mixed with the fluffy part of the sediment, although the initial dispersion of chromatin occurred. These observations suggest that the decondensation of sperm nuclei involves a rapid dispersion of chromatin by soluble cytoplasmic components, but formation of a nuclear envelope and swelling of the nuclei requires the presence of cytoplasmic vesicles.

Thus cell-free preparations of amphibian egg cytoplasm induce nuclear envelope formation, nuclear decondensation, initiation of DNA synthesis, and chromosome condensation in lysolecithin-treated sperm. These changes in nuclear morphology and activity, observed in vitro may represent the behavior of the sperm nucleus during fertilization and the mitotic cycle in the early amphibian embryo. Therefore, the biochemical basis for the cytoplasmic control of nuclear behavior may be examined with this cell-free system.

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References and Notes

1. F. J. Longo, *Biol. Reprod.* **9**, 149 (1973); F. J. Longo and M. Kunkle, *Curr. Top. Dev. Biol.* **12**, 149 (1978).
2. S. Subtelny and C. J. Bradt, *J. Morphol.* **112**, 45 (1963).
3. C. J. Graham, *J. Cell Sci.* **1**, 363 (1966); C. J. Graham, K. Arms, J. Gurdon, *Dev. Biol.* **14**, 349 (1966); M. N. Skoblina, *Ontogenez* **5**, 334 (1974); C. Katagiri and M. Moriya, *Dev. Biol.* **50**, 235 (1976).
4. J. M. Barry and R. W. Merriam, *Exp. Cell Res.* **71**, 90 (1972).
5. R. M. Benbow and C. C. Ford, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2437 (1975).
6. M. Kunkle, B. E. Magun, F. J. Longo, *J. Exp. Zool.* **203**, 381 (1978).
7. Testes were dissected from sexually mature *X. laevis* males 1 hour after injection of 100 I.U. of human chorionic gonadotropin (hCG), washed, and incubated overnight at 18°C in Ringer solution containing antibiotics and hCG (10 I.U./ml). Sperm, released by gently squeezing the testes, were centrifuged, treated for 5 minutes at 22°C with nuclear isolation medium (NIM) [D. H. Ziegler and Y. Masui, *Dev. Biol.* **35**, 283 (1973)] containing 0.05 percent lysolecithin and soybean trypsin inhibitor (1 μg/ml), and washed once in NIM and 3 percent bovine serum albumin (BSA) and three times with NIM and 0.4 percent BSA. For some experiments lysolecithin-treated sperm were stored at −80°C in 30 percent (by volume) glycerol in NIM. Lysolecithin-treated sperm were washed thoroughly with buffer (see below) before use.
8. *Rana pipiens* eggs from which the jelly was removed enzymatically were placed in Ringer solution and activated by electric shock (80 V for 200 msec), incubated in 10 percent Ringer solution at 19° ± 1°C for 1 hour and washed in an ice-cold buffer consisting of 250 m*M* sucrose, 200 m*M* KCl, 1.5 m*M* $MgCl_2$, 2.0 m*M* β-mercaptoethanol, and 10 m*M* tris-HCl at *p*H 7.5. Eggs were transferred to 5-ml centrifuge tubes containing buffer on ice and crushed by centrifugation at 9000*g* for 15 minutes. The band of heavier supernatant above the packed pigment and yolk was transferred to a 0.6-ml centrifuge tube and centrifuged at 9000*g* for 15 or 30 minutes to remove most of the pigment. Lysolecithin-treated sperm were incubated at 18°C in 200 μl of this supernatant to give a concentration of 5×10^4 to 1×10^5 sperm per milliliter.
9. The cytoplasmic preparation (100 μl) was mixed with 100 μl of 33 percent buffer containing [^{3}H]dTTP (44 Ci/mmole; Amersham) at a concentration of 80 μCi/ml. In some experiments, aphidicolin [from a stock solution dissolved at 5 mg/ml in dimethyl sulfoxide (DMSO)] was added to the 33 percent buffer to obtain a concentration of 10 μg/ml. Addition of DMSO alone had no effect on incorporation. Squash preparations were washed with cold 5 percent trichloroacetic acid, coated with Kodak NTB2 emulsion, and exposed for 17 days at 4°C.
10. S. Ikegami, T. Taguchi, M. Ohashi, M. Oguro, H. Nagano, Y. Mano, *Nature (London)* **275**, 458 (1978).
11. M. J. Lohka and Y. Masui, unpublished results.
12. T. Hultin, *Experientia* **17**, 410 (1961); E. B. Wagenaar and D. Mazia, in ICN-UCLA Symposium on Molecular and Cellular Biology: Cell Reproduction, E. R. Dirksen and D. Prescott, Eds. (Academic, New York, 1978), pp. 539–545.
13. Portions of the incubation mixture were fixed overnight on ice in 2 percent glutaraldehyde in 0.05*M* phosphate buffer at *p*H 7.4, washed three times with the same buffer, postfixed in 1 percent osmium tetroxide for 2 hours at room temperature, dehydrated through a graded series of ethanol concentrations to propylene oxide, and embedded in Epon 812.
14. M. J. Lohka and Y. Masui, in preparation.
15. Portions of the incubation mixture were fixed in a mixture of ethanol and acetic acid (3:1) and stained by the Feulgen procedure. Squash preparations [D. H. Ziegler, P. G. Meyerhof, Y. Masui, *J. Ster. Biochem.* **11**, 715 (1979)] were stained with 2 percent Giemsa.
16. We thank J. Rossant for the gift of aphidicolin; R. Valladiego for sectioning material for electron microscopy; and H. Clarke, M. Miller, and E. Shibuya for encouragement and helpful discussions. Supported by a grant from the Natural Science and Engineering Research Council of Canada (to Y.M.).

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Ectopic Pro-Opiolipomelanocortin: Sequence of cDNA Coding for β-Melanocyte–Stimulating Hormone and β-Endorphin

Abstract. *A recombinant bacterial plasmid, pMS1, was constructed that contains 318 nucleotides complementary to a portion of pro-opiolipomelanocortin (proOLMC) messenger RNA from an ectopic adrenocorticotropin-producing tumor. The cloned complementary DNA insert, which contains the sequence that codes for all of the β-melanocyte–stimulating hormone and β-endorphin portions of proOLMC, as well as the 3′ nontranslated section, is identical to the genomic sequence. Hybridization of tumor proOLMC complementary DNA to RNA subjected to electrophoresis and transferred to a nitrocellulose filter revealed two proOLMC messenger RNA species in the tumor polyadenylated RNA, but only one in pituitary polyadenylated RNA. At least one of the tumor proOLMC messenger RNA's is similar, if not identical, to human pituitary proOLMC messenger RNA.*

The syndrome later to be associated with nonpituitary, or "ectopic," secretion of adrenocorticotropin (ACTH) was reported by Brown in 1928 (*1*) and was the first of the ectopic humoral syndromes to be described. The ectopic ACTH syndrome was also the first to be satisfactorily explained, with the discovery of ACTH-like biologic activity in the plasma and tumor tissue of patients with this disorder (*2–5*). Since that time, ectopic production of a variety of peptide hormones has been reported [see (*6*) for review].

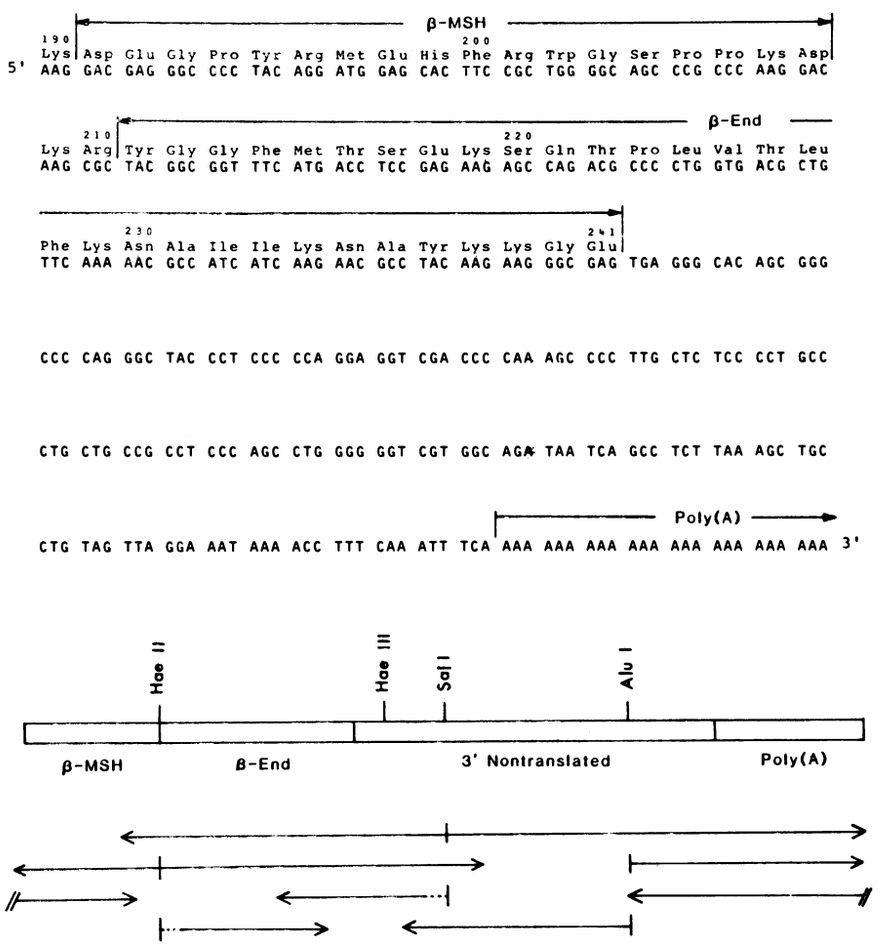
Extensive studies of ectopically produced hormones indicate that they are similar if not identical to the corresponding eutopic hormones, but the amino acid sequence of an ectopic hormone has yet to be determined and compared with that of the eutopic molecule. The nearest approximation was based on amino acid composition and suggested that an ACTH-like peptide lacking the COOH-terminal and NH_2-terminal amino acids was the major product of a thymic tumor (*7*). Ectopic ACTH-producing tumors also produce peptides similar to β-lipotropin (β-LPH), γ-LPH, β-endorphin (β-End), and γ-melanocyte–stimulating hormone (γ-MSH) (*8–10*), suggesting that they arise from a common precursor, pro-opiolipomelanocortin (proOLMC), as they do in the pituitary gland (*11, 12*). RNA from an ACTH-producing tumor was shown to direct the synthesis, in a cell-free translation system, of a large immunoreactive ACTH protein with a size similar to that of the pituitary proOLMC precursor protein (*13*). Hybridization of bovine pituitary proOLMC complementary DNA (cDNA) to tumor RNA revealed two species of messenger RNA (mRNA): a predominant species that appeared to be similar in size to the single species of human pituitary proOLMC mRNA detected, and a minor species of higher molecular weight (*13*).

The RNA used in our experiments was isolated from a malignant ACTH-producing carcinoid tumor removed from the pancreas but thought to be a metastasis from a preexisting thymic carcinoid tumor causing Cushing's syndrome in a 64-year-old man. The tumor was thinly sliced shortly after removal, frozen immediately, and stored at −56°C until extraction. RNA was extracted by the sodium dodecyl sulfate–phenol procedure (*14*), and polyadenylated [poly(A)] RNA was isolated by oligodeoxythymidylate-cellulose chromatography (*15*); 273 μg of poly(A) RNA was prepared from 10.8 g of tumor tissue. From 100 μg of tumor poly(A) RNA, 595 ng of S1 nuclease–treated double-stranded cDNA

was prepared by the procedure of Norgard *et al.* (*16*) and inserted into the Pst I site of plasmid pBR322 by the G-C (G, guanine; C, cytosine) tailing method. The recombinant plasmids were used to transform *Escherichia coli* strain RR-1. One colony of 474 transformants hybridized (*17*) with a 144–base pair (bp) cDNA probe that is complementary to the β-MSH and β-End sequences of AtT20/D16v mouse pituitary tumor cell line proOLMC mRNA (*18*). The probe was prepared by separating Hind III–cleaved fragments of plasmid pBRmel50 that had been labeled at the 5′ end with adenosine [γ-^{32}P]triphosphate and polynucleotide kinase (*19*), on a 5 percent acrylamide gel. A plasmid, pMS1, that contained a 424-bp insert was isolated from the colony. The insert was sequenced with fragments generated by digests with Alu I, Hinc II, Hae III, Pvu I, or Sal I, 5′-end labeling, and the method of Maxam and Gilbert (*19*). The 424-bp cDNA insert contained a 156-bp segment coding for all of β-MSH and β-End regions of proOLMC, a 162-bp segment corresponding to the 3′-nontranslated section, a poly(A) region of 72 nucleotides, and 5′ and 3′ ends consisting of poly(G) and poly(C) tails of 18 and 16 nucleotides, respectively (Fig. 1). Both strands were completely sequenced, and the sequence at all labeling sites was read through on another fragment.

The predicted amino acid sequence of the translated portion is the same as the corresponding pituitary peptide sequence (*20, 21*), and the entire nucleotide sequence is identical to previously published sequences for human genomic proOLMC from placenta (*22*) and fetal liver (*23, 24*). Therefore, this report, which presents the nucleotide sequence of an mRNA that directs the synthesis of an ectopic hormone, demonstrates that a major portion of ectopic proOLMC is identical to the eutopic molecule.

A cDNA clone derived from human proOLMC mRNA has not been reported previously. The sequence of pMS1 indicates that the site of poly(A) attachment is 162 bases from the 3′ end of the translated portion. Compared to bovine proOLMC cDNA, with which there is moderate homology in this region, the attachment site is lacking the two terminal nucleotides (*25*). This different attachment site may be of significance or may simply represent one of several



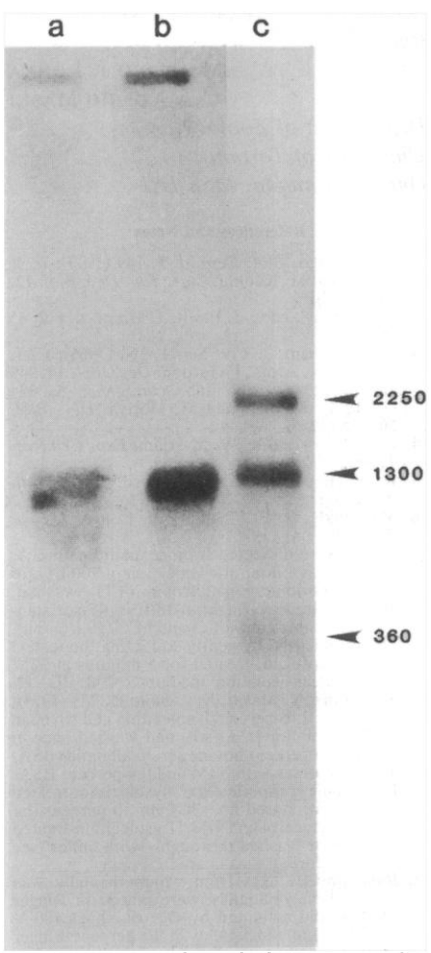


Fig. 1 (left). Nucleotide sequences of 318 bases of the 3′ portion of human ectopic proOLMC cDNA. The sequence shown is that corresponding to the mRNA. The portions coding for β-MSH and β-End are indicated. The direction and extent of sequence determinations are shown by horizontal arrows. The sites of 5′-end labeling are indicated by short vertical lines. The slash marks at the end of arrows indicate that the site of 5′-end labeling was on the vector. The broken segments on two of the arrows indicate segments for which the sequences were not determined. Fig. 2 (right). Autoradiogram of blot hybridization of poly(A) RNA. Pituitary poly(A) RNA (35 μg) was prepared from 1.76 g of human pituitary tissue by the procedure used for the tumor poly(A) RNA. (Lane a) 5 μg of tumor poly(A) RNA, (lane b) 5 μg of human pituitary poly(A) RNA, and (lane c) 1 μg of Hae II–digested plasmid Col E1 were denatured with 1*M* glyoxal and 50 percent dimethylsulfoxide in 10 m*M* Na_2HPO_4, *p*H 7.0, at 50°C for 1 hour (*28*), subjected to electrophoresis on a 1 percent agarose gel in 10 m*M* Na_2HPO_4, *p*H 7.0, and transferred to a nitrocellulose filter (Schleicher & Schuell, PH79, 0.1 μm) that had been briefly heated to boiling in distilled water and then cooled in 3*M* NaCl and 0.3*M* sodium citrate at room temperature just before transfer. The hybridization probe, pMS1, was labeled by nick translation (*29*) with deoxycytosine [α-^{32}P]triphosphate to a specific activity of 2×10^7 cpm/μg. Hybridization was carried out at 42°C for 18 hours. The filter was transferred, prehybridized, hybridized, and washed (*30*). The filter was exposed for 46 hours at −70°C with XR-5 film and a DuPont Cronex Lightning-Plus intensifying screen; the marker lane was exposed for 3 hours.

poly(A) addition sites, as occur in bovine prolactin mRNA (*26*).

Glyoxal-treated poly(A) RNA's from the tumor and from human pituitary glands were separated on an agarose gel, transferred to a nitrocellulose filter, and hybridized with ^{32}P-labeled plasmid pMS1. Two hybridizing species of about 1150 and 1300 bases were identified in the tumor poly(A) RNA, but only one species was observed in the pituitary poly(A) RNA (Fig. 2), even after different exposure times. It was not possible from the results of our experiments to determine whether the pituitary pro-OLMC mRNA corresponds to either of the two tumor species because of their similarity in size. These results are similar to those of Tsukada *et al.* (*13*) and demonstrate that another ACTH-producing thymic carcinoid tumor contains two proOLMC mRNA species. The additional tumor mRNA may be the product of a different proOLMC gene, or it may be a different product of the same gene because of incomplete or altered processing.

It is uncertain which of the two tumor proOLMC species we have cloned and what, if any, is the difference between it and the pituitary proOLMC mRNA. However, since the cloned sequence—including the 3′ nontranslated region, which is highly variable among species (*25, 27*)—is the same as the known genomic sequence, it is probable that the rest of this ectopic proOLMC mRNA is similar, if not identical, to the pituitary proOLMC mRNA.

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References and Notes

1. W. H. Brown, *Lancet* **1928-II**, 1022 (1928).
2. C. Meador, D. Island, W. Nicholson, J. Nuckton, C. Lucas, J. Luetscher, G. Liddle, Abstract, in *Program of the 43rd Meeting* (Endocrine Society, Bethesda, Md., 1961), p. 162.
3. D. A. Holub and F. H. Katz, *Clin. Res.* **9**, 194 (Abstr.), (1961).
4. N. P. Christy, *Lancet* **1961-I**, 85 (1961).
5. C. K. Meador, G. W. Liddle, D. P. Island, W. E. Nicholson, C. P. Lucas, J. G. Nuckton, J. A. Luetscher, *J. Clin. Endocrinol. Metab.* **22**, 693 (1962).
6. D. N. Orth, in *Endocrinology and Metabolism*, P. Felig, J. Baxter, A. E. Broadus, L. A. Frohman, Eds. (McGraw-Hill, New York, 1981), p. 1191.
7. P. J. Lowry, L. H. Rees, S. Tomlin, G. Gillies, J. Landon, *J. Clin. Endocrinol. Metab.* **43**, 831 (1976).
8. D. N. Orth, R. Guillemin, N. Ling, W. E. Nicholson, *ibid.* **46**, 849 (1978).
9. X. Y. Bertagna, W. E. Nicholson, G. D. Sorenson, O. S. Pettengill, C. D. Mount, D. N. Orth, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5160 (1978).
10. X. Y. Bertagna, F. Girard, D. Seurin, J.-P. Luton, H. Bricaire, R. E. Mains, B. A. Eipper, *J. Clin. Endocrinol. Metab.* **51**, 182 (1980).
11. B. A. Eipper and R. E. Mains, *Endocr. Rev.* **1**, 1 (1980).
12. D. N. Orth and W. E. Nicholson, *Ann. N.Y. Acad. Sci.* **297**, 27 (1977).
13. T. Tsukada, Y. Nakai, H. Jingami, H. Imura, S. Taii, S. Nakanishi, S. Numa, *Biochem. Biophys. Res. Commun.* **98**, 535 (1981).
14. D. Shields and G. Blobel, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2059 (1977).
15. H. Aviv and P. Leder, *ibid.* **69**, 1408 (1972).
16. M. V. Norgard, M. J. Tocci, J. J. Monahan, *J. Biol. Chem.* **255**, 7665 (1980).
17. M. Grunstein and J. Wallis, *Meth. Enzymol.* **68**, 379 (1979).
18. J. L. Roberts, P. H. Seeburg, J. Shine, E. Herbert, J. D. Baxter, H. M. Goodman, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2153 (1979).
19. A. M. Maxam and W. Gilbert, *ibid.* **74**, 560 (1977).
20. C. H. Li and D. Chung, *Int. J. Peptide Protein Res.* **17**, 131 (1981).
21. K. L. Hsi, N. G. Seidah, C. L. Lu, M. Chrétien, *Biochem. Biophys. Res. Commun.* **103**, 1329 (1981).
22. H. Takahashi, Y. Teranishi, S. Nakanishi, S. Numa, *FEBS Lett.* **135**, 97 (1981).
23. A. C. Y. Chang, M. Cochet, S. N. Cohen, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4890 (1980).
24. M. Cochet, A. C. Y. Chang, S. N. Cohen, *Nature (London)* **297**, 335 (1982).
25. S. Nakanishi, A. Inoue, T. Kita, M. Nakamura, A. C. Y. Chang, S. N. Cohen, S. Numa, *ibid.* **278**, 423 (1979).
26. N. L. Sasavage, M. Smith, S. Gillam, R. P. Woychik, F. M. Rottman, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 223 (1982).
27. J. Drouin and H. M. Goodman, *Nature (London)* **288**, 610 (1980).
28. G. G. Carmichael and G. K. McMaster, *Methods Enzymol.* **65**, 380 (1980).
29. P. W. J. Rigby, M. Dieckmann, C. Rhodes, P. Berg, *J. Mol. Biol.* **113**, 237 (1977).
30. P. Thomas, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5201 (1980).
31. We thank C. Toker for the fresh tumor tissue and J. L. Roberts for plasmid pBRmel50. Supported in part by NIH research grants 5-R01-CA11685, 5-R25-CA19429, and 2-R01-GM25526 and by NIH research fellowship award 5-F32-CA06939 (to C.R.D.).

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Formation of an Adenine-Thymine Photoadduct in the Deoxydinucleoside Monophosphate d(TpA) and in DNA

Abstract. *A photoadduct is formed between the adenine (A) and thymine (T) bases of the deoxydinucleoside monophosphate d(TpA) when it is irradiated at 254 nanometers in aqueous solution. Treatment of the photoadduct with acid converts it specifically into a fluorescent hydrolysis product, $C_7H_7N_3O$, incorporating the position-8 carbon of adenine and the methyl group of thymine. Isolation of the fluorescent hydrolysis product from acid hydrolyzates of oligo- and polydeoxyribonucleotides has shown that the photoadduct is formed by ultraviolet irradiation of d(pTpA), d(TpApT), d(TpApTpA), poly(dA-dT), and both single- and double-stranded DNA.*

The deleterious biological effects caused by exposure of living cells to ultraviolet (UV) light originate mainly from photochemical modifications to the cellular DNA molecules. Extensive studies have shown that the pyrimidine bases are the major targets of photochemical damage, and several photoproducts derived from thymine and cytosine have been identified in UV-irradiated DNA (*1–3*). The most abundant of these are pyrimidine photodimers, which can have lethal consequences (*2*), and bipyrimidine (6–4) photoadducts, which appear to act as mutagenic lesions (*4*). In contrast, no substantive evidence has hitherto been obtained for the formation of any photoproducts derived from adenine (*5*) or guanine when DNA is irradiated in vitro or in vivo. Consequently, it is generally assumed that the purines have comparatively little significance in photobiology (*1, 3*). We describe the formation of an adenine-thymine photoadduct in the deoxydinucleoside monophosphate d(TpA) (T, thymine; A, adenine) and present definitive evidence to show that it is also formed on UV irradiation of native and denatured DNA. To our knowledge, this represents the first reported occurrence of a photoreaction

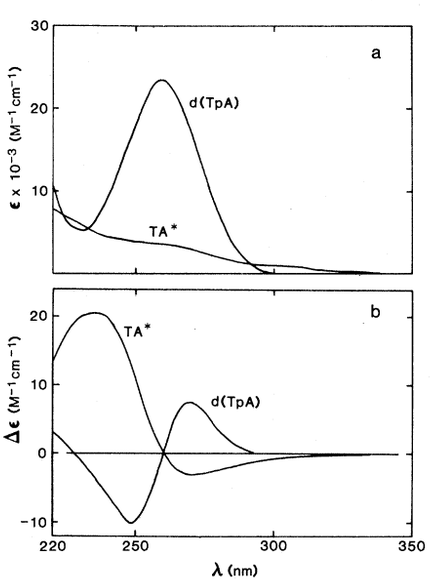

Fig. 1. Ultraviolet (a) and circular dichroism (b) spectra of d(TpA) and its photoproduct TA* at *p*H 7.0.