Biochemical and Biophysical Research Communications xxx (2015) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Characterization of DNA binding and pairing activities associated with the native SFPQ·NONO DNA repair protein complex

Durga Udayakumar [a, 1], William S. Dynan [a, b, *]

[a] Institute of Molecular Medicine and Genetics, Georgia Regents University, Augusta, GA 30912, USA
[b] Departments of Radiation Oncology and Biochemistry, Emory University School of Medicine, Atlanta, GA 30322, USA

ARTICLE INFO

Article history:
Received 30 April 2015
Available online xxx

Keywords:
DNA repair
DNA binding
Nonhomologous end joining
SFPQ
NONO

ABSTRACT

Nonhomologous end joining (NHEJ) is a major pathway for repair of DNA double-strand breaks. We have previously shown that a complex of SFPQ (PSF) and NONO (p54^{nrb}) cooperates with Ku protein at an early step of NHEJ, forming a committed preligation complex and stimulating end-joining activity by 10-fold or more. SFPQ and NONO show no resemblance to other repair factors, and their mechanism of action is uncertain. Here, we use an optimized microwell-based assay to characterize the *in vitro* DNA binding behavior of the native SFPQ·NONO complex purified from human (HeLa) cells. SFPQ·NONO and Ku protein bind independently to DNA, with little evidence of cooperativity and only slight mutual interference at high concentration. Whereas Ku protein requires free DNA ends for binding, SFPQ·NONO does not. Both Ku and SFPQ·NONO have pairing activity, as measured by the ability of DNA-bound protein to capture a second DNA fragment in a microwell-based assay. Additionally, SFPQ·NONO stimulates DNA-dependent protein kinase autophosphorylation, consistent with the ability to promote formation of a synaptic complex formation without occluding the DNA termini proper. These findings suggest that SFPQ·NONO promotes end joining by binding to internal DNA sequences and cooperating with other repair proteins to stabilize a synaptic pre-ligation complex.

1. Introduction

Nonhomologous end joining (NHEJ) is the default pathway for DNA double-strand break repair in mammalian cells. In classical, or canonical, NHEJ, Ku protein recognizes broken DNA ends and recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and other DSB repair factors (reviewed in Refs. [1–3]). Previously, we observed that purified core NHEJ factors were insufficient for DNA end joining *in vitro*, and that activity was greatly stimulated by a heterodimer of SFPQ (also known as polypyrimidine tract binding protein-associated splicing factor, or PSF) and NONO (also known as 54 kDa nuclear RNA binding protein, or p54^{nrb} [4,5]. Both SFPQ and NONO have since been found to participate in the DNA damage response *in vivo* [6–10]. They also have independent functions in RNA biogenesis and the regulation of gene expression (for example [11–13], reviewed in Refs. [14,15]).

SFPQ and NONO are part of a small family of tandem RNA recognition motif-containing proteins, with no evident sequence similarity to other known repair factors, and their mechanism of action is hence not clearly understood. Here we describe use of a novel binding assay to characterize DNA interaction and pairing activities of native SFPQ·NONO isolated from cultured mammalian cells. Data are consistent with a model where SFPQ·NONO and Ku protein interact with substrate DNA simultaneously at non-overlapping sites, stabilizing assembly of a repair complex.

2. Materials and methods

2.1. DNA substrates

A 2686 bp plasmid (pUC19, Invitrogen, Carlsbad, CA) was linearized with HindIII. Following phenol:$CHCl_3$ extraction and EtOH precipitation, DNA was incubated with 40 μM biotin-7-dATP, 100 μM each of dTTP, dCTP, and dGTP, 50 mM Tris–HCl pH 7.9, 10 mM $MgCl_2$, 100 μM dithiothreitol, 50 μg/ml bovine serum albumin, and 2 Units of Klenow fragment DNA polymerase at 37 °C for 1 h. The reaction was heated to 70 °C for 20 min and products were isolated using a G50 Sephadex spin column (Roche

* Corresponding author. Department of Biochemistry, 4121 Rollins Research Center, 1510 Clifton Rd. NE, Atlanta, GA 30322, USA.
E-mail address: wdynan@emory.edu (W.S. Dynan).
[1] Present address: Department of Radiation Oncology, Houston Methodist Research Institute, Houston, TX 77030, USA.

http://dx.doi.org/10.1016/j.bbrc.2015.05.024
0006-291X/

Diagnostics, Indianapolis, IN). Biotin labeling was confirmed as described [16]. DNA was digested with BamHI, PstI, or ScaI to create single free 5′ protruding, 3′ protruding, or blunt ends, respectively, and then gel purified.

2.2. Protein purification

Ku protein was expressed using baculovirus vectors and purified as described [17]. SFPQ·NONO were prepared to near homogeneity from human (HeLa) cells as described [5]. Fractions from the final Mono S chromatographic step were used except where otherwise indicated. SFPQ and NONO are the major polypeptide components of the purified fraction (refer to Fig. 1 of Ref [5]) and have been shown to be essential for its end-joining stimulatory activity. DNA-PKcs was prepared as described except that the phenyl-Superose and Mono S chromatography steps were omitted [18].

2.3. DNA binding assays

DNA binding assays were performed in Reacti-BindT streptavidin-coated polystyrene strip plates with Super BlockT (Pierce Biotechnology, Rockford, IL). All incubations were performed in a volume of 50 μl. Wells were incubated with 3% bovine serum albumin (Fisher Scientific, fraction V) in Buffer DB (100 mM KOAc, 20 mM Tris−HCl (pH 7.9), 0.5 mM EDTA, 10% glycerol, 10 μg/ml phenymethylsulfonylfluoride, and 1 μg/ml each of soybean trypsin inhibitor, aprotinin, leupeptin, pepstatin A) for 2 h at room temperature. Wells were washed 3 times with Buffer DB buffer, and biotinylated DNA (0.12 pmol) was added and incubated for 1 h. In assays using doubly-blocked DNA, additional streptavidin (0.5 μg) was added and incubation was continued for 30 min. Test proteins were added and incubated for 1 h. After washing to remove unbound proteins, primary antibody, diluted in blocking buffer (1% bovine serum albumin, 0.02% γ-globulin in buffer DB) was added and incubated for 3 h at room temperature or overnight at 4 °C. Primary antibody was either monoclonal anti-Ku80 (mAb 111, Abcam, Cambridge, MA) or and anti-SFPQ·NONO patient serum (kind gift of Dr. Yoshihiko Takeda, Georgia Regents University). Wells were washed three times with buffer DB containing 0.05% Tween 20. Alkaline phosphatase-conjugated secondary antibody (anti-mouse IgG, or anti-human IgG, Sigma Chemicals, St. Louis, MO), diluted 1:30,000 in blocking buffer, was added and incubation was continued for 3 h at room temperature or overnight at 4 °C. Wells were washed three times in buffer DB with Tween 20 and twice in buffer DB without Tween 20. Wells were developed with a freshly prepared solution of 100 μl of KPL Bluephos substrate (KPL laboratories). Incubation was continued for 30 min and absorbance was measured at 595 nm. Assays were performed in duplicate and error bars indicate range.

2.4. DNA capture assay

Assays were performed using Reacti-BindT streptavidin-coated polystyrene strip plates. Radiolabeled DNA substrate was the same as described previously for end-joining reactions [5] and was prepared by digestion of pUC19 with BamHI, treatment with alkaline phosphatase, and 5′-end labeling with polynucleotide kinase and [γ-^{32}P] ATP. Microwells were loaded with DNA as described above. Separately, a mixture of proteins and radiolabeled capture DNA substrate was prepared in buffer DB. The mixture was transferred into the microwell and incubated 30 min at room temperature. Wells were washed three times with 0.5X buffer DB and retention of label was measured by liquid scintillation counting and reported as counts per minute (cpm).

2.5. DNA-PKcs activity assay

DNA-PKcs activity assays were performed essentially as described [19]. Phosphorylation reactions contained, in a volume of 10 μl, 25 mM Tris−HCl, pH 7.9, 25 mM $MgCl_2$, 1.5 mM DTT, 50 mM KCl, 10% glycerol, 20 nM pUC19 plasmid digested with BamHI, 0.16 μM [γ-32 P] ATP (6000 Ci/mmol), 10 nM DNA-PKcs, 20 nM Ku protein, and purified SFPQ·NONO complex as indicated in the figure legend. Reactions were incubated for 30 min at 30 °C.

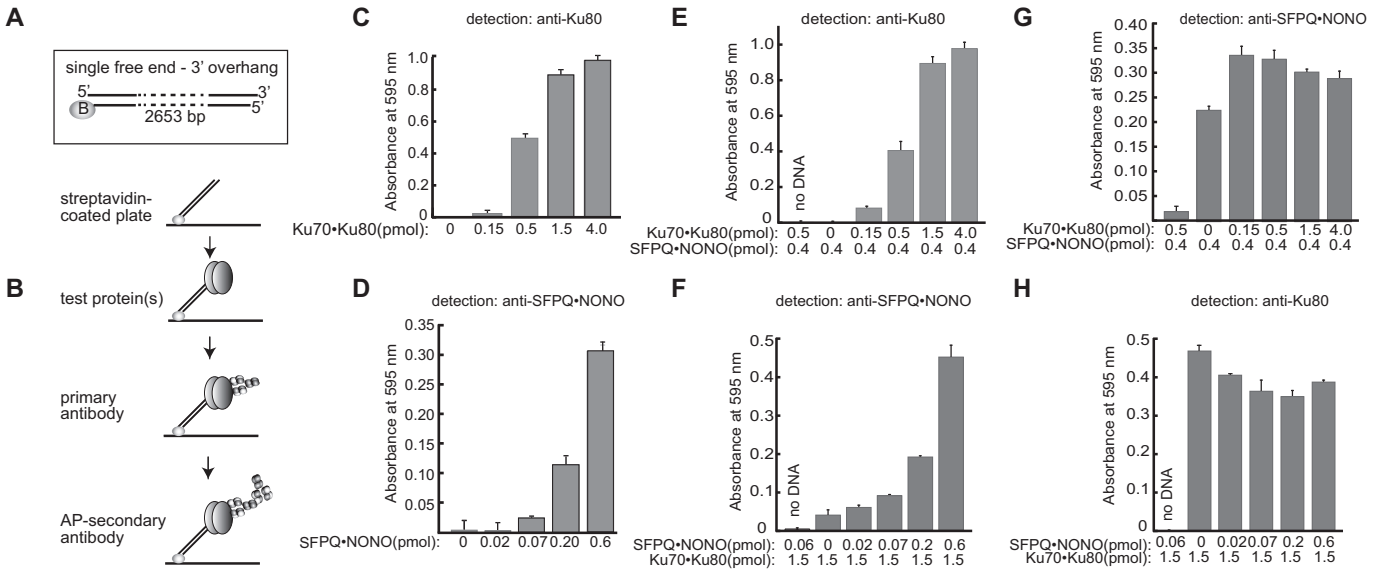

Fig. 1. Assay design and validation A. Linearized, singly biotinylated plasmid substrate used in this experiment. **B**. Assay schematic. Biotinylated DNA was bound to streptavidin-coated microwells, proteins were added, and bound proteins were detected by ELISA. Preliminary experiments (not shown) were performed to identify optimal conditions for blocking the microwells, washing, and immunodetection of bound proteins. **C, D**. Binding assays using 0.12 pmol DNA and either Ku70·Ku80 or SFPQ·NONO (amounts in pmol). Detection antibody indicated. **E, F, G, H**. Binding assays using mixtures of Ku70·Ku80 and SFPQ·NONO (amounts in pmol). Detection antibody indicated. DNA was omitted from some reactions where indicated. Experiments in different panels were performed in parallel and are thus directly comparable.

Please cite this article in press as: D. Udayakumar, W.S. Dynan, Characterization of DNA binding and pairing activities associated with the native SFPQ·NONO DNA repair protein complex, Biochemical and Biophysical Research Communications (2015), http://dx.doi.org/10.1016/j.bbrc.2015.05.024