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Developmental methylation of the coding region of c-*fos* occurs perinatally, stepwise and sequentially in the liver of laboratory mouse

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ABSTRACT

We have studied the dynamics of de novo DNA methylation of 16 contiguous CpGs in the non-CpG island-coding region of the proto-oncogene c-*fos* during mouse development by Na-bisulfite sequencing. Methylation commences from 16.5 dpc and occurs in stepwise-manner. In liver 7 sites are methylated between 16.5 dpc and day 5 after birth, but all the sites are completely methylated on 20 dpp and remain so in the adult liver. The present study provides evidence that (1) pattern of methylation of c-*fos* is distinct from those DNA sequences which methylate pre- and post-implantation, both in terms of the timing and spreading, and (2) spacing of CpGs is an important factor in determining the course of methylation. We suggest that there could be other isoforms of Dnmtases for the c-*fos* like embryonic genes, not only because they methylate later in development but also because of the difference in kinetics of the reaction, and that the nucleation of certain methylated sites facilitate methylation of neighbouring sites and their maintenance in subsequent cell generations.

1. Introduction

In mammalian genome, cytosine, when present as a CpG doublet, is frequently modified to 5′-methylcytosine causing methylation of DNA. Association of DNA methylation with regulation of gene expression is known in a range of normal and abnormal cellular processes, including the maintenance of X-inactivation (Riggs and Pfeifer, 1992), genome imprinting (Reik et al., 1987; Li et al., 1993) and carcinogenesis (Baylin et al., 1998). Methylation of cytosine occurs postreplicationally, and one of the striking features of DNA methylation patterns is that once introduced the same pattern is retained in subsequent cell divisions. However, the initiation of methylation and its maintenance in subsequent cell divisions is brought about by two sets of enzymes, respectively de novo and maintenance DNA methyltransferases. After several years of efforts, a variety of de novo (e.g. Dnmt3a and Dnmt3b) and maintenance methyltransferases have been identified and structurally and functionally characterized (Yoder et al., 1997; Okano et al., 1999).

Prior to fertilization both the gametes, sperm and the ova, develop a characteristic pattern of CpG methylation. However, in mouse and several other mammals, the preimplantation embryo undergoes a rapid wave of demethylation, causing global erasure of methylation patterns inherited from the gametes (Sanford et al., 1987; Monk et al., 1987; Howlett and Reik, 1991; Kafri et al., 1992). The male gamete loses its methylation in the pronuclear stage itself while the egg methylation disappears by the morula stage (Mayer et al., 2000; Oswald et el., 2000). By the time blastocyst is ready to implant, the genome is almost totally demethylated barring the imprinted sites (Olek and Walter, 1997; Warnecke et al., 1998). How do these demethylated genes get methylated during development and differentiation is not well understood. Monk et al. (1987) and Razin and Kafri (1994) were among the first to study de novo methylation in genome as a whole in post-implantation embryos and showed that major part of the genome gets remethylated between implantation and gastrula. However, the gene level dynamics of methylation during the development has been studied only sporadically, and that displays heterogeneity (see Table 2). *ApoA1* (Shemer et al., 1991) and *Oct3/4* is methylated by 6.5 dpc, the latter taking only a few hours (Gidekel and Bergman, 2002). In contrast, the embryonic/fetal genes, α-fetoprotein (Vedel, 1983), and *HoxA5* and *HoxB5* (Herschko et al., 2003, Sachan and Raman, 2006) methylate relatively late in fetal life and postnatally. The imprinted genes which undergo de novo methylation only in the germ cells, methylate postnatally: in oocytes between pachytene and

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Abbreviations: dpc, days post coitum; dpp, days post partum; Dnmt, DNA methyltransferase; Sp1, specificity protein 1; nt, nucleotides.

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metaphase II and in testis between prospermatogonia and the spermatid (Davis et al., 1999, 2000; Lucifero et al., 2004). Obviously methylation does not occur all at once throughout the genome, instead different genes methylate at different stages of development.

The present investigation is addressed to understanding the initiation, expansion and fixation of methylation in the coding region of the murine proto-oncogene c-*fos* during development and tissue differentiation in the mouse. This 3.5 kb gene (Beveren et al 1983) is a cellular homologue of the transforming gene *v-fos*. Earlier restriction enzyme-based studies have revealed that its upstream regulatory region and the 1st exon, which form CpG islands, do not undergo methylation at any stage of development (Uehara et al 1989), while 3rd and 4th exons take a long time to get methylated in tissue-specific manner (Chandrasekhar and Raman, 1997). In the present paper the course of methylation of 16 CpG sites, spread through a 692 bp region spanning exons 3 and 4 (Fig. 1) has been studied by bisulfite genomic sequencing at different stages of fetal and neonatal development in the mouse liver.

2. Materials and methods

2.1. Genomic DNA isolation

Random bred Parks strain mice, maintained in our lab, were used in this study. DNA was extracted from the fetal, neonatal and adult liver by the standard method of SDS-ProteinaseK followed by phenol/phenol:chloroform:isoamyl alcohol/chloroform: isoamylalcohol extraction and ethanol precipitation in presence of salt.

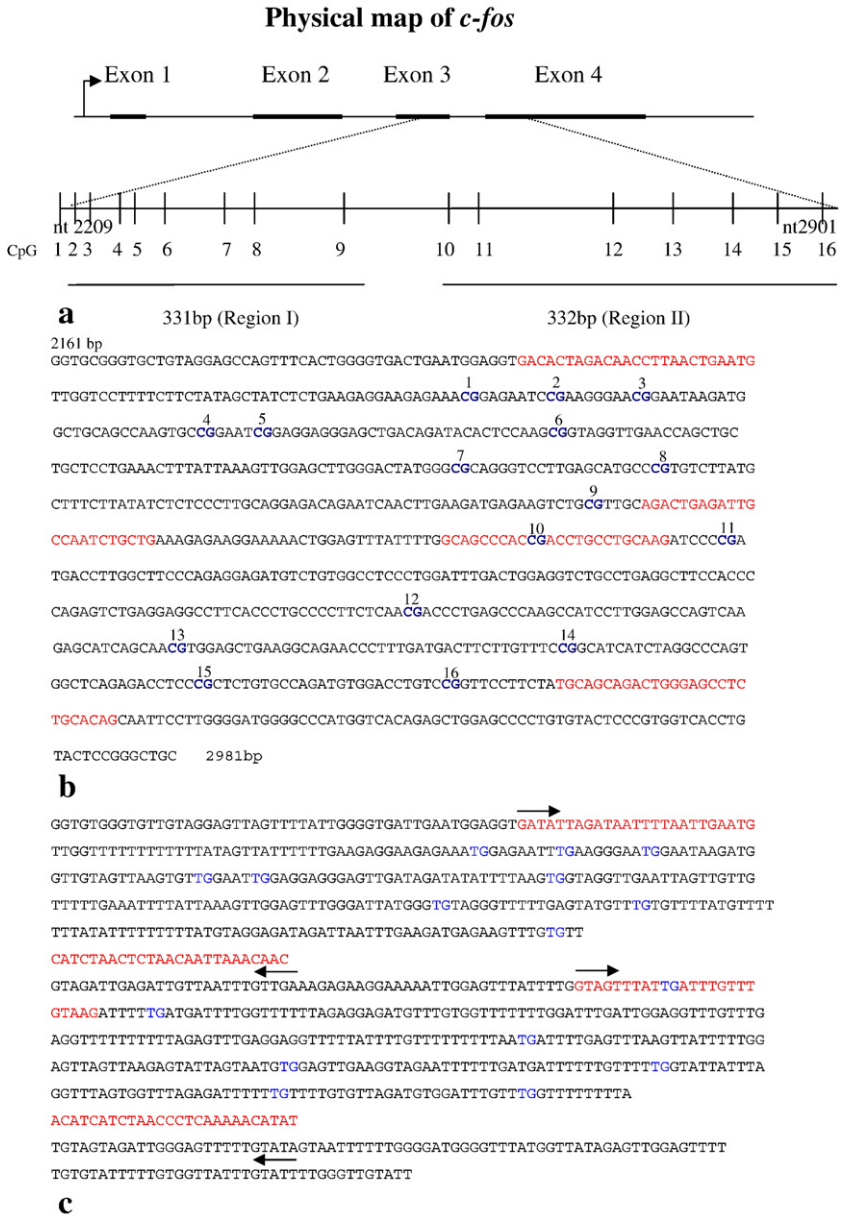

Fig. 1. (a) Cartoon showing the physical map of coding region of c-*fos* gene. A 700 bp region between exon 3 and exon 4 has been analysed for the methylation pattern. This region harbors 16 CpG sites that are indicated with vertical bars. (b) The complete nucleotide sequence of the analysed region. 16 CpG sites are indicated and numbered. (c) Bisulfite converted nucleotide sequence. Sequences in red are the primers designed for the coding strand amplification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)