Short Communication

Induction of *recA* in prespore cells in *Dictyostelium discoideum* slug following UV-light irradiation

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SUMMARY

Dictyostelium discoideum amoebae grow as unicellular microorganisms until nutrients are depleted, while they aggregate and form multicellular structures under a starved condition. We established *D. discoideum* expressing a green fluorescent protein (GFP) under control of an upstream sequence of the gene for RecA, a mitochondria-localizing DNA repair enzyme. Multicellular structures formed by the strain were irradiated by 50 J/m^2 UV light (254 nm) and GFP expression was analyzed. Fluorescence of GFP was observed not in the prestalk cells but in the prespore cells in the multicellular structures. The prestalk cells are programmed to die, while the prespore cells differentiate into viable spores. *D. discoideum* is thought to repair DNA damage in the prespore cells and maintain genomic integrity using a limited energy source.

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Organisms are continuously exposed to DNA-damaging agents such as UV light and chemicals, but they have a network of mechanisms by which DNA damage is identified and repaired. This network is thought to be essential for organisms to maintain genomic integrity. We have analyzed the molecular basis of DNA repair in the cellular slime mold *Dictyostelium discoideum*, which has been shown to be highly resistant to DNA-damaging agents (Deering, 1988; Yu et al., 1998). We have shown in a previous study that *D. discoideum* encodes RecA, an ortholog of *Escherichia coli* RecA. *D. discoideum* RecA localizes in mitochondria and a *recA*-deficient mutant is hypersensitive to DNA-damaging agents, indicating that RecA is responsible for repair of damage in mitochondrial DNA of *D. discoideum* (Hasegawa et al., 2004).

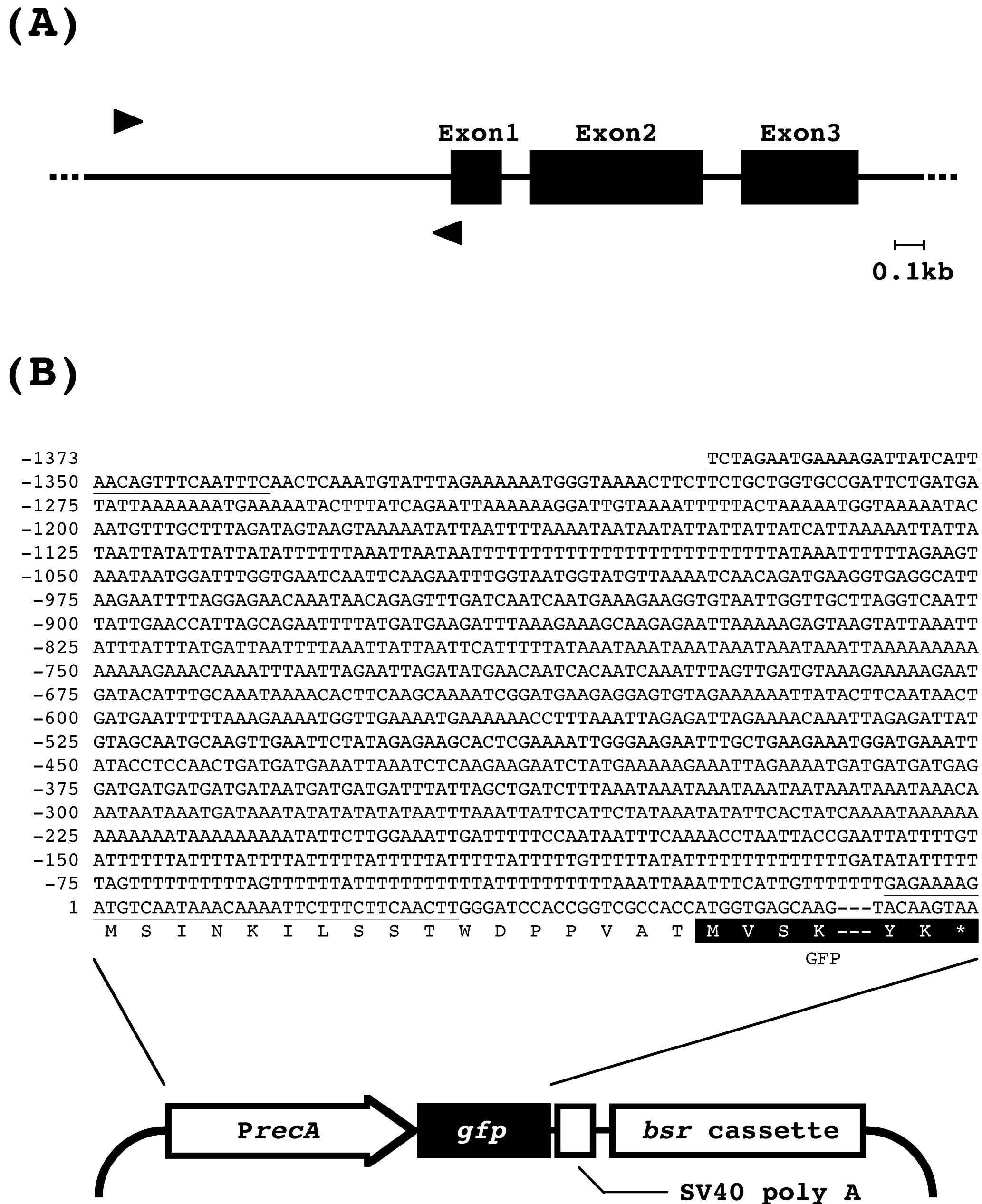

Fig. 1. Nucleotide sequence of the upstream region of *D. discoideum recA*. (A) Illustration of *recA* on *D. discoideum* genome. Exons are indicated by black boxes. Primers (5'-TCTAGAATGAAAAGATTATCATTAACAGTTTCAATTTC and 5'-AAGTTGAAGAAAGAATTTTGTTTATTGACATCTTTTCTC) used in polymerase chain reaction (PCR) are indicated by arrowheads. (B) Nucleotide sequence of the upstream region and illustration of the plasmid pDREPORT2. The upstream sequence was amplified by PCR using Pyrobest DNA polymerase (Takara Bio, Shiga, Japan). Primer annealing sites are underlined. The reaction products were inserted into a *Sma*I site of a pEGFP-N (Clontech, CA, USA). Then the expression cassette consisting of the upstream sequence of *recA* (P*recA*), GFP coding sequence (*gfp*) and SV40 polyadenylation sequence (SV40 poly A) was cloned on the *D. discoideum* extrachromosomal vector.

D. discoideum amoebae grow as unicellular microorganisms until nutrients are depleted. Under a starved condition, amoebae aggregate and form a multicellular structure consisting of approximately 10^5 cells. Cells in a multicellular structure differentiate into two major cell types, prespore cells and prestalk cells. The prespore cells differentiate into viable spores during the developmental process. The prestalk cells are programmed to form stalk, basal disc, upper cup and lower cup, which are required to hold the spore mass high off the ground. Cells consisting of these compartments die during the development (reviewed by Kessin, 2001).

D. discoideum completes the developmental process in approximately 24 h without incorporation of an exogenous energy source. The issue we wanted to resolve in this study is whether *D. discoideum* repairs damage in all cells of its body. This organism might have an order of priority in which cells are repaired because it has a limited energy source. To address this issue, we analyzed the expression level of *recA* in cells of *D. discoideum* multicellular structures.

We constructed a plasmid carrying green fluorescent protein (GFP) under control of the *recA* promoter and established *D. discoideum* harboring the plasmid. A DNA fragment (1404 bp) containing a 1373-bp upstream region of the *recA* structural gene was amplified from the *D. discoideum* genome for plasmid construction (Fig. 1). The plasmid constructed in this study, pDREPORT2, carried a gene cassette for blasticidin S (BS) resistance (a *bsr* cassette). This gene cassette consisting of *actin15* promoter, BS resistance gene and transcription terminator confers BS resistance on *D. discoideum* (Adachi et al., 1994). The backbone of this plasmid is an extrachromosomal plasmid, pTIKL-Bsr-Exp (Larochelle et al., 1997). *D. discoideum* Ax2 grown in an HL5 medium (Watts and Ashworth, 1970) was transformed by pDREPORT2 and cultured in the medium supplemented with 5 mg/l BS for selection.

D. discoideum amoebae harboring pDREPORT2 were irradiated by 50 J/m^2 UV light (254 nm) and incubated in an HL5 for 6 h. Then, expression levels of GFP were analyzed under a microscope. Exposure of *D. discoideum* to 50 J/m^2 UV light was sublethal, resulting in 97% viability (Hasegawa et al., 2004). Amoebae that had not been irradiated expressed a low level of GFP, while irradiated amoebae expressed GFP at a high level (Fig. 2A). The expression level of GFP was increased in almost all cells, indicating that all

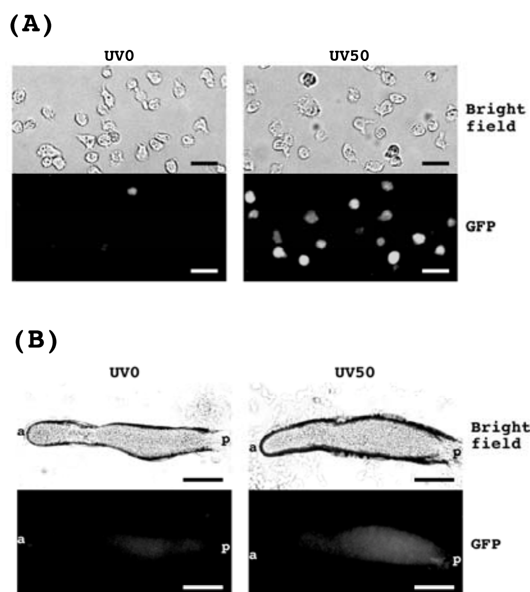

Fig. 2. Expression of GFP following UV-light irradiation. (A) GFP expression in amoebae following irradiation. *D. discoideum* amoebae carrying pDREPORT2 were cultured in HL5 supplemented with 5 mg/l BS, washed with a buffer (1.44 g/l NaH_2PO_4, pH adjusted to 6.1 with KOH), irradiated by 50 J/m^2 UV light (254 nm) using a UV cross-linker (UVP, CA, USA), and incubated. Then the expression levels of GFP were analyzed under a BHS-RFK microscope (Olympus, Tokyo, Japan). Scale bars: 20 µm. (B) GFP expression in the slug irradiated by UV light. *D. discoideum* amoebae carrying pDREPORT2 were collected, washed with the buffer, plated on a non-nutrient agar plate at a density of 1 x 10^6 cells/cm^2, and incubated. Multicellular structures formed on the plate were irradiated by 50 J/m^2 UV light (254 nm) and incubated, and then the expression levels of GFP were analyzed. a, anterior ; p, posterior. Scale bars: 100 µm.

cells induced RecA to repair DNA damage.

We next analyzed GFP expression in multicellular structures irradiated by UV light. *D. discoideum* cells aggregate and organize into a structure with polarity. Spatial organization of differentiating cells is apparent at the slug stage; prestalk cells and prespore cells localize to the anterior 20% and posterior 80% of the slug, respectively. We therefore analyzed GFP expression at the slug stage. The transformed amoebae were washed with a buffer, plated on a non-nutrient agar plate, and incubated. Multicellular structures formed on the plate were irradiated by 50 J/m^2 UV light and incubated for 6 h. Then, expression levels of GFP were analyzed. Intensity of the fluorescence of GFP in the region of prespore cells was higher than that in the region of prestalk cells (Fig. 2B).

Our experimental results suggest that *D. discoideum* has an order of priority in which cells are repaired. Prespore cells differentiate into viable spores, while prestalk cells are programmed to die. Under a starved condition, *D. discoideum* would not have a sufficient energy source to save all cells. Thus, this organism repairs damage in prespore cells using the limited energy source.

We are planning to examine whether other enzymes responsible for DNA repair are also induced in prespore cells.

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