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Gradient expression of *Cdx* along the rat intestine throughout postnatal development

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Rat genomic DNA was isolated by homology with *Cdx1*, a murine homeogene selectively expressed in intestinal cells of endodermal origin. Southern blot analysis indicated that the rat genome contains a single or a small number of closely related *Cdx* gene(s). A major 1.7 kb *Cdx* mRNA was detected in neonate, suckling and adult rats whereas a 6.5 kb mRNA was restricted to sucklings and adults. Both transcripts showed decreasing concentration from the colon towards the proximal part of the small intestine. No obvious correlation could be established with the patterns of expression of transcripts corresponding to markers of cell proliferation and cell differentiation during postnatal development.

Homeogene; *Caudal* gene; mRNA expression; Gene; Sequence; Rat

1. INTRODUCTION

During fetal development, the intestinal tract of rodents organizes from the association of embryonic sheets of endodermal and mesodermal origin. Ontogenic modifications then occur as an adaptation to neonatal, suckling and adult life [1–3]. In neonates protruding villi with digestive enzymes are present in the small intestine and in the colon. Soon after birth the colonic mucosa flattens and specializes in water and electrolyte conservation. At weaning, the small intestine undergoes a maturation which is characterized by changes in the expression of digestive functions and by the emergence of structural and functional differences along the jejunoileum axis.

From the pioneer work performed in *Drosophila*, a key role has been attributed to homeogenes in ontogeny. One of these genes, *caudal*, participates in the definition of the longitudinal axis of the fruit fly embryo and is later expressed in posterior structures such as intestinal endodermal cells [4–6]. *Cdx1*, the first murine gene isolated by homology with *caudal*, is selectively expressed in intestinal cells of endodermal origin in embryonic and adult mice [7]. The closely related *Cdx2* gene is also transcribed in the adult murine intestine [8].

The purpose of the present study was to analyze *Cdx* mRNA expression along the intestinal tract of rats and to look whether there might be some correlation with the functional changes that occur during postnatal development.

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2. MATERIALS AND METHODS

The proximal jejunum, the distal ileum and the colon of seventy-four 3–4-day-old neonates, of sixteen 14-day-old sucklings and of four 90-day-old adults was resected as previously described [9,10]. In sucklings and adults, the mucosa was scraped with glass slides. Cytoplasmic RNA was prepared using the LiCl procedure [11], separated by oligo-dT cellulose chromatography and analyzed by Northern blotting [12].

Sprague–Dawley rat genomic DNA blotted onto Hybond filters as well as a rat genomic library cloned into the *Eco*RI site of bacteriophage Charon4A were purchased from Clonetech Inc. (Palo Alto, CA). Screening the DNA library, subcloning of fragments into the vector Bluescript pSK$^-$ (Stratagene, La Jolla, CA), and DNA sequencing was performed as described by Sambrook et al. [12].

DNA–DNA and RNA–DNA hybridizations were carried out in 5 × SSC, 50% formamide, 10 mM phosphate buffer, pH 7.5, 0.1% SDS, 0.02% polyvinylpyrolidone, 0.02% Ficoll, 10% dextran sulfate at 42°C. Filters were washed under moderate (0.5 × SSC, 0.1% SDS at 42°C) or high stringency (0.2 × SSC, 0.1% SDS at 60°C). We used as probes plasmid pBH3523, spanning nucleotides 340–1602 of the published murine *Cdx1* cDNA sequence [7], as well as plasmids pSI [13], pRLU6 [14], p3PCNA [15], pSHGlul [16] and pCAD142 [17] that contain cDNAs for sucrase-isomaltase (SI), lactase-phlorizin hydrolase (LPH), proliferating cell nuclear antigen (PCNA), glucagon (GLUC) and carbamylphosphate synthetase-aspartate transcarbamylase-dihydroorotase (CAD), respectively. Double- or single-stranded DNA was labelled as already described [10,18]. The synthetic oligonucleotide MF61 was used to detect villin mRNA [19].

3. RESULTS

Rat genomic DNA cut with several restriction enzymes was probed with the 830 bp *Eco*RI/*Xba*I restriction fragment of plasmid pBH3523 that contained 389 bp of the murine *Cdx1* open reading frame. Single *Bam*HI, *Eco*RI and *Hin*dIII fragments but two *Bgl*II fragments of 5 kb and 5.3 kb hybridized to the probe under moderate stringency (Fig. 1). Screening 10^6

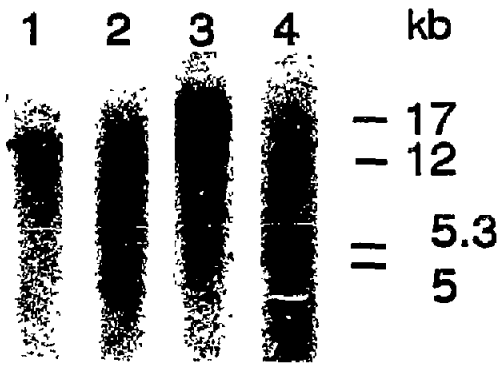

Fig. 1. *Cdx*-like sequences in the rat genome. Rat genomic DNA was cut with the restriction enzymes *Bam*HI (1), *Eco*RI (2), *Hin*dIII (3) or *Bgl*II (4), and probed with the murine *Cdx1* cDNA. Molecular weights are given in kilobases (kb).

plaques of a rat genomic library with the murine *Cdx1* cDNA led to the isolation of 11 recombinant phages that either contained a 12 kb or a 6.5 kb *Eco*RI hybridizing fragment. They were subcloned into the *Eco*RI site of vector Bluescript pSK⁻ to give plasmids pRC20E and pRC018E, respectively. pRC20E exhibited two contiguous 5 kb and 5.3 kb *Bgl*II fragments whereas pRC18E contained a single 5 kb *Bgl*II fragment whose restriction map was identical to that of the 5 kb fragment of pRC20E (not shown). The *Bam*HI, *Eco*RI, and *Hin*dIII fragments of pRC20E recognized by the murine probe showed sizes similar to those of the fragments revealed by genomic Southern blotting.

We determined the sequence of a 1143 bp DNA segment on pRC18E and pRC20E (Fig. 2). These sequences were identical, indicating that both types of phages selected above originated from the same genomic locus. Intervening sequences flanked by canonical intron/exon junctions were defined on the rat genomic segment to maximize alignments with the mouse *Cdx1* cDNA. The amino acid sequence conceptually deduced from the rat coding sequence exhibited 93% homology with the murine CDX1 protein, inside as well as outside the homeodomain [7]. It is worth noting that the rat sequence was strictly identical to the sequence of the murine CDX1 protein in the region known to differ for three residues with CDX2 [8].

Polyadenylated RNA was extracted from the proximal jejunum, from the distal ileum, and from the colon of neonate, suckling and adult rats. RNA samples probed with plasmids pRC20E, pRC18E or with the murine *Cdx1* cDNA, revealed a major 1.7 kb mRNA in each intestinal segment, whatever the developmental stage (Fig. 3). A second transcript of 6.5 kb was selectively detected in suckling and adult animals. This transcript was at least ten times less abundant than the 1.7 kb mRNA, and hybridization with single-stranded DNA probes indicated that it was colinear with the major one (not shown). We emphasize that both *Cdx* mRNAs followed a gradient of concentration along the intestinal tract at each stage during postnatal development. They predominantly accumulated in the colon compared to the small intestine and in the ileum compared to the jejunum.

In parallel, the distribution of several transcripts was analyzed to characterize the functional status of each intestinal segment during the consecutive stages of postnatal development (Fig. 4). RNAs coding for cytoskeleton proteins such as actin and villin were detected all along the intestinal tract, consistent with the presence of brush-border membranes at the apex of the epithelial cells. SI mRNA was restricted to the small intestine and appeared at weaning. Another disaccharidase, i.e. LPH, exhibited a typical pattern characterized by the step-by-step disappearance of the mRNA in the colon a few days after birth, and in the distal ileum at weaning. GLUC mRNA slightly rose in sucklings and in adults, and it was more abundant toward the distal part of the intestinal tract. Finally mRNAs encoding proteins involved in cell proliferation, namely PCNA and CAD, were barely detected in neonates.

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1     ccaagtcaggtgtacccttcgtgacttatgctcagactttatggtctggggaccctgagtcaaaacccatctggaagtttgttaccacacggctctgaggccctccgagtgaacagtcaggggaatctcctgaggcctcacttt

144   cacagctgacatagctggcttcagaccacaaccttgtcatcagcttcctgccagaggtttcctggtcctcttgagcatccactacacttgccttccctcattgcccttctgctcctcag GT AAG ACT CGG ACC AAG
                                                                                                                                  Lys Thr Arg Thr Lys

281   GAC AAG TAC CGC GTT GTC TAT ACA GAC CAC CAA CGT CTA GAG CTG GAA AAG GAG TTT CAC TAC AGT CGG TAC ATC ACC ATT CGG CGC AAG TCA GAG CTG GCT GCT AAC
      Asp Lys Tyr Arg Val Val Tyr Thr Asp His Gln Arg Leu Glu Leu Glu Lys Glu Phe His Tyr Ser Arg Tyr Ile Thr Ile Arg Arg Lys Ser Glu Leu Ala Ala Asn

389   TTG GGT CTC AGA GAG CGG CAG gtatgtgggccctccggctcagctcaagtagttgggctttcaggttgccatgctggacagagcccacaaaaaaaaaaataaaaaaaaggtagtctccagactactggcacacccccc
      Leu Gly Leu Thr Glu Arg Gln

525   cactgaaccactgccatacccaccaactaaaccccaacattatttccatacacacaaacagacatatgtatgtgtgcgcacacacacacacacacacacacacacacacacacacacacacacacgcacgcacagaggctacagtggc

668   tggtgatcaatgctattcaaaccctgtggatgggcagtcacaggaacatgggggctctggaggactgggcttgtaggagtgttcagagaaggggtgtctagagcacagggagaatgtggtgactggagcagaaagtggatcat

811   gaagaccacctctcccagcccagctccttcctaaaatctgcaggataaaatctctgcaatccccacag GTA AAG ATC TGG TTC CAG AAC CGT CGG GCC AAG GAG CGT AAA GTA AAC AAG AAG AAA
                                                                            Val Lys Ile Trp Phe Gln Asn Arg Arg Ala Lys Glu Arg Lys Val Asn Lys Lys Lys

935   CAG CAG CAG CAG CAG CAG CAA CAG CAG CAG CCC ATG CCT CCC ACA CAG TTG CCC CTG CCC CTG GAT GGC ACC CCC ACA CCA TCG GGC CAC CCT GGG AGT CTA TGC CCC
      Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Pro Met Pro Pro Thr Gln Leu Pro Leu Pro Leu Asp Gly Thr Pro Thr Pro Ser Gly His Pro Gly Ser Leu Cys Pro

1043  ACC AAT GCT GGT CTT CTG GGC ACC CCC TCC CCA GTG CCG GTC AAG GAG GAG TTT TTA CCC TAGCCCCTCCCAGCCTGGGGTCCAGGATCTAGGGACTTGAA
      Thr Asn Ala Gly Leu Leu Gly Thr Pro Ser Pro Val Pro Val Lys Glu Glu Phe Leu Pro stop
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Fig. 2. Nucleotide sequence of part of the rat *Cdx1*-like gene, and deduced amino acid sequence. Exon nucleotides are in capital letters and intron nucleotides are in small letters. Typical nucleotides of intron/exon junctions are underlined. Amino acids encoded by the homeobox are in boldface type. Identical residues in the rat and murine CDX1 proteins are labeled with *. Bars denote the three residues known to differ between the murine CDX1 and CDX2 proteins. The accession number of the nucleotide sequence in the EMBL/GenBank data libraries is M91450.

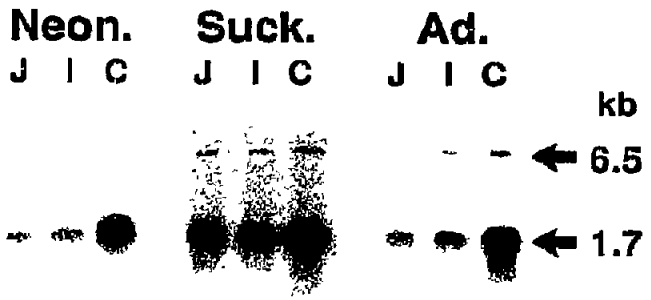

Fig. 3. Expression of *Cdx* transcripts during rat postnatal development. Polyadenylated RNA (5 μg/lane) from the proximal jejunum (J), from the distal ileum (I) and from the colon (C) of 3–4-day-old neonates, of 14-day-old sucklings and of 90-day-old adults, was hybridized to plasmid pRC20E. Molecular weights are given in kilobases (kb).

4. DISCUSSION

We have isolated rat chromosomal DNA highly homologous to the murine *Cdx1* homeogene. This DNA segment hybridized to single or to pairs of adjacent restriction fragments in the rat genome, suggesting that the corresponding gene is single copy. Yet, we cannot rule out the existence of closely related gene(s) as reported in mice [8]. By comparison to the murine *Cdx1* cDNA sequence [7], the rat homeogene contains at least two introns. One is located a few nucleotides upstream from the homeobox, at the same position as the 10.5 kb intron described in the *Drosophila caudal* gene [6]. In the rat, this intron should be more than 7 kb in length, because we failed to detect any sequence of the upstream exon in plasmid pRC20E (unpublished results). The second intron maps near the 3′ end of the homeobox and has no counterpart in the *Drosophila* gene.

Rat DNA hybridized to a single transcript in neonates and to two mRNAs in suckling and adult animals. A similar situation has been reported in mice, which express one transcript in fetuses and two transcripts in adults [7]. The murine genome contains two closely-related *Cdx* homeogenes, and the corresponding cDNAs cross-hybridize with both intestinal transcripts [8]. In the rat, it is not known whether the 1.7 kb and 6.5 kb mRNAs are encoded by a single gene or by two homeogenes belonging to the same family.

Evidence that homeogenes are involved in controlling development and cell identity came from investigations carried out in fruit flies and in nematodes. A similar role has been attributed to the mammalian counterpart genes according to specific spatial and/or temporal patterns of expression during ontogeny. Direct evidence of their function has recently been provided [20,21]. In the murine embryo, *Cdx1* was the first homeogene whose expression was restricted to the endoderm, in the region yielding the intestinal epithelium [7]. Consistent with this, we detected *Cdx* mRNAs in the rat intestine but not in the stomach (unpublished results). Thus, a role for *Cdx* may consist in specifying the intestinal epithelium as opposed to other endoderm derivatives. Moreover, we found rat *Cdx* transcripts to follow an anteroposterior gradient of concentration, the highest level occurring in the colon. This result corroborates data obtained in adult mice [8], and it further indicates that the gradient is already established in neonates and maintained throughout postnatal development. Hence, by analogy to the functional significance attributed to the gradient expression of *caudal* in *Drosophila* [5,22], it is conceivable that *Cdx* may be involved in identifying segments along the intestinal tract of mammals. Expression up to the adult stage would be required to maintain proper regional identity, because the intestinal epithelium is continuously renewed from stem cells.

Changes occurring during postnatal development along the intestinal tract of the rat have been characterized in this study with respect to the pattern of expression of several mRNAs. In the colon of neonates, the transient expression of a small intestinal-like phenotype is marked by the presence of LPH mRNA [9]. Later at weaning, small intestinal maturation shows modifications in the expression of digestive enzymes and emergence of jejuno-ileal differences, as exemplified by the rise in SI mRNA [13] and by the specific drop of LPH mRNA in the ileum compared to the jejunum [10]. At this stage, epithelial cell renewal is accelerated. Consistently, we found a rise in the concentration of mRNAs coding for CAD and PCNA, two proteins respectively involved in pyrimidine biosynthesis [17,18] and in DNA replication [15]. This study also confirms the profile of GLUC mRNA [23]. Unlike the modifications of expression of the markers of cell proliferation and cell differentiation during postnatal development, we emphasize that the profile of expression of the 1.7 kb *Cdx* mRNA was apparently unchanged. In particular, the gradient

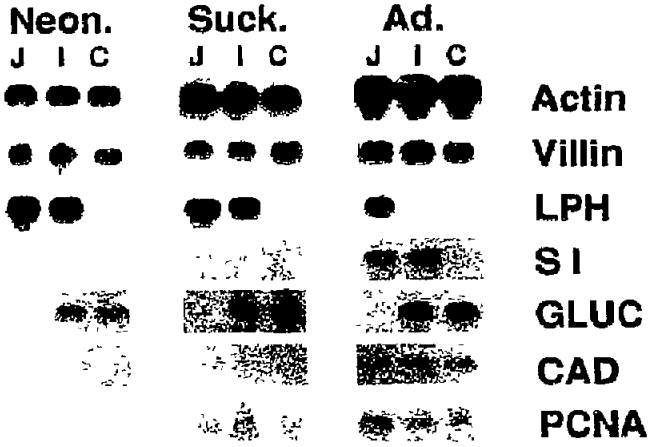

Fig 4. RNA expression of several intestinal markers. The same RNA samples as in Fig. 1 were hybridized to nucleotide probes for actin, villin, LPH, SI, GLUC, CAD and PCNA.

the colon of neonates lost small intestinal-like properties nor when the small intestine matured and acquired regional specificities at weaning. On the other hand, the onset of expression of the 6.5 kb *Cdx* transcript in sucklings may constitute a preliminary step for small intestinal maturation. Yet this is debatable, because this mRNA also appears in the colon although functional adaptation does no longer occur in this segment at weaning.

In conclusion, *Cdx* expression may be required to specify the intestinal epithelium from other endoderm derivatives, while defining functional regions along the intestinal tract. However the participation of *Cdx* homeogene(s) in the maturation of the intestinal tract during postnatal development remains an open question. Our results, combined with the fact that the *Drosophila caudal* gene controls the expression of another homeogene [24], may be a clue suggesting a primordial role for *Cdx* homeogene(s) in hierarchy of the gene expression during intestinal ontogeny in mammals.

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