Poly(ADP-ribosylation) of DNA Topoisomerase I: A Nuclear Response to DNA-strand Interruptions

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DNA in eukaryotic chromosomes is organized differently from prokaryotic DNA. It seems highly probable that mechanistic adjustments have been made in macromolecular DNA metabolism as a consequence of the chromatin structure of the eukaryotic chromosome (Igo-Kemenes et al. 1982)). This paper deals with an enzyme from eukaryotes, poly(ADP-ribose) synthetase, which may have a role in chromatin metabolism, and, in particular, we focus on the interaction between this enzyme and another enzyme that affects the structure of DNA, type-I DNA topoisomerase.

The enzyme poly(ADP-ribose) synthetase, ubiquitously found in most eukaryotic nuclei, is inactive unless stimulated by a DNA strand break (Yamada et al. 1971; Yoshihara 1972; Benjamin and Gill 1980). In response to an activating DNA structure, the enzyme covalently attaches chains of a polyanionic polymer, poly(ADP-ribose), both to sites on the enzyme itself, as well as to a small class of exogenous protein acceptors (for review, see Hayaishi and Ueda 1982). The enzyme synthesizes poly(ADP-ribose) using NAD as a precursor: nNAD→[ADP-ribose]$_n$ + nNm, where Nm is nicotinamide.

A biochemically related, but metabolically distinct, phenomenon is mono(ADP-ribosylation) (see Hayaishi and Ueda 1982). Poly(ADP-ribosylation) occurs in the nucleus and mono(ADP-ribosylation) appears to be mainly cytoplasmic. In cases so far investigated, poly(ADP-ribose) residues are linked to glutamate and mono(ADP-ribose) is linked to arginine (Fig. 1).

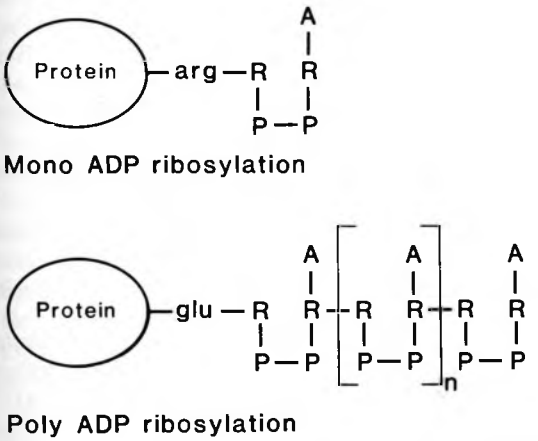

Figure 1. Poly(ADP-ribosylation) versus mono(ADP-ribosylation). A, R, and P are adenine, ribose, and phosphate moieties, respectively.

The covalent addition of poly(ADP-ribose) chains to a protein is perhaps the most drastic protein modification known. In vitro, the enzyme can carry out an automodification reaction in which up to 600–1000 residues of ADP-ribose are added per protein molecule (Yoshihara et al. 1977; Kawaichi et al. 1981). Since each ADP-ribose moiety carries a charge of −2, this means that the protein accumulates over 1000 negative charges as a result of automodification.

A large body of previous work is consistent with the model shown in Figure 2 for the auto(ADP-ribosylation) reaction in vitro (Yoshihara et al. 1981; Ferro and Olivera 1982; Zahradka and Ebisuzaki 1982; de Murcia et al. 1983). The complex kinetics of the reaction can be explained adequately by a mechanism that is based on charge repulsion. The enzyme is activated by DNA and continues to synthesize enzyme-bound poly(ADP-ribose) until the repulsion between the poly(ADP-ribose) chains and the activating DNA become so great that the enzyme is no longer able to bind to the DNA. When this "repulsion point" is reached, the enzyme is effectively inactivated (Ferro and Olivera 1982). As might be expected from this model, the poly(ADP-ribosylation) reaction is sensitive to ionic strength. In the presence of Mg^{++} ions or polyamines (Kawamura et al. 1981), which shield the negative charges in DNA from the negative charges on poly(ADPR), a much higher extent of polymerization is observed.

In addition to the automodification reaction, poly(ADPR) synthetase will covalently attach poly(ADP-ribose) to a small set of exogenous protein acceptors. These include histone H1 (Wong et al. 1977), a Ca^{++}-Mg^{++} endonuclease (Yoshihara et al. 1975), and high mobility group (HMG) proteins (Poirier et al. 1982; see Hayaishi and Ueda 1982). It recently has been discovered that DNA topoisomerase I is modified by the synthetase, resulting in inactivation of the topoisomerase (Ferro et al. 1983; Jongstra-Bilen et al. 1983; Ferro and Olivera 1984). The reaction is a highly efficient one, and all DNA topoisomerase I can be rapidly poly(ADP-ribosylated) in the presence of activating DNA and poly(ADP-ribose) synthetase; indeed DNA topoisomerase I is the best exogenous acceptor of poly(ADP-ribose) yet described (Ferro and Olivera 1984).

The biological role of poly(ADP-ribosylation) has not been definitely established. One strategy for defining this role is to use inhibitors of poly(ADP-ribosyla-

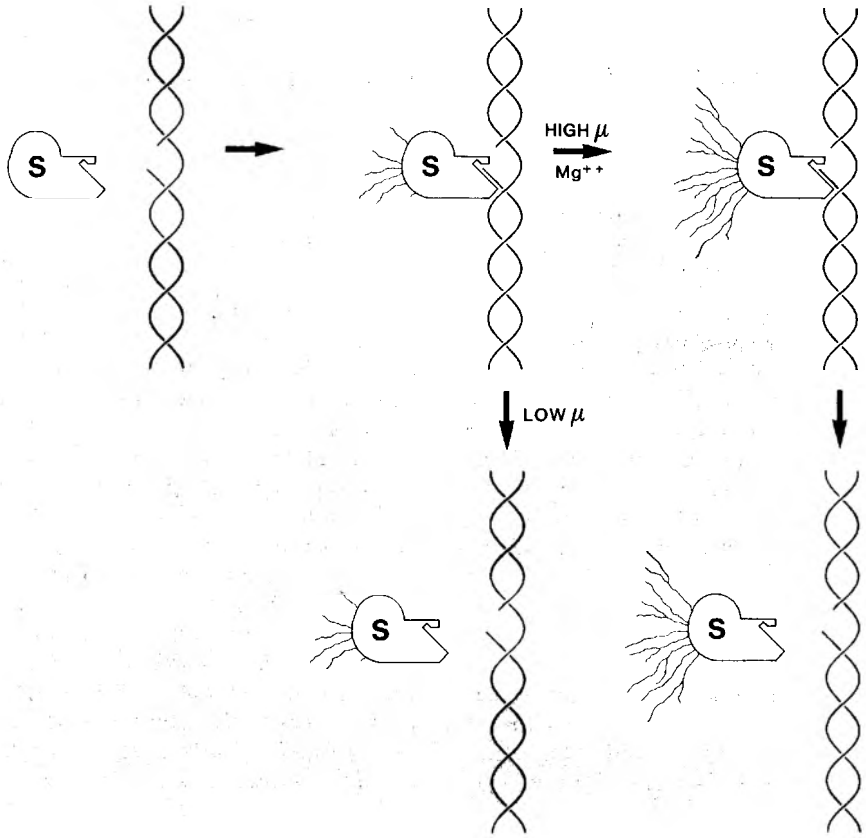

Figure 2. The auto-poly(ADP-ribosylation) reaction. The synthetase (S) attaches poly(ADP-ribose) chains (represented by "hairs") in the presence of an activating DNA site. The reaction ceases in vitro when the repulsion between DNA and poly(ADP-ribose) chains prevents enzyme binding to DNA. μ, Ionic strength.

tion) (Purnell and Whish 1980) and to investigate effects of such inhibitors in vivo. If mammalian cells in culture are treated with an inhibitor of poly(ADP-ribose) synthetase, 3-aminobenzamide, there is no effect on viability (Durkacz et al. 1981); however, among the most striking effects that have been observed is a dramatic increase in the frequency of sister chromatid exchange (SCE) (Utakoji et al. 1979; Oikawa et al. 1980). This result suggests that the normal occurrence of poly(ADP-ribosylation) depresses the rate of strand-exchange events in mammalian cells in culture.

We previously suggested that poly(ADP-ribosylation) may affect the probability of DNA strand-exchange events in mitotic cells (Ferro et al. 1984). This possibility prompted us to begin a study of the effects of poly(ADP-ribosylation) inhibitors on a system where the biological effects of strand crossing over could be more precisely defined, i.e., mitotic recombination in *Drosophila melanogaster* (Becker 1976).

EXPERIMENTAL PROCEDURES

Enzymes. Highly purified preparations of poly(ADP-ribose) synthetase and Type-I DNA topoisomerase ($M_r = 100{,}000$) from calf thymus were used in all of the experiments. These enzymes were obtained following procedures described previously (Ferro and Olivera 1984).

DNA preparations. A preparation of the plasmid pRW751 was obtained from M. Kilpatrick and R.D. Wells of the University of Alabama at Birmingham; this plasmid is a derivative of pBR322 which contains a 157-bp insert (Klysik et al. 1981). The pBR322 and calf thymus DNA preparations used were described previously (Ferro and Olivera 1984). The nicked pBR322 DNA was prepared by Todd Mangum. pBR322 plasmid DNA (60 μg) was nicked with 10 ng of bovine pancreatic DNase I (Sigma Type 1, 3300 units/mg) in a reaction mixture (1.1 ml) that contained 45 mM Tris-Cl (pH 8.0), 9 mM $MgSO_4$, 0.1 mM DTT, 45 μg/ml BSA, and 91 μg/ml ethidium bromide (Shortle and Nathans 1978). The mixture was incubated 2 hr at 37°C, and the reaction was terminated by extraction with phenol. Analysis on agarose gel electrophoresis indicated that the preparation was 95% nicked circles.

Assays for poly(ADP-ribosylation); modification of DNA topoisomerase I by poly(ADP-ribosylation). Automodification reactions and assays for poly(ADP-ribose) synthetase activity were carried out as previously described (Ferro et al. 1983). In experiments involving the modification of the topoisomerase, puri-

fied synthetase (0.16 μg) was preincubated with topoisomerase (0.028 μg) in the presence of pRW plasmid DNA (0.34 μg) in a reaction mixture (0.05 ml) that contained 25 mM Tris-Cl (pH 8), 2.5 mM $MgCl_2$, 2.5 mM DTT, 2.5 mM spermidine, 50 μg/ml bovine serum albumin (BSA), 9 mM KCl, 0.2 mM [^{32}P]NAD, and either ± 10 mM nicotinamide. Reaction mixtures were incubated at 23°C for 60 min during which time, 1.45 nmoles of NAD were incorporated. The reaction mixture was then assayed for DNA topoisomerase using the relaxation of supercoiled pBR322 DNA (Ferro et al. 1983). In the assay described (Ferro et al. 1983), the KCl concentration was erroneously reported as 72 mM. The correct concentration was 18 mM.

***In vivo experimentation with* Drosophila.** Strains and genetic markers used were: (1) wild-type, Oregon R; (2) *y w*; *mwh*: *y* (yellow), and *w* (white) are markers on the first (X−) chromosome; *mwh* (multiple wing hair) is scored on the wing as short multiple trichomes extending from a single cell (in contrast to long single trichomes in wild type) and is on the third chromosome with a meiotic map position 3-0.0; (3) f^{36a} (forked) is a marker on the X chromosome. The phenotypes for *y*, *w*, and *f* are described in Lindsley and Grell (1968).

Experiments were done to test the effects of 3-aminobenzamide on viability. Flies were allowed to deposit eggs, for 12 hr, on instant *Drosophila* medium (Carolina Biological) made with various concentrations of 3-aminobenzamide or 3-aminobenzoic acid. The parents were removed and the larvae reared on the food and scored as adults. These experiments were done with wild-type flies.

Experiments to study the effects of 3-aminobenzamide on the rate of development of larvae were also done with wild-type flies. Eggs were laid for 12 hr on a filter paper disc. Sectors of the filter paper containing a certain number of eggs (approximately 40) were transferred to vials containing instant *Drosophila* medium which had been mixed with either 4 mM 3-aminobenzamide or 3-aminobenzoic acid. At various times (58, 106, and 130 hr) after the start of egg laying (0 hr), all larvae in a given vial were collected and their developmental stage determined by a microscopic (100×) examination of mouth hooks (Bodenstein 1965).

Experiments to determine the effects of 3-aminobenzamide on mitotic recombination were done with the female offspring of a cross in which the parents were *y w*; *mwh* ♀♀ × f^{36a} ♂♂. The F_1 offspring were therefore heterozygous for the *mwh* marker on the third chromosome. The parents laid eggs for 12 hr on instant food made with 0.5 mM 3-aminobenzamide, 0.5 mM 3-aminobenzoic acid or H_20. The parents were removed, and ^{60}Co γ-rays were used to administer 1425 Rems (95 R/min; 15 min) 48 hr after the beginning of the egg laying period. Irradiation was therefore done at a time in which the larvae were in the transition between the first to second instar. The wings of the adult F_1 females were mounted in Euparal (G.B.I. Laboratories) and scored for *mwh* clones (Garcia-Bellido and Merriam 1971).

RESULTS

Activation of Poly(ADP-ribose) synthetase by DNA

The synthetase is absolutely dependent upon an activating DNA structure for the synthesis of poly(ADP-ribose) from NAD (Yamada et al. 1971; Yoshihara 1972; Benjamin and Gill 1980). Figure 3 shows the stimulation by calf thymus DNA compared with intact and nicked pBR322 DNA. The pBR322 was nicked by pancreatic DNase in the presence of ethidium (Shortle and Nathans 1978); presumably the nicked pBR322 has simple nicks as the major stimulatory structure. It is clear that as the amount of nicked DNA is increased, a saturating level of stimulation is produced. However, as the amount of calf thymus DNA is increased in the same range, the stimulatory capacity of the DNA continues to increase beyond the saturating level of the nicked pBR322. This result indicates that calf thymus DNA has structures that are more stimulatory than simple nicks.

The results shown in Figure 3 provide a basis for defining the degree of stimulation by any particular DNA structure. The DNA stimulation by nicked DNA, a homogeneous stimulating structure, shows characteristics similar to an enzyme saturation curve. This would therefore allow quantitation of both the affinity of a particular type of DNA structure for poly(ADP-ribose) synthetase, as well as the maximal stimulation that can be achieved by that structure. In addition, since at low levels of DNA, there is a linear relationship between the amount of the stimulatory DNA structure and the rate of poly(ADP-ribosylation), synthetase activity can be used as an assay for such struc-

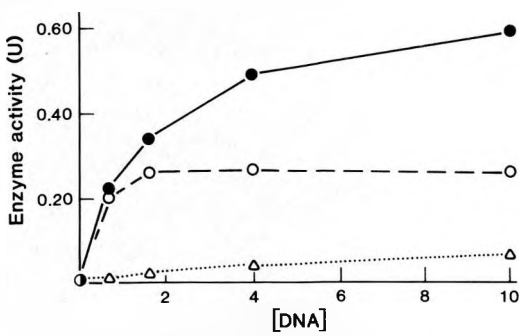

Figure 3. Stimulation of poly(ADP-ribose) synthetase by DNA. Reaction mixtures (50 μl) contained 50 mM Tris-Cl (pH 8.0), 10 mM $MgCl_2$, 1 mM DTT, 5 mM spermidine, 0.2 mM [^{32}P]NAD (specific activity 8.8×10^4 cpm/nmole) 1.25 μg enzyme/ml, and DNA as indicated. Mixtures were incubated for 5 min at 23°C, and acid-insoluble radioactivity was determined as described previously (Ferro et al. 1983). Enzyme activity refers to units of synthetase/ml reaction, and [DNA] refers to μg DNA/ml reaction. (●) Calf thymus; (○) nicked pBR322; (△) pBR322. One unit of synthetase incorporated 1 nmole of NAD into an acid-insoluble form per minute.

tures. Such studies would be confusing unless homogeneous structures were used. In the activation of the synthetase by calf thymus DNA, the total stimulation observed is a composite of that provided by the different stimulatory structures, which may be present at different concentrations.

Modification of DNA Topoisomerase I

As we have shown previously, the modification of DNA topoisomerase I by poly(ADP-ribose) synthetase leads to an inhibition of topoisomerase enzymatic activity. In our previous published work (Ferro and Olivera 1984), only 90% inhibition was achieved using purified enzymes; in the experiment in Figure 4, there is an essentially total inhibition of the DNA topoisomerase I.

This efficient inhibition, which occurs even though both enzymes are present at submicromolar concentrations, suggests either strong direct interaction between the two proteins, affinity for the same DNA site, or both. There is, in fact, evidence that the topoisomerase and the synthetase may well have an affinity for the same sites on DNA. The apparent activity of DNA topoisomerase decreases when assayed on plasmid preparations that have been stored, probably because inhibitory DNA structures are formed. The addition of poly(ADP-ribose) synthetase in the absence of NAD stimulates DNA topoisomerase activity under these conditions (Fig. 5). Synthetase that had been heat inactivated (92°C, 3 min), however, did not stimulate the topoisomerase, and the addition of calf thymus DNA (10 μg/ml) to the system prevented the stimulation of the topoisomerase by the synthetase (data not shown). The most straightforward explanation for these observations is that poly(ADP-ribose) synthetase competes with DNA topoisomerase I for the inhibitory sites on the DNA. Synthetase binding would release the DNA topoisomerase so that enzymatic activity could be raised to the levels that would be expected if DNA topoisomerase were assayed using freshly prepared preparations of plasmid. Even though the enzymes may have affinity for the same DNA sites, it seems probable that there will nevertheless be a highly specific protein–protein interaction involved in the topoisomerase modification reaction (see Fig. 6 for a model incorporating these ideas).

The In Vivo Effects of Inhibitors of Poly(ADP-ribosylation)

One of the most effective inhibitors of poly(ADP-ribose) synthetase is 3-aminobenzamide (Purnell and Whish 1980). We have studied the effects of this inhibitor on *Drosophila melanogaster* in vivo. To make it more likely that any effects observed are in fact due to inhibition of poly(ADP-ribosylation) and not to some other metabolic effects of 3-aminobenzamide, control experiments were performed in parallel using 3-aminobenzoic acid, which is a much weaker inhibitor of poly(ADP-ribose) synthetase (Purnell and Whish 1980). The most likely metabolic fate of the amide would be deamidation to 3-aminobenzoic acid.

When *Drosophila melanogaster* larvae were fed pro-

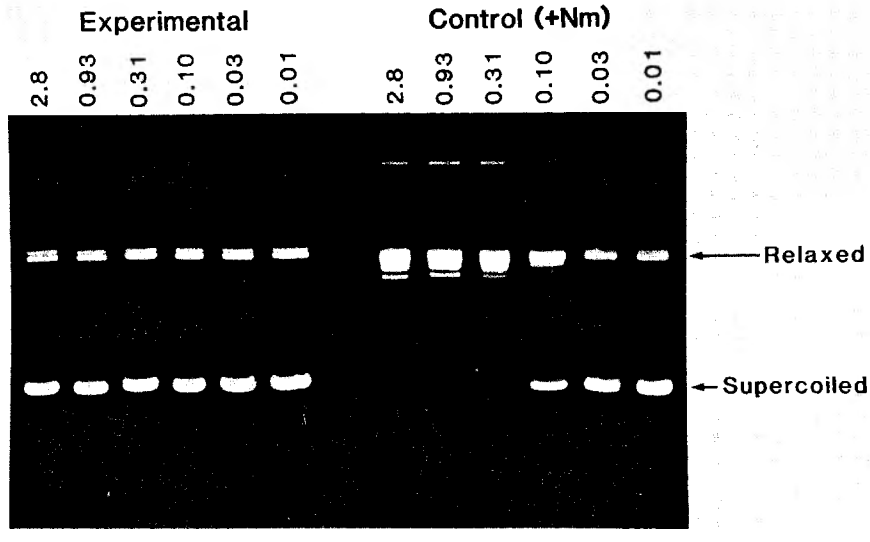

Figure 4. Inhibition of DNA topoisomerase I by poly(ADP-ribosylation). Highly purified DNA topoisomerase I was modified by purified poly(ADP-ribose) synthetase using the pRW751 plasmid to stimulate the synthetase as described under Methods. The topoisomerase activity was determined as previously described (Ferro et al. 1983) using agarose gel electrophoresis as an assay for the conversion of supercoiled pBR322 to the relaxed form. The numbers at the top refer to nanograms of topoisomerase present per 0.03-ml reaction mixture containing 0.6 μg of pBR322 DNA. The control series was identical to the experimental series except that nicotinamide was added in the preincubation mixture. There was a ca. 200-fold inhibition of topoisomerase in the experimental compared with the control series. Preliminary results indicated that the stimulatory DNA was not the major component of the plasmid preparation (supercoiled pRW751), but a minor species that could be separated from the intact plasmid using a cesium chloride-ethidium bromide gradient. The supercoiled preparation was relaxed prior to being used to stimulate poly(ADP-ribose) synthetase in this experiment.

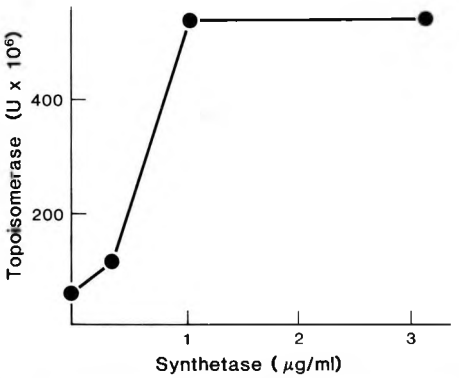

Figure 5. Apparent stimulation of DNA topoisomerase I by poly(ADP-ribose) synthetase in the absence of NAD. Purified DNA topoisomerase ($M_r = 100{,}000$) was serially diluted into 50 mM Tris-Cl (pH 8.0), 5 mM $MgCl_2$, 5 mM DTT, 5 mM spermidine, 100 μg/ml BSA, 18 mM KCl, 20 μg/ml pBR322 (supercoiled substrate), and various amounts of purified poly(ADP-ribose) synthetase, in a final volume of 50 μl. The reaction mixtures were incubated at 30° for 1 hr and analyzed using agarose gel electrophoresis (see Fig. 4).

gressively higher concentrations of 3-aminobenzamide, significant lethality was observed. At the comparable concentrations of 3-aminobenzoic acid, the flies were viable. The data for the lethal effects of 3-aminobenzamide on *Drosophila melanogaster* are shown in Table 1.

One interesting feature of 3-aminobenzamide toxicity was that the flies were not killed immediately, but instead appeared to be blocked in their development. In the presence of elevated concentrations of 3-aminobenzamide, larvae were markedly retarded in their development and could not make the transition to the pupal stage. Even with sublethal concentrations of 3-aminobenzamide, a significant proportion of individuals were still larvae at times when control adults had emerged (Fig. 7). Such an experiment at a 3-aminobenzamide concentration in which 75% of the flies failed to emerge is shown in Figure 7. With lethal levels of the drug, the larvae linger without further differentiation and finally die.

In the experiments in Figure 7 and Table 1, when an analysis of the emerging adults was carried out, preferential survival by males was observed. This effect was even more marked in crosses involving strains with the *white* locus. In crosses where white-eyed males and phenotypically wild-type females were produced (e.g., in the cross done for the study of mitotic recombination; see Experimental Procedures), there was a greater than 10-fold preferential survival of males at high concentrations of 3-aminobenzamide (results not shown). The basis of this sexual bias is presently being investigated.

The Effects of Inhibition of Poly(ADP-ribosylation) and Mitotic Recombination

We have also scored the effect of 3-aminobenzamide on the appearance of mwh clones in flies heterozygous at the *mwh* locus; this is commonly used as an assay for mitotic recombination (Postlethwait 1978). Preliminary data are shown in Table 2. In these experiments, mitotic recombination was induced by γ-irradiating larvae at the transition from first to second instar (Garcia-Bellido and Merriam 1971). γ-Irradiation induces mitotic recombination, which in this experiment was reflected in the increased number of large *mwh* clones on the wing. The induction of *mwh* clones by γ-irradiation was intensified if the larvae had been fed 3-aminobenzamide. These preliminary results suggest that 3-aminobenzamide increases the frequency of mitotic recombination. It should be emphasized that this is not the only interpretation of this assay; any mechanism leading to homozygosity or hemizygosity could yield mwh clones (Becker 1976).

DISCUSSION

Although the phenomenon of poly(ADP-ribosylation) was discovered two decades ago, the metabolic significance of this modification is still unclear. However, it is worth reviewing certain general features (for

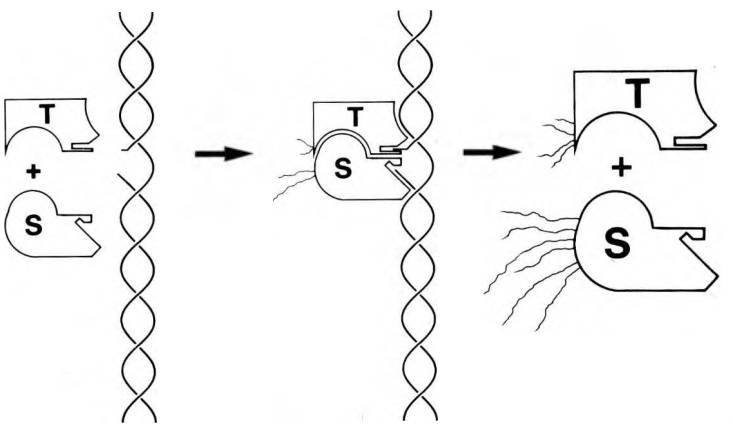

Figure 6. Poly(ADP-ribosylation) of DNA topoisomerase I. A model for the modification of DNA topoisomerase I (T) by the synthetase (S). Other symbols are as in Fig. 2.

Table 1. Effects of 3-Aminobenzamide on Wild-type *Drosophila melanogaster*

Treatment	Concentration (mm)	Total flies	♂♂	♀♀	♂♂/♀♀
3-Aminobenzoic acid	0	246	109	137	0.79
	1.0	255	112	143	0.78
	2.5	185	92	93	0.98
	4.0	253	119	134	0.88
	5.5	248	124	124	1.00
	7.0	283	136	147	0.92
3-Aminobenzamide	0	220	103	117	0.88
	1.0	279	140	139	1.0
	2.5	101	55	46	1.2
	4.0	23	15	8	1.87
	5.5	19	14	5	2.8
	7.0	13	8	5	1.6

overview, see Hayaishi and Ueda 1982) that have been established:

1. Poly(ADP-ribose) synthetase is found ubiquitously in the nuclei of multicellular eukaryotes. The enzyme is usually present in relatively high levels; from crude extracts of calf thymus, for example, a 1000-fold purification yields homogeneous enzyme. If maximally activated, the enzyme could consume the entire cellular pool of NAD in a few seconds.
2. The enzyme is activated by breaks in the DNA, and absolutely requires such interruptions in the double-helical structure of DNA for activity.

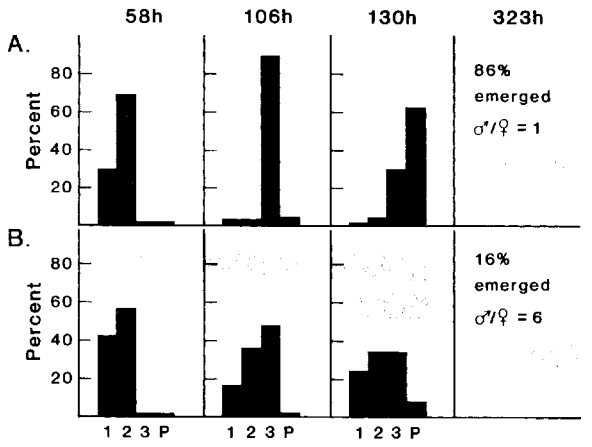

Figure 7. Effects of 3-aminobenzamide on larval development. Eggs were transferred to food made with either 4 mM 3-aminobenzoic acid (*A*) or 3-aminobenzamide (*B*), as described under Methods. The developmental pattern of flies reared on H_20 was equivalent to that of flies grown on 3-aminobenzoic acid. Larvae were harvested at various times after the beginning of the 12-hr egg laying period as indicated on top of each graph. The developmental distribution of all larvae that were recovered is shown for first, second, and third instar, and pupae (indicated as 1, 2, 3, P). At 323 hr, emergence was complete in the control and experimental populations. For the flies reared on 3-aminobenzoic acid, 36 adults emerged from 42 eggs (86%) whereas in the experimental population on 3-aminobenzamide, 6 adults emerged from 42 eggs (16%).

3. The poly(ADP-ribose) that is synthesized can be attached to three types of acceptors: (a) the enzyme itself, in an auto(ADP-ribosylation) reaction; (b) a set of exogenous proteins including histone HI, HMG proteins, a Ca^{++}-Mg^{++}-dependent deoxyribonuclease, and DNA topoisomerase I; and (c) the small nucleotide Ap_4A (Yoshihara and Tanaka 1981).

In trying to evaluate the metabolic role of poly(ADP-ribosylation), it is perhaps useful to consider the potential effects of poly(ADP-ribosylation) on one of the target proteins, DNA topoisomerase I. Of all of the targets of the enzyme, DNA topoisomerase I has a well-studied enzymatic activity (for reviews, see Wang 1981; Gellert 1981). The inhibition of topoisomerase activity by modification with poly(ADP-ribose) presumably occurs for one of three possible consequences: (1) to prevent the unwinding of positive supertwists that might accumulate ahead of the replication fork, (2) to prevent the unwinding of negative supertwists, and (3) to prevent the formation of covalent complexes of topoisomerase and DNA which may produce an interruption in the DNA structure.

These possibilities should be evaluated together with in vivo experiments using inhibitors. Poly(ADP-ribosylation) may not be directly essential for cell viability, since the addition of high levels of a poly(ADP-ribose) inhibitor such as 3-aminobenzamide does not decrease the viability of mammalian cells growing in culture (Durkacz et al. 1981). However, the addition of poly(ADP-ribosylation) inhibitors to such a mammalian cell culture increases the rate of SCE (Oikawa et al. 1980). The inhibition of DNA topoisomerase by poly(ADP-ribosylation) could be relevant to the in vivo observations on SCE in one of two ways. The first possibility is that inhibition of DNA topoisomerase I prevents the release of positive supercoils produced by replication forks. Poly(ADP-ribosylation) may be a signal to stop replication forks in the vicinity of a DNA strand interruption. Replication forks close to DNA strand breaks may increase the probability of strand-exchange events and thus the failure to stop such forks

Table 2. Induction of *mwh* Clones in the Wings of *Drosophila*

Population	Compound in food[a]	γ-Irradiation[b]	Wings scored	Total clones	Large clones[c]	Large clones/wing
1	none	–	73	8	1	0.014
2	none	+	48	6	2	0.042
3	3AB	–	48	5	0	0.0
4	3AB	+	64	27	6	0.094
5	acid	–	56	5	0	0.0
6	acid	+	48	9	2	0.042

[a]3AB, 0.5 mM 3-aminobenzamide; acid, 0.5 mM 3-aminobenzoic acid.
[b]+, 1200 Rems at $t = 48$ hr (see Methods).
[c]Clones greater than 100 cells.

would lead to higher rate of strand exchange. A second possibility is the more direct involvement of the enzyme in strand exchange. It has been observed in vitro that under certain conditions, DNA topoisomerase I can directly catalyze strand-exchange events, and the suggestion has been made that these strand-exchange events are a consequence of an attack by the end of a DNA strand on the relatively long-lived covalent topoisomerase I–DNA complex (Halligan et al. 1982). The distinction between the two hypotheses is that in the first, replication is a necessary element in the observed strand exchanges.

In our in vivo experiment using *Drosophila* to evaluate effects on mitotic recombination, a striking and unexpected biological effect was observed. At high concentrations of 3-aminobenzamide, larvae that are feeding on the drug fail to pupate. Furthermore, there is preferential male survival under these conditions. Although considerable caution should be exercised in the interpretation of inhibition studies, these results indicate that while poly(ADP-ribosylation) may not be essential for simple proliferation of cells, it is necessary for the viability of a whole organism such as *Drosophila*.

In this paper, we have probably raised more questions than provided answers. However, poly(ADP-ribosylation) appears to be an enzymatic phenomenon that modulates chromatin metabolism whenever certain types of DNA structures are produced. From this point of view, poly(ADP-ribosylation) might be viewed as a nuclear alarm signal. We will explore the hypothesis that one function of this signal is to reduce the probability of strand-exchange events when interruptions occur in the double-helical structure of DNA. That a system to suppress illicit recombination events may be crucial to the survival of multicellular organisms was suggested by Kinsella and Radman (1978); these workers presented evidence that tumor promoters may act by inducing aberrant mitotic segregation events leading to the expression of recessive mutations. An equally exciting possibility is that this nuclear alarm signal is important in programmed switches for cellular differentiation during development. The poly(ADP-ribosylation) of DNA topoisomerase I may serve as a key mechanistic component for achieving such metabolic objectives.

Note Added in Proof

A recent analysis of the mitotic chromosomes in the neuroblasts of *Drosophila* larvae strongly suggest that poly(ADP-ribosylation) plays a role in heterochromatin condensation (A.M. Ferro and B.M. Olivera, in prep.). The primary chromosomal aberration seen in neuroblasts taken from larvae fed 3-AB is a specific pattern of undercondensation in various heterochromatic regions.

ACKNOWLEDGMENTS

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