conceivable that the saturation density of *T. pyriformis* is determined by this collision effect.

A medium from which previously grown cells had been removed by filtration was designated as conditioned medium and was used for the following experiments. The growth curve of *T. pyriformis* after inoculation into a medium conditioned for 240 h, gave a lag time of 22 h and a saturation density of 350,000 cells/ml. When 160,000 hollow glass beads/ml and 240,000 hollow glass beads/ml were suspended in the medium conditioned for 240 h, the saturation densities decreased by about 150,000 cells/ml and 250,000 cells/ml, respectively.

When *Tetrahymena* cells were inoculated into media containing a significant amount of the floating glass beads, as previously shown in figure 1, the lag time before cell division was initiated was significantly lengthened. Namely, the higher the amount of glass beads in the medium was, the longer was the lag time of cell division. Furthermore, the higher the amount of glass beads in the medium was, the lower was the saturation density of *Tetrahymena,* until the point at which growth no longer occurred. However, the growth rate of *Tetrahymena* during the logarithmic growth phase was not dependent upon the amount of hollow glass beads present. The relationship between the saturation density and the number of floating glass beads is shown in figure 2. This plot suggests that saturation density is determined by the frequency of collisions between cells and glass beads or between the cells themselves. When the ratio of the number of hollow beads, in the presence of the hollow beads, to the saturation density in the absence of beads in fresh medium, or in conditioned medium, was less than 50%, no decrease in the saturation density was observed. The growth-promoting and growth-inhibiting substances are already present in a conditioned medium. Thus, it is possible that the influence of the growth-inhibiting factors in the conditioned medium is more effective than that of growth-promoting factors and that this results in a slight reduction of the saturation density (70%) in the conditioned medium (see the legend of fig. 2).

The saturation density of protozoa such as *Tetrahymena* is generally less than the saturation density of bacteria. It is known that the saturation density of bacteria is determined by the concentrations of oxygen, nutrients and end-products in the medium. In contrast, we have shown here that, in the case of *T. pyriformis,* the maximal cell density which can be reached in a *Tetrahymena* culture seems to be regulated, at least in part, by the frequency of collisions and not by nutrient effects.

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Visualization of centromeric spots in the chromosomes by UV-light exposure

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Summary. When exposed to UV-light in the presence of Hoechst 33258, chromosomes of *Allium cepa* were progressively photolyzed with increasing length of exposure; they retained their delineated contours, centromeric spots and sometimes secondary constriction bands.

Visible- or UV-light exposure following BUdR incorporation and Hoechst 33258 treatment has been successfully used for differential staining of sister chromatids[1–4]. Goto et al.[3] and Sugiyama et al.[5] proposed that differential staining produced by this technique depended on dye concentration and light-exposure time, and suggested that preferential photolysis of BUdR-substituted DNA was responsible for differential staining. It is reported that no degradation has been demonstrated with BUdR-unsubstituted DNA bound to Hoechst 33258 and exposed to UV-light[6,7]. These data suggest that an interaction of BUdR and Hoechst 33258 is related to photolysis of the chromosomes. However, the present authors have investigated the effects of UV-light on BUdR-unsubstituted chromosomes to study the organization and chemical properties of chromosomes, and found that UV-light could drastically photolyze the chromosomes under appropriate conditions without BUdR labeling. The present paper introduces a new procedure for photolysis of the chromosomes and describes chromosome architectures produced by this technique.

Material and methods. Actively growing roots of *Allium cepa* L. were excised and pretreated in aqueous 0.025% colchicine at 18 °C for 3.5 h and fixed overnight in 1:3 glacial acetic acid – 99.5% ethanol at 5 °C. The chromosomes were prepared by the enzyme maceration and flame-drying technique[8]. The chromosomes on the glass slides were soaked in McIlvain buffer (pH 8.0) for 5 min. Then, the slides were drained, blotted with paper towels and immediately stained for 5 min with freshly-prepared 50 µg/ml Hoechst 33258 (Wako Pure Chem. Ind. Ltd, Japan) dissolved in the same buffer. After a brief rinse in the buffer, the chromosomes were mounted in the buffer and sealed with paraffin. UV-light was provided by a fluorescence microscope, equipped with an 40 times objective lens (Nikon, UV-F) and a Nikon epiilluminator (Osram HBO 50 W mercury vapor lamp, Germany). The filter combination used for Hoechst 33258 fluorescence yields UV-light with a predominant wave length of 365 nm. The exposure was carried out in the dark room, focusing on the metaphase chromosomes for various times at 25–28 °C. After UV-light exposure, the coverslips were gently removed from the slides with a razor blade. Immediately, the slides were restained for about 20 min in 15–20% Giemsa solution, made up in 1/15 M phosphate buffer (pH 7.0).

Results and discussion. The effects of UV-light on the

Effect of pH of the buffer on chromosome extraction

pH	Exposure time (min) 10	30	60	90
8.0	d	b	b	a
7.0	d	c	b	a
4.0	d	d	d	d

a, remarkable; b, medium; c, slight; d, no extraction.

chromosomes were determined at various intervals, by comparing micrographs of Hoechst 33258 fluorescence with Giemsa staining of the same specimens (fig. 1). As a rule, at 20 min exposure, the fluorescence of the chromosomes was slightly quenched, and they stained with Giemsa a little less than the controls. During approximately 30–60 min of exposure, the centromeric regions and, sometimes, secondary constrictions became discernible (fig. 1, a–d). The centrometric regions fluoresced more brightly than other chromosome regions and were stained densely with Giemsa. The secondary constrictions were also stained densely with Giemsa although they did not show bright fluorescence. At 60 min exposure, most chromosomes were surrounded by haloes of diffused chromatin and, therefore, looked ghost-like (fig. 1, d). This phenomenon clearly indicates that chromosome materials are destroyed and extracted from the chromosomes during exposure. After 90 min exposure, most chromosomes were conspicuously bleached but retained their delineated contours and centromeric spots although the latter were sometimes lost on a few chromosomes (fig. 1, e and f). The centromeric spots were apparently located on each chromatid (fig. 2). They seem to be the kinetochores or probably to contain them, at least in part, since most paired spots were situated on the outside of the chromosomes.

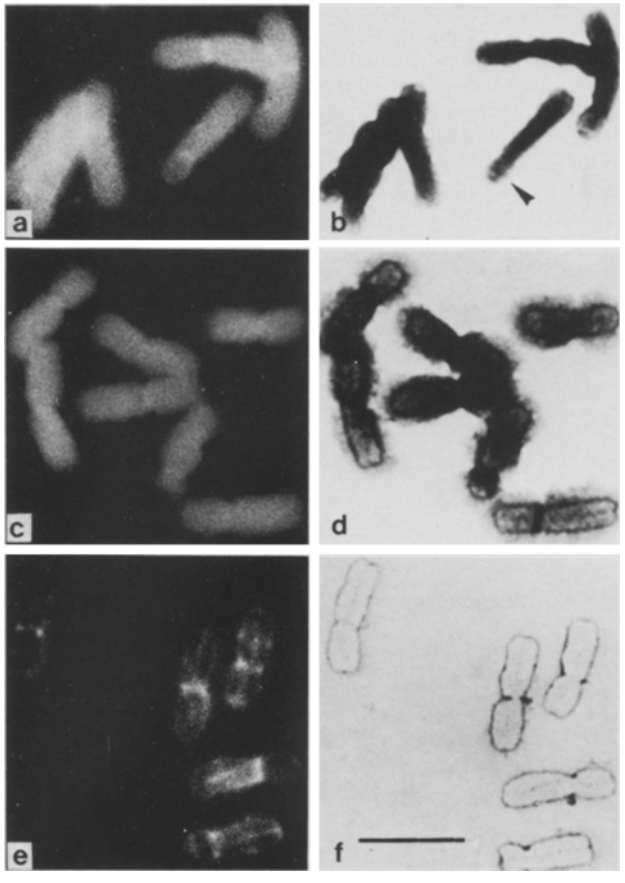

Figure 1. Effects of UV-light exposure on *Allium cepa* chromosomes, illustrated by a comparison of Hoechst 33258 fluorescence (left) and Giemsa stainability (right), sequentially obtained on the same chromosomes. *a* and *b* 30 min exposure; *c* and *d* 60 min exposure. Note that the chromatin is dispersing out from the chromosomes. *e* and *f* 90 min exposure. The chromosomes are remarkably bleached. Note the paired spots on the chromosomes. Arrow indicates the secondary constriction. Bar 10 µm.

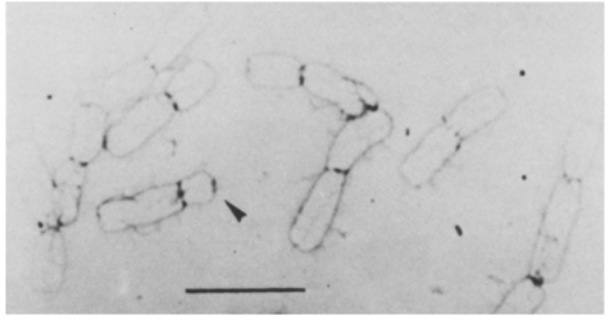

Figure 2. Metaphase chromosomes after 75 min exposure to UV-light in the presence of Hoechst 33258, followed by Giemsa staining. Note the paired spots and secondary constriction (arrow). Bar 10 µm.

No extraction occurred on UV-light exposed chromosomes in the absence of Hoechst 33258. The pH of the buffer proved to affect chromosome extraction considerably (table). Hoechst 33258 fluorescence of the chromosomes was somewhat weaker at acidic pH than at alkaline pH. The alkaline pH may be important for tight binding of Hoechst 33258 to DNA. Therefore, an argument may be presented in favor of the interpretation that Hoechst 33258 is a sensitizer for UV-light and enhances photolysis of the chromosomes[6].

The contour-delineated chromosomes produced by the present method are very similar to those obtained by treatment with chemical reagents[9] and by prolonged exposure to visible light in the presence of dilute methylene blue[10]. It is suggested that chromosome delineation, caused by these techniques, also depends on the extraction of DNA from the chromosomes. However, neither kinetochore-like spots nor secondary constriction bands could be produced by these techniques. The silver staining technique has revealed that there are acidic proteins involved with the transcriptive activity of rDNA in the secondary constrictions[11–14]. The results obtained by the EDTA method suggest that non-DNA materials, which may be, at least in part, basic proteins and ribonucleoproteins, are part of the constitution of the kinetochore[15–17]. Accordingly, the centromeric spots and secondary constrictions may show a reduced binding of the DNA-specific Hoechst 33258. This may be responsible for their strong resistance to UV-light exposure.

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