excluding the PRPP synthetase gene from the region of the X chromosome distal to the point of translocation, namely Xq26.

Assignment of the structural locus for PRPP synthetase to a position on the X chromosome segment between the centromere and the locus for HGPRT, closer to the locus for HGPRT than other known markers in this segment, raises interesting possibilities concerning a functional role for this arrangement of loci coding for sequential enzymes of PRPP metabolism. On the basis of studies on the PRPP synthetase levels in fibroblasts deficient in HGPRT, it has previously been proposed that the HGPRT locus may have some regulatory effect on the production of PRPP synthetase (*17*). Unfortunately, since the clones used in the present study were not a random set, but were specially chosen, it is not possible to apply the method of Goss and Harris (*10*) to estimate the distance between the PRPP synthetase and HGPRT genes with any reliability. Only further studies will make it possible to judge whether the apparent proximity of these two genes could be important in the regulation of PRPP synthesis and utilization.

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Methylation of Mouse Liver DNA Studied by Means of the Restriction Enzymes Msp I and Hpa II

Abstract. *The restriction enzymes Hpa II and Msp I both recognize the sequence 5'-CCGG (C, cytosine; G, guanine). However, Hpa II cuts mouse liver DNA to fragments four times larger than does Msp I. The size of DNA cut by Msp I is close to that predicted from base composition and nearest neighbor analysis. The most probable explanation of these results is that in mouse the site 5'-CCGG is highly methylated.*

5-Methylcytosine (5-MeCyt) is the only minor base found in vertebrate DNA (3 to 10 percent of cytosine), and is known to result from the enzymatic methylation of cytosine at the DNA level (*1*). The function of this modified base is not known although there has been speculation that it may be involved in differentiation (*2*).

Nearest neighbor and pyrimidine tract analysis have shown that 5-MeCyt occurs most often in the dinucleotide CpG (C, cytosine; G, guanine) (*3*), but little more is known about the sequence specificity of DNA methylation. Several groups have used restriction enzymes to probe the distribution of 5-MeCyt in eukaryotic DNA, the rationale being that 5-MeCyt in the site recognized by a DNA restriction enzyme would prevent cutting of DNA at that site (*4-6*). Gautier *et al.* (*5*) found that the restriction enzymes Hha I, Hpa II, and Sma I cut calf thymus satellite DNA only after the DNA had been cloned, thereby altering its pattern of methylation. Bird and Southern (*4*) similarly showed that the enzymes Hha I, Hpa II, and Ava I do not cut highly methylated (14 percent 5-MeCyt of the total cytosine) somatic ribosomal DNA of *Xenopus*, but do cut

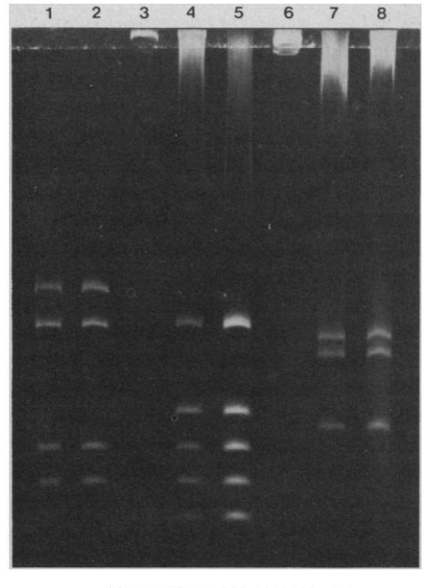

Fig. 1. Comparison of Hpa II and Msp I. One microgram each of ϕX174 pBR333 and pBR345 was treated with 2 units of Hpa II or Msp I (*8*). Mixtures were then subjected to electrophoresis on a 5 percent acrylamide gel, stained with ethidium bromide, and photographed (*10*). (Channel 1) pBR345 + Msp I; (Channel 2) pBR345 + Hpa II; (Channel 3) pBR345 uncut; (Channel 4) pBR333 + Msp I; (Channel 5) pBR333 + Hpa II; (Channel 6) pBR333 uncut; (Channel 7) ϕX174 plus Msp I; (Channel 8) ϕX174 + Hpa II.

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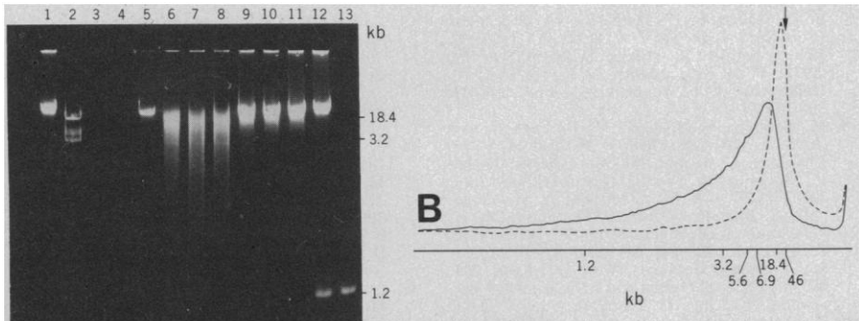

Fig. 2. Size of mouse liver DNA treated with Hpa II or Msp I. (A) Gel pattern. Mouse liver DNA (2 μg) was treated with either Msp I or Hpa II and incubated for 1.5 hours at 37°C in the appropriate buffer (*8*). Mixtures were then applied to a 1.6 percent agarose gel, subjected to electrophoresis, stained with ethidium bromide, and photographed (*10*). Mouse liver DNA: (channel 1) uncut; (channel 6) +Msp I (4 units) + pBR345; (channel 7) +Msp I (8 units); (channel 8) +Msp I (4 units); (channel 9) +Hpa II (4.5 units) +pBR345; (channel 10) + Hpa II (9 units); (channel 11) +Hpa II (4.5 units); (channel 12) uncut + pBR345. Standards: (channel 2) λplac5 + Eco R1; (channel 3) pBR345 + Msp I; (channel 4) pBR345 + Hpa II; (channel 5) λplac5 uncut. (B) Densitometer tracing of gel-fractionated DNA, cut with Hpa II (dotted line) or Msp I (solid line). Arrow indicates the position of uncut DNA. Sizes shown are those of λplac5 and fragments pBR345 cut by Eco R1 (*14*).

unmethylated oocyte ribosomal DNA. We report our studies on mouse liver DNA showing that, where unmethylated DNA is not available as a control, the enzymes Hpa II and Msp I can be used in conjunction to probe for methylation of the sequence 5′-CCGG.

Hpa II recognizes the sequence 5′-CCGG, but does not cleave it when the internal cytosines are methylated (*7*). Msp I is sold by New England Biolabs as an isoschizomer of Hpa II. The data in Fig. 1 confirm that the two enzymes recognize the same sequence; they give identical results when they cut the DNA of bacteriophage ϕX174 or the plasmids pBR333 or pBR345 (*8*). However, as described below, they differ in the way they cut mouse liver DNA.

The DNA we used was of high molecular size [50 kilobase pairs (kbp)], isolated from females of strain CF 1 (*9*). The DNA was treated with Hpa II or Msp I and fractionated according to size by electrophoresis on agarose gels (*10*). To determine the size distribution of the DNA, the gels were stained with ethidium bromide and photographed onto Polaroid type 55 positive-negative film. DNA treated with Hpa II (Fig. 2A, lanes 9 to 11) yields fragments much larger in size than those cut by Msp I (Fig. 2A, lanes 6 to 8). The difference is shown more clearly by densitometer tracings of the negatives (*10*) (Fig. 2B). The completeness of digestion with each enzyme was ascertained in two ways. (i) A twofold increase in enzyme concentration gave the same DNA fragment size distribution (Fig. 2A, lanes 7 and 8 and 10 and 11); (ii) when the plasmid pBR345 was added to reaction mixtures as an internal control, the uncut plasmid completely disappeared (Fig. 2A, lanes 6 and 9; the largest restriction fragment of pBR345 is visible at the bottom of each lane). In addition, when plasmid pBR345 was purified together with mouse DNA, the plasmid DNA was still completely cut by Hpa II (results not shown), demonstrating that digestion by Hpa II was not being limited by an inhibitor copurified with the DNA.

To quantify the difference in size between Hpa II and Msp I fragments, we developed a method in which the densitometer tracings of film negatives were used. After confirming that the area under the peaks of the densitometer tracings was proportional to DNA concentration (*4*), we determined the weight average length of DNA ($\bar{L}_w$) as follows. First, we determined the axis bisecting each peak into equal areas by cutting and weighing. We then found the corresponding value of $\bar{L}_w$ by use of a "standard curve" consisting of a semilog plot of the distance migrated by fragments of known size (Eco R1–treated λplac5) subjected to electrophoresis on the same gel. The $\bar{L}_w$ was determined in this way for several DNA's restricted by different enzymes; the method was reproducible, the maximum standard deviation being approximately 10 percent.

The $\bar{L}_w$ of DNA cut by Msp I (calculated from nine experiments) is 4.5 ± 0.5 kbp while that of Hpa II cut DNA (calculated from six experiments) is 17.8 ± 0.3 kbp, a fourfold difference in size. These values for mouse DNA were compared with that predicted from base composition, nearest neighbor analysis, and assumed random sequence distribution. The base composition of mouse DNA is 21 percent each of C and G (*11*), and its nearest neighbor frequencies are CpC and GpG, .05; CpG, .009 (*12*). Therefore, the sequence 5′-CCGG should occur with a frequency of (.21) × (.05/.21) × (.009/.21) × (.05/.21) = .0005. This is equal to 25 cuts for every 50 kbp (the average size of our uncut DNA). Since n cuts yield $(n + 1)$ fragments, the expected number average size ($\bar{L}_n$) of DNA after treatment with Hpa II is 1.9 kbp. As was shown by Tanford (*13*), $\bar{L}_w$ is twice $\bar{L}_n$ for a randomly cut polymer. Therefore the predicted length, expressed as $\bar{L}_w$, is 3.8 kbp.

Thus, calculations based on nearest neighbor analysis as well as the size of fragments cut by Msp I indicate that, in mouse liver DNA, the site 5′-CCGG is present in the genome much more frequently than it is cut by Hpa II. Assuming that Msp I cuts at every 5′-CCGG site, we calculate (from $\bar{L}_w$ = 4.5 kbp) that each 50 kbp DNA molecule contains an average of 21.2 such sites. Since an average of only 4.9 of these sites are cut by Hpa II ($\bar{L}_w$ = 17.8) the remaining sites (77 percent of the total 5′-CCGG) are modified to protect them from the enzyme. Since 5-MeCyt is the only modified base so far found in vertebrate DNA, and since it has been shown to protect DNA from cutting by Hpa II (*7*), we conclude that most 5′-CCGG sequences in mouse DNA probably contain at least one 5-MeCyt.

Note added in proof. After this manuscript was submitted a similar finding was reported by Waalwijk and Flavell (*15*).

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9. Mice were supplied by F. Luthardt. DNA was purified [J. Marmur, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1963), vol. 5, p. 726] with modifications [N. Blin and W. Stafford, *Nucleic Acid Res.* **3**, 2303 (1976)].
10. Five percent acrylamide gels (4.87 percent acrylamide and 0.13 percent bis) were cast and electrophoresis was performed at room temperature in tris-borate buffer (0.05*M*; *p*H 8.3), and 0.001*M* EDTA. Agarose gels (1.1 to 3 percent, depending on the size of DNA to be fractionated) were cast and run in buffer E [tris-acetate (*p*H 7.8), 0.04*M*; sodium acetate, 0.005*M*; and Na_2EDTA, 0.001*M*] [G. S. Hayward and M. G. Smith, *J. Mol. Biol.* **63**, 383 (1972)]. After electrophoresis, gels were stained in ethidium bromide (0.75 μg/ml), for at least 30 minutes, and were then destained in water. They were photographed in long-wave ultraviolet light with a Polaroid MP3 Land camera equipped with a Tiffen 29A red filter. The light source was a Chromato-Vue Transilluminator Model C-62 (Ultraviolet Products, Inc.). Negatives were scanned with a Joce-Loebl microdensitometer.
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Feathers of *Archaeopteryx*: Asymmetric Vanes Indicate Aerodynamic Function

Abstract. *Vanes in the primary flight feathers of* Archaeopteryx *conform to the asymmetric pattern in modern flying birds. The asymmetry has aerodynamic functions and can be assumed to have evolved in the selective context of flight.*

Was *Archaeopteryx* terrestrial or arboreal? Was it able to fly by flapping its wings or by gliding? Description of new specimens of *Archaeopteryx* (*1*) has led to a reevaluation of the ancestry of birds (*2*) and to reassessment of the general behavior of *Archaeopteryx* (*2*). It has been suggested that *Archaeopteryx* was strictly terrestrial and could not fly and that its wing feathers were therefore used, perhaps, as insect traps (*2*). We now present evidence that the primary feathers on the manus of *Archaeopteryx*, like those of modern flying birds, show an asymmetry that can be associated with an aerodynamic function. *Archaeopteryx* was therefore at least able to glide.

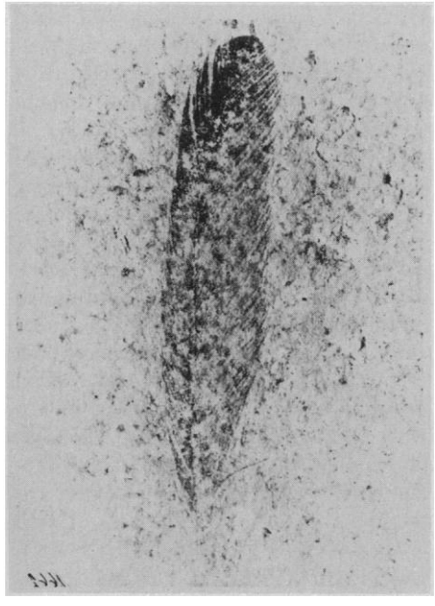

Fig. 1. The counterslab of the isolated feather attributed to *Archaeopteryx* by Hermann von Meyer in 1861. The asymmetric vanes are clearly seen and prove an aerodynamic function; thus, evidence for flight in *Archaeopteryx* has been available for more than 100 years. [Courtesy of Dr. Hermann Jaeger, Humboldt Museum für Naturkunde, East Berlin]

Arguments have been proposed to explain the early evolution of feathers from scales in the context of flight (*3*), heat shields (*4*), and as heat-retaining insulation for endothermic dinosaurs (*2*, *5*). However, the function of feathers on the wings and tail of *Archaeopteryx* can be discussed independently of the origin of feathers (*2*, *4*).

The long, tapered central support of a typical feather is termed the rachis; it separates interlocking barbs on each side which constitute a sheet known as a vane. Typical body contour feathers have symmetrical vanes (or nearly so). Asymmetry in modern birds is strong in the primary wing feathers and is somewhat less pronounced on the secondary wing feathers and usually all but the central pair of tail feathers. The rachis in asymmetric feathers lies toward the leading edge, which is thicker, stiffer, and narrower, rather than at the middle of the feather. Asymmetry is thus found in feathers that have their leading edges in close contact with the flow of air in flight. In some strong flyers, the outer vane is reduced almost to absence. The asymmetry gives each feather an airfoil cross section. In most birds the outer primary feathers function as individual airfoils, each of which produces lift in flapping flight. The asymmetry in the inner primaries, secondaries, and tail feathers stiffens the leading edge of each feather and thus improves the aerodynamic functioning of the wings and tail. Asymmetry also provides differential pressure on the two vanes, acting as "valves" to allow a surface formed by adjacent overlapping feathers to open or close as required by flapping flight.

In the Berlin specimen of *Archaeopteryx* the wings are preserved in a spread posture, and the primaries are clearly asymmetric with the outer vanes reduced as in modern flying birds; the secondary wing feathers and the tail feathers are not so easily seen. However, the first specimen of *Archaeopteryx*, a single feather discovered in 1861, clearly exhibits asymmetric vanes (Fig. 1).

As a test of the hypothesis that asymmetry in primary feathers evolved on the context of an aerodynamic function we have examined the feathers of a variety of birds. In strong fliers such as swifts,

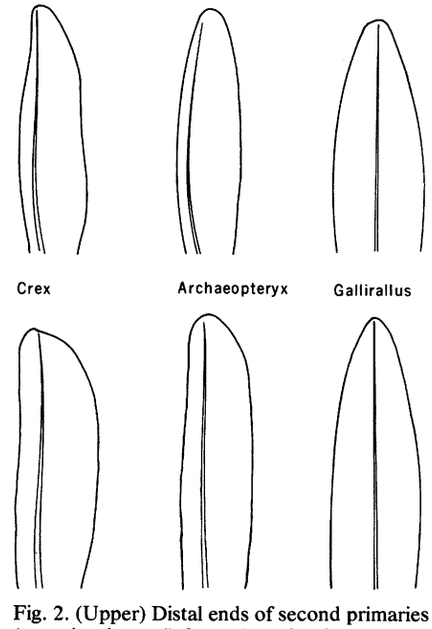

Fig. 2. (Upper) Distal ends of second primaries (counting inward) from the left wings of (left to right) *Crex crex* (a flying rail), *Archaeopteryx*, and *Gallirallus australis* (a flightless rail). (Lower) Similar views of the sixth primaries of the above forms. All drawn to scale.

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