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Glimpses of Dictyostelid research in India

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ABSTRACT Simple organisms are preferred for understanding the molecular and cellular function(s) of complex processes. *Dictyostelium discoideum* is a lower eukaryote, a protist and a cellular slime mould, which has been in recent times used for various studies such as cell differentiation, development, cell death, stress responses etc. It is a soil amoeba (unicellular) that undertakes a remarkable, facultative shift to multicellularity when exposed to starvation and requires signal pathways that result in alteration of gene expression and finally show cell differentiation. The amoebae aggregate, differentiate and form fruiting bodies with two terminally differentiated cells: the dead stalk (non-viable) and dormant spores (viable). In India, starting from the isolation of *Dictyostelium* species to morphogenesis, cell signalling and social evolution has been studied with many more new research additions. Advances in molecular genetics make *Dictyostelium* an attractive model system to study cell biology, biochemistry, signal transduction and many more.

KEY WORDS: *lower eukaryote, protist, amoeba, Dictyostelium*

Introduction

Dictyostelium discoideum was first isolated from the forest litter of North Carolina, USA by Kenneth Raper in 1935. Later, John Bonner and Maurice Sussman contributed to the research, which later attracted many researchers to this field. Many different species of *Dictyostelium* were isolated but the laboratory model *Dictyostelium discoideum* was used for further studies, which largely was for the understanding of the life cycle and spatial patterning that was observed in migrating slugs.

Dictyostelium has gained much attention as a significant model system for analysing the molecular basis of various stress responses, cell death, gene regulation, as well as pathogenesis and treatment of several human infectious diseases. Small genome size and haploid or diploid states of *Dictyostelium* facilitate the mutant screening fast and allow straightforward mapping by complementation (Loomis, 2016). The extraordinary life cycle, which completes over a period of 24 hours, offers accessible phenotypes involving cell-cell adhesion, chemotaxis, cell differentiation and intercellular signalling pathways (Fig. 1). While growing, the amoebae ingest bacteria and multiply by mitosis but when the population reaches a certain density the quorum sensors act to reduce growth and initiate post-mitotic development. Upon starvation, the cells begin to both accumulate and secrete a chemoattractant cyclic AMP (cAMP). The initially separated amoebae aggregate in response to pulses of cAMP. These amoebae enter multicellularity, undergoes various morphogenetic movements to ultimately form a fruiting body comprising of dead vacuolated stalk cells and viable spores. In slugs, the anterior ¼ comprises of the prestalk cells while the posterior ¾ is composed of prespore cells. This ratio of prestalk:prespore is always maintained during its life. The abundance of phenotypes makes *Dictyostelium* a valuable model for studying important cellular processes.

Research on Dictyostelids carried out in India

Presently, *Dictyostelium* is being used as a model for understanding molecular processes in the cell and molecular biology. Here, we provide glimpses of the research areas covered in India under the following heads:

Isolation of *Dictyostelium* species from India

Agnihothrudu first discussed the existence of Dictyosteliaceae in the rhizosphere of growing plants in Southern India (1956). He could report the occurrence of *D. mucoroides*, *Polysphondylium pallidum* and *D. discoideum*. Later, Rai and Tewari (Lucknow University) isolated *D. mucoroides* and *Polysphondylium violaceum*

Abbreviations used in this paper: cAMP, cyclic AMP; PARP, poly (ADP-ribose) polymerase; PKS, polyketide synthase.

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from soil fill (1961). Both studies showed genetic diversity of social amoebae in the same neighbourhood. Later, Cavender and Lakhanpal (1986) identified 12 (*D. purpureum, D. giganteum, D. mucoroides, D. minutum, P. pallidum, P. violaceum. D. aureo-stipes, D. sphaerocephalum, D. polycephalum, D. tenue, D. vinaceo-fuscum, A. subglobosum*) Dictyostelid slime mold species from five vegetational-climatic zones of West-Central Himalaya as well as from Tropical forests in Peninsular India which show similarities to those of East Africa than Southeast Asia.

Multicellular development and morphogenesis

Dictyostelium is used as a model organism of choice for studying cellular differentiation and development, which requires various molecular players and complex regulatory events for the transition from unicellularity to multicellularity (Mir *et al*., 2007).

After a long gap, research in *Dictyostelium* flourished in the country. Nanjundiah and his group at the Indian Institute of Science, Bangalore focused on intercellular interactions and cooperative behaviour in *Dictyostelium*, at first from the point of view of development and then later as a problem in evolution. During spatial patterning in developing systems, time required for the regeneration to occur in cellular slime moulds depended largely on the relative size of the amputated fragment but was independent of the total slug size (Lokeshwar and Nanjundiah, 1981; 1983). Nanjundiah and his group mainly focused on pattern formation especially with respect to intracellular free calcium levels. They showed heterogeneity in a seemingly identical freshly starved cell population with respect to calcium levels, where the cells made use of this heterogeneity to differentiate and show specific cell-type patterning (Tirlapur *et al.,* 1991; Saran *et al*., 1994a,b; Azhar *et al*., 1996; Baskar *et al*., 2000). Sistla *et al.,* (2012) further showed that the speed of pre-aggregating cells distributed bimodally, with prestalk cells moving faster than the prespore cells. Further, calcium levels were found to correlate with cell cycle phase and affected the cyclinB transcript levels (Azhar *et al*., 1998; Saran, 1998). In continuation, they analyzed expression of genes (eg. for asparaginyl tRNA synthetase) that were dependent on calcium levels (Jaiswal and Nanjundiah, 2003). Sawarkar *et al.,* 2009) showed that histone deacetylase activity regulated developmental timing and social interactions in *Dictyostelium*. Also, ribosomal S4 gene of *D. discoideum* could rescue yeast bud formation mutant because of the presence of common shared motif in the predicted protein (Amarnath *et al*., 2012).

Shweta Saran, a post-doctoral fellow of Nanjundiah works largely on developmental cell death at Jawaharlal Nehru University, New Delhi. Her work on polyamines (putrescine, spermidine, and spermine) showed them to be involved in the regulation of cellular proliferation. The work on ornithine decarboxylase (ODC), a rate-limiting enzyme in the polyamine synthesis pathway showed it to be expressed more in the prestalk cells and when overexpressed, it increased the cellular putrescine levels resulting in inhibition of cell proliferation but the changes in the developmental patterns were largely due to spermidine and spermine levels (Saran, 1998; Kumar *et al*., 2014). Also, the spermidine levels were modulated by overexpression of the *S-adenosyl methionine decarboxylase* (*samdc*) gene and/or treating the cells with MGBG, an inhibitor of SAMDC. In *Dictyostelium*, overexpression of *SAMDC* slowed cell proliferation, delayed development and arrested cells in S-phase of the cell cycle. Treatment with MGBG reduced cell proliferation and accelerated development but in *samdc*^{OE} cells, it increased cell proliferation suggesting critical levels of spermidine to be important (Sharma *et al*., 2018). In conclusion, they showed that a narrow range of spermidine levels must be maintained for proper growth and development in *Dictyostelium*.

Several small metabolites play important role(s) during morphogenesis. The two important factors regulating development are differentiation-inducing factor (DIF) and 4-methyl-5-pentylbenzene-1,3- diol (MPBD), which are produced by large multifunctional polyketide synthases (PKSs). Gokhale *et al.,* (2007) showed the functional significance of the large repertoire of PKSs in *D. discoideum.* Later, Ghosh *et al.,* (2008), on the basis of computational, biochemical and gene expression studies, proposed the multifunctional *Dictyostelium* PKS (DiPKS) protein DiPKS1 to be implicated in the biosynthesis of MPBD. Further, they also characterized an

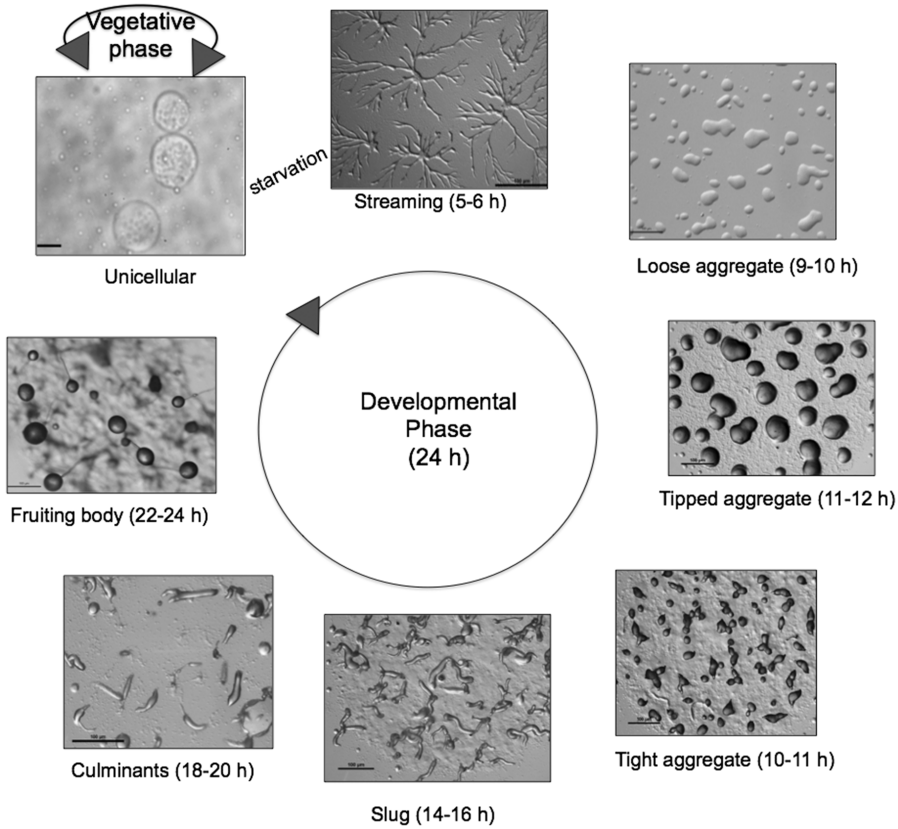

Fig. 1. Life cycle of *Dictyostelium discoideum*. *There are two phases: unicellular (vegetative) and multicellular (developmental) incorporated in its life cycle. The developmental phase completes within 24 hours when they form the fruiting body consisting of two terminally differentiated cell types: stalk (dead vacuolated) and spore (viable). The figure shows different developmental stages and the time taken after starvation (t_0). Scale bar, 100 μm; Magnification, 4X.*

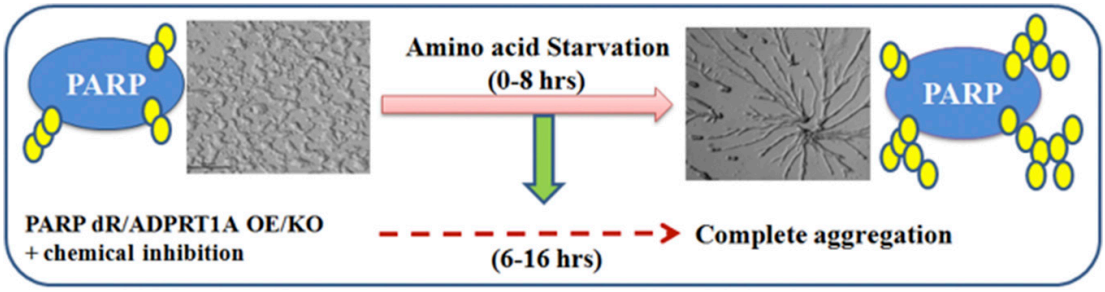

Fig. 2. Role of poly (ADP-ribose) polymerase (PARP) in development. *Chemical inhibition and genetic alterations in PARP cause a delay in developmental morphogenesis, particularly affecting the aggregation stage of* D. discoideum *that suggests regulated levels of PARP are requisite for complete development. PARP's activity or its PARylation (shown in yellow round bodies) affect development delaying the aggregation stage by ~6-16 hours which usually occur at ~0-8 hours in control cells.*

O-methyltransferase (OMT12) that has the competence to produce a variant of MPBD. Their studies provided a new perception in understanding the metabolic diversity produced by combining the existing functional scaffolds. Phosphopantetheinyl is a post-translational modification that is important for the activity of PKSs. A group of enzymes known as phosphopantetheinyl transferases (PPTases) are essential for this modification. Nair *et al.,* (2011) analysed the functions of the two PPTase (DiAcpS and DiSfp) homologues and showed them to be functionally distinct in nature. Biochemical analysis showed that DiSfp was essential for triggering of multifunctional PKS/FAS, whereas, DiAcpS could modify only the stand-alone ACP. Both the PPTases were expressed in all stages of development suggesting their importance in the developmental program. The *trishanku* (*tri*) gene encodes a nuclear protein having a Broad complex Tramtrack bric-a-brac (BTB) domain. It is highly expressed in prespore cells and also during the late G2 to S phase of the cell cycle. Deletion of *triA* (*tri*$^-$) resulted in fruiting bodies having a thick stalk and small spore mass but the ratio of prestalk to prespore in the slugs remained unaltered except that their locations were altered (Jaiswal *et al.,* 2006; Mazumdar *et al*., 2009). They observed that genes that were active in one cell type could influence the other cell type resulting in enhancement of the reproductive fitness in the first cell type showing cooperative behaviour. Later, Mazumdar *et al.,* (2011) showed that both autonomous and non-autonomous traits mediated social cooperation in *D. discoideum* where the cells of the upper cup (prestalk cells) could lift prespore and spore cells. Such observations on cooperative behaviour allowed them to look into social evolution in cellular slime moulds (Kawli and Kaushik, 2001).

Rasheedunnisa Begum and her group from the Department of Biochemistry, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara showed the functions of PARP [Poly (ADP-ribose) polymerase] during cellular differentiation and development in *D. discoideum*. PARP-1, an abundant nuclear enzyme, catalyzes the formation of poly (ADP-ribose) polymers (PAR) on accepter proteins by using NAD^+ as a substrate. PARP and PARylation control a variety of cellular processes such as DNA repair, chromatin remodelling, transcriptional regulation, cell death, etc. (Mir *et al*., 2012; Rajawat *et al*., 2014a). However, the function of PARP is still not completely understood. Eight potential *PARP* genes are annotated in *D. discoideum* (Kawal *et al*., 2011). Earlier, the regulated expression of *PrpA* (PARP homolog) was shown to be essential for asexual development in *A. nidulans* (Semighini *et al*., 2006). Supporting this study, elevated PARP activity was found in *D. discoideum* under developmental stimuli (Jubin *et al*., 2016a). A classical experiment by Rajawat and colleagues (2007, 2011) has demonstrated that chemical inhibition by benzamide and *PARP* down regulation arrested the development at aggregate stage. Overexpression of *ADPRT1A* (PARP-1 in human) also affected the developmental morphogenesis in *D. discoideum* mainly at the aggregation stage, prolonging the duration of fruiting body formation. Moreover, PARP activity was found to be elevated under developmental stimuli (Jubin *et al*., 2016a), signifying that PARP-1 activity may be developmentally regulated. To strengthen this observation, *ADPRT1A* gene expression pattern was analyzed in *D. discoideum.* The study found that *ADPRT1A* transcript levels were increased at the loose aggregate stage while a drop was observed at the tight aggregation stage. This result was further complemented by an interesting observation of increased and reduced *ADPRT1A* transcript levels at the slug stage and fruiting body stages, respectively (Jubin *et al*., 2016a). In addition, *ADPRT1A* knockout *(ADPRT1A*$^-$*)* cells also exhibited defective chemotaxis and delay in development, affected prestalk and prespore (*ecmA* and *d19,* respectively) gene expression and altered the transcript profile of cAMP production and signalling. However, the exogenous cAMP pulses could rescue these defects in the *ADPRT1A*$^-$ (Jubin *et al*., 2019a). Altered *PARP-1* levels in fungi have been shown to manifest defective development and decreased life span (Kothe *et al*., 2010). The regulated levels of ADPRT1A (PARP-1) are essential for cellular growth, differentiation and developmental morphogenesis *via* cAMP signalling and chemotaxis (Jubin *et al*., 2016b, 2017, 2019b) (Fig. 2). Thus, PARP being a multifunctional protein may be essential for complex multicellular differentiation by interacting with promoters of developmental genes or by PARylation of transcription factors. Nevertheless, future studies need to be focused on the investigation of PARP interacting proteins during development, thereby elucidating the functional link between PARP and multicellularity.

In a different study, Saran and her group classified the 14-homeobox containing proteins of which (Hbx3, Hbx4, Hbx9, Hbx11 and Hbx12) were in the TALE class, PBX (pre B-cell leukemia homeobox- Hbx3, Hbx11 and Hbx12), IRX (Iroquois homeobox- Hbx9) and CUP (Hbx4) families. Absence of *hox* genes in *Dictyostelium* implied a Hox-independent function for the *TALE* class genes (Mishra and Saran, 2015). TALE class members play prominent roles in pattern formation, axis maintenance and cell-fate decisions during embryogenesis. The spatial expression of *hbx9* during development was largely limited to the prestalk/stalk

cells. In spite of a slow cellular proliferation, *hbx9*⁻ cells completed fruiting body formation, suggesting that Hbx9 promotes cAMP signalling-dependent growth-to-development transition. cAMP being a primary morpho-regulatory signal, regulates chemotaxis, gene expression and cell differentiation during developmental morphogenesis. Deletion of *Hbx9* impaired cAMP signalling and delayed development. Hbx9 regulates prestalkA cell patterning without affecting its differentiation by regulating *cadA* expression via modulation of cAMP signalling during growth-to-development transition (Mishra *et al*., 2017).

Baskar carried out his doctoral studies with Nanjundiah at the Indian Institute of Science, Bangalore, and still continues to work with *Dictyostelium* at the Indian Institute of Technology, Madras. The aggregate size in *Dictyostelium* is largely determined by secreted counting factors called Countin and SmlA. *countin* mutants form large aggregates and this group size defect could be restored to parental wild type size by adding caffeine in the medium. Another group size defective mutant, *smlA,* forms small aggregates by fragmentation, which could be restored to wild type size by adding adenosine in the extracellular environment (Jaiswal *et al*., 2012a) suggesting that adenosine affected group size by impairing cAMP signalling. Both the compounds, adenosine as well as caffeine, rescue mutants defective in streaming (*pde4*⁻ and *pdiA*⁻) implying that adenosine affects both streaming and other stages of aggregation in *Dictyostelium*. Further, when slugs were transferred to caffeine-containing plates, several tips arise along with the slug axis, each forming a fruiting body of its own (Jaiswal *et al*., 2012b). Caffeine-induced multiple tip formation is conserved across other slime moulds (Jaiswal *et al*., 2012b). As caffeine is an antagonist to adenosine, the current view is that excess adenosine in the slug front suppresses another tip organizing center along the slug length and caffeine overrides the action of adenosine, inducing the formation of multiple organizing centers. Of the several hundred genes differentially regulated in response to caffeine treatment, the expression of thyroxine 5'deiodinase (dio3) is also high (unpublished data). Dio3 is important for normal development and differentiation of *Dictyostelium* as *dio3* knockout results in defective aggregation and group size, suggesting it to maintain aggregate size by suppressing multiple signalling centers (Singh *et al*., 2014). The functional activity of a number of proteins is determined by phosphorylation events carried out by protein kinase C. *Dictyostelium* contains a protein that contains a PKC domain (*pkcA*). The *pkcA* mutant aggregates are small with impaired group size (Mohamed *et al.,* 2015). As visualized by dark field optics, *pkcA*⁻ mutants have multiple signalling centers from a single aggregate. The impaired tissue or aggregate sizes in both *dio3*⁻ and *pkcA*⁻ mutants in *Dictyostelium* are a combinatorial effect of cell signalling, cell-cell adhesion and processes affecting group size.

Aruna Naorem has initiated *Dictyostelium* research at the University of Delhi. She has worked on DdRPB4, a subunit of RNA PolII with Sadhale at Indian Institute of Science (Naorem and Sadhale, 2008). RPB4 is well conserved and essential for growth under extreme temperatures and for proper response to nutrient starvation. The characterization of a parvulin-type peptidyl prolyl cis-trans isomerase, PinA was also reported in *Dictyostelium*. Deletion of *pinA* (*pinA*⁻) led to reduced growth rate and spore formation with abnormal prespore:prestalk patterning (Haokip and Naorem, 2017).

Developmental cell death

Cell death is a normal phenomenon occurring during multicellular development. ~15-20% of the total population of *D. discoideum* go through developmental cell death. Kawli *et al.,* (2002) analyzed stalk cell death during the multicellular development and reported that not all but few features of apoptosis could be observed. Later when the genome was sequenced, it was clear that *D. discoideum* followed a caspase-independent cell death mechanism (Eichinger *et al*., 2005) that was termed as 'paraptosis' (Katoch *et al*., 2002). Among the other paraptotic features, release of 'Apoptosis Inducing Factor' (AIF) from mitochondria and its translocation to the nucleus during developmental cell death was observed (Cande *et al.,* 2002). Nonetheless, the significance of AIF-mediated cell death during development is poorly characterized. It is suggested that AIF is a downstream effector in PARP-mediated cell death in *D. discoideum* (Rajawat *et al*., 2014b). In a well-laid-out study by Kadam *et al.,*2017), it was demonstrated that altered gene expression of prestalk [*ecmB* (Extracellular matrix B)] and prespore (*d19*) markers caused delayed development of *AIF* down-regulated cells. AIF, being a mitochondrial protein, is also involved in maintaining mitochondrial functions and hence, contributes to cell growth and development. These studies indicate the role of AIF during cell differentiation and developmental cell death. However, further studies are warranted to unravel the underlying mechanism.

Shweta Saran and her group are interested in the developmental cell death in *Dictyostelium,* which has a component of autophagy. Autophagy is a eukaryotic catabolic pathway that degrades and recycles the cellular components to maintain homeostasis. It targets dysfunctional or damaged organelles and protein aggregates. During the development of *Dictyostelium,* cells depend on autophagy to get resources (energy and metabolites) that are required for aggregation and differentiation. Using different strategies to measure autophagic flux, they have analysed the genes involved in the nutrient-signalling pathway that follows the mTOR (target of rapamycin) signal transduction pathway. *TOR* is an essential gene as *TOR* knockout cells were not viable. They showed that overexpression of *TOR* inhibits cell proliferation (Swer *et al*., 2016). Swer *et al.,* (2014b) also characterized the Rheb protein from *Dictyostelium* and showed that rapamycin could induce autophagy in *Rheb* knockout cells (Swer *et al*., 2014a; 2014b). Sirtuins (Sirts) belong to class III histone deacetylases and require NAD^+ for their activity, which is associated with the nutritional status of the cell and they directly connect cellular metabolic signalling to the post-translational modifications of protein. Sirts play an important role in healthy aging, longevity and age-related diseases, as well as in cell survival mechanisms, such as autophagy (Jain *et al*., 2016; 2018). Both Sir2A and Sir2D are shown to be involved in the induction of autophagy in *D. discoideum* (Lohia *et al*., 2017; 2018). AMPK is a serine/threonine protein kinase, an energy sensor, which is similar to human AMPK. Deletion of the *AMPK* gene results in the formation of numerous small-sized aggregates that develop asynchronously to form few fruiting bodies having small sori and long stalks. For the first time, they showed that apart from being an energy sensor, AMPK also has a role in aggregate-size determination and cell patterning (Maurya *et al*., 2018). Saran's interest in the etoposide-induced 2.4 kb transcript (*ei24*) gene that is induced both by p53 and etoposide, an anti-cancer tumour drug allowed to understand its role in *D. discoideum* which does

not possess a p53 protein (Gupta and Saran, 2018). Their work shows that EI24 (an autophagy associated transmembrane protein) is a key protein involved in autophagy (Gupta and Saran, 2019).

Vesicles and trafficking

During the vegetative stage, amoebae engulf bacteria by phagocytosis or, in the case of axenic medium, take up nutrients by pinocytosis. Ingested materials that reach the intracellular acidic compartments get delivered to lysosomes for further acidification by vacuolar proton pumps. *D. discoideum* shows a very high rate of endocytosis and contains acidic phagosomes or food vacuoles that are ejected after the onset of starvation (Gross, 1994). As development proceeds, the food vacuoles are replaced by autophagic vacuoles and their number decreases in prespore cells while it accumulates further in prestalk cells (Gross, 1994). It is well established that the choice between slug migration and the culmination is largely dependent on the amount of the unprotonated form of ammonia (a weak base) present in the aggregates. Moreover, various evidences suggest that ammonia works not by raising cytosolic pH but by increasing the pH of a few components of the widespread acidic vesicle system. Hence, the acidic vesicles/acidic compartments are requisite for the developmental effects of weak bases. However, the underlying mechanism remains obscure. It is usually supposed that primary phagosomes combine directly with lysosomes for the acidification. It was observed that prelysosomal acidic vacuolar compartments provide the proton pumps to acidify both phagosomes and pinosomes. Moreover, neutral post-lysosomal vacuoles were also observed in *D. discoideum* in which undigested endocytic load is stored and returned to the cell surface. An acidosome has been characterized in *Dictyostelium* and is rich in vacuolar proton pumps (V-H^+-ATPases) required for the acidification of endocytic vacuoles. Electromagnetic purification of endocytic vacuoles and acidosomes was carried out and shown to be physically associated in a Mg^{2+}-dependent manner. In addition, the specific functional association of acidosomes and endocytic vacuoles could be reconstituted *in vitro* in the presence of soluble proteins and Mg^{2+} (Padh, 1995). Besides the acidification function, acidosomes were found to be associated with a cyclic AMP receptor (cAR1), signifying that it is a pre-lysosomal compartment, possibly for early/recycling endosomes (Padh and Tanjore, 1995). Therefore, future studies should elucidate the functional link between acidic vesicles/acidosomes and adenylyl cyclase activity to understand the spectacular transformation of prestalk and prespore cells into a mature fruiting body. Upon returning to India, Harish Padh worked on the endo-lysosomal circuit in *Dictyostelium*. Later his research was more focused on pharmacogenetics, occasionally working with *Dictyostelium*.

Social evolution

Nanjundiah and his group are also involved in the evolution of multicellularity and social patterns using *Dictyostelium* as a model system. There are alternating solitary and social phases, which involve division of labour and shows “altruism” that raises questions on the evolution of origin and maintenance of sociality (Zahavi *et al.,* 2018). They showed the presence of *D. giganteum* and *D. purpureum* in the soils of southern states of India whose dispersal depended largely on dispersal from a nutrient-poor environment (Matapurkar and Watve, 1997; Sathe *et al*., 2010; 2014). According to them, sociality probably evolved in the amoeba as an adaptation for helping dispersal from a low nutrient environment. In their latest study, they have showed that polyclonal multicellularity evolved in cellular slime molds because environments were resource poor (Hamant *et al*., 2019).

Sathe *et al.,* (2014) showed that multicellularity possibly evolved via self-organization based on the genetic and behavioural repertoire of unicellular ancestors. The occurrence of plasticity in the development process that results in the expression of various phenotypes has implications on the theory of evolution. With the understanding of genotype-phenotype relationship, concepts of robustness and changes during development, increase the chance of plasticity. Sathe and Najundiah (2018) using *D. giganteum* have shown that complex interactions strengthen social behaviours. Although plasticity has a longer history in the behavioural sciences, it is gaining new ground in this field as well, in considering the development and evolution of behaviour (Nanjundiah and Newman, 2009).

Behera and Nanjundiah have discussed the phenotypic plasticity that could modify evolutionary pathways and accelerate the course of evolution, though it is not very straight forward with realistic fitness schemes. Later, they showed that under given parameters, the adaptation by a population that depended on regulatory genes could accelerate the rate of evolution, suggesting that phenotypic selection favoured better adaptation (1995; 1996; 1997; 2004).

An interesting observation made by Chopra and Nanjundiah (2013) was that in starved single cells, accuracy of chemotactic response to cAMP goes up with time, i.e. variance in response falls during interphase.

Using *Dictyostelium* as a model system to understand a variety of processes

Like many other systems, the amoeba also allows various biochemical, cellular and molecular biology techniques to be performed, which can help understand different cellular processes. Also, both unicellular and multicellular stages are present within one organism and can be studied independent of each other, allowing it to become a favourable model system.

Plant-microbe interactions

Kasbekar and his group studied the response of isoflavonoids on Dictyostelids. Isoflavonoids are antimicrobial compounds present in leguminous plants where microbial infection and injury is observed. They show a unique plant-microbe interaction where the isoflavonoids attract the amoebae to help clear the bacteria from the root lesions of the leguminous plants (Kasbekar and Papavinasasundaram, 1992a; Prasanna *et al*., 1998). *D. discoideum* amoebae attain a non-degradative resistance to pisatin (pea phytoalexin) which could be attributed to *nysB sunD* as observed in the double knockouts. With the help of parasexual genetics, they show that *sunD* is a recessive mutation linked to *nysB* on linkage group VI (Kasbekar and Prasanna, 1992b).

Molecular motor complex-based studies

An important study on motor-based transport using an *in vitro* setting has been carried out by Roop Mallik and his group at the

Tata Institute of Fundamental Research, Mumbai. They analysed the intracellular transport of organelles in terms of measurable quantities. The intracellular transport of cargos is largely dependent on the presence of kinesin and dynein motor proteins present on organelles but its regulatory mechanism is still not understood. They observed reversals during endosome motion and attributed it to the existing opposing movements between kinesin and dynein proteins (Soppina *et al*., 2009; Bhat and Gopalakrishnan, 2012). They have also described a protocol to purify latex bead phagosomes (LBPs) from *Dictyostelium* cells, which could be used for various *in vitro* functional assays (D Souza *et al*., 2016). Their studies indicated that ceramide enrichment possibly was evolutionarily conserved and an important step in the maturation of the phagosomes (Pathak *et al*., 2018).

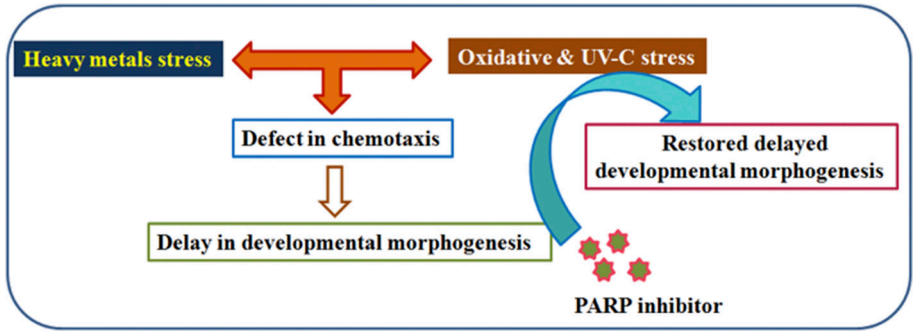

Fig. 3. Stress response to development. *Various types of stresses such as heavy metals, oxidative and UV-C cause a developmental delay that can be restored upon poly (ADP-ribose) polymerase (PARP) inhibitor treatment.*

Stress response

Rasheedunnisa Begum and her group also studied the stress response with *Dictyostelium* cells. Many human diseases are reported to root from cellular stress such as oxidative and UV exposure leading to genomic instability and cell damage (Mir *et al*., 2012). *Dictyostelium* in this context serves as an apt model to explore the complex signalling machinery that helps it combat various stress factors and facilitate the identification of possible therapeutic molecular targets. Several studies have demonstrated the effect of oxidative stress and UV-C on growth and development of *D. discoideum*. Their study indicates the resistance to oxidative stress was not just by virtue of a high level of antioxidant response but the response to UV exposure was independent of the response to oxidative stress (Katoch and Begum, 2003). The study reported that a 10.4 J/m^2 UV-C dose caused a delay in development while higher doses inhibited development due to altered expression levels of *yakA, car1, aca, csA* and *regA* genes during UV-C stress (Mir *et al*., 2015). Interestingly, treatment with hydroxylamine (HA) (for *in situ* H_2O_2 generation) resulted in a dose-dependent reduction in the number and size of fruiting bodies formed as compared to untreated cells (Rajawat *et al*., 2007). To explore the role of PARP in oxidative and UV stress in development, the cells were treated with benzamide, a PARP inhibitor, prior to oxidant and UV-C treatment and PARP inhibition could restore the delay in development of *Dictyostelium* cells under oxidative and UV-C stress. Thus, PARP inhibitors can be potential therapeutic candidate molecules for treating the diseases induced by DNA damage.

Identification of novel drugs

Saran and her group have exploited *Dictyostelium* to identify novel potent inhibitors of AMPK (Kumar *et al*., 2018). They identified two novel drugs (ZINC11784276 and ZINC12247658) having higher binding affinity over the known drugs. Similarly, they have identified novel inhibitors of TCTP (Translationally Controlled Tumor Protein). TCTP is a highly conserved multifunctional protein preferably expressed in mitotically active tissues and is used as a biomarker and target for lung cancers. They identified two novel compounds (ZINC12863423 and ZINC12657067) with the help of homology modelling and molecular dynamics (Kumar and Saran, 2017). STRAP (Serine Threonine kinase Receptor Associated Protein) is a WD40 containing protein that provides a platform for protein interactions during cell proliferation and development. Overexpression and mis-regulation of *STRAP* contributes to various carcinomas and are now recognized as therapeutic targets especially for colorectal and lung cancer (Kumar and Saran, 2018).

Toxicology studies

Heavy metal toxicity has been established as a major risk when present in larger amounts. Its long-term exposure hampers metabolic processes and damages the proper functioning of the human body. Toxicants are able to induce oxidative stress that may lead to various disorders such as Parkinson's disease, multiple sclerosis, Alzheimer's disease etc. Earlier, *D. discoideum* was identified as a model to analyze the toxicity tests of broad range of carcinogenic, xenobiotic chemicals etc. exploring basic cellular and developmental processes in the context of biomedical research. It allows the investigation of the various effects of toxicants on homogenous and large populations and *Dictyostelium* could be a suitable biomonitoring organism for toxicants (Amaroli, 2011). Samar Chatterjee from Jawaharlal Nehru University, New Delhi studied the toxicity effects of various heavy metals using *Dictyostelium* as a model system. A number of toxico-genomic studies have endowed large amount of work towards a better understanding of toxicological cellular effects in *D. discoideum* (Fig. 3). Long-term exposure to lead and cadmium treatments cause inhibition of cell growth, distorted cell morphology, delayed development with abnormal streaming, slugs and fruiting body formation and defects in cAMP chemotaxis. It was found that extracellular cAMP dependent phosphodiesterase activity was reduced upon exposure to lead/cadmium, which may have caused defective chemotaxis and development (Gurumurthy, Ph.D. thesis JNU, 2001). Several reports suggested the efficacy of *D. discoideum* to monitor pesticide cytotoxic effects (Gayatri and Chatterjee, 1991; Gayatri and Chatterjee, 1993a). In particular, carbamate/carbaryl, dichlorodiphenyl trichloroethane (DDT) and cisplatin [cis-diammine dichloro platinum (II)] pesticide treatments were shown to affect colony morphology, dose-dependent inhibition of pinocytic activity and chemotaxis of the cellular slime mould (Reddy and Chatterjee, 1999; Gurumurthy and Chatterjee, 2002; Gayatri and Chatterjee, 1994a). Moreover, carbaryl-treated cells showed larger aggregate formation, inhibition of chemotaxis

and cAMP-dependent extracellular phosphodiesterase activity which contributed to delayed development (Mukhopadhyay and Chatterjee, 1994). A high concentration of lindane (100mg/ml) was also reported to completely block morphogenesis along with chemotaxis, whereas a lower concentration of lindane (60mg/ml) delayed development with fewer and smaller aggregates, slugs and fruiting bodies (Gayatri and Chatterjee, 1992). The cytotoxicity of benzene hexachloride (BHC), an organochlorine pesticide, is associated with enhanced acid and alkaline phosphatase activities and thereby hampered growth and development of *D. discoideum* (Gayatri and Chatterjee, 1994b). Recent studies revealed that these heavy metal-induced defects in cellular processes could be due to mitochondrial dysfunction (Amhold *et al*., 2015; Boatti *et al*., 2017). However, despite of recognized toxicity, molecular signalling mechanisms involved in the action of these heavy metals need to be further investigated to understand the effective solution against heavy metals/pesticides risk.

Production of heterologous proteins

The recent advanced genetic engineering tools have made possible the production of recombinant proteins as diagnostics and therapeutics for humans and animals. The selection of the best expression system requires evaluating the options from yield to post-translational modifications to the economics of scale-up (Rai and Padh, 2001; Arya *et al*., 2008). The widely used expression systems are bacterial, yeast, baculovirus and mammalian. Each system has its own advantages and disadvantages but mammalian systems are the most favoured host for numerous eukaryotic proteins with pharmaceutical importance because of the post-translational modifications that can take place. In recent times, *D. discoideum* has emerged as an important host for the expression of heterologous recombinant proteins, because of the complex cellular machinery that is required for post-translational modifications which are similar to the one observed in higher eukaryotes.

The simple and inexpensive growth medium and its potential for large-scale production of proteins render *D. discoideum* as an alternative eukaryotic expression system for production of recombinant proteins. However, due to sub-cellular organelles like nucleopores or cellular processes like endocytosis in eukaryotes, introduction of foreign DNA becomes quite difficult. The endocytosis involves several steps *viz*. binding, internalization, formation of endosomes, fusion with lysosomes, and hydrolysis (Neekhra and Padh, 2004). It was suggested that during endocytosis of DNA–polycation complexes, the DNA might be entrapped and degraded in endolysosomes, which impedes efficient gene transfection. Previously, the capability of *D. discoideum* to produce erythropoietin was exploited by Vats and Padh (2007). Furthermore, the same group demonstrated an enhancement in the transfection efficiencies by inhibiting endocytosis after DNA uptake (Vats and Padh, 2009). Arya *et al.,* (2008a; b) successfully purified human recombinant proteins, PDE4 and PDE7 (both phosphodiestreases), in *D. discoideum* and similarly human spleen tyrosine kinase and GNE proteins were also purified (Singh *et al*., 2010; Grover *et al*., 2014). Future studies should be focused on optimizing heterologous protein production and studying the bioassay of recombinants proteins.

Summarizing, the *Dictyostelium* research field is not over-populated and definitely not in India. We need many more researchers to join and answer questions raised by the organism itself. The development of improved instrumentation and computational tools, transcriptome and proteome analysis will possibly influence this field of biological research in the future for accelerating and lead to new insights into the fundamental processes that are involved in *Dictyostelium*.

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