Induction of a special Balbiani ring by position effect in the salivary gland chromosomes of *Acricotopus lucidus*

W. Staiber

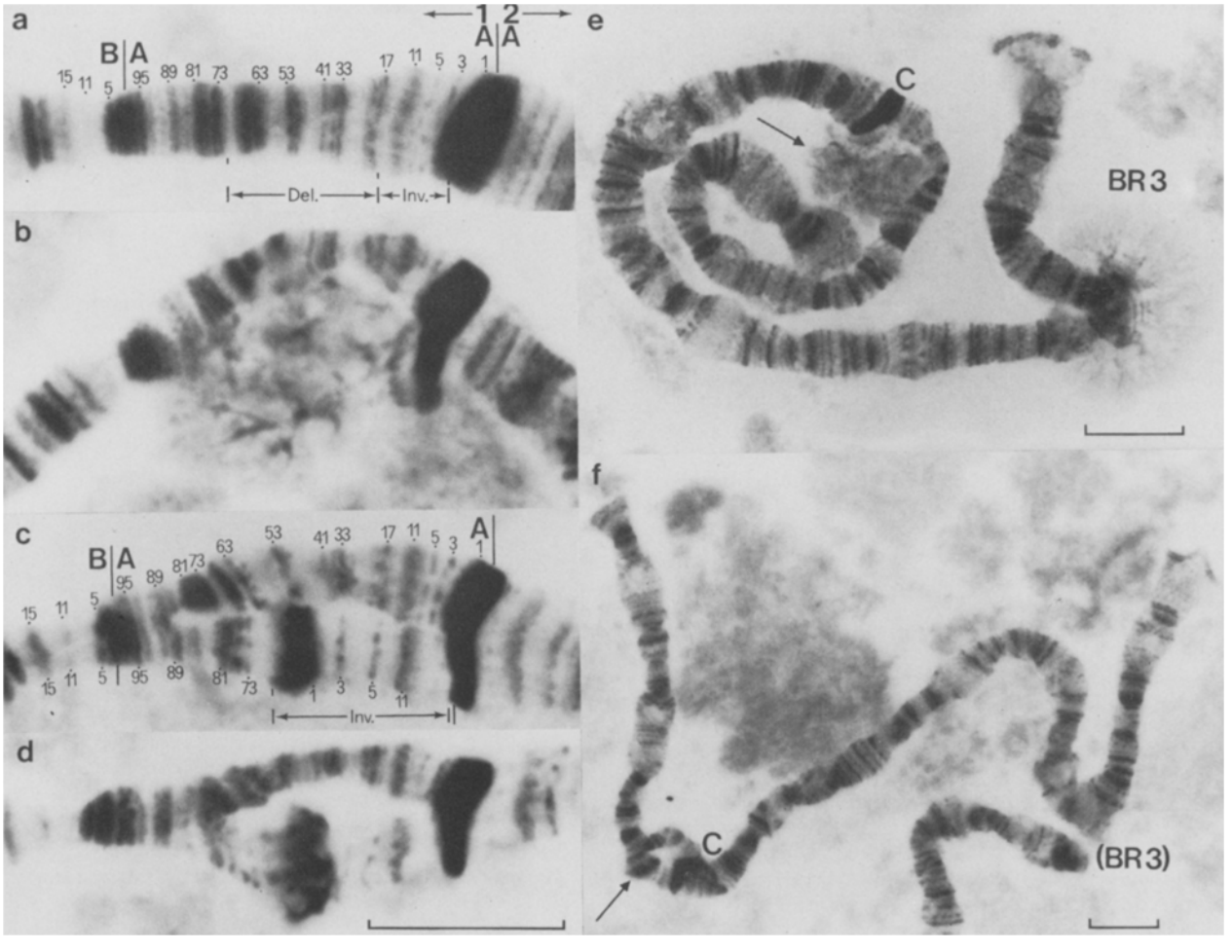
Institut für Allgemeine Genetik der Universität Hohenheim, D-7000 Stuttgart (Federal Republic of Germany), 26 April 1982

Summary. Chromosome mutations were induced by X-ray treatment of males of *Acricotopus lucidus.* One of the larvae of the F_1-generation showed a dislocation of a segment of the centromeric region into an euchromatic part of the same chromosome as a consequence of mutation. In the new position, in some salivary gland cells, this segment expressed a distinct Balbiani ring. In other cells this Balbiani ring regressed, probably through the influence of ecdysone.

Male imagines of *Acricotopus lucidus* (Diptera, Chironomidae, Orthocladiinae) were exposed to X-rays and then paired with non-irradiated females. The radiation dose applied was about 1500–2000 R using a Seifert X-ray machine 'Eresco 200' at 200 kV, 5 mA. The examination of the salivary gland chromosomes of the F_1-generation showed that 57 of the 300 larvae checked had chromosome mutations (19%).

In the somatic cells of *A. lucidus* there are $2n=6$ chromosomes. They form 3 giant chromosomes in the salivary glands. The animals of the P-generation belonged to an inbred line (more than 12 generations) with the chromosomal constitution CF[1]. The salivary glands were fixed in ethanol-acetic acid (3:1) and stained according to the method described by Beermann[2].

The larval salivary gland of *A. lucidus* consists of 3 morphologically different lobes: anterior lobe, main lobe and side lobe[3]. Each lobe exhibits a specific Balbiani ring pattern[4]. During the transition from the 4th larval instar to the prepupal stage the Balbiani rings (BR) BR 3 and BR 4 (both specific to the anterior lobe) regress in a gradient from the top to the basis of the anterior lobe through the increasing concentration of the moulting hormone ecdysone[5]. Within the anterior lobe of an animal at such a transition stage (4th larval instar to prepupa) there are cells which have already reacted with respect to their puffing



a–d Division 1A of the left arm of chromosome I. *a* Homozygous banding pattern with mapping of the rearrangements; anterior lobe; Del., deletion; Inv., inversion. *b–d* Heterozygous division 1A. *b* Special BR expanded, BR 3-situation like in *e*; anterior lobe. *c* Regressed special BR, BR 3-situation like in *f*; anterior lobe. *d* Situation in all chromosomes of the main and side lobes. *e* Chromosome I with expressed special BR (arrow) and BR 3; anterior lobe. *f* Chromosome I with condensed locus of special BR (arrow) and completely regressed BR 3; anterior lobe. *b–f* Micrographs of the same salivary gland; BR, Balbiani ring; C, centromere. Scale bars = 10 µm.

activity (BR regressed) to the secretion of the ecdysone, and others with still-active puffing (BR expanded). The centromeres of the salivary gland chromosomes are represented by blocks (C) of extremely condensed chromatin. They divide the chromosomes into a short left and a long right arm.

One of the 57 mutants, in the transition stage, showed structural heterozygosity in the left arm of chromosome I in all salivary gland cells (fig., b-f) for a complex of an adjoining deletion and an inversion. The location of these rearrangements is shown in the figure, a. The deletion includes 28 bands (1A17-1A71; according to the unpublished chromosome map by Staiber and Behnke, bands with uneven and interbands with even numbers). One of the inversion breakpoints is located within the centromere. As a consequence, a part of the centromeric region is separated and transposed into a euchromatic part of the left arm (adjacent to band 1A73; fig., c). The anterior lobe of the salivary gland of *A. lucidus* consists of 12–16 cells. In one of the salivary glands of the mutation carrying F_1-animal all 16 cells of the anterior lobe could be examined. In 10 cells, in which the BR 3 and BR 4 were fully expanded, the segment formed a BR (fig., b and e), whereas in 6 cells, in which BR 3 and BR 4 were regressed, the segment was present in a slightly puffed or condensed form respectively (fig., c and f). Apparently the segment concerned has the same pattern of activity as the loci of BR 3 and BR 4. So one can suppose that in the new position its activity is under the control of ecdysone.

In all the cells of the main and side lobes the segment was still somewhat disaggregated like a regressing structure of a formerly expanded BR (fig., d).

In the normal position, integrated in the centromere, the segment never shows any puffing or RNA-synthesis (no incorporation of ^{3}H-uridine, no specific RNA-staining by toluidine-blue- or methylgreen-pyronin-staining).

The expression of this special BR is interpreted as a position effect, due to a change of chromosomal structure[6-8].

Evidently, the locus is not able to express a BR in the regular position; in the new position, however, the locus is puffed to a BR. Another case of a position effect in *A. lucidus* has been described by Mechelke[9].

1 Wobus, U., Serfling, E., Baudisch, W., and Panitz, R., Biol. Zbl. *90* (1971) 433.
2 Beermann, W., Chromosoma *5* (1952) 139.
3 Mechelke, F., Chromosoma *5* (1953) 511.
4 Mechelke, F., in: Funktionelle und morphologische Organisation der Zelle, p. 15. Wiss. Konferenz Ges. dtsch. Naturf. u. Ärzte, Rottach-Egern 1962. Springer, Berlin/Göttingen/Heidelberg 1963.
5 Panitz, R., Biol. Zbl. *83* (1964) 197.
6 Sturtevant, A. H., Genetics *10* (1925) 117.
7 Lewis, E. B., Adv. Genet. *3* (1950) 73.
8 Hannah, H., Adv. Genet. *4* (1951) 87.
9 Mechelke, F., Naturwissenschaften *47* (1960) 334.

Host cell reactivation of ozone-treated T3 bacteriophage by different strains of *Escherichia coli*

P. L'Hérault[1] and Y. S. Chung

Département des Sciences Biologiques, Université de Montréal, C.P. 6128, Succursale A, Montréal (Québec, Canada H3C 3J7), 1 July 1982

Summary. Host cell reactivation capacity for ozone T3 phage was determined for different *E. coli* strains deficient in one or more of the DNA repair mechanisms. The results indicate that DNA polymerase I appears to play a key role in the repair of damage produced on the DNA by ozone while the *lex*A gene product seems to play a minor one.

In the last few years considerable data have been accumulated suggesting that ozone may be radiomimetic[2,3], mutagenic[4,5] and can cause some DNA degradation in different strains of *E. coli*[6-8]. Furthermore, ozone has been reported to cause chromosome breakage in *Vicia faba*[9] and in mammalian cell cultures[10]. There is still however very little information available on the ozone DNA-damage and the repair mechanism(s) in the cell.

Previous workers have defined host cell reactivation (HCR) by the fact that UV-damaged phage lambda has different plating efficiency depending on the genetic background of the host cell in which it multiplies[12]. With the need to determine rapidly the DNA damage repair capacity of different bacterial strains, many researchers have applied this process with T1, T3 and T7[13,14]. Since ozone inactivation of T3 phage is caused in part by DNA damage[15,16], this technique seemed very useful in the possible determination of the ozone DNA-damage repair mechanism(s).

Material and methods. Stocks of T3 phage were prepared by the confluent lysis method with *E. cole* B251[17]. Water suspensions of T3 phage were exposed to 10 ppm of ozone for 10 min as described elsewhere[5]. Samples (0.1 ml) were removed from the phage suspensions before and after treatment and proper dilutions were mixed with a log phase of different bacterial strains (m.o.i = 0.02) and plated on nutrient agar plates (Difco) by the layer method[17]. Plates were incubated at 37 °C and counted the next day. We calculated the fraction of survivors (S/S°) and the reactivation factor (fraction of survivors with the mutant strain/fraction of survivors with the wild type strain) for each bacterial strain. Ozone inactivation curve was done on nutrient agar plates (Difco) as previously described[5,17] and control experiments were carried out with clean air instead of ozone.

Results and discussion. The ozone inactivation curve of T3 phage to 10 ppm of ozone and for different intervals of treatment-time is represented in the figure. According to these results, the inactivation capacity of ozone on this bacteriophage increases with increasing treatment time. This type of linear decrease through the origin during ozonation has been observed with different types of virus[15,18,19].

The host cell reactivation capacity obtained with different strains of *E. coli* are shown in table 2. The host cell strains with no deficiency in any of the DNA repair mechanisms, B251, JG139 and HMS49, should theoretically reactivate the ozone-treated phage. Based on this assumptions, we calculated the host cell reactivation factor for different