Interaction of Oct-1 and automodification domain of poly(ADP-ribose) synthetase

Jing Nie, Shuji Sakamoto, Demao Song, Zhiqiang Qu[1], Katsuya Ota[2], Taketoshi Taniguchi*

Laboratory of Molecular Biology, Medical Research Center, Kochi Medical School, Okoh, Nankoku, Kochi 783-8505, Japan

Received 25 December 1997; revised version received 26 January 1998

Abstract We isolated several clones from a matchmaker two-hybrid system human lymphocyte cDNA library using an automodification domain of poly(ADP-ribose) synthetase (PARS) as a probe. A DNA sequence (~1 kbp) of the clone was identical to part of the Oct-1 DNA sequence. We then constructed either a His-tagged or GST fusion protein of the inserted cDNA from the clone and the fusion protein was shown to interact with PARS by far-Western blot analysis and co-precipitation with affinity resin. Furthermore, the His-tagged Oct-1/POU-homeo fusion protein interacted weakly with the octamer motif of the DRa promoter and the addition of PARS fusion protein greatly increased the DNA binding activity. These results suggest that PARS interacts with Oct-1 and stabilizes the binding of Oct-1 to the octamer motif.

Key words: Poly(ADP-ribose) synthetase; Oct-1; Two-hybrid system

1. Introduction

Poly(ADP-ribose) synthetase (NAD^+ ADP-ribosyltransferase, EC 2.4.2.30, PARS) is a chromatin-bound enzyme that catalyzes the transfer of the ADP-ribose moiety of NAD to nuclear proteins including the enzyme itself [1–4]. PARS was proposed to mediate stress-induced signaling and also function in DNA repair processes [5]. Although poly(ADP-ribosyl)ation takes place under conditions in which chromatin is damaged, a highly limited amount of poly(ADP-ribosyl)ated nuclear protein was detected in intact cells [6]. Therefore, the physiological roles of PARS in intact cells are still unclear. PARS is also suggested to be involved in differentiation. PARS content critically changes during cell differentiation [7–9]. PARS mRNA level decreased during granulocytic differentiation of HL-60 cells [10], erythrocytic differentiation of K562 [11] and neutrophilic differentiation of acute promyelocytic NB4 leukemia cells [12]. The neutrophilic differentiation of NB4 cells was inhibited by overexpression of PARS [13]. However, when an increase in PARS expression was prevented in stably transfected 3T3-L1 preadipocyte cells by induction of PARS antisense RNA synthesis, the cells did not differentiate [14]. Thus, the amount of PARS or activity of the enzyme may associate with the signaling pathway of differentiation. A periodic repetition of leucine residues has been proposed to mediate homo- and hetero-dimer formation involved in the DNA binding and function of these leucine zipper proteins [15,16]. The presence of such phylogenetically conserved motifs in the automodification domain of PARS has been suggested to be responsible for the protein-protein interactions involving PARS homo- and/or hetero-dimerization with other nuclear leucine zipper proteins [17]. Thus, PARS possibly interacts with a nuclear protein which is involved in differentiation processes and therefore we searched for a protein(s) which interacted with PARS. Here we report that Oct-1 interacts with the automodification domain of PARS and binding of PARS stabilizes the binding of Oct-1 to the octamer sequence.

2. Materials and methods

2.1. Yeast strains and screening of two-hybrid B-cell cDNA library

Matchmaker Two-Hybrid System was obtained from Clontech (Clontech Laboratories, Inc., USA). Yeast strain HF7c was used for screening the human B-cell cDNA library. The pGBT-PARS/AMD (automodification domain of PARS) was constructed by insertion of the *Eco*RI/*Pst*I 0.7 kbp fragment of PARS cDNA (pcD-ARS) [18] into the *Eco*RI and *Pst*I site of pGBT 9. pGBT-PARS/AMD and the pACT-cDNA fusion library were cotransformed into HF7c using the lithium acetate procedure. Double transformant cells grown on Leu^-, Trp^-, His^- plates were incubated for 5 days at 30°C. Positive colonies were picked up, and assayed for the lacZ phenotype. The selected clones were tested for their ability to transactivate with the following non-specific partners: pGBT 9, pGBT-PARS/AMD, pLAM5′ or pVA3. Filter assay for β-galactosidase activity was performed according to the manufacturer's protocols.

2.2. DNA sequencing

The library clones that grew up in the Leu^-, Trp^-, His^- plates only in the presence of pGBT-PARS/AMD were chosen for DNA sequencing. DNA sequence was determined using sequencing primers 5′-TACCACTACAATGGATG (reading toward the junction of the GAL4 activation domain and the cloned candidate interacting protein) or 5′-ACGATGCACAGTTGAAG (reading reversely from downstream of the multi-cloning site) with the ABI PRISM Genetic Analyzer Model 310 (Applied Bio-systems), using the ABI PRISM dye-terminator cycle sequencing ready reaction kit (Applied Biosystems).

2.3. Construction of fusion proteins

His-tagged Oct-1/homeo was constructed by insertion of the *Xho*I fragment of the 2-5-2 clone from the two-hybrid cDNA library into the *Sal*I site of pQE30 or the *Sal*I site of pGST-5X-1. The Oct-1/POU-homeo cDNA was synthesized by RT-PCR and subcloned into the *Kpn*I and *Bam*HI site of pQE31 expression vector. His-tagged PARS/AMD was constructed by subcloning of the *Kpn*I/*Pst*I fragment of pcD-ARS [18] into the *Kpn*I and *Pst*I site of pQE32. The

*Corresponding author. Fax: (81) (888) 80-2431.
E-mail: taniguch@pop.med.kochi-ms.ac.jp

[1]Present address: Cell Biology Laboratory, National Institute of Bioscience and Human Technology, AIST, MITI, Tsukuba Science City, Ibaragi 321-02, Japan.

[2]Present address: Department of Biochemistry, Nara Medical University, Nara 630, Japan.

Abbreviations: PARS, poly(ADP-ribose) synthetase; PARS/AMD, automodification domain of PARS; EMSA, electrophoretic mobility shift assay

PII S0014-5793(98)00131-8

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HSOCT1     1021:TCAGCCAAACTACCATCTCTCGATTTGAAGCCTTGAACCTCAGCTTTAAGAACATGTGCA 1080
2-5-2.Seq     1:----------------------------AAGCCTTGAACCTCAGCTTTAAGAACATGTGCA 33
                                            *********************************

HSOCT1     1081:AGTTGAAGCCACTTTTAGAGAAGTGGCTAAATGATGCAGAGAACCTCTCATCTGATTCGT 1140
2-5-2.Seq    34:AGTTGAAGCCACTTTTAGAGAAGTGGCTAAATGATGCAGAGAACCTCTCATCTGATTCGT 93
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HSOCT1     1141:CCCTCTCCAGCCCAAGTGCCCTGAATTCTCCAGGAATTGAGGGCTTGAGCCGTAGGAGGA 1200
2-5-2.Seq    94:CCCTCTCCAGCCCAAGTGCCCTGAATTCTCCAGGAATTGAGGGCTTGAGCCGTA-GAGGA 152
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HSOCT1     1201:AGAAACGCACCAGCATAGAGACCAACATCCGTGTGGCCTTAGAGAAGAGTTTCTTGGAGA 1260
2-5-2.Seq   153:AGAAACGCACCAGCATAGAGACCAACATCCGTGTGGCCTTAGAGAAGAGTTTCTTGGAGA 212
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HSOCT1     1261:ATCAAAAGCCTACCTCGGAAGAGATCACTATGATTGCTGATCAGCTCAATATGGAAAAAG 1320
2-5-2.Seq   213:ATCAAAAGCCTACCTCGGAAGAGATCACTATGATTGCGGATCAGCTCAATATGGAAAAAG 272
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HSOCT1     1321:AGGTGATTCGTGTTTGGTTCTGTAACCGCCGCCAGAAAGAAAAAAGAATCAACCCACCAA 1380
2-5-2.Seq   273:AGGTGATTCGTGTTTGGTTCTGTAACCGCCGCCAGAAAGAAAAAAGAATCAACCCACCAA 332
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HSOCT1     1381:GCAGTGGTGGGACCAGCAGCTCACCTATTAAAGCAATTTTCCCCAGCCCAACTTCACTGG 1440
2-5-2.Seq   333:GCAGTGGTGGGACCAGCAGCTCACCTATTAAAGCAATTTTCCCCAGCCCAACTTCACTGG 392
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HSOCT1     1441:TGGCGACCACACCAAGCCTTGTGACTAGCAGTGCAGCAACTACCCTCACAGTCAGCCCTG 1500
2-5-2.Seq   393:TGGCGACCACACCAAGCCTTGTGACTAGCAGTGCAGCAACTACCCTCACAGTCAGCCCTG 452
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HSOCT1     1501:TCCTCCCTCTGACCAGTGCTGCTGTGACGAATCTTTCAGTTACAGGCACTTCAGACACCA 1560
2-5-2.Seq   453:TCCTCCCTCTGACCAGTGCTGCTGTGACGAATCTTTCAGTTACAGGCACTTCAGACACCA 512
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HSOCT1     1561:CCTCCAACAACACAGCAACCGTGATTTCCACAGCGCCTCCAGCTTCCTCAGCAGTCACGT 1620
2-5-2.Seq   513:CCTCCAACAACACAGCAACCGTGATTTCCACAGCGCCTCCAGCTTCCTCAGCAGTCACGT 572
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HSOCT1     1621:CCCCCTCTCTGAGTCCCTCCCCTTCTGCCTCAGCCTCCACCTCCGAGGCATCCAGTGCCA 1680
2-5-2.Seq   573:CCCCCTCTCTGAGTCCCTCCCCTTCTGCCTCAGCCTCCACCTCCGAGGCATCCAGTGCCA 632
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HSOCT1     1681:GTGAGACCAGCACAACACAGACCACCTCCACTCCTTTGTCCTCCCCTCTTGGGACCAGCC 1740
2-5-2.Seq   633:GTGAGACCAGCACAACACAGACCACCTCCACTCCTTTGTCCTCCCCTCTTGGGACCAGCC 692
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HSOCT1     1741:AGGTGATGGTGACAGCATCAGGTTTGCAAACAGCAGCAGCTGCTGCCCTTCAAGGAGCTG 1800
2-5-2.Seq   693:AGGTGATGGTGACAGCATCAGGTTTGCAAACAGCAGCAGCTGCTG-CCTTCAAGGAGCTG 751
                ********************************************* ***************

HSOCT1     1801:CACAGTTGCCAGCAAATGCCAGTCTTGCTGCCATGGCAGCTGCTGCAGGACTAAACCCAA 1860
2-5-2.Seq   752:CACAGTTGCCAGCAAATGCCAGTTTTGCTGCCATGGCAGCTGCTGCAGGACTAAACCCAA 811
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HSOCT1     1861:GCCTGAT-GGCACCCTCA-CAGTTTGC-GGCTGGAGGTGCCTTACTCAGTCTGAA-TCCA 1916
2-5-2.Seq   812:GCCTGATGGGCACCCTCACCAGTTTGCGGGCTGGAGGTGCCTTACTCAGTCTGAATTCCA 871
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HSOCT1     1917:GGGACCCTGAGCGGTGCTCTCAGCCCAGCTCTAATGAGCAACAGTACACTGGCAACTATT 1976
2-5-2.Seq   872:GGGACCCTGAGCGGTGCTCTCAGCCCAGCTCTAATGAGCAACAGTACACTGGCAACTATT 931
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HSOCT1     1977:CAAGCTCTTGCTTC-TGGTGGCTCT                                    2000
2-5-2.Seq   932:CAAGGT-TTGCTTCATGGTTTGGAG                                    955
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Fig. 1. Comparison of the DNA sequence of clone 2-5-2 and human Oct-1 (A) and schematic presentation of the corresponding part of clone 2-5-2 (Oct-1/Homeo) and Oct-1/POU-homeo synthesized by RT-PCR (B).

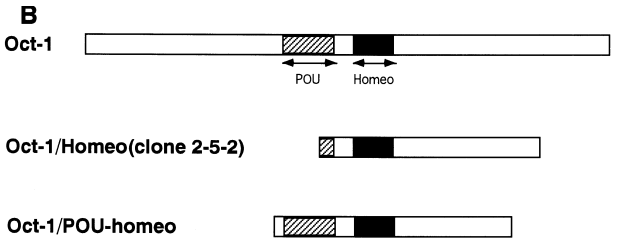

Fig. 1. (*continued*)

His-tagged fusion protein was purified on a Ni^{2+}-NTA resin column (Qiagen), dialyzed in a buffer (20 mM Tris-HCl, pH7.5, 50 mM NaCl, 1 mM EDTA, 10% glycerol, 5 mM $MgCl_2$) and used directly for experiments. The pGST/Oct-1/homeo expression vector was transformed into *Escherichia coli* BL21. The fusion protein was purified on a glutathione-Sepharose column (Pharmacia) from the extract of IPTG-induced transformant.

2.4. Far-Western blot analysis

His-tagged Oct-1/homeo fusion protein was prepared as described above, separated on 10% SDS-PAGE and then transferred onto a PVDF membrane. After soaking in 5% skim milk-TBBN (20 mM Tris-HCl pH 7.5, 50 mM NaCl, 10 mM $MgCl_2$, 0.1% NP-40) for 1 h at 25°C, the membrane was incubated overnight with POD-labeled His-tagged PARS/AMD in 0.1% skim milk-TBBN at 4°C [19]. Finally the band was visualized by a chemiluminescence detection system (ECL) according to the manufacturer's instructions (Amersham).

2.5. In vitro binding studies

Glutathione *S*-transferase (GST) or GST-Oct1-homeo (5 μg each) was incubated with His-tagged PARS/AMD (10 μg) in binding buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$, 10% glycerol, 0.5 mg/ml BSA, 5 mM 2-mercaptoethanol) at 4°C overnight. 20 μl of 50% glutathione-Sepharose 4B slurry (Pharmacia) was added and after rotation for 1 h at 4°C, the beads were sedimented and washed 4 times with 400 μl of binding buffer. The bound proteins were eluted from the beads with 1 mg/ml glutathione in the same binding buffer. The proteins were separated by SDS-PAGE and then transferred to a PVDF membrane. The His-tagged PARS/AMD fusion protein was detected by incubation with a polyclonal anti-bovine PARS antibody, followed by POD-labeled second antibody and then visualized by the ECL detection system (Amersham).

2.6. Electrophoretic mobility shift assay (EMSA)

For the Oct-1 binding reaction, a probe was synthesized using ^{32}P-labeled primer to amplify the HLA-DRα promoter region in pAKR5214-3 which contains the human genomic HLA-DRα gene [20,21]. His-tagged Oct-1/POU-homeo protein (1.2 μg) or His-tagged PARS/AMD protein (0.5 μg) was preincubated with 0.05 μg poly(dI-dC) (Pharmacia) for 30 min at 25°C in a buffer containing 10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM $MgCl_2$, 5 mM $CaCl_2$, 5% glycerol, 1 mM EDTA. An aliquot of 5000 cpm (~6 ng) of the ^{32}P-labeled double strand DNA probe was thereafter added in a final reaction volume of 10 μl and the incubation was continued for another 30 min. Reaction components were separated on a 3.5% polyacrylamide (30:1 Bis) gel in 0.5×TBE buffer at 100 V for 60 min. The DNA-protein complex was visualized by autoradiography. Anti-PARS protection assay was performed as follows: PARS/AMD was incubated with anti-PARS antibody (3 or 6 μg of IgG) at 25°C for 30 min, and then Oct-1/POU-homeo and ^{32}P-labeled DRα DNA probe were added. The complex was analyzed as described above.

3. Results

3.1. Screening of cellular proteins that interact with the automodification domain of PARS

The yeast two-hybrid system used to screen for cDNA encoding cellular proteins able to interact with a target protein of interest is well known [22–24]. To identify proteins that interact with the automodification domain of PARS, this region was subcloned into plasmid pGBT9 to be expressed as a fusion protein with the DNA binding domain of the Gal 4 protein (pGBT-PARS/AMD). The cDNA library proteins were constructed with a pACT plasmid such that fusions between the cDNA-encoding proteins and the Gal 4 activation domain were generated. The two types of hybrid plasmids were then cotransformed into the yeast host strain HF7c. About 1.5×10^5 pGBT-PARS/AMD and pACT-cDNA double transformants were screened for His-independent growth. 33 clones were isolated as His^+ transformants. His^+ transformants were further assayed for color development in the β-galactosidase assay and 12 transformants were found among 33 His^+ transformants to be β-galactosidase-positive. As some pACT-cDNA-expressed proteins may interact non-specifically with fusion proteins, expressed by pGBT9 plasmids, false-positive clones frequently appear in the screening procedure. To eliminate false-positive clones, a confirmation test was performed. pACT-cDNA plasmids were cotransformed with either pGBT9, pLAM5′, pVA3 or pGBT-PARS/AMD into HF7c and then tested for His-independent growth. In this test, clones that grew in His-deficient medium only in the presence of pGBT-PARS/AMD were considered to be true positive clones. Only six clones survived all the genetic tests and were analyzed further.

3.2. Sequence analysis of the positive pACT-cDNA clone 2-5-2

The pACT-cDNA plasmids were recovered from the six selected clones and sequenced. Sequence homology searches in GenBank revealed that the sequence of a clone, named 2-5-2 (approximately 1 kbp insert DNA), was almost identical to the human lymphoid-specific Oct-1 from bp 1048 to 1995 (Fig. 1) [25]. Oct-1 has a unique DNA binding POU domain (894–1121) and a homeo domain (1194–1373). The predicted amino acid sequence of the 2-5-2 plasmid contains the whole homeo domain and a part of the POU domain, lacking the N-terminal 330 amino acids.

3.3. Interaction of Oct-1 and automodification domain of PARS in vitro

To demonstrate the direct interaction between PARS and Oct-1/homeo, we constructed a His-tagged fusion protein by ligating the *Xho*I fragment of the 2-5-2 plasmid with the *Sal*I site of pQE vector. The fusion protein was induced by IPTG and isolated with Ni-NTA resin column. The affinity-purified fusion protein was analyzed by far-Western blot analysis with the POD-labeled His-tagged PARS/AMD protein as a probe. The fusion protein migrated with an apparent molecular weight of 38 kDa, which was in agreement with the size deduced from the cDNA sequence. An identical gel was transblotted onto a PVDF membrane and subsequently incubated with POD-labeled His-tagged PARS-AMD. The band corresponding to His-tagged Oct-1/homeo was visualized by ECL (Fig. 2A). The result indicates that the Oct-1/homeo fusion protein interacted with His-tagged PARS-AMD.

In the far-Western blot analysis, one of the two interaction partners was denatured and subsequently renatured. Therefore, we further decided to examine whether the two proteins would also be able to interact in solution when they are present as a native conformation in solution. Thus, we constructed a GST fusion protein with the *Xho*I fragment of the 2-5-2 plasmid and performed an in vitro binding assay. The

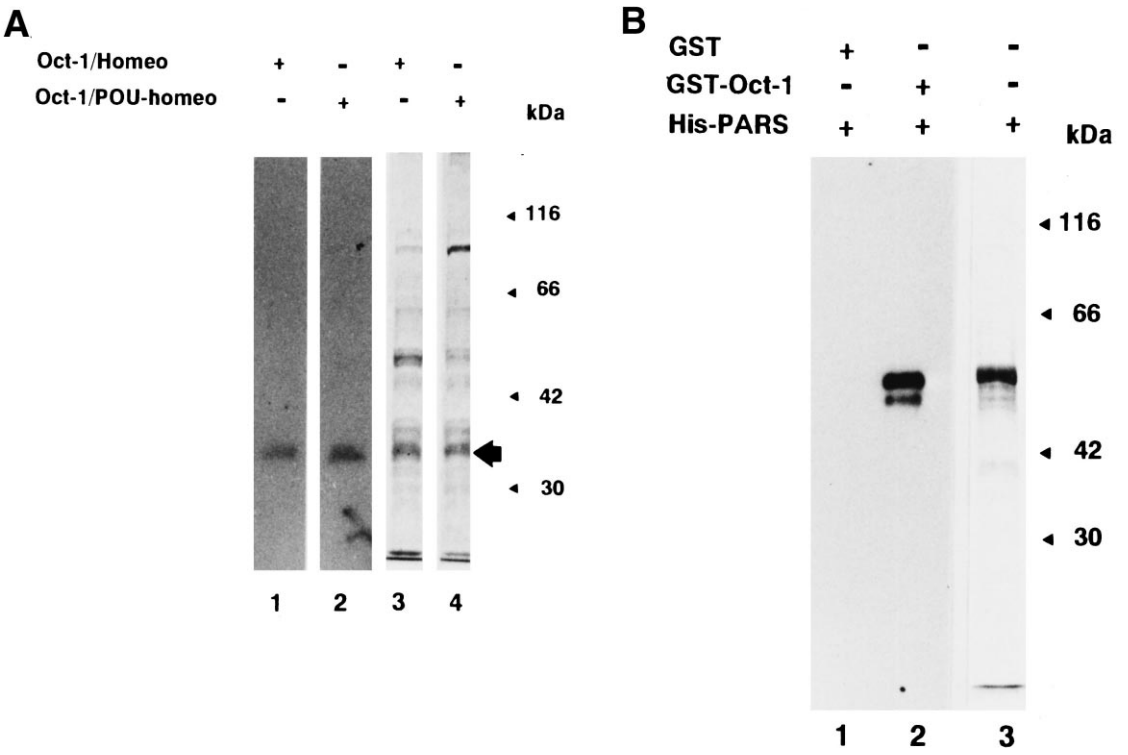

Fig. 2. A: Interaction of Oct-1/homeo or Oct-1/POU-homeo with PARS/AMD analyzed by far-Western blot analysis. The affinity-purified His-tagged Oct-1/homeo (4.6 μg) (lane 1) or Oct-1/POU-homeo (18 μg) (lane 2) was subjected to SDS-PAGE and transferred onto a PVDF membrane. The protein on the membrane was reacted with POD-labeled His-tagged PARS/AMD and visualized with ECL kit (Amersham). Lanes 3 and 4 shows SDS-PAGE protein-stained pattern of the affinity-purified His-tagged Oct-1/homeo and Oct-1/POU-homeo fusion proteins, respectively. An arrowhead indicates the position of purified His-tagged Oct-1/homeo. Standard molecular sizes of marker proteins are shown on the right. B: Interaction of GST-Oct-1/homeo with PARS/AMD, analyzed by co-precipitation with an affinity resin. His-tagged PARS/AMD was incubated with either GST control protein (lane 1) or GST-Oct-1/homeo (lane 2) and the complex was precipitated with a glutathione-affinity resin. The bound proteins were resolved by SDS-PAGE, transferred onto a PVDF membrane and then reacted with anti-PARS antibody and POD second antibody followed by visualization using an ECL kit. Lane 3 shows CBB-stained His-tagged PARS/AMD.

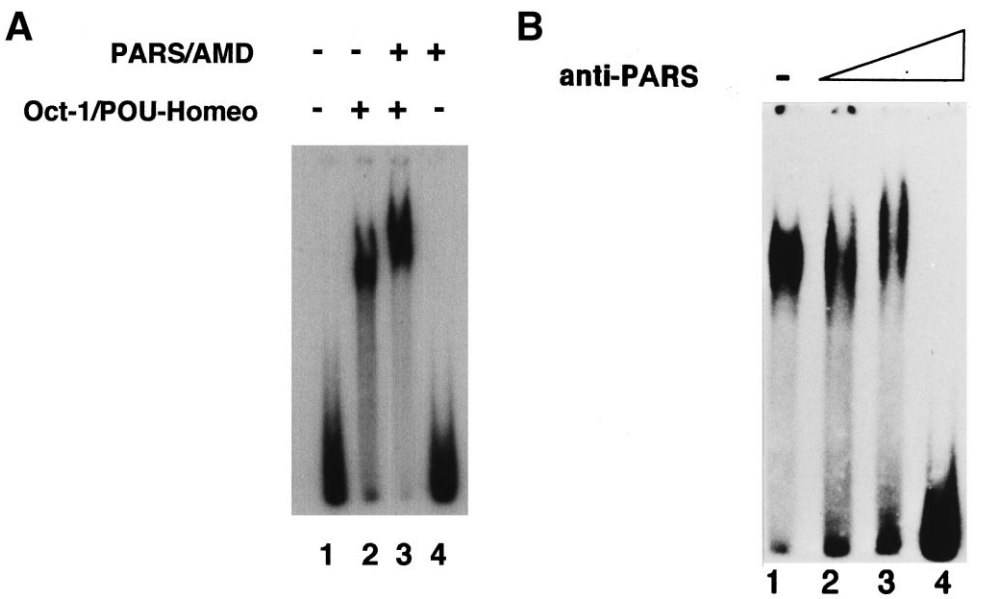

Fig. 3. A: EMSA was performed with DRα promoter DNA, which contains the typical octamer sequence as a probe as described in Section 2. Lane 1: no protein; lane 2: His-tagged Oct-1/POU-homeo (1.2 μg); lane 3: His-tagged Oct-1/POU-homeo (1.2 μg) and His-tagged PARS/AMD (0.5 μg); lane 4: His-tagged PARS/AMD (0.5 μg). White arrow and black arrow indicate Oct-1/POU-homeo-DRa DNA complex and Oct-1/POU-homeo-PARS-DRa DNA complex, respectively. B: Effect of anti-PARS antibody on the binding of OCT-1/POU-homeo and PARS/AMD complex to the DRα promoter DNA. PARS/AMD was incubated in the absence of (lane 1) or in the presence of anti-PARS antibody (0.8, 1.6 or 3.2 μg of IgG for lanes 2, 3 or 4, respectively) for 30 min at 25°C, and then Oct-1/POU-homeo and ^{32}P-labeled DRα DNA probe were added.

purified GST protein or the GST-Oct-1/homeo fusion protein was incubated with His-tagged PARS/AMD at 25°C, and then glutathione-Sepharose resin was added to the reaction mixture. After washing, the affinity resin complex was incubated in an elution buffer containing glutathione. The eluate was subjected to SDS-PAGE and transferred onto a PVDF membrane. The membrane was stained by anti-PARS antibody and POD-labeled second antibody. The His-tagged PARS/AMD was recovered with the GST-Oct-1 fusion protein but not with GST control (Fig. 2B). These observations show that Oct-1/homeo binds to PARS/AMD without the intermediary of third proteins or DNA and does so in a cell-free system as well as in yeast.

3.4. The PARS/AMD and Oct-1/POU-homeo complex bind DNA cooperatively

A question to be solved was whether the physical interaction between Oct-1 and PARS would have any functional consequences. We therefore investigated whether the interaction of these two proteins would affect the sequence-specific DNA binding of the Oct-1/POU-homeo protein. Comparison of the 2-5-2 sequence with intact Oct-1 sequence indicated that the 2-5-2 clone was lacking a part of the helix-loop-helix POU domain as described above. To perform EMSA, we synthesized an upper primer designed in upstream of POU domain and a lower primer designed near the end of the 2-5-2 cDNA and produced 1 kbp Oct-1/POU-homeo cDNA by RT-PCR. The cDNA was inserted into pQE vector and His-tagged Oct-1/POU-homeo fusion protein was purified from *E. coli* M15. The octamer sequence element of the HLA-DRa promoter was used as a probe. Under those conditions of limiting amounts of purified Oct-1/POU-homeo protein, a weak but significant interaction was observed between Oct-1/POU-homeo protein and the octamer sequence of the DRα promoter (Fig. 3A, lane 2). When the His-tagged PARS/AMD was added to the Oct-1/POU-homeo protein, a prominent retarded complex was observed (Fig. 3A, lane 3). The PARS/AMD protein itself did not interact with the probe (Fig. 3A, lane 4). The result indicates that the complex of Oct-1/POU-homeo and PARS/AMD associates with the octamer sequence more tightly than free Oct-1/POU-homeo. To confirm whether the further shifted band was due to the binding of PARS/AMD, we incubated PARS/AMD with antibody against His-tagged PARS/AMD, and then Oct-1/POU-homeo and ^{32}P-labeled DRα DNA probe was added. An excess amount of the antibody diminished the shifted band (Fig. 3B, lane 4). The same amount of pre-immune IgG did not affect the binding (data not shown). These results indicate that Oct-1/POU-homeo interacts with PARS/AMD and the complex associates with the octamer sequence cooperatively.

4. Discussion

We have demonstrated that Oct-1 interacts with the automodification domain of PARS (PARS/AMD) and this interaction increased the affinity of Oct-1 for the octamer motif of the DRα promoter. Octamer consensus sequence has been located in the promoters of several genes, some of which play an important role in cell cycle and differentiation. Among these genes, the binding of Oct-1 to the octamer motif stimulated the transcription of the immunoglobulin gene [26] and the histone H2B gene [27]. Emerging evidence suggests that transcriptional activation via Oct-1 is dependent upon interactions with additional proteins such as Pit-1 [28], OAP [29] or VP16 [30]. In contrast, Oct-1 has also been shown to act as a transcriptional repressor for a number of regulatory regions such as the IL-8 promoter [31]. It has also been reported that Sp1 and Oct-1 interact physically to regulate human U2 snRNA gene expression [32]. Thus, Oct-1 is a target for both positive and negative regulation by protein-protein interaction. The overall level of transcriptional activity is determined by the balance between the binding of Oct-1 and the particular transcriptional activator [33]. Recently Roeder and his group reported that PARS is one functional component of the positive cofactor activity and it enhanced transcription by acting during preinitiation complex formation, but at a stage after binding of transcription factor IID [34]. Considering these observations, the association between Oct-1 and PARS seems to affect, either positively or negatively, essential transcription or tissue-specific transcription. If PARS competes with these essential transcriptional factors to bind to Oct-1, it is possible that transcription is regulated by the interaction with PARS.

Acknowledgements: This work was supported in part by Grants-in-aid for scientific and cancer research from the Ministry of Education, Science and Culture, Japan. We thank Dr. Louise E. Johnstone, Department of Physiology, Edinburgh University for her help with the manuscript.

References

[1] Hayaishi, O. and Ueda, K. (1977) Annu. Rev. Biochem. 46, 95–116.
[2] Purnell, M.R., Stone, P.R. and Whish, W.J. (1980) Biochem. Soc. Trans. 8, 215–227.
[3] Hilz, H. (1981) Hoppe-Seyler's Z. Physiol. Chem. 362, 1415–1425.
[4] Lautier, D., Lagueux, J., Thibodeau, J., Menard, L. and Poirier, G.G. (1993) Mol. Cell. Biochem. 122, 171–193.
[5] Satoh, M.S. and Lindahl, T. (1992) Nature 356, 356–358.
[6] Cole, G.A., Bauer, G., Kirsten, E., Mendeleyev, J., Bauer, P.I., Buki, K.G., Hakam, A. and Kun, E. (1991) Biochem. Biophys. Res. Commun. 180, 504–514.
[7] Ohashi, Y., Ueda, K., Hayaishi, O., Ikai, K. and Niwa, O. (1984) Proc. Natl. Acad. Sci. USA 81, 7132–7136.
[8] Corominas, M. and Mezquita, C. (1985) J. Biol. Chem. 260, 16269–16273.
[9] Taniguchi, T., Morisawa, K., Ogawa, M., Yamamoto, H. and Fujimoto, S. (1988) Biochem. Biophys. Res. Commun. 154, 1034–1040.
[10] Suzuki, H., Uchida, K., Shima, H., Sato, T., Okamoto, T., Kimura, T. and Miwa, M. (1987) Biochem. Biophys. Res. Commun. 146, 403–409.
[11] Tomoda, T., Kurashige, T., Yamamoto, H., Fujimoto, S. and Taniguchi, T. (1991) Biochim. Biophys. Acta 1088, 359–364.
[12] Bhatia, M., Kirkland, J.B. and Meckling, G.K. (1995) Biochem. J. 308, 131–137.
[13] Bhatia, M., Kirkland, J.B. and Meckling, G.K. (1996) Cell Growth Differ. 7, 91–100.
[14] Smulson, M.E., Kang, V.H., Ntambi, J.M., Rosenthal, D.S., Ding, R. and Simbulan, C.M. (1995) J. Biol. Chem. 270, 119–127.
[15] Landschulz, W.H., Johnson, P.F. and McKnight, S.L. (1988) Science 240, 1759–1764.
[16] O'Shea, E.K., Klemm, J.D., Kim, P.S. and Alber, T. (1991) Science 254, 539–544.
[17] Uchida, K., Hanai, S., Ishikawa, K., Ozawa, Y., Uchida, M., Sugimura, T. and Miwa, M. (1993) Proc. Natl. Acad. Sci. USA 90, 3481–3485.
[18] Taniguchi, T., Takahashi, S., Yamamoto, H., Fujimoto, S. and Okayama, H. (1991) Eur. J. Biochem. 195, 557–562.

[19] Harlow, E. and Lane, D. (1988) in: Antibodies – A Laboratory Manual (Harlow, E. and Lane, D., Eds.), pp. 344–348, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[20] Schamboeck, A., Korman, A.J., Kamb, A. and Strominger, J.L. (1983) Nucleic Acids Res. 11, 8663–8675.
[21] Inoko, H., Ando, A., Ito, M. and Tsuji, K. (1986) Hum. Immunol. 16, 304–313.
[22] Durfee, T., Becherer, K., Chen, P.L., Yeh, S.H., Yang, Y., Kilburn, A.E., Lee, W.H. and Elledge, S.J. (1993) Genes Dev. 7, 555–569.
[23] Fields, S. and Song, O. (1989) Nature 340, 245–246.
[24] Harper, J.W., Adami, G.R., Wei, N., Keyomarsi, K. and Elledge, S.J. (1993) Cell 75, 805–816.
[25] Sturm, R.A., Das, G. and Herr, W. (1988) Genes Dev. 2, 1582–1599.
[26] Pierani, A., Heguy, A., Fujii, H. and Roeder, R.G. (1990) Mol. Cell. Biol. 10, 6204–6215.
[27] Das, G. and Herr, W. (1993) J. Biol. Chem. 268, 25026–25032.
[28] Voss, J.W., Wilson, L. and Rosenfeld, M.G. (1991) Genes Dev. 5, 1309–1320.
[29] Ullman, K.S., Flanagan, W.M., Edwards, C.A. and Crabtree, G.R. (1991) Science 254, 558–562.
[30] O'Hare, P. and Goding, C.R. (1988) Cell 52, 435–445.
[31] Wu, G.D., Lai, E.J., Huang, N. and Wen, X. (1997) J. Biol. Chem. 272, 2396–2403.
[32] Strom, A.C., Forsberg, M., Lillhager, P. and Westin, G. (1996) Nucleic Acids Res. 24, 1981–1986.
[33] Sibbet, G.J., Cuthill, S. and Campo, M.S. (1995) J. Virol. 69, 4006–4011.
[34] Meisterernst, M., Stelzer, G. and Roeder, R.G. (1997) Proc. Natl. Acad. Sci. USA 94, 2261–2265.