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Vectors as Tools for the Study of Normal and Abnormal Growth and Differentiation

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THE ALBINO PERINATAL LETHAL MUTATION: IDENTIFICATION OF AFFECTED mRNAs AND MAPPING OF THE LOCUS BY PULSED-FIELD GEL ELECTROPHORESIS

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Summary

The analysis of chromosomal deletions overlapping at the albino locus in the mouse has led to the postulation of a number of loci in this region essential for viability. One of these, the perinatal survival locus, is thought to be important for the differentiation of the liver since its deletion results in the deficiency of a set of liver-specific enzymes and ultrastructural abnormalities in the hepatocyte. By differential cDNA screening we have isolated and examined the expression of a panel of mRNAs influenced by deletion of the locus. This has been done in an attempt to elucidate the role of the normal gene product. In addition, as a prelude to cloning the perinatal survival locus, a long-range restriction map has been established onto which the extents of albino locus deletions are being placed.

Introduction

The classical genetic approach to understanding development and differentiation is the generation of mutations which arrest or alter these processes. The isolation and characterization of the affected genes may follow, according to the mutagen employed. The albino locus, designated c, on chromosome 7 in the mouse, was an early target owing to the ease of identifying mutational events through changes in coat colour. From the pleiotropic effects of a set of radiation induced c alleles it was inferred that the mutations represented deletions of various sizes overlapping at c (Gluecksohn-Waelsch et al., 1974; Gluecksohn-Waelsch, 1979). Subsequently, thirty-seven deletions have been assigned to twelve groups on the basis of detailed complementation analysis of the phenotypes they engender (Russell et al., 1979; 1982). Amongst the phenotypes are recessive lethalities (Russell and Raymer, 1979) and at least three regions linked to c essential for early embryonic development and one for survival beyond birth have been

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proposed from the complementation map (Russell et al., 1982; Niswander et al., 1988). The perinatal lethality has been investigated extensively by Gluecksohn-Waelsch and her colleagues, and the observations of the phenotype would seem to suggest that the perinatal survival locus is required for the full biochemical differentiation of the hepatocyte (Gluecksohn-Waelsch, 1979; 1987).

The perinatal lethal phenotype is associated most strikingly with a reduction in activities of a number of enzymes in the liver and with ultrastructural abnormalities of hepatocytes and cells of the proximal convoluted tubules of the kidney (Trigg and Gluecksohn-Waelsch, 1973). The deficient enzymes include glucose-6-phosphatase (G6Pase), tyrosine aminotransferase (TAT), serine dehydratase (SDH) and phosphoenol-pyruvate carboxykinase (PEPCK) (Erickson et al., 1968; Thorndike et al., 1973; Gluecksohn-Waelsch et al., 1974; Loose et al., 1986). A large number of other enzymes remain uninfluenced by the mutation, attesting to its specific nature (Thorndike et al., 1973; Gluecksohn-Waelsch, 1979). This accords with the results of two-dimensional gel electrophoresis of liver extracts which have shown that very few polypeptides are visibly altered in abundance (Baier et al., 1984). In the case of PEPCK and TAT it has been established that the decreased enzyme activities result from lower levels of steady-state mRNA (Schmid et al., 1985; Loose et al., 1986) and impaired transcription has been imputed from nuclear run-on assays partly to be responsible for this (Loose et al., 1986; E. Schmid, S.R. et al., unpublished observations). Aldolase B and metallothionein I have recently been added to the list of mRNAs reduced in abundance (Sala-Trepat et al., 1985; DeFranco et al., 1988). The synthesis of serum proteins by the liver is also diminished (Garland et al., 1976). However, this has no basis in decreased transcriptional rates (Sala-Trepat et al., 1985) and may be a consequence of the accompanying ultrastructural lesions (see below). The absence of a gene dosage effect on enzyme activities in heterozygotes was taken as an early indication that the perinatal survival locus acted in _trans_ (Russell et al., 1969). Subsequently, cell fusion experiments (Cori et al., 1981; 1983), the mapping of the genes encoding the affected enzymes to chromosomes other than 7 (Lem and Fournier, 1985; Müller et al., 1985; Peterson et al., 1985), or the direct demonstration that their structural loci were not deleted in the mutant mice (Schmid et al., 1985; Loose et al., 1986) came as further evidence that the locus acted in _trans_ and might be regulatory in nature.

Most of the enzymes influenced by the mutation play a role in gluconeogenesis and the deficiency of G6Pase contributes to the intractable hypoglycaemia regarded as the cause of death of the newborn homozygotes (Erickson et al., 1968). These enzymes are controlled by dietary status and their expression is regulated by glucagon, via cAMP, or glucocorticoids, or both (Greengard, 1970; Granner and Beale, 1985). In

addition, although their onsets of expression may differ, enzyme activities rise in the immediate post-natal period (Greengard, 1970), presumably in response to the hormonal changes occurring at that time. The precocious induction observed in fetuses after in utero administration of glucagon or cAMP underlines the importance of hormones in the developmental activation (Greengard, 1970; Garcia Ruiz et al., 1978). It was an early observation that in the albino lethal mice G6Pase activity was not inducible in late gestation fetuses (Erickson et al., 1968). TAT and PEPCK mRNA levels, likewise, fail to respond to their activators in livers of the mutant mice (Schmid et al., 1985; Loose et al., 1986) but, whilst the characteristic post-natal increase is abolished, pre-natal mRNA levels are identical to wild-type mice (Donner et al., 1988). Taken together, these observations have given rise to the hypothesis that the locus encodes a factor that confers on the set of genes the competence to respond to hormonal activation (Gluecksohn-Waelsch, 1987).

A second feature of the perinatal lethal phenotype is the disruption of the integrity of some intracellular membranes, specifically of the hepatocyte and cells of the proximal convoluted tubule of the kidney, as observed by electron microscopy. The membranes of the nucleus, rough endoplasmic reticulum and Golgi apparatus are involved. There is a tendency to dilation and vesiculation of these membrane structures and loss of membrane-bound ribosomes. The abnormalities are first detectable at day 18 of gestation, and are displayed by an increasing number of cells until birth (Trigg and Gluecksohn-Waelsch, 1973). Analysis of microsomal polypeptide populations failed to reveal a significant difference between wild-type and mutant material, however (Erickson et al., 1974). The relationship the two characteristics of the phenotype, the biochemical and ultrastructural abnormalities, have to one another is not yet clear, especially in view of the fact that the deficient liver enzymes are localized in different sub-cellular compartments (Gluecksohn-Waelsch, 1979).

In order to understand the perinatal lethal phenotype more fully and to elucidate the nature and function of the product of the perinatal survival locus two experiments are underway. Firstly, we have attempted to identify a broader set of genes influenced by the mutation than was previously possible: by differential screening we have tried to collect cDNAs for affected mRNAs whose selection is not limited by the availability of enzyme assays. This has been done to identify more rigorously a common denominator for the affected genes. Secondly, since the ultimate demonstration of the function of the gene depends upon its isolation, we are attempting to clone it from the knowledge of its chromosomal location. In keeping with the theme of this book, this communication is a review of our progress in both these areas.

Methods

Differential screening of a newborn mouse liver cDNA library. A full treatment of the construction and differential screening of the newborn mouse liver cDNA library is to appear elsewhere (Ruppert et al., 1989). The inserts of the recombinant phage isolated were subcloned into Bluescript $M13^+$ (Stratagene) for the purpose of generating hybridization probes and partial sequence analysis. Sequencing was performed by the chain-termination method using T3 or T7 promoter-specific oligonucleotides to prime Klenow polymerase (Lim and Pène, 1988). The sequences were compared to those in the GEN-Bank data bank.

Northern analysis. RNA was prepared from various tissues of newborn albino lethal (genotype c^{14CoS}/c^{14CoS}) and wild-type littermates (c^{ch}/c^{14CoS} and c^{ch}/c^{ch}) according to the method of Krieg et al. (1983). 2S FAZA (Brown and Weiss, 1975) cells were maintained in DMEM/HAM F12 (1:1) medium containing 10% fetal calf serum. Two days before harvesting for RNA, cells were washed with PBS and induced in complete medium in the presence of $3x10^{-6}$ M dexamethasone in ethanol or ethanol alone as control. Five microgram total RNA was fractionated per lane on 1% agarose/formaldehyde gels (Lehrach et al., 1977) followed by transfer in 10x SSC onto Gene Screen membranes (NEN), baking and UV-crosslinking. Filters were hybridized at 65-70°C in 50% formamide; 5x SSC; 50 mM sodium phosphate pH 6.5; 8x Denhardt's solution; 1% SDS and 500 μgml^{-1} yeast RNA containing ^{32}P labelled antisense RNA probes. Post-hybridization washes were 0.1x SSC; 1% SDS at 65-80°C. Probes were synthesized from appropriate templates by SP6, T3 or T7 polymerases (Melton et al., 1984). Additional probes used were the mouse TAT cDNA (S.R., unpublished); the rat SDH cDNA (Noda et al., 1985); a mouse transferrin cDNA (S.R., unpublished, identified by homology to the human sequence of Yang et al., 1984) and rat glyceraldehyde-3-phosphate dehydrogenase (Fort et al., 1985).

Pulsed-field gel electrophoresis. Very high molecular weight DNA suitable for pulsed-field gel electrophoresis was prepared from newborn and adult liver by embedding cells in low-melting point agarose. The procedure was that of Herrmann et al. (1987), with the exceptions that fresh tissues were used and that each 80 µl block contained the equivalent of $0.5x10^6$ cells. For restriction enzyme digestions, blocks were rinsed extensively in TE and placed in 120 µl reaction mix containing the appropriate digestion buffer and incubated with up to 20U enzyme (New England Biolabs) for a minimum of 6 h. In the case of double digestions, blocks were rinsed briefly in water before the second incubation. Blocks were loaded directly into the wells of agarose gels without further treatment. Pulsed-field gel electrophoresis was carried out in an LKB Pulsaphor

apparatus equipped with OFAGE or hexagonal electrode arrays. Gels were 1% agarose, except for when resolution of fragments greater than 3000 kb was desired when the percentage was reduced to 0.6%. Electrophoresis was conducted in 0.25x TBE (TBE = 89 mM Tris base; 89 mM boric acid; 2 mM EDTA), with running conditions based on those of Vollrath and Davis (1987) and Birren et al. (1988). Size markers were multimers of γ, and chromosomes of Saccharomyces cerevisiae strain AB972 and Schizosaccharomyces pombe. DNA was transferred to Gene Screen (NEN) filters in alkali (Jantzen et al., 1987) and immobilized by baking and UV-crosslinking. Hybridizations and washings were performed according to Church and Gilbert (1984). Probes were labelled either with [^{32}-P]dCTP by random priming (Feinberg and Vogelstein, 1984) or with [^{32}-P]UTP by transcription of appropriate templates by SP6, T3 or T7 RNA polymerases (Melton et al., 1984). Probes used were the tyrosinase cDNA or subfragments thereof (Ruppert et al., 1988); a 2.2 kb EcoRI:XhoI genomic fragment encompassing exon I of the tyrosinase gene (Ruppert et al., 1988); and 12A (Disteche and Adler, 1984).

Animals. Mouse strains carrying the albino lethal deletions c^{3H} and c^{14CoS} were obtained from S. Gluecksohn-Waelsch (Albert Einstein College of Medicine, Bronx, New York), and c^{15R60L} was provided by L.B. Russell and E.M. Rinchik (Oakridge National Laboratory, Tennessee). Each was maintained as a separate line as heterozygotes with c^{ch}.

Results

Isolation and characterization of cDNAs representing mRNAs affected by deletion of the perinatal survival locus.

A cDNA library was prepared from poly(A$^+$) RNA from normal newborn mouse liver and duplicate filters were screened with probes representing the newborn liver mRNA population of normal or albino lethal mutant (genotype c^{3H}/c^{3H}) mice. Fifty plaques out of a plating of 5×10^5 showed a more intense hybridization to wild-type than mutant probes. Twenty-one of these signals remained differential through subsequent rounds of plaque purification and could be grouped into nine families, X1 to X9, on the basis of cross-hybridization of their inserts. None proved to represent DNA from the albino deletion complex, indicating that a cDNA for the perinatal survival locus had not itself been recovered (Ruppert et al., 1989).

Partial sequence analysis indicated that cDNA X3 represented the mouse PEPCK message, by homology to that of the rat (Beale et al., 1985); X4 showed substantial homology to human and rat α-fibrinogen cDNAs (80 and 90% identity, respectively;

Rixon et al., 1983; Crabtree et al., 1985); X6 to rat serine protease inhibitors (Le Cam et al., 1987; Yoon et al., 1987); and the X8 sequence had 90% identity with the rat aldolase B cDNA (Tsutsumi et al., 1984). The expression of both the aldolase B and PEPCK genes have previously been shown to be influenced by the albino perinatal lethal deletions (Sala-Trepat et al., 1985; Loose et al., 1986). The identification of these cDNAs in our set indicates the success of the differential screening approach. The isolation of the cDNAs encoding α-fibrinogen and a serine protease inhibitor amongst our set demonstrates that the lesion at the level of the mRNA is not restricted to metabolic enzymes.

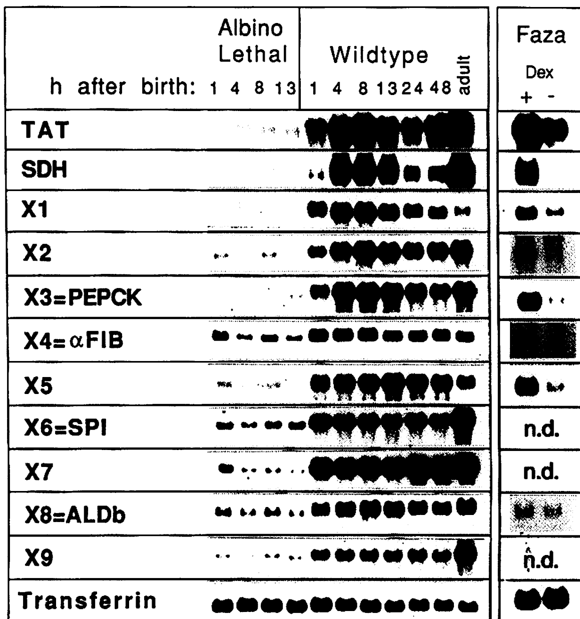

FIG. 1. Postnatal regulation and hormone induction of mRNAs influenced by the perinatal lethality. Total RNA (5 μg) isolated from livers of albino lethal mice and newborn littermates at the indicated time points after birth was analyzed by Northern blot hybridization. Filters were hyridized with antisense RNA probes derived from the cDNAs isolated by differential screening, X1 to X9, and with TAT and SDH cDNAs. In the right-hand panel the same cDNAs were used to investigate the expression of the homologous rat mRNAs in the rat hepatoma cell line 2S FAZA (Faza) in the presence (+) or absence (-) of the synthetic glucocorticoid dexamethasone (Dex). **n.d.** indicates no expression detected. RNA quality and loading was controlled by rehybridizing all filters to transferrin or glyceraldehyde-3-phosphate dehydrogenase probes. One result with transferrin is shown here.

Northern blot analysis has been used to investigate the expression of the set of mRNAs in liver and other tissues, to establish their onset of expression and to determine in which tissues the mutation has an effect. In addition to the cDNAs X1 to X9, probes for the mouse TAT (S.R., unpublished) and rat SDH (Noda et al., 1985) have been used. From Fig. 1 it is evident that the level of each of the mRNAs is sub-

stantially reduced in livers of the mutant mice in comparison to that seen in their normal newborn littermates. Some of the mRNAs, such as X1, X2 and X3 (= PEPCK), behave like TAT and SDH and show an increase in abundance during the first hours after birth, followed by a decline.

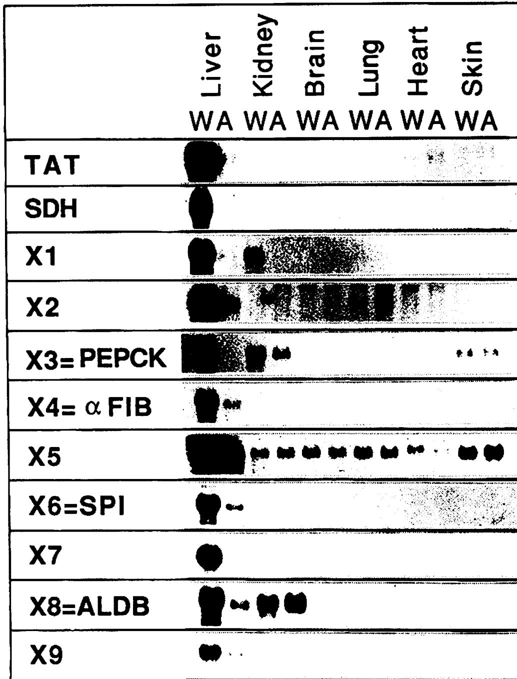

FIG. 2. Tissue-specific manifestation of the phenotype at the level of mRNA. RNA (total, 5 µg) from various tissues from albino lethal mice (A) and their wild-type (W) littermates was analyzed by Northern blotting. The autoradiographs were exposed for different times. RNA quality and loading was controlled as in Fig. 1. The apparent reduction in the lane marked 'Heart A' is due to underloading.

The mRNAs have the property in common that the highest steady-state levels are attained in liver in comparison with other organs (Fig. 2). The spectrum of expression, however, differs from the apparently liver-specific, as in the case of SDH, X4, X6, X7 and X9, to ubiquitous amongst the organs tested, e.g., X5. The influence of the mutation clearly extends only to liver and kidney, however. Intriguingly, those mRNAs that are expressed in the kidney exhibit different behaviours. Whereas X3/PEPCK and X1 mRNAs are decreased in kidney of the mutant, others, aldolase B and X5, escape the influence. Detection of the mRNAs by in situ hybridization to tissue sections has indicated that this difference cannot solely be attributed to expression of the genes in different cell populations in the kidney (Ruppert et al., 1989). However, it might be relevant that in the rat PEPCK expression is activated by glucocorticoids in the kidney (Meisner et al., 1985), whereas aldolase B is not (Munnich et al., 1985). This finding has a parallel in the expression of metallothionein I whose mRNA is reduced in liver but not in kidney of albino lethal mice, and glucocorticoid responsiveness is apparent in the liver but not the kidney (DeFranco et al., 1988).

The enzymes traditionally known to be deficient in the albino lethal mice are normally subject to regulation by glucagon or glucocorticoids. To begin to examine whether the additional mRNAs we have isolated have in common induction by hormones, the expression of their rat homologues was analysed in the rat hepatoma cell line 2S FAZA. For those species for which expression could be detected, the abundance of the mRNA was clearly elevated after a 46-h treatment with the synthetic glucocorticoid, dexamethasone (Fig. 1).

Long range restriction mapping around the perinatal survival locus.

We are attempting to isolate the perinatal survival locus from the knowledge of its chromosomal location. This has been made possible with the isolation of probes mapping to the c locus. Recently, we and others have cloned the cDNAs encoding human and mouse tyrosinase (Kwon et al., 1987; Yamamoto et al., 1987; Ruppert et al., 1988), which is the enzyme encoded at the c locus (Müller et al., 1988) and essential for melanin production, and hence crucial for coat colour. This has, in turn, led to the cloning of the entire structural gene, which has proven to occupy 70 kb (Ruppert et al., 1988). Probes from the tyrosinase gene have been used to construct a long-range restriction map around c by employing restriction enzymes that cut very rarely in the mammalian genome (Brown and Bird, 1986) in combination with pulsed-field gel electrophoresis by which very large fragments of DNA can be resolved (reviewed by Barlow and Lehrach, 1987). The aim is to superimpose the genetic map of the albino deletions onto the molecular map in such a way as to define the minimal region in which the perinatal survival locus is located. The following information is being procured: the mapping of the position and extent of the deletions that do and do not remove the perinatal survival locus; the orientation of the derived map on the chromosome and within it the orientation of the tyrosinase gene so as to indicate the direction for chromosomal jumping and walking.

The various levels of the mapping exercise are illustrated in Fig. 3. Fig. 3A provides a map of some of the relevant complementation groups (modified from Russell et al., 1982). Group A, for example, involves the perinatal survival locus and tyrosinase but no other known markers. The positions of two deletions belonging to this group, deletions c^{14CoS} and c^{15R60L}, have been derived from the use of a probe, 12A, that was previously isolated from a library of sorted chromosomes (Disteche and Adler, 1984). 12A proved to map within the albino deletion complex, but the sequence it recognizes is not removed by these two deletions. In addition, the probe must map distal to c, from genetic considerations. A crude map, covering approximately 5000 kb, of some very large restriction fragments detected by the tyrosinase and 12A probes has been produced. The limits of the two deletions can be roughly placed within this (Fig. 3B).

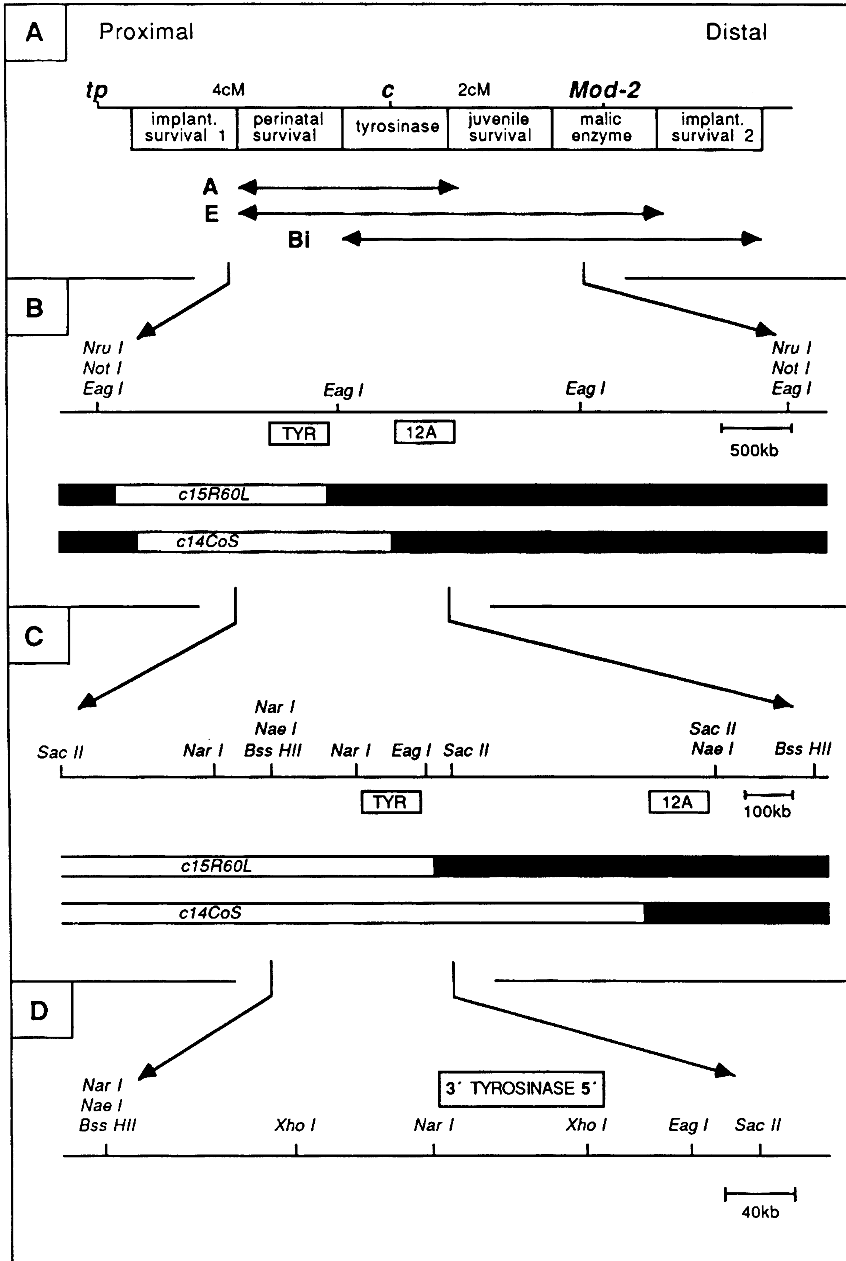

Figure 3

Legend to FIG. 3. Summary of the long range restriction mapping around the perinatal survival locus. **A.** Complementation map of a set of the deletions at the c locus (modified from Russell et al., 1982). For simplicity, only three of the twelve complementation groups are illustrated. Of the deletions referred to in this work, c^{14CoS} and c^{15R60L} belong to group A and c^{3H} to E. The boxes indicate six of the loci predicted in this region. **tp** is the marker taupe and **Mod-2** the structural locus for the mitochondrial malic enzyme. **B.** Restriction map of ≈ 5000 kb surrounding the tyrosinase gene (TYR) and the approximate location of two A group deletions. The deletions are represented by open regions within the filled bars. 12A is an anonymous DNA sequence mapping distal to c (Disteche and Adler, 1984). **C.** Restriction sites within 1500 kb of the tyrosinase gene, interpreted from the data in Table 1. The positions of the distal breakpoints of the illustrated deletions were inferred from information gained with additional enzymes. **D.** The orientation of the tyrosinase gene within the mapped region derives from the presence of an XhoI site in the first exon and an NarI site in the cloned region 3' to the gene.

This would indicate deletions of about 1500 kb, which is in accord with genetic and cytogenetic considerations which put the c^{14CoS} deletion at 1cM, or 2000 kb.

Table.1

	Fragments Recognized By	
ENZYME	**TYR**	**12A**
Sac II	850kb	580kb
Sac II + Bss HII	380kb	580kb
Bss HII	1200kb	1200kb
Sac II +Nae I	380kb	580kb
Nae I	950kb	950kb
Sac II + Nar I	180, 380, 520kb	580kb
Nar I	>1200kb	>1200kb
Nar I + Xho I	90kb	n.t.
Xho I	170kb	n.t.

Restriction fragments detected by the tyrosine (TYR) and 12A probes by pulsed-field gel electrophoresis analysis contributed to the maps shown in Fig. 3C and 3D. **n.t.** not tested.

A higher resolution mapping has been achieved of the 1500 kb surrounding the tyrosinase gene and extending towards the perinatal survival locus. The sizes of restriction fragments hybridizing to tyrosinase probes is given in Table 1, and the derived map is shown in Fig. 3C. The orientation of the map on the chromosome was deduced by linking up the mapping information for the 12A probe (Table 1). Both probes detected BssHII and NaeI fragments of similar sizes, but distinct SacII fragments. To demonstrate that the BssHII fragments were indeed the same and that the SacII fragments were adjacent, products of complete BssHII followed by partial SacII digests were compared. Fig. 4 illustrates the cleavage of the common 1200 kb BssHII fragment, via a common 1000 kb intermediate, to the ultimate BssHII:SacII and SacII fragments recognized by the tyrosinase and 12A probes have been defined from digests with additional, more frequently cutting enzymes (data not shown).

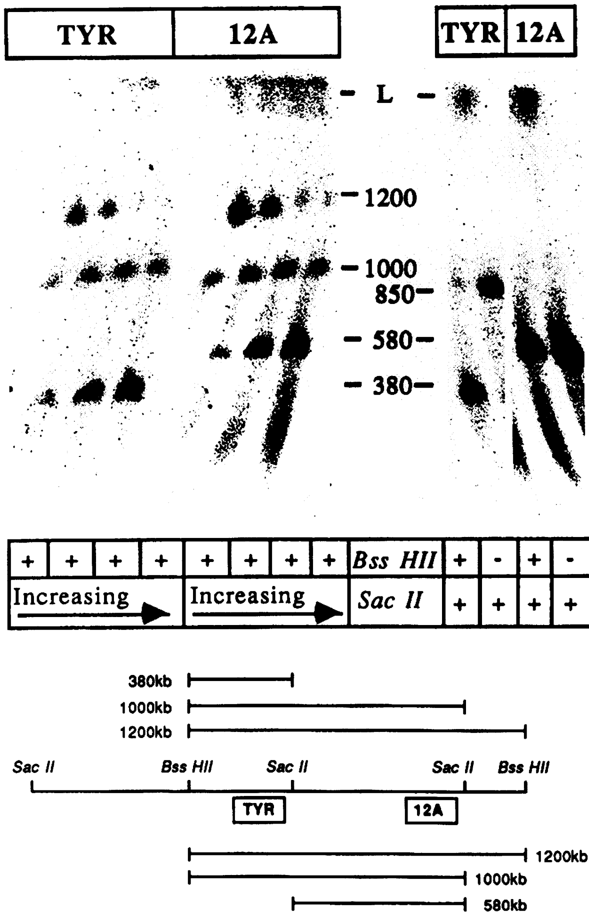

FIG. 4. Linking of the tyrosinase and 12A probes to adjacent SacII fragments. In the left-hand panel, DNA was digested to completion with BssHII, and then with increasing amounts of SacII to achieve partial digestion. In the right-hand panel, DNA was digested with SacII alone or in combination with BssHII. The map below is the interpretation of the bands detected. DNA was resolved by OFAGE (250 V, 120 sec pulse, 47 h) and a Southern blot hybridized sequentially with a tyrosinase cDNA (TYR) and the 12A probe. Probe was removed between hybridizations. Fragment sizes given in kb were estimated from the migration of chromosome of S. cerevisiae AB972. L indicates the limiting mobility region of the gel.

In order to be able to use tyrosinase probes as start points for chromosomal jumps to progress towards the locus, information concerning the orientation of the

tyrosinase gene and restriction sites within and flanking it is required. Fig. 3D depicts sites close to tyrosinase, taken from the data in Table 1. Since the location of the XhoI and NarI sites within the 100 kb cloned around the gene are known, the transcriptional orientation can be inferred.

Conclusions and Perspectives

The properties of a set of mRNAs whose abundance is reduced in the livers of albino lethal mice have been examined in order to come to an understanding of the nature of the perinatal lethal phenotype. The mRNAs do not constitute a homogeneous collection: they encode polypeptides of diverse function; they possess different tissue-specificities, although all are most abundant in liver; the kinetics of their expression in newborn liver are not identical, although a subset exhibit a transient induction soon after birth. It should be stressed, however, that it cannot be assumed at this stage that each mRNA is influenced in the same way by the mutation, and this must lead to caution in drawing conclusions. Nuclear run-on assays are presently in progress to determine whether the mRNAs are all affected at the transcriptional level, as is the case of PEPCK and TAT (Loose et al., 1986; E. Schmid, S.R., unpublished observations), and to what extent. Similarly, it is not yet possible to distinguish between primary and secondary effects of the mutation on these mRNAs. The recent observation that the abundance of a mRNA encoding a putative transcription factor, the CAAT-box/enhancer binding protein (C/EBP, Landschulz et al., 1988), is also reduced in livers of albino lethal mice might imply a hierarchy of events (Ruppert et al., 1989).

A common property of the mRNAs remains their regulation by hormones, glucocorticoids and/or cAMP. Thus, in agreement with Gluecksohn-Waelsch (1987), a failure in the induction process appears to be the underlying lesion in these mice. The observation that in the kidney PEPCK can be regulated by glucocorticoids and is influenced by the mutation whilst aldolase B and metallothionein I are not regulated and not influenced is an interesting coincidence that needs further investigation. The response of all of the mRNAs to hormonal stimuli in vivo is being analysed in order to test how general this correlation is. Important for any model for the function of the product of the perinatal survival locus is whether all or only a subset of glucocorticoid and cAMP regulated genes are deficient in the affected tissues. We do not detect a difference in the expression of mRNAs encoding the glucocorticoid receptor or cAMP-dependent protein kinase subunits between wild-type and mutant mice (Ruppert et al., 1989).

The question as to where the primary lesion in the perinatal lethality lies remains open and, hence, the function of the product of the perinatal survival locus remains elusive. Two possibilities may be envisaged. The factor encoded by the locus might have a direct effect on the transcription of the set of genes. For example, by operating at the level of DNA:protein interaction or chromatin structure it might confer to the genes the competence to respond to their activators, as advanced by Gluecksohn-Waelsch (1987). Alternatively, the effect of the mutation might be less direct in that the absence of the normal gene product may interfere with some other stage of the pathway of transducing a signal from the extracellular domain to the responsive genes in the nucleus. An argument in favour of the latter is the altered properties of selected membranes in the affected cells (Trigg and Gluecksohn-Waelsch, 1973). Any model must account for the fact that the phenotype is cell-specific, and the mutation has pleiotropic effects within the cell and interferes with more than one induction process.

Ultimately, the understanding of the gene product, and associated deficient phenotype, lies in the isolation of the perinatal survival locus. This is being approached by the generation of a molecular map of the region of the chromosome surrounding the locus and by positioning the various c locus deletions within it. The map is being used to guide the construction of chromosome jumping libraries (Poustka et al., 1987). For example, a jump of 230 kb has been made from a SmaI site within the tyrosinase gene to a SmaI site associated with the BssHII/NaeI/NarI cluster (see Fig. 3C and D) proximal to the gene (A.S., G.K., unpublished observations). This cluster could then be shown to be retained in two out of five deletions of the Bi complementation group. These deletions remove c but not the perinatal survival locus (Fig. 3A; Russell et al., 1982). This new probe, therefore, allows the locus to be excluded from a further region of the map. Given that it does not presuppose a mechanism of action of the normal gene, this approach to pinpoint the locus is a promising one.

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