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msg1, a novel melanocyte-specific gene, encodes a nuclear protein and is associated with pigmentation

(differential display/melanoma/5′-rapid amplification of cDNA ends)

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ABSTRACT **Messenger RNA transcripts of the highly pigmented murine melanoma B16-F1 cells were compared with those from their weakly pigmented derivative B16-F10 cells by differential display. A novel gene called *msg1* (melanocyte-specific gene) was found to be expressed at high levels in B16-F1 cells but at low levels in B16-F10 cells. Expression of *msg1* was undetectable in the amelanotic K1735 murine melanoma cells. The pigmented murine melanocyte cell line melan-a expressed *msg1*, as did pigmented primary cultures of murine and human melanocytes; however, seven amelanotic or very weakly pigmented human melanoma cell lines were negative. Transformation of murine melanocytes by transfection with *v-Ha-ras* or *E1a* was accompanied by depigmentation and led to complete loss of *msg1* expression. The normal tissue distribution of *msg1* mRNA transcripts in adult mice was confined to melanocytes and testis. Murine *msg1* and human *MSG1* genes encode a predicted protein of 27 kDa with 75% overall amino acid identity and 96% identity within the C-terminal acidic domain of 54 amino acids. This C-terminal domain was conserved with 76% amino acid identity in another protein product of a novel human gene, *MRG1* (*msg1*-related gene), isolated from normal human melanocyte cDNA by 5′-rapid amplification of cDNA ends based on the homology to *msg1*. The msg1 protein was localized to the melanocyte nucleus by immunofluorescence cytochemistry. We conclude that *msg1* encodes a nuclear protein, is melanocyte-specific, and appears to be lost in depigmented melanoma cells.**

Benign and malignant melanocytes represent one of the best-characterized model systems for the study of tumor progression (1–5) and differentiation (6–15). Close correlation between aberrant differentiation of cultured melanocytes and malignancy has been well demonstrated (2, 3, 6, 7, 9, 11, 16). In human melanoma cells it has also been reported that there is a decrease in the expression of genes that are related to cell cycle and cell growth (e.g., cdc2, cyclin A, p53, and c-*myc*) with experimentally induced terminal differentiation (15). Therefore, it is important to understand the molecular mechanism of melanocyte differentiation and its aberration in malignancy.

In general, pigmentation decreases with melanoma progression and/or loss of several other differentiated properties of melanocytes (2, 6–9, 11, 16, 17). At least, loss of pigmentation reflects some aberrations in melanocytic differentiation. Therefore, it is reasonable to hypothesize that information on the mechanism(s) of aberrant differentiation of melanoma may be obtained by comparing gene expression between well-pigmented and poorly pigmented melanoma cells.

Based upon this concept, we attempted to isolate genes that are differentially expressed between B16-F1 and B16-F10 murine melanoma cells, which, under our culture conditions, showed different degrees of pigmentation (B16-F1 ≫ B16-F10). We report here the isolation of a novel gene, *msg1* (murine melanocyte-specific gene), expressed at high levels in B16-F1 cells but at low levels in B16-F10 cells.

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MATERIALS AND METHODS

Cell Culture. Murine melanoma cells B16-F1, B16-F10 (18), and K1735 (19) were provided by I. J. Fidler. Human melanoma cell lines (20) were provided by R. H. Byers. All melanoma cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Normal human epidermal melanocytes isolated from neonatal foreskin were from Clonetics (San Diego). Normal murine melanocyte cell line melan-a (17) was provided by D. C. Bennett. Murine primary culture epidermal melanocytes were prepared from newborn C57BL/6 mice as described (21). Differentiated status of normal melanocytes used was confirmed by pigmentation and morphology as described (16, 17).

Northern Blot Hybridization and Differential Display. Extraction of total RNA and Northern blot hybridization were performed as described (22). Total RNA of L-B10.BR normal murine melanocytes and those oncogene-transfected variants (16) were gifts from C. Missero and P. G. Dotto. Poly(A)-rich RNA blot of normal BALB/c mouse tissues was from CLONTECH. Differential display was performed as described (23, 24).

Cloning and Characterization of a Full-Length cDNA of Murine *msg1*. A cDNA library was constructed from poly(A)-rich RNA of B16-F1 cells and screened with the 3′-end cDNA fragment of *msg1* obtained by differential display. The transcription start site of *msg1* mRNA in B16-F1 cells was determined both by the primer extension (25) using a primer 5′-ATAAATCGGTGGCAGTTGAT and the 5′-rapid amplification of cDNA ends (5′-RACE) (26) using a primer 5′-ATAAATCGGTGGCAGTTGAT. *In vitro* synthesis of the protein products of human *MSG1* gene and murine *msg1* gene was performed using an *in vitro* transcription/translation kit from Promega.

Human *MSG1* Clones. Plasmids harboring partial human *MSG1* cDNA were IMAGE (Integrated Molecular Analysis of Genomes and their Expression) Consortium (Lawrence Livermore National Laboratory) cDNA clones (ID no. 265596 and 270311) (27). A complete human *MSG1* cDNA sequence was reconstituted from expressed sequence tag (EST) sequences of GenBank (accession nos. EST-N21409, -N21574, -N27472, -N29413, -N31118, -N31365, -N38759, and -N41476). Accuracy of the coding sequence was confirmed by at least three EST sequences listed above and sequences of the plas-

Abbreviations: *msg1*, murine melanocyte-specific gene 1; msg1, protein product of *msg1*; *MSG1*, human melanocyte-specific gene 1; MSG1, protein product of *MSG1*; 5′-RACE, rapid amplification of cDNA ends; HA, influenza virus hemagglutinin; IMAGE, Integrated Molecular Analysis of Genomes and their Expression.

Data deposition: The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U65091–U65093).

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mids. We found several mutations in the IMAGE plasmids and repaired those by site-directed mutagenesis.

Western Blotting and Immunoprecipitation. Rabbit polyclonal antibody I51904K was raised against a synthetic peptide for a part of the msg1 protein (NH_2-LGQNEFDFTADFPSG) and affinity-purified using an antigen-fixed column. Western blot analysis was performed following a standard protocol (28). For immunoprecipitation, normal human epidermal melanocytes were metabolically labeled with [^{35}S]Met and subjected to a standard protocol (28). Rabbit anti-human von Willebrand factor polyclonal antibody (Sigma) was used for a negative control.

Immunofluorescence Cytochemistry. Cells were fixed by 4% paraformaldehyde, permealized by 1% Nonidet P-40, and subjected to immunostaining. The primary antibody was I51904K anti-msg1 antibody (0.6 μg/ml), and the secondary antibody was goat anti-rabbit IgG antibody conjugated with Rhodamine (Jackson ImmunoResearch; 1.3 μg/ml). Intracellular localization of the msg1 protein was determined by fluorescence microscopy. For influenza virus hemagglutinin (HA)-tagging, a part of the HA sequence (NH_2-MAYPYDVPDYASLGI) was fused to the N terminus of msg1. The expression plasmid for HA-tagged msg1 protein with cytomegalovirus promoter was transiently introduced into COS-1 cells. The HA-tagged msg1 protein was detected 48 h after transfection with anti-HA monoclonal antibody (12CA5) and goat anti-mouse IgG antibody conjugated with Rhodamine (Jackson ImmunoResearch) by fluorescence microscopy.

RESULTS

Isolation of Transcripts That Are Differentially Expressed Between Highly and Weakly Pigmented B16 Melanoma Cell Variants by Differential Display. Under our cell culture conditions, B16-F1 and B16-F10 murine melanoma cells were highly and weakly pigmented, respectively (Fig. 1*A*). To isolate transcripts that are differentially expressed in these cells, we used the differential display technique (23, 24). By comparing about 15,000 displayed cDNA fragments, 54 differentially expressed bands were identified. cDNA fragments were iso-

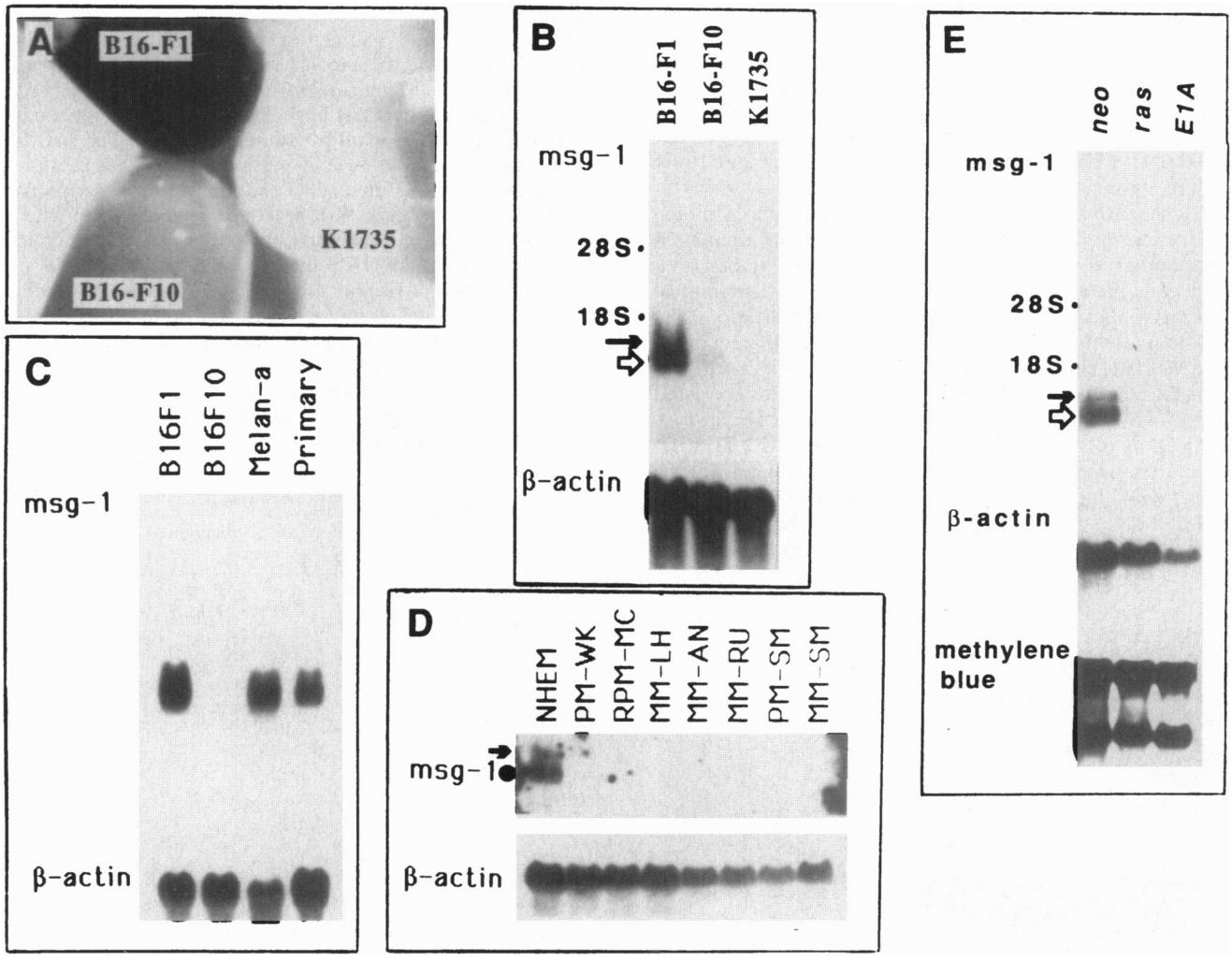

FIG. 1. Expression of *msg1* mRNA transcripts in melanocytes and melanoma cells. Expression of *msg1* mRNA transcripts was analyzed by Northern blot hybridization using a *msg1* cDNA probe. (*A*) Pigmentation of murine melanoma cells. Cells were pelleted to the bottom of microcentrifuge sample cups. (*B*) Expression of *msg1* mRNA transcripts in murine melanoma cells. Open and narrow arrows indicate 1.0 kb and 1.3 kb *msg1* mRNA transcripts, respectively. (*C*) Expression of *msg1* mRNA transcripts in the normal murine melanocyte cell line, melan-a, and primary culture murine epidermal melanocytes (Primary), compared with those in B16-F1 and B16-F10 cells. (*D*) Expression of *MSG1* mRNA transcripts in normal human epidermal melanocytes (NHEM) and melanoma cells (PM, primary melanoma; RPM, recurrent primary melanoma; MM, metastatic melanoma; followed by patients' initials). Two molecular species of *MSG1* mRNA transcripts [1.3 kb (arrow) and 1.0 kb (dot)] were detected in normal human epidermal melanocytes. (*E*) expression of *msg1* mRNA transcripts in L-B10.BR murine melanocytes transfected with *neo* control vector, expression plasmid for *v-Ha-ras*, or adenovirus *E1A*. Open and narrow arrows indicate the 1.0 kb and 1.3 kb *msg1* mRNA transcripts, respectively. Methylene blue staining of RNA fixed on the blot is to show equal amounts of RNA loading.

lated and examined by Northern blot hybridization to confirm differential expression (i.e., greater than 10-fold) between B16-F1 and B16-F10 cells; 13 such transcripts were found to be differentially expressed. Positive cDNA fragments were subjected to an additional Northern blot hybridization to RNAs of B16-F1 and B16-F10 cells that had been maintained in two other independent laboratories (R. Tepper and I. Stamencovic, Massachusetts General Hospital Cancer Center) to eliminate candidates with poor reproducibility. A gene, denoted as *msg1*, which reproducibly showed differential expression (B16-F1 ≫ B16-F10), was selected for further characterization.

Expression of *msg1* mRNA Transcripts in Melanocytes and Melanoma Cells. Messenger RNA transcripts of *msg1* were present at a high level in B16-F1 cells, but at only a low level in B16-F10 cells, and undetectable in K1735 amelanotic murine melanoma cells (Fig. 1*B*); this correlated with the degree of pigmentation of the three cell types (Fig. 1*A*). Neither 12-*O*-tetradecanoylphorbol 13-acetate (TPA) nor culture cell density affected *msg1* mRNA levels in these cells. Two molecular species of *msg1* mRNA transcripts, a prevalent species of 1.0 kb and less-abundant species of 1.3 kb, was observed. No gross DNA rearrangement was noted by Southern blot analysis (data not shown).

Messenger RNA transcripts of *msg1* were detected in a pigmented murine melanocyte cell line, melan-a (17), as well as in the pigmented primary culture epidermal melanocytes isolated from C57BL/6 mice, at a level comparable to B16-F1 cells (Fig. 1*C*).

Messenger RNA transcripts of *MSG1* (the human homolog of the murine *msg1* gene) were also detected in the pigmented normal human epidermal melanocytes, while no human melanoma cells tested by us expressed detectable amounts of *MSG1* mRNA transcripts by Northern blot hybridization (Fig. 1*D*). The human melanoma cells studied were all amelanotic except MM-AN cells, which were very weakly pigmented. The mRNA transcripts of *MSG1* in human melanocytes consisted of the 1.3-kb minor species and the 1.0-kb major species.

Expression of *msg1* mRNA transcripts in the L-B10.BR pigmented murine melanocyte cell line was completely lost by transfection with either *v-Ha-ras* or adenovirus *E1A* (Fig. 1*E*). Rapid and drastic depigmentation of L-B10.BR melanocytes by these transfections were described by Dotto *et al.* (16).

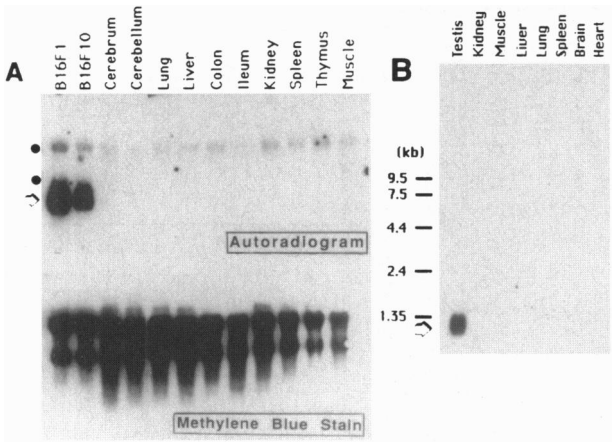

FIG. 2. Tissue distribution of *msg1* mRNA transcripts in mice. Total RNAs (10 μg per lane) from tissues of C57BL/6 mice (*A*) or poly(A)-rich RNAs (2 μg per lane) from tissues of BALB/c mice (*B*) were analyzed by Northern blot hybridization using a full-length murine *msg1* cDNA probe. Open arrows indicate the 1.0-kb *msg1* mRNA transcript, and dots indicate positions of ribosomal RNAs. The autoradiogram of *A* was overexposed to show the absence of *msg1* mRNA transcripts in any murine tissues.

Expression of *msg1/MSG1* mRNA transcripts and pigmentation of melanocytes and melanoma cells are summarized in Table 1. Based on these results we concluded that expression of *msg1/MSG1* mRNA transcripts is associated with pigmentation.

Melanocyte-Specific Expression of *msg1* mRNA Transcripts. We analyzed the tissue distribution of *msg1* mRNA transcripts in C57BL/6 mice (Fig. 2). Messenger RNA transcripts of *msg1* were not readily detectable in any major murine organs or tissues except testis. Prolonged exposure of the poly(A)-rich RNA blot revealed very low levels of *msg1* mRNA transcripts in the brain. Messenger RNA transcripts of *msg1* were not detected in BALB/3T3 murine fibroblasts. Thus, these data demonstrate the melanocyte-specific expression of *msg1* (melanocyte-specific gene).

Molecular Cloning of Murine *msg1* cDNA. We constructed a cDNA library for B16-F1 cells and screened it with the 3′-end fragment of *msg1* cDNA obtained by differential display. Two independent cDNA clones were isolated that contained the full-length murine *msg1* cDNA (Fig. 3*A*) which was 883 nt long with an open reading frame encoding a polypeptide of 203 amino acids (estimated molecular weight of 20,787). The peptide sequence of the msg1 protein did not correspond to any previously described proteins or contain described functional domain structures in various data bases. The transcription start site of the murine *msg1* gene (shown as −195 nt in Fig. 3*A*) was confirmed by both sequencing nine independent 5′-RACE clones (26) and by primer extension. The first Met codon (nt 196–198) was assigned as the translation initiator because (*i*) it was the first ATG codon in the cDNA occurring in the context of the Kozak consensus initiation site of eukaryotic mRNA translation (29), and (*ii*) it was conserved in the human *MSG1* cDNA sequence (Fig. 3*B*).

Identification of Human *MSG1* cDNA Clones. By data base searching for genes homologous to murine *msg1* cDNA, we could identify two human genes that were deposited to GenBank data base without information of the encoded proteins. One of those genes revealed it to be *MSG1*, the human homolog of murine *msg1* gene; and the other was a related but different gene described below. The human *MSG1* cDNA was

Table 1. Expression of *msg1* in melanocytes and melanoma cells

Cells	Transformation*	Pigmentation†	*msg1/MSG1* mRNA‡
NMEM§	−	+++	+++
melan-a	−	+++	+++
B16-F1	+	+++	+++
B16-F10	+	+	+
K1735	+	−	−
L-B10.BR/*neo*	−	+++	+++
L-B10.BR/*ras*	+	−	−
L-B10.BR/*E1A*	+	−	−
NHEM¶	−	++	+++
PM-WK	+	−	−
PM-SM	+	−	−
RPM-MC	+	−	−
MM-LH	+	−	−
MM-AN	+	+/−	−
MM-RU	+	−	−
MM-SM	+	−	−

*Data from refs. 16, 17, and 20.
†Evaluated by the color of cell pellets. L-B10.BR cell variants data were from ref. 16. +++, Black; ++, dark gray; +, light gray; +/−, brown; −, white.
‡Northern blot hybridization of total cellular RNA. −, Undetectable; +, weak expression less than 10% of that in B16-F1 cells; +++, strong expression comparable to that in B16-F1 cells.
§Normal murine epidermal melanocytes (C57BL/6 mice).
¶Normal human epidermal melanocytes.

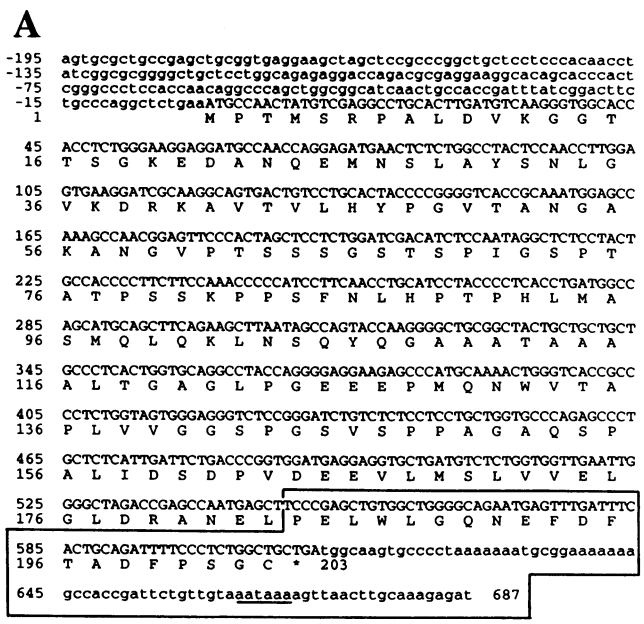

FIG. 3. Sequences of murine *msg1* and human *MSG1* cDNA clones. Underlining indicates putative polyadenylylation signals, and stars indicate the stop codons. (*A*) Full-length murine *msg1* cDNA clone. The 3′-end fragment of *msg1* cDNA isolated by differential display (box) was used for screening a cDNA library of B16-F1 cells. (*B*) Human *MSG1* cDNA clone.

found in two plasmids of the IMAGE. cDNA clones (ID nos. 265596 and 270311) (27) that are derived from a cDNA library from human foreskin melanocytes. The human *MSG1* cDNA was 845 nt long with a complete open reading frame encoding a polypeptide of 193 amino acids (estimated molecular weight of 19,865) (Fig. 3*B*). Estimated amino acid sequences of human MSG1 and murine msg1 proteins showed 75% overall identity and 96% identity in the C-terminal 54 amino acids (Fig. 4). This highly conserved C-terminal sequence showed remarkable acidity with 22% of the amino acids being Asp or Glu.

Molecular Cloning of Human *MRG1* cDNA, an *msg1*-related Gene. As mentioned above, we identified another gene with significant homology to murine *msg1* cDNA and deposited in the GenBank data base without protein information [accession nos. Z46096 and EST-17101 (27)]. This gene, *C-2JA11* (280 nt) showed 77% nucleotide sequence identity with the 3′ end of

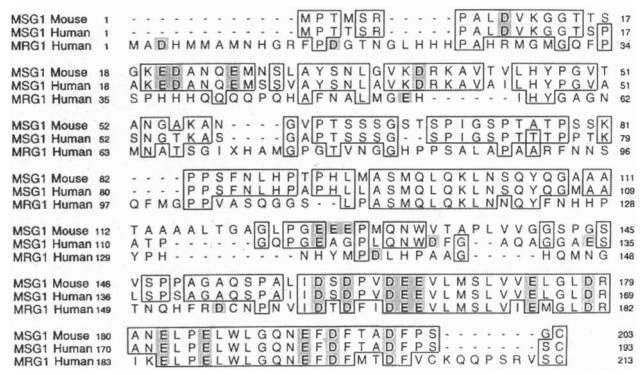

FIG. 4. Comparison of estimated amino acid sequences of human MSG1 and murine msg1 proteins and human MRG1 protein. Amino acid sequences were aligned by PILEUP software. Identical amino acids are framed, and acidic amino acids are shaded.

the murine *msg1* cDNA. A cDNA clone that contained a complete estimated open reading frame and the *C-2JA11* sequence was cloned from cDNA of human melanocytes by 5′-RACE. The resulting human cDNA clone was denoted as *MRG1* (*msg1*-related gene). The details of the cloning procedures and the nucleotide sequence of the *MRG1* cDNA will be described elsewhere. The predicted amino acid sequence of MRG1 (estimated molecular weight of 23,715) is shown in Fig. 3*B* together with the human MSG1 and murine msg1 proteins. There are two highly conserved regions between them (Fig. 4); a region between amino acids 110–123 (numbers of the MRG1 protein; 86% identity) and the C-terminal acidic region described above for the MSG1 and msg1 proteins (amino acid 161–209 of MRG1, 82% identity). Data base searching for the human MRG1 revealed no homologous proteins or functional domains. Messenger RNA of *MRG1* was detected ubiquitously in murine tissues, and at high levels in B16-F1, B16-F10, and K1735 cells.

Immunological Detection of Cellular msg1 and MSG1 Proteins. A rabbit anti-msg1 polyclonal antibody (I51904K) was raised against a synthetic peptide as described in *Materials and Methods*. Using this antibody, the msg1/MSG1 proteins in melanocytes and melanoma cells was analyzed by Western blotting (Fig. 5 *A* and *C*) and immunoprecipitation (Fig. 5*B*). Both methods revealed the molecular mass of the cellular msg1/MSG1 proteins to be 27 kDa, which was similar to that obtained by *in vitro* translation of murine *msg1* cDNA and human *MSG1* cDNA. Differential expression of the msg1 protein in B16-F1 and B16-F10 cells (Fig. 5*C*) was consistent with the levels of *msg1* mRNA transcripts in those cells (Fig. 1*B*). The anti-msg1 antibody I51904K cross-reacted with murine msg1 protein and human MSG1 protein (Fig. 5*C*). The high specificity of this antibody for the msg1/MSG1 proteins was confirmed by staining human melanoma cells and K1735 murine melanoma cells transiently transfected with expression plasmids for human MSG1 protein and murine msg1 protein, respectively. Absence of cross-reactivity to the MRG1 protein was confirmed by staining MM-SM human melanoma cells that express very high levels of *MRG1* mRNA transcripts.

Intracellular Localization of the msg1/MSG1 Proteins. As an initial step in the characterization of the msg1/MSG1 proteins, their intracellular localization was determined by immunofluorescence cytochemistry using I51904K anti-msg1 antibody. Murine melan-a melanocytes showed either dendritic, spindle-shaped morphology or flattened shape in the presence or absence, respectively, of phorbol ester in the culture medium as described (17). Both cell phenotypes clearly showed nuclear staining of the msg1 protein. Only the staining of the flat-shaped cells is shown in Fig. 6 to demonstrate the predominant nuclear staining of the msg1 protein; there was

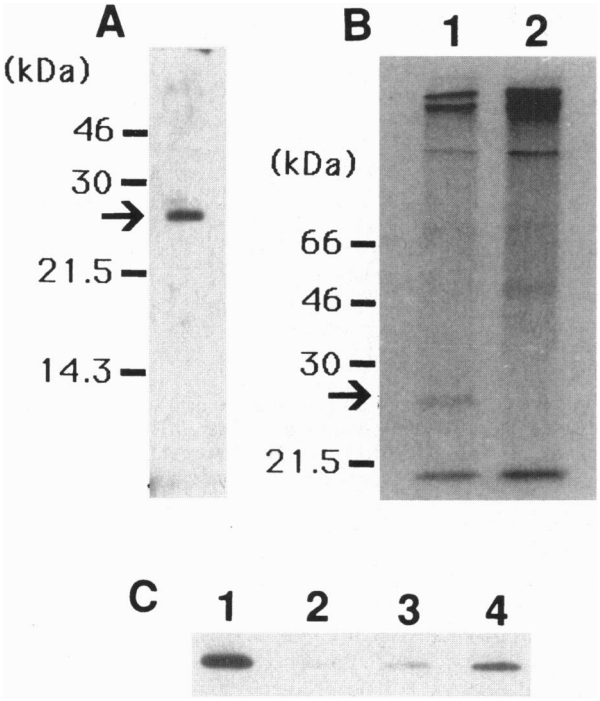

FIG. 5. Immunological detection of the msg1/MSG1 proteins expressed in melanocytes and melanoma cells. The msg1/MSG1 proteins were detected by an anti-msg1 polyclonal antibody (I51904K). (*A*) Western blot analysis of B16-F1 cell lysate for the msg1 protein. Arrow indicates the 27-kDa msg1 band. (*B*) Immunoprecipitation of the MSG1 protein from lysate of normal human epidermal melanocytes. Cellular total proteins were metabolically labeled by [^{35}S]Met and subjected to immunoprecipitation using anti-msg1 antibody (lane 1) or unrelated control antibody (lane 2). Arrow indicates the 27-kDa MSG1 band. (*C*) Comparison of expression levels of the msg1/MSG1 proteins in melanocytes and melanoma cells. The same amount (10 μg) of the whole cell lysates were subjected to Western blot analysis as described in *A*. Lanes: 1, B16-F1 cells; 2, B16-F10 cells; 3, C57BL/6 murine primary culture epidermal melanocytes; 4, normal human epidermal melanocytes.

no staining of melanosomes. The nuclear localization of the msg1 protein was confirmed with B16-F1 cells, normal human epidermal melanocytes, and primary culture murine epidermal melanocytes. B16-F10 cells showed weaker but detectable nuclear staining of the msg1 protein compared with B16-F1 cells, and there was no significant staining for K1735 cells. Nuclear staining was not observed with nonimmunized rabbit IgG nor with anti-msg1 antibody in the presence of the antigen peptide (4 μg/ml), which was confirmed for all cells mentioned above.

Nuclear localization of the msg1/MSG1 proteins was confirmed by transiently transfecting human melanoma cells and K1735 murine melanoma cells (negative for *msg1* mRNA) with expression plasmids for human MSG1 protein and murine msg1 protein, respectively, followed by immunofluorescent staining with anti-msg1 antibody. All cell lines tested accepted the expression plasmids and revealed nuclear staining of the msg1/MSG1 proteins above the clear background of nontransfected cells (data not shown).

To further confirm the nuclear localization of the msg1/MSG1 proteins, COS-1 cells and RPM-MC human melanoma cells were transiently transfected with expression plasmids for the msg1/MSG1 proteins fused to the HA-tag at its amino terminus. The HA-tagged msg1/MSG1 proteins were detected in the transfected cells by an anti-HA monoclonal antibody, again demonstrating the nuclear localization of the msg1/MSG1 proteins. Based on these data, we concluded that the msg1/MSG1 proteins were localized in the nuclei.

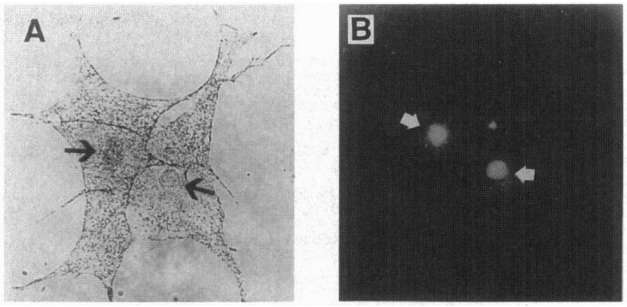

FIG. 6. Nuclear localization of the msg1 protein in melan-a murine melanocytes. Cells were immunostained with the anti-msg1 antibody I51904K (*B*). Intracellular localization of the msg1 protein was visualized with Rhodamine-conjugated secondary antibodies against rabbit or mouse IgG. (*A*) Phase contrast image of the same cells shown in *B*, exhibiting well-developed melanosomes as black dots in the cytoplasm. Arrows indicate the nuclei.

DISCUSSION

We have isolated a novel gene, *msg1*, whose expression is confined to melanocytes and testis and is associated with pigmentation of melanocytes and melanoma cells. The protein product, msg1, is a 27-kDa nuclear protein.

Because pigmentation is a highly characteristic and distinguishing property of differentiated melanocytes, loss of pigmentation should, at least, reflect some aberrations in the mechanism of melanocytic differentiation. Therefore, the fact that the loss of *msg1* expression correlated with the loss of pigmentation (Fig. 1 and Table 1) suggests that it may contribute to the differentiated properties of melanocytes. The confined expression of *msg1* mRNA transcripts to melanocytes and testis (Fig. 2) lends support for this hypothesis. Since testis expresses some mRNAs without apparent biological significance, one needs to be cautious in interpreting the significance of the presence of *msg1* mRNA transcripts in the testis.

The expression of the *msg1* gene in normal melanocytes was completely suppressed by transfection of these cells with the activated *ras* oncogene (Fig. 1*E*). Frequent mutation of *ras* has been reported for spontaneous human melanoma lesions, and the potential contribution of activated *ras* to melanoma progression has been suggested (8, 30–32). Analyzing *ras* activation and differentiation markers for many human melanoma cell lines, Albino *et al.* (8) specifically emphasized the correlation between *ras* activation and loss of pigmentation associated with a shift of cell surface markers from the late (well differentiated) to the early-intermediate (less differentiated) set of the differentiated antigens. Therefore, together with other markers of late-stage melanocytic differentiation, *msg1* expression in melanoma lesions may be lost concomitantly with *ras* activation. However, we have not yet characterized the status of *ras* activation in the human and murine melanoma cell lines used in the present study.

To our knowledge, the msg1 protein is the first example of a nuclear protein whose expression is confined predominantly to melanocytes. Two genes have been reported that encode nuclear proteins and regulate expression of several melanocyte-specific genes—namely, *mi* and *brn-2*. Messenger RNA of *mi* encodes a basic helix-loop-helix-zipper transcription factor mi (33–35), which binds to the "M-box" element that is conserved in the promoters of the genes of the major pigmentation-related enzymes and trans-activates the transcription of those genes (35, 36). Although it has been speculated that *mi* provides a melanocyte-specific signal that activates the pigmentation program (35), its expression is not confined to melanocytes but is also found in the uterus, lung, and heart (33). The other gene, *brn-2*, encodes N-Oct-3 and N-Oct-5 octamer-binding transcription factors that have the POU DNA-binding domain (37), and it has been demonstrated

recently that *mi* gene expression is downstream of *brn-2* expression (37). Although the importance of *brn-2* in melanocytic differentiation has been clearly demonstrated (37), its expression also is not restricted to melanocytes but is widely distributed within the central nervous system (38). Recently, Eisen *et al.* (39) have shown that the Brn-2/N-Oct3 transcription factor suppressed the tyrosinase promoter instead of activating it, suggesting that the regulation of melanocyte-specific gene expression is complex. Therefore, it will be desirable to investigate whether *msg1* gene expression is downstream of *brn-2* expression and/or *mi* expression.

The msg1 protein does not contain any described functional domain structures such as nuclear import signals (40) or DNA-binding domains (41). To obtain insights about possible functional domains in the msg1 protein, we compared its amino acid sequence with the MRG1 protein. We found that the strong homology between the msg1 protein and the MRG1 protein was confined to two regions, while the other regions showed only marginal similarity (Fig. 4). Interestingly, all acidic amino acids in the C-terminal region were strictly conserved in human MSG1 protein, murine msg1 protein, and human MRG1 protein, suggesting their possible importance. By the Chou and Fasman algorithm (42) and the helical wheel projection (43), it was predicted that a part of the conserved C-terminal acidic region forms an amphipathic α-helical structure, where all acidic amino acid residues face one side of the helix with the hydrophobic amino acids residing on the other side. Because some transcription factors have so-called "acidic" activator domains whose structures are predicted to be amphipathic α-helices (reviewed in refs. 41 and 44), it is tempting to speculate that the msg1 protein might be able to activate transcription.

In summary, we have isolated a novel gene *msg1* that encodes a nuclear protein whose expression is confined to pigmented melanocytes. Our current studies are aimed at elucidating the function(s) of this gene and specifically its possible role in melanocyte differentiation.

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1. Clark, W. H., Jr., Elder, D. E., Guerry, D., IV, Epstein, M. N., Greene, M. H., van Horn, M. & Guerry, D. (1984) *Hum. Pathol.* **15,** 1147–1165.
2. Herlyn, M., Kath, R., Williams, N., Valyi-Nagy, I. & Rodeck, U. (1990) *Adv. Cancer Res.* **54,** 213–234.
3. Kerbel, R. S. (1990) *Adv. Cancer Res.* **55,** 87–132.
4. Clark, W. H. (1991) *Br. J. Cancer* **64,** 631–644.
5. Reed, J. A., Loganzo, F., Jr., Shea, C. R., Walker, G. J., Flores, J. F., Glendening, J. M., Bogdany, J. K., Shiel, M. J., Haluska, F. G., Fountain, J. W. & Albino, A. P. (1995) *Cancer Res.* **55,** 2713–2718.
6. Kreider, J. W. & Schmoyer, M. E. (1975) *J. Natl. Cancer Inst.* **55,** 641–647.
7. Bennett, D. C., Dexter, T. J., Ormerod, E. J. & Hart, I. R. (1986) *Cancer Res.* **46,** 3239–3244.
8. Albino, A. P., Nanus, D. M., Mentle, I. R., Cordon-Cardo, C., McNutt, N. S., Bressler, J. & Andreeff, M. (1989) *Oncogene* **4,** 1363–1374.
9. Bennett, D. C. (1991) *J. Cell Sci.* **98,** 135–139.
10. Ortonne, J. P. (1992) *Br. J. Dermatol.* **127,** Suppl. 41, 43–47.
11. Bennett, D. C. (1993) *Int. Rev. Cytol.* **146,** 191–260.
12. Soballe, P. W. & Herlyn, M. (1994) *Melanoma Res.* **4,** 213–223.
13. Jiang, H., Lin, J., Su, Z.-Z., Herlyn, M., Kerbel, R. S., Weissman, B. E., Welch, D. R. & Fisher, P. B. (1995) *Oncogene* **10,** 1855–1864.
14. Jiang, H., Lin, J. J., Su, Z.-Z., Goldstein, N. I. & Fisher, P. B. (1995) *Oncogene* **11,** 2477–2486.
15. Jiang, H., Lin, J., Young, S.-M., Goldstein, N. I., Waxman, S., Davila, V., Chellappan, S. P. & Fisher, P. B. (1995) *Oncogene* **11,** 1179–1189.
16. Dotto, P. G., Moellmann, G., Ghosh, S., Edwards, M. & Halaban, R. (1989) *J. Cell Biol.* **109,** 3115–3128.
17. Bennett, D. C., Cooper, P. J. & Hart, I. R. (1987) *Int. J. Cancer* **39,** 414–418.
18. Fidler, I. J. (1973) *Nature (London) New Biol.* **242,** 148–149.
19. Fidler, I. J., Gruys, E., Cifone, M. A., Barnes, Z. & Bucana, C. (1981) *J. Natl. Cancer Inst.* **67,** 947–956.
20. Byers, H. R., Etoh, T., Doherty, J. R., Sober, A. J. & Mihm, M. C., Jr. (1991) *Am. J. Pathol.* **139,** 423–435.
21. Tamura, A., Halaban, R., Moellmann, G., Cowan, J. M., Lerner, M. R. & Lerner, A. B. (1987) *In Vitro Cell. Dev. Biol.* **23,** 519–522.
22. Shioda, T., Ohta, T., Isselbacher, K. J. & Rhoads, D. B. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11919–11923.
23. Liang, P. & Pardee, A. B. (1992) *Science* **257,** 967–970.
24. Liang, P., Averboukh, L. & Pardee, A. B. (1993) *Nucleic Acids Res.* **21,** 3269–3275.
25. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
26. Frohman, M. A., Dush, M. K. & Martin, G. R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8998–9002.
27. Lennon, G. G., Auffray, C., Polymeropoulos, M. & Soares, M. B. (1996) *Genomics* **33,** 151–152.
28. Harlow, E. & Lane, D. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
29. Kozak, M. (1989) *J. Cell Biol.* **108,** 229–241.
30. van't Veer, L. J. V., Burgering, B. M. T., Versteeg, R., Boot, A. J. M., Ruiter, D. J., Osanto, S., Schrier, P. I. & Bos, J. L. (1989) *Mol. Cell. Biol.* **9,** 3114–3116.
31. Yasuda, H., Kobayashi, H., Ohkawa, A. & Kuzumaki, N. (1989) *J. Invest. Dermatol.* **93,** 54–59.
32. Shukla, V. K., Hughes, D. C., Hughes, L. E., McCormick, F. & Padua, R. A. (1989) *Oncogene Res.* **5,** 121–127.
33. Hughes, M. J., Lingrel, J. B., Krakowsky, J. M. & Anderson, K. P. (1993) *J. Biol. Chem.* **268,** 20687–20690.
34. Hodgkinson, C. A., Moore, K. J., Nakayama, A., Steingrímsson, E., Copeland, N. G., Jenkins, N. A. & Arnheiter, H. (1993) *Cell* **74,** 395–404.
35. Hemesath, T. J., Steingrímsson, E., McGill, G., Hansen, M. J., Vaught, J., Hodgkinson, C. A., Arnheiter, H., Copeland, N. G., Jenkins, N. A. & Fisher, D. E. (1994) *Genes Dev.* **8,** 2770–2780.
36. Bentley, N. J., Eisen, T. & Goding, C. R. (1994) *Mol. Cell. Biol.* **14,** 7996–8006.
37. Angus, J., Thomson, F., Murphy, K., Baker, E., Sutherland, G. R., Parsons, P. G. & Sturm, R. A. (1995) *Oncogene* **11,** 691–700.
38. He, X., Treacy, M. N., Simmons, D. M., Ingraham, H. A., Swanson, L. W. & Rosenfeld, M. G. (1989) *Nature (London)* **340,** 35–42.
39. Eisen, T., Easty, D. J., Bennett, D. C. & Goding, C. R. (1995) *Oncogene* **11,** 2157–2164.
40. Görlich, D. & Mattaj, I. W. (1996) *Science* **271,** 1513–1518.
41. Mitchell, P. J. & Tjian, R. (1989) *Science* **24,** 371–378.
42. Chou, P. Y. & Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47,** 251–276.
43. Schiffer, M. & Edmundson, A. B. (1967) *Biophys. J.* **7,** 121–135.
44. Frankel, A. D. & Kim, P. S. (1991) *Cell* **65,** 717–719.