ANALOGUE AND DRUG-INDUCED CHANGES IN THE TEMPORAL DEVELOPMENT OF *DICTYOSTELIUM DISCOIDEUM*

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Analogue and Drug-induced Changes in the Temporal Development of *Dictyostelium discoideum*

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Abstract:

The temporal development of *Dictyostelium discoideum* is altered by the disruption of pyrimidine metabolism in amoeba during mitotic cell division or following nutritional starvation. The well-defined differentiation process is accelerated by exposure to specific biosynthetic intermediates, degradative products, uridine analogues, and drugs affecting pyrimidine biosynthesis or nucleotide interconversion. These perturbations appear to be stage specific and are induced by specific effectors. Artifical elevation of orotate and dihydroorotate pools or uridine pools by medium supplement (50 mg/ml) causes the abrupt cessation of amoeboid growth for 14-18 hours followed by a resumption of normal growth rates. Folate analogues, allopurinol, and barbiturates induce similar changes. A variety of pyrimidine analogues independent of nitrogenous base cause multifarious effects. However, enhanced differentiation resulting from exposure to naturally occurring pyrimidine metabolites is not observed with cytidine and thymidine derivatives.

Introduction:

D. discoideum has become increasingly popular as a model system for the analysis of the biochemical mechanisms involved in eukaryotic differentation (Loomis, 1975). The complex development of the cellular slime molds (See Fig. 1) was described using a film by G. Gerisch (1972) distributed by Bailey Films Associates.

Materials and Methods:

Organism: Dictylostelium discoideum AX2 is a cellular slime mold which forms no syncytial plasmodium upon social aggregation. D. discoideum AX2 is a strain capable of axenic growth derived from NC4. Growth: AX2 is grown on Escherichia coli B/r on BACTO Minimal Broth Davis containing 0.2% glucose or axenically in medium (HL-5) of Cocucci and Sussman (1970). Growth was monitored by haemocytometer count. Various effectors were added to HL5 liquid culture at 50 mg/ml final concentration and growth in parallel cultures was compared. Differentiation: The development of differentiated fruiting bodies was obtained by either prolonged growth on minimal medium using E. coli B/r as a nutrient source or by placing washed amoeba on solid, non-nutritive millipore filters as described by Sussman (1967).

Results and Discussion

Vegetative growth may be interrupted by nutrient starvation and a well-defined developmental process is initiated. Social aggregation of 10^3-10^5 amoeba occurs following a seven hour orientation toward differ-

entiation (see Fig. 1). During this initial period RNA and protein degradation is stimulated and a unique regime of gene expression begins (Firtel, 1972). Aggregation occurs between hours 7 and 10 and the formation of an immobile aggregate is complete at twelve hours. Coordinated migration of the cellular aggregate begins as anteriorly positioned cells assume chemotactic and phototactic function. Differentiation is completed by the establishment of a complex fruiting body containing 10^3-10^4 spores within the sorus (for review see Loomis, 1975). This developmental scheme has been analyzed and four classes of dysfunction have been identified as follows:

germination inhibitors:	eg. 3, 5-dibenzoyl-thymidine
differentiation enhancement:	eg. trimethoprim, barbiturates, pyrimidine precursors from de novo synthesis, degradative products
differentiation inhibitors:	eg. 6-azauridine, 5-fluorodeoxycytidine, allopurinol
aggregation inhibitors:	eg. caffeic acid

Approximately 20% (21/110) of the analogs tested as either positive or negative effectors on the rate of differentiation. Several of the compounds which enhance the rate of differentiation have been analyzed according to the scheme in Figure 2.

These compounds do not affect the rate of bacterial growth but stop amoeboid growth in axenic medium and appear to provide some early transition signal for differentiation. Mitotic cell division of amoeba in axenic medium will be stopped by the addition of 50 µg uridine, barbiturate, or dihydrouracil per ml (Figure 3). Immediate cessation of growth occurs upon addition of these compounds (A, 12 hours) and growth is inhibited for 12-16 hours. The initial growth rate (8.3 hours per generation) is abruptly re-established following inhibition. Mitotic cell division can be interrupted a second time (B, 40 hours) and this inhibition does not seem to be affected by the first disruption. All of the normal pyrimidine

metabolites (eg. uridine, anabolic intermediates, and degradative products) and the antimetabolites (eg. 6-azauridine) which cause developmental dysfunction result in similar inhibition of amoeboid growth. Pyrimidines which do not affect differentiation (eg. 2-thiouridine), do not affect cellular growth.

The intermediates of de novo biosynthesis are effectively channeled to UMP by a multifunctional enzyme complex in eukaryotes (Shoaf and Jones, 1973). Six enzymes involved in de novo synthesis of pyrimidines from glutamine and ATP may form a facilitated aggregate which effectively prohibits the formation of intracellular pools of carbamyl aspartate, dihydroorotate, orotate, and orotidylate (OMP) (Fig. 4). Artificial elevation of any of those pools might signal metabolic dysfunction for pyrimidine biosynthesis. Allopurinol mimicks the effects of de novo biosynthetic intermediates in Dictyostelium and has been shown to inhibit OMP pyrophosphorylase or OMP decarboxylase activities in vitro (Foster et al. 1973). Since these enzymes function subsequent to orotate in the biosynthetic pathway, allopurinol may produce its effect by causing an increase in orotate and/or dihydro-orotate pools. The effects of degradative intermediates may result directly from a similar mechanism or may feedback effect the biosynthetic pathway.

Furthermore, barbiturate, uridine, and dihydrouracil shorten the normal differentiation cycle from 24-26 hours to 10-14 hours. Figure 5 represents the variation observed in the developmental cycle of D. discoideum. Although most of the distinct stages are observed, the rate of aggregation is enhanced and several intermediate stages appear to be abbreviated. Figure 5A represents the normal time-developmental sequence when amoeba are removed from HL-5 growth medium and placed on a solid, non-nutritive substratum described by Sussman (1967). Figures 5B and 5C

represent the types of temporal changes that result from the addition of uridine or barbiturate to actively growing cultures. Those compounds which produce elevated intracellular pools of pyrimidine precursors or produce high uridine or pyrimidine degradative pools enhance development. Several pyrimidine metabolites involved in the de novo synthesis of pyrimidines (eg. dihydroorotate and orotate), salvage pathway (eg. uridine and uracil), and degradation (barbiturate and dihydrouracil) disrupt the developmental stages of D. discoideum. These dysfunctions may result from the temporary production of abnormal intercellular pools of metabolites.

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Legends to Figures

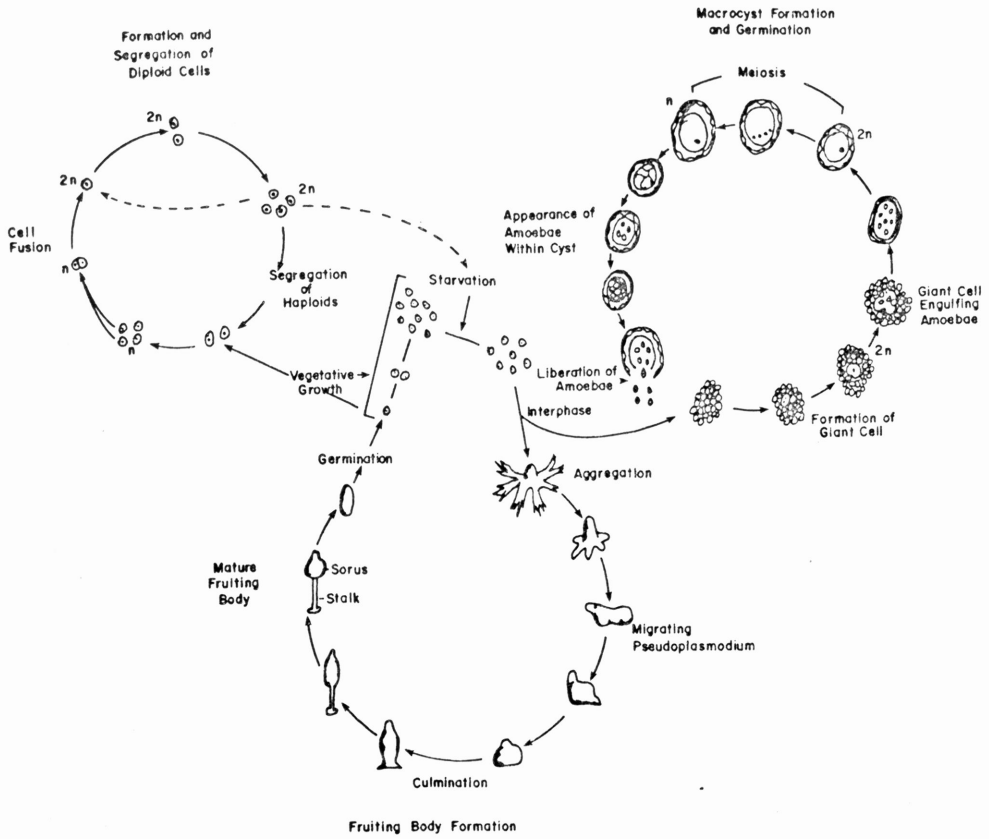
Figure 1. Life cycle of Dictyostelium discoideum. Three distinct morphological cycles comprise the life cycle of D. discoideum when haploid vegetative growth is disrupted. Fruiting body formation results from the aggregation of up to 10^5 amoeba followed by pseudoplasmodial formation and cellular differentiation. The life cycle possesses no obligate sexual phase but pairs of haploid cells can fuse and undergo karyogamy to produce true diploids at a frequency of 1/10,000 cells. Alternatively, cells may aggregate in submerged culture in the absence of nutrients and form macrocysts containing diploid cells (modified from Jacobson and Lodish, 1975).

Figure 2. Developmental Scheme of D. discoideum. Following heat shock the spore case splits longitudinally during germination and the liberated amoeba grow by mitotic cell division. Upon nutrient deprivation mitotic cell division ceases and chemotactic aggregation produces a pseudoplasmodium capable of directed migration. Following a variable period of migration extensive cellular differentiation produces a complex fruiting body topped by a sorus containing 10^3-10^4 encapsulated spores.

Figure 3. Effect of pyrimidines on mitotic cell division in axenic medium. Growth of AX2 on HL5 medium was monitored through the addition of several pyrimidines at concentrations of 50 µg uridine (−▲−) and barbiturate (−●−) per ml compared to the control without additional pyrimidines (−○−). Identical concentrations were added at 12 hours (A) and at 40 hours (B). Similar results have been obtained with dihydrouracil and orotate.

Figure 4. Generalized scheme of pyrimidine biosynthesis.

Figure 5. Effect of barbiturate and uridine on non-nutritive differentiation of D. discoideum. Several distinct morphological stages are observed during fruiting body formation and the stages are temporally determined under defined conditions as referenced in materials and methods. Barbiturate and uridine alter the normal temporal development represented in A by abbreviating the sequence as shown in B and C. Dihydrouracil has similar effects.



SPORES

heat shock

GERMINATION

add bacterial source

INFINITE MULTIPLICATION → NO DIFFERENTIATION

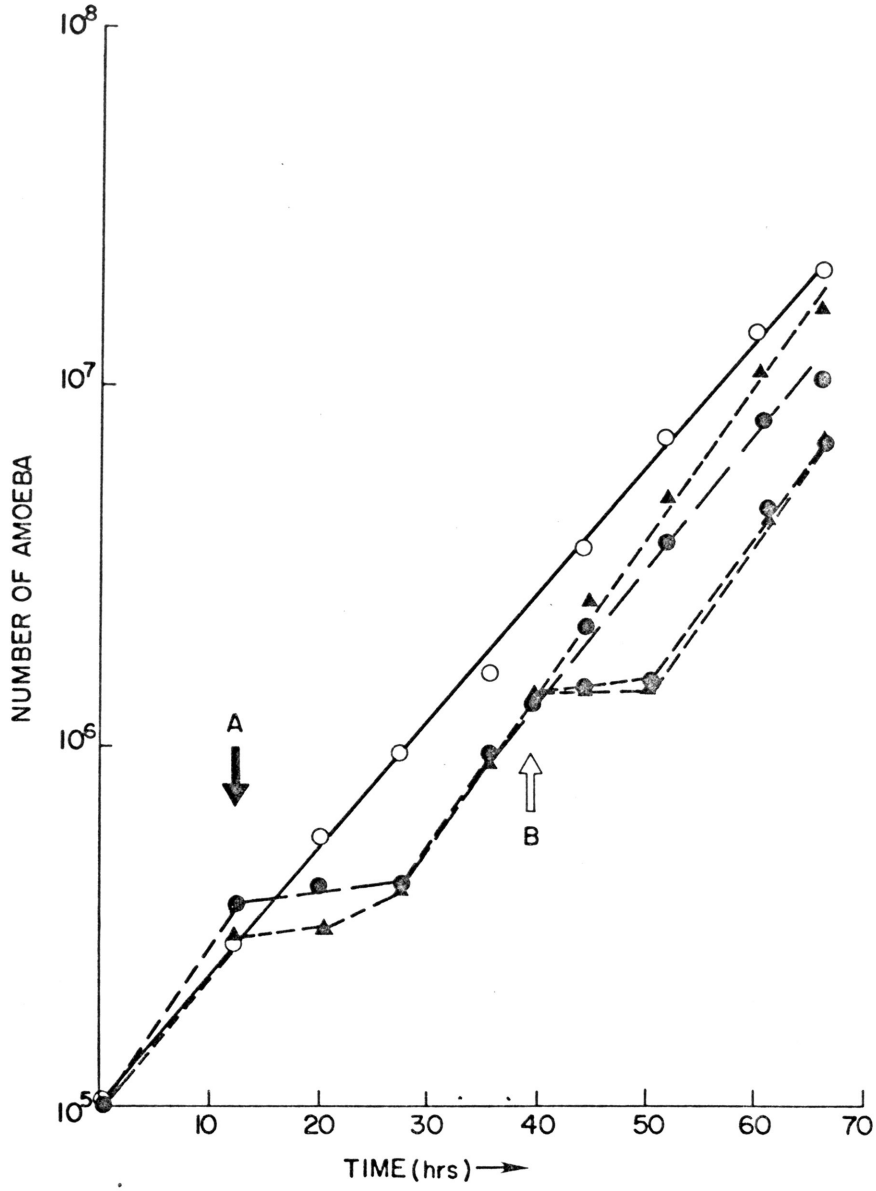
deplete nutrient

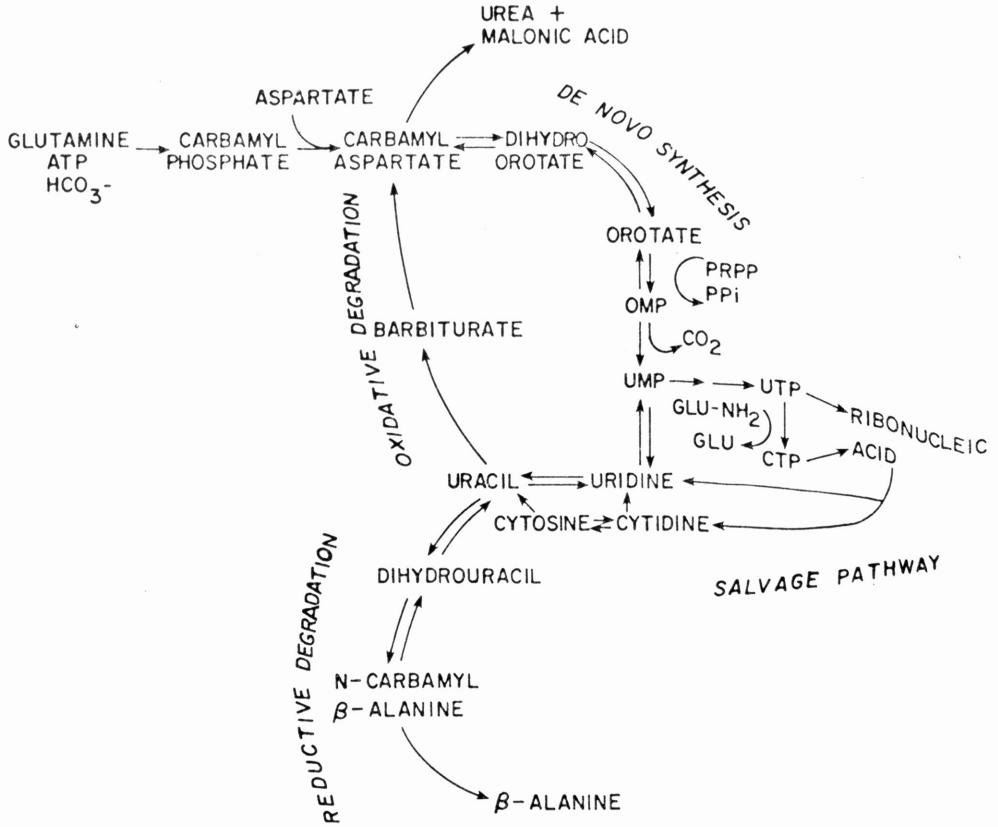
MITOTIC DIVISION CEASES

NO CHEMOTACTIC AGGREGATION → DEATH

CHEMOTACTIC AGGREGATION → MATURATION AND DIFFERENTIATION

SORUS FORMATION


10^8
10^7
10^6
10^5
NUMBER OF AMOEBA
A
B
10
20
30
40
50
60
70
TIME (hrs) ⟶



GENERALIZED SCHEME OF PYRIMIDINE BIOSYNTHESIS