Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Occurrence and Transmission of a Pattern of DNA Methylation in Xenopus laevis Ribosomal DNA

A. P. Bird

Phil. Trans. R. Soc. Lond. B 1978 **283**, 325-327
doi: 10.1098/rstb.1978.0032

Email alerting service Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1978 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

Phil. Trans. R. Soc. Lond. B. **283**, 325–327 (1978) [325]
Printed in Great Britain

The occurrence and transmission of a pattern of DNA methylation in *Xenopus laevis* ribosomal DNA

By A. P. Bird
M.R.C. Mammalian Genome Unit, King's Buildings, West Mains Road, Edinburgh EH9 3JT, U.K.

Even before the double helical structure of DNA had been put forward, it was known that DNA often contains bases in addition to adenine, thymine, guanine and cytosine (Wyatt 1951). In multicellular organisms the rare base is invariably 5-methyl cytosine (5MC), and few species that have been examined in sufficient detail have failed to show its presence (Shapiro 1968). We now know that 5MC arises by direct methylation of cytosine within the DNA duplex (Sneider & Potter 1969; Burdon & Adams 1969), and that in animals the methylated C is next to G on its 3′ side (Grippo, Iaccarino, Parisi & Scarano 1968). Beyond this our knowledge is rather limited, and the functional rôle played by DNA methylation remains a complete mystery.

In prokaryotes more is known. In particular, the discovery of bacterial DNA restriction and modification has for the first time suggested a function for the modified bases in micro-organisms (Meselson, Yuan & Heywood 1972). Appropriate methylation of either DNA adenine or cytosine renders the short sequence normally recognized and cleaved by a restriction endonuclease immune to cleavage. Such a crucial rôle in determining the interaction of a protein with DNA has fed speculation that perhaps eukaryotic DNA methylation is also involved in permitting or preventing protein–DNA interactions (Riggs 1975; Holliday & Pugh 1975).

Unfortunately our scanty knowledge of the distribution of 5MC within the genome places few restraints on the kinds of model which can be put forward. Furthermore, what information we have about the overall level of 5MC in different tissues suggests that variations are too slight to be of significance in cell differentiation.

It has been our purpose to obtain more detailed information about DNA methylation, first of all by studying a homogeneous and well defined fraction of the genome rather than total DNA, and secondly by applying a technique capable of distinguishing methylated from unmethylated C in duplex DNA (Bird & Southern 1978). Since we know that the methylated sequence is CpG in animals, any restriction enzyme whose recognition sequence contains this sequence, and whose normal associated modification enzyme confers resistance to cleavage by methylating the C of CpG, should be able to make the distinction by its ability or inability to cleave at a known recognition site. This can be tested by comparing the digestion of methylated and unmethylated DNA fractions with likely enzymes. Fortunately the ribosomal RNA genes of *Xenopus laevis* (Birnstiel *et al.* 1968; Brown, Wensink & Jordon 1972) are ideal for this purpose since in blood and other somatic cells they are heavily methylated, while in the oocyte extrachromosomal copies contain no detectable 5MC (Dawid, Brown & Reeder 1970). Comparative restriction enzyme digests using these DNAs show that sites for three enzymes (HpaII, AvaI and HhaI) are largely blocked in blood cell rDNA by the presence of 5MC. We refer to these enzymes as C*pG enzymes.

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to
Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.
www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

Having established that our probe for methylation is effective, the next step was to look for any specificity in the distribution of methyl groups within somatic cell rDNA. By combined digests with a C*pG enzyme and other restriction enzymes whose cleavage sites in rDNA are well known, it was possible to identify a strikingly undermethylated site at a unique location within the rDNA repeat unit (HhaI hotspot, figure 1). Whereas most CpGs are methylated to a very high level (99%), this site is only methylated in about half of rDNA repeat units. There is another, more extensive, preferentially unmethylated region which maps at the right hand end of the spacer near the origin of rRNA transcription. Despite an intriguing location, however, this 'hotspot' is less easy to characterize. At the moment we cannot say whether it appears because of undermethylation of the region, or because of a reiterated sequence containing an abnormally high concentration of CpGs.

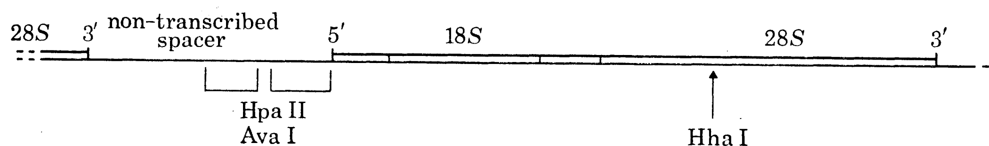

FIGURE 1

To summarize, the rDNA repeat unit in blood cells is heavily methylated, but there is at least one specifically located 'gap' where methylation is about 50-fold lower than normal. Since our selection of C*pG enzymes detects about one quarter of all CpGs in each rDNA repeat unit (280 out of 1000) we do not know if other 'gaps' are present, though it is already possible that one or more undermethylated sites occur in the HpaII/AvaI hotspot (figure 1).

So far we have not considered that CpG is one of four possible dinucleotide sequences which must be paired with an identical sequence on the opposite strand of the double helix. Thus CpGs occur in pairs and the question arises: are both CpGs in a pair methylated $\begin{pmatrix}C^*G \\ G\ C^*\end{pmatrix}$ or can half methylated pairs exist $\begin{pmatrix}C^*G \\ G\ C\end{pmatrix}$? This question was answered by denaturing a methylated, ^{32}P-labelled rDNA fragment, reassociating it again in the presence of a large excess of unmethylated fragment, and comparing the C*pG enzyme susceptibility of hybrid counts to that of the native (^{32}P) rDNA. If half-methylated sites are present, then reannealing with unmodified DNA exposes them to C*pG enzyme attack resulting in an increased susceptibility compared with the native DNA. If, on the other hand, all methylated sites are symmetrically methylated, then susceptibility to C*pG enzymes before and after reannealing must be the same. The result supports symmetrical methylation and the sensitivity of the assay suggests that fewer than 2% of paired CpGs can be half-methylated (Bird 1978).

The significance of this result becomes apparent when it is considered that after DNA replication, new methyl groups are added to the progeny DNA strand but not to the parental strand. We can now see that the methylase, being confronted with half-methylated DNA immediately after DNA replication, efficiently restores each half-methylated site to the symmetrically methylated condition. However, unmethylated sites, which are found at the 'hotspots' and rarely at other sites within the repeat unit, persist, implying that the methylase is unable to begin methylation at an unmodified site. It follows directly from these properties

Downloaded from rstb.royalsocietypublishing.org

that the pattern of methylated and unmethylated CpGs is passed on by a cell to its daughter cells in the manner suggested by Riggs (1975) and Holliday & Pugh (1975).

In summary, the above experiments have uncovered a pattern of methyl groups on rDNA which, while having no effect on its coding properties, may somehow affect protein–DNA interactions. Since the only specificity detected resides in the absence rather than the specific presence of methylated C, it is possible, by analogy with restriction–modification systems, that unmethylated 'hotspots' are access points for proteins. Furthermore, it now seems that the methylation pattern can be copied by a 'maintenance methylase' at each DNA replication thereby permitting a clone of cells, all bearing the same pattern, to arise. If indeed the distribution of 5MC has informational significance for the cell, the copying mechanism provides an appealingly simple means of stably perpetuating the information through cell divisions.

This work was funded by the Medical Research Council (U.K.).

References (Bird)

Bird, A. P. 1978 *J. molec. Biol.* (In the press.)
Bird, A. P. & Southern, E. M. 1978 *J. molec. Biol.* (In the press.)
Birnstiel, M. L., Speirs, J., Purdom, I., Jones, K. & Loening, U. E. 1968 *Nature, Lond.* **219**, 454–464.
Brown, D. D., Wensink, P. C. & Jordon, E. J. 1972 *J. molec. Biol.* **63**, 57–73.
Burdon, R. H. & Adams, R. L. P. 1969 *J. molec. Biol.* **42**, 271–284.
Dawid, I. B., Brown, D. D. & Reeder, R. H. 1970 *J. molec. Biol.* **51**, 341–360.
Grippo, P., Iaccarino, M., Parisi, E. & Scarano, E. 1968 *J. molec. Biol.* **36**, 195–208.
Holliday, R. & Pugh, J. R. 1975 *Science, N.Y.* **187**, 226–232.
Meselson, M., Yuan, R. & Heywood, J. 1972 *A. Rev. Biochem.* **41**, 447–466.
Riggs, A. D. 1975 *Cytogenet. cell. Genet.* **14**, 9–25.
Shapiro, H. S. 1968 In *Handbook of biochemistry* (ed. H. A. Sober). The Chemical Rubber Co.
Sneider, T. W. & Potter, V. R. 1969 *J. molec. Biol.* **42**, 271–284.
Wyatt, G. R. 1951 *Biochem. J.* **48**, 581–583.