more random steady-state patterns have been proposed for other forest ecosystems (*3, 16*). Inherent in the steady-state hypothesis is the idea that postdisturbance changes such as accelerated decomposition, mineralization, and nitrification are normal aspects of ecosystem maintenance and may even be components of a homeostatic response leading to rapid recovery.

DOUGLAS G. SPRUGEL
Department of Forestry, Michigan State University, East Lansing 48824

F. H. BORMANN
School of Forestry and Environmental Studies, Yale University, New Haven, Connecticut 06511

References and Notes

1. F. E. Clements, *J. Ecol.* **24**, 252 (1936).
2. E. W. Jones, *New Phytol.* **44**, 130 (1945); P. S. White, *Bot. Rev.* **45**, 229 (1979).
3. W. S. Cooper, *Bot. Gaz. (Chicago)* **55**, 1 (1913); A. S. Watt, *J. Ecol.* **35**, 1 (1947); H. E. Wright, *Science* **186**, 487 (1974).
4. P. M. Vitousek and W. A. Reiners, *BioScience* **25**, 376 (1975).
5. D. G. Sprugel, thesis, Yale University, New Haven, Conn. (1974).
6. ———, *J. Ecol.* **64**, 889 (1976).
7. Y. Oshima, M. Kimura, H. Iwaki, S. Kuroiwa, *Bot. Mag. Tokyo* **71**, 289 (1958); H. Iwaki and T. Totsuka, *ibid.* **72**, 255 (1959); S. Kuroiwa, *ibid.*, p. 413.
8. K. Kai, *Geogr. Rev. Jpn.* **47**, 709 (1974).
9. We studied the bands by laying transects through 16 different bands on Whiteface Mountain, New York, and on various mountains in New England. Each transect began in the mature forest uphill from the zone of death and extended to the mature forest on the other side. At 10-m intervals along each transect we chose five trees at random and determined their ages. We also periodically inspected trees at or near the wave edges and repeatedly photographed marked plots.
10. The direction of wave movement on Whiteface Mountain was estimated on the ground and then checked and corrected if necessary by referring to aerial photographs. Wave speed was estimated by calculating the slope of a graph of the age of the trees plotted against their distance from the wave edge.
11. S. Nicholson and J. T. Scott, in *Vegetation-Environment Relations at Whiteface Mountain in the Adirondacks*, J. G. Holway and J. T. Scott, Eds. (State Univ. of New York, Albany, 1969), p. 149; E. W. Holroyd, *For. Sci.* **16**, 222 (1970).
12. G. S. Raynor, *For. Sci.* **17**, 351 (1971).
13. J. Warren Wilson, *J. Ecol.* **47**, 415 (1959); R. Geiger, *The Climate Near the Ground* (Harvard Univ. Press, Cambridge, Mass., 1966), p. 350.
14. R. F. Morris, *For. Chron.* **24**, 106 (1948).
15. F. H. Bormann, G. E. Likens, D. W. Fisher, R. S. Pierce, *Science* **159**, 882 (1968); W. H. Smith, F. H. Bormann, G. E. Likens, *Soil Sci.* **106**, 471 (1968); G. E. Likens, F. H. Bormann, N. M. Johnson, D. W. Fisher, R. S. Pierce, *Ecol. Monogr.* **40**, 23 (1970); F. H. Bormann and G. E. Likens, *Sci. Am.* **223**, 92 (October 1970); A. S. Dominski, thesis, Yale University, New Haven, Conn. (1971); F. H. Bormann, G. E. Likens, T. G. Siccama, R. S. Pierce, J. S. Eaton, *Ecol. Monogr.* **44**, 255 (1974); W. W. Covington, thesis, Yale University, New Haven, Conn. (1976); G. E. Likens, F. H. Bormann, R. S. Pierce, J. S. Eaton, N. M. Johnson, *Biogeochemistry of a Forested Ecosystem* (Springer-Verlag, New York, 1977).
16. F. H. Bormann and G. E. Likens, *Pattern and Process in a Forested Ecosystem* (Springer-Verlag, New York, 1979).
17. H. Hesselman, *Medd. Statens Skogsforsoeksans.* **13–14**, 923 (1917); L. G. Romell, *Cornell Univ. Agric. Exp. Stn. Mem. 170* (1935); R. F. Wright, *Ecology* **57**, 649 (1976).
18. R. G. Woodmansee, *BioScience* **28**, 488 (1978).
19. W. J. Mattson and N. D. Addy, *Science* **190**, 515 (1975).
20. R. H. Whittaker and G. M. Woodwell, in *Productivity in Forest Ecosystems*, P. Duvigneaud, Ed. (Unesco, Paris, 1971), p. 159.
21. S. A. Wilde, S. K. Voigt, J. G. Iyer, *Soil and Plant Analysis for Tree Culture* (Oxford and IBH, New Delhi, 1972), pp. 56–63.
22. C. J. Krebs, *Ecology: The Experimental Analysis of Distribution and Abundance* (Harper & Row, New York, ed. 2, 1978), pp. 455–456.
23. G. E. Likens and F. H. Bormann, *Yale Univ. Sch. For. Bull.* **79**, (1970).

24. Supported in part by grants from the National Science Foundation to the Hubbard Brook Ecosystem Study. We thank G. E. Hutchinson, R. S. Miller, R. N. Muller, and D. M. Smith for helpful suggestions; S. MacIntyre and J. Ginsberg for assistance in the field; and the Atmospheric Sciences Research Center of the State University of New York, Albany, for the use of their facilities.

21 February 1980; revised 6 October 1980

Reactivation of an Inactive Human X Chromosome: Evidence for X Inactivation by DNA Methylation

Abstract. *A mouse-human somatic cell hybrid clone, deficient in hypoxanthine-guanine phosphoribosyltransferase (HPRT) and containing a structurally normal inactive human X chromosome, was isolated. The hybrid cells were treated with 5-azacytidine and tested for the reactivation and expression of human X-linked genes. The frequency of HPRT-positive clones after 5-azacytidine treatment was 1000-fold greater than that observed in untreated hybrid cells. Fourteen independent HPRT-positive clones were isolated and analyzed for the expression of human X markers. Isoelectric focusing showed that the HPRT expressed in these clones is human. One of the 14 clones expressed human glucose-6-phosphate dehydrogenase and another expressed human phosphoglycerate kinase. Since 5-azacytidine treatment results in hypomethylation of DNA, DNA methylation may be a mechanism of human X chromosome inactivation.*

According to the Lyon hypothesis, one of the two X chromosomes is inactivated in the somatic cells of the normal mammalian female (*1*). This event occurs early in embryonic development, and either the paternal or maternal X is randomly inactivated. Once established, the pattern of inactivation remains the same for that cell and all of its descendants. This inactivation process achieves dosage compensation between males and females for X-linked gene products. The main features of the X-inactivation theory have been amply verified by genetic, cytologic, and biochemical investigations (*2*). The female embryo initially has two active X chromosomes and one X is inactivated in somatic cells later in development (*3*). The entire human X is not inactivated, since the locus for microsomal steroid sulfatase (STS) escapes inactivation (*4–6*). The gene locus for STS is at the distal end of the short arm of the human X chromosome (*5, 7*).

Table 1. Production of HAT-resistant subclones from clone 37-26R-D after 5-azacytidine treatment. Clone 37-26R-D contains the structurally normal inactive human X chromosome. Cells were plated into 60-mm dishes (10^5 cells per dish) and 24 hours later, they were treated with 5-azacytidine at the concentration indicated. After a 24-hour exposure, the cultures were washed and maintained in normal growth medium for 72 hours. The medium was then changed to HAT (*15*), and after maintenance in HAT for 2 weeks, colonies were visible. Cells were washed with isotonic saline and fixed with methanol and acetic acid (3:1). Cells were stained with 1 percent Giemsa, and colonies were counted. The average number of colonies per treatment is derived from five dishes at each concentration.

Concentration of 5-azacytidine (μM)	HAT-resistant clones per 10^5 cells	
	Average	Range
Control	0	
0.1	0	
0.5	1	0–3
1	26	23–30
2	142	133–151
5	107	90–138
10	41	35–50

Although several models have been proposed to explain the mechanism of X inactivation at a molecular level (*8*), experimental evidence in support of these theories is lacking. One hypothesis suggests that DNA modification by methylation could be a mechanism of X inactivation (*9*); this model could be verified by modifying or reversing X inactivation by interfering with DNA methylation. The only methylated base found in mammalian DNA is 5-methylcytosine (*10, 11*), and 5-azacytidine (5-azaC) is a cytidine analog that can be integrated into DNA (*12, 13*). Incorporation of 5-azaC leads to hypomethylation of DNA (*13*), which is attributed to impaired methylation at the sites of substitution of 5-azaC as a result of the presence of nitrogen in place of carbon at the fifth position in the pyrimidine ring. Treatment of mouse fibroblasts with 5-azaC not only significantly reduces DNA methylation but also induces formation

0036-8075/81/0123-0393$00.75/0

Table 2. Frequency of HPRT-positive clones in three cell lines. Cells were plated, treated, and evaluated as described in the legend to Table 1. Duration of each treatment was 24 hours. The mouse parental line is A9; 37-26R-A is a hybrid clone with a human chromosome constitution identical to that of 37-26R-D except for the absence of the inactive human X. Values designated I and II are from two different experiments. Average numbers of colonies in experiments I and II were derived from ten and five dishes, respectively. For plating efficiency determinations, 200 cells of 37-26R-D were seeded in a 60-mm dish in experiment II and, after treatment, were maintained in normal growth medium for 10 days. Cells were then fixed and stained, and the colonies were counted. The plating efficiency was derived from five dishes per treatment, and the relative plating efficiency (given as percentage) was determined by taking that of the control to be 100. The absolute plating efficiency of the control averaged 20 percent.

Treatment and concentration	Frequency of HPRT-positive clones (per 10^5 cells)						
	37-26R-D			A9		37-26R-A	
	I	II	Relative plating efficiency (%)	I	II	I	II
Control	0.1	0	100	0	0	0	0
Cytidine (10 μM)	0.1	0	136	0	0	0	0
6-Azacytidine (10 μM)	0						
6-Azacytidine (2 μM)		0	80				
5-Azacytidine (10 μM)	28.3			0	0	0	0
5-Azacytidine (5 μM)	74.5						
5-Azacytidine (2 μM)		117.4	63		0		0
5-Azacytidine (2 μM) + cytidine (10 μM)		0	61				
5-Azacytidine (2 μM) + cytidine (20 μM)		0.4	63				
Arabinosylcytosine (2 μM)		0	5				
5-Bromodeoxyuridine (10 μM)		0	45				

of differentiated cellular phenotypes not previously expressed, such as myotubes (*13, 14*). We therefore assessed the effects of 5-azaC on the expression of genes located on an inactivated human X chromosome.

Somatic cell hybrids were derived in hypoxanthine, aminopterin, thymidine, and glycine (HAT) medium (*15*) by fusing an established mouse line (A9) deficient in hypoxanthine-guanine phosphoribosyltransferase (HPRT) to fibroblasts from a human female carrying a balanced X/11 translocation [46,X,t(X;11)(p21;q13); GM1695, Institute for Medical Research, Camden, New Jersey] (*6*). In the human parental cells, the distal half of the short arm of an X chromosome is translocated to the long arm of chromosome 11, and a portion of the long arm of chromosome 11 is in turn translocated to the short arm of the X chromosome. As in most carriers of balanced X/autosome translocations (*16*), the structurally normal X chromosome is genetically inactive (X^{in}) in these human cells (*17*). Thus, there is a morphological difference between the active and inactive X chromosomes in the human parental cells. The translocated X chromosome containing the X long arm [der(X)] carries the active human HPRT locus. Among the primary hybrid clones isolated from the fusion experiment, one contained the X^{in} and der(X), as shown by cytogenetic analysis. This hybrid clone was treated with 8-azaguanine to select secondary clones that had lost the human der(X) chromosome carrying the active HPRT gene. Secondary clones were obtained with and without the inactive human X chromosome. Hybrid clones with only the X^{in} did not express human glucose-6-phosphate dehydrogenase (G6PD), phosphoglycerate kinase (PGK), or HPRT, but did express human STS, since the STS locus escapes X inactivation (*4*). Thus, the presence of X^{in} in hybrid clones could be monitored by evaluation of STS, as well as by cytogenetic analysis.

Two secondary clones and the mouse parental line A9 were used in the experiments reported. Clone 37-26R-D retained human chromosomes 3, 4, 5, 7,

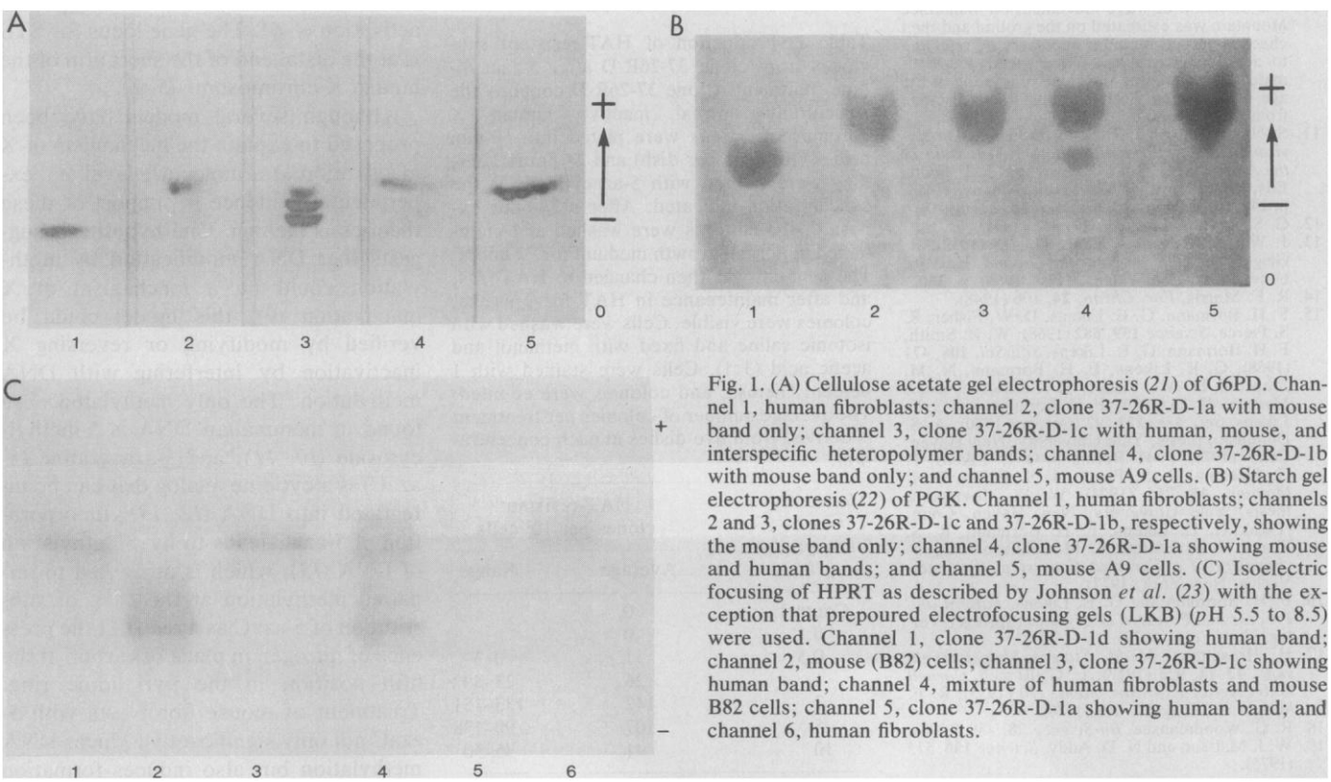

Fig. 1. (A) Cellulose acetate gel electrophoresis (*21*) of G6PD. Channel 1, human fibroblasts; channel 2, clone 37-26R-D-1a with mouse band only; channel 3, clone 37-26R-D-1c with human, mouse, and interspecific heteropolymer bands; channel 4, clone 37-26R-D-1b with mouse band only; and channel 5, mouse A9 cells. (B) Starch gel electrophoresis (*22*) of PGK. Channel 1, human fibroblasts; channels 2 and 3, clones 37-26R-D-1c and 37-26R-D-1b, respectively, showing the mouse band only; channel 4, clone 37-26R-D-1a showing mouse and human bands; and channel 5, mouse A9 cells. (C) Isoelectric focusing of HPRT as described by Johnson *et al.* (*23*) with the exception that prepoured electrofocusing gels (LKB) (*p*H 5.5 to 8.5) were used. Channel 1, clone 37-26R-D-1d showing human band; channel 2, mouse (B82) cells; channel 3, clone 37-26R-D-1c showing human band; channel 4, mixture of human fibroblasts and mouse B82 cells; channel 5, clone 37-26R-D-1a showing human band; and channel 6, human fibroblasts.

10, 13, 14, 15, 18, 21, and X^{in}. Clone 37-26R-A had an identical human chromosome constitution except that X^{in} was not retained. Data summarized in Tables 1 and 2 show that 5-azaC treatment yielded HPRT-positive clones from 37-26R-D cells, but not from 37-26R-A or A9 cells. The frequency of HAT-resistant colonies under optimal conditions (2 μM 5-azaC for 24 hours) was approximately one per thousand, compared with approximately one per million in untreated cells of 37-26R-D. Several of the HPRT-positive colonies were isolated and analyzed for the expression of human X markers. All of the clones expressed human HPRT and STS (Table 3 and Fig. 1C). The expression of human STS indicates the presence of the structurally normal X chromosome in these clones, and this was verified in one clone by cytogenetic analysis (Table 3). One of the independent clones isolated (37-26R-D-1c) expressed human G6PD, and another (37-26R-D-1a) expressed human PGK (Table 3 and Fig. 1, A and B). The expression of human G6PD was also observed in pooled cells from HPRT-positive clones after 5-azaC treatment, thus indicating the reactivation of the G6PD locus in additional clones. Serial dilution experiments on mouse-human hybrid cells with and without an active human X chromosome showed that at least 3 percent of the cells must have an active X for our technique to detect the expression of human G6PD. Expression of human PGK was not detected in these pooled cells, but the sensitivity of our electrophoretic technique for the detection of human PGK is much lower than that for G6PD.

Clone 37-26R-D-1c expressed human G6PD and HPRT after continuous growth either in HAT or nonselective medium (MEM) for seven passages (at least 15 doublings during 5 weeks). Five independent subclones derived from this clone also expressed human G6PD and HPRT, an indication that the changes in X-chromosomal gene expression induced by 5-azaC are stable and are maintained in the absence of 5-azaC or selective pressure. Treatment of hybrid clone 37-25R-D with 6-azacytidine, arabinosylcytosine, and 5-bromodeoxyuridine did not yield any HPRT-positive clones. Simultaneous treatment of 37-26R-D cells with 5-azaC and cytidine (five- or tenfold molar excess) inhibited the production of HPRT-positive clones. The relative plating efficiency values indicate that this inhibition was not due to cytotoxicity (Table 2).

Earlier attempts have failed to induce reactivation of the inactive X in human somatic cells (*18*) although spontaneous reactivation of the HPRT locus from the inactive human X in mouse-human hybrid cells had been observed (*19*). In the latter studies HAT selection was used to isolate hybrid cells with a reactivated human HPRT locus. However, reactivation was not reported for any other human X markers, and HPRT-positive clones were found at a frequency comparable to that observed for untreated hybrids. This is one-thousandth of the frequency observed after 5-azaC treatment.

The proposal that DNA methylation is a mechanism of cellular differentiation (*11, 20*) has recently been reviewed (*10*). A key feature of such models is the maintenance of the methylation pattern through successive cell divisions. Methylcytosine usually occurs in specific palindromic sequences, and whenever a relevant cytosine residue is methylated, the cytosine in the symmetrical position on the complementary strand of DNA is also methylated. This is believed to be achieved by the presence of a DNA methylase (maintenance methylase) that rapidly methylates the second cytosine in a half-methylated DNA palindrome after DNA replication. It is also assumed that the maintenance methylase cannot methylate completely unmethylated sites. This provides for the stability of a given pattern of DNA methylation through many cycles of DNA replication and cell division.

Our studies indicate that human gene loci on the inactive X chromosome can be reactivated, by treatment with 5-azaC. Our interpretation of these results is the following. We will consider the sequence

. . . CCGG . . .

. . . GGCC . . .

as an example of a typical methylation site, and assume that the internal cytosine residues of this palindrome are methylated. We will further assume that during a DNA replication cycle 5-azaC replaces cytosine at random at one of these two critical sites in a daughter molecule. When this DNA molecule undergoes another round of replication in the absence of 5-azaC, one of the two resulting molecules will be half-methylated and the other will be completely unmethylated but will have a 5-azacytosine residue in one strand. The latter DNA molecule would not be a substrate for the maintenance methylase, which

Table 3. Biochemical and cytogenetic analyses of hybrid clones; + indicates presence and − indicates absence of the human chromosome or enzyme marker. Hybrid clone 37-26 was isolated in HAT medium after fusion of HPRT-deficient A9 mouse cells and human cells carrying an X/11 translocation (*6*). The subclones of 37-26, 37-26R-D, and 37-26R-A were isolated in medium containing 8-azaguanine. Clones 37-26R-D-1a, -1b, -2a, -2b, -2c, and -1c were isolated in HAT medium after treatment of 37-26R-D with 5-azacytidine, and 37-26R-D-1d was isolated in HAT after spontaneous reactivation. Clone 37-26R-D-1c was analyzed at passages 4 and 11 (grown in HAT) and passage 11 (grown in MEM from passage 4 onward); 37-26R-D-1c-1, -2, -3, -4, and -5 are subclones of 37-26R-D-1c isolated in HAT medium. Chromosome analyses of hybrid clones were done with the aid of Q banding as described in (*24*). Enzyme markers G6PD (*21*) and PGK (*22*) were evaluated by electrophoresis, and HPRT was evaluated by isoelectric focusing (*23*). Microsomal steroid sulfatase (STS) activity was measured as in (*25*). The STS activity detected in these hybrid clones is human (*7*). Eight additional independent HPRT-positive clones were found to have no expression of human G6PD and PGK.

Hybrid clone	Human X markers				Human X constitution		
	G6PD	PGK	HPRT	STS	X	der(X)*	der(11)†
37-26	+	+	+	+	+	+	−
37-26R-D	−	−	−	+	+	−	−
37-26R-A	−	−	−	−	−	−	−
37-26R-D-1a	−	+	+	+			
37-26R-D-1b	−	−	+	+			
37-26R-D-1d	−	−	+	+			
37-26R-D-2a	−	−	+	+			
37-26R-D-2b	−	−	+	+			
37-26R-D-2c	−	−	+	+			
37-26R-D-1c/4	+	−	+	+	+	−	−
37-26R-D-1c/11 (HAT)	+	−	+	+			
37-26R-D-1c/11 (MEM)	+	−	+	+			
37-26R-D-1c-1	+	−	+	+			
37-26R-D-1c-2	+	−	+	+			
37-26R-D-1c-3	+	−	+	+			
37-26R-D-1c-4	+	−	+	+			
37-26R-D-1c-5	+	−	+	+			

*Translocation chromosome: Xqter → Xp21 :: 11q13 → 11qter. †Translocation chromosome: Xpter → Xp21 :: 11q13 → 11pter.

recognizes only a half-methylated site. Therefore, all subsequent copies of this DNA will no longer be methylated at the designated sequence. If a given site is unmethylated after 5-azaC incorporation, a specific gene could be reactivated. Those cells in which the HPRT locus was reactivated by incorporation of 5-azaC at the necessary site grew in HAT medium. The stability of the reactivated gene loci in our experiments is also in keeping with the model.

It appears from the evaluation of human G6PD and PGK that demethylation at a specific site (or sites) does not reactivate the entire X chromosome, since G6PD and PGK were not expressed in all clones in which HPRT was reactivated. We interpret this as evidence for the inactivation of the X in discrete units. It may be that the three X markers we have evaluated, G6PD, PGK, and HPRT, are in differentially regulated segments. Such a mechanism is compatible with the existence on the human X chromosome of loci such as that for STS, which may be in a segment that is never inactivated. However, the frequency of reactivation of HPRT is at best one per thousand, whereas the expression of human G6PD or PGK was detected in 1 of 14 independent HPRT-positive clones isolated. Also, the expression of human G6PD was detectable in pooled samples of reactivated clones. Thus, coordinate reactivation of HPRT and G6PD or PGK appears to occur at a frequency greater than that expected by chance.

Our results thus provide evidence that DNA methylation plays a role in inactivation of the X chromosome and that inactivation occurs on the human X in a segmental fashion. It is well established that DNA-protein interactions are altered when DNA is methylated (*9*, *10*). Such changes could account for the inactivation of X-linked genes and heterochromatization of the X chromosome. The exact nature of this alteration must be resolved by further experimentation.

T. MOHANDAS
Division of Medical Genetics, Department of Pediatrics, Harbor-University of California Medical Center, Torrance 90509
R. S. SPARKES
Division of Medical Genetics, Departments of Medicine, Pediatrics, and Psychiatry, Center for the Health Sciences, University of California, Los Angeles 90024
L. J. SHAPIRO
Division of Medical Genetics, Department of Pediatrics, Harbor-University of California Medical Center

References and Notes

1. M. F. Lyon, *Nature (London)* **190**, 370 (1961).
2. ———, *Biol. Rev. Cambridge Philos. Soc.* **47**, 1 (1972); S. M. Gartler and R. J. Andina, *Adv. Hum. Genet.* **7**, 99 (1976).
3. P. G. Kratzer and S. M. Gartler, *Nature (London)* **274**, 503 (1978); C. J. Epstein, S. Smith, B. Travis, G. Tucker, *ibid.*, p. 500; G. R. Martin *et al.*, *ibid.* **271**, 329 (1978).
4. L. J. Shapiro, T. Mohandas, R. Weiss, G. Romeo, *Science* **204**, 1224 (1979).
5. C. R. Muller, A. Westerveld, B. Migl, W. Franke, H. H. Ropers, *Hum. Genet.* **54**, 201 (1980).
6. T. Mohandas, R. S. Sparkes, B. Hellkuhl, K. H. Grzeschik, L. J. Shapiro, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
7. T. Mohandas L. J. Shapiro, R. S. Sparkes, M. C. Sparkes, *ibid.* **76**, 5779 (1979).
8. M. M. Grumbach, A. Morishima, J. H. Taylor, *ibid.* **49**, 581 (1963); M. F. Lyon, *Nature New Biol.* **232**, 299 (1971); S. Ohno, *Annu. Rev. Genet.* **3**, 495 (1969); *Cold Spring Harbor Symp. Quant. Biol.* **38**, 155 (1974); P. R. Cook, *Nature (London)* **245**, 23 (1973).
9. A. D. Riggs, *Cytogenet. Cell Genet.* **14**, 9 (1975).
10. A. Razin and A. D. Riggs, *Science* **210**, 604 (1980).
11. J. H. Taylor, in *Molecular Genetics*, part III, *Chromosome Structure*, J. H. Taylor, Ed. (Academic Press, New York, 1979), pp. 89–115.
12. A. Cihak, *Oncology* **30**, 405 (1974).
13. P. A. Jones and S. M. Taylor, *Cell* **20**, 85 (1980).
14. P. G. Constantinides, P. A. Jones, W. Geners, *Nature (London)* **267**, 364 (1977); P. G. Constantinides, S. M. Taylor, P. A. Jones, *Dev. Biol.* **66**, 67 (1978); S. M. Taylor and P. A. Jones, *Cell* **17**, 771 (1979).
15. J. W. Littlefield, *Science* **145**, 709 (1964).
16. R. L. Summitt, R. E. Tipton, R. S. Wilroy, P. R. Martens, J. P. Phelan, *Birth Defects, Orig. Artic. Ser.* **14**, 219 (1978).
17. R. M. Greenstein, M. P. Reardon, T. S. Chan, *Pediatr. Res.* **11**, 457 (1977).
18. D. E. Comings, *Lancet* **1966-II**, 1137 (1966); B. R. Migeon, *Nature (London)* **239**, 87 (1972).
19. B. Kahan and R. DeMars, *Proc. Natl. Acad. Sci, U.S.A.* **72**, 1510 (1975); B. Hellkuhl and K.-H. Grzeschik, *Cytogenet. Cell Genet.* **22**, 527 (1978).
20. R. Holliday and J. E. Pugh, *Science* **187**, 226 (1975).
21. R. S. Sparkes, M. C. Baluda, D. E. Townsend, *J. Lab. Clin. Med.* **73**, 531 (1969).
22. E. Beutler, *Biochem. Genet.* **3**, 189 (1969).
23. G. G. Johnson, L. R. Eisenberg, B. R. Migeon, *Science* **203**, 174 (1979).
24. T. Mohandas, R. S. Sparkes, M. C. Sparkes, J. D. Shulkin, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5628 (1977).
25. L. J. Shapiro *et al.*, *Lancet* **1978-II**, 756 (1978).
26. We thank M. C. Sparkes, R. Weiss, and M. B. Passage for technical assistance and K. Nelson for assistance in preparation of the manuscript. Supported in part by grants from the National Institute of Child Health and Human Development (HD-12178, HD-05615 and HD-04612) and from the National Foundation (1-639). L.J.S. is the recipient of a research career development award from NICHD.

15 August 1980

Mouse Oocytes Transcribe Injected *Xenopus* 5S RNA Gene

Abstract. *Transcripts produced after injection of the* Xenopus *5S RNA gene into oocyte germinal vesicles of mice migrate electrophoretically with the 5S RNA marker, an indication that the gene is transcribed and processed with considerable accuracy. Approximately two 5S RNA molecules are transcribed per gene per hour. This system may be useful in studying DNA processing and gene regulation by the mammalian ovum and might be modified to allow permanent incorporation of specific genes into mice.*

The *Xenopus* oocyte has been used extensively to study the biological activity of macromolecules introduced by microinjection (*1–3*). The oocyte translates numerous types of injected messenger RNA (mRNA) (*2*) and transcribes several types of DNA with great fidelity (*4–5*). This system has been extraordinarily useful in studying the processing of injected mRNA and DNA by a normal living cell (*5*). Globin mRNA microinjected into the mouse oocyte or into a one-cell fertilized ovum is translated to globin protein (*6*). The translation characteristics of the mouse oocyte are different from those of the *Xenopus* oocyte, and therefore injection of the mouse ovum provides a valuable additional technique for studying mRNA processing in the intact cell. We now report that the mouse oocyte is also capable of transcribing foreign genes introduced by microinjection and can thus be used to study DNA processing by the mammalian egg cell.

Growing mouse oocytes (50 to 60 μm in diameter) were dissected from the ovaries of 14-day-old hybrid C57 × SJL females. The oocytes were collected in Brinster's medium for ovum culture (BMOC) (*7*) modified by the addition of bovine serum albumin (4 mg/ml), glucose (1 mg/ml), Eagle's essential and nonessential amino acids, and 10 percent fetal calf serum (BMOC-2-M). Mature oocytes (just prior to ovulation) were dissected from the ovaries of 6- to 8-week-old mice. The medium used for collection and maintenance was BMOC-2 (*7*). Fertilized ova were flushed from the oviduct with BMOC-2 on day 1 of pregnancy. The gene used in these experiments was for somatic *5S* RNA from *Xenopus borealis*; two repeating units were cloned in plasmid pBR322. The characteristics of the cloned sequence and gene (pXbs1) have been described (*8*). The concentration of the DNA was 1 mg/ml in a dilute salt solution (0.015*M* NaCl, 0.5 m*M* EDTA, and 5 m*M* tris at *p*H 7.8). The injection procedure was similar to that previously employed for injecting cells into blastocysts (*9*) and mRNA into one-cell fertilized ova (*6*). Growing oocytes were placed on a depression slide in BMOC-2 containing cytochalasin B (5 to 10 μg/ml) and held with a blunt pipette (*6*). The tip of the injector pipette was filled with the DNA

0036-8075/81/0123-0396$00.50/0