The Direct Experimental Observation of Cells in Phenomic Lag

Abstract. By employing cells mutating at a very high spontaneous rate and a grid plate containing a medium totally selective against mutant cells, direct microscopic observation was made of mutant cells of recent origin passing through a limited number of phenomic lag divisions.

The concept that cells immediately following mutation are phenotypically normal and require several generations for mutant phenotype expression has been invoked to interpret discrepancies in mutation rates estimated by various methods (1), and to interpret the effect of intermediate cultivation after exposure to mutagens, on the number of mutants expressed (2,3). Ryan (4) reviewed the evidence for phenomic lag and concluded that it was not compelling.

The likelihood of direct observation of a newborn mutant in phenomic lag is unreasonably small where the mutation rate is low. Where the rate is high (e.g. 10-40% as in the cytoplasmic mutation to respiration deficiency in some yeast strains) the direct observation of phenomic lag becomes possible on a medium totally selective against the mutant. Under these conditions one predicts that normal cells should form colonies (complete development), old mutant cells or dead cells should fail to divide (no development), but mutant cells of recent origin should exhibit only one or a few divisions (limited development), if phenomic lag does in fact occur.

Intermittent observation of the development of many individual cells on an agar surface may be made if each cell can be relocated on a grid. A convenient grid plate may be constructed as follows: Ten razor blades are clamped together with the edges aligned on a plane surface. The clamped blade edges are used to cross stamp the dry agar surface of a two day old plate lightly, yielding a grid of squares approximately 0.1mm on edge. One drop of a cell suspension (ca 1 x 10^4 per ml) is placed directly on the grid. If the agar is dry the drop is rapidly absorbed and microscopic observation may be begun almost immediately. The mechanical stage is modified to accomodate a petri dish, the corner of the grid is located under low and then high power and, as the grid is scanned, each cell or configuration (singlet, doublet, triplet etc.) is drawn on a paper facsimile of the grid. At timed intervals the grid is scanned and the number of cells in each configuration is again recorded. Intermittent microscopic observation may be made of the open plate for 48 hours without visible interference from airborne contaminants. The tendency of growth to become confluent interferes with longer periods of observation.

Two plating media have been employed. One, a glucose nutrient agar allows both respiration deficient (aer) and respiration sufficient (AER) cells to form colonies. The other, a lactate nutrient agar allows only AER cells to form colonies (5). When a respiration deficient clonal isolate of strain 14940 (6), growing in exponential phase in glucose nutrient broth was transferred to glucose grid plates, essentially all cells or configurations developed to form colonies. A low frequency (less than 1%) failed to produce a single bud and were considered dead cells. When aliquots of the same culture were studied on lactate grid plates, not a single new bud was formed in more than 1000 configurations examined. To test whether the aer cells had died on the lactate agar a doughnut shaped ring of filter paper impregnated with glucose was placed on the agar surface surrounding the grid. After some lag essentially all configurations budded on the plates to which glucose had been added. The aer cells were therefore still viable. Although previous observations (5) had indicated that aer cells were incapable of forming macrocolonies on lactate agar, the observation of the complete failure to form new buds on the lactate grid plate is more critical evidence for the totally selective character of the medium against aer cells. It does not, however, yield any information concerning the ability of aer cells recently arisen from AER cells to produce buds in the selective medium.

Strain 14940 exhibits a high spontaneous mutation rate to respiration deficiency when growing exponentially in lactate nutrient broth at 35°C(ca 40%). The population consists principally of doublets (cells with attached buds) with a lower frequency of singlets, triplets, and quadruplets. When samples of this culture were transferred to glucose grid plates, essentially all configurations developed to microcolonies within 24 hours and to macrocolonies by 48 hours. A small number (less than 1%) failed to develop at all and were considered dead cells. On the lactate grid plate, however, many of the configurations exhibited limited development. In a population consisting of 36.8% singlets, 54% doublets, 7.8% triplets, 1% quadruplets and only 0.3% of any larger configurations, 61% of the singlets, 23% of the doublets, and 17% of the triplets showed limited development. To test whether all the cells in a configuration exhibiting limited development had died, glucose was added to some of the lactate grid plates after 24 hours via the filter paper ring described above. By 48 hours the cells which had shown limited development had resumed their growth. On control plates the cells which had shown limited development for 24 hours produced few additional buds between 24 and 48 hours.

A strain (14716 x 8256) characterized by a low spontaneous mutation rate exhibited limited development in only a small proportion of its cells.

The prediction of limited development for considerable number of cells is thus fulfilled only with a strain showing a high mutation rate on a medium totally selective against the mutant. Further development is produced by delayed supplementation of the medium with a nutrilite which sustains mutant growth, indicating that development is not limited by death.

These observations are interpreted as the direct experimental observation of phenomic lag (7). When a AER cell produces a aer bud the latter still retains residual respiratory enzyme capacity and can proceed through a number of divisions until its respiratory capacity is diluted to the level of the aer cell. Since the medium is totally selective against cells which lack respiratory ability, mutant cell division ceases when the phenomic lag is completed.

If one assumes essentially equal partition of the residual respiratory capacity during divisions of a newborn aer mutant, no more than 4 or 5 phenomic lag generations should be possible. The grid plate experiments indicate that no configurations exhibiting limited development reached a terminal size compatible with more than 4 phenomic lag generations. These configurations exhibiting fewer than 4 phenomic lag generations probably arose from mutations which occurred one or more generations before plating.

Maurice Ogur
Ralph St.John
Sylvia Ogur
Department of Microbiology and Biological Research Laboratory
Southern Illinois University, Carbondale

References and Notes

(1) H.B. Newcombe Genetics 33, 447 (1948)

(2) M. Demerec Proc. Nat. Acad. Sci. Wash. 32, 36 (1946)

(3) B.D. Davis J. Am. Chem. Soc. 70, 4267 (1948)

(4) F.J. Ryan The American Naturalist 89, 159 (1955)

(5) M. Ogur and R. St.John J. Bacteriology 72, 500 (1956)

(6) The yeast strains employed are from the Carbondale collection.

(7) This work was supported in part by grants from the American Cancer Society and the National Science Foundation. We are indebted to Drs. J. Lederberg and C.C. Lindegren for their helpful comments.

Ogen

$$\frac{dm}{dt} = aN \qquad \frac{dn}{dt} = kN$$

For m/N at equilibrium,

$$\frac{d\ m/N}{dt} = 0 = N\ dm/dt - m\ dN/dt$$

$$\frac{m}{N} = \frac{dm/dt}{dN/dt} = \quad = aN/kN = a/k$$ obviously, then, $m/m+N = a/a+k$

Watch the defibition of k: it is the rate at which N cells produce new N cells. The totall instantaneous growth rate is a+k