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Characterization of rat and human c-MYC gene expression

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ABSTRACT

Rat genomic regions covering cellular myelocytomatosis oncogene (c-MYC) were cloned from the DNA of both normal liver and two lines of Morris hepatomas, one of which had c-MYC amplification. The three restriction maps showed perfect agreement within the overlapping regions. The 7 kilo base pair (kbp) regions, which included the entire normal rat c-MYC and the region 2.2 kbp upstream, and one from the hepatomas, were sequenced and found to be identical. The coding regions of exons 2 and 3 were highly conserved between rat, mouse and human, but some differences in amino acids of c-MYC were noted. Exon 1 and the non-coding region of exon 3 showed limited homology between the three species. Rat exon 1 contained several nonsense codons in each frame and no ATG initiation codon, indicating there to be no coding capacity in this exon. The 2.2 kbp upstream regions and the introns compared showed unusual conservation between the rat and human c-MYC genes. Some motifs, previously proposed as having a functional role in human c-MYC, were also found in equivalent positions of the rat sequence. S1 Nuclease protection mapping revealed the second promoter of c-MYC gene to be preferentially used in most tissues or in hepatoma cells, and the second poly A addition signal to be the only one functional in all the c-MYC mRNA sources examined

KEYWORDS :

INTRODUCTION

Myelocytomatosis oncogene (MYC) was initially discovered in the form of a viral oncogene of an avian myelocytomatosis virus, MYC 29, and subsequently identified in various vertebrate genomes in the forms of its cellular counterpart, c-MYC, and transducing viral MYC oncogene homologue (v-MYC) in several oncogenic retroviruses (Duesberg PH. et.al. 1977, Kan NC. et.al. 1983). The structure of c-MYC is highly conserved through evolutionary stages, and the product, c-MYC protein, is known to be localized in the nucleus and to bind to DNA. Several cellular functions, including growth competence or differentiation, seem to require the expression or suppression of c-MYC, although the precise role which the gene has in these functions is not yet clear. Various types of cancer cells overexpress *c-MYC* mRNA, some of them as a result of rearrangement or amplification (Lovén J. et.al. 2012). Such gene overexpression or deregulation is suspected to be a cause of the unlimited growth of the cancer cells. Expression of *c-MYC* gene seems to be regulated in a complicated manner involving transcription initiation, transcript elongation and mRNA degradation (Dang CV. 2013, Bentley DL, Groudine M. 1988). Such regulation requires sequences of functional significance to be located upstream, and within exons and introns. Recent studies using human c-MYC have suggested both positive and negative regulatory sequences to be located upstream and within exon 1 and intron 1 (Blanchard J-M. et.al. 1985). Rearrangements resulting from translocations or retroviral insertions far upstream of the gene have been reported in some B-cell lymphomas, suggesting the importance of remote sequence elements in the regulation of the gene (Lovén J. et.al. 2012). One approach towards identifying such elements is the search for evolutionally conserved sequences in the gene and its neighboring regions. To date, an 8 kb sequence of the entire human c-MYC and its flanking regions has been reported. Another mammalian c-MYC sequence available is that of the mouse, although, in this case, information on the upstream region is limited and the sequence of intron 2 is unavailable.

In the present papar we report the cloning of normal rat c-MYC and that of the Morris hepatomas, 5123D and 7794A. Overexpression of the *c-MYC* mRNA in both hepatomas, and amplification of the gene in the latter, have been reported previously (Dang CV. 2013). Approximately 7 kbp of both normal rat and Morris hepatoma 7794A, that including the gene and the 2.2 kbp upstream region, were sequenced. The sequences from the two sources were exactly the same. Comparison between rat and human sequences revealed remarkably conserved stretches distributed throughout the 2.2 kbp upstream region and both introns, as well as in the exons. It is shown that the second of the two promoters is very much preferred as the *c-MYC* mRNA initiation site in various tissues and cultured hepatoma cells, and also that, in all tissues and cells examined, only the signal downstream of the dual termination (poly A addition) is used.

We cloned the c-MYC of a normal rat and two lines of transplanted hepatomas, one of which contained the gene amplified 10 fold (Dang CV. 2013). Restriction maps and the sequences determined from the independent sources agreed, suggesting no mutations nor rearrangements to have occurred in the amplified gene and its flanking regions (Shaw G. 1986). This is in agreement with earlier notions of no significant structural changes being found in the amplified *c-MYC* genes of some human malignant tumour cells (Kuzyk A, Mai S. 2014).

Apart from the exon sequences, homologous stretches between rat and human c-MYC were found throughout the compared region. The evolutionary distance between rat and man is estimated to be approximately 100 million years. Considering the changing rate of sequences with no selective pressure to be 0.66% per million years (Barlow JH. et.al. 2013), the "meaningless" sequences should diverge to give almost no homology between the two species. The remarkable conservation in the surrounding regions of c-MYC, therefore, suggests: there to be many sites, in the region several kbp upstream and in the introns of c-MYC, where transacting factors bind and contribute to gene regulation, such regions to have some biological functions not directly related to c-MYC(Duesberg PH. et.al. 1977, Kan NC. et.al. 1983). In this regard, it is of interest that recent reports have suggested a new transcriptional activity that seems to start from heterogeneous sites in c-MYC and extend upstream of the gene (Dang CV. 2013, Bentley DL, Groudine M. 1988).

Research report have suggested that human *c-MYC* exon 1 codes for a protein which reacts with antibodies raised to synthetic peptides having amino acid sequences deduced from regions of the nucleotide sequence of the exon. They assumed a minor mRNA species starting upstream of the two major promoters of *c-MYC* gene, and the ATG initiation codon provided by the upstream sequence. In the corresponding region, we could find no amino acid sequences possibly related to those used in raising the antibodies. Such a protein cannot, therefore, be coded in the rat c-MYC region.

It is known that *c-MYC* mRNA turns over rapidly (half life 15 - 30 min) in various cells *in vivo* and *in vitro* experiments (12, authors' unpublished data). The short life time is one of the characteristics of some mRNAs involved in cell cycling, e. g. those of cellular FBJ murine osteosarcoma viral oncogene homologue (c-FOS), cellular Avian myeloblastosis virus oncogene homolog (c-MYB), and some growth factors(Kruijer W. et.al. 1984, Thompson CB. 1986). These *c-MYC* mRNAs have a region in common containing ATTTA and T clusters. Shaw et al. have shown one such regions from *Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF)* mRNA to be responsible for the instability of the mRNA (Shaw G. et.al. 1986). An RNase, possibly, specifically recognizing this sequence motif, could be involved in the metabolism of the short lived mRNAs. One candidate for this enzyme

is RNase L, which is known to degrade U-rich sequences preferentially (Floyd-Smith G. et.al. 1981). Accelerated degradation of *c-MYC* mRNA by interferon (IFN) also supports the idea of the enzyme's involvement, since IFN causes a rapid synthesis of 2'-5' oligo A, an activator of this RNase (Einat M. et.al. 1985). We found the non-coding region in exon 3 to be responsible for the rapid turnover of rat *c-Myc* mRNA (manuscript in preparation), and this region to contain two sets of ATTTA and T-clusters. Mouse and human *c-MYC* genes also have two motifs at identical positions in the non-coding regions of exon 3. In all cases, these motifs of *c-MYC* gene were located approximately 60 nucleotides upstream of the poly A addition signals as termination signal.

Inhibition of protein synthesis by cycloheximide is known to stop *c-MYC* mRNA degradation, and result in *c-MYC* mRNA accumulation in various types of cell (Blanchard J-M. et.al. 1985). One interpretation of this effect of cycloheximide is that the inhibitor "freezes" ribosomes on the *c-MYC* mRNA, thus sterically protecting the *c-MYC* mRNA from RNase attack. The destabilizing sequence of *c-MYC* gene, which is likely to be the site recognised by the involved RNase is, however, located downstream of the termination codon and expected to be exposed whether or not the ribosomes were frozen on the upstream sequence of the *c-MYC* mRNA. Perhaps the effect of cycloheximide should be otherwise explained, such as the drug inactivating the RNase. Interestingly, poly A addition at the upstream signal, if such occurs, removes one of the destabilizer sequences from the *c-MYC* mRNA. Such shortened *c-MYC* mRNA may have a longer life and, therefore, may accumulate even in the absence of transcriptional activation. Although we have not yet found such a shortened *c-MYC* mRNA version, the conservation of the poly A addition signal's duality hints at such a regulatory mechanism being required at some stage of the life cycle (Marcel V. et.al. 2015).

Finally, we are tempted to emphasize that the clones and the rat c-MYC sequence data could be useful in studying the possible involvement of this gene in tumor progression and other disorders since the rat is the most widely used animal in experimental carcinogenesis.

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References

Barlow JH, Faryabi RB, Callén E, Wong N, Malhowski A, Chen HT, Gutierrez-Cruz G, Sun HW, McKinnon P, Wright G, Casellas R, Robbiani DF, Staudt L, Fernandez-Capetillo O, Nussenzweig A. Identification of early replicating fragile sites that contribute to genome instability. Cell 152(3): 620-632, 2013.

Bentley DL, Groudine M. Sequence requirements for premature termination of transcription in the human c-myc gene. Cell. 53(2):245-256, 1988.

Blanchard J-M, Piechaczyk M, Dani C, Chambard J-C, Franchi A, Pouyssegur J, Jeanteur P. c-myc gene is transcribed at high rate in G0-arrested fibroblasts and is post-transcriptionally regulated in response to growth factors. Nature 317: 443-445, 1985.

Dang CV. MYC on the path to cancer. Cell 149(1): 22-35, 2013.

Duesberg PH, Bister K, Vogt PK. The RNA of avian acute leukemia virus MC29. Proc. Nat. Acad. Sci. USA 74: 4320-4324, 1977.

Einat M, Resnitzky D, Kimchi A. Close link between reduction of c-myc expression by interferon and, G0/G1 arrest.Nature 313: 597-600, 1985.

Floyd-Smith G, Slattery E, Lengyel, P. Science 212: 1030-1032, 1981.

Kan NC, Flordellis CS, Garon CF, Duesberg PH, Papas TS. Avian carcinoma virus MH2 contains a transformation-specific sequence, mht, and shares the myc sequence with MC29, CMII, and OK10 viruses. Proc.. Nat. Acad. Sci. USA 80: 6566-6570, 1983.

Kruijer W, Cooper JA, Hunter T, Verma I. M. Platelet-derived growth factor induces rapid but transient expression of the c-fos gene and protein. Nature 312: 711-716, 1984.

Kuzyk A, Mai S. c-MYC-induced genomic instability. Cold Spring Harb Perspect Med 4(4): a014373, 2014.

Lovén J, Orlando DA, Sigova AA, Lin CY, Rahl PB, Burge CB, Levens DL, Lee TI, Young RA Revisiting global gene expression analysis. Cell 151(3): 476-482, 2012.

Marcel V, Catez F, Diaz JJ. p53, a translational regulator: contribution to its tumour-suppressor activity. Oncogene 34(44): 5513-5523, 2015

Shaw G, Kamen R. A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell 46: 659-667, 1986.

Thompson CB, Challoner PB, Neiman PE, Groudine M. Expression of the c-myb proto oncogene during cellular proliferation. Nature 319: 374-380, 1986.