Original Article

HMG-17, a chromosomal non-histone protein, shows developmental regulation during organogenesis

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ABSTRACT We used the differential hybridization technique for isolating developmentally regulated genes from the mouse metanephric kidney. In this screening, we identified the cDNA encoding high-mobility-group protein 17 (HMG-17), a chromosomal non-histone protein which modulates the conformation of transcriptionally active chromatin. Using Northern blot analysis, the HMG-17 mRNA was strongly expressed during embryogenesis and downregulated in various adult murine organs. At the histological level, the transcript localized to differentiating tissue regions and was apparently downregulated in mature structures indicating that HMG-17 expression is linked to cell differentiation. HMG-17 can thus be regarded as a general marker for tissues or cells undergoing differentiation during organogenesis.

KEY WORDS: proliferation, cell differentiation, high-mobility-group proteins, differential hybridization

Introduction

The developing kidney offers an excellent model system for cloning developmentally expressed genes involved in the epithelial-mesenchymal interactions that guide the development and in the epithelial cell differentiation that follows these interactions. The development of the mouse metanephric kidney starts on embryonic day 11 when an epithelial ureter bud bulges from the Wolffian duct and invades the metanephrigenic mesenchyme. The subsequent events depend on reciprocal inductive interactions between these two tissue types. The mesenchymal cells surrounding the ureter bud induce the bud to branch, and these branches eventually form the collecting duct system of the kidney. In response to signals from the ureter bud, the mesenchymal cells undergo mesenchymal-to-epithelial transition: They aggregate and, through several intermediate stages, form the epithelium of the nephrons, the secretory units of the kidney (Saxen, 1987).

Expression of various types of molecules, including growth factors and their receptors, signaling molecules, second messengers, transcription factors and various intra- and extracellular structural proteins has been reported at different timepoints throughout the induction, cell differentiation and morphogenesis of the kidney (Bard et al., 1994,1996; Lechner and Dressler, 1997; Vainio and Müller, 1997). Molecules essential for early kidney development include Wilms tumor suppressor gene 1 (WT-1) (Kreidberg et al., 1993), the glial-cell-line-derived neurotrophic factor (GDNF) and its tyrosine kinase receptor c-Ret (Durbec et al., 1996; Treanor et al., 1996). WT-1 and GDNF are expressed in the metanephric mesenchyme at the onset of kidney development (Pritchard-Jones et al., 1990; Armstrong et al., 1992; Hellmich et al., 1996), and c-Ret is abundant in the epithelial ureter bud (Pachnis et al., 1993; Avantaggiato et al., 1994). The knock-out mice of these molecules show a similar phenotype: The kidneys are either totally missing or severely abnormal, leading to death during embryogenesis or soon after birth (Kreidberg et al., 1993; Schuchardt et al., 1994,1996; Moore et al., 1996; Pichelsky et al., 1996; Sanchez et al., 1996). Some molecules, e.g., the transcription factor Pax-2, are required only for a certain phase of kidney development whereas they are downregulated. In transgenic mice, which constantly express Pax-2, the nephrons develop abnormally (Dressler et al., 1993,1995; Dressler and Douglass, 1992).

Several methods have been employed for cloning developmentally regulated genes (Maser and Calvet, 1995; Wan et al., 1996). These include differential cDNA library screening.

Abbreviations used in this paper: BrdU, bromodeoxyuridine; EDTA, ethylenediaminetetraacetic acid; EST, expressed sequence tag; FCS, foetal calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GDNF, glial-cell-line-derived neurotrophic factor; HMG-17, high-mobility-group protein 17; MEM, Eagle's Minimal Essential Medium; PBS, phosphate-buffered saline; PC7, polymerase chain reaction; SDS, sodium dodecyl sulphate; WT-1, Wilms tumor suppressor gene 1.

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Results

**HMG-17 is downregulated during organogenesis**

A CDNA library prepared from 17-day embryonic mouse kidney was screened for genes expressed differentially at different developmental stages of the kidney. The cDNAs were characterized by determining their nucleotide sequence and by confirming the differential expression of the potentially interesting ones by Northern blotting using total RNA derived from undifferentiated metanephric mesenchymes and embryonic day-17 and adult kidneys. In this way, the cDNA encoding HMG-17 was identified as one displaying clear developmental regulation: the HMG-17 mRNA of 1.4 kb was expressed in the undifferentiated metanephric mesenchyme and the 17-day embryonic kidney and then downregulated during adulthood (Fig. 1A). Northern blot analysis of adult tissues revealed, however, a low level of HMG-17 expression in heart and brain, hardly any signal was detectable in kidney, lung and intestine (Fig. 1B).

**Localization of the HMG-17 transcript in the 14-day embryo**

In situ hybridization of 14-day whole embryo sections revealed wide HMG-17 expression although the levels of the mRNA in different organs varied (Fig. 2A-D). Hybridization with the sense control probe gave no signal (Fig. 2E-F). In the liver, the transcript concentrated in the differentiating hepatocytes whereas the hematopoietic cells showed hardly any signal (Fig. 2A-B). In the intestine and pancreas both the epithelial and mesenchymal cell components were positive for HMG-17 (Fig. 2A-B). Also in the heart the transcript was evenly distributed (Fig. 2C-D). In the lung, the bronchial and respiratory systems start to develop on embryonic day 14 (Ten Have-Oppbroek, 1991). At this stage HMG-17 was abundant both in the bronchial branches and the mesenchymal cells with the exception of the mesenchymal cells surrounding the central bronchioles which were negative (Fig. 2C-D).

**Localization of the HMG-17 transcript in embryonic day-17 organs**

On embryonic day 17, the expression of HMG-17 in several organs was more restricted than on embryonic day 14. In the kidney, the transcript was abundant in the cortical collecting ducts and in the nephrogenic region, which contains undifferentiated mesenchymal cells and differentiating epithelial structures, such as comma- and S-shaped bodies (Fig. 3A-B). In the more mature structures of the nephron, the proximal tubules and glomeruli, as well as in the stromal mesenchyme and the epithelial cells of the pelvis, HMG-17 was already downregulated.

In the heart, the myocardium of both the ventricles and the aatri expressed HMG-17 (Fig. 3C-D). The endocardium was also positive. This was well presented in the atrioventricular valve, where the endocardial cells showed a strong signal. The myocardium of the valve, which contains more dense connective tissue than cardiac muscle, was only weakly labeled (Fig. 3C-D).

In the lung, marked developmental changes occur after embryonic day 16 (Ten Have-Oppbroek, 1991). The bronchial and respiratory systems develop further and formation of the alveolar ducts and terminal alveolar sacs begins. The ciliated columnar epithelial cells of the terminal bronchioles and low columnar or cuboidal epithelial cells of the respiratory bronchioles were strongly positive for the HMG-17 mRNA (Fig. 3E-G). When the bronchioles...
Fig. 2. Localization of the HMG-17 transcript in day 14 mouse embryos. In situ hybridization with HMG-17 antisense (A-D) and sense (E-F) probes. A, C, and E are dark-field images and B, D, and F the corresponding bright-field images (A-B). The liver, intestine (I), and pancreas (p) show evenly distributed HMG-17 signal (C-D). In the lung (h), HMG-17 is abundant in the mesenchymal cells and branches of the bronchioles (arrowhead). Mesenchymal cells surrounding the central branch of the bronchiole (+) have ceased to express HMG-17. In the heart (h), HMG-17 is expressed homogeneously (E-F). A section hybridized with the sense probe shows no signal. (i), lung; (h), heart. Bar, 200 μm.

continue as alveolar ducts and alveolar sacs, the epithelial cell morphology changes to a flattened one (Fig. 3H-I), which is better suited for the exchange of gases taking place in the alveoli after birth. These flattened cells showed hardly any HMG-17 expression. Adjacent alveolar sacs are separated by the interalveolar septum, which consists of supporting connective tissue and three main types of cells, viz., type I cells, type II cells and endothelial cells. Type I cells or flattened surface epithelial cells line all alveolar spaces. Type II cells are able to proliferate; they can also transform to type I cells and thus are the main source of renewed cells lining alveoli (Ten Have-Opproek, 1981, 1991). The interalveolar septa showed weak HMG-17 expression except in the periphery of the lung, i.e., in the region of alveolization, where the transcript was abundant.

In the embryonic day-17 intestine, the HMG-17 transcript concentrated in the developing crypts between the villi and the underlying muscular and connective tissue layers (Fig. 4A-B). In some villi, the HMG-17 signal extended a few cell layers along the villus whereas the tips were consistently negative. The cells of the villi differentiate from stem cells located in the crypts. While differentiating, these cells migrate along the villus and finally bud off from the tip (Gordon and Hermiston, 1994). The expression of HMG-17 correlated well with this differentiation gradient.

In the liver, the HMG-17 mRNA was abundant in the differentiating hepatocytes whereas the hematopoietic cells contained hardly any transcript (Fig. 4C-D). Cells surrounding the hepatic and central veins were positive. Some of these small groups of cells resembled ductal structures.

At this stage of development the spleen contains both developing hematopoietic and lymphatic tissue. The latter concentrates in the periphery of the organ and around the large vessels. The HMG-17 mRNA mainly localized to the lymphatic tissue whereas the hematopoietic cells showed hardly any signal (Fig. 4E-F).

In embryonic day-17 testis the HMG-17 probe hybridized to the developing tunica albuginea and the condensing mesenchymal cells underlying it (Fig. 4G-H). Expression was also observed in the developing interstitium, which at this stage consists of differentiating mesenchymal and Leydig cells, still indistinguishable from each other. The seminiferous tubules were also positive.

Proliferation during organogenesis

To find out whether HMG-17 expression correlates with proliferation, we labeled embryonic day-14 and day-17 embryos in utero with bromodeoxyuridine (BrdU). In the embryonic day-14 lung both the epithelial and mesenchymal tissue components were positive for BrdU (Fig. 5A). In the liver (Fig. 5B) and the heart (not shown), proliferating cells were also present all over the organ. In the intestine the epithelial cells were at this stage dividing slightly more actively than the cells of the muscular layer (not shown). Also, in the pancreas the epithelial branches contained more dividing cells than the surrounding mesenchymal tissue (Fig. 5B). In the lung on embryonic day 17 the cells of the
interalveolar septum, evidently the developing type II cells (Ten Have-Opbroek, 1981, 1991), were proliferating most actively, especially around the newly forming alveoli (Fig. 5C). The epithelial cells of the developing bronchial system showed very little BrdU signal; these cells were, however, strongly positive for the HMG-17 mRNA (Fig. 3E-I). In the intestine, strong BrdU labeling indicating rapid cell division was detected in the crypts between the villi and also in the underlying muscle and connective tissue layers (Fig. 5D). These cells and, differing from the region of BrdU-positivity, often also a few cells extending along the villi were positive for the HMG-17 transcript (Fig. 4A-B). In the heart and liver, proliferation was observed almost uniformly throughout the organs (not shown). In comparison, the HMG-17 signal in the liver concentrated in the hepatocytes and cells surrounding the hepatic and central veins (Fig. 4C-D).

**Discussion**

**Differential expression of HMG-17**

In this study we utilized the differential hybridization technique to isolate developmentally regulated cDNAs from the mouse kidney. HMG-17 was identified as a clone expressed at a high level during embryonic kidney development and downregulated in the adult organ. HMG-17 is a chromosomal non-histone protein, which has previously been shown to function in maintaining the chromatin conformation of transcriptionally active genes (Albanese and Weintraub, 1980; Weisbrod et al., 1980; Schröter and Bode, 1982, Weisbrod, 1982). HMG-17 binds to core histones and arranges chromatin into a more open conformation, thereby facilitating the access of the transcriptional machinery to the target sequence; it also increases the turnover of the transcriptionally
HMG-17 concentrates in differentiating cells during organogenesis

Organogenesis is characterized by strictly controlled up- and downregulation of genes, some of which are expressed in a wide range of tissues and cell types, while others are active in very limited regions or specific cellular populations. The function of HMG-17 in regulating the structure of transcriptionally active genes suggests that it could be expressed in actively differentiating parts of the embryonic organs. This led us to study the HMG-17 mRNA localization in various organs at different developmental stages. The stages chosen for the analysis were embryonic day 14, when most of the organs undergo active differentiation, and embryonic day 17, when both mature and still differentiating structures are present.

In situ hybridization of 14-day embryos revealed that the HMG-17 transcript was widely expressed in the embryo, although the expression level varied between and within different organs. For example, the heart showed a rather weak signal as compared to the lung. In the lung the bronchial branches and mesenchymal cells in the periphery exhibited abundant HMG-17 expression whereas the mesenchymal cells surrounding the more central branches of the bronchioles showed weak or negligible expression.

At a later developmental stage, on embryonic day 17, the differences were more dramatic. In some organs, such as the kidney, lung and intestine, it is possible to distinguish actively differentiating regions from mature structures, which renders these organs particularly suitable for studying the correlation between cell differentiation, proliferation and expression of a specific molecule. In the kidney, HMG-17 was abundant in the cortical collecting ducts and the nephrogenic region whereas the more mature structures had already ceased to express HMG-17. Also the lung is differentiating actively at this stage, especially the bronchial and alveolar systems, which were positive for HMG-17. In the intestine HMG-17 expression concentrated in the crypts harboring the stem cells, which divide and differentiate into epithelial cells, as well as the underlying muscular and connective tissue layers. In the embryonic day-17 liver HMG-17 localized rather to differentiating hepatocytes and small ductal type structures than to hematopoietic cells. The heart showed fairly ubiquitous signal throughout the organ. In the spleen the signal concentrated in the lymphatic cells. On embryonic day 17 the testes is differentiating actively. This holds true both for the interstitium and the seminiferous tubules, both of which were found to express HMG-17.

These data suggest that HMG-17 is expressed predominantly in cell types which are undergoing active cell differentiation. This
accords with the suggested function of HMG-17 in regulating the structure of transcriptionally active chromatin (Albanese and Weintraub, 1980; Weisbrod et al., 1980; Schröter and Bode, 1982; Weisbrod, 1982). When the tissues reach a mature state during embryonic development or adulthood, the expression of HMG-17 is downregulated.

**Relationship between HMG-17 expression and proliferation**

During embryonic development, cell differentiation and division typically proceed synchronously. To see if HMG-17 expression correlates with proliferation, we labeled various organs by BrdU. In the embryonic day-14 tissues, active cell division was taking place virtually ubiquitously. On the embryonic day-17, however, comparison of HMG-17 expression and BrdU-positivity yielded interesting results. In some tissues, such as the intestine, proliferation and HMG-17 expression were seen in the same cell types, such as the intestinal crypt cells which both divide actively and differentiate. Importantly, HMG-17 expression extended higher up along the villus-crypt axis than dividing cells. In the lung, abundant expression of HMG-17 similarly included structures displaying very low BrdU incorporation. In the liver, proliferating cells were present throughout the organ whereas HMG-17 concentrated only to a limited cell population. These observations thus suggest that HMG-17 expression is coupled to cell differentiation rather than to proliferation. The results are in accordance with the earlier studies performed on cultured cells (Pash et al., 1990; Shakoori et al., 1993). HMG-17, suggested to function in regulating the structure of transcriptionally active chromatin (Albanese and Weintraub, 1980; Weisbrod et al., 1980; Schröter and Bode, 1982; Weisbrod, 1982), can thus be regarded as a general marker for regions undergoing cell differentiation in developing organs.

**Materials and Methods**

**Tissue material**

Organs were dissected from (CB6xNMRI)F1 mouse embryos and NMRI adult males (testis) or females (other organs). The day of the appearance of the vaginal plug was designated as embryonic day 0. For isolating undifferentiated metanephrogenic mesenchymes, embryonic day 11 kidneys rudiments were treated with 2.25% pancreatin, 0.75% trypsin in Tyrode's solution at room temperature for 20 seconds thereafter the mesenchymes were mechanically separated from the ureter buds. The mesenchymes were let to recover in Eagle's Minimal Essential Medium (MEM) supplemented with 10% foetal calf serum (FCS) at 37°C for a few hours.

**Differential hybridization**

The library used for the hybridization was prepared from 17-day embryonic mouse kidney. The cDNA first strand was synthesized from 150 μg of total RNA by using an oligo-dT-primer and AMV-RT (Life Science Inc.) for reverse transcription. Second strand synthesis and cDNA ligation with the lambda arms were performed according to the Stratagene lambda ZAP-cDNA Synthesis protocol. The Giga Pack Gold Kit (Stratagene) was used for packaging the library into phage particles. The unamplified cDNA library was plated on the E. coli strain PLK-F. Duplicate filters of each dish were hybridized with [α-32P]dCTP-labeled first strand cDNA probes representing embryonic day 11 unduced metanephrogenic mesenchyme or 17-day embryonic kidney. The filter hybridized with the 17-day kidney probe was stripped and reprobed with an adult kidney cDNA probe. The probes were made from five micrograms of total RNA labeled by reverse transcription in the presence of [α-32P]dCTP (essentially as in the first strand cDNA synthesis). Hybridization was performed in 5xSSC (NaCl/NaCit buffer), 0.02% sodium dodecyl sulphate (SDS), 0.1% N-laurylsarcosine, 1.5% DIG blocking reagent (Boehringer Mannheim) containing the labeled probes at the concentration 0.22x106 cpm/ml at 68°C for 48 h. The filters were washed twice for 30 min in 0.1xSSC, 0.5% SDS at 60°C and exposed on Agfa X-ray films with intensifying screens at 80°C for 22 h. The clones that showed differential expression were isolated, and the inserts were recovered in pBluescript SK(-) by the Stratagene in vivo excision protocol. The cDNAs were analyzed by dideoxy DNA sequencing using the T7 Sequencing Kit (Pharmacia) and the expression patterns of the potentially interesting cDNAs were confirmed by Northern blot analysis.

**RNA isolation and Northern blot**

Total RNA of mouse embryonic day-11 undifferentiated metanephrogenic mesenchymes, 17-day embryonic kidney and different adult mouse organs was isolated by the guanidinium isothiocyanate method (Sambrook et al., 1989). The RNAs were separated in 1.6% agarose-formaldehyde
In situ hybridization

In situ hybridization was performed essentially as described (Lütske et al., 1993). The pBluescript SK(-) carrying the 900-bp HMG-17 cDNA was linearized either with EcoRI or XhoI for the production of antisense or sense probes, respectively. The single-stranded RNA probes were labeled with [35S]UTP (Amersham) by the SP6/7T run off transcription method (Riboprobe II Core System, Promega). Embryonic day 14 whole embryos or embryonic day 17 organs were fixed with 4% paraformaldehyde and embedded in paraffin. Five-micrometer sections were hybridized with the antisense and sense probes in parallel in 60% deionized formamide, 0.3 M NaCl, 20 mM Tris-HCl (pH 8.0), 5 mM ethylenediaminetetraacetic acid (EDTA, pH 8.0), 10% dextran sulphate (Mw 500 000), 1X Denhardt’s solution, 0.5 mg/ml torula yeast RNA and 0.1 M dithiothreitol (DIT) at 52°C for 15-20 h. After hybridization the sections were washed in high stringency conditions (50% deionized formamide, 2XSSC, 30 mM DTT, 65°C) twice for 30 min. dipped in Kodak NTB-2 autoradiography emulsion and exposed at 4°C for seven days.

Brdu-labeling and Immunohistology

NMRI females on the 14th or 17th day of pregnancy were injected intraperitoneally with BrdU (Amersham). After two and a half hours the animals were sacrificed, whole 14-day embryos or dissected organs of 17-day embryos were fixed in 3.5% paraformaldehyde and embedded in paraffin. Five-micrometer sections were deparaffinized, rehydrated in descending series of ethanol, and the endogenous peroxidase blocked by treating the sections with 0.5% hydrogen peroxide (H2O2) in methanol for 30 min. To increase the permeability and denature the DNA the sections were incubated in 0.1% Triton X-100 in phosphate-buffered saline (PBS) at room temperature for 15 min and then in 2 N HCl at 37°C for one hour (isolated embryonic day-17 organs) or in 0.25% trypsin followed by 4 M HCl at 37°C for 15 min each (day-14 whole embryos). HCl was neutralized by several washes with 0.1 M sodium borate buffer, pH 8.5. After rinsing with PBS the sections were incubated with 0.1 µg/ml anti-BrdU antibody (Boehringer) diluted in 5% FCS, 0.5% saponin in PBS at 4°C overnight. Detection was carried out using the Vectastain ABC Peroxidase Mouse IgG kit (Vector Laboratories, Inc.) according to the manufacturer's instructions.

Acknowledgments

We thank Ms. Anja Tuomi and Ms. Ulla Kiiski for excellent technical assistance and Prof. Lauri Saxén and Dr. Päivi Mettinen for critical reading of the manuscript. This study was supported by the Research Council of the Central Hospital of the University of Helsinki (E.L.), the Medical Research Council of the Academy of Finland (E.L.; grants 44426, 45877 and 48709) Whun Foundation (E.L.), Emil Aaltonen Foundation (S.L.), Paulo Foundation (S.L.) and the University of Helsinki, Finland (S.L., E.L.). V.M.O. was a recipient of a fellowship from the European Molecular Biology Organization and research grants from the Genome Research Program of the Academy of Finland (grants 29222 and 42163).

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Received: April 1998
Accepted for publication: April 1998.