# Localization of amplified DNA in nuclei of the orchid Cymbidium by in situ hybridization

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*Summary.* In situ hybridization of $^3$H-polyuridylic acid of low specific activity to somatic nuclei of Cymbidium protocorms is suggested to indicate the location of the highly amplified AT-rich DNA fraction.

The nuclear DNA of the orchid genus Cymbidium is unique among monocots in showing an AT-rich satellite in neutral CsCl density gradients[1]. In situ hybridization of radioactive satellite DNA and complementary RNA to nuclei, as well as staining of nuclei with AT-specific fluorochromes, showed that the AT-rich DNA is located within the heterochromatin (chromocenters)[2,3].

The nuclei of certain cells of protocorms and roots of Cymbidium show extra replication (amplification) of portions of the heterochromatin during cytodifferentiation. This was demonstrated by cytophotometry, autoradiography, and analysis of derivative melting profiles of the DNA[4–6]. On the basis of CsCl ultracentrifugation and quantitative light and electron microscopy, it has been suggested that not the satellite DNA, but a DNA fraction which is located within the superficial region of the chromocenters becomes amplified, while the central, very AT-rich DNA does not[1,3]. To prove this suggestion the following experiment was devised.

*Material and methods.* As the nuclear DNA of Cymbidium can be fractionated into a number of relatively AT-rich fractions[6], it was necessary to choose the appropriate conditions for this experiment. The appearance of silver grains in in situ hybridization slides depends on both the specific activity of the radioactive nucleic acid and the degree of repetitiveness of the complementary DNA sequence in the nuclei[7,8]. As the number of copies of the amplified DNA sequence in Cymbidium must be several orders higher than the non-amplified DNA[4], labelling of the amplified DNA should occur under conditions which do not yield silver grains of the non-amplified DNA. Therefore, $^3$H-polyuridylic acid of relatively low specific activity (50 Ci/mM) was hybridized in situ to the denatured DNA of mechanically isolated nuclei and chromocenters of the Cymbidium hybrid 'In memoriam Cyrill Strauss', which was aseptically cultured as described earlier[5]. Hybridization was performed in $2 \times$ SSC[14] with 1 μCi/ml of $^3$H-poly(U)[9] at 36 °C for 2 h[9]. The slides were covered with Ilford L4 emulsion diluted 1:1 with aqua distillata and exposed for 28 days.

*Results.* The figure shows the result of the experiment. Most of the label is found on the surface of the chromocenters, or around them. This labelling pattern was, however, only found in nuclei which apparently belong to the DNA amplifying population[4], while meristematic and non-amplifying nuclei were always unlabelled. These findings strongly suggest that hybridization occurred preferentially between poly(U) and the amplified DNA of the nuclei.

*Discussion.* The regular appearance of silver grains in the surroundings of the chromocenters cannot be explained by inappropriate resolving power of the light microscope autoradiographs, because satellite DNA and complementary RNA always yield label over the central area of the chromocenters[2]. I prefer to believe that the present findings indicate the release of amplified DNA from the chromocenters. This suggestion is confirmed by pulse-chase experiments employing $^3$H-thymidine, which showed that the extra replicated DNA copies are released from the nuclei after some time (W. Nagl, unpublished). The results of the present hybridization experiments are consistent with earlier suggestions that 1) the chromocenters of Cymbidium nuclei are composed of various types of heterochromatin[3], 2) the amplified DNA of this

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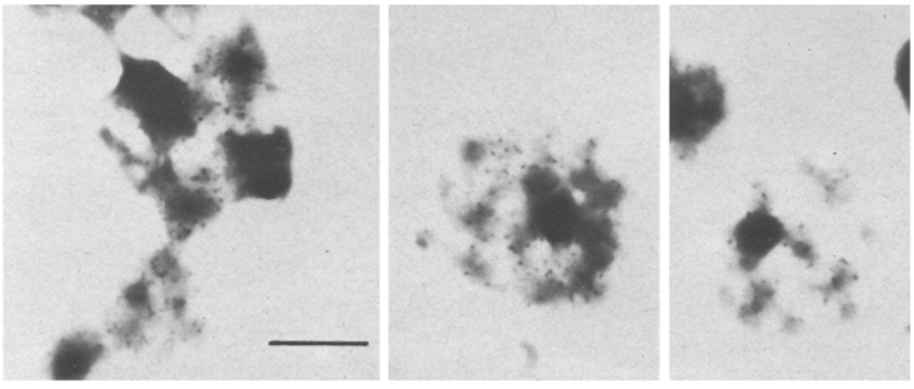

Autoradiographs of mechanically isolated nuclei and chromocenters of Cymbidium; hybridization with $^3$H-polyuridylic acid; toluidine blue. Note the preferential location of silver grains in the periphery of the chromocenters. The bar indicates 10 μm. As the distribution of silver grains can hardly be seen in the figures, original prints will be provided on request.

genus is AT-rich[6], 3) it is located peripherically in the chromocenters[6], and 4) is not completely identical with the satellite DNA fraction[6]. The satellite DNA is composed of 2 components, which are reiterated respectively $2.2 \times 10^5$ and $1.4 \times 10^5$ times[10]. This is, with respect to the conditions of hybridization not enough to yield the silver grain number as shown in the figure. In addition, these reiteration values may represent an over-estimation, because they were obtained through analysis of the DNA extracted from nuclei regardless of their content of amplified DNA. Without knowing the genome organization in Cymbidium, it is difficult to say what kind of DNA is amplified. At present we know that it is a AT-rich fraction and probably a repetitive sequence (because of its location in heterochromatin).

The functional significance of the amplification of AT-rich DNA in somatic nuclei of Cymbidium is not yet clear. With respect to a hypothesis put forward earlier[11], it may be argued that this 'lateral reiteration' must substitute for too low 'tandem reiteration' during the evolution of non-coding DNA, which is probably involved in the control of gene activity and cytodifferentiation[12]. A similar interpretation has recently been given for the amplification of intermediately repetitive DNA in chicken cartilage cell differentiation[13], and is very likely to apply to the situation in Cymbidium, as phytohormone-controlled changes in the amplification process are accompanied by significant changes in morphogenesis[5].

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14 Abbreviations used: poly (U) = polyuridylic acid; SSC = 0.15 M NaCl + 0.015 M sodium citrate.

## Effects of $\gamma$-rays on DNA-cholesterol complex

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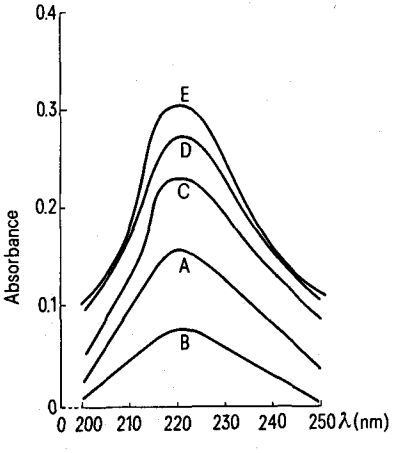
*Summary.* Effects of $\gamma$-rays on the DNA-cholesterol complex have been studied. Radiation-induced changes are found and compared with those on DNA-dye or drug complexes.

The effects or $\gamma$-rays on the complexes of DNA with multiferious dyes and drugs[2,3] have been studied. It has been known that cholesterol, which is a lipid, is complexed with DNA. Moreover steroid hormone[4] binds to cytoplasmic receptor proteins. To investigate the effects on the complex with an agent (with probable different binding mechanism), dissimilar to that of earlier studies[2,3], the effects of $\gamma$-rays on the DNA-cholesterol complex, formed under varied conditions have been studied spectrophotometrically.

*Materials and methods.* Highly polymerized calf thymus DNA (Sigma), as sodium salt, cholesterol (Merck) and for solvent 0.01 M Tris-HCl buffer at pH-7.4[5], found free from imparting radiation effects were used, unless otherwise specified. Utilizing the property of slight[6] solubility of cholesterol in water, solutions in water and by the same method in Tris-HCl buffer were made: $\times 5$ mg of cholesterol was stirred with 100 ml of water or buffer and heated for 10 min on water-bath. Part of the sample did not dissolve and was filtered off. For verification, part of the filtrate was evaporated to dryness on water-bath; the residues were dissolved in chloroform, and presence of cholesterol was confirmed by Lieberman-Burchard reaction. Cholesterol solution of arbitrary concentration, having OD (stated later), was thus prepared in buffer to use as parent solution. The spectrum of the parent solution, mixed with equal volume of the solvent, served as reference, and that with equal volumes of DNA and the parent solutions as control. All measurements, including control, were carried out between 200 and 250 nm, using the concentration of reference. For test samples, equal volumes of $1.5 \times 10^{-5}$ M, $3 \times 10^{-5}$ M, $6 \times 10^{-5}$ M, $9 \times 10^{-5}$ M, $12 \times 10^{-5}$ M and $18 \times 10^{-5}$ M DNA and parent solutions were mixed. The complexes formed after incubation at room temperature for 30 min were irradiated to different doses, in $CS^{137}$ source at a dose rate of 245 R/min; for the slow oxidation[7] of cholesterol, in presence of air and light,




Effect of $\gamma$-rays on DNA-cholesterol Complex. A-cholesterol; B-reference; C – $9 \times 10^{-5}$ M DNA + cholesterol; D-solution of C + 9000 R. E – Solution of C + 15,000 R.

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