μ*M*), an estrogen-competitive antagonist, elicited a small (about twofold) increase in progesterone receptors. When tamoxifen was included with partially purified ligand (50 ng/ml), the induction of progesterone receptors was reduced from a sevenfold increase by ligand alone to almost the increment seen with tamoxifen alone. On the other hand, when the ligand concentration was raised to 500 ng/ml, the tamoxifen blockade was overcome and near maximal induction of progesterone receptors was achieved.

Numerous other experiments were carried out with ligand preparations at steps 2 and 4 of purification as well as with step 3 material. In various experiments estradiol at 0.1 n*M* induced a 6- to 14-fold increment in progesterone receptors in MCF-7 cells. Responses to 10 to 50 ng of partially purified yeast ligand (step 3) were, in general, equivalent to those seen with 0.01 to 0.1 n*M* estradiol. Fractions of HPLC eluate that did not exhibit [^{3}H]estradiol displacement potency had minimal bioactivity.

We next used ovariectomized mice to ascertain whether the estrogenic activity of partially purified yeast ligand could be demonstrated in vivo. To conserve our limited supply of ligand we used small mice (~ 20 g) and performed only two experiments at the highest ligand dose. As shown in Fig. 3, estradiol, administered on three successive days, elicited a minimal uterotrophic response at a dose of 1 ng per mouse but induced a doubling of uterine weight at 5 ng. Partially purified ligand (step 3 material), at a dose of 2.5 μg per mouse for 3 days, caused a detectable increase in uterine weight. The response was significant at 10 μg and was even more substantial in the two mice that received 40 μg. Induction of progesterone receptors was a more sensitive measure of estrogenic activity than the uterotrophic response. A significant effect could be detected when mice were given 1 ng of estradiol for 3 days, with substantial increments in receptors detected at higher doses. Partially purified yeast ligand, at a dose of 2.5 μg per mouse, caused a minimal increase in progesterone receptors. At 10 μg per mouse the ligand induced a significant response midway between the responses to 1 and 5 ng of estradiol. The response to 40 μg of ligand per mouse was substantial. In all these experiments the ligand preparation used was far from pure. If the active principle in the crude material represented 0.1 percent of the mass of total ligand added, then the ligand would be as potent as estradiol itself.

To determine whether an estrogenic substance might be contaminating the commercially obtained yeast, experiments were performed with various starting materials, including several different batches of dried yeast, bakers' yeast, and α cells grown in our laboratory (*2*) in medium shown not to possess ligand activity. In all cases ligand activity could be demonstrated in extracts prepared from these starting materials, strongly indicating that the ligand was not an extraneous contaminant. We believe that these results demonstrate that a lipid-extractable product of *S. cerevisiae* has estrogenic activity in mammalian systems. The next effort should be to purify this yeast substance and identify its chemical nature and structure.

The presence of an estrogen-binding protein and an estrogenic substance that has [^{3}H]estradiol displacement potency supports our hypothesis that these molecules represent a yeast hormone receptor system (*1*). The functions in the yeast that are regulated by this system remain to be defined.

Substances with estrogenic activity have been described as constituents or contaminants of plants and plant products (*6*). Zearalanones, produced by the fungus *Fusarium*, contaminate feed grain and cause an estrogenic response in swine, apparently acting through the estrogen receptor (*7*). However, since *S. cerevisiae* is the yeast used extensively in the baking and fermentation industries, it is possible that this estrogenic substance may enter the human food supply and affect public health. Our findings indicate that the *S. cerevisiae* ligand is estrogenic in mammalian systems, probably acting through estrogen receptors. Further study will be required to define the structure of this yeast substance, to elucidate its physiological role in *S. cerevisiae*, and to determine whether it is a significant source of environmental estrogens affecting humans.

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Synthesis of a Cyclic Melanotropic Peptide Exhibiting Both Melanin-Concentrating and -Dispersing Activities

Abstract. *A putative melanin-concentrating hormone was synthesized. This peptide, H-Asp-Thr-Met-Arg-Cys-Met-Val-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Glu-Val-OH, stimulates melanin granule aggregation within teleost melanocytes at nanomolar concentrations as does the natural purified teleost pituitary preparation. In addition, this peptide stimulates melanin granule dispersion within melanocytes of frogs and lizards. The peptide has about one six-hundredth of the activity of α-melanocyte–stimulating hormone on frog and lizard melanocytes and is a full agonist.*

Several melanotropins that stimulate pigment dispersion within integumental melanocytes have been identified within the intermediate lobe of the pituitary and the hypothalamus of vertebrates. These peptides include α-melanocyte–stimulating hormone (α-MSH) as well as other structurally homologous peptides, such as β-MSH, γ-MSH, and ACTH. Evidence suggests that α-MSH is the only physiologically relevant melanotropin that regulates skin coloration in tetrapod vertebrates (*1*). In addition to skin pigmentogenesis, other peripheral and neuroregulatory effects of α-MSH have been reported (*2, 3*). The mechanism of action of α-MSH on melanophores has been elucidated (*4, 5*). After hormone-

receptor binding, a signal is transduced to adenylate cyclase, and intracellular cyclic adenosine monophosphate (AMP) concentrations are elevated, which leads to melanin granule dispersion. The melanin-dispersing action of MSH can be reversed by norepinephrine or by melatonin. The mechanism of melanin concentration is unknown but may be due to lowered cyclic AMP levels within melanophores.

Many lower vertebrates are believed to exhibit color changes through dual hormonal control by two antagonistic pituitary melanophorotropic hormones (*6*). An antagonist hormone, melanin-concentrating hormone (MCH), was found in the pituitary gland of teleost fishes in 1955 (*7*). Teleost MCH was separated from fish pituitary gland extracts by absorption to aluminum oxide followed by elution of the hormone with decreasing concentrations of acetone (*8*). The highest concentrations of MCH were found in the neurointermediate lobe of the pituitary gland (*9*). This hormone induces blanching of the skin by perinuclear aggregation of melanin granules within melanophores when injected into certain species of teleost fishes. Teleost MCH has been isolated in pure form from chum salmon pituitaries and was characterized as a cyclic heptadecapeptide (*10*). The primary structure of this peptide was found to be H-Asp-Thr-Met-Arg-Cys-Met-Val-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Glu-Val-OH. At concentrations as low as 1 n*M*, salmon MCH was effective in causing melanin concentration in 50 percent of the melanocytes of a fish (*Tilapia*) scale. We

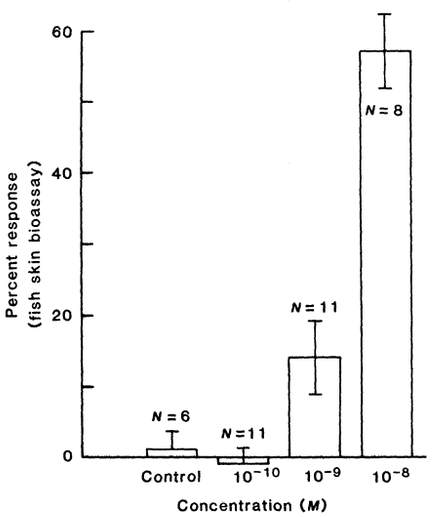

Fig. 1. Melanin-concentrating activity of MCH as determined in vitro with the skin of the fish *Gobeisox pinniger*. Each bar represents the mean ± standard error of the mean of the responses (skin lightening) of the skins to the putative hormone, MCH.

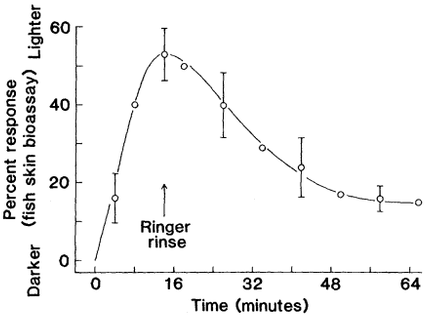

Fig. 2. Demonstration of the melanin-concentrating action of MCH (○) in vitro on skins of the fish *Gobeisox pinniger*. At time zero the peptide ($10^{-8}M$, final concentration) was added to the skins ($N = 5$), which became maximally light by 14 minutes. The skins darkened again when they were placed in a bathing medium lacking the peptide (Ringer rinse). Each value represents the mean ± standard error of the mean of the responses (reflectance) of the skins at the times noted.

have determined the structure-function relations of a number of melanotropins (*5*). Therefore, we were interested in the use of MCH as a possible antagonist, particularly since no peptide antagonist of MSH has been discovered.

The total synthesis of salmon MCH was achieved by solid-phase peptide synthetic methods (*11, 12*). Purification was performed by column chromatography on Sephadex G-25, carboxymethyl-cellulose, and by reversed-phase high-performance liquid chromatography (HPLC). The elution profiles obtained from these chromatographic methods were in agreement with the elution patterns used in the isolation of naturally occurring salmon MCH (*10*). The overall synthetic yield of the purified heptadecapeptide was 14 percent. The peptide was characterized by amino acid analysis, thin-layer chromatography in five solvent systems, HPLC, paper electrophoresis at two different *p*H's, and optical rotation.

The frog (*Rana pipiens*) and lizard (*Anolis carolinensis*) skin bioassays were performed in vitro (*13, 14*). In these assays, melanin granule (melanosome) dispersion into the dendritic processes of melanocytes resulted in darkening of the skin, whereas melanosome aggregation (concentration) to a perinuclear position within the cell resulted in a lightening in skin color. The entire skin of a teleost (*Gobeisox pinniger*) was assayed in the same manner as the frog and lizard skin.

The synthetic hormone caused melanosome aggregation at nanomolar concentrations when bioassayed in the fish *Gobeisox* (Fig. 1). This resulted in the skin turning from a dark (brown-black) color to a light (yellow-tan) color. When the medium bathing the skins was replaced by a medium lacking the MCH (Ringer rinse) (Fig. 2), the skins returned to the initial (base) dark color. Microscopic examination of the skins revealed that lightening of the skins caused by MCH was a result of melanosome concentration within the individual melanophores, whereas darkening resulted when the melanin dispersed back into the dendritic processes of the cells. Addition of α-MSH ($10^{-7}M$) partially reversed the lightening response induced by MCH (data not shown). The synthetic MCH ($10^{-9}M$) also induced melanosome aggregation within melanophores of the following teleosts: *Xiphophorus helleri, Carassius auratus, Lebistes reticulatus*, and *Pimephales promelas*.

The MCH peptide was a full agonist of melanosome dispersion when added to both frog (Fig. 3A) and lizard (Fig. 3B) skin melanophores. MCH was about 600 times less potent than α-MSH in both assays (Fig. 3, A and B). Concentrations of MCH that failed to darken frog ($10^{-8}M$) or lizard ($10^{-7}M$) skins also failed to antagonize or reverse the melanotropic action of α-MSH when used at a submaximal concentration ($10^{-10}M$).

These results show that a synthetic preparation of MCH has melanin-concentrating activity in teleosts at concentrations similar to those reported for the purified pituitary preparation. In addition, synthetic MCH is an agonist of frog and lizard skin melanophores. α-MSH

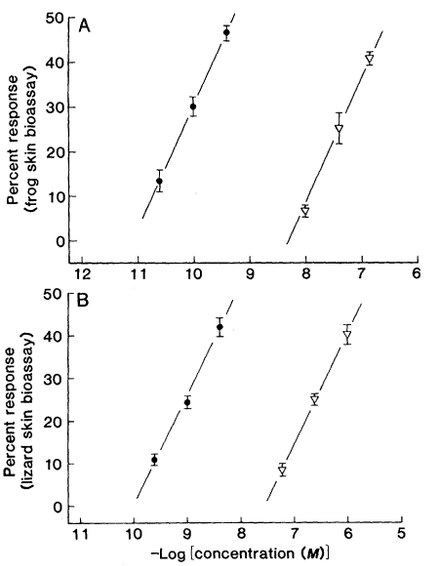

Fig. 3. Relative potency of MCH (▽) compared to α-MSH (●) as determined in (A) frog (*Rana pipiens*) and (B) lizard (*Anolis carolinensis*) skin bioassays in vitro. Each value represents the mean ± standard error of the mean of the responses (darkening) of the skins ($N = 12$) to the melanotropins at the concentrations noted.

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.

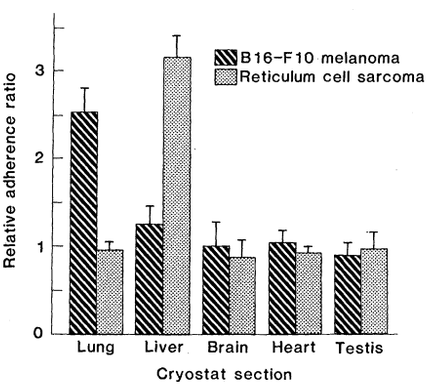

Fig. 1. Organ-specific binding of murine B16-F10 melanoma and reticulum cell sarcoma cells in vitro. Tumor cells were labeled with ^{51}Cr and then incubated on fresh cryostat sections of tissues obtained from C57BL/6 mice (*7*). The relative adherence ratio (*4*) is the ratio of the number of adherent 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-