Molecular Cloning and DNA Sequence Analysis of Preproinsulin Genes in the NON Mouse, the Animal Model of Human Non-Obese, Non-Insulin-Dependent Diabetes Mellitus

（ヒト非肥満インスリン非依存型糖尿病の動物モデルＮＯＮマウスのプレプロインスリン遺伝子のクローニングとその構造解析）

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Molecular Cloning and DNA Sequence Analysis of Preproinsulin Genes in the NON Mouse, an Animal Model of Human Non-Obese, Non-Insulin-Dependent Diabetes Mellitus

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Running title: Insulin Genes in the NON Mouse with NIDDM

ABSTRACT

We have proposed in the previous study that the NON mouse is an animal model suitable for studying human non-obese, non-insulin-dependent diabetes mellitus, and we have shown that hypo-insulinemia in the NON mouse was associated with reduced levels of insulin mRNA in their pancreas. These results suggest that structural alternation of the insulin gene may occur in this mouse. In the present study, to corroborate this possibility, we attempted to isolate and characterize the insulin gene of the NON mouse. Comparison with structures of the insulin I and II genes of the BALB/c mouse demonstrated that the NON mouse has two non-allelic insulin genes, encoding separately insulin I and II. The nucleotide sequences of the NON mouse insulin genes, including 5'-transcriptional regulatory region and exon/intron organization, do not differ from the reported sequences of the insulin I and II genes of the BALB/c mouse. We can, however, point out that about 100 base pairs additional sequence and 7 point mutations are involved in further upstream 5'-flanking region of, and 3 point mutations in 3'-flanking region of the insulin II gene of the NON mouse. We conclude that the reduced expression of the insulin genes in the NON mouse is due to neither the structural change in the known transcriptional

regulatory elements nor occurrence of the abnormal and unstable insulin mRNA. Role of the 5'-upstream additional sequence for the insulin II gene expression remains to be elucidated.

INTRODUCTION

Diabetes mellitus is not a single disease but a group of heterogeneous disorders with common features due to deficiency of insulin action(World Health Organization, 1985). Non-insulin-dependent diabetes mellitus (NIDDM) is one of major types of diabetes mellitus. NIDDM is expected from several clinical findings to have a strong genetic basis in addition to environmentally affected conditions. Its exact pathogenesis, however, remains to be elucidated, because of the difficulty to clarify extent of contribution of both factors to an individual case of NIDDM(Rossini, Mordes & Handler, 1988).

The NON mouse is a subline separated from the Jcl-ICR mouse in the course of isolation of the NOD mouse that is known as an animal model for insulin-dependent diabetes mellitus (IDDM) (Makino, Kunimoto, Muraoka et al. 1980; Tochino, 1986). In the previous study, we have characterized the NON mouse as follows; (1)no obesity, (2)glucose intolerance but not overt diabetes, (3)hypoinsulinemia, (4)no pathological findings including autoimmune changes in pancreatic islets and the thyroid gland. These characteristics appear to be similar to those of non-obese human NIDDM, we have proposed that the NON mouse is an animal model suitable for studying non-obese human NIDDM (Ohgaku, Morioka,

Sawa et al. 1988). Therefore, we anticipate that the study using the NON mouse will be a major breakthrough to clarify the pathogenesis of NIDDM. Furthermore we have demonstrated the remarkably reduced levels of both insulin and preproinsulin mRNA in pancreas of the NON mouse. In the analysis of restriction fragment length polymorphism (RFLP) of insulin gene in the NON, NOD, and Jcl-ICR mouse, we have observed an extra and weak signal only in the BamHI-treated genomic DNA of the NON mouse(Ohgaku et al. 1988). From these results, we suggested that glucose intolerance in the NON mouse results from the reduced expression of the insulin gene which may have an altered structure especially in the transcriptional regulatory region. In the present study, we isolated two non-allelic insulin genes from the NON mouse, determined their nucleotide sequences, and discussed about their features.

MATERIALS AND METHODS

Animal strains.

NON mice were provided from Shionogi Research Laboratories, Osaka, Japan.

Materials.

N-Lauroylsarcosine, sodium salt, was obtained from Sigma, St Louis, MO, U.S.A. Proteinase K was from Merck, Darmstadt, F.R.G. [α-^{32}P]dCTP (111 TBq/mmol) was purchased from ICN Chemicals, Irvine, CA, U.S.A., and [α-^{35}S]dATP (24.1 TBq/mmol) was purchased from Amersham International plc, Amersham, Bucks, U.K. Deoxyribonuclease I was from Pharmacia LKB Biotechnology AB, Upsala, Sweden. MboI and BstXI were from New England Biolabs, Beverly, MA, U.S.A. Other restriction endonucleases, DNA modifying enzymes were from NIPPON GENE, Toyama, Japan. Rat preproinsulin I cDNA was a generous gift from Dr. Hiroshi Okamoto, Department of Biochemistry, Tohoku University School of Medicine, Japan, who cloned and analyzed its sequence(unpublished data).

Extraction of high-molecular-weight DNA.

High-molecular-weight genomic DNA was isolated from the mouse liver as described elsewhere (Cox, Damjanov, Abanobi et al. 1973) with slight modifications. After sacrifice of animal,

liver was immediately excised and rinsed in 0.9% (w/v) NaCl. About 1 g of the tissue was put into nylon mesh(sample pack; EIKEN, Tokyo, Japan), immersed in 7 ml of ice-cold 0.5 M EDTA, (pH 8.0) and squashed gently with the blunt end of a spatula on ice. Liver cells were suspended in 10 ml of 0.5 M EDTA, pH 8.0, in a 50 ml Falcon polypropylene tube (#2070), and lysed by addition of 0.1 ml of 10 mg/ml proteinase K and 0.25 ml of 20% (w/v) sodium Lauroyl sarcosinate. After overnight incubation at 50°C, DNA was extracted 3 times with phenol/chloroform/isoamyl alcohol (25:24:1, v/v), and subjected to dialysis until OD_{270} of the dialysate is less than 0.05.

Construction of the genomic library of the NON mouse.

After partial digestion of high molecular weight genomic DNA with MboI, DNA fragments having the sizes of about 20 kilobase pairs (kbp) were isolated by sucrose gradient ultracentrifugation, ligated with the BamHI-treated Lambda Dash arms (Stratagene, San Diego, CA, U.S.A.) and assembled into phage using the *in vitro* packaging kit (Amersham) by a standard protocol(Maniatis, 1982).

Isolation and subcloning of the genes encoding insulin I and II from the genomic library of the NON mouse.

Using the nick-translated rat preproinsulin I cDNA as a probe,

approximately 6 X 10^5 phages of the amplified genomic library was screened by plaque hybridization (Benton & Davis, 1977) with slight modification as follows: nitrocellulose replicas were incubated at 45°C overnight with prehybridizing solution containing 50% (v/v) deionized formamide, 50 mM Tris-HCl (pH 8.0), 1 M NaCl, 0.1% (w/v) sodium dodecyl sulfate (SDS), 5 mM EDTA, 4 mM sodium phosphate, 5x Denhardt's solution (1x is 0.02% (w/v) polyvinylpyrrolidone, 0.02% (w/v) Ficoll, 0.2% (w/v) bovine serum albumin), and 200 µg of sonicated, heat-denatured salmon testes DNA/ml. They were then hybridized with the probe in the solution at 45°C for 24 h, and washed at 45°C in a solution containing 10 mM Tris-HCl(pH 8.0), 25 mM NaCl, 0.1% SDS, 1 mM EDTA, 1 mM sodium phosphate, and 1x Denhardt's solution for 6 h with several changes of the washing solution. Phage DNA was prepared by glycerol step gradient centrifugation(Maniatis, 1982) and digested with EcoRI and BamHI. After agarose gel electrophoresis, phage DNA was transferred to nitrocellulose filter. The filter was hybridized to rat preproinsulin I cDNA probe as described above. DNA fragment containing putative preproinsulin gene were subcloned into Bluescript KS(+) plasmid (Stratagene, San Diego). The recombinant plasmid was propagated in Escherichia coli MC1061,

and isolated by alkaline lysis and polyethylene glycol precipitation(Brush, Dodgson, Choi et al. 1985).

Construction of deletion mutants and nucleotide sequence determination.

Plasmid carrying the genomic DNA in both orientations were isolated and subjected to preparation of serial deletion mutants according to the method of Henikoff(1984). The appropriately deleted inserts were selected and sequenced by the dideoxy chain termination method(Sanger, Nicklen & Coulson, 1977). Synthetic primers complementary to T3 and T7 promoter sequences, alkali-denatured plasmid DNAs, and [α-^{35}S] dATP were used in the reaction. 7-Deaza-dGTP (Boehringer Mannheim GmbH, Mannheim, F.R.G.) was used instead of dGTP in the sequencing to avoid the secondary structure problems.

RESULTS

After the screening, we obtained 5 positive clones. We subjected these clones to Southern analysis using the radioactive cDNA probe. One clone, λNins-I, showed 3 EcoRI fragments and a 1.4-kbp fragment was hybridized with the probe. In the BamHI-digestion, this clone showed a 5.3-kb fragment, which was formed from the internal BamHI sites, and this fragment was hybridized. Another, λNins-II, displayed a 2.5-kb BamHI fragment, and this fragment was hybridized with the cDNA probe(Fig. 1). From the comparison of restriction maps of the BALB/c mouse preproinsulin I and II genes(Wentworth, Schaefer, Villa-Komaroff et al. 1986), we suggested that λNins-I and λNins-II clones carry putative NON mouse preproinsulin I and II genes respectively.

For further restriction mapping and sequencing analysis, we constructed plasmid subclones: pNins-I for the 1.4-kb EcoRI fragment from λNins-I, and pNins-II with the 2.5-kb BamHI fragment from λNins-II. The restriction maps and sequencing strategies for the NON mouse pNins-I and II subclones are shown in Fig. 2.

Figure 3 shows the nucleotide and deduced amino acid sequences of the pNins-I. Comparing with the preproinsulin I gene of the BALB/c mouse, we suggested that putative trans-

cription initiation site was present at A of position 648. Sequences for exon 1 (position 648 to 693), exon 2 (812 to 1209) including polyadenylation signal (position 1190), intron (694 to 811), were identical to that of the BALB/c mouse preproinsulin I gene. Thus, pNins-I encodes the NON mouse preproinsulin I gene. The 5'-flanking sequence(position 1 to 647) was identical to that of the BALB/c mouse preproinsulin I gene. This region includes general promoter and enhancer elements: the TATA box (position 624-629), the CAAT box (position 579-588), enhancer core sequence (position 339-345), and also the beta cell-specific enhancer elements: "Nir box", and "Far box" like sequences (positions 542-549 and 416-423, respectively) as previously reported (Karlsson, Edlund, Moss et al. 1987). The sequence for 3'-flanking region was also identical with that of the BALB/c mouse preproinsulin I gene.

The structure of the pNins-II is shown in Fig. 4. The putative transcription initiation site was present at A of position 1061. Sequences for exon 1 (position 1061 to 1106), exon 2 (1225 to 1427), and exon 3 (1916 to 2115) including the polyadenylation signal (position 2096), and intron 1 (position 1107 to 1224), and intron 2 (1428 to 1915) were all identical to the reported structures of the BALB/c mouse preproinsulin II

gene. Thus, pNins-II encodes the NON mouse preproinsulin II gene. The 5'-transcriptional regulatory region (from transcription initiation site to about 350bp upstream) was identical with that of the BALB/c mouse preproinsulin II gene. But further upstream, near the BamHI subcloning site, 7 point mutations were found: 5 insertions at position 156, 237, 264, 361 and 374, 1 substitution at 315, 1 deletion at between 124 and 125. And from 1 to 113 (1061bp to 948bp upstream of transcriptional initiation site), the NON mouse preproinsulin II gene had 113bp additional sequence was not reported in the BALB/c mouse preproinsulin II gene. Three point mutations were also found in the 3'- flanking region: 1 insertion at 2495, 1 substitution at 2365, 1 deletion at between 2391 and 2392.

Furthermore, as would be expected from these results, the deduced amino acid sequences of the NON mouse preproinsulins I and II were identical with those of BALB/c mouse preproinsulins I and II, respectively.

DISCUSSION

In the previous studies, we have presented that the NON mouse is an animal model suitable for studying non-obese human NIDDM. Furthermore, from the evidence for the reduced level of preproinsulin mRNA in the pancreas of the NON mouse, we have proposed a hypothesis that glucose intolerance in the NON mouse is caused by the reduced synthesis of insulin due to possible defect(Ohgaku et al. 1988). Several responsible sites of defects may be possible: gene defects, reduced mRNA stability due to changes in the exon/intron organization, the abnormalities of splicing in maturation of mRNA, and decrease in transcriptional efficiency due to an altered structure in cis- or trans-acting factors.

In this study, we have shown that the NON mouse has two non-allelic preproinsulin genes, I and II, and that their exon/intron organizations are identical to those of the BALB/c mouse. Thus, we exclude possibilities of gene defects and of changes in the exon/intron organization.

Several cases of human diabetes, induced by the abnormal insulin due to the point mutation of insulin gene, have been reported (Kwok, Steiner, Rubenstein et al. 1983; Haneda, Chan, Kwok et al. 1983; Nanjo, Sanke, Miyano et al. 1986). In the

preliminary study, we have observed that the NON mouse insulin I and II proteins are identical with those of normal Jcl-ICR mouse with reversed-phase high-performance liquid chromatography (HPLC)(data not shown). Further confirmation of the results was obtained from the present study, where no substitutions were observed in the deduced amino acid sequences of NON mouse insulin I and II (Figs.3 and 4).

In mammals, insulin gene expression is restricted to beta cells of the pancreatic islet. The cis-acting regions of the rat insulin genes have been located within about 350 bp of their 5'-flanking region, 5'-transcriptional regulatory region(Walker, Edlund, Boulet et al. 1983; Hanahan 1985). Two cell-specific regulatory elements, an enhancer and a promoter, appear to be operative in this region. Recent reports have demonstrated that reductions of insulin gene expression result from mutations in these elements(Karlsson et al. 1987, Crowe & Tsai, 1989; Hwung, Crowe, Peyton et al. 1989). From these studies, it was possible that mutations in 5'-transcriptional regulatory region can reduce expression of the insulin gene in the NON mouse. Indeed our RFLP study of NON mouse preproinsulin gene has demonstrated that NON mouse preproinsulin gene has a unique RFLP in the BamHI-digested DNA, which seemed to offer indirect evidence for

this possibility. In this study, we observed no structural change in the known 5'-transcriptional regulatory elements in both NON mouse preproinsulin genes. Accordingly, the reduced expression of NON mouse preproinsulin genes is not the result of the mutation in these elements. Since the NON mouse has no internal BamHI site in preproinsulin I and II genes, the 1.7-kb extra band with weak intensity observed in the NON mouse is false positive.

Several mutations are found in the further 5'-upstream flanking region and 3'-flanking region. Especially, an additional 5'-flanking sequence which is not reported in BALB/c mouse calls our attention. This sequence seems to be similar to polymorphic region as reported in human insulin gene(Bell, Karam & Rutter, 1981; Bell, Selby & Rutter, 1982) and in rat preproinsulin I gene(Winter, Beppu, Maclaren et al. 1987). The relationship between the length of polymorphic region of insulin gene and the occurrence of diabetes in human has been discussed(Bell, Horita & Karam, 1984; Rotwein, Chyn, Chirgwin et al. 1981). But this polymorphic region is reported not to be essential for insulin gene expression (Hanahan, 1985) and does not have functional role for overall insulin secretion(Permutt, Rotwein, Andreone et al. 1985). The possibility of the poly-

morphic region of preproinsulin gene to function as a cis-acting factors, however, has not been entirely excluded in human(Takeda, Ishii, Seino et al. 1989). In rat, it has been demonstrated that negative transcriptional regulatory ("silencer") element is present between 2.0 and 4.0 kbp upstream of rat preproinsulin I sequence (Laimins, Holmgren-Koenig & Khoury, 1986). It is possible that the additional 113 bp sequence is a "silencer" element. To elucidate this possibility, it is important to know the affected component of the two insulin proteins in the NON mouse. Thereafter, expression study using the reported additional region is needed. element or not.

We also found 3 point mutations in the 3'-flanking region of preproinsulin II gene. From previous studies(Walker et al. 1983; Hanahan 1985), 3'-flanking region of the insulin gene does not appear to have the enhancer activities to the gene expression. We need, however, to examine enhancer activities in this region and effect of 3 mutations. Furthermore, we cannot exclude possibilities that abnormalities of the splicing or an altered structure in trans-acting factor takes part in reduced expression of the insulin gene expression and glucose intolerance in the NON mouse. Thus, we need to elucidate these possibilities in further study.

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REFERENCES

Bell, G.I., Karam, J.H., Rutter W.J. (1981). Polymorphic DNA region adjacent to the 5' end of the human insulin gene. Proceedings of the National Academy of Sciences of the U.S.A. 78, 5759-5763.

Bell G.I., Selby, M.J., Rutter, W.J. (1982). The highly polymorphic region near the human insulin gene is composed of simple tandemly repeating sequences. Nature 295, 31-35.

Bell, G.I., Horita, S., Karam, J. H. (1984). A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. Diabetes 33, 176-183.

Benton, W.D., Davis, R.W. (1977). Screening λgt recombinant clones by hybridization to single plaques in situ. Science 196, 180-182.

Brush, D., Dodgson, J.B., Choi, O.-R., Stevens, P.W., Engel, J.D. (1985). Replacement variant histone genes contain intervening sequences. Molecular and Celluar Biology 5, 1307-1317.

Cox, R., Damjanov, I., Abanobi, S.E., Sarma, D.S.R. (1973). A method for measuring DNA damage and repair in the liver in vivo. Cancer Research 33, 2114-2121.

Crowe, D.T., Tsai, M.-J. (1989). Mutagenesis of the rat insulin II 5'-flanking region defines sequences important for

expression in HIT cells. Molecular and Cellular Biology 9, 1784-1789.

Hanahan, D. (1985). Heritable formation of pancreatic β-cell tumours in transgenic mice expressing recombinant insulin/ simian virus 40 oncogenes. Nature 315, 115-122.

Haneda, M., Chan, S.J., Kwok, S.C.M., Rubenstein, A.H., Steiner, D.F. (1983). Studies on mutant insulin genes: Identification and sequence analysis of a gene encoding [Ser^{B24}] insulin. Proceedings of the National Academy of Sciences of the U.S.A. 80, 6366-6370.

Henikoff,S. (1984). Unidirectional digestion with exonuclease creates targeted breakpoints for DNA sequencing. Gene 28, 351-359.

Hwung, Y.-P., Crowe, D.T., Peyton, M., Tsai, S.Y., Tsai, M.-J. (1989) Regulation of rat insulin II gene expression: cis- and trans-acting factors. In Perspectives on the Molecular Biology and Immunology of the Pancreatic β Cell. pp.55-59. Eds. Hanahan, D., McDevitt, H.O., Cahill, Jr., G.F. New York: Cold Spring Harbor Laboratory.

Karlsson, O., Edlund, T., Moss, J.B., Rutter, W.J., Walker M.D. (1987). A mutational analysis of the insulin gene transcription control region: Expression in beta cells is dependent

on two related sequences within the enhancer. Proceedings of the National Academy of Sciences of the U.S.A. 84, 8819-8823.

Kwok, S.C.M., Steiner, D.F., Rubenstein, A.H., Tager, H.S. (1983). identification of a point mutation in the human insulin gene giving rise to a structurally abnormal insulin (Insulin Chicago). Diabetes 32, 872-875.

Laimins, L., Holmgren-Koenig, M., Khoury, G. (1986). Transcriptional "silencer" element in rat repetitive sequences associated with the rat insulin 1 gene locus. Proceedings of the National Academy of Sciences of the U.S.A. 83, 3151-3155.

Makino, S., Kunimoto, K., Muraoka, Y.Mizushima, Y., Katagiri, K., Tochino, Y.(1980). Breeding of a non-obese, diabetic strain of mice. Experimental Animal 29, 1-13.

Maniatis, T. (1982). Molecular Cloning: A Laboratory Manual. New York: Cold Spring Harbor Laboratory.

Mordes, J.P., Rossini, A.A. (1985). Animal models of diabetes mellitus. In Joslin's DIABETES MELLITUS 12th Edition, pp.110-117. Eds. Marble, A., Krall, L.P., Bradley, R.F., Christlieb, A.R., Soeldner, J.S. Philadelphia: Lea & Febiger.

Nanjo, K., Sanke, T., Miyano, M., Okai, K., Soma, R., Kondo, M., Nishimura, S., Iwo, K., Miyamura, K., Given, B.D., Chan, S.J., Tager, H.S., Steiner, D.F., Rubenstein, A.H. (1986).

Diabetes due to secretion of a structurally abnormal insulin (Insulin Wakayama). Journal of Clinical Investigation 77, 514-519.

Ohgaku, K., Morioka, H., Sawa, T., Yano, S., Yamamoto, H., Okamoto, H.,Tochino, Y. (1988). Reduced expression and restriction fragment length polymorphism of insulin gene in NON mice, a new animal model for nonobese, noninsulin-dependent diabetes. In Frontiers in diabetes research: lessons from animal diabetes II pp.319-323. Eds. Shafrir, E. and Renold, A.E. London: John Libbey & Company Ltd.

Permutt, M.A., Rotwein, P., Andreone, T., Ward, W.K., Porte, Jr., D. (1985). Islet β-cell function and polymorphism in the 5'-flanking region of the human insulin gene. Diabetes 34, 311-314.

Rossini, A.A., Mordes, J.P., Handler, E.S.(1988). Speculations on etiology of diabetes mellitus: Tumbler hypothesis. Diabetes 37, 257-61.

Rotwein, P., Chyn, R., Chirgwin, J., Cordell, B., Goodman, H.M., Permutt, M.A. (1981). Polymorphism in the 5'-flanking region of the human insulin gene and its possible relation to type 2 diabetes. Science 213, 1117-1120

Sanger, F., Nicklen, S., Coulson, A.R. (1977). DNA sequencing

with chain-terminating inhibitors. Proceedings of the National Academy of Sciences of the U.S.A. 74, 5463-5467.

Takeda, J., Ishii, S., Seino, Y., Imamoto, F., Imura, H. (1989). Negative regulation of human insulin gene expression by the 5'-flanking region in non-pancreatic cells. FEBS Letters 247, 41-45.

Tochino, Y. (1986). Discovery and Bleeding of the NOD mouse. In Insulitis and type I diabetes: lessons from the NOD mouse pp.3-10. Eds. Tarui, S., Tochino, Y., Nonaka, K. Tokyo: Academic Press.

Walker, M.D., Edlund, T., Boulet, A.M., Rutter, W.J. (1983). Cell-specific expression controlled by the 5'-flanking region of insulin and chymotrypsin genes. Nature 306, 557-561.

Wentworth, B.M., Schaefer, I.M., Villa-Komaroff, L., Chirgwin, J.M. (1986). Characterization of the two nonallelic genes encoding mouse preproinsulin. Journal of Molecular Evolution 23, 305-312.

Winter, W.E., Beppu, H., Maclaren, N.K., Cooper, D.L., Bell, G.I., Wakeland, E.K. (1987). Restriction-fragment-length polymorphisms of 5'-flanking region of insulin I gene in BB and other rat strains: Absence of association with IDDM. Diabetes 36, 193-198.

World Health Organization (1985). Report of a WHO Study Group. Technical Report Series 727. Geneva: WHO.

Legends for Figures

Figure 1:

Electrophoresis and Northern blot analysis for genomic clones λNins-I and λNins-II. The λNins-I digested with EcoRI(lane 1) or BamHI(lane 2), and the λNins-II digested with BamHI(lane 3) are analyzed by electrophoresis on 0.8 percent agarose gel, which was stained with ethidium bromide, and then photographed under UV illumination. lanes 4, 5 and 6 are the analysis of lane 1, 2 and 3, respectively, by Northern blot hybridization using rat preproinsulin I cDNA probe. Molecular size markers are indicated in kbp on the left side of the figure.

Figure 2:

Restriction maps and sequencing strategies of the subclones pNins-I (a) and pNins-II (b). Nucleotide numbers are given at the top. The deduced restriction endonuclease sites are shown. Exons are indicated by solid boxes. Horizontal arrows indicate the sequencing strategy.

Figure 3:

Nucleotide and deduced amino acid sequence of NON mouse preproinsulin I gene. Capital letters indicate exons and lowercase

letters are used for intron and 5'- and 3'-flanking sequences. The number of nucleotide residues is indicated at the end of each line. The deduced amino acid sequence is given below the nucleotide sequence. Stop codon is indicated by "***". The sequences of putative promoters, enhancers and the poly A signal are underlined.

Figure 4:

Nucleotide and deduced amino acid sequence of NON mouse prepro-insulin II gene. Capital letters, lowercase letters, and the numbering of nucleotide residues are used as described in Fig.2. The deduced amino acid sequence is given below the nucleotide sequence. Stop codon is indicated by "***". Nucleotide differences found in the BALB/c sequence are displayed beneath the NON mouse sequence, and indicated by vertical arrows. Deletions are indicated by dashes. The sequences of putative promoters, enhancers and the poly A signal are underlined.

top

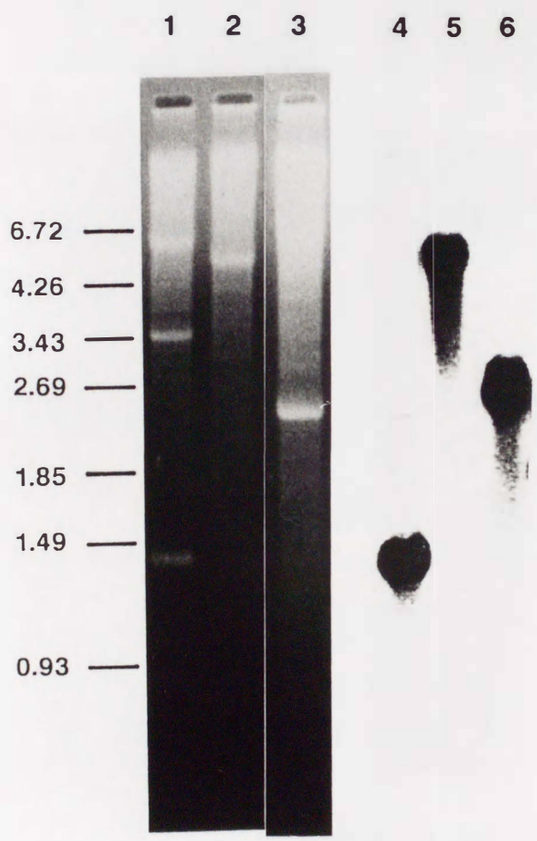

Figure 1

Figure 2

(a)
EcoRI
HincII
ApaI
PstI
PstI
PstI
SmaI
HindIII
EcoRI
0
200
400
600
800
1000
1200
(bp)
(b)
BamHI
SacI
KpnI
PstI
SacI
PstI
EcoRI
ApaI
HincII
PstI
PstI
EcoRI
ApaI
KpnI
PstI
SmaI
KpnI
BamHI
0
300
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1200
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1800
2100
2400
(bp)

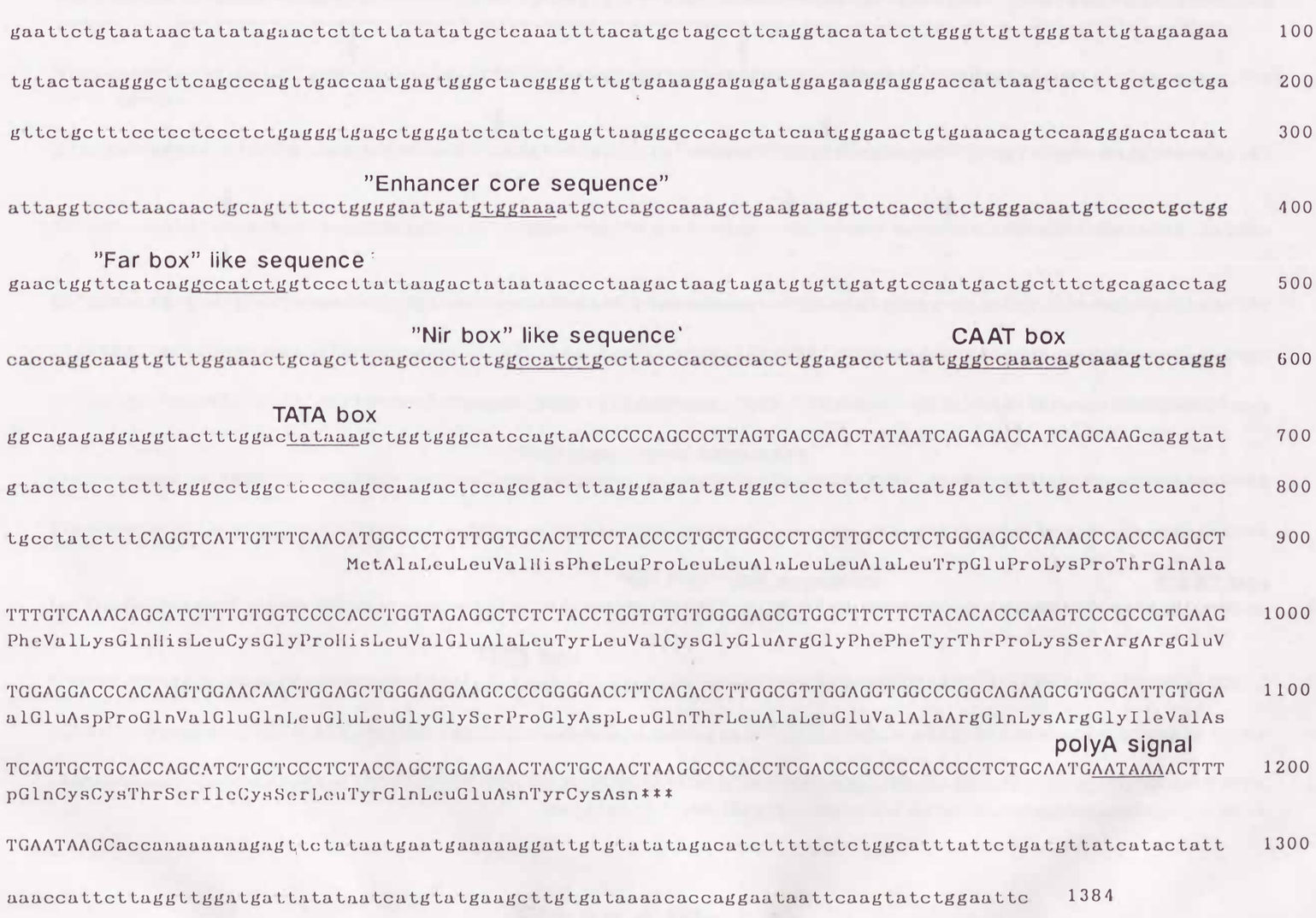

"Enhancer core sequence"
"Far box" like sequence
"Nir box" like sequence
CAAT box
TATA box
polyA signal

Figure 3

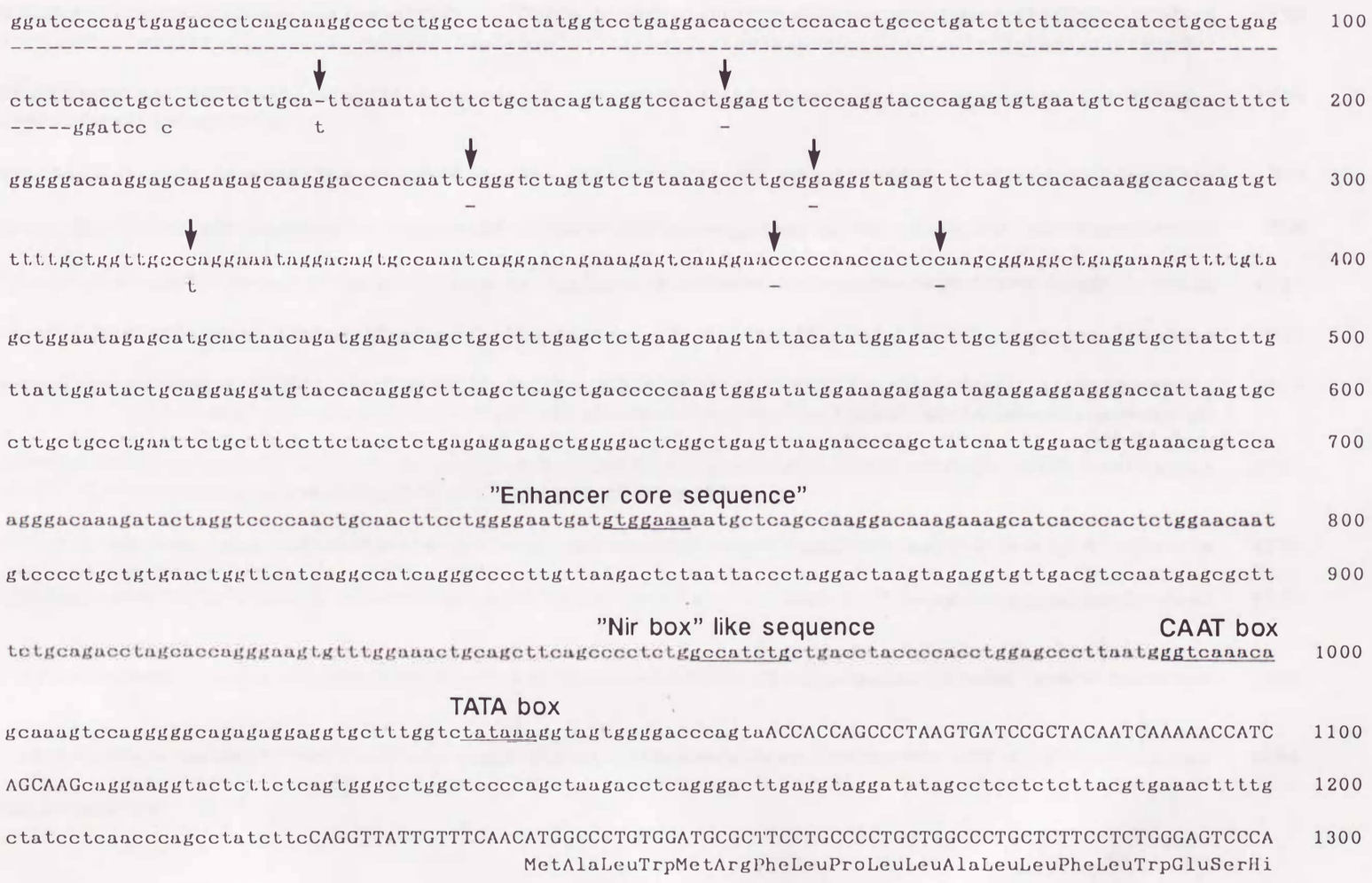
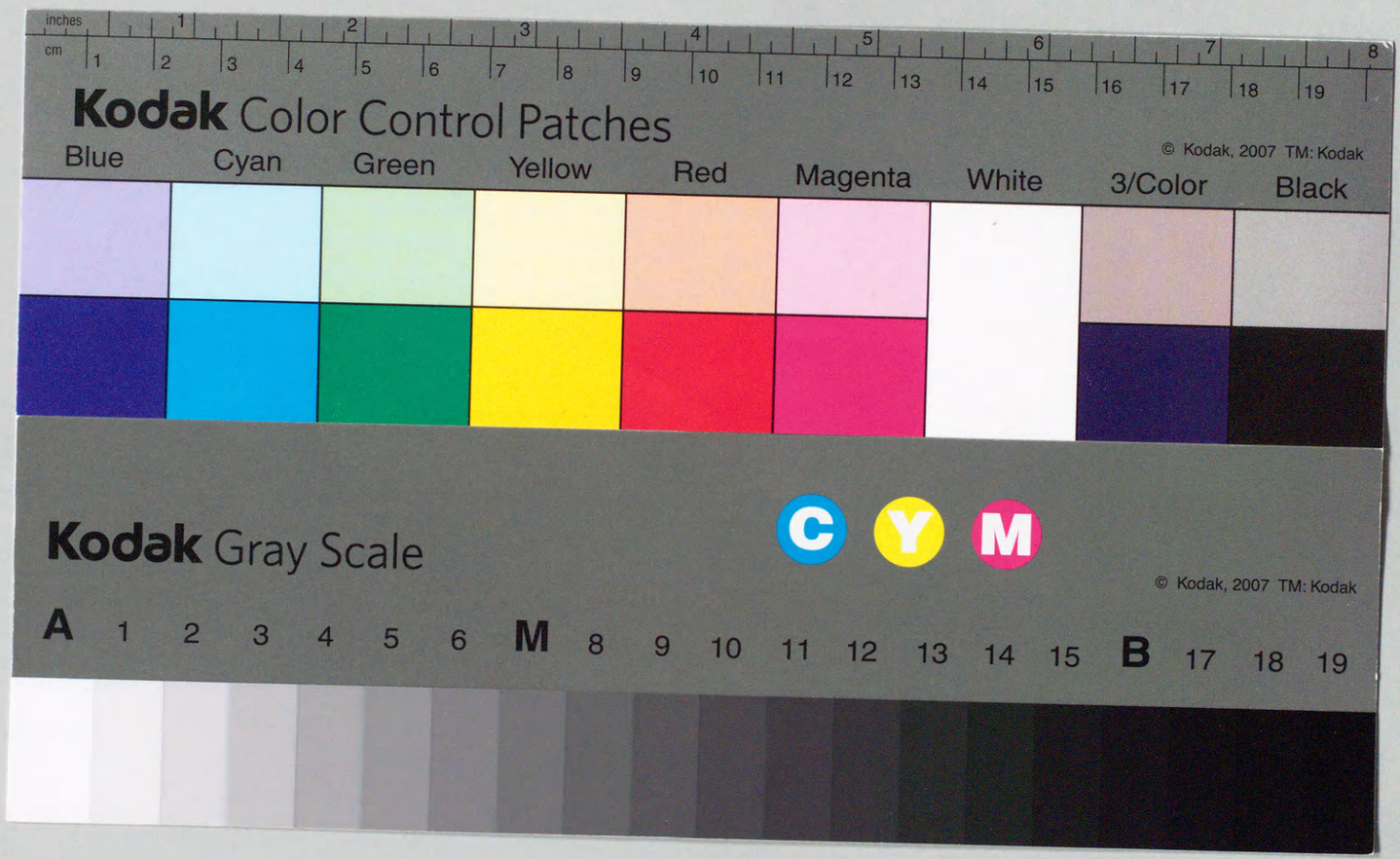


Figure 4 (Part 1)

Figure 4 (Part 2)


inches
cm
Kodak Color Control Patches
© Kodak, 2007 TM: Kodak
Blue
Cyan
Green
Yellow
Red
Magenta
White
3/Color
Black
Kodak Gray Scale
C
Y
M
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A 1 2 3 4 5 6 M 8 9 10 11 12 13 14 15 B 17 18 19