rescence scattered from the deeper retinal layers). If the cone is treated for 2 minutes with a hypotonic solution containing 50 m*M* NaCl, however, reapplying 10 μM DDC now stains the mitochondrial membranes as well as the outer segments. Thus, the presumed topological difference between rod and cone outer segment membranes suggested by electron microscopic and electrical evidence is confirmed by the DDC staining test.

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References and Notes

1. R. W. Young, *J. Cell Biol.* **33**, 61 (1967) ——— and D. Bok, *ibid*, **42**, 392 (1969).
2. A. I. Cohen, *ibid.* **37**, 424 (1968); *Vision Res.* **10**, 445 (1970).
3. W. A. Hagins and H. Rüppel, *Fed. Proc.* **30**, 64 (1971); H. Rüppel and W. A. Hagins, in *Biochemistry and Physiology of Visual Pigments*, H. Langer, Ed. (Springer, New York, 1973), pp. 257–261.
4. T. Tomita, *Q. Rev. Biophys.* **3**, 179 (1970); W. A. Hagins, *Annu. Rev. Biophys. Bioeng.* **1**, 131 (1972); R. D. Penn and W. A. Hagins, *Biophys. J.* **12**, 1073 (1972).
5. Dansyl is an abbreviation for 5-dimethylamino-1-naphthalenesulfonyl.
6. Fluorescein and rhodamine G were obtained from Fisher Scientific Co. Fluorescein isothiocyanate (isomer I) and rhodamine B isothiocyanate were purchased from Nutritional Biochemicals and were used without purification after testing for reactivity with bovine serum albumin. Procion yellow M4RAN was a gift of W. Stewart. *N*-dansyl glucosamine was synthesized by reacting 10 mmole of dansyl chloride (Pierce Chemical Co.) with 0.1 mole of glucosamine in 20 ml of H_2O-ethanol (3 : 1) and extracting the dansyl derivative in $CHCl_3$ in a continuous extractor. The purity of the product and of the other commercially prepared *N*-dansyl amino acids (Pierce Chemical Co.) was checked by thin-layer chromatography in butanol-H_2O-acetic acid (80 : 20 : 20).
7. A. M. Laties and P. A. Liebman, *Science* **168**, 1475 (1970).
8. Our attempts to demonstrate selective staining of cone outer segments confirmed the previous report (*7*), but such preparations also showed many rod and cone nuclei stained with the fluorochrome in cells whose outer segments were unstained. We do not know if this result is due to variation in the staining properties of different batches of Procion yellow dyes. Until their structures are published and pure samples are available, the Procion dyes will be difficult to use in reproducible experiments.
9. J. Korenbrot and R. A. Cone, *J. Gen. Physiol.* **60**, 20 (1972).
10. Photographs of the microscopic fields were made on Polaroid type 55 P/N sheet film before (B) and during (D) exposure of the layer of rods in agarose to hypertonic Ringer solution. A contact print of the negative of B was made on a Kodak Lantern slide plate developed to a contrast of $\gamma = 1$. The exposure of the plate was adjusted so that when it was superimposed on the negative of B, a uniform gray appearance almost devoid of contrast was seen. The plate was then superimposed on the negative of D and printed on grade 4 (high contrast) photographic paper.
11. C. W. Wu and L. Stryer, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1104 (1972).
12. The fluorescence emission maximum of DDC dissolved in Ringer solution is at 550 ± 5 nm while that of DDC bound to rod outer segments is at 505 ± 5 nm.
13. We thank W. H. Cobbs for discussion.

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Centric Fusion, Satellite DNA, and DNA Polarity in Mouse Chromosomes

Abstract. *A fluorescent staining technique has demonstrated a contralateral arrangement of fluorescent spots in the centromeric region of mouse metacentric chromosomes which have resulted from centric fusion. The results suggest that centric fusion involves the maintenance of DNA polarity through the centromere and that the thymidine-rich chain of satellite DNA in the centromeric region is associated with the same DNA chain in every mouse autosome.*

There is a fluorometrically detectable lateral asymmetry in the centromeric region of mouse (*Mus musculus*) chromosomes (*1*). This asymmetry was detected through the use of a newly developed chromosome staining technique based on the quenching by 5-bromodeoxyuridine (BrdU) of the fluorescence of the dye 33258 Hoechst (*2*). In the absence of BrdU treatment, the centromeric region of mouse chromosomes (except the Y chromosome) fluoresces brightly and uniformly when stained with 33258 Hoechst (*3*). In contrast, when the chromosomes of mouse cells grown for one generation in the presence of BrdU are stained with 33258 Hoechst, there is a marked difference in the fluorescence between the halves of the centromeres, such that half of the centromeric region of each chromosome (except the Y chromosome) shows a brightly fluorescent spot (*1*). The staining properties and pattern of segregation during cell division of this fluorescent spot suggest that it is associated with the thymidine-rich chain of satellite DNA in the centromeric region. [One chain of mouse satellite DNA contains 45 percent thymidine, and the other chain contains 22 percent (*4*).]

The mouse cells used in the previous experiments involving the BrdU-Hoechst treatment were cells of permanent lines (RAG and NCTC 2472), and they contained a few metacentric chromosomes not present in diploid mouse cells. After one cycle of replication in BrdU, two brightly fluorescent spots were observed in each metacentric chromosome. The spots were arranged contralaterally, one on either side of the centromere and located on the opposite arms of the chromosome (*1*). Although the results led to speculations concerning DNA polarity and the arrangement of satellite DNA, no firm conclusions could be drawn because it was not known whether the metacentric chromosomes in these cells had arisen by misdivision of the centromere or by Robertsonian centric fusion of telocentric chromosomes.

We now describe the centromeric staining properties of metacentric chromosomes known to be the result of Robertsonian centric fusion of telocentric chromosomes. The cells for this study were derived from two different strains of mice. One of these strains is the tobacco mouse (*Mus poschiavinus*), which contains seven pairs of metacentric chromosomes resulting from centric fusion (*5*). The other strain is the 36(TW/TW TA/TA) strain which contains two pairs of metacentric chromosomes resulting from centric fusion (*6*) (these mice are referred to below as 36T mice). The tobacco mice were provided by Dr. Alfred Gropp, and the 36T mice were provided by Dr. Beverly White.

Male tobacco mice were bred with female mice of the CD-1 strain (Charles River), which contain no metacentric chromosomes. (The mice resulting from this cross will be referred to as TM/C mice.) Male and female 36T mice were bred with each other. At 15 to 18 days after fertilization, the embryos were removed and minced into small fragments, which were further dissociated by trypsinization. The cells were then cultured in F10 medium supplemented with 15 percent fetal calf serum. For the chromosome analyses, the cells were grown in medium containing $10^{-5}M$ BrdU for either 17 or 31 hours—that is, one or two cycles of DNA replication, respectively. The cells were harvested, fixed, stained with 33258 Hoechst, and photographed (*2*). The 33258 Hoechst was generously provided by Dr. H. Loewe, Hoechst AG, Frankfurt.

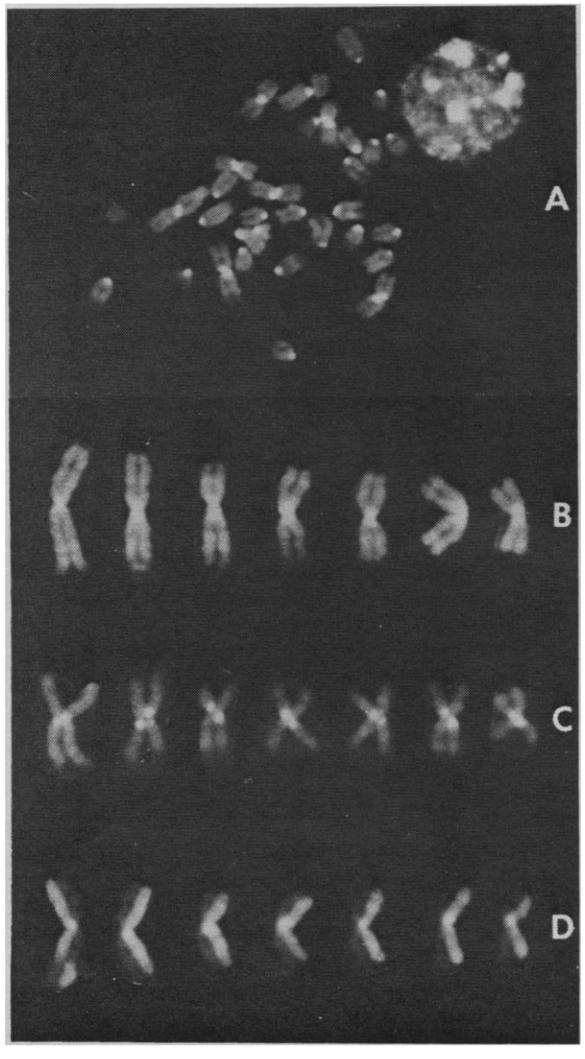
The fluorescent staining patterns of the chromosomes of TM/C cells are shown in Fig. 1. These cells are heterozygous for each of the seven metacentric chromosomes of the tobacco mouse. After one cycle of replication in BrdU, the centromeric region of each metacentric chromosome exhibits two brightly fluorescent spots, arranged one on either side of the centromere and on opposite arms of the chromo-

some. After two cycles of replication in BrdU, there is only one brightly fluorescent spot in the centromeric region of each metacentric chromosome. Also, after two cycles of replication in BrdU, there is a difference in fluorescence between the sister chromatids, as previously described for both mouse and human cells (*1, 2*). In these chromosomes the fluorescent centromeric spot is always associated with one of the brightly fluorescent chromatids.

The fluorescent staining patterns of the metacentric chromosomes of 36T cells are shown in Fig. 2. These cells are homozygous for each of two metacentric chromosomes. The arrangement of the fluorescent spots in the chromosomes of 36T cells grown for one or two generations in BrdU is identical to the arrangement in the TM/C cells.

The arrangement of the fluorescent centromeric spots in the metacentric chromosomes of TM/C and 36T cells grown in BrdU is the same as the arrangement of the centromeric spots in the metacentric chromosomes of the mouse cells of permanent lines already analyzed (*1*). However, in contrast to the uncertain origin of the metacentric chromosomes in the cells of the permanent lines, it is known that the metacentric chromosomes in the TM/C and 36T cells resulted from Robertsonian centric fusion between telocentric chromosomes. This permits us to make several suggestions concerning chromosome structure.

As was proposed (*1*), the brightly fluorescent spot in the centromeric region after replication in BrdU is probably associated with the thymidine-rich chain of satellite DNA. (The unequal content of thymidine in the two chains would lead to an unequal incorporation of BrdU into new chains, and therefore an unequal quenching of fluorescence after replication in BrdU.) The fluorescent spot therefore provides a marker for the "old" thymidine-rich DNA chain in the centromeric region after 1 and 2 cycles of replication in BrdU. Furthermore, the difference in fluorescence between the sister chromatids after two cycles of replication in BrdU provides a marker for the "old" DNA chain in the noncentromeric region. In the metacentric chromosomes of cells grown for two generations in BrdU the two brightly fluorescent nonsister chromatids and the fluorescent centromeric spot are all located on the same side (except where sister chromatid exchange has occurred). This indicates that the "old" DNA chains in the chromatids on both sides of the centromere and the "old" chain of satellite DNA in the centromeric region segregate together.



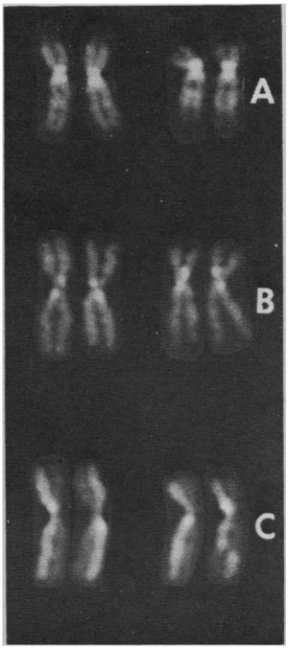


Fig. 1 (left). Fluorescent staining of TM/C chromosomes. (A) Complete metaphase of TM/C cell grown for one cycle of replication in $10^{-5}M$ BrdU. Metacentric chromosomes of cells grown for: (B) no, (C) one, and (D) two cycles of replication in $10^{-5}M$ BrdU. Fig. 2 (above). Fluorescent staining patterns of 36T chromosomes. Metacentric chromosomes of 36T cells grown for: (A) no, (B) one, and (C) two cycles of replication in $10^{-5}M$ BrdU.

The observation that the fluorescent centromeric spots are always on the opposite arms of the metacentric chromosomes after one cycle of replication in BrdU indicates that the centric fusion resulting in the formation of the metacentric chromosomes in these cells occurs with a definite polarity. Such polarity of centric fusion could result if the orientation of the chromosomes during centric fusion were dependent either on the maintenance of the polarity of the DNA through the centromere or on the differing base composition between the two chains of satellite DNA. The fact that the "old" chains of DNA in the chromatids and in the centromeric region segregate together in the fused chromosomes favors the hypothesis that the polarity of the DNA is maintained through the centromere; that is, that the DNA is continuous through the centromere, and that DNA polarity determines the orientation of centric fusion. It has been suggested that DNA polarity also is involved in determining the orientation of rejoining of sister chromatids after damage induced by x-rays (*7*). Continuity of the DNA through the centromere has been recently suggested also for *Drosophila* chromosomes (*8*) on the basis of biochemical experiments, and for a human translocation (*9*) on the basis of cytological experiments similar to those described above.

If it is correct that the polarity of the DNA is maintained through the centromere in the fused chromosomes, then the contralateral arrangement of the fluorescent centromeric spots in the metacentric chromosomes after one cycle of replication in BrdU suggests

that the thymidine-rich chain of satellite DNA is associated with the DNA chain of the same polarity in each pair of telocentric chromosomes which fuse. Since this arrangement was observed for nine different metacentric chromosomes, involving 15 of the 19 autosomes, it is likely that this is the general rule for centric fusion in the mouse.

The published data for the association of telocentric into metacentric chromosomes in the mouse allow the construction of a "chain" of centric fusions which links 18 of the 19 autosomes (*6, 10*). (No centric fusion with chromosome number 18 was reported.) For example, chromosome 15 has been observed to fuse with chromosomes 5 and 6, which have been observed to fuse with chromosomes 4 and 19. If it is assumed, as suggested above, that the thymidine-rich chain of satellite DNA is associated with the DNA chain of the same polarity in every pair of telocentric chromosomes that fuse to form a metacentric chromosome, then these results suggest that the thymidine-rich chain of satellite DNA is associated with the same DNA chain (in terms of polarity) in every mouse autosome. This finding may be relevant to the evolution of the chromosome complement in the mouse.

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References and Notes

1. M. S. Lin, S. A. Latt, R. L. Davidson, *Exp. Cell Res.* **86**, 392 (1974).
2. S. A. Latt, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3395 (1973).
3. I. Hilwig and A. Gropp, *Exp. Cell Res.* **75**, 122 (1972).
4. W. G. Flamm, M. McCallum, P. M. B. Walker, *Proc. Natl. Acad. Sci. U.S.A.* **57**, 1729 (1967).
5. A. Gropp, U. Tettenborn, E. von Lehmann, *Cytogenetics* **9**, 9 (1970).
6. B. J. White, J.-H. Tjio, L. C. Van de Water, C. Crandall, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2757 (1972).
7. J. G. Brewen and W. J. Peacock, *ibid.* **62**, 389 (1969).
8. R. Kavenoff and B. Zimm, *Chromosoma* **41**, 1 (1973).
9. S. A. Latt, R. L. Davidson, M. S. Lin, P. S. Gerald, *Exp. Cell Res.*, in press.
10. A. Gropp, H. Winking, L. Zech, H. Muller, *Chromosoma* **39**, 265 (1972); L. Zech, E. P. Evans, C. E. Ford, A. Gropp, *Exp. Cell Res.* **70**, 263 (1972).
11. Supported by NIH grants HD04807 and HD06276. We thank Drs. Samuel A. Latt and Park S. Gerald for suggestions and Dr. Latt for the use of his fluorescence microscope. We also thank Dr. Alfred Gropp for the tobacco mice, Dr. Beverly White for the 36T mice, and Dr. H. Loewe for the 33258 Hoechst.

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Pulmonary Alveolar Hypoxia: Release of Prostaglandins and Other Humoral Mediators

Abstract. *Hypoxic ventilation of isolated perfused cat lungs caused the frequent appearance in pulmonary perfusates of biologically active substances, which included prostaglandins or prostaglandin-like compounds. In anesthetized cats, inhibition of prostaglandin biosynthesis with infusions of aspirin (more than 50 milligrams per kilogram) reduced the pulmonary vasoconstrictor and bronchoconstrictor responses to hypoxic breathing.*

It has long been recognized that hypoxia induces constriction of pulmonary vessels, but the mechanisms of this reaction have remained incompletely understood (*1*). Recent evidence has suggested the possibility that the pulmonary vascular response to hypoxia may be mediated by chemical substances released from the lung. Such evidence includes: (i) the presence of lung tissue is required for the pressor response; pulmonary vessels stripped of lung tissue fail to constrict when exposed to low Po_2 (*2*); (ii) hypoxic pulmonary hypertension is reduced or abolished by cooling (*3*); and (iii) hypoxic ventilation is accompanied by morphologic signs of secretory activity in certain cells of the lung (*4*). We now report that alveolar hypoxia may provoke the synthesis and release of prostaglandin-like compounds and other mediators from isolated cat lungs, and that inhibition of prostaglandin biosynthesis in intact cats reduces the pulmonary vasoconstrictor and bronchoconstrictor responses to hypoxia.

We used two experimental approaches. (i) We perfused isolated cat lungs, allowing the perfusate from the lung to superfuse isolated smooth-muscle organs, for continuous detection and assay of any released biologically active material. (ii) We assessed the effect of metabolic inhibition of prostaglandin (PG) biosynthesis on the pulmonary vascular (and airway) reactions to hypoxia in the intact cat.

Perfusion of isolated cat lungs was carried out in 32 experiments. The lungs were perfused in situ (*5*) with 5 percent dextran in Krebs solution (19 experiments) or with a 1 : 1 mixture of this solution with the cat's own blood (13 experiments). The perfusing fluid was prewarmed to 37°C and pumped at a constant flow rate of 10 ml/min. Pulmonary venous pressure was maintained at 3 mm-Hg, and the outgoing perfusate constantly superfused (*6*) a series of up to four isolated smooth-muscle organs, selected from: trachea, ileum, and gallbladder of guinea pig; stomach and colon of rat; and chick rectum. The specificity of the responses of isolated organs was enhanced by pretreating them, in 22 instances, with agents which blocked the effects of histamine, serotonin, catecholamines, and acetylcholine (a solution containing 10^{-7} g of mepyramine maleate per milliliter, 2×10^{-7} g of methysergide bimaleate per milliliter, 2×10^{-6} g of propranolol HCl per milliliter, 10^{-7} g of phenoxybenzamine HCl per milliliter, and 10^{-7} g of hyoscine HBr per milliliter). The tissue responses were measured with Harvard isotonic transducers and continually recorded by a Beckman Dynograph. Perfusion (pulmonary arterial) pressure also was continually recorded. Samples of outflow perfusate also were collected during the experiment for subsequent assay and confirmation of the "on line" results. The lungs were ventilated with 21 percent O_2, 5 percent CO_2 in N_2, or with 2 percent O_2, 5 percent CO_2 in N_2, at predicted normal tidal volume and frequency. Hypoxic breathing was maintained for at least 10 minutes, and was repeated after the vascular and isolated tissue responses had returned to control range, or had stabilized at new levels.

A pressor response (mean pulmonary arterial pressure increase of at least 1 mm-Hg) was observed during hypoxia in 24 of the 32 experiments and averaged 31.6 percent for Krebs-dextran perfusions, or 38.7 percent for perfusions where blood was added. Whenever pulmonary hypertension occurred, it was accompanied by contraction of one or more of the assay organs (Fig. 1) in all but four cases. The contractions were independent of the presence of blood in the perfusate, and occurred in the following percentages of times in which the respective tissue was used: guinea pig trachea, 71; guinea pig gallbladder, 54; rat stomach strip, 38; rat colon, 31; guinea pig ileum, 29; chick rectum, 19. In 21 of these 24 experiments, the responses