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Overexpression of S-adenosylmethionine decarboxylase impacts polyamine homeostasis during development of *Dictyostelium discoideum*

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ABSTRACT The polyamines putrescine, spermidine and spermine are essential polycations involved in the regulation of cellular proliferation. They exert dynamic effects on nucleic acids and macromolecular synthesis *in vitro* but their specific functions *in vivo* are poorly understood. Here, we have modulated the spermidine levels either by overexpressing the *S-adenosylmethionine decarboxylase (samdc)* gene or treating the cells with methylglyoxal-bis (guanylhydrazone) (MGBG), an inhibitor of SAMDC. In *Dictyostelium*, overexpression of SAMDC slowed cell proliferation, delayed development and arrested cells in the S-phase of the cell cycle. Treatment with MGBG reduced cell proliferation and stimulated development, but in *samdc*^{OE} cells, it increased cell proliferation suggesting critical levels of spermidine to be important. In *samdc*^{OE} cells, spermidine levels remained high throughout development but only small changes in the spermine levels were observed. Initial putrescine levels did increase but reverted to wild-type levels after the mound stage. As tight regulation of polyamine homeostasis is required, we identified genes that could be involved in its maintenance. In conclusion, we characterised *samdc*^{OE} cells and observed the maintenance of polyamine homeostasis during the development of *Dictyostelium* cells.

KEY WORDS: *Dictyostelium, SAMDC, polyamine, transporter, homeostasis*

The naturally abundant polyamines (PAs), putrescine (put, diamine), spermidine (spd, triamine) and spermine (spm, tetramine) are involved in various cellular processes like modulation of chromatin structure, gene transcription and translation, DNA stabilization, signal transduction, cell growth and proliferation (Handa *et al.,* 2018). Increased PA levels are coupled with increased cell proliferation, decreased apoptosis and increased expression of genes associated with tumor invasion and metastasis, thus making their metabolism a target for cancer treatment and prevention (Ye *et al.*, 2016). The free cellular PA pools are maintained within a very narrow range and is regulated in a precise manner, which is achieved at four levels: *de novo* synthesis, inter-conversion, terminal degradation and transport (Wallace, 1996).

Dictyostelium discoideum is a good model system to analyse the functions related to growth and differentiation. It lives as a unicellular amoeba when feeding on bacteria but upon starvation they aggregate at common collecting points to undergo development into multicellular structures. Muticellularity arises due to the coming together of the spatially segregated cell-types, which undergo various morphogenetic movements to finally culminate into a fruiting body consisting of viable spores and dead stalk cells (Tarnita *et al.*, 2015).

Earlier, we showed the involvement of put in growth control and spd in development of *Dictyostelium* (Saran, 1998; Kumar *et al.*, 2014). Here, we modulated the levels of spd, (which in turn modulates other PA levels) by constitutively expressing the *samdc* gene (*samdc*^{OE}) and/or by treatment with exogenous methylglyoxal-bis (guanylhydrazone) (MGBG), an inhibitor of SAMDC (Regenass *et al.*, 1992). During PA biosynthesis, S-adenosyl-L-methionine decarboxylase (SAMDC, EC 4.1.1.50) converts the methyl donor and ethylene precursor S-adenosyl-L-methionine (SAM), to decarboxylated S-adenosyl-L-methionine. Decarboxylated SAM serves as an aminopropyl donor in the synthesis of higher PAs, spd and spm from the put (Moschou *et al.*, 2008) (Supplementary Fig S1).

Abbreviations used in this paper: MGBG, methylglyoxal-bis (guanylhydrazone); ODC, ornithine decarboxylase; PA, polyamine; put, putrescine; SAMDC, S-adenosylmethionine decarboxylase; spd, spermidine; spm, spermine.

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We show *samdc*^{OE} cells are slow growers, arrest in S-phase and has a bias towards stalk pathway. Also, the absolute spd level was high during terminal differentiation, no significant change in spm levels but initial put level was increased. This prompted us to observe the developmental changes in catabolic, anabolic and transporters involved in PA homeostasis.

Results

Overexpression of samdc (samdc^{OE}*)*

The phylogenetic tree shows DdSAMDC to be closer to *Heterostelium album* (*Heterostelium* gen. nov. Acytosteliaceae) and shares 45% similarity with humans (Supplementary Fig. S2A, B). We earlier expressed the *samdc* gene (DDB_G0275567) as a fusion protein with the eYFP reporter at the C-terminal under *actin15* promoter (Supplementary Fig. S2C; Kumar *et al.*, 2018). The *samdc* transcript levels increased till the slug stage, after which it decreased till fruiting body formation. The fusion protein showed largely cytosolic localization (Supplementary Fig. S3A, B).

Effect of methylglyoxal-bis (guanylhydrazone) (MGBG)

The intracellular spd levels were modulated both at the genetic level by creating *samdc*^{OE} and biochemically by treating with MGBG. Interestingly, the level remained higher than that observed in Ax2 cells upon treatment with MGBG, in case of *samdc*^{OE} cells (Supplementary Fig. S2D).

Fig. 1A shows cell proliferation in Ax2 and *samdc*^{OE} cells both in the presence and absence of MGBG. Both control and MGBG treated Ax2 cells showed maximum cell density of 13.5 x10^6 ± 0.81 and 7.1x10^6 ± 0.26 cells/ml, respectively at t72 after which it declined. On the other hand, *samdc*^{OE} cells showed a longer lag period of 24h and reached maximum cell density of 13.93 x10^6 ± 0.7 cells/ml by t96. Interestingly the decline was not pronouced till t120. Upon treatment with MGBG we observed a longer lag period with maximum cell density of 12.63 x10^6 ± 0.17 cells/ml at t72 and a decline thereafter. The size of Ax2 and *samdc*^{OE} + MGBG cells were more or less similar, while MGBG treated Ax2 cells and the *samdc*^{OE} cells had increased (Fig. 1B).

Cell-viability during cell proliferation was examined through cell regrowth and MTT assay (Supplementary Fig. S3 C,D). Cell regrowth was analysed in aging population where t0 indicates the time from midpoint of stationary culture (Jain *et al.,* 2016). A sharp decrease upon MGBG treatment of Ax2 cells as compared to untreated was observed. Nearly 50 ± 0.38% cell death after 72h in both the cases was observed. Cell survival was better in *samdc*^{OE} cells but reduced to nearly 50 ± 2.62% by 72h. Upon MGBG treatment the survival was comparable to Ax2 cells alone. Both Ax2 and *samdc*^{OE} cells showed a decline after 96h but upon MGBG treatment, it reduced significantly after 24h and reached nearly 50 ± 1.63% by 120h in Ax2 cells but the decline was not to

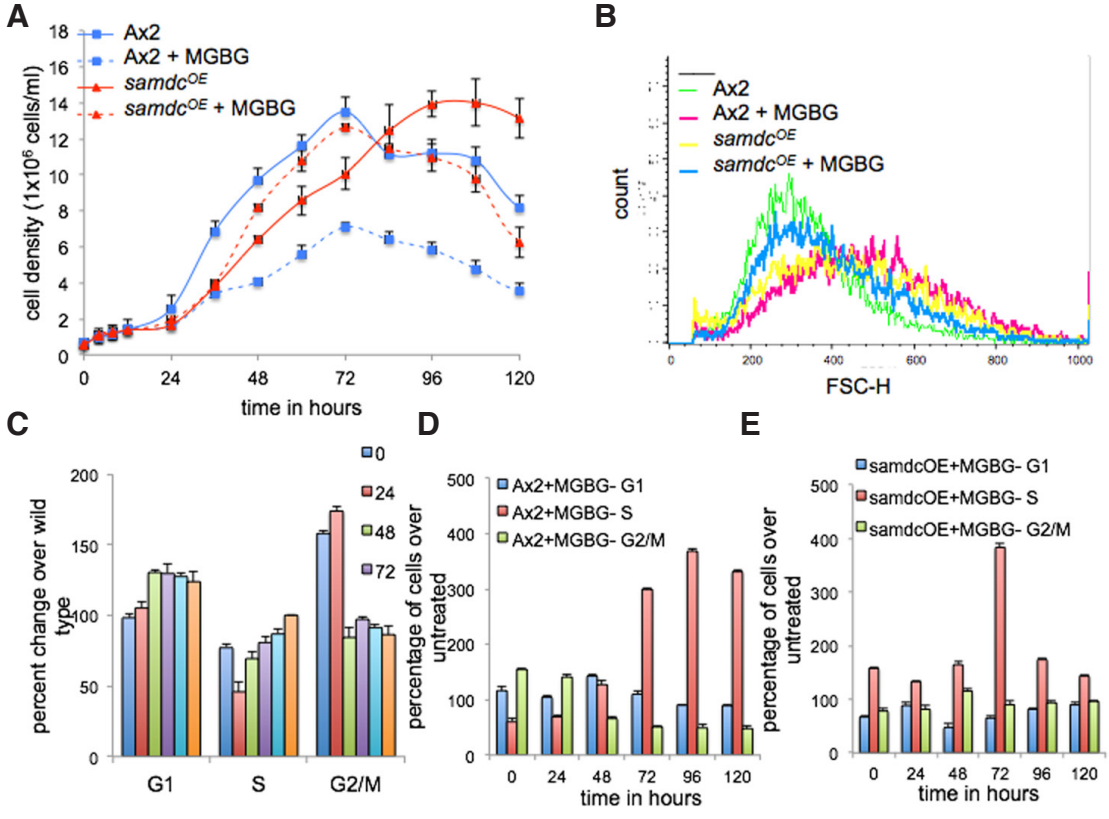

Fig. 1. Effect of methylglyoxal-bis (guanylhydrazone) (MGBG) on cell proliferation, cell size and cell cycle. (A) *Cell proliferation studies with Ax2,* samdc^{OE} *in the presence and absence of MGBG. The* samdc^{OE} *cells showed slow cell proliferation.* **(B)** *Cell-size analysis of wild-type and* samdc^{OE} *cells in the presence and absence of MGBG (1 mM) at different time-points (0, 24, 48, 72, 96 and 120 h).* **(C)** *Bar diagram represents relative percent over the wild-type in* samdc^{OE} *cells at different time points. Data represents relative percentage of cells after treatment with MGBG and normalization with untreated of the same time-point for* **(D)** *Ax2 and* **(E)** samdc^{OE}. *(n=3).*

the same extent for *samdc*^{OE} cells.

The differences in cell proliferation prompted us to analyze cell cycle progression. Fig. 1C shows the percent change over Ax2 cells at the given time-points for *samdc*^{OE} cells. An increase in S-phase over time and a corresponding decrease in G2/M-phase was observed. Similar results were obtained with MGBG treatment in Ax2 cells (Fig. 1D). MGBG treatment showed an increase in S-phase till 72h and a decline thereafter over untreated *samdc*^{OE} cells (Fig. 1E). Insignificant changes in the G2/M-phase and a small decrease in G1-phase was observed.

Further, cells were plated at equal densities (5x10^7 cells/ml) and allowed to complete development after synchronization (Fig. 2A). Ax2 cells completed their development by forming aggregation territories by 12h, slugs at 16h and culminant by 24h. All three, Ax2 + MGBG, *samdc*^{OE} and *samdc*^{OE} + MGBG showed asynchrony and developed small-sized territories as compared to untreated Ax2 cells. All *samdc*^{OE} aggregates did not culminate into fruiting bodies and showed aborted intermediates. Fig. 2B shows enlarged images of the fruiting bodies formed. MGBG treated Ax2 cells formed fruiting body with small sorus and stouter stalk and basal-disc. The *samdc*^{OE} cells formed very long stalks with small sorus but upon MGBG treatment, stalks became stouter but shorter than the untreated cells. Further, upon MGBG treatment, spore-viability of Ax2 cells decreased (35.33 ± 4.04 %) while no change (89.6 ± 2.51%) was observed with *samdc*^{OE} cells (Supplementary Fig. S4A). Upon MGBG treatment, the number of aggregates formed by Ax2 cells increased (nearly 40%) and a very small decline was observed with *samdc*^{OE} cells (Supplementary Fig. S4B). The size of the aggregates formed after MGBG treatment, decreased (nearly 5 folds) in the Ax2 cells but increased in *samdc*^{OE} cells (Supplementary Fig. S4C). The stalk length was higher (nearly two-folds) in case of *samdc*^{OE} cells but decreased upon MGBG treatment. A decrease in Ax2 cells was also observed (Supplementary Fig. S4D).

We observed increased spd and put levels in the *samdc*^{OE} cells (Fig. 3A). Upon MGBG treatment, put and spd levels decreased in both Ax2 and *samdc*^{OE} cells. Further, MGBG treatment decreased the levels of put and spd in both strains though the levels in *samdc*^{OE} cells remained higher than that observed in Ax2 cells. Nearly 2.7-folds increase in spd levels was observed in the overexpressing cells. Further, we observed an increase in put and spd but no change in spm levels during growth and development of *samdc*^{OE} as compared to Ax2 cells (Fig. 3B-D).

Maintenance of polyamine homeostasis during development

The mRNA levels of the anabolic enzyme *spd/spm synthase* and the catabolic enzymes like *spd/spm oxidase* and *SSAT* showed a significant increase, decrease and insignificant change, respectively during development of the *samdc*^{OE} cells (Fig. 4A-C; Supplementary Fig. S5A-D, S6 A, B).

Next, we analysed the mRNA levels of few transporters (Supplementary Fig. S6C, D –S8) during development (Fig. 5) of the above strains. The mRNA levels of both *DDB0185190* and *DDB0233584*

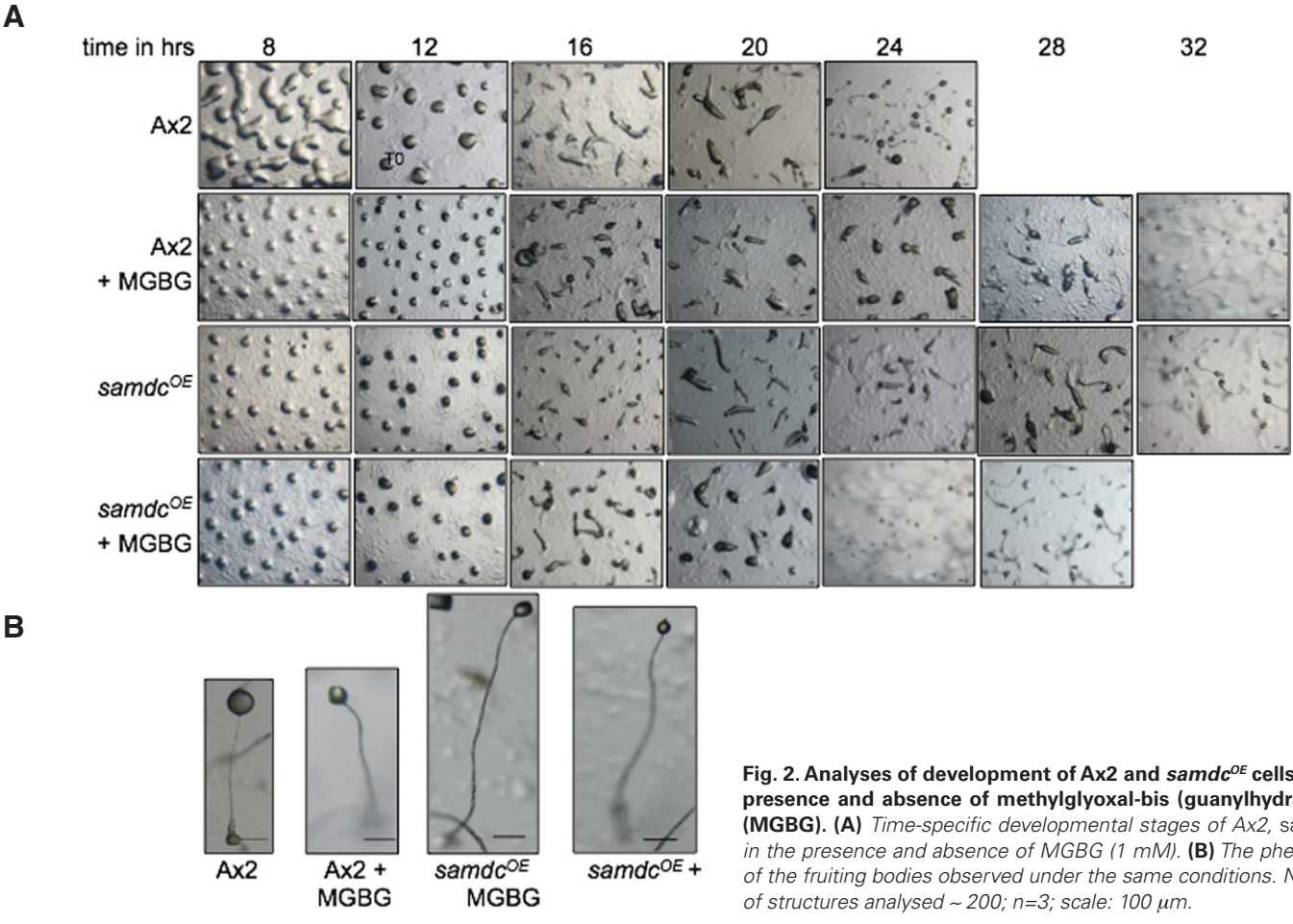

Fig. 2. Analyses of development of Ax2 and *samdc*^{OE} cells in the presence and absence of methylglyoxal-bis (guanylhydrazone) (MGBG). (A) *Time-specific developmental stages of Ax2,* samdc^{OE} *in the presence and absence of MGBG (1 mM).* **(B)** *The phenotype of the fruiting bodies observed under the same conditions. Number of structures analysed ~ 200; n=3; scale: 100 μm.*

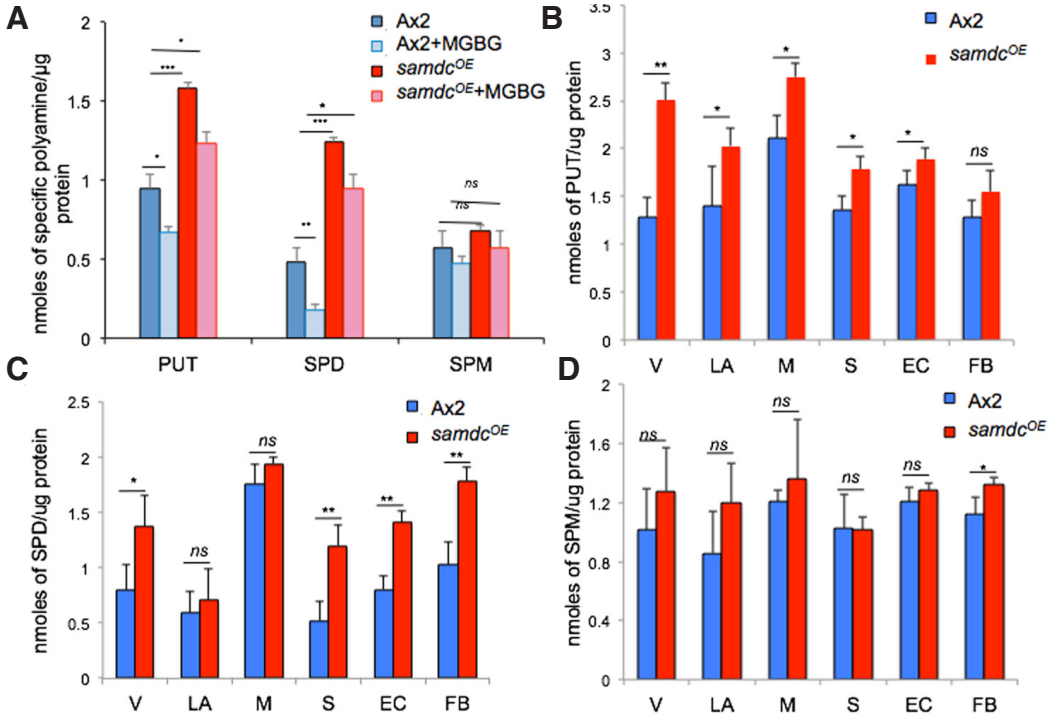

Fig. 3. Levels of polyamines in Ax2 and *samdc^{OE}* cells in the presence or absence of methylglyoxal-bis (guanylhydrazone) (MGBG) during development. (A) *Levels of polyamines in Ax2 and* samdc^{OE} *cells.* samdc^{OE} *cells showed increased polyamine levels while MGBG treatment decreased the levels.* **(B,C,D)** *Levels of put, spd and spm in the various developmental stages. The values representing mean ± standard error (S.E.); n = 3; Student t-test, p-value ≤0.05, ≤0.01 and ≤0.001 represented as *, ** and ***, respectively. Abbreviations: EC, early culminant; FB, fruiting body; LA, loose agreggate; M, mound; S, slug; V, vegetative.*

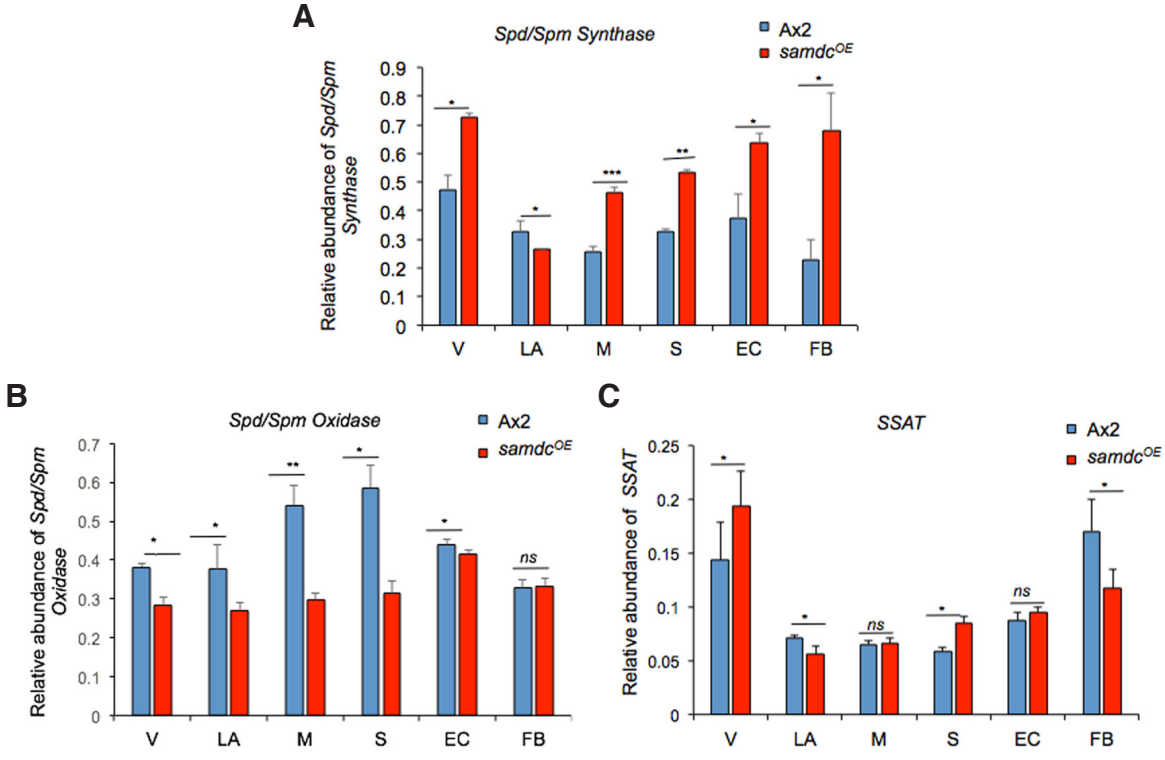

Fig. 4. *samdc^{OE}* cells show altered mRNA expression of biosynthetic genes involved in polyamine homeostasis. *RT-PCR analyses of anabolic and catabolic genes,* **(A)** Spd/Spm synthase*;* **(B)** Spd/spm Oxidase*;* **(C)** SSAT *during development of Ax2 and* samdc^{OE} *cells after normalization to* ig7 *are shown. n=3; Student t-test, p-value ≤0.05, ≤0.01 and ≤0.001 represented as *, ** and ***, respectively. Abbreviations, as in Fig. 3.*

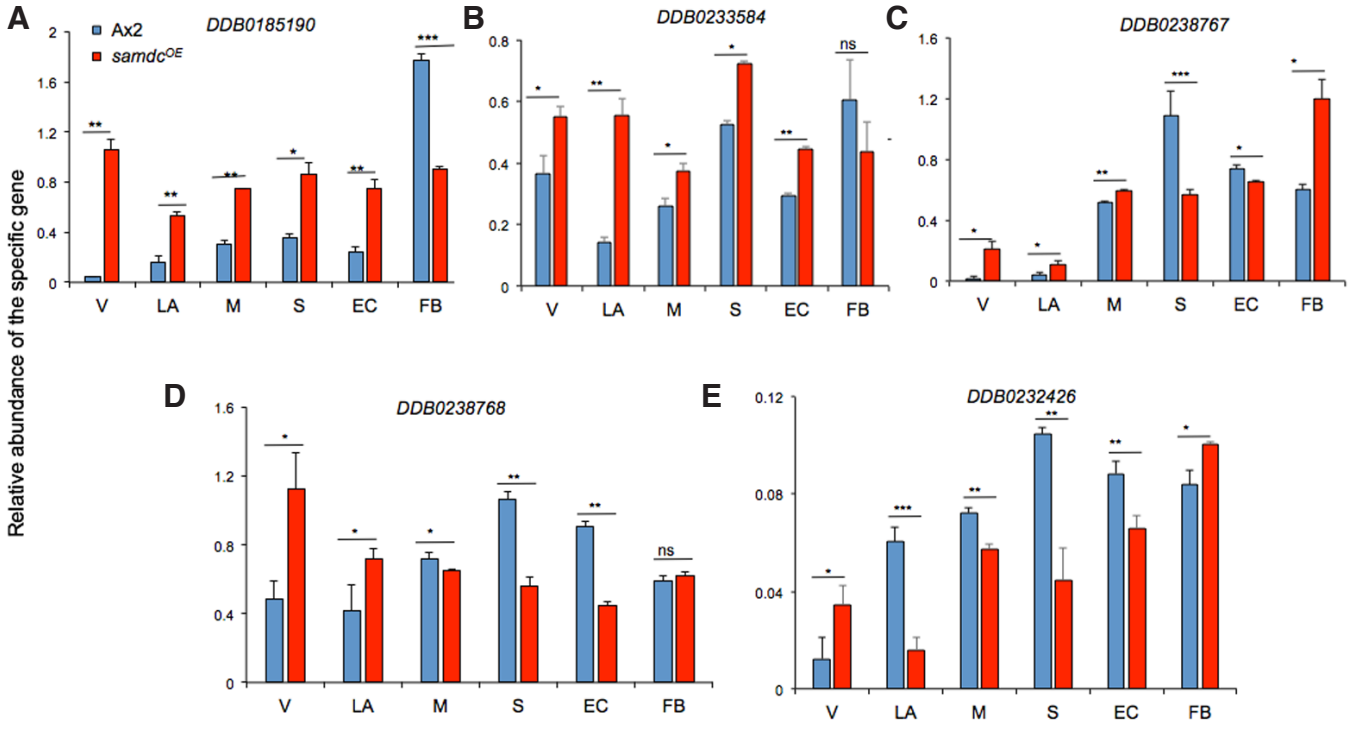

Fig. 5. *samdc*^{OE} cells show changes in mRNA expression of putative transporter genes involved in polyamine homeostasis. *RT-PCR analyses of transporter genes:* **(A)** DDB0185190, **(B)** DDB0233584, **(C)** DDB0238767, **(D)** DDB0238768, **(E)** DDB0232426 *during development of Ax2 and* samdc^{OE} *cells after normalization to* ig7 *are shown. n=3; Student t-test, p-value ≤0.05, ≤0.01 and ≤0.001 represented as *, ** and ***, respectively; Abbreviations, see Fig. 3.*

during development showed an increase except in the fruiting body (Fig. 5 A,B). *DDB0238767* did not show much change during development except for an abrupt two-folds increase in fruiting body (Fig. 5C). *DDB023768* transcript was high till aggregation and decreased thereafter till it reached the Ax2 levels in fruiting body (Fig. 5D). *DDB0232426* mRNA expression was decreased almost throughout development (Fig. 5E).

Discussion

Here, we succefully modulated the spd levels either by overexpressing the *samdc* gene or treating with its inhibitor MGBG. Our results showed spd levels could be brought down by MGBG but an effective amount of spd/PAs was required for growth to proceed. The *samdc*^{OE} cells showed better cell proliferation with a possibility of increased put due to increased spd levels. Both MGBG treated Ax2 and the *samdc*^{OE} cells are of larger size suggesting it to be the possible reason for the slower cell proliferation. It suggests that both extremes: low (Ax2 + MGBG) and high (*samdc*^{OE}) levels of spd were not beneficial for cell proliferation. Cell cycle data suggests a prestalk bias under different conditions, showing any alteration in spd levels lead towards the death/stalk pathway.

The put levels revert back to near normal with development but a significant increase in the spd levels was observed in *samdc*^{OE} cells. The initial increase in put may be involved in cell proliferation, as with development the levels declined and was comparable to wild-type in fruiting bodies suggesting conversion of put to spd or the efflux during development. The phenotype aberrations observed in *samdc*^{OE} cells may not be due to put levels but to the increased spd levels observed during terminal differentiation. Also, the contribution of put during early development in influencing aggregation cannot be ruled out.

Reduction in PA levels lead to growth arrest (Imai *et al.*, 2004), whereas surplus can cause cytotoxicity (Tobias and Kahana, 1995). The transcript levels of genes encoding proteins involved in PA biosynthesis (catabolic and anabolic) and the transporters (importers and exporters) were examined in both Ax2 and *samdc*^{OE} cells. Increased *spd synthase* transcript level especially during terminal differentiation, indicates the conversion of put into spd, which may be required to maintain high spd levels in *samdc*^{OE} cells. It is possible that this enzyme is required to overcome the efflux to maintain Spd/Spm intracellular levels. The mRNA levels of catabolic enzymes, *spd/spm oxidase* and *SSAT* were studied to analyse the feedback mechanism of PA level maintenance. Results with *spd/spm oxidase* suggests that during early development, the conversion to Spd/Spm does not take place as the levels of the intermediates may not be high but by the time of culmination when the actual levels of spd increased this enzyme reached wild-type levels. *SSAT* that is required to convert the intermediates to Spd/Spm did not show much difference during development. This enzyme showed a significant decrease in fruiting body suggesting that it was not required for the maintenance of high spd levels observed in *samdc*^{OE} cells during terminal differentiation.

Increase in *DDB0185190* mRNA levels during development (except in fruiting body) suggests it maybe an exporter helping maintain PA levels. Due to overexpression, spd levels remain high in *samdc*^{OE} cells. We observed normal levels of spm and put in fruiting body, it is possible that it helps in the efflux of PAs. Similar

mRNA expression pattern was observed for *DDB0233584* suggesting it to be involved in efflux. *DDB0238767* did not show much change during development except in fruiting body and since the levels of put and spm was significantly high in fruiting body this may be active in efflux. *DDB0232426* mRNA expression was decreased almost throughout development suggesting it to be a putative importer rather than an exporter. We could speculate it to be a spd importer, which is not required during the development of *samdc*^{OE} cells as they themselves maintain high spd levels.

In conclusion, a narrow range of spd levels must be maintained by the cell for proper growth and development. An increase/ decrease in the effective amount of PA is not good for the cell. A closer look at the biosynthetic and transporters genes of PA shows to be pretty conserved among different species. The results reveal a complex homeostatic mechanism at work for PA metabolism involving several parts of the pathway operating in a coordinated manner.

Materials and Methods

Strains and development

Cells were cutured in axenic medium HL5 at 22°C (with or without G418) under shaken conditions according to Maurya *et al.,* (2017) and proliferation was monitored according to Mishra *et al.,* (2017).

Treatment with MGBG and spermidine

Logarithmic phase cells were cultured in the absence and presence of 1mM (MGBG) (Sigma-Aldrich) or spd for 48h at 22°C and developed.

Cell-viability assay

MTT (3- [4, 5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) reduction assay was performed according to Swer *et al.,* (2014) to monitor cell-viability.

Cell cycle analysis by flow cytometry

This was performed using FACS Calibur (Becton Dickinson, USA) according to Swer *et al.,* (2014) both in the presence and absence of MGBG. Cell Quest software for acquisition and analysis was used.

Measurement of various polyamine levels

Polyamine was estimated as described previously (Saran, 1998; Kumar *et al*., 2014).

Identification of genes involved in polyamine homeostasis

To identify the genes involved in the polyamine homeostasis homologous genes of polyamine anabolism, catabolism and transporters were searched in the genomic database. The functional domains of these protein sequences were then run on BLASTp using *Dictyostelium* database to identify the proteins involved (Kumar, PhD thesis, JNU, 2012).

RT-PCR

Protocols for RNA isolation and RT-PCR (Reverse transcriptase-polymerase chain reaction) were followed as previously described (Maurya *et al*., 2017). Following cDNA synthesis, RT-PCR reactions were performed using gene-specific primer pairs (Supplementary Table S1) for various genes. *rnlA* was used as an internal control (Gosain *et al*., 2012).

Statistical analyses

The statistical analyses were performed (mean standard deviation and standard error) and values were plotted in graph using Microsoft Excel-2013. Levels of significance were calculated using Student's t-test and p-values of less than 0.05 were considered as significant.

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References

GOSAIN A, LOHIA R, SHRIVASTAVA A, SARAN S (2012). Identification and characterization of peptide: N-glycanase from *Dictyostelium discoideum*. *BMC Biochem*. 8: 13-19.

HANDA AK, FATIMA T, MATTOO AK (2018) Polyamines: Bio-Molecules with Diverse Functions in Plant and Human Health and Disease. *Front Chem*. 6: 10.

IGARASHI K, KASHIWAGI K (1999). PA transport in bacteria and yeast. *Biochem J* 3: 633–642.

IMAI A, MATSUYAMA T, HANZAWA Y, AKIYAMA T, TAMAOKI M, SAJI H, SHIRANO Y, KATO T, HAYASHI H, SHIBATA D, TABATA S, KOMEDA Y, TAKAHASHI T (2004). Spd synthase genes are essential for survival of *Arabidopsis*. *Plant Physiol* 135: 1565-1573.

JAIN P, SHARMA P, SHRIVASTAVA A, SARAN S (2016). *Dictyostelium discoideum*: A Model System to Study Autophagy Mediated Life Extension. In *Topics in biomedical Gerentology.* (Eds. Pramod C Rath, Ramesh Sharma, S. Prasad). Springer, pp 35- 56. (ISBN: 978-981-10-2154-1).

KUMAR R (2012). Role of ornithine decarboxylase in the development and differentiation programme of *Dictyostelium discoideum.* Ph.D Thesis submitted to Jawaharlal Nehru University.

KUMAR R, RAFIA S, SARAN S (2014). Cloning, expression and characterization of the ornithine decarboxylase gene from *Dictyostelium discoideum*. *Int J Dev Biol* 58: 669-676.

KUMAR R, SHARMA P, SARAN S (2018). Identification and cloning of S-adenosylmethionine decarboxylase (*Samdc*) gene from *Dictyostelium discoideum. Int. J. Adv. Res.* 6: 791-798.

MAURYA R, KUMAR R, SARAN S (2017). *Dictyostelium* AMPKa regulates aggregate-size and cell-type patterning. *Open Biol* 7: 170055.

MISHRA H, SARAN S (2015). Classification and expression analyses of *homeobox* genes from *Dictyostelium discoideum*. *J Biosci* 40: 241-255.

MOSCHOU PN, SANMARTIN M, ANDRIOPOULOU AH, ROJO E, SANCHEZ-SERRANO JJ, AND ROUBELAKIS-ANGELAKIS KA (2008). Bridging the gap between plant and mammalian polyamine catabolism: a novel peroxisomal polyamine oxidase responsible for a full back-conversion pathway in *Arabidopsis*. *Plant Physiol* 147: 1845-1857.

REGENASS U, CARAVATTI, G, METT H, STANEK, J, SCHNEIDER P, MULLER M, MATTER A, VERTINO P, PORTER CW (1992). New S-adenosylmethionine decarboxylase inhibitors with potent anti tumor activity. *Cancer Res* 52: 4712-4718.

SARAN S (1998). Changes in endogenous PA levels are associated with differentiation in *Dictyostelium discoideum. Cell Biol Int* 22: 575-580.

SWER PB, LOHIA R, SARAN S (2014). Analysis of rapamycin induced autophagy in *Dictyostelium discoideum. Indian J Exp Biol* 52: 295-304.

TARNITA CE, WASHBURNE A, MARTINEZ-GARCIA R, SGRO AE, LEVIN SA (2015). Fitness tradeoffs between spores and non-aggregating cells can explain the co-existence of diverse genotypes in cellular slime molds. *Proc Natl Acad Sci USA* 112: 2776-2781.

TOBIAS KE, KAHANA C (1995). Exposure to ornithine results in excessive accumulation of put and apoptotic cell death in ornithine decarboxylase overproducing mouse myeloma cells. *Cell Growth Differ* 10: 1279-1285.

WALLACE HM (1996). PAs in human health. *Proc Nutr Soc* 55: 419-431.

YE C, GENG Z, DOMINGUEZ D, CHEN S, FAN J, QIN L, LONG A, ZHANG Y, KUZEL TM, ZHANG B (2016). Targeting Ornithine decarboxylase by α-Difluoromethylornithine inhibits tumor growth by impairing myeloid-derived suppressor cells. *J Immunol* 196: 915-923.

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