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REPAIR AND MUTATION INDUCTION IN MOUSE
GERM CELLS: A SUMMARY AND SOME THOUGHTS

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Evidence for repair of premutational lesions induced in mammalian germcells has come from several directions, and some of it has been around for quite some time. It is only as a result of relatively recent work that we now have the opportunity, with experimental mammals, to penetrate into areas of research that have already yielded rich rewards in yeast and *Drosophila*, namely the genetics of repair capacity. This opportunity is particularly welcome in view of the fact that experimental mammals, which obviously could provide animal models for human genetic disease, have lagged behind man in the study of repair-deficiency states. Several such states are known in humans; in the mouse, we are only now on the threshold of discovering some.

The various lines of evidence for repair of premutational damage in mouse germcells were presented at this symposium. The first indication of repair came, over 20 years ago, from the radiation dose-rate effect on mutations induced in both spermatogonia and oocytes (14, 15). W. L. Russell (13) reviewed results from a variety of specific-locus experiments, involving protracted or fractionated doses, that point strongly to the existence of a repair process. He believes that some premutational lesions -- most of which result from single-track events -- can be repaired at low doses and dose rates in certain germcell stages; but that, at high doses and dose rates, the repair system is either damaged or saturated. Some others have interpreted Russell's data on the basis of a linear-quadratic function, which assumes that a large proportion of the mutations found after high-dose-rate irradiation are two-track events (1). W. L. Russell's paper at this symposium demonstrated that curve fitting alone can give few clues as to the nature of the induced

mutations: thus, models involving a single-track hypothesis fit the data as well as (actually better than) does the linear-quadratic model. The decisive evidence must come from an analysis of the nature of the mutations. Such analyses as deficiency and complementation mapping have indicated that the bulk of the specific-locus mutations induced in spermatogonia are either intragenic or deficiencies so small as to be almost certainly one-track in origin (8, 10).

More evidence for repair comes from the finding of unscheduled DNA synthesis (UDS) in mouse germcells. UDS was discussed for the male by Sega (16), and for the female by Pedersen (7).

In the female, UDS has now been demonstrated for all stages of oocyte development following U.V. irradiation. If, as Pedersen believes, UDS is a reflection of excision repair, such repair capacity appears to be highest in the growing oocyte; and, though lower, still quite sizeable in the neighboring stages, i.e., the earlier resting oocyte and the later germinal-vesicle stage (mature oocyte). Unfortunately, no female mutation-rate data are available for any agent that, like U.V., induces long-patch repair, and it is therefore not yet possible to relate oocyte long-patch repair capacity to mutational endpoints. Only X-ray mutagenesis has been extensively studied. This gives evidence for some sort of repair (dose-rate effect for specific-locus mutations) in mature and maturing oocytes. It also suggests that there might exist a highly efficient repair mechanism in resting oocytes, since the mutation rate, even after high-dose-rate irradiation, drops to control levels during the greater-than-six-week post-irradiation intervals when such oocytes are being utilized (11). If this phenomenon is, in fact, due to repair rather than to other possible causes

(which Russell has enumerated), then the resting oocyte could have the greatest capacity for short-patch repair. The maturing oocyte presumably also possesses considerable short-patch repair capabilities, as indicated by the major dose-rate effect on transmitted mutations [low dose rates yielding no increase over control levels (12)]. In addition, UDS reveals the maturing oocyte to have the greatest capacity for long-patch repair.

It is somewhat troublesome that Pedersen finds the lowest levels of UDS in the very stage for which we now have some of the most intriguing evidence for repair of premutational damage, namely, the fertilized egg (see below). However, though low at that stage, UDS is still clearly detectable. Further, we do not yet know whether any of the agents for which genetic differences in repair capacity of the fertilized egg have been demonstrated in fact induce long-patch repair of the type presumably revealed by U.V.-stimulated UDS.

Sega's work with mouse males raises some basic questions about the interpretations to be placed on UDS endpoints in chemical mutagenesis. How is UDS in mammalian germcells related to removal of DNA lesions? And how are these lesions related to transmitted genetic damages of various types? There are, for example, very marked differences in UDS between mature sperm and late spermatids on the one hand, and earlier post-leptotene germcell stages on the other, whereas the initial levels of DNA alkylation are roughly the same for both sets of stages. Sega finds that the rate of loss of alkylated bases varies in the same direction as does UDS, i.e., both endpoints are much lower in mature sperm and late spermatids than in earlier germcells. On the basis of this stage correspondence, he suggests

that excision of bases is associated with the UDS response, and that low response of the stages that have condensed chromosomes is the result either of nonproduction of repair enzymes or of inaccessibility of lesions to the enzymes.

Sega arrives at another conclusion primarily on the basis of stage correspondence, and that is that UDS may not have a significant effect on the lesions that lead to chromosomes breakage. He believes that, in the case of EMS, the primary target for such lesions is not DNA, but protamine, since, with respect to stage of EMS treatment, the ethylation curve for protamine matches the dominant-lethal curve, while the ethylation curve for DNA does not. Similar relations will have to be shown for a variety of agents before such a conclusion can be broadened, especially since, for a number of chemicals, dominant lethals are readily inducible in germcell stages that have chromatin devoid of protamine (2, 3).

If UDS is, in fact, not related to chromosome-breakage-derived genetic endpoints, such as dominant lethals, is it related to point mutations, as measured in the specific-locus test? Sega's conclusion, that it is not, is based on a comparison of specific-locus mutation frequencies for irradiated postspematogonial stages with relative UDS levels for these same stages: the latter increase during weeks 3 and 4 after treatment, while the former not only do not decrease, but increase slightly. Sega's conclusion thus rests on the assumption that UDS indicates restoration of a pre-damage condition; if, in fact, UDS were related to error-prone repair processes, the observed results would not be out of line. The standard specific-locus test is capable of detecting a wide array of changes involving the loci -- from

intragenic alterations not associated with absence of material to deletions long enough to involve neighboring markers (8, 10). Even with radiation, a sizeable proportion of the mutations induced (though not necessarily at the germcell stages at which UDS is induced) are the result of intragenic changes; UDS will have to be compared with specific-locus mutations resulting from chemicals known to produce primarily "subtle" changes before any conclusions can be drawn about its relation to the specific-locus endpoint.

The challenge of building the bridge between initial lesion and genetic endpoint is being approached in two ways. UDS observed in male or female germcell stages is presumably an indication of events that affect the initial lesion. However, the relation between UDS and measurable genetic endpoints still requires considerable exploration. Another study of repair in mammalian germcells (4) takes the opposite direction, i.e., it starts with genetic endpoints and attempts to trace back to events affecting the initial lesions. This is the approach coming out of Generoso's finding that the genetic constitution of the female can strongly affect the level of dominant lethals induced by exposure of the male to various mutagens, but especially to isopropyl methanesulfonate, IMS (an agent that produces a high ratio of O-6 to N-7 alkylation of guanine). Possible alternative explanations for this phenomenon have been eliminated (5), leaving only the interpretation that the penetrated oocytes of certain strains are repair competent, while those of others are repair deficient. A similar condition has already been shown to exist in *Drosophila* [see Würgler (18)]; and I had early indications that paternal sex-chromosomes loss might be affected by the strain of

female (9). Generoso's work, however, provides the first well-documented evidence for an association between repair and induction of genetic damage in mammalian germcells.

Much remains to be done, as Generoso himself points out: different genetic endpoints and different mutagens must be studied. However, the advantages of having gained a genetic handle on repair processes are manifold. The study of repair-deficiency states will now be possible in a laboratory mammal, and these states may provide experimental models for human genetic diseases. To elucidate the genetics of repair processes, it will be necessary to develop tests by which individuals can be classified as deficient or competent; presently, only population parameters are available (e.g., dominant-lethal frequencies). Explant tissue cultures may be made to serve this purpose if the genetic differences demonstrable in fertilized eggs also apply to somatic cells. The mammalian work must also attempt to discover whether there is an association between mutagen sensitivity and repair deficiency as in *Drosophila*, and such experiments have been initiated by Generoso. Another avenue of attack (which is also in progress at Oak Ridge) is to compare the effect of known repair inhibitors on the fertilized egg of repair-competent and -deficient strains.

Generoso proposes an hypothesis to account for the finding that the agent, IMS, that gives the greatest strain differences in repair capacity is also the one that gives the lowest frequency of translocations for a given level of dominant lethals. He suggests that lesions produced by IMS persist until the time of pronuclear DNA synthesis, at which time they are converted into chromatid or

isochromatid deletions; whereas lesions induced by some other agents (e.g., EMS, TEM, X-rays) are converted to breaks and interchanges prior to pronuclear DNA synthesis.

Although it will be important to see how many other agents fit this pattern, the hypothesis already suggests testable cross references to Sega's UDS work. For example, by Generoso's hypothesis, lesions induced in sperm or late spermatids by EMS (and perhaps MMS?) might become "fixed" even before sperm entry into the oocyte, as indicated by the almost negligible differences between stocks of females in repair capacity for EMS-induced damage. On the other hand, such lesions could be available for possible repair for a short period between sperm entry into the oocyte and sometime prior to pronuclear DNA synthesis [which begins about 6 hours after sperm entry (6)]. By Sega's evidence, probably no repair of these lesions occurs prior to sperm entry. Would this brief period of reparability of the male genome in the oocyte be detectable by UDS? That lesions induced in the male genome by EMS or MMS are, in principle, detectable by UDS is known from the treatment of postleptotene spermatocytes and early spermatids. However, UDS studies of eggs fertilized with MMS-treated sperm were negative (17). It would seem important to do similar work with EMS-treated sperm, since Generoso's hypothesis predicts that lesions in such cells should be available for repair during the entire period between sperm entry and pronuclear DNA synthesis. This should result in UDS.

Other experiments, too, would provide cross references between UDS and genetic indicators. For example, UDS determinations in various oocyte stages, such as were summarized by Pedersen for U.V.

treatment, should be done following IMS treatment of strains identified by Generoso as repair competent or deficient, respectively. In the male (early spermatid), Sega has identified high- and low-UDS strains following MMS exposure, and he suggests that the differences may be related to the relative efficiencies with which these strains remove the alkylation product. It will be of interest to check UDS in early spermatids exposed to IMS, specifically comparing those strains whose females (penetrated eggs) have shown high or low repair capacity in Generoso's experiments. Sega has such tests in progress.

One important implication of the probable existence of genetic repair deficiency that has been pointed out by Generoso is a new relation between mutagenicity and carcinogenicity. No longer is mutagenicity of an agent merely an indication that such an agent also has a good probability of being an initiator of cancer. Mutagenicity per se achieves an added dimension of importance in that a presumably large number of loci in the germline could mutate to repair-deficiency alleles which, even in the heterozygous state (i.e., F_1) could increase susceptibility to carcinogenic agents. In addition, there is the possibility that a "self-feeding" process could be initiated with respect to mutagenicity. Thus, existing genetic differences (i.e., repair-competency versus deficiency) apparently determine the level of probability at which new genetic differences (induced mutations) come about; and some of these new genetic states, in turn, could affect future mutability.

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