10/1/52

Lederberg

Problem: If the units (mutons) of an allele each produce a small part of an enzyme and they are put together in a particular sequence, it is understandable why the mechanism is stopped if one muton in the same chromosome is defect. The problem is whether, in case the defect muton is supplied from a virus (λ), some synthesis of complete enzyme can take place, i.e., without recombination to a cisform. In other words, is it possible to devise more sensitive methods than cell division and coloniformation for the study of enzyme synthesis? According to Jacob, at least 10^{10} molecules of glucose are needed to make one bacterial cell divide, whereas less than 2×10^9 molecules are needed to produce 50 phages. The following method is proposed.

Kurahashi has found that Lederberg's number W3091 and W3094 of $K_{12}\lambda$ are PGal uridyl transferase-less. Cross λ 3091 with K_{12} 3094, wash and starve; then induce with U.V. light, take an aliquot containing about 10^3 cells and plate on galactose agar which has in advance been inoculated with a very large amount of Gal + E. coli to which λ^s is virulent. In a population of about 1000 $K_{12}\lambda$ there

should be between 0 and 5 big plaques due to recombinants. If the transduced but non-recombined K_{12} also can produce enzyme, but much less effectively, a number of several hundred small plaques should appear after an induction period of 1 to 3 hours, for instance. Since many enzymes have rate constants (number of moles substrate reacted per mole enzyme protein) amounting to between 10^6 to 10^9 substrate molecules per hour, only between 10 and 1000 protein molecules of galactokinase need be produced during 1 hour to make enough UDPGal to start a small plaque. This is a true trigger reaction in the sense that one induced $K_{12}\lambda$ being lysed will lysogenize about 5 to 50 surrounding sensitive E. coli and so on.

Controls: Transduced W3091/W3091 (λ) or W3094/ W3094, i.e. 'crosses' of same muton should not give any plaques. An experiment of the same type, as well as on controls, could actually also be run on the mutants of galacto kinase. The outlined techniques might also be used on mixtures of protoplasts inside a cellophane container, using heavy inoculation both of induced $K_{12}\lambda$, Gal- and of sensitive

E. coli, gal+.

This principle should also be considered for use in trying to pick up phenotypic segregation of β-glucuronidase in spermatozoos from hybrid mice. Spermatozoos are incubated with glucose-glucuronide at pH 4.3. About 200 spermatozoos are spread on agar without any carbon source and plated with starved U.V. induced $K_{12}\lambda$ as indicator strain (heavy bacterial inoculate). The spermatozoo is the feeder of the $K_{12}\lambda$. In a hybrid population one should observe two types of plaques: large and small (difference in size: 1 to 3 or more). In homozyg., GG (i.e. high β-glucuronidase titer) only large plaques should be present, in homozyg., gg (low glucuronidase titer) only small plaques should appear. If the spermatozoo population of a single individual show only small fluctuations, such a technique should be able toreveal phenotypic segregation in heterozygotes (Gg) i.e., 50% large plaques and 50% small. One prerequisite which is important is that the substrates used, whether it is galactose or cellobiuronic acid, must be very pure. Any trace of glucose would

disturb greatly.

* Another variation of this experiment would be to use K_{12} gal + ($\vec{s}$ λ) as the indicator strain on the galactose agar and induce with U.V. several times after plating. This would in effect make the Gal+ sensitive after lysogenisation. If a *E. coli* strain which is sensitive to λ without any induction, it would obviously be preferable).