding site spacings while the distance between the POU_S and POU_H domains belonging to one promoter increases. Different spacings can be adopted by the flexible linker (not shown) between the two domains.

sticking into a hydrophobic pocket of the partner domain [12]. In contrast to this, the protein–DNA interactions of the two POU_S and POU_H sub-domains of Oct-1 with the corresponding DNA site is virtually identical in both dimeric structures.

The available structural data do not reveal unambiguous preference for one protein–protein interface vs. the other in the two different Oct-1 dimers, for instance in terms of surface area or counting statistics on specific interactions. On the other hand, in both complexes, the protein–DNA complex boundaries are defined by specific, distal interactions between the POU_H domains and the AT-motifs, with different spacings in the MORE vs. PORE, and by specific binding sites for the two POU_S domains at proximal positions to each DNA response motif. Modeling of the ‘wrong’ dimers (a MORE-type dimer on PORE, and *vice versa*) indicates that each DNA sequence only allows one type of fitting dimer associate, thus introducing the concept of DNA-mediated transcription factor dimerization, which is determined by the spacings and the sequence of the respective binding motifs. The biological significance of this concept is underlined by differential coactivator interaction of a series of POU dimers bound to the MORE and the PORE sites. Comparison of the two Oct-1 dimer/DNA structures with a ternary complex crystal structure containing a 44-residue fragment of OBF-1 bound to the Oct-1/octamer motif complex [15] revealed the structural basis for this differential coactivator interaction. OBF-1 binds to the DNA and to both the POU_S and POU_H domains of the Oct-1 monomer, thereby forming a clamp-like contact between them through the major groove. Since POU/OBF-1 interaction requires a strict spatial arrangement between POU_S and POU_H, the observed different quaternary arrangements in the two POU dimer/DNA structures explain the selectivity of the OBF-1 interaction. In addition, the Oct-1/MORE complex shows that the same surface patch of the POU_H and POU_S domain that binds OBF-1 in the monomeric Oct-1/octamer is used in the POU_S–POU_H interface of the POU/MORE complex, thus blocking this coactivator binding site. In conceptual terms, this comparison demonstrates that the two DNA-binding domains of this POU family member not only possess redundant surface paths capable of mediating POU dimer formation but these surface patches may also serve multiple functions, such as being employed for coactivator binding, as noted here. Hindrance of such an interface by POU dimer formation may lead to an inability to bind to other coregulators.

2. Differential Pit-1 dimer activities by selective recruitment of a corepressor

Studies conducted in parallel on another factor of the POU family, Pit-1, have demonstrated how the same transcription factor can act as either an activator or repressor of cell type specific genes depending on the nature of its

binding site. Pit-1 plays a key role in the development of the pituitary gland and is required for the activation of genes encoding growth hormone, prolactin and thyrotropin in somatotrope, lactotrope, and thyrotrope cell types, respectively. Recently, a mechanism has been proposed by which expression of prolactin and growth hormone is reciprocally activated and restricted in lactotrope and somatotrope pituitary gland cells, respectively [11]. This model provides a concept how Pit-1 may be involved in differential functions in the spatial-temporal context of pituitary gland development. The structures of Pit-1 bound to two related DNA response elements, within the *prolactin* (*Prl*) and the *growth hormone* (*GH*) promoters, revealed that these two enhancers bind Pit-1 dimers *via* the same protein–protein interface [11] which matches that of the MORE-mediated interface in Oct-1. The POU_S and the POU_H domains of the bipartite DNA binding segment of Pit-1 are accommodated on the same face of the DNA on the growth hormone element, while they are bound to perpendicular faces of the prolactin element DNA. These two enhancer motifs are almost identical, apart from a TT insert between the two half-sites in the GH element. Only the extended conformation adopted by Pit-1 on GH enables recruitment of a corepressor complex containing N-CoR, leading to repression of GH transcription in lactotrope pituitary tissues. Although the molecular basis for differential recruitment of N-CoR remains unknown (in the absence of direct structural information on N-CoR) it is assumed to employ a different switch mode for binding than that observed for the OBF-1/Oct-1 interaction which is regulated by protein–protein dimerization interface swapping in Oct-1/PORE vs. Oct-1/MORE. In contrast, differential recognition of the N-CoR corepressor appears to be regulated mainly by different spacings and orientations of the two Pit-1 dimer binding sites in GH and *Prl*, using different versions of the MORE dimer arrangement.

3. POU dimer-specific coregulators of transcription: a general model?

The functional properties of a wide range of transcription factors are altered by conformational changes induced by activators, repressors, and specific receptor ligands [16]. At the structural level, these changes often lead to the formation of additional helices, reorientation of loops, and rearrangements of hydrophobic cores. Members of the POU family lack such a structural flexibility within their POU sub-domains. Nevertheless, they have evolved structural flexibility, which relies mainly on the independent positioning of their sub-domains, directed by the architecture and overall length of targeted DNA sequences. These DNA response elements not only induce either POU factor binding as monomers or dimers, but also mediate the assembly of different quaternary arrangements stabilized by specific protein–protein interactions.

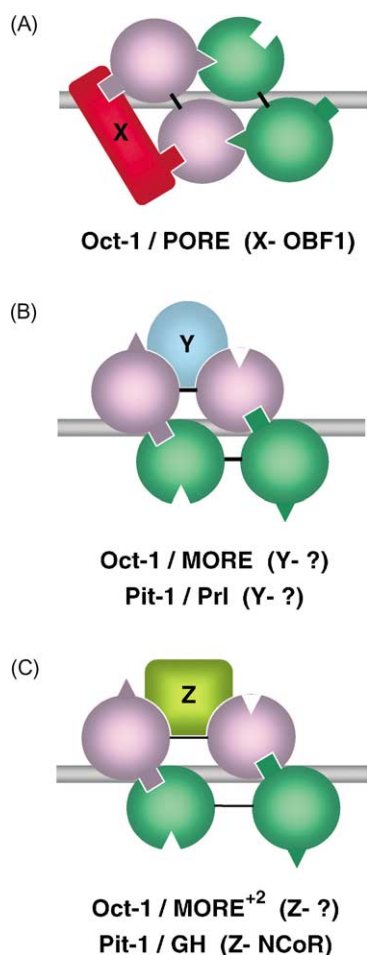


Fig. 3. Model for selective recruitment of cofactors by POU dimers. Schematic representation of POU dimer arrangements bound to: (A) the PORE; (B) the MORE (Oct-1) or Prl (Pit-1); (C) the MORE⁺² (Oct-1) or GH (Pit-1) DNA response elements, which contain a two base pair insertion between the two half-sites when compared to MORE/PrI. The different quaternary arrangements of POU sub-domains (indicated by spheres) either expose or bury the MORE- or the PORE-type dimerization interfaces. The MORE-type interface is indicated by the rectangular indentations (on the surface of POU_S) and protrusions (on the surface of POU_H). The PORE-type interface is indicated by triangles. The open MORE-type interface is used for binding of OBF-1 in the Oct-1/PORE dimer. On the other hand, the PORE-type interface could be potentially engaged for specific cofactor recruitment (“Y” and “Z”), which could be selective with respect to either the type of dimer configuration (MORE vs. PORE) or to the spacing of half-sites within one configuration. The second type of selectivity is only applicable for the MORE configuration, as the PORE configuration does not allow different spacing of DNA half-sites. The N-CoR corepressor complex selectively binds to Pit-1 in complex with GH (“Z”) but not with PrI (“Y”).

The distinct dimeric arrangements allow differential recruitment of cofactors, which exert diverse effects on transcription (outlined schematically in Fig. 3). This way of binding is not limited to Oct-1 and Pit-1 but could be regarded as a general POU factor property, since all members of the POU transcription factor family are capable of dimerizing on DNA response elements in a versatile manner. According to our model, only a specific configuration of POU dimers provides protein surfaces accessible

for interaction with a cofactor. For example, the OBF-1 coactivator utilizes the accessible Oct-1 POU sub-domain surfaces in the PORE-type configuration that are inaccessible in the MORE-type configuration (Fig. 3). Therefore, the MORE-type configuration fails to recruit OBF-1. However, the opposite situation is also conceivable, namely that the POU Oct-1 dimer forms on the MORE and mediates the recruitment of a yet unknown specific cofactor (“Y”, Fig. 3B). Furthermore, differences within the same POU dimer configuration, generated by different spacing arrangements of half-sites, may also lead to selective cofactor recruitment, as shown for the pituitary-specific POU factor Pit-1 [11].

4. Perspectives

This review has focused on the recent structural studies that have shed light onto novel mechanisms of how members of the POU transcription factor family, with only few members, ally with diverse DNA response elements to fulfill their very complex regulatory tasks. Their DNA sequence-dependent exposure or burial of interaction interfaces with transcriptional coregulators is likely to contribute to their biological function: the proper, error-free manifestation of the DNA encoded genetic program during embryonic development. We predict that this yet undescribed property of DNA binding proteins is also found in proteins from other transcription factor families, for example members of the PAX family. Modulation of protein–protein interactions—which are so central to this elegant regulation—rather than the more elusive protein–DNA interactions will provide us strategies in the future that are sufficiently specific and selective for benevolent interference with pathological processes of transcription.

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