**Materials and methods**

**Cell culture.** *Dictyostelium discoideum* Ax2 cells (wild type) and REMI mutant GWDI\_488\_A\_5 cells were from the *Dictyostelium* Stock Center (Chicago, IL) [1]. Cells were cultured in HLG0102 HL5 (Formedium, Hunstanton, UK) at 21 ˚C on a rotary shaker at 175 rpm.

**cDNA synthesis and normalization.** Total RNA was extracted from 5 x 106 cells at mid-log phase (~1 x 106 cells/ml) using a R1054 RNA prep kit (Zymo Research, Irvine, CA). 3 µg RNA was used for directional double-strand cDNA (ds-cDNA) library synthesis with the primers 5’ - AAGCAGTGGTATCAACGCAGAGT***ACTAGT***GGGG - 3’ (*SpeI* site bolded) and 5’ - AAGCAGTGGTATCAACGCAGAGT***AGATCT***TTTTGTTTTTTCTTTTTTTTTTTTTVN -3’ (*BglII* site bolded) using a Mint-2 cDNA synthesis kit (Evrogen, Moscow, Russia). The resultant cDNA has a *SpeI* site on the end corresponding to the 5’ end of the mRNA and a *BglII* site on the other. 1.2 µg of this ds-cDNA library was then normalized using the Trimmer-2 kit (Evrogen). For the normalization, the double-stranded cDNA library was denatured to single-stranded DNA at 98 °C for 2 minutes and then allowed to hybridize to double-stranded DNA again at 68 °C for 5 hours. This hybridization process generated two populations of cDNA, successfully re-paired double-stranded DNA and unpaired single-stranded DNA. Abundant transcripts hybridize to double-stranded DNA more efficiently than rare transcripts [2]. The double-stranded cDNA, composed primarily of abundant transcripts, was then degraded by the duplex-specific nuclease (provided from the kit). All procedures followed the manufacturers’ instructions.

**Construction of a normalized antisense cDNA library.** The remaining single stranded cDNA, corresponding to a cDNA library with many of the abundant transcripts removed, was amplified by PCR with the primer 5’ - AAGCAGTGGTATCAACGCAGAGT - 3’ following the Trimmer-2 kit instructions. The enriched PCR product and the vector plasmid pDM326 containing the blasticidin resistance cassette [3] were digested for 4 hours at 37 ˚C with the restriction enzymes BglII and SpeI. Digested PCR products larger than 200 bp were purified and enriched using a Select-a-Size DNA Clean & Concentrator kit (Zymo). Digested pDM326 was gel purified using a D2500-01 Gel Extraction kit (Omega, Norcross, GA). 100 ng digested cDNA and 50 ng digested pDM326 were then ligated at 16 ˚C overnight using T4 ligase (NEB, Ipswich, MA). The ligation was concentrated with a DNA Clean & Concentrator-5 kit (Zymo) to a volume of 10 µl. The ligation was then used to transform 5-alpha electrocompetent *E. coli* cells (NEB) following the manufacturer’s protocol with 1 µl purified ligation product and 25 µl competent cells per transformation. 1/10 of the total transformed cells from one transformation were plated to one LB/AMP agar plate (100 µg/ml ampicillin) to determine the library size. The total transformed cells from 10 µl ligation product were plated on 35 LB/AMP agar plates and incubated at 37 ˚C overnight. Colonies were collected using a plate scraper with 3 ml LB per plate. The collected cells were grown in 500 ml of LB to an OD 600 of ~3, and 25 ml of this culture was mixed with 25 ml of 50% glycerol in H2O, and aliquots were stored at -80 ˚C. The remaining culture was used for a plasmid DNA maxi-prep using a D4203 Maxi-prep kit (Zymo) and the plasmid DNA was used to transform *D. discoideum* cells.

**Transformation of *Dictyostelium discoideum*.** Wild-type Ax2 cells were transformed by electroporation following [4]. For each electroporation, 4 x 107 cells in 180 µl H50 buffer (20 mM HEPES, 50 mM KCl, 10 mM NaCl, 1 mM MgSO4, 5 mM NaHCO3, 1 mM NaH2PO4, pH 7.0) were mixed with 20 µl DNA (500 ng) and incubated on ice for 5 minutes. This mixture was then loaded into an EC2L 2-mm electroporation cuvette (Midsci, Valley Park, MO) and electroporated with a GenePulser Xcell electroporator (Bio-Rad, Hercules, CA) at 850 V and 25 µF, with 2 pulses with a 5 second gap. Electroporated cells were then cultured in 10 ml HL5/ 100 µg/ml ampicillin in a 100-mm tissue culture petri dish. 10 µg/ml blasticidin (GoldBio, St. Louis, MO) was added 16-20 hours later for selection of transformed cells. Colonies normally appeared after 5-7 days and were transferred to shaking culture for screen assays. Each plate typically contained 800 colonies.

**Screen for transformants resistant to polyphosphate proliferation inhibition**. Wild-type Ax2 cells and the pool of transformants were cultured in HL5 (containing 10 µg/ml blasticidin for transformants) with a cycle of 2 days of 150 µM SO169 polyphosphate (Spectrum, New Brunswick, NJ) and two days of no polyphosphate. Each cycle started with cells at 5 x 105 cells/ml, and after two days, cells were washed with HL5, counted, and diluted to 5 x 105 cells/ml again. Cell densities were counted daily to calculate proliferation rates. After 4 to 5 cycles, compared to Ax2 cells, transformants pools which had a significantly faster proliferation rate in the presence of polyphosphate were plated on lawns of *E. coli* on SM/5 (2 g/L glucose, 2 g/L bacto peptone, 0.2 g/L yeast extract, 0.2 g/L MgSO4·7H2O, 1.9 g/L KH2PO4, 1 g/L K2HPO4, 15 g/L agar) plates for single clone selection. Cells in plaques on the lawns were then picked and cultured in HL5 plus polyphosphate to verify the phenotype of abnormally fast proliferation in the presence of polyphosphate. Clones passing this verification were selected for further analysis.

**Isolation and cloning of antisense cDNAs.** The antisense cDNA plasmid was extracted from 3 x 106 mid-log phase transformed *Dictyostelium* cells using a ZR plasmid miniprep kit (Zymo) and then used to transform chemical competent *E. coli* (Lucigen, Middleton, WI) on the same day. After selection and growth, the antisense cDNA plasmid was then extracted from the transformed *E. coli* using the same kit as above. The antisense cDNA was digested with BglII and SpeI, gel purified, and ligated into a modified pGEM-T vector (BglII site incorporated, a gift from Dr. Beiyan Nan, Texas A&M University). The ligation was then used to transform DH5-alpha *E. coli.* ThepGEM-antisense cDNA plasmid was isolated from these transformed cells and used for sequencing.

**Construction of *sodC* knock-down strain by antisense repression.** PCR was done using the cDNA library to generate the full-length fragment of *sodC* with the primers forward 5’- CGC***ACTAGT***ATGAGACTTTTATCTGTATTAG -3’ and reverse 5’- GCG***AGATCT***TTAAAGCAAAGCAAAGATAAT -3’, and a truncated version of *sodC* with the primers forward 5’- GCG***ACTAGT***GATGGATACTGGTTACTA -3’ and reverse 5’- GCG**AGATCT**TTAAAGCAAAGCAAAGATAAT -3’. PCR products were digested with BglII and SpeI and ligated into the pDM304 vector [3] in the antisense direction. pDM304-antisense-sodC was used to transform Ax2 cells by electroporation and transformants were selected with 10 µg/ml G418.

**Proliferation inhibition assay.** Cells at 1.5 x 106/ml were cultured with 0, 125 and 150 µM polyphosphate in HL5 for 24 hours and cells were then counted. The data were plotted as 100 x (density with polyphosphate – 1.5 x 106/ ml) / (density with no added polyphosphate – 1.5 x 106/ ml). Data were analyzed by t-test using Prism 7 (Graphpad, La Jolla, CA). Significance was defined as p < 0.05.

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2. Hames BD, Higgins SJ. *Nucleic acid hybridisation: a practical approach*. IRL Press, (1985).

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4. Gaudet P, Pilcher KE, Fey P, Chisholm RL. Transformation of Dictyostelium discoideum with plasmid DNA*.* *Nature protocols* 2(6), 1317 (2007).