branes[4] with the radical-initiated lesion of hepatocellular membranes in mammals by tetrachloromethane indicate that irrespective of the mode of generation of the primary radicals the secondary reactions may be very similar, i.e. cross-linking and oligomerization of unsaturated fatty acids and direct and indirect effects on vital membrane functions[24].

These observations also suggest that uptake and photodegradation in the phytosphere contribute strongly to the elimination of anthropogenic chlorocarbons from the environment.

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Photodynamic effect of the porphyrin derivative meso-tetra (4-N-methylpyridyl) porphine on sister chromatid exchanges in meristematic cells

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Summary. The porphyrin derivative meso-tetra (4-N-methyl-pyridyl) porphine tetraiodide (TMpyP) was tested for its ability to induce sister chromatid exchanges (SCE) in *Allium cepa* roots. A significant increase in the SCE frequency was observed when BrdU-substituted chromosomes were treated with TMpyP during the G_1 period of the second-division cycle and also when treatment with TMpyP was followed by blue light irradiation.

Key words. Allium cepa chromosomes; meso-tetra (4-N-methylpyridyl) porphine; sister chromatid exchanges; photodynamic effect.

Sister chromatid exchanges (SCE) have been shown to be sensitive indicators of the effects of chemical mutagens and carcinogens on eucaryotic chromosomes[1-3]. It is known that certain drugs and dyes (e.g. psoralens[4-7], mitomycin C[8], pyronin Y[9]) enhance their ability as inducers of SCE when treatments are followed by irradiation with a suitable light. Porphyrin derivatives are photosensitizing agents[10], and several authors have proved that treatment with hematoporphyrin or a hematoporphyrin derivative, followed by various irradiation treatments, increases the SCE frequency in animal cells[11,12]. The aim of this paper was to determine for the first time the combined effect of a basic porphyrin derivative and light in an in vivo plant system. The synthetic porphyrin derivative meso-tetra (4-N-methylpyridyl) porphine tetraiodide (TMpyP) (fig. 1) was chosen because it has been identified as a photosensitizing agent[13] and DNA intercalator[14-17].

Material and methods. Root meristems of *Allium cepa* bulbs (15–30 g in weight) were grown in the dark at a constant temperature of 25 ± 0.5 °C in tap water (renewed every 24 h) and aerated by continuous bubbling at a rate of 10–20 cm^3/min. BrdU-substitution of DNA was achieved throughout the first 20 h in 10^{-4} M 5-bromo-2′-deoxyuridine (BrdU) and 10^{-7} M 5-fluor-2′-deoxyuridine, after which the bulbs were placed in 10^{-4} M unlabeled thymidine (dT) for an equal period. Treatments with 5×10^{-8} M TMpyP (Ventron) were performed in the presence of dT throughout the G_1 period of the second-division cycle (4 h). The duration of interphase periods corresponding to the cycle time was estimated according to González-Fernández et al.[18]. The treatment with TMpyP throughout the G_1 period alone was performed in order to study the effect of a postirradiation at the beginning of the S period, when cells are highly sensitive[9].

The irradiation was applied for 30 min at the end of the G_1 period (4 h after the start of dT treatments). The growing roots were placed in glass receptacles containing tap water at a mean distance of 5 cm from the light source. A 60 W incandescent blue

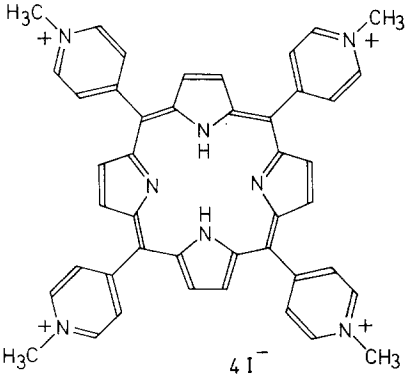

Figure 1. Chemical structure of meso-tetra (4-N-methylpyridyl) porphine tetraiodide (TMpyP).

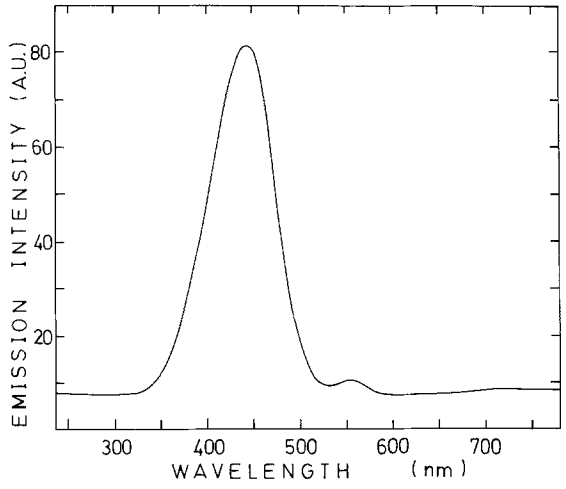

Figure 2. Spectral characteristics of emission showed by the blue glass lamp used in this study. The emission peak corresponds to a wavelength of 445 nm.

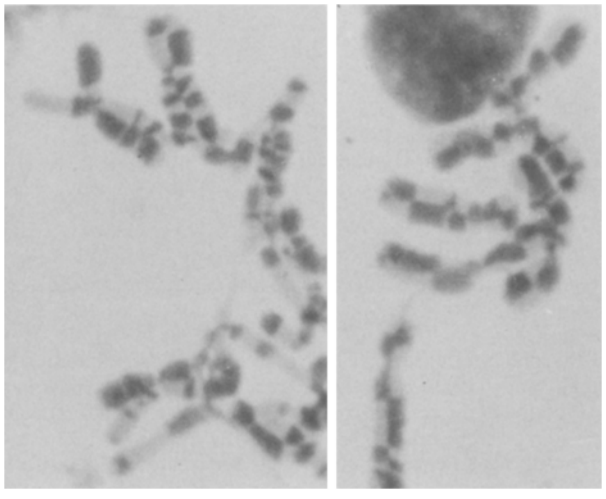

Figure 3. *Allium cepa* chromosomes stained by the FPG technique after the incorporation of BrdU throughout the first cycle and a treatment with TMpyP 5×10^{-8} M during the second G_1 period followed by 30 min irradiation with blue light at the end of the drug incorporation.

glass lamp with an intensity of 0.2 mW/cm^2 (Spectra Physics 404) was used. The spectral characteristics of the blue lamp were determined using a Perkin-Elmer 650-10 S fluorescence spectrophotometer[9] and the lamp as the light source (fig. 2). Finally, roots were treated with 0.05% colchicine for 3 h and their tips were fixed overnight in ethanol-acetic acid (3:1) at 4 °C. After fixation, roots were processed by a modified fluorescence-plus-Giemsa (FPG) technique in which 33258 Hoechst is replaced by eosin Y[19].

Results and discussion. Photochemical effects of several porphyrins[10,20] involve the production of an excited and highly reactive (cytotoxic) state of molecular oxygen[21], which induces DNA breaks[22,23]. The photosensitizing agent TMpyP binds to DNA by an intercalative mechanism[14–17], and it is known that most intercalating agents are potent SCE inducers. As far as we know there are no references about the induction of sister chromatid exchanges in any eukaryotic system by TMpyP alone or combined with light. The number of SCE increased the closer the irradiation time was to the beginning of the S phase[25]. This relationship between the increased SCE yield and the S period has also been reported using other agents[7,9,26].

Frequency of SCE in controls and after treatments with TMpyP and blue light irradiation

Treatments on BrdU-substituted chromosomes	Dose (M)	SCE/chromosome[a,b]
Control	–	2.81 ± 0.17
TMpyP	5×10^{-8}	4.02 ± 0.22
Irradiation	–	3.04 ± 0.20
TMpyP + Irradiation	5×10^{-8}	4.57 ± 0.24

[a] Mean ± SE; [b] 300 chromosomes were studied in each case.

The table summarizes the effect of TMpyP and the influence of the irradiation with blue light on the frequency of SCE in BrdU-substituted chromosomes. A significant increase of SCE ($p < 0.01$, Student's t-test) compared with controls was observed when meristematic cells were exposed to TMpyP during the second G_1 period. SCE values were even higher when TMpyP treated roots were subjected to irradiation with blue light (fig. 3). The excitation wavelength (emission maximum at 445 nm, fig. 2) was chosen because of its proximity to the characteristic Soret band (425 nm) of the porphyrin derivatives. An increase in the SCE frequency has been reported in mammalian cells using other porphyrins (hematoporphyrin or hematoporphyrin derivative) in combination with light at different wavelengths[11,12]. Experiments with DNA in vitro[23] indicate the occurrence of single and double strand breaks provoked by TMpyP. Taking into account our results, TMpyP is able to induce a DNA damage in vivo, which is greater when the treatment with this porphyrin derivative is followed by blue light irradiation at low intensity.

In view of the general assumption that photodynamic carcinogenesis results from biologically significant damage to DNA, and that TMpyP appears as a genotoxic agent in our plant system, it should be an interesting compound in the research on photoradiation therapy of tumors[27]. Further studies to analyze more precisely the photodynamic action induced by this basic porphyrin derivative are under way.

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Numerical changes of nucleolar organiser regions and nucleolar activity in *Lathyrus*

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Summary. The transcriptional activity of the nucleolar organiser regions was compared in 3 *Lathyrus* species having different numbers of secondary constrictions in their complements. The 3 species differed significantly. It is also shown that the distribution of ribosomal RNA gene copies varied between pairs of satellited chromosomes within a complement.
Key words. NOR; nucleolar activity; ribosomal DNA; chromosomal distribution; *Lathyrus*.

The nucleoli which appear in the interphase and prophase nuclei of eukaryotic cells are rich in RNA and are formed by one or more specific regions in the haploid complement called nucleolar organisers. The nucleolar organiser regions (NORs) are well differentiated regions of chromatin DNA which transcribes 5.8S, 18S and 28S ribosomal RNA sequences. At metaphase the nucleoli disappear and the NORs appear as constrictions (secondary constrictions) at the distal end of metaphase chromosomes. The NOR is highly variable in number and location among species but within a complement it is relatively constant[1]. The genes for 5.8S, 18S and 28S rRNA occur in the form of tandemly repeated units separated by transcribed spacer regions[2]. While the rRNA genes are highly conserved among higher organisms the spacer sequences may be heterogeneous within a species and highly diverged among related species.

A survey among *Lathyrus* species has shown large variation in the number of secondary constrictions in the cromosome complements. The number ranged from one pair to six pairs among the satellited complements even though all species investigated were diploids with the same chromosome number, 2n = 14. The aim of this investigation was to find out a) whether the numerical changes of secondary constrictions has any significant effect upon the nucleolar activity at interphase and prophase and b) whether the distribution of ribosomal RNA gene copies varies among satellited chromosomes within a complement.

Materials and methods. *L. articulatus* L. and *L. tingitanus* L., were from the Aberystwyth collection. *L. pisiformis* L. was supplied by Hortus Botanicus, Sweden.

1) Silver staining of interphase nuclei. The procedure for staining interphase nuclei was essentially the same as described by Bloom and Goodpasture[3]. Cytological preparations were made from the meristematic tissue dissected out from root tips which were fixed in 1:3 acetic alcohol. Squash preparations were stored in absolute ethanol at −20 °C until use. Slides were air dried and 2–3 drops of freshly prepared silver nitrate solution (75%, w/v) was applied to each slide. The coverslips were sealed with rubber solution and incubated in a moist black box at 50 °C for 24 h. The slides were rinsed in distilled water and treated with 3% (v/v) formaldehyde for 20–40 min. The slides were rinsed briefly first in distilled water and then in 0.04 M phosphate buffer (equimolar mixture of Na_2HPO_4 and NaH_2PO_4, pH 6.8) and stained in 1% Giemsa solution (Gurr's improved, R66). After differentiating in 0.04 M phosphate buffer the slides were made permanent by mounting in euparal.

2) Silver staining of metaphase chromosomes. The method of Howel and Black[4] was used for staining metaphase chromosomes. Two stock solutions of reagents were made and stored in dark bottles in a refrigerator. They were: a) a solution containing 1% (w/v) gelatin and 1% (v/v) formic acid in distilled water, b) 50% (w/v) silver nitrate made in 0.04% (v/v) formaldehyde. Metaphase chromosome preparations were made from meristematic tissue of root tips as described above. Two drops of solution (a) and 4 drops of (b) were placed on each slide. The slides were warmed for 3–5 sec on a hot plate maintained at 50 °C. The slides were rinsed in deionised distilled water and transferred to 'photo fix' (1:3 dilution of Kodak fixative) for 1 min. The slides after washing in distilled water were air dried and mounted in euparal. The chromosomes were observed under oil immersion using a phase contrast microscope.

3) Measurement of 2C nuclear DNA amount and chromosome volume. The methods have already been reported[5]. 2C nuclei in root meristems quantitatively stained with Feulgen were measured in a Vickers M85 microdensitometer. The 2C DNA values are the means of at least 3 replications. *Allium cepa* (2C = 33.5 pg) was used as a standard to convert DNA estimates to absolute amounts (pg). At least 4 Feulgen-stained full metaphase plates at c-mitosis were used for measuring chromosome volume and the mean values are given in the text.

4) C-banding of metaphase chromosomes. The method suggested by Vosa[6] was used in this investigation.

5) In situ hybridisation. The cloned *Pisum sativum* ribosomal DNA probe used in this experiment was a gift from Dr Jan-Peter Nap, Department of Molecular Biology, Agricultural University, Wageningen, The Netherlands. The procedure for the transcription of complementary RNA (cRNA) and in situ hybridisation has already been reported[7]. H^3-labeled ATP (32 Ci/mMol) and UTP (46 Ci/mMol) were purchased from Amersham Radiochemicals, UK. *E. coli* RNA polymerase was from Sigma Ltd. The unlabeled nucleotides were from Boehringer Mannheim Ltd. The H^3-labeled cRNA had a specific activity of 10^8 cpm/µg. It was hybridised in situ to the chromosomal DNA using the method of Dennis et al.[8]. 8 µl of cRNA (100,000 cpm) in 3 × SSC and 50% formamide was placed under a coverslip on each slide. It was sealed with rubber solution. The chromosomal DNA was denatured by dipping the slides for 3 min in a water bath maintained at 75 °C. The slides were then incubated for 24 h at 37 °C. After removing the coverslips the excess cRNA was removed by extensive washing in several changes of SSC (0.15 M NaCl+0.015 M Na citrate pH 7). Non-specifically bound cRNA