INTRODUCTION
The most outstanding feature of living organisms is, their capacity to reproduce themselves for generations with highest fidelity. With series of discoveries, now we know that the genetic information is contained in the linear sequence of nucleotides, deoxyribonucleic acid (DNA) (though some viruses have RNA instead of DNA as genetic material). DNA was first isolated by Miescher from the pus cells who called it nuclien. Structural elucidation of DNA by Watson and Crick (1953) is considered as one of the major milestones of modern biology. But it was Avery, MacLeod and MacCarthy’s (1944) experiment which proved Griffith’s transforming principle as same as DNA. Later, Messelson and Stahl proved (1958) the hereditary role of DNA at the molecular level. Today, it is proved beyond doubt that the genetic information is contained in and transmitted by DNA. So it is identical to say that understanding DNA is understanding life.

A variety of criteria is now applied for the studies of cellular functioning and its behaviour. One such method is to alter the DNA structure thereby, in turn, altering the functions of DNA. There are certain groups of chemicals which are known to affect specific phases of cell division e.g., decision to enter cell division, formation of spindle and initiation of cytokinesis.

1. DNA ACTING AGENTS

Chemicals specific for DNA synthesis inhibition and others which modify the structure are also known. It is understood that the mechanism could be different but the ultimate results may be the same e.g., inhibition of cell division, alteration of gene expressions in growing cell population or in differentiating cells (Kihlman 1966).
These compounds can be classified broadly into a few groups:

1. Nucleic acid base analogues
2. Nucleoside antibiotics
3. Alkylating agents
4. Nitroso compounds
5. Miscellaneous compounds.

1.1 NUCLEIC ACID BASE ANALOGUES

Purines and pyrimidines are basic components of nucleic acids. In both normal and altered growth of living systems these universal components are involved in cellular functions and multiplications.

Base analogue should be sufficiently similar to one of the four normal bases of DNA or ribonucleic acid (RNA), so that it can get incorporated into DNA during replication or in RNA during transcription by competing with the endogenous ones. Such a substance should be able to base pair into the template strand. However, if a base analogue has more than one mode of hydrogen bonding it could be mutagenic. Most of the analogues can participate in many reactions of their normal counterparts and may act on multiple loci (Kihlman 1966, Roy-Burman 1970).

Nucleic acid base analogues can be categorized into two classes:

(a) Purine Analogues

These analogues are structurally and functionally similar to naturally occurring purines like adenine and guanine. The examples are: 6-mercaptopurine,
6-azathiopurine, 6-methylthiopurine, 6-thioguanine, Allopurinol etc (Roy-Burman, 1970).

(b) **Pyrimidine Analogues**

These analogues are similar to naturally occurring pyrimidines, structurally as well as functionally. The examples are, 5-azacytidine, 5-azauridine, 5-azafluorouridine, 5-bromo-2-deoxyuridine, arabinosyl cytosine etc (Roy-Burman, 1970).

### 1.2 NUCLEOSIDE ANTIBIOTICS

The antibiotics which can resemble nucleoside in their structure have been termed as nucleoside antibiotics. They can fall into two major groups.

(a) **Amino Acid Linked Compounds**

They interfere with protein synthesis in various biological systems and do not function as structural analogues of nucleoside. The examples are: puromycin, lysylamino adenosine, homocitrullylamino adenosine, blastidin etc. (Roy-Burman 1970).

(b) **Adenosine Like Compounds**

They do not contain amino acids and with few exceptions they are converted into nucleotide derivatives in cells and participate in many reactions of adenosine and its nucleotides. These adenosine like antibiotics can be considered as both structural and functional analogues of nucleic acid components. Some examples are tubermycin, cordycepin, showdomycin (Roy Burman, 1970).
1.3 ALKYLATING AGENTS

They all carry one, two or more alkyl group in reactive forms. The action of alkylating agents on DNA is very complex. They are known to react with purine bases particularly with guanine at N-7 atom and the bifunctional alkylating agent (those with two alkyl reactive groups) may thus bring about cross linking between the opposite strands in the DNA molecules. Alkylation of purines in position N-7 also gives rise to unstable quaternary nitrogens so that the alkylated purines may separate from the deoxyribose leaving a gap which might interfere with DNA replication or cause the incorporation of wrong bases. The phosphate group may also be alkylated. The phosphate triester so formed is unstable and may hydrolyse the sugar and the phosphate so that the DNA chain is broken. Alkylating agents promote mutagenic and carcinogenic feature (Murray & Meyn, 1984, Davidson et al 1976). Some examples of alkylating agents are nitrogen mustard, melphalan, chlorambuol, cyclophosphamide, and arecoline etc.

1.4 NITROSO COMPOUNDS

Nitroso compounds are proved to be carcinogenic (Drukrey et al, 1961 a b & c Wary & Sharan, 1991). Presence of atleast one alkyl group in the molecule is necessary for its carcinogenic activity, the chromosome breaking effects. The carcinogenic effects requires the presence of oxygen. It is believed that the species responsible for such effects are alkylating agent formed by enzyme catalyzed oxidative dealkylation (Kihlman, 1966). The examples of nitroso compounds are
1.5 MISCELLANEOUS COMPOUNDS

Some compounds other than mentioned above too have chromosome damaging activity, for example:

Maleic hydrazide, structural isomer of uracil, is reported to produce chromosomal breaks in root tips of *Vicia faba* (Darlington and McLeish, 1951).

Potassium cyanide also causes chromosomal breakage in *Vicia faba* root tip chromosome. It brings about the effect by inhibiting the enzyme containing heavy metals (Lilly and Thoday, 1956).

Hydroxylamine is a potent mutagen which reacts specifically with cytosine and also reacts albeit less strongly with thymine and uracil. Chromosomal aberrations were reported by hydroxylamine (Somers & Hsu, 1962) in Chinese hamster ovary cells.

Formaldehyde reacts with amino group of free bases or single stranded nucleic acids (Wilhelm, 1971).

Structural changes in chromosome by acridine orange and visible light are reported (Kihlman, 1959, Ronchi & D'Amato, 1961). *In vitro* experiments have shown that irradiation of DNA with visible light in the presence of acridine orange resulted in breakage of chemical bonds in DNA. The requirements for this is oxygen and binding of dye to DNA (Freifelder *et al.*, 1961).
2. MODIFICATION OF CHROMATIN

2.1 Modification of DNA

(a) Methylation of Cytosine

Methylation of cytosine at 5th position of cytidine is a post synthetic modification of DNA. It can be maintained by methyl maintenance enzymes and can be inherited (Adams, 1974, 1982).

The DNA of many higher eukaryotes including man has a small proportion of 5-methyl cytosine. The distribution of 5-methyl cytosine in DNA is not random, most of it occurs in the sequence CpG. Since CpG is a self complementary sequence, fully methylated DNA would contain pairs of 5-methyl cytosine residue at opposite sites of the double helix (Adams & Burdon, 1985).

In general, the DNA of inactive genes are found to be heavily methylated than the DNA of active genes (Taylor & Jones, 1979, Davis et al, 1987, Monk, 1986, Monk et al, 1987) Moreover, in several cases inactive genes containing methylated cytosine have been shown to become demethylated during gene activation (Adams & Burdon, 1985, Compere & Palmiter, 1981).

One of the evidence of correlation between gene activity and demethylation comes from the experiments where base analogue 5-azacytidine is used. 5-Azacytidine can not be methylated because it has nitrogen at the 5th position of pyrimidine ring instead of carbon. In this case selective genes were shown to be activated (Taylor & Jones, 1979).
It is well known that cytosines were heavily methylated on the inactive X-chromosome than those on the active ones. In fact in this case timing of DNA methylation of several genes on inactive X chromosome has been shown to follow X chromosome inactivation quite closely (Jones et al, 1982, Jones & Taylor, 1980, Tribioli et al, 1992) but this observation leaves open the possibility of reduced DNA methylation as an effect rather than a cause of gene-activation. Moreover, organisms like Drosophila and Caenorhabditis elegans do not have any known DNA methylation pattern.

(b) Methylation of Adenine


Engel & Von Hippel (1978) have shown that presence of 6- methyl adenine results in destabilization of the DNA helix presumably because N1-cis orientation of the methyl group is favoured. Such effects could be significant in protein-DNA interactions (Sterglanz & Bugg, 1973, Cheng et al, 1985).

2.2 Modifications of histone

Nucleosome, the basic subunit of chromosome, contain 160-240 base pairs of
DNA complexed with histone. Histones are relatively small proteins with a very high proportion of positively charged amino acids. Histone binds very tightly to DNA and probably it plays an important role in many genomic activities.

There are five types of histones in the eukaryotic cells which fall into two categories. The first group or the nuleosomal histones comprises H2A, H2B, H3 and H4. They are responsible for folding the DNA into nucleosomes. These four histones are among the most highly conserved of all known proteins.

The second group consists of H1 which except for a central core region the amino acid sequence of H1 has been much less conserved.

There is a strong evidence that the modification of the histones are essential for chromatin condensation especially during mitosis (Mathews, 1977).

(a) Histone Acetylation

In this case lysine side chains are acetylated. Acetylation is thought to be enhanced in the chromatin of active genes (Allfrey, 1980, Johnson & Allfrey, 1978).

(b) Histone Phosphorylation

In this case serine residues are phosphorylated. Phosphorylation of H1 appears to be essential for the cyclic condensation of chromatin during the mitotic cycle. Though phosphorylation of H1 is required but it is not sufficient to trigger condensation (Darzynkiewicz, 1986, Gurley, 1981).

(c) Histone Methylation

Histone H3 & H4 are methylated on the amino groups. It takes place predominantly during G2 and mitosis (Darzynkiewicz, 1986).
(d) **Poly ADP Ribosylation**

It involves addition of ADP ribosyl subunit to H1 & H2B. The enzyme, poly (ADP) ribose polymerase, which catalyzes the reaction, is bound to the chromatin. The function of poly (ADP) ribosylation is not clear (Darzynkiewicz, 1986).

(e) **Ubiquitination**

The covalent linkage of ubiquitin, a highly conserved 74 amino acid long protein, to a lysine side chain of H2A is called ubiquitination. The role of ubiquitination is unclear. This may have an essential role in a continuous modulation of nucleosome structure and in packing of metaphase chromosome (Darzynkiewicz, 1986).

In view of profound alteration in DNA structure and function caused by above mentioned chemicals, we have investigated the actions of two potent DNA acting agents viz., arecoline and 5-azacytidine on cell growth and differentiation of a well-known eukaryotic microbe *Dictyostelium discoideum*.

3. **THE CHEMICALS**

3.1 **ARECOLINE** (arc)

Epidemiological studies have demonstrated a clear cut association between chewing of betel quid containing areca nut and an increased risk of oral cancer (Atkinson *et al*, 1964, Samuel *et al*, 1969, Jussawala & Deshpande, 1971, IARC, 1985).

Areca nut is often a major ingredient of betel quid but relatively less
information is available about the effects of areca nut alone (IARC, 1985).

Areca nut contains several alkaloids, tannin, fat and free fatty acids as well as polysaccharides. Arecoline is one of the major alkaloid which may comprise up to 0.8% by weight (Goswami & Ahmad, 1956). Arecoline is a vermifuge which exerts, cholinergic, and other effects similar to those produced by Pilocarpine, muscarine and nicotine (Burkhill, 1935, Muir & Kirk, 1960, Nieschulz & Schmersahl, 1968, Bhide et al, 1979, Choudhuri & Ganguly, 1974).

Arecoline (fig. 1) has been reported for carcinogenicity and mutagenicity (Boyland & Nery, 1969, Shirname et al, 1983, 1984) clastogenicity (Panigrahi & Rao, 1982, 83) teratogenicity (Sinha & Rao, 1985a) and immunosuppressive abilities (Selvan et al, 1991).

3.1.1 Various effects of arecoline

(a) Carcinogenicity

The ability of arecoline to react with thiol group shows that it is a alkylating agent (Boyland & Nery, 1969). This is considered as a feature of many chemical carcinogens. Alkylating agents, after reacting with thiol group can lead to uncontrolled cell proliferation (Harrington, 1967). But there is no report available showing any interaction of arecoline with any of the four bases of nucleic acids.

(b) DNA strand Breaks

Arecoline is known to cause single strand breaks in human buccal epithelial cells (Sundqvist et al, 1989, Wary & Sharan, 1988, 1991) and in bronchioepithelial cells (Grafstrom et al, 1986).
(c) **Effects on Macromolecular Synthesis**

The rate of protein and DNA synthesis was reduced in mammalian cells treated with arecoline (Wary & Sharan, 1991).

(d) **Cytotoxicity**

Cytotoxic nature of arecoline has been proved in mammalian cells in a dose dependent manner (Sundqvist et al, 1989, Wary & Sharan, 1988, 1991).

(e) **Micronuclei formation**

One of the major ways to determine the mutagenic activity of the given compound is to test for micronuclei formation. Many plant alkaloids have the capability to act like a spindle poison and thereby, facilitate micronuclei formation. Arecoline is reported to induce micronuclei formation in mice (Sinha & Rao, 1985b) and buccal mucosa of betel quid chewers (Stich et al, 1982a).

(f) **Genotoxicity**

Mice cells showed increased frequency of sister chromatid exchanges after treatment with arecoline. Caffeine along with arecoline exerts an additive effect on sister chromatid exchanges (Panigrahi & Rao, 1982, 1983).

(g) **Clastogenicity**

The clastogenic properties of arecoline has been reported in mouse bone marrow cells in vivo (Panigrahi & Rao, 1982, 1983) and in Chinese hamster cells in vitro (Stich et al, 1981).

(h) **Immunosuppressive ability**

Selvan et al (1991) have demonstrated the immunosuppressive ability of
arecoline in mammals.

(i) Modulation of Drug Metabolising System

Recently Singh and Rao (1993) have shown the ability of arecoline in increasing the activity of Cyto b5 and Cyto p-450 glutathione S transferase, malondialdehyde etc.

3.1.2 MODE OF ACTION

Arecoline under mild acidic condition forms N-nitroso compounds under \textit{in vitro} (Wenke & Hoffman, 1983) and \textit{in vivo} conditions (IARC 1985): such as 3-N-nitrosomethylamino propionaldehyde (NMPA) and 3-(N-nitrosomethylamino) propionitrile (NMPN). In rat, NMPN is proved to be a potent and complete carcinogen (Prokopczyk \textit{et al}, 1987, Wenke \textit{et al}, 1984) which can methylate as well as cyanoethylate DNA in target tissue (Prokopczyk \textit{et al}, 1987). The nitroso compounds are known to be strong carcinogens (Castegnaro, 1988).

In rat, arecoline reacts with N acetyl cysteine and gets converted into N-acetyl-S-(3-carboxy-1 methylpiperil-4-yl)-L-cysteine. It reacts similarly with glutathione. In fact the ethylene bond of arecoline reacts with the thiol group (Ashby \textit{et al}, 1979) under \textit{in vivo} and \textit{in vitro} conditions (Boyland & Nery, 1969). The mechanism of addition of thiols to the ethylenic bond of arecoline is complex and not very clear.

The reaction of arecoline with thiol group shows that it is an alkylating agent (Boyland & Nery, 1969). This is a common feature of many chemical carcinogens with or without metabolic activation. However, this serves no proof of its having a
carcinogenic potential but it was shown that such interactions with thiol groups could lead to uncontrolled cell proliferation ultimately leading to cancer (Harrington, 1967).

3.2 5-AZACYTIDINE (azaC)

5-azacytidine (fig. 1) is a triazine nucleoside analogue of cytidine. It was synthesized first by Piskala and Sorm in 1964 but later, was discovered in actinomycetes *Streptoverticillium ladakanus* (Hanka *et al*, 1966, Bergy & Herr, 1966)

5-Azacytidine is considered to be a powerful bacteriostatic, antitumour and mutagenic agent. It has also been proved to be antimitotic, immunosuppressive, radioprotective and virostatic agent (Vesely & Cihak, 1978).

It is a very potent DNA hypomethylating agent due to the presence of nitrogen at 5th position in place of carbon in its pyrimidine ring. It has profound effects on cellular ageing and also induces altered pattern of development (Sorm *et al*, 1964).

In various differentiated cells transcriptionally active chromatin is characterized by hypomethylation, sensitivity to non specific DNase, presence of sites hypersensitive to DNAse I. From these alterations hypomethylation appears to be primary one triggering the sequence ultimately leading to gene expression. There are many such reports showing the increased cellular activities due to hypomethylation, brought by 5-azacytidine (Bird *et al*, 1981, Christman *et al*, 1980, Constantinides *et al*, 1977, Groudine *et al*, 1981, McGhee & Ginder, 1979, Taylor & Jones, 1979)

3.2.1 Various effects by 5-azacytidine

(a) Carcinogenicity

5-azacytidine has been reported to increase the frequency of large plague
mutant of *Venezuelan equine encephalomyelitis virus* (Halle, 1968). The temperature sensitive mutant of an *Avian sarcoma viruses* is obtained following 5-azacytidine treatment (Toyoshima & Vogt, 1969).

(b) **Cytotoxic & Cytostatic Effects**

The cytotoxic nature of 5-azacytidine was reported by Constantinides et al in 1977. In *E.coli* azacytidine arrested cell division completely (Doskocill and Sorm, 1970).

(c) **Interference with Macromolecular Synthesis**

Protein synthesis was completely inhibited in *E.coli* whereas RNA synthesis was much less affected. Surprisingly, the rate of DNA synthesis was not affected though the growth was arrested completely (Doskocil et al, 1967, Doskocil and Sorm, 1973).

(d) **Radioprotective effects**

Vesely et al (1969) have shown that when azaC is administered at an appropriate dosage and time schedule, it exerted a considerable degree of radioprotection in mice.

(e) **Immunosuppressive ability**

Immunosuppression by azaC is reported in mice (Vadlamudi et al, 1969, 1970b, Fischer et al, 1966).

(f) **Embroytoxicity**

AzaC causes developmental abnormalities in the cranial parts and in the liver of treated fetuses of mice (Seifertora et al, 1968).
(g) **Modulation of induced liver enzymes**

AzaC blocks the hormonal induction of tryptophan oxygenase in rats. AzaC has been shown to stimulate the basal levels as well as tryptophan and casein hydrolysate induced levels of rat liver tyrosine aminotransferase (Cihak et al., 1973a, Vesely & Cihak, 1978).

### 3.2.2 Mode of Action

Upon entering the cell, azaC may get deaminated to 5-azauridine and either of these can be phosphorylated and gets incorporated into DNA and all forms of RNA. This causes a rapid breakdown in protein synthesis and polysome synthesis (Cihak et al., 1973b).

Azacytidine can kill the cell after a lag of several hours as a result of their incorporation into DNA (Li et al., 1970, Zain et al., 1973, Adams et al., 1982).

Some of the deleterious effects of azaC are caused by the breakdown of the triazine ring. In this case chromosome damage can be seen (Karon & Benedict, 1972, Li et al., 1970, Harrison et al., 1983).

Effects of azaC on protein synthesis has been studied in great detail. It was observed that 5-azauridine containing mRNA is non translatable but azaC allows translation with frequent miscoding. The polysome containing azaC incorporated rRNA show less affinity for mRNA during translation while tRNA does not show much variation from normal ones (Paces et al., 1968a, b).

DNA methyltransferase binds irreversibly to DNA containing 5-azacytosine (Santi et al., 1984). They propose that enzyme via -SH group acts at 5:6 double bond
and become bound to the 6th position thereby activating the otherwise inert carbon in 5th position. Addition of a methyl group and β elimination release the enzyme again. The presence of nitrogen in place of carbon in position 5 will lead to accumulation of DNA enzyme intermediate or more likely the breakdown of triazine ring to release a formylated enzyme. Proteins other than DNA methylase may also bind to azacytosine containing DNA and formation of any stable DNA-protein complexes can alter gene expressions.

4. THE ORGANISM: *DICTYOSTELIUM DISCOIDEUM*

*Dictyostelium* was first discovered in 1869 by German mycologist Brefeld but its asexual life cycle, the most unique feature of *Dictyostelium* was understood only in 1890 by Ph. Van Teighem. It was only after discovery of a new species *Dictyostelium discoideum*, by Raper in 1935, developmental biologists paid more attention to this organism.

Taxonomy of *Dictyostelium* still remains uncertain due to its striking similarity with organisms in different kingdom (Grell, 1935). Some mycologists consider it as non-hyphal fungi and include it under special division Myxomycota, while protozoologists classify it under Rhizopoda or Sarcodina among protozoa. The developmental processes of this organism are quite similar with higher metazoan though in a much simplified manner. Despite of its controversial position *Dictyostelium* remain a very popular model system among investigators to answer various problems in biological sciences (Loomis, 1982).
4.1 Life Cycle of *Dictyostelium discoideum*

*Dictyostelium discoideum* has two alternate life cycles

(a) Asexual (b) Sexual

(a) Asexual

*Dictyostelium discoideum* popularly known as cellular slime mould is unicellular, eukaryotic, haploid organism which is abundant in soil. The natural food for *Dictyostelium* is bacteria (Pann *et al*, 1972) but the mutants like Ax1, Ax2 etc can be cultured in a defined media.

As long as food is available *Dictyostelium* grows and divides by binary fission. Depletion of food leads to differentiation. At first few cells start secreting cAMP which is sensed by distal cells. In response they move towards the source and make aggregates. The movement of cells is periodic and highly organized. During signal relay many cAMP receptors are expressed on the surface of aggregation competent cells (Siu, 1990). When cAMP binds to the surface receptors it activates adenylate cyclase and the activated cells in turn, release pulses of cAMP (Klein, 1976). In *D. discoideum* the cAMP pulse is maintained by adaptation of receptors and the enzyme extracellular phosphodiesterase (ePDE) which attenuates the signal by cleaving cAMP. The ePDE level is regulated by ePDE inhibitors (Janssens & Van Haastert, 1987, Frank & Kessin, 1981).

Aggregate is first loosely made then it becomes a tight mound like structure (Bonner, 1971). Only during differentiation specific adhesion molecules are synthesized (Bozarro *et al*, 1987, Siu, 1990). The EDTA sensitive adhesion molecule
is 24 kD glycoprotein which appears maximal during the first 4 hours of development. Another glycoprotein gp 80 is expressed during aggregation but drops rapidly in the post aggregating stages. Subsequently a tip appears on the top of the mound and moves upward to give finger like structure which is called slug. The slug is motile and has worm like appearance. Slug stage shows specific surface molecule gp 95. It migrates and continuously secretes slimy sheath material, and subsequently culminates into fruiting body consisting mainly of two cell types, stalk cells made up of dead vacuolated cells and spores mass, the dormant form to start the new cycle upon germination (Loomis, 1982).

(b) Sexual Life Cycle

*Dictyostelium* cells of opposite mating types or heterothallic strains can form zygote which is a giant cell. The zygotes are surrounded by many amoebae and ultimately by a wall. Then the giant cell phagocytoses the other cell and eventually it is the only cell remains within the wall which is known as macrocyst. Subsequently zygote undergoes series of reduction divisions, forming many cells within the macrocyst derived from zygote. On favourable condition, cells germinate from macrocyst and begin the new cycle (Loomis, 1982).

4.2 *Dictyostelium* Genome

*Dictyostelium* is a haploid eukaryote. It has 7 chromosomes. The genome size is roughly 10 times larger than *E.coli* and 10 times smaller than human. It has unique nonrepetitive portion which makes up 70-75% of the genome. The remaining 25-30% of the *Dictyostelium* genome is composed of sequences that are repeated an average
100 times per haploid cell (Cockburn et al, 1976, Maizels, 1976).

The G+C content of the nuclear genome is only 22%, one of the least G+C rich source known (Sussman & Ruyner, 1971, Firtel & Bonner, 1972). The base composition of protein coding region of some genes reveals G+C content to be as much as 30-40% (Jacobson et al, 1974, Firtel et al, 1979). Interestingly, the G+C residues are distributed in a very non uniform pattern.

In contrast, the non coding region contains much lower 5-10% G+C contents. The A+T rich region includes the 5’ and 3’ untranslated sequence of mRNA, the introns and rDNA space sequence (Firtel et al, 1976, Firtel et al, 1979, Mckeown and Firtel, 1981).

4.3 Some unique features of Dictyostelium

In Dictyostelium the growth and development are mutually exclusive unlike most plant and animals where growth is a major factor in differentiation. For example in plants and in animals fertilized eggs or spores grow as the same time as they differentiate and undergo morphogenesis.

In Dictyostelium only two differentiated cell types are present, stalk and spore cells though the process of development and pattern formation occurs as in many higher organisms (Bonner, 1967, Williams et al, 1989, Inouye, 1992). It was considered that there is very little or no interchange or interactions between two cell types (Devine & Loomis, 1985) but later on interconversion of cells were observed (Sternfeld,1992). Whatever changes or interconversion takes place, the prespore/prestalk ratio remains same. In Dictyostelium during slug stage, a clear cut
pattern formation exists which makes it an excellent system to study differentiation and pattern formation which is still an enigma to developmental biologists.

Though the cAMP signal transduction during aggregation in Dictyostelium has been studied extensively, but cell interactions in slug stage and during culminations are still not clear where cAMP plays a dual role, as a signal transduction molecule as well as morphogen.

The Dictyostelium amoebae are haploid possessing only 7 chromosomes. It makes Dictyostelium an ideal system for genetical studies and mutagenesis. Moreover, it is very easy to grow and handle in the lab.

The unique asexual life cycle has made Dictyostelium one of the most favourite model system to answer some fundamental questions regarding cell adhesion, cell movement, pattern formation, signal transduction and gene expression. The life cycle of Dictyostelium discoideum has been illustrated in fig. 2.

5. AIM AND SCOPE OF THE PRESENT INVESTIGATION

The complex arrangement of cells and tissues in a multicellular organism arise from the precise expression of different combinations of genes in different sets of cells throughout the development. One of the major aim of developmental biology is to understand time and position specific expression of genes during morphogenesis and how it is related to the formation of a distinct pattern of differentiated cell types. Separation of growth from differentiation, presence of only two types of terminally differentiated cell types, cAMP chemotaxis, highly regulated signal transduction
system, cell adhesion and pattern formation are some of the phenomenon which made
*Dictyostelium* an excellent model system to study the morphogenesis.

In Indian subcontinent and other parts of the world betel quid chewing is
associated with oral cancer. Arecoline, active principle of betel nut, is a known
mutagen and carcinogen. 5- Azacytidine a DNA hypomethylating agent has profound
effects on ageing and differentiation.

In the present investigation the effects of these two DNA acting agents
(arecoline and 5-azacytidine) have been studied on growth and differentiation of
cellular slime mould *D. discoideum*.

The present study has been undertaken with the following objectives:

1. To check the effects of arc and azaC on survival, spore germination,
endocytosis and macromolecular synthesis in *Dictyostelium* cells.

2. To check the effects of arc and azaC on the morphogenesis, pattern formation,
cAMP chaemotaxis, EDTA stable contacts formation and ePDE activity.

3. To find out the interrelationship between growth and differentiation which may
exist in the cellular slime moulds.