about 50 μm across, with individual fibers of the order of 2 to 3 μm. Figure 2 shows a section of tape after calcification. The dark areas show regions where coherent mineralization has occurred. Some spherulitic growth is evident on the outer surface of some deposits and especially in the white region where the tape has been torn apart, perhaps by stresses induced during crystallization. One band of collagen is only partially covered, showing the small striations in the underlying fiber.

Figures 3 and 4 are specimens of the apatite material after the removal of collagen by ethylenediamine treatment. Figure 3 shows a bundle of apatite fibers with a hollow in the center, which appears to have grown around one of the bands of collagen about 30 μm in diameter. Note the parallel arrangement of bundles and the curvature of segments which have grown apparently in conformity with the shape of the collagen fiber bundle. Figure 4 shows the fractured end of a piece of fibrous apatite. Beneath the snowlike fine material on the surface, this sample appears to be a mass of parallel crystallites with cross-sectional dimensions of about 1 μm or less; some of these appeared hexagonal in cross section. Parallel striations can be seen on the exposed longitudinal surfaces. The mottled appearance of the transverse surfaces indicates individual crystallite fracture surfaces whose curvature is reminiscent of that of the conchoidal fracture seen in macroscopic apatite mineral specimens. The electron microscopic observations are entirely consistent with the evidence from x-ray diffraction and polarized light microscopy.

The phosvitin-modified collagen tapes used in these experiments can only be a crude approximation to the natural collagen produced under cellular control. In the latter a complex enzyme system catalyzes the formation of precise molecular species, whose conformations lead to structures which must be even more specific in their capacity to control the orientation of crystals of apatite in growing hard tissues. We reemphasize that the only experiments yielding these fibrous deposits were those in which the phosphoprotein was added to the collagen in the presence of a known cross-linking agent.

These experiments do not establish Robison's proposed calcification mechanism. However, they indicate that the failure until now to detect a concentrated substrate for alkaline phosphatase does not, of itself, rule out such a mechanism. If alkaline phosphatase does play a part in biological calcification, it would only require the presence in the tissue of a system that would release a calcium salt of an organic phosphate at a rate appropriate to the rate of release of alkaline phosphatase. Henrichsen (*12*) has added β-glycerophosphate to a tissue culture of chicken heart fibroblasts and showed that calcification occurs only near the sites of dying cells. This could account for abnormal calcification occurring in normally noncalcifying tissues. It would be of considerable interest to carry out a similar study in cultures of osteoblasts, odontoblasts, or similar cells from normally calcifying tissues.

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References and Notes

1. A. Dutton, M. Adams, S. J. Singer, *Biochem. Biophys. Res. Commun.* **23**, 730 (1966); G. E. Davies and G. R. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **66**, 651 (1970).
2. R. Robison, *Biochem. J.* **17**, 286 (1923).
3. F. C. McLean and M. R. Urist, *Bone* (Univ. of Chicago Press, Chicago, ed. 3, 1968), p. 54.
4. H. Rasmussen and P. Bordier, *The Physiology and Cellular Basis of Metabolic Bone Disease* (Williams & Wilkins, Baltimore, 1974).
5. D. McConnell, W. J. Frajola, D. W. Deamer, *Science* **133**, 281 (1961).
6. M. J. Glimcher and S. M. Krane, in *Radioisotopes and Bone*, P. Lacroix and A. M. Budy, Eds. (Blackwell, Oxford, 1962), p. 393.
7. S. E. Allerton and G. E. Perlmann, *J. Biol. Chem.* **240**, 3892 (1965).
8. L. C. Shapiro, thesis, Polytechnic Institute of New York (1975).
9. M. J. Glimcher, A. J. Hodge, F. O. Schmitt, *Proc. Natl. Acad. Sci. U.S.A.* **43**, 960 (1957).
10. See also E. P. Katz, *Biochim. Biophys. Acta* **194**, 121 (1969).
11. R. W. G. Wyckoff, *Crystal Structures* (Wiley-Interscience, New York, ed. 2, 1965), vol. 3, p. 232.
12. E. Henrichsen, *Exp. Cell Res.* **11**, 403 (1956).
13. We thank H. Talts for infrared spectra, M. Chianelli for technical assistance, and R. L. Kronenthal of Ethicon Corp. for generously providing collagen tapes. Supported by NIH grant DE-03543.

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Localization of the Globin Gene in the Template Active Fraction of Chromatin of Friend Leukemia Cells

Abstract. *Friend leukemia cell chromatin has been fractionated into template active and inactive components. The globin gene sequence is associated with the template active component both prior to and after the cells are induced with dimethyl sulfoxide to synthesize hemoglobin and therefore appears to be in an active configuration in uninduced as well as in induced Friend leukemia cells. In cells which have lost the ability to produce hemoglobin, the globin gene sequence is not associated with the template active fraction of chromatin. These results demonstrate the success of the fractionation procedure.*

Gene regulation in higher organisms is accomplished, at least in part, by the restriction of transcription to selective regions of the genome (*1*). What specific interactions of the proteins, RNA, and DNA of chromatin are responsible for the inactivation of certain regions of the genome and activation of other regions is not known. Early cytological studies suggested that the extended regions of chromatin or euchromatin were active in RNA transcription, whereas the condensed regions or heterochromatin were transcriptionally inactive (*2*). Recent biochemical evidence showing active genes to be more readily digestible by deoxyribonuclease I (E.C. 3.1.4.5) also argues in favor of structural differences between transcriptionally active and inactive regions of the genome (*3*).

The fractionation of template active and inactive components of chromatin has been much investigated (*4–10*). One approach has been to shear chromatin to a size smaller than the average transcriptional unit and subject the material to a physical separation procedure such as differential centrifugation (*4*), sucrose or glycerol gradient centrifugation (*5*), ion exchange chromatography (*6*), selective precipitation (*7*), gel filtration (*8*), thermal chromatography on hydroxyapatite (*9*), or others (*10*). Another approach is based on a brief digestion of chromatin with deoxyribonuclease II (E.C. 3.1.4.6) followed by differential centrifugation and selective precipitation of inactive chromatin with magnesium (*11, 12*).

Although it has proved difficult to obtain a pure fraction of chromatin by any of the reported procedures, highly enriched fractions have been obtained. Indeed, the deoxyribonuclease II procedure has yielded a six- to sevenfold enriched template active fraction of rat liver chromatin (*12, 13*).

We have now used the deoxyribonuclease II procedure to gain insight into

the mechanism of gene induction. Using Friend virus transformed mouse leukemia cells, which do not transcribe globin messenger RNA (mRNA) unless induced (*14, 15*), we have localized the globin gene in the fractionated chromatin of uninduced and induced cells.

Friend leukemia cells, clone FSD-3 (*15, 16*), were grown in suspension culture as described (*15*). Cells were induced in the presence of 1.5 percent dimethyl sulfoxide for 24 hours, a time at which the maximum rate of transcription of globin mRNA is achieved (*15*). Chromatin was prepared by lysis of nuclei in a buffer of low ionic strength as described in Fig. 1. Fractionation of the chromatin from induced and uninduced FSD-3 cells was performed as described previously (*12*). The DNA was prepared from the template active fraction of chromatin as well as from whole cells and analyzed for the frequency of globin gene sequence by the following technique. The DNA to be analyzed was mixed with ^{3}H-labeled complementary DNA (cDNA) to globin in mass ratios varying from 5×10^3 to 5×10^7. The unlabeled DNA was maintained at 5 mg/ml. The samples were denatured by heating and allowed to renature to an equivalent C_0t (initial concentration versus time) of 10,000 (with respect to unlabeled DNA). As described in Fig. 1, the fraction of cDNA in hybrid at a particular mass ratio is related to the frequency of the globin gene sequence in the driver DNA. Computer analysis of the data allows a calculation of the point at which 50 percent of maximum hybridization occurs. This is the point where the concentration of cDNA is equal to the concentration of globin gene sequence in the unlabeled DNA. By this analysis, it is seen that the DNA from the template active fraction of induced FSD-3 cell chromatin is enriched sevenfold for globin gene sequence over whole cell DNA (Fig. 1), demonstrating the success of the fractionation procedure. Figure 1 also shows the result for DNA from the template active fraction of uninduced FSD-3 cell chromatin. The uninduced cell template active DNA is enriched for globin gene sequence as well, and is essentially identical to induced cell template active DNA in this respect.

These results demonstrate that not only is a gene which is actively transcribed present in a conformation that allows fractionation into the template active component, but so also is a gene with the potential to be transcribed. As a further check on the fractionation procedure, we isolated the template active DNA from Friend leukemia cells which have lost the ability to be induced (F4+ cell line) (*16*). Saturation hybridization experiments were performed on DNA from the template active fraction of uninduced FSD-3 chromatin and of F4+ chromatin and compared to the hybridization of whole cell DNA. In this experiment, increasing amounts of ^{3}H-labeled globin cDNA were hybridized to 50 μg of DNA until saturation was approached. Hybrids were detected by hydroxyapatite chromatography. As shown in Fig. 2, DNA from uninduced FSD-3 template active chromatin is again found to be enriched over whole cell DNA (four- to fivefold) while F4+ template active DNA contains barely detectable levels of globin sequence. That the F4+ cells

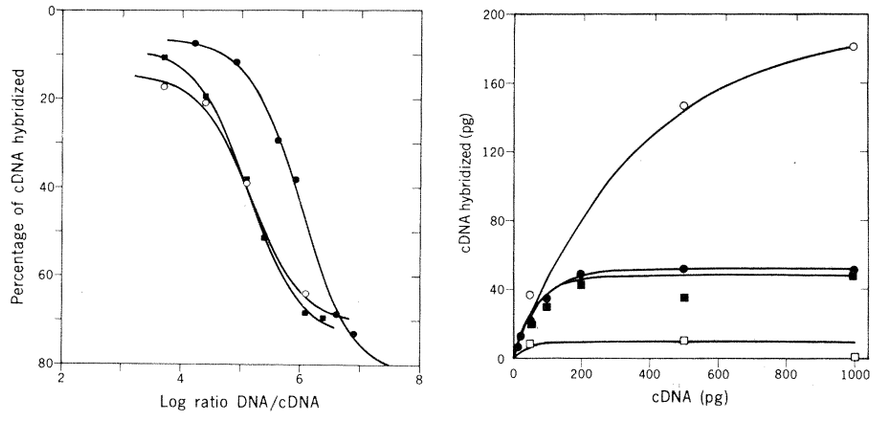

Fig. 1 (left). Titration of the globin gene sequence in DNA from template active fractions of Friend leukemia cell chromatin and DNA from whole cells. Friend leukemia cells, clone FSD-3 (*16*), were grown in suspension culture as described previously. Induced cells were cultured for 24 hours in the presence of 1.5 percent dimethyl sulfoxide. Cells were harvested, washed in phosphate buffered saline, and suspended in solution A consisting of 10 m*M* tris-HCl, *p*H 7.5, 6 m*M* KCl, 5 m*M* magnesium acetate, 1m*M* phenylmethyl-sulfonylfluoride (PMSF), and 0.1 m*M* ethylene glycol bis(aminoethyl ether)tetraacetate (EGTA) containing 0.5 percent NP-40. Pellets were formed from the nuclei by centrifugation at 1000*g* for 5 minutes; the pellets were washed once in solution A containing 0.5 percent NP-40, once in solution A, and once in 0.075*M* NaCl, 0.025*M* EDTA, *p*H 7.5, and 1 m*M* PMSF. The nuclear pellet was resuspended with a glass-Teflon homogenizer in solution B containing 10 m*M* tris-HCl, *p*H 8.0, 1 m*M* PMSF, and 0.1 m*M* EGTA. Pellets were formed from chromatin by centrifugation at 10,000*g* for 10 minutes. The chromatin was washed three times in solution B, suspended in solution B at an absorbance of 10 at 260 nm measured in 0.1*N* NaOH. The chromatin suspension was dialyzed overnight against 25 m*M* sodium acetate, *p*H 6.6, and 0.1 m*M* EGTA. Isolation of template active chromatin and preparation of DNA was performed as described (*12*). The DNA was prepared from whole cells by the procedure of Marmur (*19*) and sheared to a size of 350 base pairs in a VirTis 60 homogenizer. The ^{3}H-labeled globin cDNA was prepared as described by Harrison *et al.* (*20*). Titration of the globin gene sequence was performed as follows: DNA (5 mg/ml) in 0.4*1M* phosphate buffer, *p*H 6.8 (*21*), was mixed with ^{3}H-labeled globin cDNA [2×10^7 disintegrations per minute (dpm) per microgram] in mass ratios varying from 5×10^3 to 5×10^7. A maximum of 6250 dpm and a minimum of 250 dpm of [^{3}H]cDNA were used. The DNA was denatured by boiling for 5 minutes and was allowed to renature at 70°C for 40 hours (C_0t 10,000). Hybridization of the ^{3}H-labeled globin cDNA was detected by hydroxyapatite (HAP) chromatography as described (*21*). The fraction of ^{3}H-labeled globin cDNA in hybrids is related to the mass ratio of driver DNA to ^{3}H-labeled globin cDNA by the equation $F(R) = A + (B/1 + RC)$ where $F(R)$ is the fraction [^{3}H]cDNA (single-stranded) at mass ratio R; A is the fraction of cDNA unreactable in vast excess of whole cell driver DNA (data not shown); B is the fraction of cDNA reactable; C is the frequency of globin gene sequence in the driver DNA expressed as fractional mass; and R is the mass ratio of driver to tracer DNA's. Data were analyzed by nonlinear least-squares fitting as described (*22*). The concentration of template active DNA has been corrected for its twofold lower reactability compared with whole cell DNA. The lower reactability of template active DNA has been noted previously (*12*) and appears to be random with respect to sequence. Whole cell DNA (•); template active DNA from induced FSD-3 cells (▪); template active DNA from uninduced FSD-3 cells (○). Fig. 2 (right). Saturation hybridization of ^{3}H-labeled globin cDNA to template active DNA from uninduced FSD-3 cell chromatin and from F4+ cell chromatin and to whole cell DNA. Template active and inactive DNA was isolated as described previously (*12*) and in the legend to Fig. 1, except that the DNA was further purified by binding to HAP in 0.12*M* phosphate buffer, washing in 0.12*M* phosphate buffer, and then eluting in 0.48*M* phosphate buffer (*21*): DNA's treated in this way had a reactability up to 90 percent of whole cell DNA. Saturation hybridization experiments were performed as follows: 50 μg of DNA in 0.4*1M* phosphate buffer, *p*H 6.8, was mixed with increasing amounts of ^{3}H-labeled globin cDNA up to 1.0 ng. The DNA concentration was maintained at 5 mg/ml. The samples were denatured by boiling for 5 minutes and allowed to renature for 40 hours (C_0t 10,000). Hybridization of the ^{3}H-labeled globin cDNA was detected by HAP chromatography (*21*). The 10 percent HAP binding of the cDNA in the absence of driver has been subtracted from each point. Whole cell DNA (•); template active DNA from uninduced FSD-3 cells (○); template active DNA from F4+ cells (□); template inactive DNA from F4+ cells (▪).

have not suffered a deletion of the gene is shown by the normal saturation hybridization of the F4+ template inactive DNA with ^{3}H-labeled globin cDNA (Fig. 2). Thus, chromatin from a cell line which does not have the potential to transcribe globin sequences does not have the globin gene in a conformation which allows its fractionation into the template active chromatin fraction.

These results have three important implications. First, they show that an active gene can be localized in the template active fraction of chromatin. Second, the finding that in uninduced FSD-3 cells the globin gene is present in a conformation which allows its fractionation with template active chromatin suggests that the globin gene is switched on somewhat early in differentiation of erythropoietic cells and that prior to its involvement in actual transcription it is activated by a cytoplasmic or nuclear signal. It is not unlikely that this would be true for any inducible gene, that is, a gene which can be turned on by some specific environmental signal such as a hormone, or other chemical agent. This might differ from a differentiation process in which the cells in a lineage must undergo multiple changes in the expressed genetic program and which could, for example, require a round of cell division to achieve these changes (*17*). Finally, the localization of the globin gene in the template active fraction of FSD-3 cell chromatin argues that the target cell for Friend virus is a somewhat differentiated erythroid committed precursor cell. This supports the earlier observation that the virus needs a mature precursor cell to express its oncogenic potency (*18*).

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References and Notes

1. R. S. Gilmour and J. Paul, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3440 (1973); R. Axel, H. Cedar, G. Felsenfeld, *ibid.*, p. 2029; A. Steggles, G. Wilson, J. Kantor, D. Picciano, A. Falvry, W. F. Anderson, *ibid.* **71**, 1219 (1974); I. R. Brown and R. B. Church, *Dev. Biol.* **29**, 73 (1972); L. Grouse, M. D. Chilton, B. J. McCarthy, *Biochemistry* **11**, 798 (1972); C. P. Liarakos, J. M. Rosen, B. W. O'Malley, *ibid.* **12**, 2809 (1973); D. S. Holmes and J. Bonner, *ibid.* **13**, 841 (1974); G. A. Galau, W. H. Klein, M. M. Davis, B. J. Wold, R. J. Britten, E. H. Davidson, *Cell* 7, 487 (1976).
2. V. C. Littau, V. G. Allfrey, J. H. Frenster, A. E. Mirsky, *Proc. Natl. Acad. Sci. U.S.A.* **52**, 93 (1964); J. H. Frenster, in *The Cell Nucleus*, H. Busch, Ed. (Academic Press, New York, 1974), vol. 1, pp. 565–580.
3. H. Weintraub and M. Groudine, *Science* **193**, 848 (1976); A. Garel and R. Axel, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3966 (1976).
4. J. H. Frenster, V. G. Allfrey, A. E. Mirsky, *Proc. Natl. Acad. Sci. U.S.A.* **50**, 1026 (1973).
5. R. Chalkley and R. H. Jensen, *Biochemistry* **7**, 4380 (1968); E. C. Murphy, S. H. Hall, J. H. Shepherd, R. S. Weiser, *ibid.* **12**, 3843 (1973); E. M. Berkowitz and P. Doty, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3328 (1975).
6. R. T. Simpson and G. R. Reeck, *Biochemistry* **12**, 3853 (1973).
7. E. A. Arnold and K. E. Young, *Arch. Biochem. Biophys.* **164**, 73 (1974); G. G. Markov, I. G. Ivanov, I. G. Pashev, *J. Mol. Biol.* **93**, 405 (1975).
8. M. Janowski, D. S. Nasser, B. J. McCarthy, *Karolinska Symp. Res. Methods Reprod. Endocrinol.* **5**, 112 (1972).
9. B. L. McConaughy and B. J. McCarthy, *Biochemistry* **11**, 998 (1972).
10. D. Rickwood, A. Hell, S. Malcolm, G. D. Birnie, A. J. MacGillivray, J. Paul, *Biochim. Biophys. Acta* **353**, 353 (1974); G. Turner and R. Hancock, *Biochem. Biophys. Res. Commun.* **58**, 437 (1974).
11. K. Marushige and J. Bonner, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2941 (1971); R. J. Billing and J. Bonner, *Biochim. Biophys. Acta* **281**, 453 (1972).
12. J. M. Gottesfeld, W. T. Garrard, G. Bagi, R. F. Wilson, J. Bonner, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2193 (1974); J. M. Gottesfeld, G. Bagi, B. Berg, J. Bonner, *Biochemistry* **15**, 2472 (1976).
13. J. Bonner, W. T. Garrard, J. Gottesfeld, D. S. Holmes, J. S. Sevall, M. Wilkes, *Cold Spring Harbor Symp. Quant. Biol.* **38**, 303 (1973).
14. C. Friend, W. Scher, J. G. Holland, T. Sato, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 378 (1971); J. Ross, Y. Ikawa, P. Leder, *ibid.* **69**, 3620 (1972); J. Ross, J. Gielen, S. Packman, Y. Ikawa, P. Leder, *J. Mol. Biol.* **87**, 697 (1974); R. S. Gilmour, P. R. Harrison, J. Windass, N. A. Affara, J. Paul, *Cell Differ.* **3**, 9 (1974).
15. W. Ostertag, H. Melderis, G. Steinheider, N. Kluge, S. Dube, *Nature (London) New Biol.* **239**, 231 (1972).
16. S. K. Dube, G. Gaedicke, N. Kluge, B. J. Weimann, H. Melderis, G. Steinheider, T. Crozier, W. Ostertag, in *Proceedings of the 4th Symposium: Princess Takamatsu Cancer Research Fund, Differentiation and Control of Malignancy in Tumor Cells* (Univ. of Tokyo Press, Tokyo, 1974). pp. 103–136; F. Keppel, B. Allet, H. Eisen, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 653 (1977).
17. H. Holtzer, N. Rubinstein, S. Fellini, G. Yeoh, J. Chi, J. Birnbaum, M. Okayama, *Q. Rev. Biophys.* **8**, 523 (1975).
18. P. E. Tambourin and F. Wendling, *Nature (London)* **256**, 320 (1975).
19. J. Marmur, *J. Mol. Biol.* **3**, 208 (1961).
20. P. R. Harrison, G. D. Birnie, A. Hell, S. Humphries, B. D. Young, J. Paul, *ibid.* **84**, 539 (1974).
21. R. J. Britten, D. E. Graham, B. R. Neufeld, *Methods Enzymol.* **39**, 363 (1974).
22. W. Pearson, E. H. Davidson, R. J. Britten, *Nucleic Acids Res.* **4**, 1727 (1977).
23. We thank I. B. Pragnell and W. Ostertag for a gift of globin cDNA and helpful discussions. This research was supported by PHS grant GM-13762. S.K.D. has been supported as a Sherman Fairchild Distinguished Scholar at the California Institute of Technology. R.B.W. is the recipient of a Medical Research Council of Canada fellowship.
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Mechanism of Suppression in *Drosophila*: Control of Sepiapterin Synthase at the Purple Locus

Abstract. *The amounts of sepiapterin and red pteridine eye pigments (drosopterins) in* Drosophila melanogaster *are known to be reduced in the purple mutant and restored to normal by a suppressor mutation. We show here that sepiapterin synthase activity is 30 percent of normal in* pr *and* pr^{bw}*, two naturally occurring alleles of purple, and is restored to nearly normal levels by the suppressor* su(s)^2*. A heterozygote of two newly induced alleles of* pr *has even lower enzyme activity (< 10 percent). The sepiapterin synthase activity is proportional to the number of wild-type* pr *alleles in flies when one and two copies of the allele are present and is higher in three- than in two-dose flies. We hypothesize that the purple locus may be a structural gene for sepiapterin synthase in* Drosophila.

The purple mutant of *Drosophila melanogaster* is deficient in pteridines (*1*). The red pteridine eye pigments, drosopterins, are reduced to 30 percent of normal in two alleles of purple, *pr* and *pr^{bw}*, and are restored to normal levels in *su(s)^2; pr* and *su(s)^2; pr^{bw}* (*2*). In addition the sepiapterin pool undergoes changes that parallel those in the drosopterins (*2*). These results indicate that the biochemical defect in purple is in pteridine biosynthesis and that the defect is most probably among the early steps in the pathway.

In this report two early enzymatic activities in pteridine biosynthesis, guanosine triphosphate (GTP) cyclohydrolase and sepiapterin synthase, were measured in wild-type, purple, and suppressed purple flies. Each activity has been previously demonstrated in *Drosophila* (*3*, *4*).

Two new alleles of purple were isolated by treating the flies with the mutagen ethylmethane sulfonate. The treated *pr$^+$cn* male flies were crossed with *Sco pr cn/CyO, pr cn^2* females. Flies containing the purple mutation were selected, and stocks of the newly induced *pr* alleles were constructed. One new mutation, *pr^{c4}*, is lethal as a homozygote and behaves as the least functional of all the purple alleles, indicating that the product of the *pr* locus is required for viability of *Drosophila*. White eyes are expected if the purple mutation is extreme (not leaky) since cinnabar is included in these stocks to prevent the synthesis of brown ommochrome eye pigments. The heterozygote, *pr^{c4}/pr*, is distinctly lighter in eye color than *pr/pr*. The second new mutant, *pr^{c5}*, is associated with a translocation between the Y and second chromosome and shows a variegated position effect. The eye color of *pr^{c5} cn/pr cn* is orange like that of *pr cn/pr cn*, but that of *pr^{c4} cn/pr^{c5} cn* is white with yellow flecks. The eye color of *pr^{c4}/pr^{c5}* tends to darken with age.

Guanosine triphosphate cyclohydro-