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Chromosomal protein HMG-14 gene maps to the Down syndrome region of human chromosome 21 and is overexpressed in mouse trisomy 16

(nonhistones/gene expression/chromatin)

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ABSTRACT **The gene for human high-mobility-group (HMG) chromosomal protein HMG-14 is located in region 21q22.3, a region associated with the pathogenesis of Down syndrome, one of the most prevalent human birth defects. The expression of this gene is analyzed in mouse embryos that are trisomic in chromosome 16 and are considered to be an animal model for Down syndrome. RNA blot-hybridization analysis and detailed analysis of HMG-14 protein levels indicate that mouse trisomy 16 embryos have approximately 1.5 times more HMG-14 mRNA and protein than their normal littermates, suggesting a direct gene dosage effect. The HMG-14 gene may be an additional marker for the Down syndrome. Chromosomal protein HMG-14 is a nucleosomal binding protein that may confer distinct properties to the chromatin structure of transcriptionally active genes and therefore may be a contributing factor in the etiology of the syndrome.**

Down syndrome, one of the most prevalent human birth defects (1), results from the presence of an extra copy of chromosome 21 in cells of affected individuals (2). In some instances, the Down syndrome phenotype is associated with the triplication of only a segment of chromosome 21 (3). Recent results suggest that the region of chromosome 21 located within bands 21q22.1–21q22.3 contains genes whose overexpression may be responsible for the majority of the clinical features of the syndrome (3, 4). The Down syndrome region of chromosome 21 is partially syntenic with the distal fifth of mouse chromosome 16 (5). Because certain phenotypic aspects of mouse trisomy 16 (Ts 16) fetuses are reminiscent of those observed in human trisomy 21, mouse Ts 16 has been considered to be an animal model for Down syndrome (1). In this report we demonstrate that the human gene for high-mobility-group (HMG) chromosomal protein HMG-14 is localized on chromosome 21 in the region obligate for the development of the pathogenesis of the syndrome, and we present data that indicates that mouse Ts 16 fetuses have 1.5-fold more HMG-14 protein than their normal littermates.

Chromosomal protein HMG-14 and its close homologue HMG-17 are among the most abundant, ubiquitous, and evolutionary conserved nonhistone proteins found in all higher eucaryotes (6, 7). Each nucleosome contains two binding sites for these proteins (8, 9); however, the limited amount of HMG protein in the nucleus confines their presence to a nucleosomal subset. Although their cellular function is not fully understood, there is evidence that these proteins are part of a process that confers distinct properties to chromatin regions containing transcriptionally active genes (10–13). The location of the HMG-14 gene in the Down syndrome region of chromosome 21 and its overexpression in an animal model for this syndrome suggest that it would be worthwhile to investigate the possibility that this protein is involved in certain aspects of Down syndrome.

MATERIALS AND METHODS

Chromosome Mapping. Human prophases, prometaphases, and metaphases from methotrexate-synchronized normal peripheral lymphocytes were pretreated with ribonuclease and denatured at 70°C in 0.30 M NaCl/0.03 M sodium citrate, pH 7/70% formamide. The HMG-14 probe, a *Pst* I fragment excised from intron 4 of the human gene (14), was labeled with all four tritiated nucleotides to high specific activity (4.1×10^7 cpm/mg of DNA). *In situ* hybridization and G-banding procedures were carried out as described (15, 16).

Mouse Embryos. Normal and Ts 16 mouse 14- to 15-day embryos were obtained by breeding normal C57BL/6N female mice with Robinsonian translocation males Rb(16.17)-32Lub/Rb(11.16)2H as described (17, 18).

RNA Blot (Northern) Analysis. Poly(A)$^+$ RNA isolation and Northern analysis were performed as described (14). The Northern blots were probed with the full-length cDNA coding for either human HMG-14 (14) or human HMG-17 (19). The probes were radiolabeled with [^{32}P]dCTP by nick-translation.

Protein Preparation and Analysis. Proteins were prepared by direct homogenization of normal and Ts 16 mouse embryos in 5% perchloric acid (20). The homogenates were centrifuged at 10,000 × *g* for 10 min. The proteins contained in the supernatants were precipitated in 25% trichloroacetic acid at 4°C for 16 hr. The resulting precipitate was recovered by centrifugation; washed sequentially with 80% acetone/0.3 M HCl, 80% acetone, 100% acetone; and then dried. The pellet was dissolved in sample buffer, and the proteins were fractionated on acid–urea/acrylamide gels. Protein bands were visualized by Coomassie blue staining and quantitated by scanning in triplicate with a LKB laser densitometer.

Immunoblot Analysis. The gels were equilibrated for 1 hr in 2% sodium dodecyl sulfate/5% 2-mercaptoethanol/10% (vol/vol) glycerol/62.5 mM Tris·HCl, pH 6.8, and transferred to nitrocellulose with a semidry electroblotter (Janssen) according to the manufacturer's instructions. The nitrocellulose filter was washed with phosphate-buffered saline (PBS) for 5 min, treated with 1% glutaraldehyde in PBS for 5 min, washed with PBS, and blocked with 2% nonfat dry milk in PBS for 1 hr. The blots were incubated with a 1:300 dilution of anti-HMG-14 (21) or anti-HMG-17 (22) in 2% milk for 1 hr. Unbound antibody was removed by washing once with PBS for 5 min, once with PBS/Tween 20 for 5 min, and twice with PBS for 5 min. The location of bound antibodies

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Abbreviations: Ts 16, trisomy 16; HMG, high mobility group.
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was visualized with a 1:500 dilution of alkaline phosphatase-labeled goat anti-rabbit IgG antibody.

RESULTS

Mapping the HMG-14 Gene. The location of the HMG-14 gene was determined by *in situ* hybridization of 135 human chromosome preparations. Of these, 50 were obtained from prometaphases and prophases. During this stage of cell division, chromosomes are significantly longer than in metaphase, thereby allowing a fine-grain localization (Fig. 1). From the 131 grains examined in the 85 metaphase preparations, 30% were localized on chromosome 21. The remainder of the grains were randomly distributed over the other chromosomes (Fig. 2*C*). Within chromosome 21, 72% of the grains were at the proximal region of the long arm at band 22.3 (Fig. 2*B*). High-resolution grain localization in the 50 prometaphases and prophase chromosomes (Figs. 1 *b* and *d* and 2*A*) demonstrates that the HMG-14 gene is located at 21q22.3, a location close to that of probe *D21S55*, which recently has been localized in the Down syndrome region (4). Futhermore, recent mapping of human HMG-14 by linkage analysis with a probe obtained from another region of the gene localized the gene for HMG-14 close to the *ETS2* gene (encoding the oncogene homologue of avian erythroblastosis virus E26) and the *T1S3* probe, both of which map to band 21q22.3 (27). Therefore, we conclude that the human HMG-14 gene is located in the Down syndrome region of chromosome 21.

Overexpression of HMG-14 in Ts 16 Mice. Practically all of the Down syndrome region of chromosome 21 is syntenic with the distal segment of mouse chromosome 16 (5). Ts 16 mouse fetuses also have several phenotypic features that are similar to those present in human trisomy 21. Therefore, they are considered an animal model for Down syndrome (1). These fetuses were used to study possible gene dosage effects

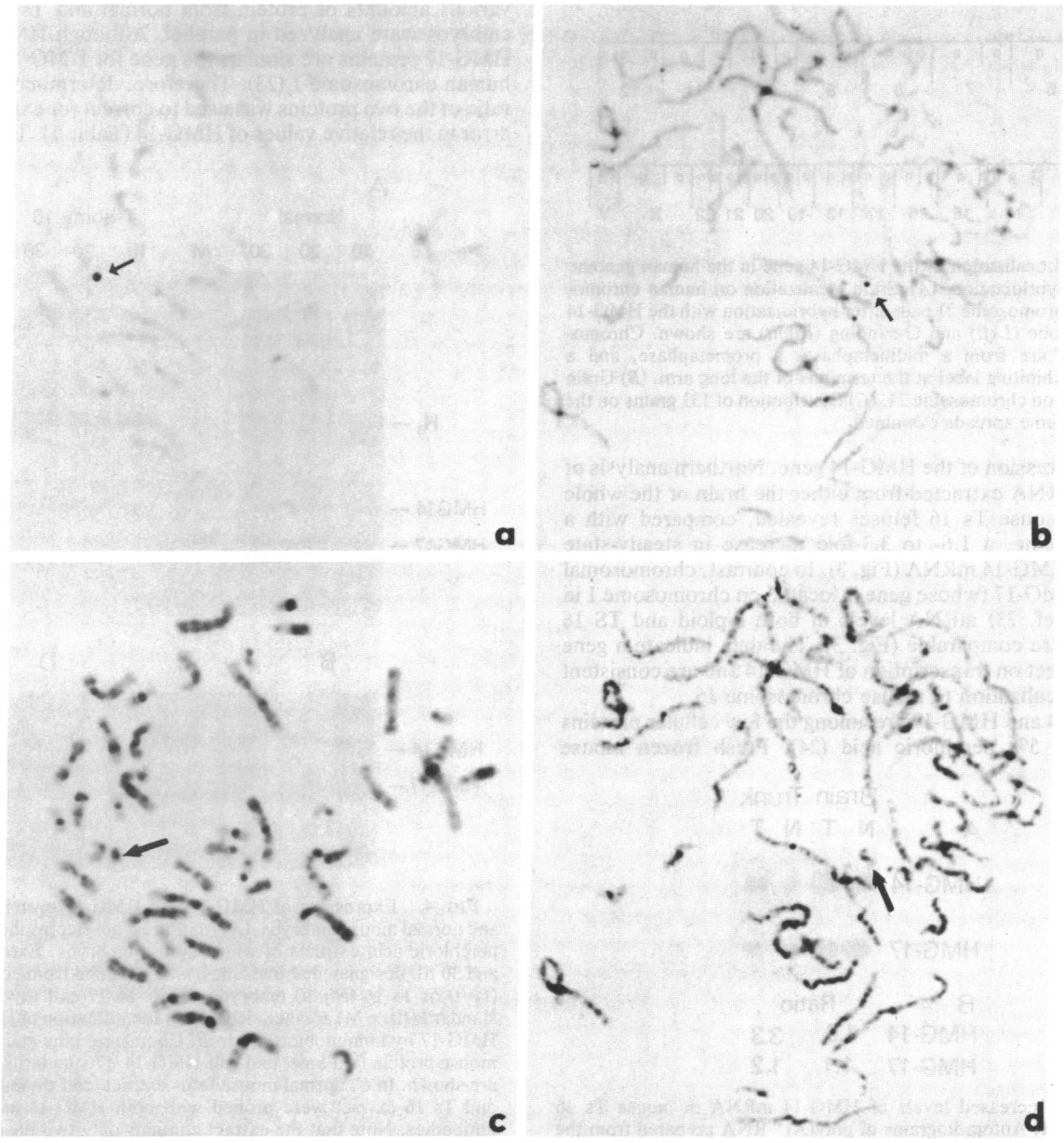

FIG. 1. Human chromosome spreads hybridized with the HMG-14 probe: human prometaphase (*a*) and prophase (*b*) from normal synchronized peripheral leukocyte cultures after *in situ* hybridization with a HMG-14 genomic probe. The label is found at the terminus of the long arm of a small acrocentric chromosome (arrows). After G-banding, the labeled chromosomes are identifiable as chromosome 21 (*c* and *d*). Examination of prophase chromosomes (*b* and *d*) allows a more precise determination of the location of the gene.

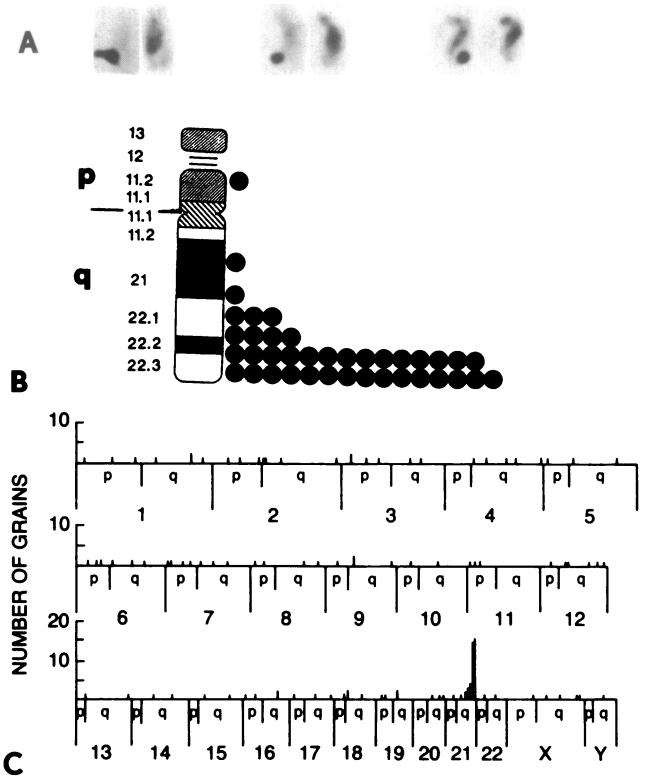

FIG. 2. Localization of the HMG-14 gene in the human genome by *in situ* hybridization. (*A*) Grain localization on human chromosome 21. Chromosome 21 pairs after hybridization with the HMG-14 genomic probe (*Left*) and G-banding (*Right*) are shown. Chromosome pairs are from a midmetaphase, a prometaphase, and a prophase exhibiting label at the terminus of the long arm. (*B*) Grain distribution on chromosome 21. (*C*) Distribution of 131 grains on the 85 chromosome spreads examined.

on the expression of the HMG-14 gene. Northern analysis of poly(A)$^+$ RNA extracted from either the brain or the whole trunk of mouse Ts 16 fetuses revealed, compared with a diploid sibling, a 1.6- to 3.3-fold increase in steady-state levels of HMG-14 mRNA (Fig. 3). In contrast, chromosomal protein HMG-17 (whose gene is located on chromosome 1 in humans; ref. 23) mRNA levels of both diploid and TS 16 fetuses were comparable (Fig. 3). The data indicate a gene dosage effect on transcription of HMG-14 and are consistent with its localization to mouse chromosome 16.

HMG-14 and HMG-17 are among the few cellular proteins soluble in 5% perchloric acid (24). Fresh frozen mouse

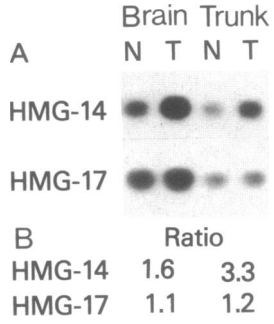

FIG. 3. Increased levels of HMG-14 mRNA in mouse Ts 16 embryos. (*A*) Autoradiograms of poly(A)$^+$ RNA prepared from the brain or trunk of normal (lanes N) or Ts 16 (lanes T) 14- to 15-day-old mouse fetuses probed for HMG-14 or HMG-17. (*B*) Ratio of Ts 16 to normal embryos for HMG-14 or HMG-17 mRNA. The values obtained by densitometric scanning of the autoradiograms shown in Fig. 3*A* are shown directly under the corresponding autoradiogram.

embryos were homogenized in 5% perchloric acid, and the acid-soluble proteins were analyzed by electrophoresis in polyacrylamide gels (Fig. 4). The electrophoretic profile of proteins extracted from normal mouse fetuses was practically indistinguishable from those extracted from Ts 16 fetuses (Fig. 4*A*). Mouse HMG-14/17 proteins were identified by immunoblot analysis (Fig. 4 *B–E*) with antibodies elicited against the calf thymus standards (21, 22). Treatment of the gel blot with both anti-HMG-14 and anti-HMG-17 antibodies identified the mouse HMG-14 and HMG-17 proteins in the gel (Fig. 4*C*). A similar gel blot to that presented in Fig. 4*C* was cut in two, splitting the calf thymus marker lane. The left half of the blot was probed with anti-HMG-14 antibody (Fig. 4*D*), and the right half was probed with anti-HMG-17 antibody (Fig. 4*E*). In this fashion the mouse HMG-14 and HMG-17 were unambiguously identified.

For quantitative analysis, gels were scanned (Fig. 5), and the bands were identified as HMG-14 and HMG-17 integrated. Three different experiments were performed; in each, various amounts of protein from normal and Ts 16 mouse embryos were analyzed in parallel. Although HMG-14 and HMG-17 proteins are similar, the gene for HMG-17 maps to human chromosome 1 (23). Therefore, determination of the ratio of the two proteins was used to correct for experimental error in the relative values of HMG-14 (Table 1). The ratio of

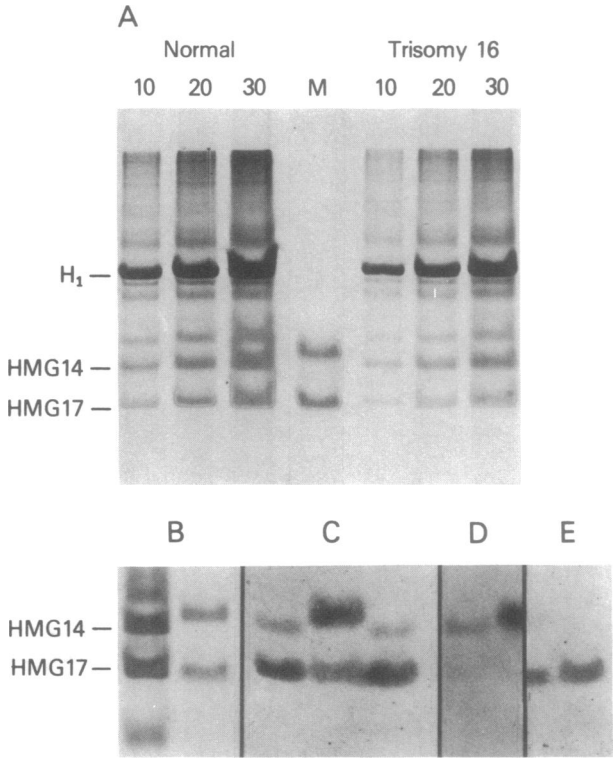

FIG. 4. Expression of HMG-14 and HMG-17 proteins in Ts 16 and normal mouse embryos. (*A*) Acid-urea/gel electrophoresis of 5% perchloric acid extracts of whole mouse embryos. Extract (10, 20, and 30 μl, designated above lanes) was obtained from either normal (*Left*) or Ts 16 (*Right*) embryos. HMG-14/17 calf thymus protein standards (lane M) are included. (*B–E*) Identification of HMG-14 and HMG-17 by immunoblotting. In *B*, Coomassie blue stain of normal mouse protein (left lane) and calf HMG-14/17 standards (right lane) are shown. In *C*, normal mouse fetus extract, calf thymus standard, and Ts 16 extract were probed with both HMG-14 and HMG-17 antibodies. Note that the extract contains only two positive bands. In *D*, normal mouse protein (left lane) and calf HMG-14/17 standard (right lane) were probed with anti-HMG-14. In *E*, normal mouse protein (right lane) and calf HMG-14/17 (left lane) were probed with rabbit anti-calf HMG-17. From *B–E*, it is apparent that the mouse proteins were identified correctly.

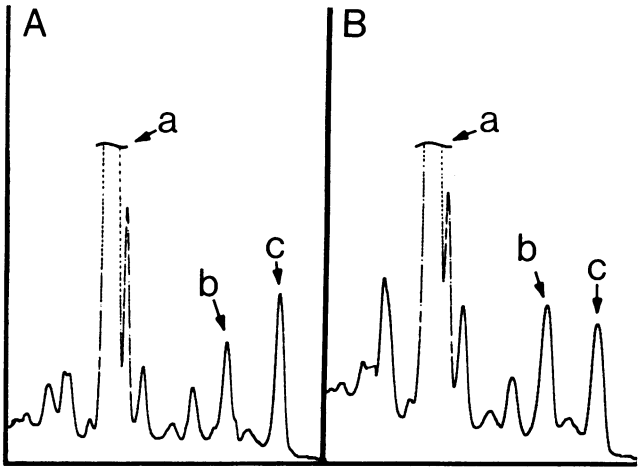

FIG. 5. Densitometric scanning of 5% perchloric acid-soluble proteins from normal (*A*) and Ts 16 (*B*) embryos. Peaks: a, Histone H1; b, HMG-14; c, HMG-17.

HMG-14 to HMG-17 varied between the experiments depending on the rate of staining and destaining of the gels (not shown). However, within each experiment, the protein ratio of HMG-14 to HMG-17 in Ts 16 embryo was 1.5 to 1.7 times higher than the ratio in normal embryo. This result correlates well with the relative levels of mRNA and supports our conclusion that mouse HMG-14 is located on mouse chromosome 16. If indeed HMG-14 maps to mouse chromosome 16, then the amount of protein correlates directly with the number of gene copies. Remarkably precise gene dose effects have been previously observed in human trisomy 21 for several other gene products (1).

DISCUSSION

Although the exact region of chromosome 21 responsible for the Down phenotype is still being defined, the present consensus is that the phenotype occurs when a region contained in bands 21q22.1–21q22.3 is triplicated (1, 3). Recently, it was suggested that the region distal to 21q22.2 but proximal to 21q22.3 is critical for the expression of the Down syndrome (4). Both linkage analysis studies (27) and the results presented here indicate that the gene coding for human chromosomal protein HMG-14 is located in that region. Pulse-field gel analysis suggests that the critical region contains between 400 and 3000 kilobases (kb) (4). Assuming that 10% of nuclear DNA codes for genes and that the average size of a gene is 10 kb, this region contains no more than 30 genes (25). Of the several probes available for the Down syndrome region only six, genes for cystathionine synthase, superoxide dismutase, α interferon, phosphofructokinase, phosphoribosylglycinamide synthetase, and *ETS2* code for known gene products (1). Of these, only *ETS2* and phosphofructokinase genes map in the 21q22.2–21q22.3 border region (26). Thus, HMG-14, located in this Down syndrome region, may be a useful marker and serve as an anchor for further analysis of sequences involved in the etiology of the disease.

Obviously, one can only speculate as to the possible involvement of chromosomal protein HMG-14 in the etiology of the syndrome. Since the gene product is expressed in a dose-dependent fashion, the fetuses contain a significant excess of this protein. Down syndrome involves several distinct physical features such as changes in several facial structures; changes in the structure of hands, teeth, and neck; low IQ; increased incidence of leukemia; increased cell susceptibility to radiation damage; increased incidence of congenital heart disease; certain immunological and hematological disorders; and the neuropathology of Alzheimer disease (1). If a single gene is involved in the etiology of the disease, it probably interferes in some metabolic function or developmental process (1). HMG-14 may modulate the structure of transcriptionally active chromatin; therefore, an imbalance of this protein could have a global effect and influence the expression of a multitude of genes.

Table 1. Ratio of HMG-14 protein to HMG-17 protein in Ts 16 and normal mouse embryos

Exp.	HMG-14/HMG-17 protein ratio		Comparison of ratios (Ts 16/normal)
	Ts 16	Normal	
1	2.19	1.66	
	2.07	1.22	
	1.54	0.99	
Mean ± SD	1.93 ± 0.28	1.29 ± 0.27	1.50 ± 0.25
2	0.97	0.45	
	0.95	0.60	
	0.98	0.66	
Mean ± SD	0.96 ± 0.01	0.57 ± 0.09	1.68 ± 0.15
3	0.58	0.38	
	0.66	0.38	
	0.64	0.39	
Mean ± SD	0.62 ± 0.03	0.38 ± 0.004	1.63 ± 0.03
		Mean ± SD	1.60 ± 0.10

In each experiment, three samples of 5% perchloric acid extracts (10, 20, and 30 μl) from either normal or Ts 16 mouse embryos were electrophoresed in acid-urea/20% gels. Gels were stained with Coomassie blue. HMG-14 and -17 protein bands were quantitated by using an LKB laser densitometer.

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