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Developmental regulation of a gene that encodes a cysteine-rich intestinal protein and maps near the murine immunoglobulin heavy chain locus

(intestinal development/coordinate gene expression/mouse chromosome 12/protein structure/evolution)

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ABSTRACT **Mouse and rat small intestinal cDNA libraries were screened for recombinants derived from mRNAs whose concentration changed during the transition from suckling to weaning. cDNAs transcribed from a 570-nucleotide-long mRNA were isolated. Dot blot hybridization analyses of RNA recovered at various stages of rat gastrointestinal ontogeny indicated that the concentration of this mRNA begins to increase during the mid-suckling period, reaching a peak during weaning. There is considerable variation in the relative amount of this mRNA in adult tissues, with highest levels encountered in the rat small intestine and colon. Its concentration in duodenum, jejunum, and ileum is approximately the same. It is more concentrated in villi than in crypts. The rat mRNA encodes a 77 amino acid, 8.55-kDa polypeptide that has seven cysteine residues. This cysteine-rich intestinal protein (named CRIP) has two internal repeated sequence blocks. Computer-assisted comparisons of CRIP to proteins of known function disclosed that it is homologous to certain ferredoxins. Southern blot analyses revealed that sequences homologous to the rat gene are present in sea squirt, fish, bird, and human DNA, indicating that this gene is highly conserved and that related proteins may be present in many if not all vertebrates. Recombinant inbred mouse strains were utilized to show that the CRIP gene is closely linked to the immunoglobulin heavy chain constant region locus, *Igh-c*, on chromosome 12. CRIP mRNA is a molecular marker for the suckling-to-weaning transition of rodent intestinal development. The cloned cDNA may be a useful probe for identifying factors that regulate intestinal development during this period.**

Remarkable alterations in structure and function occur during the ontogeny of the rat and mouse small intestine (reviewed in ref. 1). The best-described biochemical changes in these altricial species are those that occur during the postnatal period as the suckling animal adapts first to the high-fat low-carbohydrate diet of mother's milk (2) and then to a low-fat high-carbohydrate chow diet. Examples of intestinal functions that show high activity during the suckling period, but reduced activity during the weaning period, include the export of triglyceride-rich lipoproteins (3), the transport of macromolecules by pinocytosis (4), and the hydrolysis of the principal dietary carbohydrates lactose and neuraminlactose by brush border lactase (5) and lysosomal neuraminidase (6). In contrast, the brush border enzymes maltase, sucrase, and alkaline phosphatase have low activities during the suckling period and high activities during or at the conclusion of weaning (7).

The molecular events that underlie these developmental processes have not been studied in a systematic way. Recent studies have focused on developmental changes in the accumulation of the most abundant rat intestinal mRNA sequences—i.e., those specifying apolipoproteins A-I and A-IV as well as two fatty acid binding proteins (8, 9). These sequences display a sudden dramatic increase in concentration within 24 hr after birth. We have now isolated a cloned cDNA transcribed from an intestinal mRNA whose concentration increases abruptly during the weaning period. This previously unknown abundant mRNA encodes a small cysteine-rich protein that has remarkable sequence similarities with ferredoxins. Evidence is presented which indicates that the sequence encoding this polypeptide was highly conserved during chordate evolution. The cDNA clone may be a useful probe for identifying factors that modulate the coordinated morphological, physiological, and biochemical changes that occur during the second through fourth postnatal week of rodent gastrointestinal development.

MATERIALS AND METHODS

Animals. Mice were from inbred strains maintained at The Jackson Laboratory. Sprague–Dawley rats were purchased from Sasco (St. Louis, MO).

RNA Isolation. Total cellular RNA was isolated from rat tissues by extraction with guanidine thiocyanate followed by centrifugation through CsCl (10). RNA was also isolated from small intestinal mucosal fractions enriched for villi and crypt cell populations by the procedure of Dietschy and Siperstein (11). Thymidine kinase and alkaline phosphatase assays indicated that the fractionation produced a 5- to 7-fold enrichment of each cell type.

Molecular Cloning of Developmentally Regulated mRNAs. A cDNA library was prepared (12) from C57BL/6J mouse intestinal poly(A)$^+$ RNA. To eliminate abundant mRNAs that are found in many tissues (e.g., those encoding cytoskeletal proteins), 432 intestinal clones were screened by colony hybridization with a cDNA probe made from brain poly(A)$^+$ RNA of 1-day-old mice. The 154 clones (36%) that did not hybridize above background levels were then plated in duplicate and probed with cDNA made from either 9-day suckling or adult intestinal poly(A)$^+$ RNA. Twelve of the 154 clones hybridized strongly to one or both of these probes and thus represented relatively abundant intestinal mRNA sequences. Of thes 12, the 4 that gave the most intense signals (pMI1 through pMI4) were selected for plasmid purification and further characterization.

DNA Sequencing. Restriction fragments from each plasmid were subcloned in M13 mp18 and mp19 phage and sequenced by the dideoxy chain termination method (13).

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Abbreviations: CRIP, cysteine-rich intestinal protein; apoAI, apolipoprotein A-I.

Primer Extension. An oligonucleotide primer, 5'-CCTCC-TTGTCGCACTTGGGGC-3', was synthesized using phosphoramide chemistry and an Applied Biosystems (Foster City, CA) model 380A machine. The primer was labeled at its 5' end with T4 polynucleotide kinase and [γ-^{32}P]ATP. Then 0.5 pmol of radiolabeled primer was annealed with 5 μg of rat small intestinal poly(A)$^+$ RNA in a 15-μl reaction mixture containing 100 mM Tris·HCl, pH 8.3, and 100 mM KCl. After a 2-hr incubation at 46°C, this solution was added to 15 μl of solution A (35 units of reverse transcriptase/16 mM $MgCl_2$/40 mM 2-mercaptoethanol/1.4 mM of each deoxynucleoside triphosphate). The mixture was then incubated at 46°C for 2 hr and the extension products were subsequently precipitated with ethanol before being electrophoresed on DNA sequencing gels.

Blot Hybridization. Restriction endonuclease digests of cellular DNA were blotted on nitrocellulose filters and hybridized to nick-translated ^{32}P-labeled plasmid DNA (14). Zetabind (AMF, Meriden, CT) nylon filter blots were probed by using conditions specified by the manufacturer. Total cellular RNA was analyzed by dot blot and electrophoretic blot hybridization (8).

Computer-Assisted Analysis of Protein Sequence Data. A VAX 11/780 running on the VMS operating system was used. The National Biomedical Research Foundation Protein Sequence Database was searched with the programs SEARCH (15) and FASTP (16). Protein sequence similarities were evaluated by using comparison matrices (17) and the RELATE algorithm (15). Protein sequences were aligned with PRTALN (18).

RESULTS

Identification of Abundant Intestinal mRNAs. Sequential screening (see *Materials and Methods*) of a mouse intestinal cDNA library identified four clones, pMI1 through pMI4, that represented abundant intestinal mRNAs. RNA blot analyses showed that both pMI1 and pMI2 hybridized to a 790-nucleotide intestinal mRNA (data not shown). Since both clones also hybridized to identical bands on Southern blots of mouse DNA, they are probably derived from the same mRNA. pMI3 hybridized to a 570-nucleotide intestinal mRNA. Both pMI4 and an apolipoprotein A-I (apoAI) cDNA clone (19) hybridized to a 1100-nucleotide intestinal mRNA. Southern blots of mouse DNA hybridized to either of these two clones produced identical bands (data not shown). We therefore concluded that pMI4 is an apoAI cDNA clone.

Dot blots of plasmid DNAs prepared from these clones were hybridized to 9-day suckling and adult intestinal cDNA.

Table 1. Dot blots of recombinant plasmids hybridized to cDNA probes

Plasmid DNA	DNA probe bound, cpm		Ratio adult/9-day
	9-day	Adult	
pMI2	188	3222	17.1
pMI3	195	4193	21.5
pMI4 (apoAI)	10872	13282	1.2

^{32}P-labeled cDNA probes with similar specific activities (70–75 cpm/pg) were generated in parallel incubations in the presence of identical amounts of small intestinal poly(A)$^+$ RNA. Each probe was hybridized to 1.0-μg plasmid DNA "dots" blotted onto nitrocellulose filters. After hybridization and washing, the radioactivity in the blot of each plasmid DNA sample was measured in scintillation fluid. A background of 188 cpm obtained with pBR322 DNA was subtracted from each sample.

Hybridization to pMI2 and pMI3 DNA was much higher with the adult cDNA, while pMI4 showed little difference in its reactivity with these two probes (Table 1). pMI4 is a useful control in this experiment—apoAI mRNA represents ≈1% of the total mRNA in adult mouse and rat small intestine, and its concentration does not change significantly during the weaning period (9, 20). The subsequent analyses presented in this paper focus on pMI3. Its mRNA concentration increases dramatically during the transition from suckling to adulthood. Moreover, this mRNA is relatively abundant in the adult mouse small intestine (estimated from Table 1 to be ≈0.3% when compared to apoAI mRNA).

Sequence of pMI3 and Comparison to a Homologous Rat Clone. pMI3 was used to screen a rat intestinal cDNA library (12). A probe-positive rat clone, pRI3, was isolated. The nucleotide sequences of the cDNAs contained in pMI3 are shown in Fig. 1. pRI3 has a 404-base-pair DNA insert. The highly homologous 303-base-pair mouse clone "begins" at rat nucleotide 144, extends to the end of the rat sequence, and then has an additional 42 adenosine residues originating from the poly(A) tail. The conserved polyadenylylation signal, AATAAA, begins 18 nucleotides upstream from the polyadenylylation site.

Blot analysis of rat and mouse intestinal RNA indicated that both pMI3 and pRI3 hybridized to a 570-nucleotide mRNA. Since the average length of the poly(A) tail in eukaryotic mRNAs is ≈200 nucleotides, it appeared that pRI3 was a full-length cDNA clone. To prove this, an oligonucleotide was synthesized that was complementary to nucleotides 72 through 92 (see Fig. 1). The oligonucleotide was annealed with rat intestinal poly(A)$^+$ RNA and then

```
        10         20         30         40         50         60         70         80         90        100
TCCAGAGCCTACAACCTACTTCCTTCTAGCTGCAGCCACTTGTGCAGGACCAGGTGCCGCCATGCCGAAGTGCCCCAAGTGCGACAAGGAGGTGTATTTC
                                                                    MetProLysCysProLysCysAspLysGluValTyrPhe

       110        120        130        140        150        160        170        180        190        200
                                                    ▼          T
GCTGAGCGAGTGACGTCACTAGGCAAGGACTGGCATCGTCCCTGCCTGAAGTGCGAGAAATGTGGAAAGACACTGACCTCTGGGGGTCATGCTGAGCATG
AlaGluArgValThrSerLeuGlyLysAspTrpHisArgProCysLeuLysCysGluLysCysGlyLysThrLeuThrSerGlyGlyHisAlaGluHisG

       210        220        230        240        250        260        270        280        290        300
                   T                                                                 G                  C
AAGGCAAGCCCTACTGCAACCATCCCTGCTACTCCGCCATGTTTGGGCCCAAAGGCTTTGGGCGAGGTGGAGCTGAAAGCCACACTTTCAAGTAGACTGA
luGlyLysProTyrCysAsnHisProCysTyrSerAlaMetPheGlyProLysGlyPheGlyArgGlyGlyAlaGluSerHisThrPheLys*am

       310        320        330        340        350        360        370        380        390        400
                   G          ACA             AC   CG             G G A   AG                 C
GGTTGTGGAAACTCTCCCTACCCGCCCAGGCGAGTGCCAGGCCTTGTCCCTAGACAGCAGGGCTCTCCGCAGCCCTCCATGCCTTTAATAAACTTGATCT

   G(A)42
TTGG
```

FIG. 1. DNA sequences of intestinal cDNA clones pRI3 and pMI3. The entire sequence of the pRI3 clone is shown. Above the pRI3 sequence, the corresponding pMI3 sequence is shown only at positions where the two sequences are different. The pMI3 sequence begins at pRI3 nucleotide 144 (indicated by an arrow) and ends with a poly(A) tail that is 42 nucleotides in length. The amino acid sequence encoded by pRI3 is shown below the DNA sequence. *am, amber termination codon.

extended with reverse transcriptase to reach the 5′ end of its mRNA. The same oligonucleotide was used as a sequencing primer for pRI3 DNA. The primer extension and sequencing results are shown in Fig. 2. The major primer extension product comigrated with the DNA sequencing band that represents the second C residue in the poly(C) tail. A minor product comigrated with the third C residue. These results indicate that pRI3 contains most if not all of the 5′ untranslated region of its mRNA.

Identification of the Primary Translation Product. The 5′-proximal AUG triplet serves as the initiator codon in most mRNAs examined (19). In the full-length rat clone it is located at nucleotide 62, where the initiator consensus sequence CCRCCAUG (R = purine) is also present (19). Translation of the rat sequence starting with this first methionine codon yields a 77 amino acid protein with a molecular weight of 8550 (Fig. 1). The protein is unusual in that it contains 7 cysteine residues. We therefore named it cysteine-rich intestinal protein, or CRIP. Comparison of the rat and mouse cDNA sequences (Fig. 1) indicated that all of the respective codons in which nucleotide sequence differences occurred are synonymous. The degree of nucleotide sequence identity is remarkable. Within comparable areas of the coding region they have 98% identity, while in the 3′ nontranslated region the sequences are 87% homologous.

Developmental Regulation and Tissue Distribution of CRIP mRNA. The results presented in Table 1 indicated that there is more CRIP mRNA in adult compared to 9-day-old small intestine. To characterize the developmental changes in greater detail, RNA was isolated from rat intestine at various ontogenic stages and hybridized to pRI3 DNA (Fig. 3). CRIP mRNA was first detected in the 21-day fetal gut. There was a modest rise during the first 2 days after birth. However, at 14 days a more dramatic elevation in mRNA concentration began. The highest concentration occurred at 24 days and was 8-fold greater than at birth. This was followed by a 40% decrease to the adult level by 35 days.

To determine the tissue specificity of CRIP expression, the relative amounts of CRIP mRNA in a variety of fetal and adult rat tissues were measured by using dot blot hybridization. Eighteen- and 21-day fetal liver, yolk sac, and placenta had no detectable CRIP mRNA (data not shown). In the adult, highest levels were present in the small intestine, although the colon also contained a relatively high concentration (46% of the small intestine). Lower levels were observed in lung (15%), spleen (12%), adrenal (9%), and testis (8%). CRIP mRNA was not detected in adult brain, kidney,

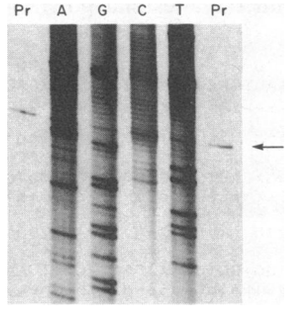

FIG. 2. Primer extension. A synthetic oligonucleotide primer was hybridized to rat intestinal poly(A)$^+$ RNA and extended in length by using reverse transcriptase. The same oligonucleotide was used as a sequencing primer for pRI3. The primer extension products (Pr) and the products from the four DNA sequencing reactions (A, G, C, T) were electrophoresed in a urea/polyacrylamide gel. The arrow marks the location of the major primer extension product. This product's termination points correspond to the second C residue in the poly(C) tail present at the 5′ end of the cDNA sequence.

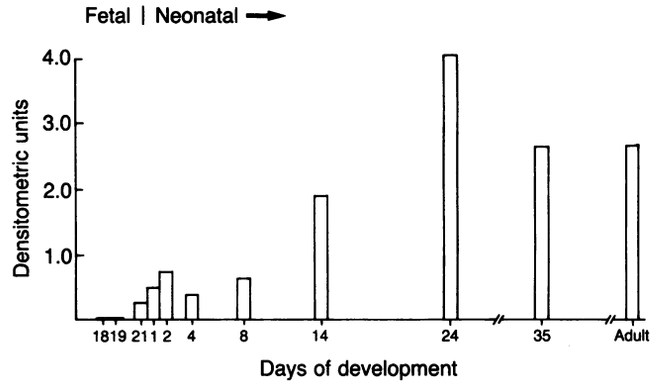

FIG. 3. Developmental regulation of CRIP mRNA accumulation in rat small intestine. For each developmental stage analyzed, *intact* intestines from 10–30 animals were pooled prior to RNA extraction. Total cellular RNA was denatured and applied to nitrocellulose filters by using a template manifold (8, 9). The dot blots contained reference standards of intestinal RNA diluted over a 100-fold concentration range (0.03–3 μg). Conditions used for hybridizing and washing the filters were previously described (8). Autoradiographs of the filters were analyzed by quantitative scanning densitometry in the linear range of film sensitivity.

or liver. These results indicate that there is considerable variation in the expression of CRIP mRNA in adult tissues.

We determined if gradients in the concentration of this mRNA existed along the length of the small bowel or between crypt and villus cells. Dot blot analyses revealed that the concentration of CRIP mRNA was the same in RNA prepared from intact adult rat duodenum, jejunum, and ileum. Hybridization analysis of RNA isolated from intestinal mucosal fractions enriched for either jejunal crypt or villus cells indicated that ≈70% of the mucosal CRIP mRNA was localized to the villus.

Computer-Assisted Analysis of CRIP. Information about the possible biological function of CRIP was obtained by identifying sequence similarities between CRIP and proteins of known function. When a search of the Protein Sequence Database was conducted using either the SEARCH (15) or FASTP (16) algorithms, a list of the 10 best sequence matches consistently contained multiple ferredoxin entries from a variety of species. The ferredoxins are small, iron-containing proteins that are found in plants and bacteria. They function as electron carriers in a wide variety of biochemical processes such as photosynthesis, oxidation–reduction respiratory reactions, nitrogen fixation, and sulfate reduction.

Fig. 4 shows an alignment of CRIP with the ferredoxin from the bacteria *Megasphaera elsdenii* (21). Particularly noteworthy is the similar spacing of cysteines in the two proteins. Each of the two domains in the classical clostridial-type ferredoxins contains a characteristic pattern of four cysteines and a proline, CXXCXXCXXXCP (22). These ferredoxins have two [4Fe-4S] clusters.

The program RELATE is a sensitive computational method for detecting and quantitating sequence similarities (15). Comparison scores are expressed in SD units and are derived from a comparison of the mean score for the real sequences with the mean score achieved from comparisons of randomized sequences that have the same amino acid composition as the real sequence. RELATE analyses of the two proteins shown in Fig. 4 gave a comparison score of 4.542 SD units, using a span of 47 amino acids and 100 runs of shuffled sequences. The probability of obtaining this SD score by chance alone is less than one in 10^6. The greatest homology was seen when residues 28 to 74 of CRIP and residues 8 to 54 of *M. elsdenii* ferredoxin were compared. This is consistent with the results of the alignment generated by PRTALN.

```
(CRIP)
         10        20        30        40        50          60        70
MPKCPKCDKEVYFAERVTSLGKDWHRPCLKCEKCGKTLTSGGHAEHEGKPYCNHPCYS--AMFGPKGFGRGGAESHTFK
                    .     :.::. :..: ..:. .: :.:    ...: .  :  .     :  .::
                    MHVISDECVKCGACASTCPTGAIEEGETKYVVTDSCIDCGACEAVCPTGAISAE
                             10        20        30         40        50
(FEME)
```

FIG. 4. Alignment of CRIP and *M. elsdenii* ferredoxin. The program PRTALN was used to align the two protein sequences (*k*-tuple = 1, window size = 20, gap penalty = 2). Amino acid identities are indicated by colons and conservative substitutions defined by the mutation data matrix (16) are indicated by periods.

Because some ferredoxins contain internally repeated sequences that define functionally important domains (22), we searched for repeats within CRIP using the comparison matrix method described by McLachlan (17). The presence of short diagonal lines offset from the main diagonal in the matrix plot indicated that CRIP contains repeated sequences (Fig. 5). When CRIP was compared to itself by using RELATE, two repeated sequence blocks were also identified:

```
    3                       27     25                    47
(A) KCPKCDKEVYFAERVTGLGKDWHRP  (B) HRPCLKCEKCGKTLTGGGHAEHE
    :: ::.:..   .....   :: . ..     ..:: .  .   :.....:: ..:.
    KCEKCGKTLTGGGHAEHEGKPYCNH      NHPCYSAMFGPKGFGRGGAESHT
    30                      54     53                    75
```

(Symbols as in Fig. 4.) The segment comparison scores were highly significant in both cases (SD = 6.220 for *A* with a span of 24 and 100 comparisons of shuffled sequences; SD = 6.084 for *B* with a span of 22). The probability of achieving these scores by chance alone is less than 1 in 10^{10}. The highly conserved sequence blocks displayed in *B* include the region of CRIP that is homologous to the ferredoxin shown in Fig. 4. These domains may have analogous structural or functional roles in the two polypeptides.

Evolution of the CRIP Gene. Since the protein sequence comparisons suggested that CRIP was related to plant and bacterial ferredoxins, we examined the possibility that the CRIP DNA sequence may be highly conserved in the phylum Chordata. Mammalian DNAs from rat, mouse, and human were blotted and hybridized to pRI3 by the Southern procedure (Fig. 6*A*). One to four bands were detected in each digest. Chicken DNA run on the same gel and washed under the same stringency conditions (52°C in 15 mM NaCl/1.5 mM sodium citrate/0.1% $NaDodSO_4$) showed the presence of one major band in each digest (Fig. 6*A*). When the filter was washed under higher stringency conditions by raising the temperature to 60°C, the chicken bands were not detectable but the mammalian bands remained unchanged (data not shown). To investigate more divergent species, another Southern blot was prepared of rat, chicken, fish (*Lebistes reticularis*), and sea squirt (*Botryllus schlosseri*) DNAs. The filter was washed at 45°C in 15 mM NaCl/1.5 mM sodium citrate/0.05% $NaDodSO_4$. Surprisingly, bands were detectable in all species (Fig. 6*B*). These results suggest that an ancestral gene existed very early in chordate evolution. The unambiguous sequence homology detected in teleost DNA indicates that related genes may have existed when fish diverged from other vertebrates 450 million years ago.

Chromosomal Location. Insight into gene function can sometimes be obtained by chromosomal mapping, especially if a sequence maps to a known mutation. The mouse gene was mapped by using pRI3 to probe Southern blots of mouse DNA from recombinant inbred (RI) strains (23). Initial screening of nine progenitor strains revealed that in C57BL/6J both *Kpn* I and *Taq* I generated restriction fragment length polymorphisms. In AKR/J, *Taq* I generated a polymorphism. Twenty-five C57BL/6J × DBA/2J, 12 C57BL/6J × C3H/HeJ, 7 C57BL/6J × BALB/cByJ, and 18

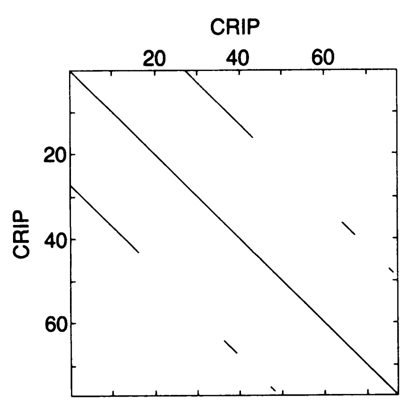

FIG. 5. Comparison matrix analysis of CRIP. The algorithm of McLachlan (17) was employed, using spans of 25 residues. Only spans achieving comparison scores with a probability of chance occurrence of 10^{-3} or less are plotted.

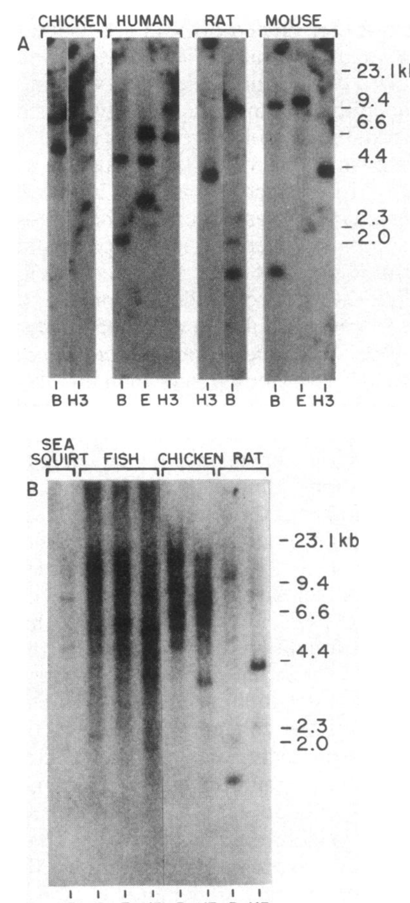

FIG. 6. Southern blot analysis of cellular DNA hybridized to pRI3. (*A*) This Zetabind nylon filter was washed at 52°C as described in *Results*. (*B*) This nitrocellulose filter was washed at 45°C as described in *Results*. DNAs were digested with *Bam*HI (B), *Eco*RI (E), or *Hin*dIII (H3).

AKR/J × C57L/J RI strains were screened. Southern blot analysis of these 62 RI strains indicated that no crossovers occurred between CRIP and the immunoglobulin heavy chain constant region locus (*Igh-c*). These data indicate that CRIP is closely linked to the *Igh-c* locus on mouse chromosome 12 even though CRIP does not have significant sequence similarities with murine immunoglobulins. The upper 95% confidence limit of the actual map distance is 1.3 centimorgans. On the basis of linkage conservation within this chromosomal segment between mouse and man (24), we predict that the homologous gene is located on the long arm of chromosome 14 in humans.

DISCUSSION

In rats, the weaning period spans the 12th through 28th day of postnatal life (25). During this interval the intestinal epithelium undergoes a period of remarkable morphological and biochemical differentiation—e.g., mucosal cell turnover increases and a number of brush border enzymes (maltase, alkaline phosphatase, sucrase, leucine aminopeptidase) begin their dramatic rise in activity towards adult levels (1, 7, 26). Although thyroid hormones and glucocorticoids have been implicated as regulators of some of these changes (1), little is known about the molecular events that produce these developmental alterations. We report that an intestinal mRNA shows significant changes in concentration during the weaning period. As such, the cloned cDNA transcribed from this mRNA now provides a molecular probe for the weaning stage of intestinal development.

Interpretation of the observed changes in CRIP mRNA concentration illustrates a fundamental problem that will be encountered as other cloned cDNAs are used as hybridization probes to quantitate alterations in the *accumulation* of specific mRNAs during the suckling-to-weaning transition. The intestinal epithelium undergoes continuous replacement owing to the proliferation of progenitor crypt cells, which are translocated towards the villus tip during their subsequent differentiation. Morphometric analyses (26) have shown that during this period there is an increase in the depth of the crypt region and a relative decrease in the population of villus compared to dividing crypt cells. This produces a decrease in the ratio of villus height to crypt depth from 6.5 to 2 (26). The absolute and relative population of proliferating cells in the crypts increases. (The turnover rate of *mucosal* cells in 21- to 32-day-old animals is 4 times greater than in 5-day-old pups.) Thus, an increase in the *accumulation* of CRIP mRNA during the weaning stage of postnatal intestinal development could result from several mechanisms: (*i*) an increase in CRIP mRNA concentration within a given cell type; (*ii*) a change in the *absolute* number of these cells without a change in their fractional representation among intestinal epithelial cells or in the concentration of CRIP mRNA per cell; (*iii*) a change in the relative representation of crypt versus villus cells; or a combination of *i–iii*. Since the dot blot hybridization analyses demonstrate an increase in the relative *concentration* of CRIP mRNA per μg of total cellular small intestinal RNA, they would not be influenced by *ii*. We believe that the increase in CRIP mRNA concentration reflects either *i* or *iii*. *In situ* hybridization techniques provide a direct way of investigating these possibilities and of confirming our data from the mucosal fraction experiments that levels of CRIP mRNA are higher in villus than in crypt cells. The factors that modulate CRIP gene expression are unknown but the similarities between the kinetics of its induction and the "adult" brush border hydrolases raise the possibility that they are regulated by similar hormonal and dietary factors (1).

We know of no other study that has described CRIP. However, it is interesting to note that cell-free translations of 17-day fetal and adult mouse intestinal RNA indicated that several abundant mRNAs encoding low molecular weight [^{35}S]-cysteine-labeled proteins were detectable in the adult but not fetal gut (25). The CRIP gene sequence appears to be highly conserved and present in many, if not all, vertebrates. This observation, coupled with the fact that CRIP mRNA is abundant in adult rodent small intestine, suggests that CRIP has an important role in nutrient absorption or intestinal metabolism. Computer-assisted analyses of the CRIP sequence indicated that it has similarities to certain ferredoxins. It is interesting to speculate that CRIP is a mammalian member of the ancient ferredoxin gene superfamily and is therefore an iron-containing protein. If so, CRIP could be involved in electron transport or iron metabolism. However, two mouse mutations, microcytic anemia and sex-linked anemia, that have abnormal intestinal iron *transport*, do not map to the CRIP locus on chromosome 12 (27). Further analysis of CRIP's function will require its isolation from gut epithelium or *Escherichia coli* after introduction of expression vectors containing CRIP cDNA.

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