

cDNA cloning of the murine 30-kDa protein homologous to the 32-kDa subunit of human replication protein A

Masashi Nakagawa, Satoshi Tsukada, Toshihiro Soma, Yoshifumi Shimizu, Seigou Miyake, Akihiko Iwamatsu[1] and Haruo Sugiyama*
The Third Department of Internal Medicine, Osaka University Medical School, Fukushima-ku, Osaka 553 and [1]Central Laboratories of Key Technology, Kirin Brewery Co., Ltd, Soujamachi 1-chome, Maebashi, Gunma 371, Japan

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DNA-binding proteins were purified by using sepharose 4B column coupled with the synthetic 53-bp nucleotide(5′-CCAG-AGACACAGTGAGGGAAGTCCAATGTGAGCCTGCACA-AATACCTCTCTGC-3′) containing a 7mer-23bp-9-mer recombination signal sequence for immunoglobulin heavy chain gene rearrangements. Nuclear extract from Abelson murine leukemia virus-transformed immature B cells (1) was applied to the DNA column, and the DNA-binding proteins were eluted with 2M KCl. Then, the DNA-binding proteins were well separated into three major bands (66-, 54-, and 30-kDa) on SDS−PAGE under reduced conditions. Since the 30-kDa protein was most abundant, this 30-kDa protein was first sequenced. The 30-kDa protein was electroblotted onto PVDF membrane, and in situ digestion was carried out with lysyl-endopeptidase. The amino acid sequences of HPLC-purified peptide fragments were determined by a gas phase sequencer. When a cDNA library prepared from Abelson murine leukemia virus-transformed immature B cells was PCR-amplified using a mixture of oligonucleotide primers synthesized on the basis of the partial amino acid sequences of the 30-kDa protein, only one 462-bp fragment was amplified. Using this 462-bp fragment as a DNA probe for plaque hybridization, a full-sized cDNA clone of 1,447 bp was isolated. This cDNA clone contained a 810-bp open reading frame, predicting that the 30-kDa protein consists of 270 amino acids. This 30-kDa protein had the same numbers of amino acids and 87% homology at amino acid level, compared to the 32-kDa subunit of human replication protein A (RP-A) (2). Therefore, we concluded that this 30-kDa protein is a mouse homologue to the 32-kDa subunit of RP-A. In recent data-base searching we could find no other proteins with significant homology to the 30-kDa protein.

RP-A, a human single-stranded DNA-binding protein, is absolutely required for semiconservative replication of SV40 DNA in vitro (3, 4). RP-A consists of three polypeptides of 70-, 32-, and 13-kDa, and acts with T antigen and topoisomerases to unwind DNA. Our 30-kDa protein is highly homologous (87%) to the 32-kDa subunit of RP-A. Therefore, our 30-kDa protein is thought to be a mouse homologue to the 32-kDa subunit of RP-A. This 30-kDa protein appears to play an important role for DNA replication in mouse. Experiments to determine the biological activities of the 30-kDa protein are currently being carried out.

In comparison with amino acid sequences, Cys^{49} and Cys^{219} are conserved in mouse and human, predicting that a disulfide bond is formed between Cys^{49} and Cys^{219}. When similar amino acids are taken into consideration, the homology is 95%, suggesting that the mouse and human genes are derived from a common ancestral gene.

Initially, this work is planned to purify IgH gene recombination signal sequence-specific DNA-binding proteins. However, under our column conditions, single-stranded DNA-binding proteins appear to be purified as described here.

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         10        20        30        40        50        60

MWNSGFESFTSSTYGGRGGYTQSPGGFGSPTPSQAEKKSRVRAQHIVP|C|TISQLLSATLT
******** ***.*** *************.*********.*******|*|**********.
MWNSGFESYTSSSYGGAGGYTQSPGGFGSPAPSQAEKKSRARAQHIVP|C|TISQLLSATLV

         70        80        90       100       110       120

DEVFRIGDVEISQVTIVGIIRHAEKAPTNIVYKIDDMTAPPMDVRQWVDTDDASGENAVV
*******.******************************* ************.*.**.**
DEVFRIGNVEISQVTIVGIIRHAEKAPTNIVYKIDDMTAAPMDVRQWVDTDDTSSENTVV

        130       140       150       160       170       180

PPETYVKVAGHLRSFQNKKSLVAFKIIPLEDMNEFTAHILEVVNSHMMLSKPNSQASAGR
**************************.*********.*****.*.**.*** *** ****
PPETYVKVAGHLRSFQNKKSLVAFKIMPLEDMNEFTTHILEVINAHMVLSKANSQPSAGR

        190       200       210       220       230       240

PSMSNPGMSESFNFSGNNFMPANRLTVVQNQVLNLIKA|C|PRPEGLNFQDLRSQLQHMPVP
  .*******. **.** ***** ***.**********|*|***********.****** *
APISNPGMSEAGNFGGNSFMPANGLTVAQNQVLNLIKA|C|PRPEGLNFQDLKNQLKHMSVS

        250       260       270

SIKQAVDFLCNEGHIYSTVDDDHFKSTDAE
*********.********************
SIKQAVDFLSNEGHIYSTVDDDHFKSTDAE
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Amino acid sequence of the 30-kDa protein (upper) in comparison with that of the 32-kDa subunit of human replication protein A (lower). Asterisks, identical amino acids; dots, similar amino acids. Boxes indicate the cysteine residues which will form a disulfide bond.