Infection Structures from Rust Urediospores: Effect of RNA and Protein Synthesis Inhibitors

Abstract. *Urediospores of* Puccinia graminis tritici, *floated on buffer, produce infection structures when subjected briefly to 30°C soon after germination. Inhibitors of RNA synthesis interfere with the differentiation of infection structures if present during this heat treatment. Inhibitors of protein synthesis prevent differentiation if present following heat treatment. Apparently infection structure formation is accompanied by synthesis of RNA, and the completion of infection structure development requires protein synthesis.*

The rust fungi are obligate parasites of higher plants. Characteristically, a colony of the fungus is initiated by a spore germinating on the leaf epidermis, and developing a germ tube which grows until it reaches a guard cell. It then gives rise to a series of structures (appressorium, vesicle, and infection hypha) collectively termed infection structures (Fig. 1) because they establish the parasitic association with the host plant. Their formation is the prelude to colony formation, and may involve changes important for the continuation of vegetative growth.

On nonliving substrates only germination and formation of an unbranched germ tube normally occur, and growth of the germ tube stops before it is a millimeter long. Development of the infection structures can be induced in various ways with different species of rust fungi (*1–3*).

For urediospores of *Puccinia graminis* Pers. f. sp. *tritici* Erikss. and E. Henn. (race 56), we used a heat shock consisting of an exposure of germinated spores to a temperature of 30°C for 1.5 hours as the inducing treatment. This results in differentiation of 80 to 90 percent of the germinated spores (*2*). Exposure to inhibitors of RNA and protein synthesis before, during, or after the heat treatment provides evidence of the nature and timing of RNA and protein synthesis in relation to the progress of differentiation.

Analogs or antibiotics known to inhibit some phase of protein or RNA synthesis were prepared in a calcium phosphate–potassium phosphate buffer at *p*H 7.0 (*2*). Their effects on germination and germ tube development were assayed in micro-Conway vessels with a loopful of spores (about 2000) floating on 0.5 ml of test solution in the center well. Inhibitors were added or removed by replacing this solution. Nearly complete germination (95 percent) in 90 minutes was assured by 0.5 ml of $1 \times 10^{-4}M$ *n*-nonyl alcohol in the outer ring. Figure 1 shows the results obtained with four of the compounds tested.

Actinomycin D and 5-fluorouracil (5-FU), inhibitors of RNA synthesis, interfered with later differentiation if present during the heat shock treatment. Actinomycin D prevented development of infection structures when present during the heat shock period only, whereas 5-FU also prevented subsequent differentiation if present only during the germination period. The action of 6-methylpurine (100 μg/ml) was the same as that of 5-FU. The presence of these compounds did not, however, interfere with germination or growth of the germ tube. Inhibition of differentiation by 5-FU (100 μg/ml) was completely overcome by uracil (200 μg/ml). Development of normal infection structures occurred in the presence of 8-azaguanine or 5-bromouracil, each at 100 μg/ml.

In contrast to the inhibitors of RNA synthesis, inhibitors of protein synthesis, such as puromycin and *p*-fluorophenylalanine (FPA), prevented the differentiation of infection structures if present after inductive treatment. Puromycin was active only during that period, and its presence during earlier stages was without effect upon subsequent differentiation. The FPA (100 μg/ml) acted in the same manner as puromycin. The action of cycloheximide is apparently more complex. This antibiotic inhibited germination and the development of infection structures if applied during any stage of differentiation. Urediospores germinated and formed normal infection structures in the presence of either streptomycin or chloramphenicol (100 μg/ml). Germination and the proportion of germ tubes forming infection structures were in these cases comparable to controls.

The inhibition by actinomycin D, 5-FU, and 6-methylpurine indicates that the initiation of infection structure formation is accompanied by the synthesis of RNA. The inhibition of differentiation in the presence of puromycin, FPA, and cycloheximide indicates that the completion of infection structure development requires protein synthesis. The failure of these inhibitors, other

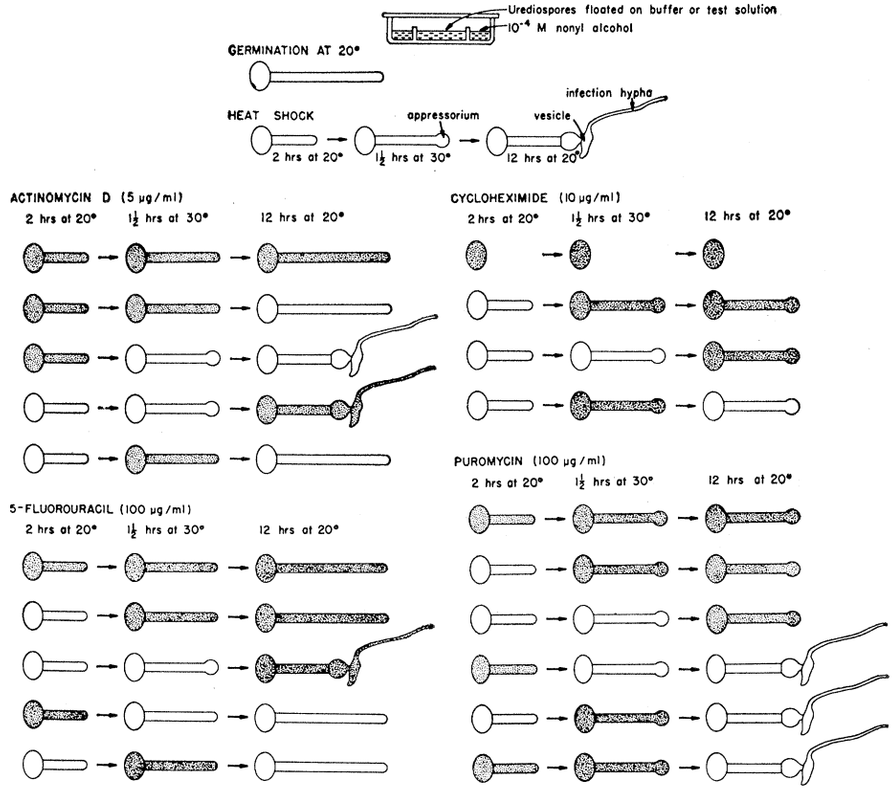

Fig. 1. Effects of various antibiotics and analogs on the development of infection structures by wheat stem rust urediospores. Stippling indicates presence of antibiotic or analog.

than cycloheximide, to inhibit germination and growth of germ tubes might indicate a limited synthesis of proteins and RNA during these earlier stages of development but does not exclude the possibility of such syntheses. Indeed, the occurrence of polyribosomes in urediospores (*4*), the incorporation of radioactive precursors into protein (*5*), and the appearance of isozymes of cytochrome oxidase and acid phosphatase (*6*) indicate that protein synthesis actually does occur in the germinating spore even though there may be no net increase in protein (*5, 7*). Permeability factors could conceivably account for some of the observed differences in sensitivity to inhibitors but do not explain the general effectiveness of inhibitors of RNA synthesis only at early stages and of protein synthesis inhibitors primarily at later stages. More probably, these effects of inhibitors, taken together with cytological evidence that DNA synthesis occurs only during differentiation (*3, 8*), indicate a general onset of protein and nucleic acid synthesis that follows the stimulus to differentiation. They suggest that new RNA must be formed before differentiation, and that new kinds of protein are then synthesized during differentiation of the infection structures.

Reports that actinomycin D inhibits infection of excised wheat leaves and bean leaf discs (*9*), and that it inhibits infection of flax by *Melampsora lini* only if applied earlier than 5 or 6 hours after inoculation (*10*) may be accounted for by the sensitivity of postgermination stages to actinomycin D.

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Lizard Reflectivity Change and Its Effect on Light Transmission through Body Wall

Abstract. *Light transmission through the body wall of living, color-labile desert iguanas* (Dipsosaurus dorsalis) *was measured by spectrophotometry. In the dark phase, the body wall's absorption of ultraviolet light and visible light was approximately twice that of the body wall in the light phase. The shorter wavelengths of ultraviolet could penetrate the body wall in the light phase but not in the dark phase. The intensity and wavelengths of light which could penetrate the body wall without pigments are potentially mutagenic when judged by bacterial standards.*

Damaging physiological effects of solar-radiant energy have been observed for years (*1*). Black peritoneums were noted in colorless fish, whereas clear peritoneums were found in dark-colored forms (*2*). Pigments occur around the central nervous system or around the gonads of many diurnal vertebrates, or in both places, but not in their related nocturnal forms (*3*). An ecological study of six species of *Anolis* (*4*) demonstrated that the degree of peritoneal pigmentation was directly related to the amount of time that the animals spent in the sun. Sunlight kills developing fish embryos (*5*) and unpigmented cave animals (*6*). Quantitative measurements of photobiological effects in specific wavelength bands of near-ultraviolet and visible light are lacking in higher vertebrates. Such photobiological effects of near-ultraviolet and visible light, however, have been measured quantitatively for *Escherichia coli* (*7, 8*). Potential specific mechanisms for the "mutations" observed by Webb and Malina (*8*) in *E. coli* include possible binding of a chromophore or chromophores through intercalation with the DNA of the cell. Riboflavin, vitamin K, or "any of several porphyrins also are possibilities for the chromophore" (*8*).

We now demonstrate that without black peritoneums color-labile desert iguanas would not be able to change color and thereby adapt to the rigors of a hot desert environment without exposing internal biochemical processes to sufficient visible and near-ultraviolet radiation to produce mutations by bacterial standards. Such biochemical reactions might easily affect gonadal maturation.

Color change typically alters reflectivity through the spectral range extending from the near ultraviolet (from about 320 nm) into the near infrared (to at least 1150 nm) (*9*). Change is thus centered within the wavelength band in which more than 80 percent of the incident energy from the sun arrives at the earth's surface. Recent investigations show that such change in the smaller desert lizards can greatly affect the animal's heating and cooling rates (*9, 10*) and suggests a thermoregulatory function (*9, 10*). The smaller reptiles showing labile reflectivity may have melanin deposits in the peritoneum, the membranous sheaths of the testes, the dura, between dorsal muscle blocks, and other tissues. Such black membranes receive their color from aggregations of melanin granules lying within fixed melanophores (*11*).

Some proposed that these black membranes protect internal organs against injurious amounts of ultraviolet light (*11, 12*), while others suggested they absorb significant amounts of heat and thus have a thermal function (*13*). The black peritoneum actually absorbs enough near-ultraviolet light from the sun (*14, 15*) during a day's exposure to prevent "mutations," as judged by bacterial standards (*7*).

Some species of diurnal lizards do not have black peritoneums; instead they have heavy concentrations of fixed melanin in the skin. Measurements of light transmission show that the body walls of such animals exclude as much or more light from the body cavity as the body wall plus the black peritoneum of reflectivity-labile lizards (*14, 15*). The coincidence of mobile dermal melanin and black peritoneums in one group of lizards and the absence of black peritoneums in other lizards with fixed dermal melanin may represent two evolutionary routes toward blockage of damaging radiation.

Reflectance and transmittance of the highly color-labile (*9*) desert iguana were measured with a spectroreflectometer. After the light-color phase had been established by heating the animal into its activity temperature range, and after reflectance had been measured (*9*),