regulation of gene transcription involves a release of chromatin template restriction by the phosphorylation of some chromosomal protein; the phosphorylation appears to be mediated by a protein kinase which has been translocated to the medullary nucleus.

Only 20 percent of the ^{32}P incorporated into chromatin in our in vitro system could be extracted with $0.4N$ H_2SO_4, which indicates that most of the phosphorylation probably was in the nonhistone protein fraction. Purified acidic nuclear phosphoproteins isolated from a number of tissues bind to homologous DNA and enhance RNA synthesis in vitro (*17*). Thus, the augmented template activity observed in our system may have resulted from the phosphorylation of acidic chromatin proteins. The lack of quantitative correlation between the extent of RNA transcription and the degree of protein phosphorylation (Fig. 1) may suggest that phosphorylation of a specific substrate is involved in gene activation. Indeed, our recent data demonstrate a differential phosphorylation of acidic chromosomal proteins under the various conditions described in Fig. 1. It will be of interest to identify chromosomal proteins functioning as a physiological substrate for the catalytic subunits of protein kinase which are translocated during the transsynaptic induction of tyrosine 3-monooxygenase in rat adrenal medulla.

D. M. CHUANG
R. HOLLENBECK
E. COSTA

Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeths Hospital, Washington, D.C. 20032

References and Notes

1. *Acta Endocrinol. Suppl. No. 168* (1972).
2. J. Axelrod, *Science* **173**, 589 (1971).
3. A. Guidotti, A. Kurosawa, D. M. Chaung, E. Costa, *Proc. Natl. Acad. Sci.* **72**, 1152 (1975).
4. A. Kurosawa, A. Guidotti, E. Costa, *Mol. Pharmacol.* **12**, 420 (1976); E. Costa, A. Kurosawa, A. Guidotti, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1058 (1976); A. Kurosawa, A. Guidotti, E. Costa, *Science*, in press.
5. D. M. Chuang and E. Costa, *Adv. Biochem. Psychopharmacol.*, in press.
6. ———, *Mol. Pharmacol.* **12**, 514 (1976).
7. D. M. Chuang, G. Zsilla, E. Costa, *ibid.* **11**, 784 (1975).
8. T. C. Spelsberg and L. S. Hnilica, *Biochim. Biophys. Acta* **228**, 202 (1971).
9. J. L. Farber, G. Rovera, R. Baserga, *Biochem. Biophys. Res. Commun.* **49**, 558 (1972).
10. A. A. Keshgegian and J. J. Furth, *ibid.* **48**, 757 (1972).
11. M. J. Tasi and G. F. Saunder, *ibid.* **51**, 756 (1973).
12. P. H. W. Butterworth, R. F. Cox, C. J. Chesterton, *Eur. J. Biochem.* **23**, 229 (1971).
13. R. A. Jungmann, P. C. Hiestand, J. S. Schweppe, *J. Biol. Chem.* **249**, 5444 (1974).
14. R. A. Jungmann and E. G. Kronias, *Adv. Biochem. Psychopharmacol.*, in press.
15. O. J. Martelo and J. Hirsch, *Biochem. Biophys. Res. Commun.* **58**, 1008 (1974).
16. O. J. Martelo, *Miami Winter Symp.* **5**, 199 (1973).
17. L. S. Teng, C. T. Teng, V. G. Allfrey, *J. Biol. Chem.* **246**, 3597 (1972); M. Shea and L. J. Kleinsmith, *Biochem. Biophys. Res. Commun.* **50**, 473 (1973); M. Kamiyama, B. Dastugue, J. Kruh, *ibid.* **44**, 1345 (1971); N. C. Kostraba, R. A. Montagna, T. Y. Wang, *J. Biol. Chem.* **250**, 1548 (1975); N. C. Kostraba and T. Y. Wang, *Biochim. Biophys. Acta* **262**, 169 (1972).
18. W. F. Marzluff, E. C. Murphy, R. C. Huang, *Biochemistry* **12**, 3440 (1973).
19. R. R. Burgess and J. J. Jendrisak, *ibid.* **14**, 4634 (1975).
20. J. F. Kuo and P. Greengard, *Adv. Cyclic Nucleotide Res.* **2**, 41 (1972).
21. U. Karsten and A. Wollenberger, *Anal. Biochem.* **46**, 135 (1972).
22. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).

19 January 1976; revised 22 March 1976

Cross-Linking of DNA in situ as a Probe for Chromatin Structure

Abstract. *The photochemical cross-linking of DNA in situ in chromatin is blocked over short intervals. Electron microscopy of DNA cross-linked in chromatin reveals the lengths of protected regions and provides a map of their sites along the DNA. Protected regions occur most frequently in tandem and have a basic length of 160 to 200 base pairs.*

A chemical probe for chromatin structure ideally should possess two properties. (i) It should be capable of permeating cells and acting in vivo to avoid isolation artifacts; and (ii) it should involve a covalent interaction with the chromatin components so that the product provides a stable record of the specificities of this interaction. Photochemical cross-linking of DNA in chromatin by derivatives of psoralen meets these criteria.

Psoralens are a class of medically important furocoumarins known for their ability to photosensitize mammalian skin (*1*) (Fig. 1). Psoralen and its derivatives in conjunction with ultraviolet light of long wavelength (320 to 380 nm) can covalently cross-link pyrimidines in opposite strands of the DNA double helix (*1, 2*). Because cellular and nuclear membranes are permeable to psoralens, this reaction can take place in DNA in situ in chromatin, isolated nuclei, or intact living cells (*1, 3*).

We have developed a method for determining the sites of cross-links in DNA by electron microscopy. The DNA is prepared for microscopy as follows: it is exhaustively denatured by heat for 2 hours at 70°C in a mixture of 10 percent formaldehyde, $0.02M$ sodium carbonate, pH 7.0, $0.005M$ EDTA. Denatured regions are prevented from renaturing by their reaction with formaldehyde (*4*). A portion of the sample is then spread on a cytochrome protein film by the Kleinschmidt technique (*5*) with the formamide conditions of Davis *et al.* (*6*) for visualizing both (denatured) single strands and double strands of nucleic acid. Cross-linked regions thus have the appearance of double-stranded DNA while regions protected from cross-linking appear as open loops with single-stranded arms (Fig. 2b).

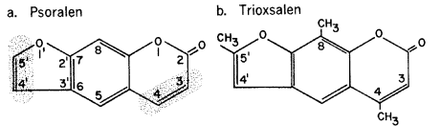


Fig. 1. (a) The general structure of psoralens. After intercalation into the DNA double helix, psoralens photoreact covalently with pyrimidines via the bonds in the shaded areas (*2*). (b) The structure of 4,5′,8-trimethylpsoralen (trioxsalen), which is the psoralen derivative used in this study.

By this technique we have observed the sites of cross-linking in DNA that undergoes photoreaction in situ and is subsequently purified. The overall design of the experiment is shown in Fig. 3. Spaced regions of each DNA molecule are often found to be so heavily cross-linked that the distance between cross-links is not detected by our technique (*7*). These regions alternate with stretches of 50 or more base pairs, which are protected from cross-linking. The protection of regions of DNA from cross-linking in situ appears not to be the result of base sequence heterogeneity since equivalent cross-linking of deproteinized DNA is uniform and nearly complete (Fig. 2a). The cross-linking pattern therefore must result from DNA conformation or interaction with proteins in chromatin.

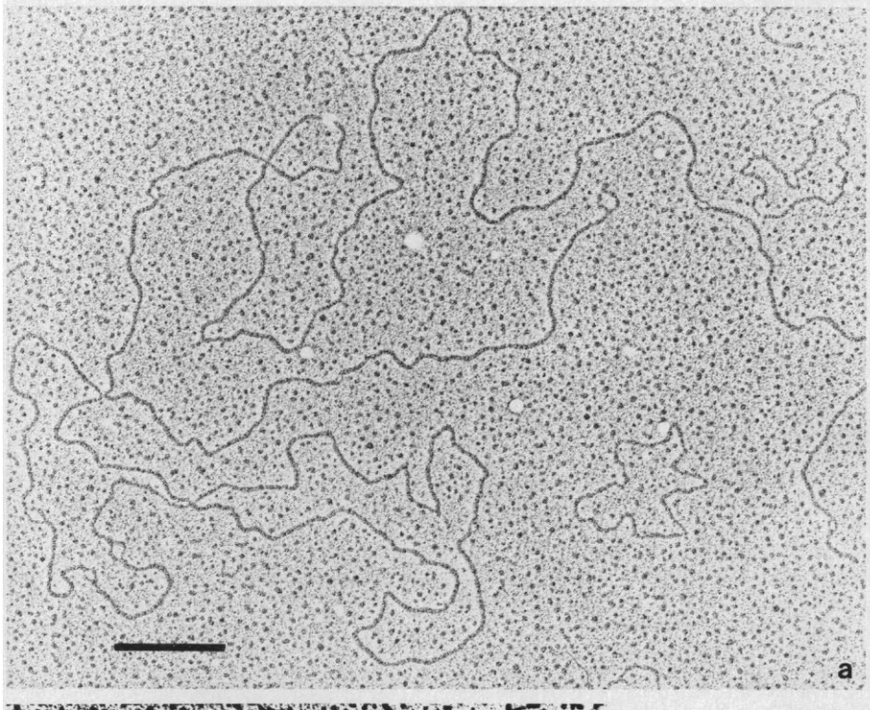
The percentage of DNA in protected regions, the lengths of such regions, and their loci along the molecule may be determined. The fraction of DNA in protected regions is dependent on the extent of photochemical cross-linking; but even when the cross-linking is taken to a saturating level, a minimum of 60 percent of the DNA remains protected. This is comparable to the 50 to 87 percent of DNA that was protected from degradation in several studies of (limit) digestion of DNA in chromatin by exogenous nuclease (*8–10*).

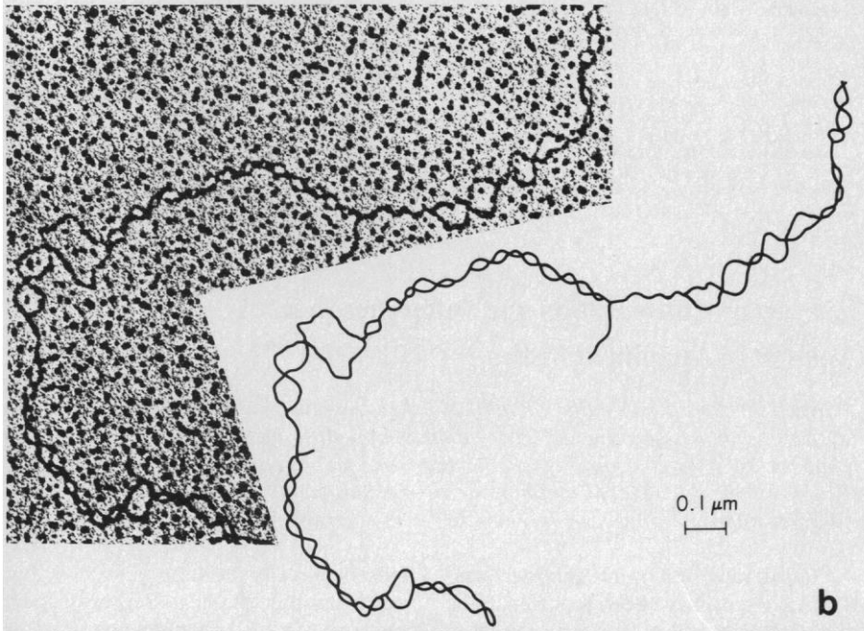
The lengths of regions protected from cross-linking form a distribution as

shown in the weight-average histograms (Fig. 2c). The principal peaks in these distributions correspond to 160 to 200 base pairs. The resolution of our technique is also sufficient to reveal a "dimer" peak at approximately twice this basic length. This result is consistent with the existence of a tandom distribution of protected regions of regular length.

Subunit structures for chromatin have been deduced from the electron microscopy of chromatin extended in media of low ionic strength (*11*), from the size of DNA fragments resistant to in situ nuclease digestion (*10, 12*), and from cross-linking and association studies of chromatin proteins (*13*). The periodicity predicted in these structures (*10–14*) ranges from 175 to 200 base pairs and thus is the same as found for the basic length of regions protected from cross-linking.

The broadness of the peaks in Fig. 2c perhaps reflects the native variation in the lengths of protected regions, but may also be influenced by (i) incomplete saturation of unprotected (interloop) regions by cross-links; (ii) migration of proteins along the chromatin axis (*8, 15*) during cross-linking; and (iii) variations due to calibration techniques in the co-mapping of regions from many separate molecules (legend to Fig. 2c). Widely varying extents of photoreaction (*16*) result in multimodal distributions with principal peaks centered at the same positions. With lower photoreaction times an expected shift of material out of the "monomer" peak may be seen.





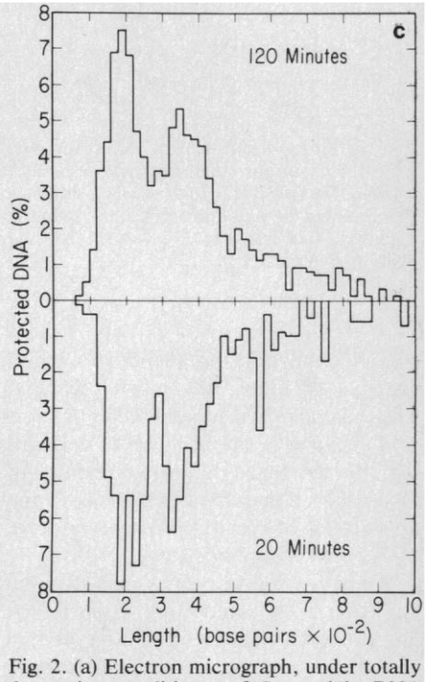


Fig. 2. (a) Electron micrograph, under totally denaturing conditions, of *Drosophila* DNA deproteinized as described in the legend to Fig. 3 and then cross-linked in vitro; *Drosophila* embryo DNA at 15 μg/ml was photoreacted with a saturated solution (6 μg/ml) of trioxsalen in standard saline citrate (SSC) buffer for 20 minutes at 18°C with long wavelength ultraviolet light from two General Electric F15T8 BLB tubes. The incident intensity was 1.4 mw/ml of reaction mixture as determined by ferrioxalate actionometry (*20*). The peak irradiation wavelengths (360 to 370 nm) correspond to those most commonly used for photoreaction of psoralens (*1–3*), and wavelengths below 350 nm were removed by a plexiglass filter. Scale bar, 1 μm. (b) Electron micrograph, under totally denaturing conditions, of DNA purified from isolated *Drosophila* nuclei that were previously photoreacted with trioxsalen under the same conditions as was the purified DNA in (a). Nuclei were isolated from 18- to 24-hour-old *Drosophila melanogaster* embryos in 0.3*M* sucrose, 0.1*M* NaCl, 0.01*M* $NaHSO_3$, 0.01*M* tris, *p*H 7.3, and photoreacted in SSC. Scale bar, 0.1 μm. (c) Weight average frequency histograms of lengths of protected regions (average of the lengths of the two branches of each loop) in DNA photoreacted in situ in *Drosophila* nuclei. Total number of loops used to construct histograms: 2260 for 120-minute experiment, 460 for 20-minute experiment. In the 20-minute experiment 62 percent of the DNA is in protected (loop) regions. (Percent protected was not determined in the 120-minute experiment.) Lengths in base pairs are calibrated by comparison with the contour length of single-stranded circular DNA isolated from fd virus and included on the electron microscope grid and assigned a length of 5740 ± 210 nucleotides (*21*).

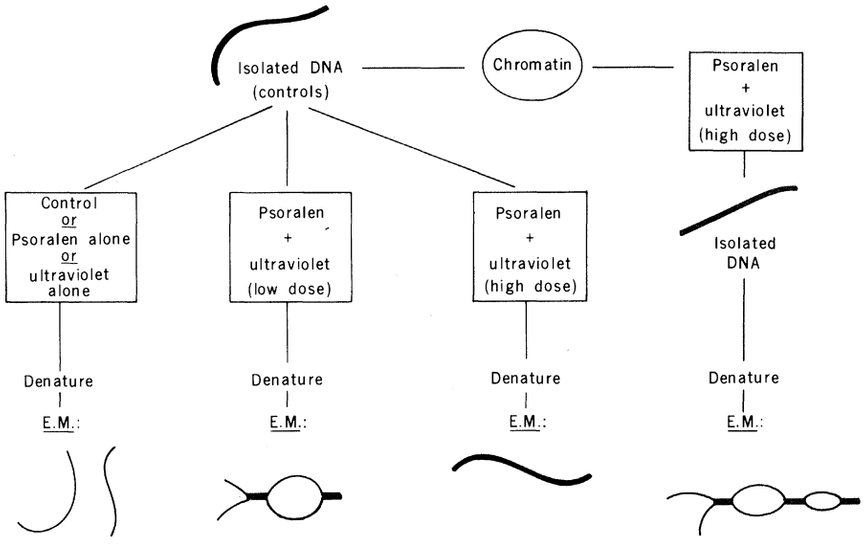


Fig. 3. Experimental design. Before or after photoreaction with trioxsalen, DNA is isolated from chromatin (or intact nuclei or cells) by incubation at 50°C for 5 to 12 hours with 1 percent sarkosyl, 0.1*M* EDTA, 0.01*M* tris, *p*H 8.6, and nuclease-free Pronase (1 mg/ml). The DNA is then banded by equilibrium sedimentation in a gradient of Cs_2SO_4 and dialyzed exhaustively against 0.01*M* tris, *p*H 7.6, 0.001*M* EDTA prior to preparation for electron microscopy as described in the text.

Histograms of the (center-to-center) spacing of loops are more complex. These data are also more difficult to interpret because of the problem of calibrating the length of interloop regions of DNA. These denatured regions have the appearance of native DNA only because of frequent cross-links.

We have observed an essentially similar pattern of alternating cross-linked and protected regions in many systems, including interphase and metaphase HeLa cells, cultured *Drosophila* cells, nuclei isolated from *Drosophila* embryos, isolated HeLa metaphase chromosomes, replicating chromosomes of SV40 virus in vivo, and isolated SV40 chromosomes in vitro.

The possible perturbation of chromatin structure by the psoralen cross-linking probe itself is unknown. However, photoreaction with trioxsalen does not alter the pattern of DNA fragment lengths obtained by subsequent micrococcal nuclease digestion of nuclei (*17*).

An example of subchromosomal variations in cross-linking pattern is found in *Drosophila* nuclei. Fractionation of *Drosophila* satellite DNA by density gradient techniques (*18*) is still possible after cross-linking in situ. The several satellites show loop patterns different from that of main-band DNA and different from those of each other. We believe that these differences result from chromatin structure in centric heterochromatin where these satellite DNA's are localized (*19*).

In conclusion, the ability of the psoralen probe to preserve a linear record of its interaction sites appears to present unique potentials in the study of chromatin structure.

CARL VEITH HANSON
CHE-KUN JAMES SHEN
JOHN E. HEARST

Department of Chemistry,
University of California, Berkeley 94720

References and Notes

1. M. A. Pathak, D. M. Kramer, T. B. Fitzpatrick, in *Sunlight and Man: Normal and Abnormal Photobiologic Responses*, T. B. Fitzpatrick *et al.*, Eds. (Univ. of Tokyo Press, Tokyo, 1975), p. 335; L. Musajo, G. Rodighiero, G. Caporale, F. Dall'acqua, S. Marciani, F. Bordin, F. Bacchetti, R. Bevilacqua, in *ibid.*, p. 369; L. Musajo and G. Rodighiero, in *Photophysiology*, A. C. Giese, Ed. (Academic Press, New York, 1972), vol. 7, p. 115; *Experientia* **18**, 153 (1962); M. A. Pathak, L. R. Worden, K. D. Kaufman, *J. Invest. Dermatol.* **48**, 103 (1967).
2. R. S. Cole, *Biochim. Biophys. Acta* **254**, 30 (1971); F. Dall'acqua, S. Marciani, L. Ciavatta, G. Rodighiero, *Z. Naturforsch. Teil B* **26**, 561 (1971).
3. M. A. Pathak and D. M. Kramer, *Biochim. Biophys. Acta* **195**, 197 (1969); R. S. Cole, *ibid.* **217**, 30 (1970).
4. R. Haselkorn and P. Doty, *J. Biol. Chem.* **236**, 2378 (1961).
5. A. K. Kleinschmidt, *Methods Enzymol.* **12B**, 125 (1968).
6. R. W. Davis, M. Simon, N. Davidson, *ibid.* **21**, 413 (1971).
7. Theoretical resolution is limited to a minimum loop size of approximately 50 base pairs by the dimensions of the stained and shadow-cast cytochrome particles.
8. R. J. Clark and G. Felsenfeld, *Nature (London) New Biol.* **229**, 101 (1971); *Biochemistry* **13**, 3622 (1974).
9. R. Axel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4101 (1974).
10. C. G. Sahasrabuddhe and K. E. VanHolde, *J. Biol. Chem.* **249**, 152 (19 → M. Noll, *Nature (London)* **251**, 249 (1974).
11. A. L. Olins and D. E. Olins, *Science* **183**, 330 (1974); J. P. Langmore and J. C. Woolev, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2691 (19 → P. Oudet, M. Gross-Bellard, P. Chambon, *Cell* **4**, 281 (1975); J. Griffith, *Science* **187**, 1202 (1975).
→ D. R. Hewish and L. A. Burgoyne, *Biochem. Biophys. Res. Commun.* **52**, 504 (1973); L. A. Burgoyne, D. R. Hewish, J. Mobbs, *Biochem. J.* **143**, 67 (1974); R. L. Rill *et al.*, *Nucleic Acids, Res.* **2**, 1525 (1975); D. K. Oosterhof, J. C. Hozier, R. L. Rill, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 633 (1975).
→ R. D. Kornberg, *Science* **184**, 868 (1974); J. O. Thomas and R. D. Kornberg, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2626 (1975); J. E. Hyde and I. O. Walker, *FEBS Lett.* **50**, 150 (1975); H. Weintraub, K. Palter, F. VanLente, *Cell* **6**, 85 (1975); R. C. Hardison, M. E. Eichner, R. Chalkley, *Nucleic Acids Res.* **2**, 1751 (1975).
14. J. E. Hyde and I. O. Walker, *Nucleic Acids Res.* **2**, 405 (1975); K. E. VanHold, C. G. Sahasrabuddhe, B. R. Shaw, *ibid.* **1**, 1597 (1974); H. J. Li, *ibid.* **2**, 1275 (1975).
15. Y. V. Ilyin and G. P. Georgiev, *Nature (London)* **250**, 602 (1974).
16. Photochemical cross-linking depends on both drug concentration and amount of irradiation. In these experiments the drug was held at a very high constant level—a tenfold excess above its solubility limit. Extent of reaction is then controlled by the duration of irradiation.
17. G. Wiesehahn and J. Hearst, in *Molecular Mechanisms of Gene Expression*, D. P. Nierlich, W. J. Rutter, C. F. Fox, Eds. (Academic Press, New York, in press).
18. W. J. Peacock, D. Brutlag, E. Goldring, R. Appels, C. W. Hinton, D. L. Lindsley, *Cold Spring Harbor Symp. Quant. Biol.* **38**, 405 (1974).
19. C. K. Shen and J. Hearst, in preparation.
→ C. G. Hatchard and C. A. Parker, *Proc. R. Soc. London Ser. A* **235**, 518 (1956).
21. S. A. Berkowitz and L. A. Day, *Biochemistry* **13**, 4825 (1974).
22. Supported by NIH grant GM-11180 and American Cancer Society grant NP-185. We thank T. Cech for suggesting use of in situ cross-linking by psoralen as a probe for DNA structures.

8 December 1975; revised 24 February 1976

Karyotype Conservation and Difference in DNA Amount in Anguilloid Fishes

Abstract. *The two Pacific anguilloid fishes* Anguilla japonica *and* Astroconger myriaster, *belonging to the different families, appear to have identical chromosome numbers (2n = 38) and karyotypes, including one pair of conspicuous heteromorphic chromosomes in females. Cytophotometric measurements, however, indicate a considerable difference in DNA content between the two species.*

Considerably different genome sizes (DNA content) have been reported in the divergent fish orders (*1, 2*). In this connection, Ohno (*1*) suggested that quantum evolution from fish to mammal was not accomplished simply by point mutations of already existing gene loci, but rather through duplicated genes which made possible the emergence of new genes with previously nonexistent functions. It is also believed that the primitive teleostean ancestor had approxi-