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One important aspect of our work has been the relationship of the inducible inhibitor, discovered in our laboratory, and postirradiation cell leakiness to the induction of phage. Two lines of work have been followed, both collaborative with other laboratories. The first was started with Dr. D. Billen and Dr. P. Achey in the University of Florida (Gainesville) and consisted of the observation of the behavior of the inducible inhibitor in four strains of Escherichia coli, all of which are auxotrophs for an amino acid. One of these cells is in strain 15 and is known to have a defective prophage; two are strains of B/r which is believed not to have such a prophage, or which, if present, is induced only to a low degree; one is strain B_{s-1}, known^{not} to have the inducible inhibitor. This work has been submitted to the International Journal of Radiation Biology and a preprint is enclosed (C00-2362-2).

In the above mentioned article it can be seen that a conclusion has been reached that the inducible inhibitor is not part and parcel of phage induction, but that it is closely related to it. In this connection a preprint of an article submitted to PNAS by Evelyn Witkin has been received. She points out that there are a number of apparently inducible factors, including filament formation, the phenomenon of reactivation of irradiated phage in irradiated host cells, and the concept of an inducible error-prone repair system. It is quite likely that our inducible inhibitor is one factor in these mechanisms; possibly it is concerned with the error-prone repair. It is very intriguing to be so close to this finding.

The work with Dr. R. P. Boyce has concentrated on observations of four strains of E. coli K12, one essentially wild type, one a recA⁻, and one lex⁻uvr⁻, with and without λ phage. It is found that in the cases of recA⁻ and lex⁻ there is really no effect of the presence of λ , which suggests

that the λ does not confer the property of inhibition, but that the inhibition is something that is suppressed by the mutations. In the wild type and uvr⁻ cases, there is a small effect of the presence of λ , but it would not be very difficult to explain this in terms of the synthesis of DNA associated with the induction of the λ itself.

We therefore have come to the conclusion that the inducible inhibitor is closely related to phage induction, but that something beyond the induction of the inhibitor occurs for the induction of phage. Suggestions for what this may be are given below.

The second line of work has been to study the role of lex (designation in K12; the equivalent in strain B is exxA). We have been concerned with the inducibility of lex⁻ cells, and while this is thought to be small, it is not nearly as small as in recA⁻. We also have looked at the development of immunity in ultraviolet-irradiated lex⁻ cells. The immunity is developed, but only to the extent that fits the induction process. It looks very much as though induction is quite possible in lex⁻ cells, but that a secondary factor sharply cuts down on the total number of cells that are actually induced. This is something we are most interested in, and we want to continue work in this area.

We have reached a concept of a two-step induction process. We have conducted some theoretical studies which lead us to believe the suggestion of Goldthwait and Jacob (C. R. Acad. Sci., Paris 259:661, 1964) that the aberrant DNA synthesis, which follows the inducing insult, causes an increase in some DNA precursor that is the effector molecule for derepression cannot possibly be correct. We base this on the rather simple idea that the repression of λ is very firm in the ordinary way, requiring tight binding of the repressor to DNA, and yet it is almost immediately removed by the

radiation insult. If the insult caused an increase in precursors, then we require some 2000 of these to cause induction whereas the cell has 6000 in its normal functioning process. We are therefore led, on the precursor hypothesis, to rather extravagant ideas about the separation between normally present precursors and the repressors, and to use the hypothesis seems somewhat far-fetched. A much more likely process is a two-step induction. Here the irradiation insult generates something which could be an effector molecule or could be an alteration in DNA, and that induces a gene, which tentatively we will call gene R (a Radiation gene). This gene R is now derepressed and produces a product which is the effector molecule for the induction of the phage. It is quite likely that the same kind of derepression which occurs here may occur for other factors; it is also possible that the gene product of gene R does more than simply combine with the λ repressor, but perhaps derepresses other components. Recent work by H. A. Echols reported at a phage symposium and available as a preprint (submitted to PNAS) describes the isolation of an inducing protein which combines with the λ repressor. It is, however, present to some extent in recA⁻ and combines also with the lac repressor, both of which are disturbing findings. It is thus likely that the two-step induction process is valid.

With this concept of two-stage induction, we have been working out theoretical curves, based on the idea that the inducing process may have considerable insensitivity in that the ionizing radiation may have to produce a relatively rare event, and that this is the inducing process. At the same time, ionizing radiation is damaging both the phage itself and the processes of the cell needed to develop the phage and this

is a process that diminishes induction. Theoretical curves fit experiments remarkably well. The experiments are based both on the induction process and on the sensitivity of a temperature-sensitive, inducible, lysogenic cell to ionizing radiation. This phage does not require the derepression by the process we have postulated, but the repressor flips as the temperature goes beyond a certain figure.

The agreement is remarkably good, and we are able even to make reasonably good fits with data taken on cells grown in different ways, giving a factor of three difference in the amount of induction.

The apparent insensitivity of the induction by ionizing radiation is a new factor. It has been accepted by many that induction is very sensitive; our findings would indicate that possibly only one primary ionization in 200 is actually able to produce the inducing event. We have looked for and found some oxygen-nitrogen effect. The data are more complete than in past work; there are detectable differences.

An interesting effect of near-violet irradiation on induction has been found. We are inclined to link this with the suggestion made by Tyrrell (Argonne National Laboratory) (reported in a symposium at Gainesville; now in press with Photochemistry and Photophysiology) that near-violet is inactivating the repair enzymes. We would like to work up a separate program for this, supported independently, but it is of clear interest and obviously fits in with this project.

Also, separately, in Dr. Ginoza's and Dr. Taylor's laboratories (this department), Deno Kazanis and David Spencer have shown that there is a considerable amount of a protein, which they call protein X, that is generated in response to ultraviolet light in cells that could be induced for the inducible inhibitor. No such protein^{is} generated in lex⁻ cells or

recA⁻ cells, but it is generated in almost all other cells. They have made the extremely interesting observation that the degradation-resistant mutant isolated in our laboratory by Paul Horan and designated PHH1 owes its insensitivity to DNA degradation to the fact that the inducible inhibitor is constitutive and does not need to be induced. They find a 30% amount of protein X in their PHH1 all the time.

Our current hypothesis for the induction of prophage by ionizing radiation is as follows: We agree that the radiation insult must cause some relatively rare event which we are tentatively proposing as a special cross-link. The response of the cell to this cross-link generates some effector molecule, which might well be a degradation product of DNA, for example a deoxymonophosphate. This normally is not present in the cell and acts to derepress the gene we are calling gene R, which generates considerable amounts of this special protein, which in some way produces the derepression of phage and also perhaps acts as the inducible inhibitor and as a factor in error-prone repair and the generation of filaments.

Studies with bromouracil substitution have shown that BrUdR substitution followed by 313 nm light produces mutations which are different in class (as studied by mutant phage response, using the techniques of Person et al. [Osborn, Person, Phillips & Funk, J. Mol. Biol. 26:437, 1967]) from normal UV or spontaneous mutations. We are currently investigating whether lex⁻ cells show a difference in BrUdR mutation character, something which directly ties in to the error-prone repair concept.

This stage has been reached by very intensive work in the laboratory and also by weekly discussions in which members of other groups have been present. These discussions have been valuable and will be continued.

Personnel on Project:

Faculty Ernest C. Pollard, Co-principal Investigator
W. D. Taylor, Co-principal Investigator
Adrian V. Rake, Faculty Associate

Postdoctoral

R. Christensen, NIH Trainee
W. Coetzee, S. African Medical Research Council Fellow

Research Aide: Kathleen Keller (part-time)

Graduate Assistants

B. K. Lydersen (other support)
J. McCloskey

Part-time undergraduate helpers.

Publications resulting from this project:

Pollard, E. C. & E. P. Randall. Studies on the inducible inhibitor of radiation-induced DNA degradation of Escherichia coli. Radiat. Res. 55:265-279 (1973). Reprints enclosed (COO-2362-3)

Christensen, R. C., K. Keller & E. C. Pollard. Degradation of cellular deoxyribonucleic acid after gamma irradiation of spheroplasted Escherichia coli CR thy cells. J. Bacteriol. 116:491-493 (1973). Reprints enclosed (COO-2362-4)

In press:

Pollard, E. C., D. Billen & P. M. Achey. Kinetic studies of the effect of restoration of a required amino acid on radiation-induced DNA degradation and cell permeability. Int. J. Radiat. Biol., in press. Preprint enclosed (COO-2362-2)

Abstracts of papers to be presented at the Biophysical Society Meeting in Minneapolis, June, 1974:

Coetzee, W. & E. C. Pollard. The effect of near-ultraviolet irradiation on E. coli tryptophanase. (COO-2362-5)

Lydersen, B. K. & E. C. Pollard. The induction of prophage λ by ionizing radiation. (COO-2362-6)