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Chromatid Breakage: Cytosine Arabinoside–Induced Lesions Inhibited by Ultraviolet Irradiation

Abstract. *Exposure to ultraviolet light can reduce the frequency of chromatid breaks induced by cytosine arabinoside in the DNA synthetic and post-DNA synthetic phases of the cell cycle. This effect can be correlated temporally with a decrease in the uptake of tritiated thymidine after ultraviolet treatment, implying that the genesis of such breakage is intimately related to DNA synthesis and that such synthesis is not confined to the DNA synthetic phase.*

Cytosine arabinoside (ara-C) inhibits DNA synthesis. The drug prevents the reduction of cytidine diphosphate (*1*), interferes with DNA polymerase (*2*), and produces fraudulent macromolecules by incorporation into DNA and RNA polynucleotides (*3*). In cultures of hamster fibroblasts, ara-C produces chromatid breaks in the pre- (G_1) and post-DNA synthetic (G_2) phases of the cell cycle as well as during the DNA synthetic (S) phase (*4*). Deoxycytidine prevents chromatid breakage when added simultaneously with ara-C and decreases the number of breaks when added after a 30-minute pulse of the drug in the S phase but not in the G_1 or G_2 phase (*4*). Since ultraviolet irradiation produces chromatid breakage (*5*), exposure to both ultraviolet and ara-C would be expected to increase the frequency of chromatid aberrations. In contrast to these expectations, exposure to ultraviolet light prior to ara-C treatment markedly decreased the number of chromatid breaks.

Asynchronous cultures of hamster fibroblasts, Don-C (T_C, generation time, 13 hours; G_1 phase, 3.9 hours; S phase, 6.2 hours; G_2 phase, 2.2 hours; and mitosis, 0.7 hour), growing in monolayers (*6*) were treated with ultraviolet irradiation (2537 Å), 3 and 9 erg/mm^2, after removal of media. Immediately thereafter, except as otherwise indicated, replicate cultures were exposed to ara-C (10 μg/ml) for 30 minutes 2.5 hours (G_2) and 4.5 hours (S) prior to harvest. After drug exposure, the cultures were washed three times with Hanks solution, fresh media not containing the drug were added, and incubation was resumed. The cultures were treated with colcemid (0.06 μg/ml) 1 hour prior to harvest to collect metaphases. Sodium citrate (0.95 percent) was added for 30 minutes, the cells were removed with a rubber policeman, and chromosome preparations were made as previously described (*4*). The cells were stained with aceto-orcein. Fifty metaphase cells were counted to estimate chromatid breakage. In this study, chromatid gaps with separations at least the width of a chromatid were considered "breaks." Only lesions which completely dissected the chromatids were counted.

Table 1. Effect of ultraviolet light on cells treated with ara-C (10 μg/ml, 30 minutes).

Ara-C (μg/ml)	Ultraviolet (erg/mm^2)	Percentage of metaphases with N breaks per cell			
		0	1 to 4	4 to 9	10+
		G_2 phase			
None	None	94	6	0	0
10	None	44	50	4	2
10	3	62	38	0	0
10	9	74	24	2	0
None	9	92	8	0	0
		S phase			
None	None	96	4	0	0
10	None	56	40	0	4
10	3	76	22	2	0
10	9	84	16	0	0
10	9*	60	34	0	6
None	9	98	2	0	0

* Ultraviolet irradiation after 30 minutes' exposure to ara-C. All other exposures were just prior to ara-C treatment.

The results of these experiments are shown in Table 1. Treatment with ara-C in the G_2 phase (2.5 hours) caused chromatid breaks in 56 percent of the metaphases. The frequency of breakage was decreased to 38 and 26 percent when the cells were exposed to 3 and 9 erg/mm^2, respectively, prior to ara-C treatment. Ultraviolet irradiation (9 erg/mm^2) did not by itself produce an increase in chromatid breaks. Treatment with ara-C in the late S phase (4.5 hours) produced breakage in 44 percent of metaphases. The frequency of metaphases containing breaks was reduced to 24 percent by ultraviolet irradiation with 3 erg/mm^2 and to 16 percent with 9 erg/mm^2 ($P < .05$). If the ultraviolet irradiation was added after the 30-minute ara-C exposure in the S phase, the number of chromatid lesions was the same as seen after a 30-minute ara-C exposure without ultraviolet irradiation, indicating that ultraviolet light must precede ara-C to interfere with chromatid breaks.

The effect of ultraviolet light was also studied after treatment of the cells in the G_2 and late S phases with 10 μg of drug per milliliter for 1.5 hours (Table 2). In the absence of ultraviolet irradiation, 74 percent of the metaphases had chromatid breaks in the G_2 and 70 percent in the S phase. When the cells

were exposed to ultraviolet light (9 erg/mm^2) just prior to the addition of the ara-C, only 40 percent of the metaphases had breaks in the G_2 and 26 percent in the S phase ($P < .05$). Of particular significance was the absence of metaphases having five or more breaks. When exposure to ultraviolet was delayed for 30 minutes after the onset of a 1.5-hour ara-C treatment in both the S and G_2 phases (Table 2), chromatid breakage was essentially the same as that observed after a 30-minute pulse of ara-C (Table 1). These findings imply that ultraviolet light can rapidly block chromatid breakage induced by ara-C and can prevent the acquisition of new lesions for at least 1 hour after exposure to ultraviolet.

The possibility that ultraviolet light merely serves to decrease cell transit time, preventing cells with lesions from reaching metaphase, is made unlikely by the fact that the mitotic index after ultraviolet exposure ranged between 0.6 and 1.2 for the first 6 hours. In addition, the "protective" effect of ultraviolet can be demonstrated in the G_2 phase where only a minimal disturbance of transit time should occur (*5–7*). Finally, exposure to ultraviolet light 30 minutes after ara-C does not diminish the frequency of chromatid breaks in the S phase (Table 1). If a decrease in transit time was the primary mechanism of ultraviolet action, fewer damaged cells would reach mitosis and less breakage would be observed.

To investigate the relationship between DNA synthesis and exposure to ultraviolet light, replicate cultures were exposed to ultraviolet, 3 erg/mm^2, then "pulsed" for 10 minutes with 2 μc of tritiated thymidine per milliliter (6.7 curie/mmole) at various intervals after irradiation, and the acid-precipitable radioactivity was determined. By 1.5 hours after irradiation, the uptake of tritiated thymidine had decreased to 30 percent of the control value (Fig. 1). The rate of DNA synthesis then gradually increased to the 80 percent level by 4 hours after exposure to ultraviolet.

The relationship of this decrease in DNA synthesis to the prevention of chromatid breakage induced by ara-C was studied. Replicate cultures were exposed to ultraviolet light, 3 erg/mm^2, hourly from 8 to 4 hours prior to harvest. We added ara-C (10 μg/ml) 4 hours prior to harvest (late S phase), and the metaphases were collected with colcemid as indicated above. The results of this experiment are also summarized in Fig. 1. The maximum inhibition of chromatid breakage induced by ara-C occurred 2 hours after exposure to ultraviolet. This period coincided with that required to produce maximum inhibition of DNA synthesis. As the capacity for DNA synthesis recovered, the protective effect of ultraviolet light decreased (Fig. 1). These changes are significant ($P < .005$).

Table 2. Effect of ultraviolet light on cells treated with ara-C (10 μg/ml, 1.5 hours).

Ultraviolet (9 erg/mm^2)	Percentage of metaphases with N breaks per cell			
	0	1 to 4	5 to 9	10+
	G_2 phase			
None	26	58	12	4
Simultaneous*	60	36	4	0
30 minutes later	54	46	0	0
	S phase			
None	30	50	14	6
Simultaneous	74	26	0	0
30 minutes later	58	40	0	2

* Irradiation just prior to ara-C exposure.

When cells are exposed to ultraviolet light, semiconservative DNA synthesis is temporarily inhibited (*8*) while "unscheduled" DNA synthesis occurs in the G_1, S, and G_2 phases and is nonsemiconservative (*9*). This type of DNA synthesis has been correlated with repair replication (*10*). Repair replication involves the excision of damaged DNA and the reinsertion of normal precursors into parental strands of DNA after exposure to ultraviolet light (*10*) and x-rays (*11*). In *Tetrahymena pyriformis*, repair replication is completed before semiconservative DNA synthesis resumes (*12*).

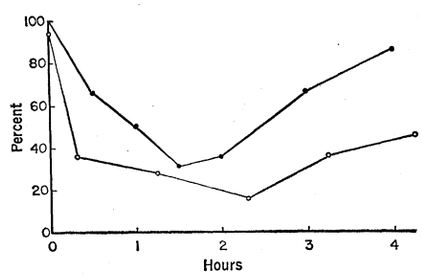

Fig. 1. The effect of ultraviolet irradiation on DNA synthesis and chromatid breakage. The rate of DNA synthesis was measured by the uptake of a 10-minute pulse of tritiated thymidine at various intervals after ultraviolet irradiation (3 erg/mm^2). Chromatid breakage was assessed as follows: replicate cultures were exposed to ultraviolet irradiation (3 erg/mm^2) 4, 3, 2, 1, and 0 hours prior to treatment with ara-C (10 μg/ml for 30 minutes) (late S phase). The cultures were rinsed free of drug and allowed to incubate in fresh media for an additional 3½ hours prior to harvest. Colcemid (0.06 μg/ml) was added to collect metaphases for 1 hour. The percentage of metaphases containing breaks was arbitrarily plotted at the midpoint of the 30-minute ara-C exposure. The shape of each curve and its relationship to the other was tested for significance by a least-squares regression analysis (*15*); P was $< .005$. ●—●, Percent of tritiated thymidine uptake in control cultures; ○—○, percent of metaphases with breaks.

Although ara-C inhibits semiconservative DNA replication, it does not prevent repair replication (*13*). Since the amount of unscheduled DNA synthesis has been correlated with survival in two closely related Chinese hamster cell lines (*14*), unscheduled synthesis presumably represents a necessary step in the repair of ultraviolet damage to DNA. The rapid ultraviolet inhibition of chromatid breakage induced by ara-C in both the S and G_2 phases may indicate that repair replication takes precedence over chromosomal synthesis. An alternate possibility would be the existence of two species of DNA with different susceptibilities to damage by ara-C and ultraviolet light.

The fact that ara-C can induce chromatid breakage in the G_1, S, and G_2 phases of the cell cycle (*4, 5*) and that the inhibition of DNA synthesis by ultraviolet light can decrease the formation of these lesions, lends support to the concept that DNA synthesis in some form is not solely a property of the S phase and that semiconservative or "scheduled" DNA synthesis is involved in the mechanism of chromatid breakage.

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Insect Control by Genetic Manipulation of Natural Populations

Abstract. *The possible use of chromosome rearrangements is considered as a means for introducing genes into insect populations for their own control. The release of laboratory-constructed strains differing from the field population for a number of chromosome interchanges should create an unstable situation leading to the rapid replacement of the field population. This replacement should allow introduction of genes for insecticide susceptibility, cold sensitivity, or the like. The process would produce sterile hybrids while the genetic displacement occurs which itself will contribute to a reduction in pest numbers.*

The ability of insects to evolve resistance to insecticides continues to pose a major problem in controlling many pest species. Furthermore the absence of selective forces to reduce sufficiently the frequency of "resistance" genes in natural populations after insecticide application prevents reuse of discarded insecticides. The often limited life of insecticides has led to attempts to modify the outmoded insecticide by altering those portions of the molecule subject to attack by the resistance mechanisms (*1*), or else by synthesizing new, and sometimes more potent, compounds. Little attention has been given to the possibility of manipulating the insect population to remove the resistance genes in a limited number of generations and thus permitting the reuse of otherwise suitable insecticides.

Meiotic drive, one suggested candidate for such a task (*2*), has become less attractive with the discovery that it may invariably cause recessive sterility (*3*), thus preventing it, along with the susceptibility genes that it would carry, from becoming fixed in a population. I now suggest a possible system using homozygous chromosomal interchanges (translocations) which allows the rapid removal of insecticide resistant genes, while, at the same time, permitting the infusion of other "useful" genes into natural populations. Since the system can engender additionally a high level of inherited sterility which by itself may reduce population numbers (*4, 5*), it is suggested that adequate control may be provided for some species of insects by a period of insecticide application until resistance evolves, followed by a period where synthetic strains are released. Thus these releases provide the twofold service of direct control via high zygotic mortality while the appropriate gene substitutions occur. The whole cycle can then be repeated once the resistance genes have been removed and effectiveness is restored to the insecticide.

The production and isolation of translocations are routine procedures, particularly in the higher Diptera—such as the housefly, the screwworm fly, the Australian sheep blowfly, and *Drosophila*—where marker genes are available and the absence of genetic crossing-over in males makes translocations easier to detect. Since some 30 percent of translocations are viable and fertile as homozygotes (*6*) and do not show any visible phenotypic effects, the collection of homozygous translocations should be quite feasible for some important pest species (*7*). By appropriate backcrossing it should be possible to have available two strains, each with similar genomes but differing for one homozygous interchange. The backcrossing (i) eliminates recessive lethals that may have been carried by the two chromosomes involved in the translocation and (ii) restores the initial variability that was present before the translocation was isolated.

A serial repetition of the procedure should allow the synthesis of a strain homozygous for several translocations but, once again, differing in no other respect to the base strain. Although this multiple translocation strain can be expected to equal the base strain in fitness in that it contains a similar spectrum of genetic variability, a translocation hybrid resulting from a cross between the two strains should be nearly sterile if sufficient translocations have been incorporated.

We can formally equate the situation where a multiple translocation strain and the base strain are mixed to the single locus case of two alleles, A and T, representing the normal and translocated sequences. Let w_1, w_2, and w_3 be the relative genetic fitness of AA, AT, and TT, respectively. Since it is assumed that w_1 and w_3 have values near 1 while w_2 is near zero, because AT is nearly sterile though viable, we have a sufficient condition for an unstable equilibrium (*8*). Thus if q is the frequency of T, we have for $q > \hat{q}$, $q \to 1$; for $q < q$, $\hat{q} \to 0$, where $\hat{q}$ is the equilibrium frequency of T and is given by

$$\hat{q} = (w_1 - w_2)/(w_1 - 2w_2 + w_3)$$

Of particular interest is the rapidity of the replacement of A by T. Suppose the frequency of T exceeds the equilibrium by as little as 0.05. Within six or seven generations A is eliminated from a finite population after a single release of the same size order as the native population (Table 1). It can also be noted from Table 1 that $\hat{q}$ depends primarily on the fitness of TT relative to AA, while the rate of displacement of A is largely a function of the level of sterility of AT. It is important to observe that a genotype can be displaced by a less fit genotype provided that the less fit genotype begins in sufficient frequency.

Thus, if we can induce a set of homozygous translocations in a strain taken from the field and then return this strain in a higher frequency to the native population, we can expect that portion of the genome of the native