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The Role of MC1R in Speciation & Phylogeny

RECOMMENDED FOR *AP Biology*

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Abstract

A point mutation in the MC1R gene, a G-protein-coupled receptor, has been found that could have led to the formation of two subspecies of Solomon Island flycatcher from a single ancestral population. I discuss the many roles that G-protein-coupled receptors play in vertebrate physiology and how one particular point mutation can have enormous evolutionary consequences.

Key Words: *Speciation; MC1R; G-protein-coupled receptor.*

The in-depth study of a single protein is an excellent educational tool that enables students to connect many areas of biology. Here, I describe the protein melanocortin-1 receptor (abbreviation: MC1R). This protein is widely distributed in vertebrates, is involved in determining the coloration of many fish, birds, and mammals, and may play an important role in speciation.

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MC1R & Speciation

Can a single-base-pair change in DNA, leading to a single-amino-acid change in a single protein, result in the formation of two species from an ancestral population? Such an example would provide powerful evidence for evolution; point mutations happen all the time, and if a point mutation could lead to a speciation event, the origin of species becomes more understandable.

Uy et al. (2009) have found just such an example in the Solomon Islands, a series of islands located in the South Pacific, east of New Guinea and northeast of Australia (Figure 1). On several of these islands, there are small birds known as "Solomon Island flycatchers" (*Monarcha castaneiventris*). Two subspecies of these birds are located on the nearby islands of Makira and Santa Ana. The birds on Makira, the larger island, are black with chestnut-colored bellies, whereas those on Santa Ana are black all over, including black bellies (Figure 2). What is the effect of this striking phenotypic difference?

Uy et al. studied the behavior of these birds by showing them taxidermic mounts of the same and different coloring. They found that each kind of bird regarded taxidermic mounts with the same kinds of bellies as a threat to their territory, but ignored taxidermic mounts with differently colored bellies. This implies that they would preferentially choose as mates birds with the bellies of the same color because they would likely be aggressive toward birds they regarded as rivals in securing mates. Over time, this could lead to reproductive isolation between chestnut- and black-bellied birds; this could eventually result in so little sharing of genes between the two populations that they would become new species. It is likely that this phenotypic difference played an important role in the initial speciating event that separated a single population of Solomon Island flycatchers into what are now two distinct subspecies.

Uy et al. (2009) studied the genes and proteins of these birds. In a stunning result, they found that the birds differ by 3 base pairs in their MC1R gene, and by 1 amino acid in their MC1R protein. This single-amino-acid difference is due to one of the mutations; the other two are silent mutations that do not change the order of amino acids in the protein. Later, we will discuss how a single-amino-acid change in one protein can cause such a dramatic phenotypic change. But first, some background on melanin and the structure and activity of the MC1R protein.

Melanin

Melanin is the pigment found in birds and mammals that gives skin, fur, and feathers coloration. There are two kinds of melanin; both are synthesized from the amino acid tyrosine. Eumelanin is black or brown, and phaeomelanin is yellow or red. The pathway of melanin

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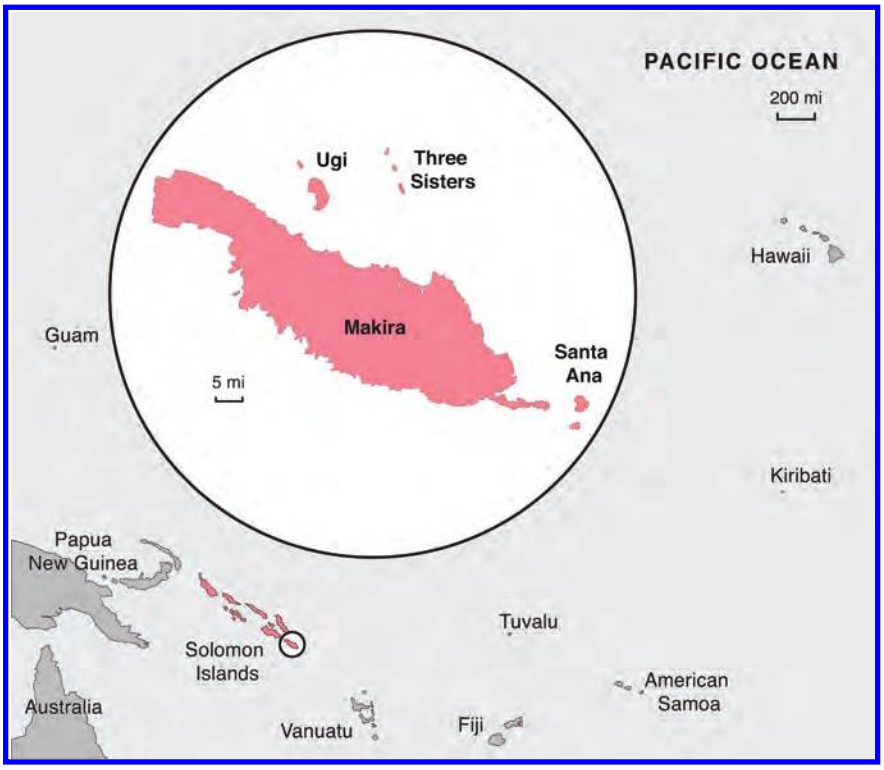

Figure 1. Map of the Solomon Islands (modified from Uy et al., 2009, and used with permission of University of Chicago Press).

synthesis is complex and not fully understood. Melanin itself is not a single molecule, but rather a mixture of pigments produced in this complex pathway. The first, and rate-limiting, step in the synthesis of both kinds of melanin requires the enzyme tyrosinase. Tyrosinase is required for two steps of the phaeomelanin (red/yellow) pathway and for three steps of the eumelanin (black/brown) pathway (Figure 3). When tyrosinase levels are low, phaeomelanin (yellow/red) is produced, and when tyrosinase levels are high, eumelanin (black/brown) is produced.

○ MC1R

We can now talk about our protein, MC1R. The human MC1R protein is 317 amino acids long. In mammals, the MC1R protein is found in the cell membranes of melanocytes, cells that produce melanin. The ligand that binds to MC1R is alpha melanocyte stimulating hormone, abbreviated α-MSH, a small peptide 13 amino acids long, produced by specialized cells in the anterior pituitary gland (de Duve, 1984).

When α-MSH binds to MC1R in the cell membrane of a melanocyte, the gene that codes

Figure 2. Two subspecies of Solomon Island flycatchers, *Monarcha castaneiventris*. The chestnut-bellied flycatcher on the left has an MC1R protein that is responsive to alpha-MSH, so black melanin is produced only when alpha-MSH is present. The black-bellied flycatcher on the right has an MC1R protein that is always active, regardless of whether alpha-MSH is present, so black melanin is always produced. This phenotypic difference is due to a single-amino-acid difference in the MC1R protein. (Photos from *Science* magazine, 2009, vol. 324, p. 1499. Reprinted with permission of AAAS. Photos kindly supplied by R. G. Moyle, Associate Professor/Curator, Department of Ecology and Evolutionary Biology, Natural History Museum and Biodiversity Institute, University of Kansas, Lawrence.)

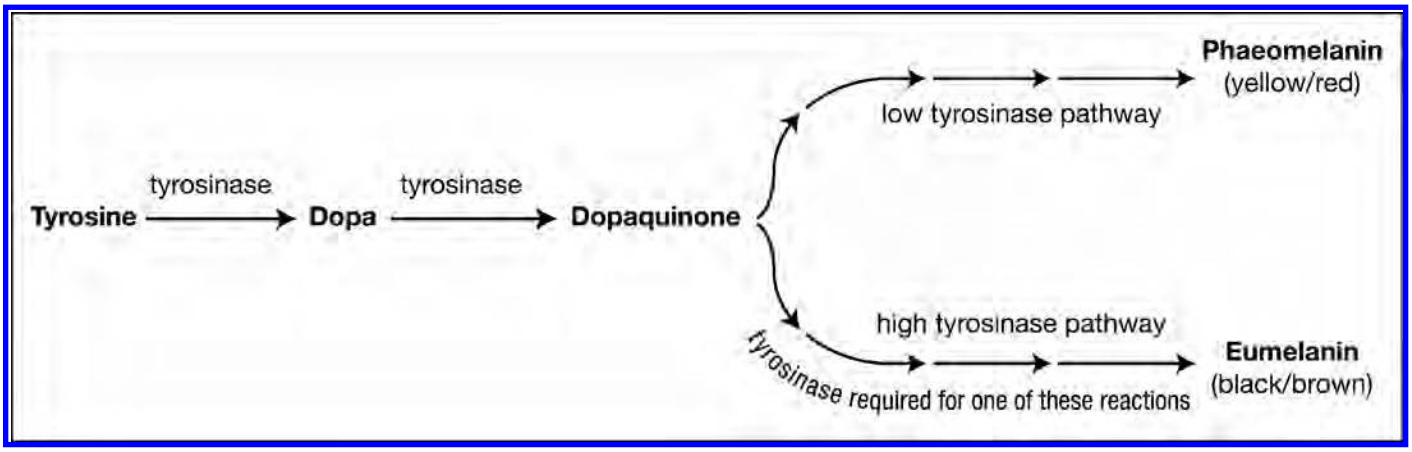

Figure 3. Schematic diagram of the pathway of melanin synthesis. All melanin is made from the amino acid tyrosine. When tyrosinase levels are low, phaeomelanin (yellow/red) is produced; when tyrosinase levels are high, eumelanin (black/brown) is produced. Individuals completely lacking in tyrosinase make no melanin of any kind and are albino. Albinism is most frequently caused by a mutation in the gene coding for tyrosinase (OMIM no. 606933).

for tyrosinase is turned on. Tyrosinase levels in the cell are high, and black pigment is produced. When α-MSH is not bound to MC1R, less tyrosinase is produced; with low tyrosinase levels, yellow pigment is produced.

Mutations can occur in the gene that codes for MC1R that cause it to always be on, regardless of whether α-MSH is present. In animals with this mutation, tyrosinase levels are always high and black pigment is always produced.

○ Speciation in the Solomon Island Flycatchers

Getting back to the Solomon Island flycatchers, the most likely scenario is that the ancestral population resembled the present chestnut-bellied flycatcher. This ancestral bird had an MC1R protein that was active only when α-MSH, its ligand, was present. In the absence of α-MSH, tyrosinase levels in the belly cells were low, and phaeomelanin was produced, giving the bird its chestnut-colored belly. Then, a mutation occurred in the MC1R gene that changed amino acid number 119 from aspartic acid to asparagine (Uy et al., 2009) (Figure 4). As a result of this mutation, MC1R is always turned on and tyrosinase levels are always high. This results in a black belly. This is a stunning example of a point mutation that can be an initial step in the splitting of a single population into two species. It is entirely reasonable to think that a bird that has been raised by parents with chestnut-colored bellies would look for a mate with a chestnut-colored belly, whereas a bird that has been raised by parents with black bellies would seek out a black-bellied mate. This is reinforced by the studies done by Uy et al. (2009) that showed that birds behaved aggressively only to other birds with the same color of belly and therefore saw only birds with the same belly color as competitors for mates. Over time, this sexual selection could lead to two populations that rarely exchanged genes and that eventually would become two species. Speciation is a process, not an event. The separation of two populations into separate species that never exchange any genes with each other typically occurs over a period of several million years (Grant & Grant, 2007). These little birds give us hard evidence for a process that scientists had long assumed occurred.

○ G-Protein-Coupled Receptors

MC1R is a G-protein-coupled receptor, a kind of signaling protein. Because these are such important proteins, it is worthwhile to describe them in more depth. These connections are useful in classroom discussions. In fact, the 2012 Nobel Prize in Chemistry was just given to Robert J. Lefkowitz and Brian K. Kobilka for their decades' long work in studying the structure and mechanism of action of G-protein-coupled receptors. There is much useful information at the Nobel Prize website (http://www.nobelprize.org).

G-protein-coupled receptors are located in the cell membrane and are associated with a G protein on the inside of the cell membrane. When a small molecule outside the cell binds to a G-protein-coupled receptor, the G-protein-coupled receptor changes shape and activates the G protein inside the cell. Typically, activation of a G protein activates the enzyme adenyl cyclase, resulting in an increase in the level of cyclic AMP (cAMP) in the cell; this causes further changes inside the cell.

A G-protein-coupled receptor is a protein about 300–500 amino acids long; it has seven transmembrane segments, crossing the cell membrane seven times. Each transmembrane segment is an alpha helix 20–22 amino acids long. The free amino end of the protein is on the outside of the cell (extracellular domain), and the free carboxyl end of the protein is on the inside of the cell (intracellular domain) (Figure 5). The extracellular domain of the protein has a specific shape that differs slightly in every kind of G-protein-coupled receptor. A small molecule, complementary in shape and charge, binds specifically to this extracellular domain to activate the protein. Because the extracellular domain of each kind of G-protein-coupled receptor has a slightly different shape, a different ligand will bind to each kind of G-protein-coupled receptor.

G-protein-coupled protein receptors are the largest protein family in the human genome. The human genome contains about 20,000 genes; 800 of these code for G-protein-coupled receptors. Odor receptors in the nose are G-protein-coupled protein receptors, as are taste receptors in the tongue and vision receptors in the rods and cones of the retina. Other G-protein-coupled receptors include receptors for marijuana, glucagon, adrenalin, glutamate, and opioids such as opium, morphine, and codeine.

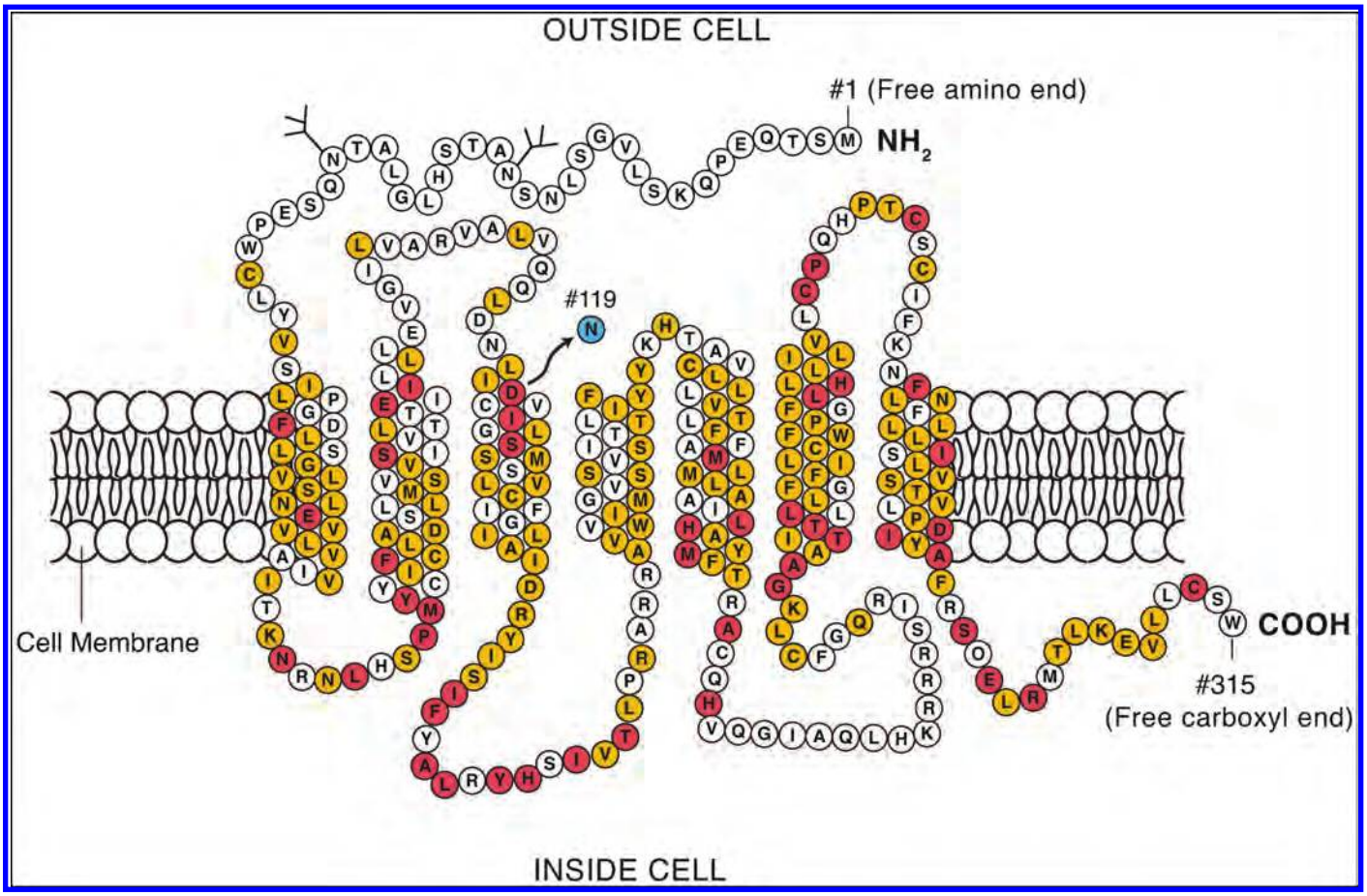

Figure 4. Comparison of MC1R protein in the two subspecies of Solomon Island flycatchers. The protein shown is the wild type MC1R protein, sensitive to alpha-MSH. This is the sequence found in the chestnut-colored birds. Amino acid no. 119 (highlighted) is aspartic acid, indicated by its single-letter abbreviation, D. In the black-bellied bird, amino acid no. 119 is changed to asparagine, indicated by its single-letter abbreviation, N. This single-amino-acid change is caused by a single-base-pair mutation in the gene coding for MC1R. The amino acids are numbered from 1 to 315, with 1 being the free amino end and 315 the free carboxyl end. The figure shows the mouse MC1R protein; it is very similar to the MC1R protein in the Solomon Island flycatchers. The protein is 315 amino acids long. It is located in the cell membrane. The free amino end is outside the cell, and the free carboxyl end is inside the cell. There are seven transmembrane segments as shown; each is an alpha helix 20 to 22 amino acids long. Single-letter abbreviations for the amino acids are as follows: A = alanine, C = cysteine, D = aspartic acid, E = glutamic acid, F = phenylalanine, G = glycine, H = histidine, I = isoleucine, K = lysine, L = leucine, M = methionine, N = asparagine, P = proline, Q = glutamine, R = arginine, S = serine, T = threonine, V = valine, W = tryptophan, and Y = tyrosine. (Modified from Lu et al., 1998. Copyright 1998, The Endocrine Society. Modified and reprinted with permission.)

Most mammals have about 1000 different kinds of odor receptors, each of which binds to one kind of odorant molecule. This gives mammals an excellent sense of smell. Primates, including humans, have only about 300 different kinds of odor receptors. It is likely that when primates developed good color vision and began using vision to find food, the selection on odor receptors was relaxed. Approximately 700 of our odor receptor genes have decayed over evolutionary time; many exist today as "pseudogenes," stretches of DNA that have recognizable "odor receptor" sequence but do not code for a functional protein, perhaps because there is a stop codon in the middle of the sequence. Because genes code for proteins, there is a different gene coding for each kind of G-protein-coupled receptor. The genomes of mammals contain about 20,000 genes; in non-primate mammals, about 1000, or an astonishing 5% of these genes, code for odor receptors, one kind of G-protein-coupled receptors.

We don't know how the mutation in the Solomon Island flycatchers affects the binding of α-MSH to MC1R, because the tertiary structure of MC1R is not known. Tertiary structures of proteins are difficult to study. To date, the tertiary structures of only 128 human proteins have been determined. It is particularly difficult to determine the tertiary structures of G-protein-coupled receptors because, like many membrane proteins, they are unstable outside of the cell membrane; as of 2010, the tertiary structures of only five human G-protein-coupled receptors were known (Ledford, 2010). A very recent study (Rasmussen et al., 2011) describes the crystal structure of a G-protein-coupled receptor and its associated G protein. However, even this extremely thorough study was able to determine the tertiary structure of the G-protein-coupled receptor only at a low resolution. In other recent work, described by Filizola and Devi (2012), the tertiary structures of four opioid receptors were reported. But

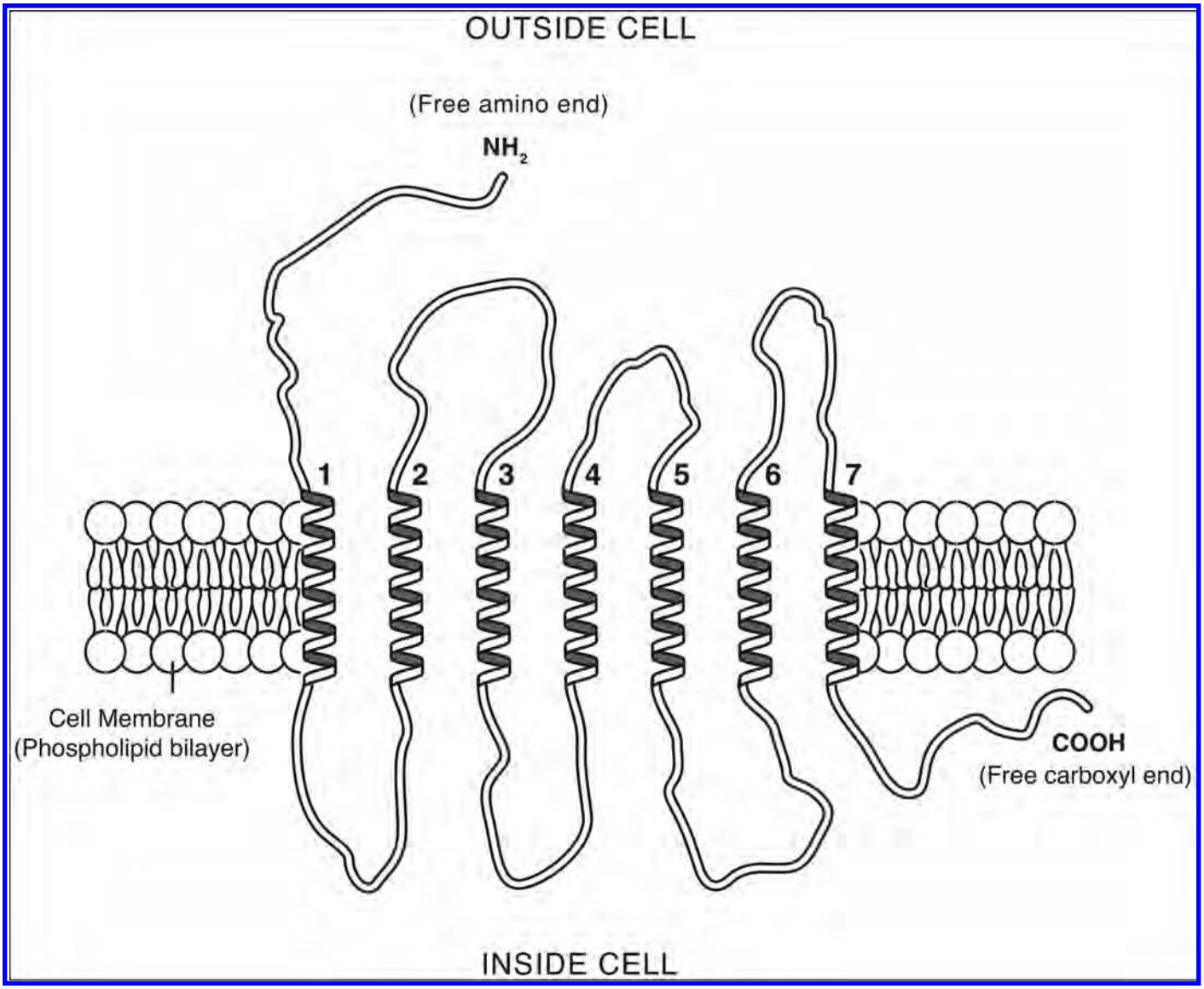

Figure 5. General structure of a G-protein-coupled receptor. A typical G-protein-coupled receptor is a single amino acid chain in the cell membrane. The free amino end of the protein is on the outside of the cell; the free carboxyl end is on the inside of the cell. The protein crosses the cell membrane seven times. The seven transmembrane segments are labeled 1–7. Each transmembrane segment is an alpha helix 20 to 22 amino acids long. At 3.6 amino acids per turn of an alpha helix, this would mean that a transmembrane domain would have approximately 6 turns of an alpha helix.

even for these receptors, they could only get structures of the proteins when they were bound to their ligands.

○ MC1R & Human Redheads

Mutations can also occur in the gene that codes for MC1R that make it insensitive to α-MSH. In this case, MC1R is never active even if α-MSH is present. As a result, tyrosinase levels are always low, and only the yellow/red phaeomelanin pigment is produced. Such a mutation was found in humans by Frändberg et al. (1998), in which amino acid number 151 was changed from arginine to cysteine. In humans, redheads are homozygous for this mutation on chromosome 16. Because they produce only yellow phaeomelanin, and no black eumelanin, they have red hair and fair skin coloring (Frändberg et al., 1998).

○ MC1R & Pigmentation in Other Organisms: The Evolution of MC1R & G-Protein-Coupled Receptors

Sean Carroll (2006) and Lu et al. (1998) give examples of animal species in which individuals can have different alleles for MC1R. The different alleles result in different colorations in animals as diverse as jaguars, brown bears, lesser snow geese, cattle, sheep, foxes, cave fish, zebrafish, frogs, and gray squirrels.

Black bears in British Columbia have long been known to have some white (not albino) bears in their populations. These white-furred black bears are called "spirit" bears by the native people. The white allele is caused by a different mutation in the MC1R gene that changes one amino acid in the MC1R protein. This shows how mutations in MC1R arise independently in many different vertebrates,

affecting their phenotypes in different ways (Hedrick & Ritland, 2012).

The MC1R protein affects fur coloring in rock pocket mice. Excellent classroom activities have been developed by the Howard Hughes Medical Institute and are available on their website, hhmi.org/biointeractive/.

MC1R and G-protein-coupled receptor proteins are conserved across hundreds of millions of years of evolution. Birds and mammals both have genes for MC1R proteins, yet birds and mammals have not had a common ancestor for 310 million years. Therefore, the gene that codes for MC1R is ≥310 million years old. The MC1R gene is also found in many fish and, therefore, goes back to the common ancestor of mammals and fish, >400 million years ago. It may be even older, but it has not been found in organisms with more distant common ancestors. Even in fish, the MC1R protein was difficult to identify because its sequence was quite divergent from the sequence in birds and mammals (Hopi Hoekstra, pers. comm.). G-protein-coupled receptors are even more ancient, occurring in all three domains and, therefore, likely to go back to the universal ancestor that lived 3.5 billion years ago.

○ Acknowledgments

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Appendix: Background Information on Membrane & Protein Structure

Proteins have a tertiary structure, a specific shape in which they exist in the cell. The tertiary structure determines the function of the protein and is determined by the order of amino acids in the protein. Small molecules called *ligands* can bind to a protein. When a ligand binds to a protein it can cause the protein to change shape; this can either activate or inactivate the protein.

The cell membrane is a phospholipid bilayer with interspersed proteins. Many different kinds of proteins are found in the membrane of a single cell; on average, about a million copies of each kind of protein are present in each cell. Many of the proteins in a cell membrane function as receptors. G-protein-coupled receptors bind ligands outside of the cell. This causes a change in conformation in the G-protein-coupled receptor, resulting in a response inside the cell. The ligand itself never enters the cell. Typically, when a ligand binds to a G-protein-coupled receptor, a G-protein inside the cell becomes activated and this, in turn, activates adenyl cyclase, resulting in the formation of cyclic AMP, a "second messenger." Recently, we have come to appreciate that many of the seven transmembrane receptor proteins activate proteins other than G-proteins inside the cell. Therefore, these proteins are beginning to be called seven transmembrane receptors (7TM receptors). Some 7TM receptors, such as MC1R, are G-protein-coupled receptors (see http:www.nobelprize.org).