weak cytokinin activity; concentrations of 50 μmole/liter or more are required for its detection in the tobacco bioassay. Such a high concentration could not have been attained in the present tests, and thus any contribution by 6-methylaminopurine to the observed activity was negligible. While bases other than 6-(γ,γ-dimethylallylamino) purine could have contributed to the total observed activity, the several purified tRNA species which were devoid of cytokinin activity have normal amounts of dihydrouridine, pseudouridine, and methylated purine bases. Also the ribosomal RNA was inactive.

Whether there is a functional relation between cytokinin molecules localized in tRNA and the growth and morphogenesis of tissue in response to administered free bases remains to be determined.

FOLKE SKOOG
DONALD J. ARMSTRONG
Department of Botany,
University of Wisconsin, Madison
JOSEPH D. CHERAYIL
ARNOLD E. HAMPEL
ROBERT M. BOCK
Department of Biochemistry,
University of Wisconsin

References and Notes

1. H. G. Zachau, D. Dütting, H. Feldmann, *Angew. Chem.* **78**, 392 (1966).
2. K. Biemann, S. Tsunakawa, J. Sonnenbichler, H. Feldmann, D. Dütting, H. G. Zachau, *ibid.* **78**, 600 (1966).
3. J. H. Rogozinska, J. P. Helgeson, F. Skoog, *Physiol. Plantarum* **17**, 165 (1964); N. J. Leonard and T. Fujii, *Proc. Nat. Acad. Sci. U.S.* **51**, 73 (1964); H. Q. Hamzi and F. Skoog, *ibid.*, p. 76.
4. J. D. Cherayil and R. M. Bock, *Biochemistry* **4**, 1174 (1965).
5. G. Bruening, thesis, Univ. of Wisconsin, Madison, 1965.
6. J. D. Cherayil and R. M. Bock, *Biochim. Biophys. Acta*, in press.
7. J. E. Bacher and F. W. Allen, *J. Biol. Chem.* **182**, 701 (1950).
8. E. M. Linsmaier and F. Skoog, *Physiol. Plantarum* **18**, 100 (1965).
9. R. W. Holley, J. Agpar, G. A. Everett, J. T. Madison, M. Marquisee, S. H. Merrill, J. R. Penswick, A. Zamir, *Science* **147**, 1462 (1965). The alanine tRNA was furnished by Dr. Holley.
10. R. H. Hall, M. J. Robins, L. Stasiuk, R. Thedford, *J. Amer. Chem. Soc.* **88**, 2614 (1966).
11. N. J. Leonard, S. Achmatowicz, R. N. Loeppky, K. L. Carraway, W. A. H. Grimm, A. Szweykowska, H. Q. Hamzi, F. Skoog, *Proc. Nat. Acad. Sci. U.S.* **56**, 709 (1966).
12. D. Klämbt, G. Thies, F. Skoog, *ibid.* **56**, 52 (1966); J. P. Helgeson and N. J. Leonard, *ibid.*, p. 50.
13. J. E. Fox, *Plant Physiol.* **41**, 75 (1966).
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Protoperithecia in Neurospora crassa: Technique for Studying Their Development

Abstract. *A technique is described which facilitates analysis of the development of individual protoperithecia in* Neurospora crassa. *The formation of this organelle proceeds in several clearly discernible steps beginning with the looping of a single hyphal filament and ending with a heavily pigmented, densely packed structure that is the mature protoperithecial structure. The effects of a number of environmental conditions on the development of protoperithecia in two wild types and in a female-sterile mutant strain, ty-1, are presented.*

Genetic and environmental factors affect the development of protoperithecia in *Neurospora crassa* (*1, 2*). However, molecular genetic studies of this relatively simple, differentiating system have been hampered in part by the difficulty of obtaining a clearcut picture of the morphological sequence of events leading to the formation of these organelles. Descriptions of the development of protoperithecia in *Neurospora* have been limited to *N. sitophila* and *N. tetrasperma* (*3, 4*), and these descriptions were not accompanied by photographs.

We report a technique permitting a more extensive study of the development of individual protoperithecia in *N. crassa*. We have extended previous observations on the effects of temperature on protoperithecial formation and shown that a female-sterile mutant (*4,*
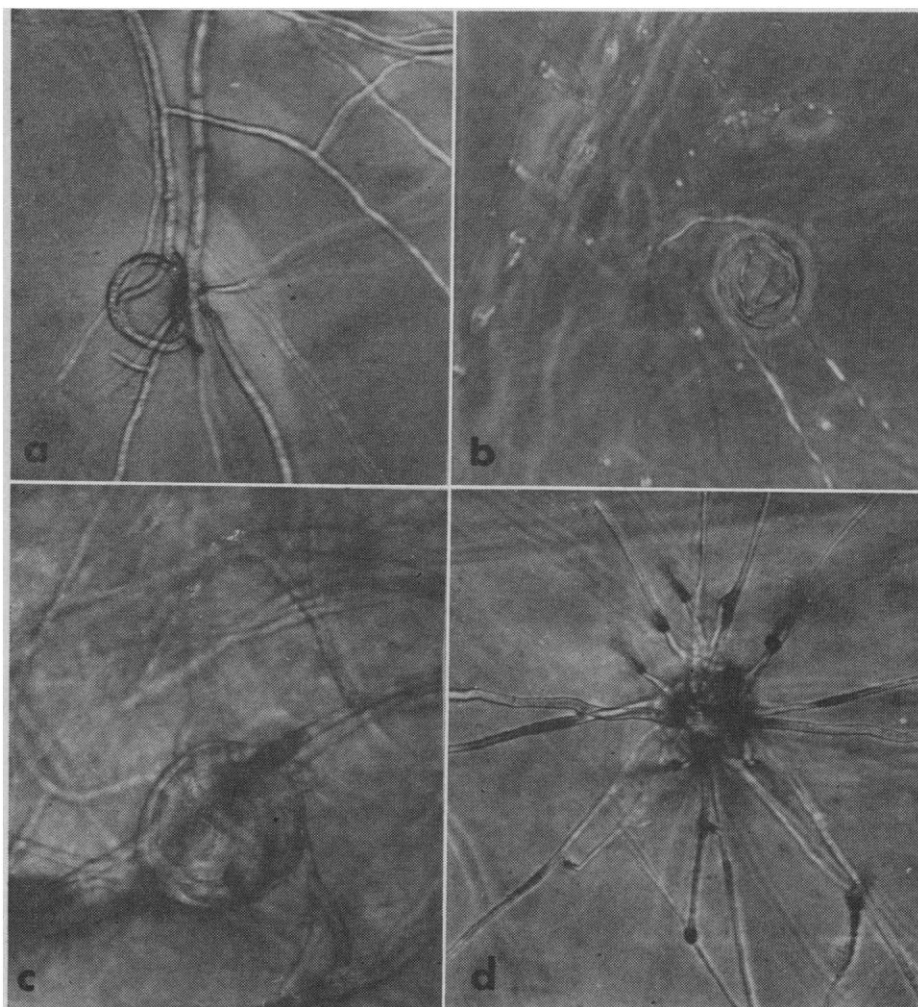

Fig. 1. Photomicrographs of the development of protoperithecia in *N. crassa*. (a) Hyphal filament forming a loop, 76 hours after transfer to Bacto-agar; (b) extension of hyphal loop to form coils, 78 hours; (c) further coiling, 84 hours; (d) filaments growing from an early protoperithecium, 88 hours ($\times$ 256). Photographs were taken on a Zeiss standard phase contrast microscope with 35-mm Zeiss attachment camera.

5) that lacks tyrosinase activity can develop protoperithecia under certain conditions.

Two wild strains of *N. crassa*, 5297a and 74A, and a mutant (ty-1) lacking tyrosinase activity were used. Westergaard and Mitchell's (*1*) crossing-media was used for maximum protoperithecial formation. However, the density of the hyphal growth on this medium prevented suitable photography of individual, developing protoperithecia. In contrast, they were readily discernible when the culture was initially grown in Petri plates on strips of sterile (autoclaved) Visking dialysis membrane cut to 4 by 1.5 cm and placed on top of Westergaard and Mitchell's medium for 4 to 6 days—that is, until protoperithecial development had been initiated. Subsequently, they were transferred to Petri plates containing 1.2 percent Bacto-agar. This procedure restricts hyphal growth sufficiently to permit accurate observation of the development and differentiation of individual protoperithecia on the agar, adjacent to the membrane, unobscured by heavy hyphal growth. Under these conditions, the protoperithecia are morphologically indistinguishable from those developing on Westergaard and Mitchell's medium under usual conditions and are normal as evidenced by mature perithecial formation and subsequent ascospore formation after fertilization.

In the wild type, about 76 hours after its transfer to the Bacto-agar, the developing protoperithecia were initially recognized by the extension of a hyphal filament to form a loop (Fig. 1a). The incipient protoperithecia usually, but not invariably, occur within a halo-like zone, and occasionally development appears to be preceded by the deposition of a granular material.

Subsequently, the hyphal loop extends to form numerous coils (Fig. 1, b and c) that become closely packed. The early protoperithecium then darkens, and hyphal filaments grow out from the organelle (Fig. 1d). The entire process takes about 12 to 18 hours.

Normally, on Westergaard and Mitchell's medium, the first protoperithecia begin to develop about 36 hours after inoculation at 25°C. Full development of a protoperithecium on Westergaard and Mitchell's medium also is complete within about 12 to 18 hours. The maximum number of protoperithecia is reached in about 6 days. At

14°C, development is normal but occurs more slowly, an 8 to 9 day lag occurring before initiation of protoperithecial formation.

Protoperithecia ordinarily do not develop at 35°C on Westergaard and Mitchell's medium. However, if wild type *N. crassa* is transferred to Bacto-agar medium by the method with Visking strips, protoperithecia will develop on the agar adjacent to the Visking membrane at 35°C after 12 to 20 days.

The female-sterile, tyrosinase deficient mutant, ty-1, has been described by Horowitz, *et al.* (*5*). Female sterility is not affected by the usual inducers and repressors of the tyrosinase. We have noted that ty-1 does develop to the coiling stage (Fig. 1, b and c) at both 14°C and 25°C on Bacto-agar during the usual period of incubation, and, furthermore, some of these structures do develop into mature protoperithecia if allowed to incubate for an extended period (25 to 35 days). With ty-1 at 35°C, neither the early nor late stages of development were noted on Westergaard and Mitchell's medium or on the Bacto-agar medium.

A variety of cross-feeding experiments with mixtures of both whole cells and extracts of wild type and ty-1 failed to provide an explanation for female sterility in terms of any readily detectable, diffusible inducers or repressors.

In summary, a new technique has permitted us to define more clearly the several stages of development of protoperithecia in *N. crassa* and to extend previous observations on the effects of temperature on the formation of this organelle. We feel this may be a useful procedure in future studies of the genetic and metabolic control of this reproductive structure.

HENRY ROTHSCHILD
SIGMUND R. SUSKIND
McCollum-Pratt Institute,
Johns Hopkins University,
Baltimore, Maryland

References and Notes

1. M. Westergaard and H. K. Mitchell, *Amer. J. Bot.* **34**, 573 (1947).
2. H. M. Hirsch, *Physiol. Plantarum* **7**, 72 (1954).
3. M. Westergaard and H. Hirsch, *Colston Res. Soc., Proc.* **7**, 171 (1954).
4. L. C. Frost and A. D. Greenhill, *Neurospora Newsletter* No. 4, 6 (1963).
5. C. L. Shear and B. O. Dodge, *J. Agr. Res.* **34**, 1019 (1927).
6. B. O. Dodge and M. E. Swift, *Torreya* **33**, 31 (1933).
7. B. O. Dodge, *Mycologia* **27**, 418 (1935).
8. B. Colson, *Ann. Bot.* **48**, 2 (1934).
9. M. P. Backus, *Torrey Bot. Club Bull.* **66**, 63 (1939).
10. N. H. Horowitz, M. Fling, H. L. MacLeod, N. Sueoka, *J. Mol. Biol.* **2**, 96 (1960).
11. N. H. Horowitz, M. Fling, H. L. MacLeod, Y. Watanabe, *Cold Spring Harbor Symp. Quant. Biol.* **26**, 233 (1961).
12. Contribution No. 485 of the McCollum-Pratt Institute. This research was supported by NIH grant CA-03080.

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Feedback Inhibition of Key Glycolytic Enzymes in Liver: Action of Free Fatty Acids

Abstract. *Increasing concentrations of sodium octanoate were progressively inhibitory to the activities of glucokinase, hexokinase, phosphofructokinase, and pyruvate kinase. Glucose-6-phosphate and 6-phosphogluconate dehydrogenases were also markedly inhibited. Other enzymes of carbohydrate metabolism such as lactate dehydrogenase, phosphohexose isomerase, and fructose-1,6-diphosphatase were not decreased. Among the key glycolytic enzymes, the inhibition of pyruvate kinase by the fatty acid was most marked. The biological significance of the inhibition of the key glycolytic enzymes is interpreted as a feedback inhibitory mechanism in regulation of fatty acid biosynthesis. The mechanism may function for rapid adaptation by which the organism can use the fatty acid level as a metabolic directional switch in decreasing glycolysis and turning on gluconeogenesis.*

Metabolic regulation in the mammalian organism is exerted through mechanisms of acute and chronic adaptation. In the control of hepatic gluconeogenesis and glycolysis we emphasized that the dynamic balance of the key rate-limiting enzymes of glycolysis and gluconeogenesis may play a crucial role in determining the direction of overall pathways (*1*). Studies on chronic adaptation showed that insulin induced the biosynthesis of the key glycolytic enzymes, glucokinase (*1, 2*), phosphofructokinase (*1, 3*), and pyruvate kinase (*1, 3, 4*), whereas it suppressed the biosynthesis of the