stimulates melanosome dispersion within melanocytes of some teleost species. Whether the actions of MCH on frog and lizard melanophores and of α-MSH on fish melanophores are mediated through the same receptor, or whether the melanotropic actions of the two peptides are mediated through separate receptors, remains to be determined.

Both melatonin (*N*-acetyl-5-methoxytryptamine) and catecholamines (norepinephrine and epinephrine) also cause melanin concentration within melanophores of most teleost species. The structural differences between these hormones and MCH are such that the actions of each agonist would be expected to be mediated through separate receptors. The actions of norepinephrine can be blocked by phentolamine, an α-adrenergic antagonist, whereas the actions of MCH are unaffected (*10*). Whether antagonists to the melanin-concentrating actions of melatonin would affect the actions of MCH is not yet known.

Comparison of the sequence of the cyclic MCH heptadecapeptide and of α-MSH shows little sequence homology of the peptides, and thus the apparent ability of the heptadecapeptide to interact with amphibian and reptilian melanocytes is perhaps surprising. The observation of low potency of the cyclic heptadecapeptide on amphibian and reptilian melanocyte receptors must be interpreted carefully. However, we reported that [Cys^4, Cys^{10}]-α-MSH and related cyclic melanotropin analogs are potent agonists of amphibian and reptilian melanophores (*5, 15*). Since the putative MCH is also a cyclic peptide, its ability to stimulate amphibian melanophores may be related to the topological three-dimensional properties inherent in the cyclic structure, which are similar to those in the cyclic α-MSH analog. A synthetic analog of MCH might prove useful in a variety of pharmacological and physiological studies. Antibodies to synthetic MCH would be useful in determining the cellular localization of the peptide within animal tissues.

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Organ-Specific Adhesion of Metastatic Tumor Cells in Vitro

Abstract. *Binding of tumor cells to cryostat sections of host organs was studied. B16-F10 melanoma cells and reticulum cell sarcoma cells demonstrated an organ specificity in their binding in vitro that reflected the organ specificity of their metastatic distribution 25 days after intravenous injection. These results provide evidence for specific binding of tumor cells to the tissues that they selectively colonize in vivo.*

Clinical and experimental studies have demonstrated that certain tumors metastasize preferentially to one or more specific organs (*1, 2*). It has been suggested that this organ selectivity is mediated by specific adhesive interactions between circulating tumor cells and the microvasculature of particular host organs (*2, 3*). Recently, a quantitative technique for demonstrating cellular adhesions to specific tissues was developed to study lymphocyte binding to specific host blood vessels in cryostat sections (*4*). We have modified this assay to study the binding of tumor cells that exhibit organ-specific metastasis. We found that murine B16-F10 melanoma cells and murine reticulum cell sarcoma (M5076) cells demonstrate a specificity in their binding to organ sections in vitro that reflects the specificity of their metastatic homing in vivo.

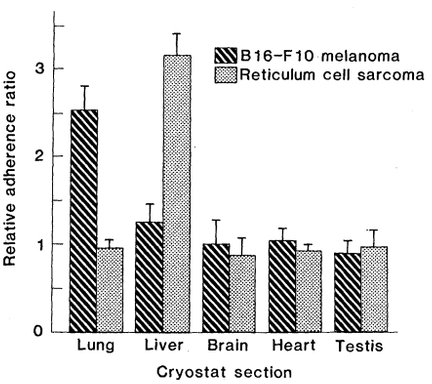

Fig. 1. Organ-specific binding of murine B16-F10 melanoma and reticulum cell sarcoma cells in vitro. Tumor cells were labeled with ^{51}Cr and then incubated on fresh cryostat sections of tissues obtained from C57BL/6 mice (*7*). The relative adherence ratio (*4*) is the ratio of the number of adherent sample cells to the number of BALB/c mouse 3T3 cells that bound to duplicate sections. Values are means ± standard deviations for at least five samples.

The behavior of the two cell lines in vivo was evaluated by injecting 3×10^5 trypsinized viable cells into the tail veins of C57BL/6 mice. At necropsy 25 days later the number of surface metastases was determined without magnification. All eight animals injected with B16-F10 melanoma cells contained numerous pulmonary tumors (112 ± 25 tumors per animal, mean ± standard deviation), whereas there was only 0.2 ± 0.7 hepatic metastasis per animal. In contrast, all eight mice injected with reticulum cell sarcoma cells contained many hepatic metastases (58 ± 16 per animal), but there was only 0.4 ± 0.5 pulmonary tumor per animal. Neither tumor produced significant numbers of metastases in sites other than the liver or lung. These findings are in accord with results showing that B16-F10 melanoma localizes preferentially in the lungs after being injected into the tail vein (*5*) and that reticulum cell sarcoma is found predominantly in the liver (*6*).

The organ specificity observed in vivo was also found in vitro. Tumor cells were labeled with ^{51}Cr and incubated with gentle agitation for 40 minutes at 5° to 7°C on fresh cryostat sections prepared from mouse brain, heart, liver, lung, and testis (*7*). After gentle washing to remove nonadherent cells, the radioactivity associated with individual sec-

tions was counted to determine the number of adherent tumor cells per section. These counts were normalized to control values for BALB/c mouse 3T3 fibroblasts. As shown in Fig. 1, B16-F10 melanoma cells, which metastasize preferentially to the lung in vivo, bound preferentially to cryostat sections of mouse lung. Similarly, reticulum cell sarcoma cells bound preferentially to cryostat sections of mouse liver. Thus the binding of the tumor cells to syngeneic tissue sections reflected their metastatic patterns in vivo.

Binding of unlabeled tumor cells to the cryostat sections was observed by light microscopy. As shown in Fig. 2, many more B16-F10 melanoma cells (27 ± 5 cells per field at ×50) than reticulum cell sarcoma cells (9 ± 3) adhered to lung sections. Conversely, many more reticulum cell sarcoma cells (51 ± 5 cells per field at ×50) than B16-F10 melanoma cells (17 ± 3) bound to liver sections. These findings are consistent with those obtained with the ^{51}Cr–labeled cells.

The adhesive determinants on the B16-F10 melanoma cell surface were characterized enzymatically (*8*). As shown in Table 1, the ability of B16-F10 melanoma cells to bind to cryostat sections of mouse lung was greatly reduced by Pronase or a combination of neuraminidase and β-galactosidase but was less affected by neuraminidase or β-galactosidase alone or by trypsin, deoxyribonuclease I, or ribonuclease A. Treatment of B16-F10 cells with the antibiotic tunicamycin, an inhibitor of glycoprotein synthesis, also reduced their binding to cryostat sections of mouse lung. These results are consistent with Irimura *et al.*'s finding (*9*) of an adhesive glycoprotein on the surface of these cells. Binding of the B16-F10 cells to liver sections was reduced by Pronase but not by neuraminidase plus β-galactosidase, indicating that the enzymatic treatments did not cause nonspecific changes in adhesiveness and that adhesion of melanoma cells in liver and lung are mediated by different classes of molecules.

Table 1. In vitro binding of B16-F10 melanoma cells treated with various enzymes or tunicamycin. After the treatments (*8*), the cells were washed with cold (4°C) medium and then incubated over cryostat sections as described in the legend to Fig. 1. Values are means ± standard deviations for at least five samples.

Treatment	Binding (percent of control)	
	Lung	Liver
Pronase	23 ± 14	36 ± 9
Trypsin	73 ± 5	86 ± 7
Neuraminidase	80 ± 15	119 ± 12
β-Galactosidase	75 ± 15	86 ± 5
Neuraminidase + β-galactosidase	47 ± 13	96 ± 7
Deoxyribonuclease I	103 ± 13	90 ± 18
Ribonuclease A	106 ± 19	105 ± 14
Tunicamycin	27 ± 4	62 ± 7

Our results demonstrate the efficacy of using cryostat sections to study tumor cell binding and provide new evidence for a correlation of organ-specific adhesion with organ-related differences in metastatic patterns. Although it is essential to establish the functional significance of such findings in vivo, this assay should be extremely useful for identifying adhesive molecules that may be involved in the metastatic process.

Note added in proof: Since this work was completed, Kieran and Longnecker (*10*) reported that avian lymphoma cells that selectively colonize liver and ovary in vivo preferentially adhere to cryostat sections of these organs in vitro.

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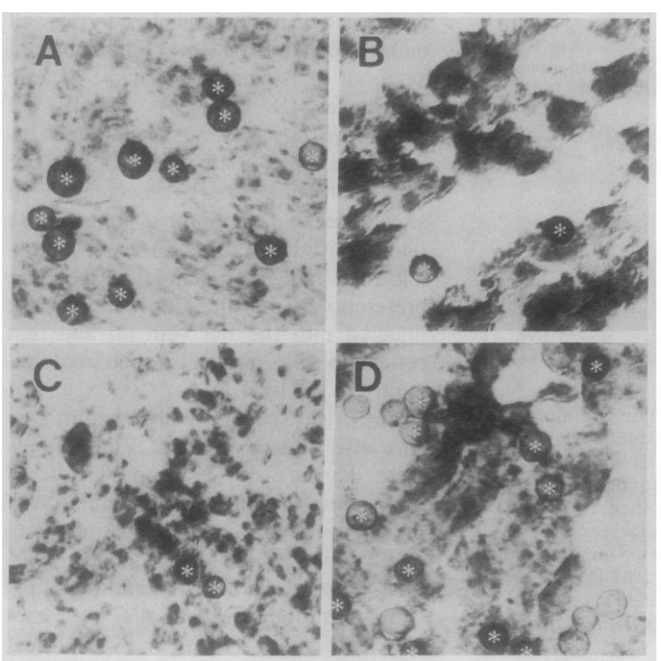

Fig. 2. Micrographs of tumor cells bound to tissue sections. B16-F10 melanoma cells were incubated with sections from mouse lung (A) and liver (B), and reticulum cell sarcoma cells were incubated with sections from mouse lung (C) and liver (D). Tumor cells are indicated by asterisks and are in the plane of focus (hematoxylin and eosin; magnification ×350).

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7. Tumor cells were labeled with $Na_2{}^{51}CrO_4$ (20 μCi/ml; New England Nuclear) for 8 hours in Eagle's minimum essential medium (Dulbecco's modification) supplemented with 10 percent calf serum. A single-cell suspension was prepared with 0.1 percent trypsin (Sigma type III) in calcium- and magneium-free phosphate-buffered saline (PBS) and then washed extensively with 0.5 percent soybean trypsin inhibitor (Gibco) in PBS. Labeled cells (7×10^5 in 100 μl of medium) were incubated for 40 minutes at 5° to 7°C on fresh, unfixed, 10-μm cryostat sections of tissues obtained from 5 to 10-week-old C57BL/6 mice and mounted on 1.2-cm cover slips in wax pencil circles. The cover slips were gently agitated (70 to 80 rev/min) during incubation. After incubation the medium was removed with adsorbant tissue and the cover slips were placed on edge in PBS containing 1 percent glutaraldehyde for at least 15 minutes and then rinsed gently with PBS. The amount of radioactivity associated with the sections was measured in a gamma counter and the number of cells bound to each section was calculated. Typically, 0.1 to 4 percent of the cells added initially remained bound after rinsing.
8. Labeled cells were treated with enzymes for 1 hour at 37°C in PBS (*p*H 7.4). Enzyme concentrations chosen were those at which the observed cell viability and inhibition of binding were maximal. The following enzymes were incubated with the cells (7×10^6 per milliliter): Pronase (100 proteolytic units per milliliter; Sigma); trypsin (100 BAEE units per milliliter; Sigma type III); neuraminidase (42 U/ml; Calbiochem); β-galactosidase (50 U/ml; (Sigma grade VI); bovine pancreas deoxyribonuclease I (50 Kunitz units per milliliter; Sigma); and bovine pancreas ribonuclease A (50 Kunitz units per milliliter; Calbiochem). Trypan blue dye exclusion tests indicated that loss of viability under these incubation conditions was negligible. Tunicamycin (Calbiochem) was solubilized in 10 m*M* sodium hydroxide at 2 mg/ml and diluted in culture medium to a final concentration of 0.5 μg/ml (*9*). Cells were treated with tunicamycin for 36 hours and labeled with ^{51}Cr during the last 8 hours of the incubation period, with a negligible loss of viability.
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Clustering of Human H1 and Core Histone Genes

Abstract. *An H1 histone gene was isolated from a 15-kilobase human DNA genomic sequence. The presence of H2A, H2B, H3, and H4 genes in this same 15-kilobase fragment indicates that mammalian core and H1 histone genes are clustered.*

The core histones, H2A, H2B, H3, and H4, are fundamental parts of the primary structural unit of chromatin, the nucleosome (*1, 2*). Also there is compelling evidence that H1 histones are involved in internucleosomal interactions and higher order structures (*1–5*). While the synthesis of core histone polypeptides is confined predominantly to the S phase of the cell cycle (*6–14*), two H1 histone subspecies, $H1^0$ and H1e, accumulate mainly in quiescent cells (*15, 16*). Analysis of cloned genomic histone sequences has shown that the genes encoding mammalian core histones are clustered but not organized as simple tandem repeats (*17–22*). The inability to identify H1 histone genes in human and murine histone gene clusters indicates that there may be functional significance to the apparent separation of H1 and core histone coding sequences. We now report the isolation of a human H1 histone gene from a λCharon 4A human genomic library. We present evidence that this human H1 histone gene resides in a 15-kilobase segment of DNA that also contains H2A, H2B, H3, and H4 histone genes.

A λCharon 4A human gene library (*23*) was screened with the use of a hybridization probe, the chicken H1 histone gene from pCH 4.8E (*24*). The 1.8-kb Sma I fragment containing the chicken H1 sequence was isolated from a low gelling temperature agarose gel and nick-translated in the presence of α-^{32}P-labeled deoxycytidine triphosphate (dCTP). Of the 12 recombinant phage originally isolated and plaque-purified, only one (λHHG 415) was eventually definitively shown to contain a human H1 histone gene.

A restriction map of λHHG 415 was developed by standard procedures with the use of restriction endonucleases Eco RI, Hind III, Xba I, Sma I, and Bam HI (Fig. 1A). The representation and localization of histone coding sequences were determined by hybridizing Southern blots (*25*) of restriction endonuclease–digested DNA with a series of homologous and heterologous histone gene probes. The core histone genes were identified by hybridization with human H2A, H2B, H3, and H4 histone genes (*18, 26, 27*) while the H1 gene was identified by hybridization with a plasmid containing a 650-base-pair (bp) chicken H1 complementary DNA (cDNA) (Fig. 1B). Confirmation of the representation and organization of human histone coding sequences was by hybrid selection and in vitro translation with either the entire λHHG 415 phage DNA or various subcloned fragments used to select polysomal messenger RNA's (mRNA) from HeLa cells. Each of the core histone genes as well as an H1 histone gene was represented in the genomic fragment in λHHG 415 (Fig. 1C).

Unequivocal identification of the H1 gene in λHHG 415 was provided by DNA sequence analysis of the 5′ region of the gene encoding the first 58 amino acids of an H1 histone protein (Fig. 2A). The amino terminal sequence determined for the human H1 histone protein was compared (Fig. 2B) with the amino acid sequences of H1 histone proteins from rabbit, *Xenopus*, and trout (*28, 29*). There was extensive cross-species sequence homology even in the amino terminal region of H1 histone protein, which is generally considered to be poorly conserved (*28*).

Hybridization of a restriction fragment containing the cloned H1 gene (^{32}P-labeled) to total genomic human DNA (restriction endonuclease–digested, fractionated electrophoretically, and immobilized on nitrocellulose) verified that this H1 containing histone gene cluster is represented in the genome of intact cells (compare Fig. 3A with Fig. 1A). Similar analyses, with the H1 gene having different lengths of 3′ and 5′ flanking sequences as ^{32}P-labeled hybridization probes, indicated that one (or more) reit-

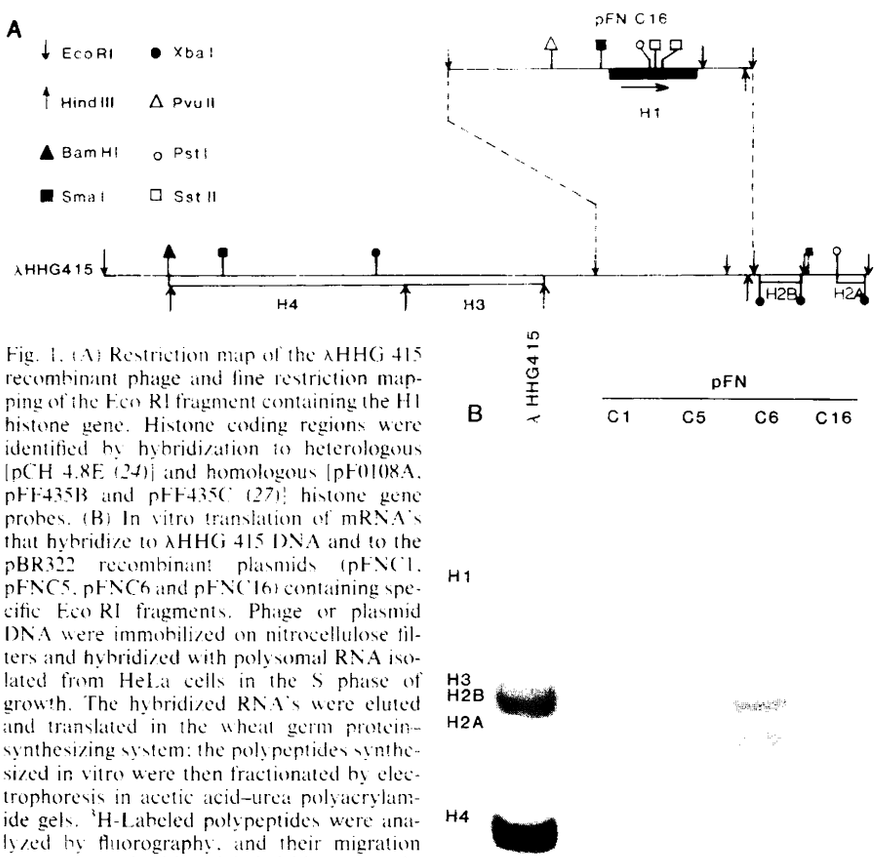

Fig. 1. (A) Restriction map of the λHHG 415 recombinant phage and fine restriction mapping of the Eco RI fragment containing the H1 histone gene. Histone coding regions were identified by hybridization to heterologous [pCH 4.8E (*24*)] and homologous [pF0108A, pFF435B and pFF435C (*27*)] histone gene probes. (B) In vitro translation of mRNA's that hybridize to λHHG 415 DNA and to the pBR322 recombinant plasmids (pFNC1, pFNC5, pFNC6 and pFNC16) containing specific Eco RI fragments. Phage or plasmid DNA were immobilized on nitrocellulose filters and hybridized with polysomal RNA isolated from HeLa cells in the S phase of growth. The hybridized RNA's were eluted and translated in the wheat germ protein-synthesizing system; the polypeptides synthesized in vitro were then fractionated by electrophoresis in acetic acid–urea polyacrylamide gels. ^{3}H-Labeled polypeptides were analyzed by fluorography, and their migration was compared with unlabeled histone markers stained with amido black.