NO EFFECT OF INHIBITION OF POLY(ADP-RIBOSYLATION) ON THE FREQUENCY OF HOMOLOGOUS RECOMBINATION. II. FLOW CYTOMETRY

Maria Wojewódzka, Teresa Bartłomiejczyk, Tomasz Ołdak[1/], Marcin Kruszewski

[1/] Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Warszawa, Poland

The preceding report of Wojewódzka *et al.* [1] presented a study carried out with the aim of explaining the role of poly(ADP-ribosylation) in repair of DNA double strand breaks (DSB). The frequency of spontaneous and X-ray induced homologous recombination (HR) in non-homologous end-joining (NHEJ)-competent (Chinese hamster cell line CHO-K1) and NHEJ-deficient (xrs-6) cells lines was estimated in transfectants containing pLrec plasmid that carries two non-functional copies of a bacterial gene, *lacZ*, (ß-galactosidase) in a tandem array [2]. The *lacZ* genes are divided by a selective marker gene, which provides resistance to the G418 antibiotic (gene *neo*). The cells were transfected with a linear plasmid by electroporation and the transfected clones were selected in the medium containing 500 µg/ml G418. The experimental schedule was presented in [1].

Table 1. Frequency of spontaneous and induced recombination of *lacZ* genes in clones of CHO-K1 cells transfected with single copy of pLrec (rate of conversion to LacZ(+) expressed as event/cell generation).

Clone	Treatment	G418	Rate of conversion to LacZ(+) (event/cell generation)
N11	control	-	3.39 E-05
	2 Gy	-	4.60 E-05
	AB	-	5.10 E-05
	2 Gy+AB	-	5.46 E-05
	control	+	3.22 E-05
	2 Gy	+	4.35 E-05
	AB	+	5.18 E-05
	2 Gy+AB	+	4.69 E-05
TK15	control	-	2.40 E-04
	2 Gy	-	2.36 E-04
	AB	-	2.46 E-04
	2 Gy+AB	-	2.32 E-04
	control	+	2.25 E-04
	2 Gy	+	2.08 E-04
	AB	+	2.17 E-04
	2 Gy+AB	+	2.34 E-04

To assess the number of LacZ expressing cells, the following procedure for flow cytometry (FACS) sorting of LacZ containing cells based on cleavage of a fluorescent substrate (fluorescein di-β-D-galactopyranoside, FDG) by β-galactosidase was used, as originally described by Nolan *et al.* [3]. Exponentially growing cells were treated with trypsin (0.25% in phosphate buffered saline) until they could be removed from the plate with mild agitation. Cells were counted and brought to $5x10^6$ per ml in MEM medium containing 2% (vol/vol) foetal calf serum. Number of β-galactosidase cells was estimated according to the following protocol: 100 µl of cell suspension was added to a 5-ml polystyrene tube and brought to 37°C in a water bath for 5 min. The cell suspension was mixed gently but thoroughly

Table 2. Frequency of spontaneous and induced recombination of *lacZ* genes in clones of xrs6 cells transfected with a single copy of pLrec (rate of conversion to LacZ(+) expressed as event/cell generation).

Clone	Treatment	G418	Rate of conversion to LacZ(+) (event/cell generation)
S9	control	-	4.11 E-03
	+2 Gy	-	5.17 E-03
	+AB	-	4.69 E-03
	+2 Gy+AB	-	5.17 E-03
	control	+	3.58 E-03
	+2 Gy	+	4.93 E-03
	+AB	+	3.19 E-03
	+2 Gy+AB	+	5.66 E-03
S11	control	-	6.46 E-03
	+2 Gy	-	8.23 E-03
	+AB	-	8.24 E-03
	+2 Gy+AB	-	8.53 E-03
	control	+	3.62 E-03
	+2 Gy	+	4.18 E-03
	+AB	+	6.12 E-03
	+2 Gy+AB	+	6.03 E-03
S15	control	-	1.17 E-03
	+2 Gy	-	1.31 E-03
	+AB	-	1.09 E-03
	+2 Gy+AB	-	1.44 E-03
	control	+	1.11 E-03
	+2 Gy	+	1.15 E-03
	+AB	+	1.00 E-03
	+2 Gy+AB	-	1.26 E-03

with 100 μl of 2 mM FDG pre-warmed to 37°C and immediately placed in 37°C water bath for exactly 1 min. FDG loading was terminated by addition of 1800 μl ice-chilled incubation medium and 1 μM propidium iodide. The cells were kept on ice for 60 min until viewed by FACS analysis.

The results, presented in Tables 1 and 2, are in agreement with the previously described enzymatic activity determinations. They show that there is no effect of poly(ADP-ribosylation) inhibition on recombination frequency in this experimental model. This result is compatible with those of other authors [4], who show that poly(ADP-ribose) polymerase (PARP) is not directly engaged in HR repair. Nevertheless, as discussed in [4,5], there are numerous data on the anti-recombinogenic role of PARP. Hence, inhibition of poly(ADP-ribosylation) should have a pro-recombinogenic effect. This, however, was not observed in our experimental system, where the substrate was the plasmid incorporated into the host cell genome. A possible reason for this is the p53 mutation in CHO and xrs6 cells [6], whereas the effect of PARP on DSB repair depends on wild type p53, as shown by Susse *et al*. [5].

Supported by the State Committee for Scientific Research (KBN) – grant No. 3 P04A 036 24.

References

[1]. Wojewódzka M., Bartłomiejczyk T., Kruszewski M.: No effect of inhibition of poly(ADP-ribosylation) on the frequency of homologous recombination. I. Enzymatic assay. In: INCT Annual Report 2004. Institute of Nuclear Chemistry and Technology, Warszawa 2005, pp.112-113.
[2]. Herzing L.B., Meyn M.S.: Gene, 137, 163-169 (1993).
[3]. Nolan G.P., Fiering S., Nicolas J.F., Herzenberg L.A.: Proc. Natl. Acad. Sci. USA, 85, 2603-2607 (1988).
[4]. Schultz N., Lopez E., Saleh-Gohari N., Helleday T.: Nucleic Acids Res., 31, 4959-4964 (2003).
[5]. Susse S., Scholz C.J., Burkle A., Wiesmuller L.: Nucleic Acids Res., 32, 669-680 (2004).
[6]. Lee H., Larner J.M., Hamlin J.L.: Gene, 184, 177-183 (1997).

ADAPTIVE RESPONSE: STIMULATED DNA REPAIR OR DECREASED DAMAGE FIXATION?

Irena Szumiel

The usual definition of the adaptive response says that this is a cellular response whereby a mild stress stimulus (called adaptive or priming) applied before a challenge treatment with a DNA damaging agent causes a decrease in the detrimental effects of that treatment. Many experimental results suggest that the priming stimulus is the source of signalling which eventually leads to expression of the adaptive response. Then, the "primed" cell is for a certain time able to respond to the challenge dose by an increased recovery, as compared to the control one. An essential part of the adaptive response is generation or receipt and transmission of a signal which is the direct cause of initiation of a cellular response that diminishes the effects of DNA damage.

The often accepted view that DNA repair is stimulated in the "primed" and challenged cell is not supported by consistent data on increase in the rate of repair or altered level of initial damage (*e.g.* [1-3]). So, the emphasis is now shifted towards fidelity of repair rather than its stimulation, as in the more recent studies of Sasaki *et al.* [4]. These authors, however, did not identify the molecular mechanism of the fidelity increase. The hypothesis which I present does not contradict that of Sasaki *et al.* [4], but rather redefines it. Instead of ascribing the radioadaptation to DNA repair stimulation or repair fidelity increase, I interpret the experimental results in terms of decreased damage fixation. This idea derives from the transcription-based model of damage fixation of Radford [5].

Taking into account the abrogation of radioadaptation by poly(ADP-ribosylation) inhibitors applied simultaneously with the challenge dose and the fact that adaptation is revealed as a decrease in chromosomal aberration frequency, one can apply to the adaptive response the same arguments as those that support the fixation model of Radford [5]. According to it, double strand break (DSB) fixation takes place in transcriptional factories due to cooperation of two molecules of topoisomerase I. In result, an exchange event takes place, bringing

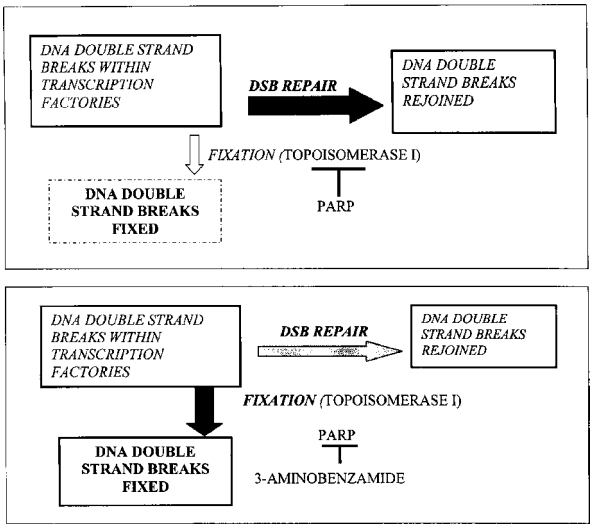

Fig. Competition between DSB repair and fixation in transcription factories, according to the model of Radford [5].

about chromosomal aberration. There is a competition between repair and fixation (Fig.); poly(ADP-ribose) polymerase (PARP)-effected inhibition of topoisomerase I prevents damage fixation whereas PARP inhibitor, 3-aminobenzamide promotes it. In conclusion, adaptive response (at least in part) is due to diminished fixation of DSBs in the transcription factories by the mechanism proposed by Radford [5].