THE EFFECT OF ACTINOMYCIN D ON DEVELOPMENT IN THE CELLULAR SLIME MOLD

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The accompanying report describes the rate of RNA turnover at various stages of development in the cellular slime mold. As a test of the biological significance of this process for differentiation we have investigated the effect of Actinomycin D (Act D) on the rate of development and RNA synthesis in this organism.

Methods

Dictyostelium discoideum, strain NC-4, was grown in the presence of E. coli, separated from the bacteria by differential centrifugation (Liddel and Wright, 1961) and spread on 2% agar plates containing 0.01 M phosphate buffer, pH 6.5, and 0.001 M EDTA, in the presence or absence of Act D.* Cells were rinsed off the plates and incubated with radioactive uracil (the incubation mixture for cells from a plate containing a given concentration of Act D also contained Act D at that concentration); the rate of RNA synthesis was based on the specific radioactivity of intracellular UTP (Pannbacker, 1966).

To test the effect of Act D at later stages of development, the cells were allowed to reach the desired stage in the absence of Act D and then re-spread on plates with and without Act D. The cells on the control plates reaggregated and developed in a normal fashion.

Results and Discussion

Fig. 1 shows that only myxamoebae, prior to the aggregation stage, are completely inhibited with respect to their subsequent morphogenesis by Act D

*The kind gift of Merck and Co.

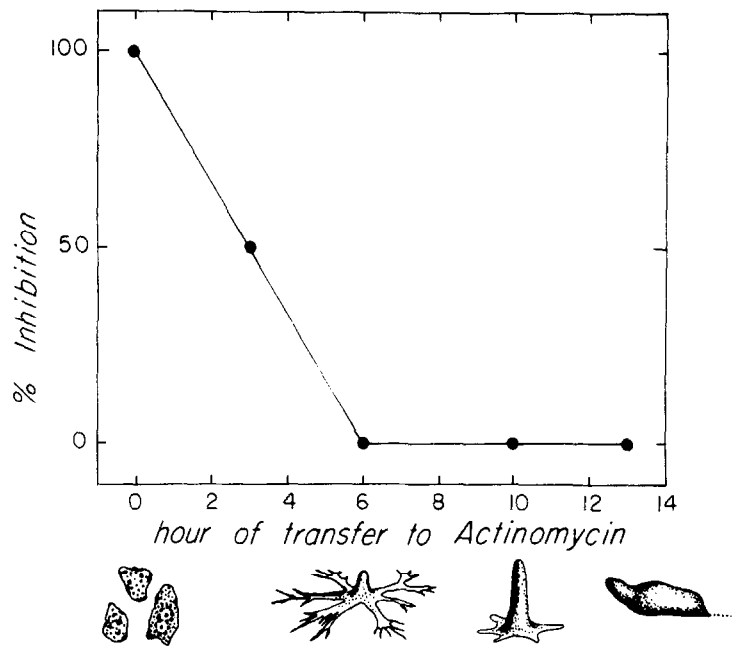

Fig. 1. Inhibition of the rate of development of cells transferred to plates containing 10 μg/ml Act D at various stages.

(10 μg/ml). Amoebae transferred to the drug at any time during the first few hours of starvation show no signs of development (i.e., do not subsequently aggregate), while cells in the early stages of aggregation show some inhibition in their developmental rate. In contrast, aggregated cells as well as those allowed to reach any later stage prior to exposure to Act D are completely unaffected (with respect to their development) by 10 μg/ml of the drug. Indeed, 30 μg/ml Act D will not affect development of aggregated cells and 60 μg/ml give only partial inhibition of development. Table I summarizes these observations and describes the effect of the drug on RNA synthesis under these various conditions. It appears that comparable degrees of inhibition of RNA synthesis may be attained whether or not differentiation is arrested.

TABLE I

Actinomycin D Effects on Development and RNA Synthesis

Stage at which cells were transferred to Act D plates	Expt. No.	Act D level (μg/ml)	Hours exposed to Act D	Effect on development	Inhibition of RNA synthesis
Amoebae	1	10	2	Complete inhibition	39%
	2	10	2	" "	53%
	2	10	6	" "	62%
Aggregation	1	10	2	None	23%
	2	10	2	"	5%
	2	10	6	"	51%
	3	10	12	"	48%
	4	10	12	"	51%
	3	30	12	"	58%
	5	60	6	Partial inhibition*	92%
	4	60	12	" "	86%

*Rate of development is lower; cells may not complete fruit formation.

The preceding paper emphasizes the importance of basing calculations of the rate of RNA synthesis on the specific radioactivity of the immediate precursor (^{14}C-UTP). This procedure is also necessary when comparing rates of RNA synthesis in the presence and absence of Act D. Table II shows typical data indicating that 10 μg/ml Act D may have pronounced, unpredictable effects on the specific radioactivity of intracellular UTP following exposure of the cells to ^{14}C-uracil. The inhibitions reported in Table I are based on endogenous ^{14}C-UTP-specific radioactivity.

In the interpretation of the data of Table I, it should be borne in mind that the amoebae may still grow to a limited extent after their transfer to 2% agar, during the early stages of the "starvation" period. Perhaps some process associated with the end of vegetative growth is the site of greatest sensitivity to Act D. Such an interpretation is consistent with data suggesting that mRNA may only be synthesized during periods of growth (Brown, 1966), and with the possibility that messages necessary for future multicellular differentiation may be present in the amoebae prior to multi-

TABLE II

The Effect of Act D on the Specific Radioactivity of Intracellular ^{14}C-UTP

	UTP Specific radioactivity (cpm/μmole)	
Expt. No.	Control	Act D
1	10,400	23,000
2	391,000	394,000
3	16,900	5,120

cellular differentiation (Wright, 1960; Wright, 1963). Cells which have reached the Act D resistant stage are disaggregated in the process of replating them on Act D plates, yet they reaggregate and continue morphogenesis in a normal manner, even though their RNA synthesis can be inhibited to the same extent as that of sensitive cells. Thus, whatever the mechanism of Act D inhibition of morphogenesis may be, aggregation *per se* is not prevented by the drug.

The lack of effect of Act D on differentiation (as opposed to its inhibition of development), is open to fewer interpretations. Clearly, that fraction of RNA which is not synthesized in the presence of the inhibitor (50%) is not critical to multicellular development after aggregation has begun.

Our data are of interest to compare with other recent studies of the effect of Act D on the slime mold. Using levels of Act D 10-fold higher than ours, Sussman and Sussman (1965) examined its effect on the appearance of an enzyme activity during development (UDP galactose polysaccharide transferase). The results indicated that sensitivity to Act D does not begin until about 7 hours after the start of morphogenesis, and lasts 8 hours (pseudoplasmodia stage). However, based on a different index of differentiation,

our data suggest a decreasing importance of RNA synthesis from the very beginning of starvation until the aggregation stage of differentiation.

The striking effect of Act D at 10 μg/ml justifies its use at this level, as opposed to higher levels, for investigations of the interdependence of differentiation and RNA synthesis at the later stages of morphogenesis. This is true particularly in view of available data demonstrating that the amoebae (as contrasted to later stages of development) are least permeable to four compounds tested (Wright, 1964a; Pannbacker, 1966) and, presumably, also to Act D. Thus, in effect, the same exogenous concentration of Act D applied at an early and at a later stage may well result in a higher intracellular level in cells at the later stage. Furthermore, higher levels of this inhibitor may arrest morphogenesis indirectly, owing to non-specific deleterious effects on metabolism. Complex phenomena such as respiration, growth, and protein synthesis, can be at least as sensitive to Act D as is RNA synthesis (Laszlo *et al.*, 1966; Reich *et al.*, 1962; Revel *et al.*, 1964). Even at the *in vitro* level, high levels of Act D have resulted in non-specific effects in some systems; such levels are required to inhibit RNA synthesis using slime mold DNA as a primer (Kahan *et al.*, 1963; Reich and Goldberg, 1964).

In conclusion, it must be realized that even if the inhibition of RNA synthesis is directly responsible for an inhibition of differentiation, a requirement for new mRNA synthesis need not be invoked. Active synthesis of mRNA may well be necessary for the maintenance of a specific enzyme in differentiating systems, particularly since such systems are frequently characterized by rapid protein turnover (Wright, 1964b). It should also be pointed out that, if the activity of such an enzyme is critical only to a later stage of morphogenesis, interference with its maintenance could be interpreted as a failure in the appearance of a new message at that time.

The results and interpretations of the effect of Act D on the slime mold serve as another example of the difficulties inherent at this time in

ascribing a definite role to mRNA in differentiating systems (Nemer and Bard, 1963; Spirin and Nemer, 1965; Gross and Cousineau, 1964; Gross et al., 1964).

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