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Light modulates the melanophore response to α-MSH in *Xenopus laevis*: An analysis of the signal transduction crosstalk mechanisms involved

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ABSTRACT

Melanin granule (melanosome) dispersion within *Xenopus laevis* melanophores is evoked either by light or α-MSH. We have previously demonstrated that the initial biochemical steps of light and α-MSH signaling are distinct, since the increase in cAMP observed in response to α-MSH was not seen after light exposure. cAMP concentrations in response to α-MSH were significantly lower in cells pre-exposed to light as compared to the levels in dark-adapted melanophores. Here we demonstrate the presence of an adenylyl cyclase (AC) in the *Xenopus* melanophore, similar to the mammalian type IX which is inhibited by Ca^{2+}–calmodulin-activated phosphatase. This finding supports the hypothesis that the cyclase could be negatively modulated by a light-promoted Ca^{2+} increase. In fact, the activity of calcineurin PP2B phosphatase was increased by light, which could result in AC IX inhibition, thus decreasing the response to α-MSH. St-Ht31, a disrupting agent of protein kinase A (PKA)–anchoring kinase A protein (AKAP) complex totally blocked the melanosome dispersing response to α-MSH, but did not impair the photo-response in *Xenopus* melanophores. Sequence comparison of a melanophore AKAP partial clone with GenBank sequences showed that the anchoring protein was a gravin-like adaptor previously sequenced from *Xenopus* non-pigmentary tissues. Co-immunoprecipitation of *Xenopus* AKAP and the catalytic subunit of PKA demonstrated that PKA is associated with AKAP and it is released in the presence of α-MSH. We conclude that in *X. laevis* melanophores, AKAP12 (gravin-like) contains a site for binding the inactive PKA thus compartmentalizing PKA signaling and also possesses binding sites for PKC. Light diminishes α-MSH-induced increase of cAMP by increasing calcineurin (PP2B) activity, which in turn inhibits adenylyl cyclase type IX, and/or by activating PKC, which phosphorylates the gravin-like molecule, thus destabilizing its binding to the cell membrane.

1. Introduction

Cells detect extracellular stimuli through membrane-bound receptors and process this information through the receptor's attendant transduction cascades. Receptor types far outnumber the classes of transduction cascades, indicating that multiple receptors must signal through the same transduction mechanisms. Adding to this complexity is the fact that different signaling cascades can interact, resulting in potentially confounding crosstalk (Hur and Kim, 2002). How useful information can be extracted from this cacophony of signals remains the subject of active study among many labs.

Intracellular signaling scaffolds are emerging as a mechanism by which extracellular signals can by segregated within microdomains of the cell. This segregation maximizes the spatial and temporal resolution of incoming signals (Force et al., 2007; Pawson and Scott, 1997). Furthermore, scaffolds provide a substrate for constructive crosstalk between different signaling cascades. Such crosstalk is critical in facilitating the integration of multiple concurrent stimuli whose temporal and spatial coincidence is informative to the cell (Schulte and Levy, 2007; Vazquez-Prado et al., 2003; Werry et al., 2003).

In this report we describe the differential response of dermal melanophores to the darkening agent α-melanocyte stimulating hormone (α-MSH). The variability in response is related to the lighting history of the cells such that pre-exposure to light diminishes the cell's response to α-MSH. α-MSH is a peptide hormone produced from the cleavage of proopiomelanocortin (POMC) in the pars intermedia of the pituitary gland. In amphibians, the hormone is a potent skin darkening agent, dispersing melanin granules (melanosomes) throughout the cytoplasm of dermal melanophores

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(Castrucci et al., 1984). Among the five melanocortin receptor subtypes only MCR1 is expressed in melanophores. MCR1 is coupled to G_s protein and its activation stimulates adenylyl cyclase, resulting in an increase of intracellular cAMP, which in turn, activates a cAMP-dependent protein kinase A (PKA) (Tsatmali et al., 2002). PKA is a tetrameric holoenzyme with two regulatory (R) and two catalytic subunits (C) (Taylor et al., 1990). Four genes (RIα, RIβ, RIIα, and RIIβ) encode the R subunits and three (Cα, Cβ, and Cγ) encode the C subunits (Taylor et al., 1990). The active kinase stimulates a cascade of specific events leading to the final melanosome dispersing response (Nery and Castrucci, 1997). Although cAMP is essential for PKA activation (Builder et al., 1980), other regulatory mechanisms dictate where and when the kinases will be activated in response to specific stimuli. Extracellular signals generated by hormones, neurotransmitters, etc., trigger a variety of cell responses, including ion channel modulation, neural excitation, growth, and differentiation (Taylor et al., 1990). Systems of intracellular transduction must act fast and precisely, compelling the cell to simultaneously manage hundreds of signals. Kinase adaptors such as anchoring kinase A proteins (AKAPs) are part of the cell strategy to deal with the problem of multiple concurrently active signaling pathways. Presently, more than 70 families of AKAPS are known, representing a functional group of molecules which precisely locates kinases and phosphatases at specific intracellular domains (Colledge et al., 2000). A series of kinases and phosphatases has been reported to be bound by AKAPs. AKAP79 has binding sites for PKC and phosphatases PP2B (calcineurin) and PP1 (Dodge and Scott, 2000). Calcineurin complexed with AKAP79 is inactive in *Xenopus laevis* oocytes (Plata et al., 2004); AKAP220 binds and inhibits phosphatase PP1 (Schillace et al., 2001); AKAP350/450 binds kinases such as PKN, a Ser/Thr kinase that possesses PKC, PP1, and PP2A homolog domains (Takahashi et al., 1999); gravin, also known as AKAP250 or AKAP12, possesses binding sites for PKC and PP2B (Lin and Gelman, 2002; Piontek and Brandt, 2003). AKAPs bind to inactive PKAs, usually at the PKA regulatory domain RII (Scott and McCartney, 1994), and less frequently at RI (Malbon et al., 2004). The complex formation does not prevent the kinase activation. More than 75% of PKAs are found intracellularly bound to at least one representative of the AKAP family. A major function of these anchoring proteins is to position the kinases at specific sites of the cell membrane. This regulatory mechanism assures the exposure of PKA to isolated cAMP gradients and a precise location in respect to PKA substrate (Wong and Scott, 2004). Despite the importance of AKAPs as a regulatory component of the cAMP pathway, little is known about their role in melanophores and whether these proteins participate in the response triggered by α-MSH.

Xenopus laevis melanophores also disperse the melanosomes in response to light, through the activation of the photopigment melanopsin (Rollag et al., 2000). However, the signaling that evokes the photo-response does not rely on cAMP increase, but on the activation of phospholipase C, cytoplasmic Ca^{2+} increase, production of IP_3, and PKC activation (Isoldi et al., 2005).

In the present study the signal transduction pathway activated by light was further characterized and the interaction of this system with α-MSH signaling was investigated. This analysis showed involvement of calcineurin and AKAP in the light signal transduction pathway and that light attenuates α-MSH action on *X. laevis* melanophores.

2. Material and methods

2.1. Characterization of the model

The melanophores were derived from *X. laevis* embryos in 1992, and have been maintained in culture as described elsewhere (Rollag et al., 2000). Briefly, the cells were kept in 60% L-15 medium, 480 mg/l galactose, 5 mg/l insulin/transferrin/selenium, 4 mg/l uridine, 87.6 mg/l L-glutamine, 25 mg/l L-asparagine, 152 mg/l $CaCl_2$, 49.6 mg/l $MgCl_2$, 51.7 mg/l $MgSO_4$, 0.4× MEM non-essential amino acids solution, 0.2× MEM amino acids solution, 0.3× MEM vitamin solution, 2× HT supplement, 0.05% penicillin/streptomycin and 5% non-inactivated fetal calf serum (all from Gibco BRL, Carlsbad, CA, USA), pH 7.5, at 25 °C.

2.2. Cloning and sequencing

Total RNA was extracted with Trizol (Invitrogen, Carlsbad, CA, USA) and reverse transcribed (Superscript™ II Reverse-Transcriptase Preamplification System, Invitrogen, Carlsbad, CA, USA). PCR was performed using primers for *X. laevis* adenylyl cyclase type 9 (Gi: 1514669): forward 5′-GTGGTTCCTGAACCGTGAGT-3′ and reverse 5′-TCATCTGTCCTTTCCCCTTG-3′, and AKAP (Gi: 12007267): forward 5′-TTCAGATGTGGAGCAAAGAGTGG-3′ and reverse 5′-CTCTTCCATCAACCATTTCTTC-3′. We designed specific primers for AC9 since it is the only adenylyl cyclase subtype which is inhibited by calcineurin. As to AKAP, the primers were designed for the one already cloned from *Xenopus*. The obtained DNA fragments were ligated into pCR II-TOPO vector using TOPO TA Cloning kit (Invitrogen, Carlsbad, CA, USA), and inserted into One Shot chemically competent cells. cDNAs were purified by miniprep (Promega, San Luis Obispo, CA, USA) and sequenced by Big-Dye Terminator Cycle Sequencing (PE Applied Biosystems, Foster City, CA, USA), to confirm their identity.

2.3. Sequence analysis

The nucleotide sequences of adenylyl cyclase and AKAP were identified by sequence homology using the Basic Local Alignment Search Tool (BLAST; GenBank (www.ncbi.nlm.nih.gov/Blast)). The complete deduced amino acid sequence of *X. laevis* identified as gravin-like protein as compared with two other known gravin sequences (*Homo sapiens* gravin GI: 2081607 and *Rattus norvegicus* AKAP12 GI: 16930812) in the Swiss Institute of Bioinformatics (SIB) site (www.expasy.ch) using ScanProsite software (Gattiker et al., 2002), and in the Massachusetts Institute of Technology (MIT) site (http://scansite.mit.edu/) using Scansite 2.0 program (Obenauer et al., 2003). Scansite identifies short protein sequence motifs that are recognized by modular signaling domains phosphorylated by protein Ser/Thr- or Tyr-kinases, or motifs that mediate specific interactions with protein or phospholipid ligands (Obenauer et al., 2003).

2.4. Second messenger determination

For cyclic nucleotide assays, *Xenopus* melanophores were seeded (10^4 cells/well) in 96-well plates in 60% L-15 medium supplemented with 2% serum and 10^{-7} M retinaldehyde (Sigma Chemical Co., St. Louis, MO, USA), and kept in the dark for 3 days. The cells were then pre-incubated with 10^{-4} M 3-isobutyl-1-methylxanthine (IBMX, Sigma Chemical Co., St. Louis, MO, USA) for 15 min, followed by 1 min, 5 min and 10 min exposures to white light (EI_{50}), in the presence of IBMX. EI_{50} (irradiance necessary to evoke 50% of the maximal pigment dispersing response in *Xenopus* melanophores) was previously determined to be 2.082×10^3 μW/cm^2 (Isoldi et al., 2005). Additional triplicates were kept in the dark and received 10^{-8} M α-MSH (Sigma Chemical Co., St. Louis, MO, USA), as a positive control for cAMP. The cells were lysed with the kit reagents and the protocol was followed as described for total cAMP measurements (Enzyme Immunoassay System, Code RPN 225, Amersham Biosciences, Buckinghamshire, England).

2.5. AKAP role

To test the effect of AKAP–PKA disruption on 10^{-8} M α-MSH- or light-induced responses, *Xenopus* melanophores were seeded in 96-well plates as described above. Prior to assaying, the cells were treated with 10^{-9} M melatonin for 60 min (to fully aggregate the melanosomes; Isoldi et al., 2005), and the AKAP–PKA disrupting peptide, St-Ht31 (Promega, San Luis Obispo, CA, USA) was added to the medium for an additional 30 min in the presence of melatonin. Plates were then exposed to white light (EI_{50}) and triplicates were kept in the dark in the absence (not shown) or presence of 10^{-8} M α-MSH. Melanosome dispersion was quantified as absolute absorbance in a plate reader (Tecan, Model Sunrise, Durham, NC, USA), after 30 and 60 min, and expressed as control-corrected optical density (OD) (dark control without α-MSH) after 60 min of exposure to the EI_{50} in the absence of the inhibitor.

2.6. Calcineurin phosphatase (PP2B) colorimetric assay

Xenopus melanophores were seeded in 6-well plates (10^6 cells/well), in 60% L-15 medium supplemented with 2% serum and 10^{-7} M retinaldehyde, and kept in the dark for 3 days. After exposure to 1 and 5 min of white light, in the absence or presence of the calcium/calmodulin kinase inhibitor KN-93 (Biomol Research Laboratories, Plymouth Meeting, PA, USA), the cells were lysed with the kit buffer (Biomol Green Cellular Calcineurin Assay Kit Plus, AK-816, Biomol Research Laboratories, Plymouth Meeting, PA, USA). The free phosphate released from a PP2B substrate provided in the kit was detected by the classic Malachite green assay. Results obtained in the presence of EGTA (total phosphatase activity minus the calcium-dependent calcineurin activity) were subtracted from total phosphatase activity (PP1, PP2A, PP2B and PP2C phosphatases), thus obtaining calcineurin activity.

2.7. AKAP immunoprecipitation and Western blot analysis of PKA

For co-immunoprecipitation assays, *Xenopus* melanophores were seeded in 6-well plates and kept in the dark or exposed to 5 min of light or to 10^{-8} M α-MSH. Cells were lysed with 200 µl of PhosphoSafe extraction buffer (Novagen, La Jolla, CA, USA) containing protease inhibitors (Roche, Nutley, NJ, USA). The lysate was centrifuged for 5 min at 12,000 *g* at 4 °C. The supernatant was incubated with goat polyclonal anti-gravin (1:100 dilution, Abcam, Cambridge, MA, USA) and rabbit polyclonal anti-β-actin (1:1000 dilution, Abcam, Cambridge, MA, USA) overnight at 4 °C. Thirty microliters of protein A beads were added to 200 µl of the samples, and the mixture was kept at 4 °C for 90 min under agitation. Immune complexes were collected by centrifugation at 12,000 *g* and the beads were washed (5×) with PhosphoSafe extraction buffer.

The protein concentration was determined by BCA protein assay (Pierce, Rockford, IL, USA), and the mixture of loading gel, antioxidant agent (Invitrogen, Carlsbad, CA, USA), and 20 µg of protein was heat denatured (70 °C for 10 min) and loaded into a 10% NuPAGE Tris–Bis gel (Invitrogen, Carlsbad, CA, USA). Electrophoresis was performed at 200 V for 50 min. After transfer (30 V) to polyvinylidene fluoride PVDF membranes, the blot was developed by using the WesternBreeze chromogenic immunodetection kit (Invitrogen, Carlsbad, CA, USA). Briefly, nonspecific sites were blocked (1 h), and membranes were incubated overnight at 4 °C in a mixture of polyclonal antibody raised against the catalytic subunit of PKA (1:1000 dilution) and a loading control antibody against β-actin (1:1000 dilution; both raised in rabbit, from Abcam, Cambridge, MA, USA). Membranes were then rinsed (4×, 5 min) with the kit washing solution, incubated in the alkaline phosphatase-conjugated anti-rabbit secondary antibody for 1 h at room temperature, rinsed again (4×, 5 min), and finally placed in a solution of 5-bromo-4-chloro-3-indolyl-1-phosphate and nitro blue tetrazolium (BCIP/NBT) substrate for alkaline phosphatase until bands appeared. After rinsing in water, the membranes were air-dried, digitalized and analyzed by Scion Image (Scion Corporation, Frederick, MD, USA). The densitometry values of actin were used to normalize any variation in the amount of protein per lane. This experiment was performed in triplicate.

2.8. Statistical analyses

The data were compared by one-way ANOVA followed by Student–Newman–Keuls and considered significantly different at $p < 0.05$. For the experiments with AKAP–PKA disrupting agent, Student's *t*-test was used to compare the results in the absence and in the presence of St-Ht31 in each time point.

3. Results

It has been previously shown that *Xenopus* melanophores disperse melanosomes in response to α-melanocyte stimulating hormone (α-MSH) through a cAMP-dependent signaling pathway (Sugden and Rowe, 1992). As expected, α-MSH promoted a 6-fold increase in cAMP as compared to basal levels. But curiously, although light alone did not directly affect cAMP concentration, pre-exposure of *Xenopus* melanophores to light diminished the MSH-induced increase of cAMP for all light durations tested (Fig. 1).

Comparison of the deduced peptide sequence of the only known adenylyl cyclase (AC) found in *Xenopus* melanophores with those sequences in GenBank revealed that the melanophore AC belongs to the type IX family of adenylyl cyclases (Gi: 1514669). The catalytic sites are quite conserved as demonstrated by the high homology of these domains between *Xenopus* and human AC IX (Gi: 50959205) sequences (Fig. 2). As already reported (Antoni et al., 1998) AC IX may be negatively regulated by calcineurin, a phosphatase 2B.

We then hypothesized that light would activate calcineurin, thus inhibiting the production of cAMP by α-MSH. In fact, the hypothesis was fully confirmed by calcineurin enzymatic assay; upon light exposure, an increase of enzyme activity was observed (Fig. 3). This finding led us to investigate the presence of regulatory complexes between kinases, particularly PKA and PKC, in addition to phosphatases, such as calcineurin. Cloning and sequencing demonstrated that the anchoring protein expressed in *Xenopus* melanophores had already been sequenced from various non-pigmentary *X. laevis* tissues (GenBank Gi: 12007267). It is similar to human

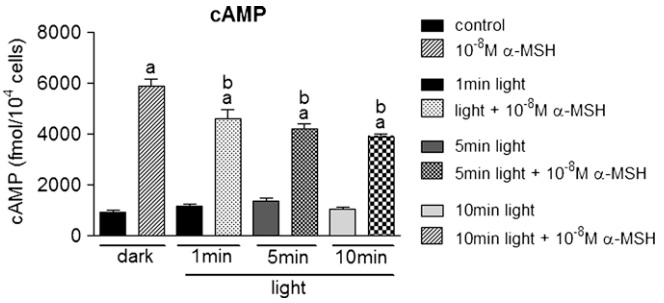

Fig. 1. *Xenopus* melanophores were seeded in 96-well plates and kept in the dark for a minimal of 3 days. cAMP was determined in *Xenopus* melanophores in the dark and after 1, 5, and 10 min light exposure, in the absence or presence of 10^{-8} M α-MSH. Each bar is the mean ($n = 6$), ±SE, fmol/10^4 cells. (a) Significantly different from the respective control in the absence of the hormone. (b) Significantly different from 10^{-8} M α-MSH in the dark. $p < 0.01$.

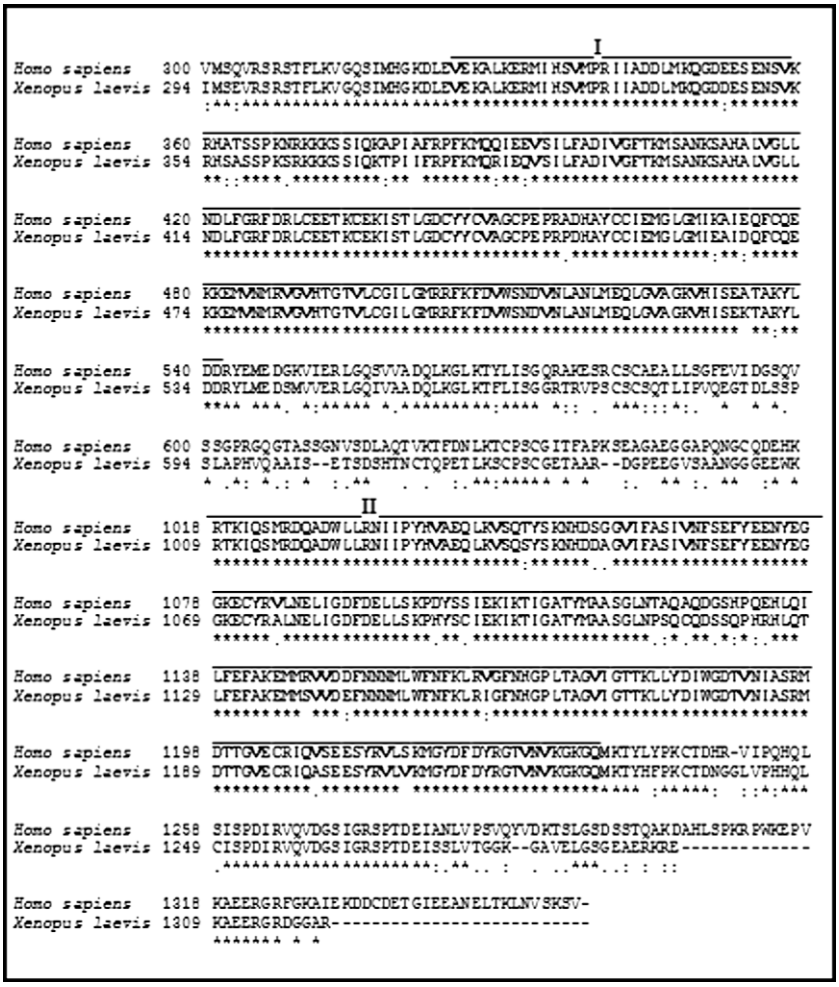

Fig. 2. Partial amino acid sequences of *Xenopus laevis* and human adenylyl cyclase IX (AC9). Identical residues in bold constitute the catalytic domains I and II (CLUSTALW 1.83).

gravin, also known as AKAP250 or AKAP12, and to other mammalian AKAPs. The comparative analysis of *Xenopus*, human and *Rattus* AKAPs performed by Scansite 2.0 software revealed several sites for serine/threonine phosphorylation known to be involved in the modulation of AKAP activity. In addition, several putative binding sites for the various PKC isoforms were found in all three gravins. It also called our attention the existence of binding sites for cell cycle related kinases, in agreement with what has been known for PKA adaptor molecules. The analysis performed with the ScanProsite program also detected putative sites for PKC in the three sequences, in addition to the gravin characteristic N-myristoylated sites. Sites for caseine kinases II and PKA were detected by both programs.

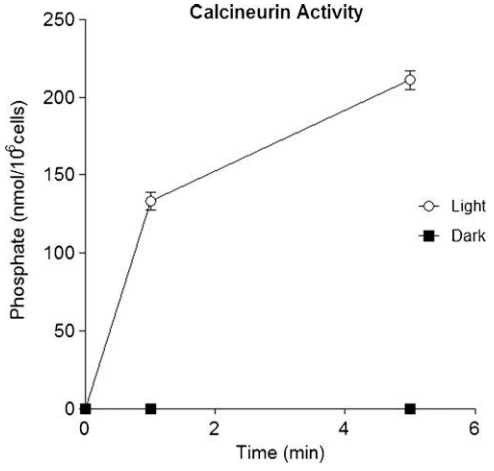

Fig. 3. Calcineurin activity in *Xenopus* melanophores in the dark, and after 1 and 5 min light exposure. Each point is the mean ($n = 6$), ±SE, phosphate nmol/10^6 cells, at the times noted.

To confirm AKAP participation in α-MSH mechanism of action, we used a specific AKAP–PKA disrupting agent, the peptide St-Ht31, which completely blocked the hormone-induced melanosome dispersion in *Xenopus* melanophores. The peptide did not affect the response to light (Fig. 4). In the same way, immunoprecipitation of *Xenopus* AKAP, followed by Western blot detection of the catalytic subunit of PKA (Fig. 5), demonstrated that PKA is associated with AKAP and it is released in the presence of α-MSH.

4. Discussion

Melanosome dispersion within *X. laevis* melanophores is a common response to either light or α-MSH stimulation (Rollag et al., 2000). Until recently it was accepted that both responses were signaled by cAMP elevation (Daniolos et al., 1990). We have, however, demonstrated that the initial biochemical steps evoked by light and α-MSH are distinct (Isoldi et al., 2005) because the increase

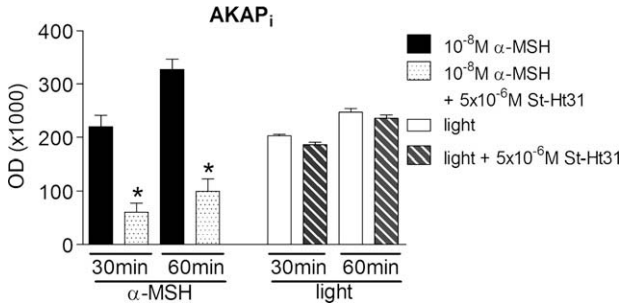

Fig. 4. Light or 10^{-8} M α-MSH-evoked pigment granule dispersion in *Xenopus* melanophores in the absence or presence of 5×10^{-6} M St-Ht31, the disrupting agent of AKAP–PKA complex. Each bar is the mean ($n = 6$), ±SE, absorbance (1000×) of light- or MSH-stimulated cells. *Significantly different ($p < 0.001$) from MSH-evoked response in the absence of St-Ht31.

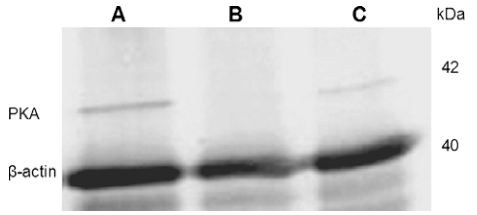

Fig. 5. Western blot of PKA catalytic subunit from *Xenopus* melanophores, previously co-immunoprecipitated with gravin. (A) Dark control, (B) 5 min 10^{-7} M α-MSH, and (C) 5 min of light exposure.

in cAMP observed in response to the hormone was not seen after light exposure. Nevertheless, time-course determinations of cAMP in response to α-MSH were significantly lower, in a time-dependent manner, in cells also exposed to light as compared to dark-adapted melanophores. AC modulation by light could be due to a light-induced increase of cytoplasmic Ca^{2+} (Isoldi et al., 2005) inhibiting a Ca^{2+} sensitive AC. Nine isotypes of AC are presently known, and this classification is mainly based on the cyclase regulation by Ca^{2+} or by PKC. Ca^{2+} stimulates isoforms AC1 and AC8 (Xia and Storm, 1997), whereas AC2, AC4, and AC7 are activated by PKC (Jacobowitz et al., 1993). Among those inhibited by low Ca^{2+} concentrations are AC5 and AC6 (Yoshimura and Cooper, 1992; Premont et al., 1992). On the contrary, AC3 and AC9 are enzymes negatively regulated by high concentrations of Ca^{2+}. AC3 activity can be blocked up to 50% by the elevation of cytoplasmic Ca^{2+}. This inhibition results from AC3 phosphorylation by CaMKII (Wei et al., 1996). On the other hand, AC9 is negatively regulated by calcineurin, which in turn is activated by Ca^{2+}/calmodulin (Antoni et al., 1998). AC9 is the most abundant AC isoform in the brain (Premont et al., 1996).

The cloned *Xenopus* melanophore AC exhibits high homology to the mammalian type IX, which is inhibited by Ca^{2+}/calmodulin-activated phosphatase. This is consistent with the hypothesis that the cyclase could be modulated by Ca^{2+} increase promoted by light. Calcineurin is a Ser–Thr phosphatase PP2B activated by Ca^{2+}/calmodulin, and extremely conserved evolutionarily from fungi to humans (Hemenway and Heitman, 1999). Furthermore, inhibition of the phosphatase PP2B (calcineurin) decreases light-induced inward currents in *Limulus* eye, enhancing arrestin phosphorylation (Kass et al., 1998). These facts led us to determine calcineurin activity in *Xenopus* melanophores.

The kinetic assay of the phosphatase showed a dramatic increase of activity in cells exposed to light. This finding is consistent with our demonstration that Ca^{2+}/calmodulin inhibitors do not block pigment dispersion, but actually induce such a response in the melanophore model system (Cozzi and Rollag, 1992). The increase of calcineurin activity demonstrated that light would, among other effects, activate the phosphatase, which in turn would inhibit AC9, thus decreasing the response to α-MSH. AC9 is also downregulated by PKC activation (Cumbay and Watts, 2004). As PKC is activated by light in *Xenopus* melanophores (Isoldi et al., 2005), light-induced inhibition of AC9 may also be promoted by PKC increased activity.

cAMP elevation usually leads to PKA activation. Protein kinases normally function in specific sites within the cells, and displacement to another location results in phosphorylation of a different substrate, inducing a different cell response (Beene and Scott, 2007). The importance of PKA adaptors, also known as AKAPs, has been recognized in recent years, despite being known to exist for decades. These molecules were always found associated with PKA regulatory domains RII, but believed to be contaminants during extraction (Dodge and Scott, 2000). Cloning and sequencing of these adaptors by different groups of researchers, led to the development of an inhibitory peptide, St-Ht31, and motivated us to investigate the participation of adaptor proteins in the response promoted by α-MSH. This peptide is the esterified form of Ht31 derived from the human thyroid AKAP, and prevents AKAP binding to PKA regulatory subunit RII. St-Ht31 blocked the melanosome dispersion response to α-MSH, but did not impair the photo-response in *Xenopus* melanophores.

After cloning and sequencing, the comparison of the melanophore AKAP fragment with GenBank sequences showed that the anchoring protein was a gravin-like adaptor previously sequenced from *X. laevis* non-pigmentary tissues. This protein is highly expressed in embryonic tissues of *X. laevis*, in which it was originally detected and exhibited a distinct expression profile (Klingbeil et al., 2001). Using ScanProsite (Gattiker et al., 2002) and Scansite (Obenauer et al., 2003) algorithms, which search kinase binding sites, we confirmed the theoretical possibility of AKAP phosphorylation by PKC, since the molecule possesses several putative phosphorylation sites for the various PKC isoforms.

Coprecipitation of gravin and PKA demonstrated that AKAP protein is expressed in *X. laevis* melanophore, that PKA is associated to AKAP and that the catalytic subunits of PKA are released in the presence of α-MSH.

Gravin is a myristoylated protein of high molecular weight expressed in many tissues, and is orthologous to the human protein SSeCKS (Src-suppressed C kinase substrate). It was initially identified as an auto-antigen in myasthenia gravis patients (Gordon et al., 1992), and is highly expressed in the human brain (Grove et al., 1994). Gravin is an anchoring molecule for PKA and PKC, and it has been shown that gravin-bound kinases are inactive (Piontek and Brandt, 2003). For PKA, it is known that gravin, as well as the other AKAPs, binds the inactive holoenzyme (Colledge and Scott, 1999). For PKC, its binding to gravin inhibits the activity of PKC βII in human erythroleukemia (Nauert et al., 1997). Therefore, besides restricting the kinase intracellular position, the interaction with gravin may also render the kinase inactive (Piontek and Brandt, 2003). Gravin is also able to associate with actin, through the myristoylated N-terminal site of the adaptor, thus allowing gravin to be the link with cytoskeleton components. Among others, gravin may function in cell adhesion, mitosis and oncogenesis (Gelman, 2002).

We have elucidated the biochemical routes activated by light to trigger melanosome dispersion in *Xenopus* melanophores (Isoldi et al., 2005). We suggested that melanopsin activates phospholipase C through the activation of a Gq protein, producing inositol trisphosphate, elevating cytoplasmic Ca^{2+} and activating PKC.

We propose that light diminishes the α-MSH-induced increase of cAMP by increasing calcineurin (PP2B) activity. Active calcineurin in turn inhibits AC9, and/or by activating PKC, which phosphor-

ylates the anchoring protein AKAP12, thus destabilizing its binding to the cell membrane. This mechanism is similar to that described in human neurons NT2-N (Piontek and Brandt, 2003). Although *Xenopus* melanophore gravin (AKAP12) possesses PKC phosphorylation sites, this hypothesis still needs experimental confirmation.

Melanopsin (Opn4) is also expressed in intrinsically photosensitive retinal ganglion cells (ipRGC, Provencio et al., 2002); gravin, calcineurin, and adenylyl cyclase IX are highly expressed in neurons. In dissociated ipRGCs, the photo-response persists in excised, inside-out membrane patches, demonstrating that the signaling components are associated with the membrane (Graham et al., 2008), suggesting the participation of scaffold proteins.

Our results demonstrate that light and α-MSH induce melanosome dispersion by signaling through different initial routes. To our knowledge this is the first report of AKAP12 (also known as AKAP250 or gravin) participation in α-MSH-evoked response, and in the modulation of the α-MSH signaling pathway by light in *X. laevis* melanophores. While the functional significance of this signaling crosstalk remains to be determined, it is likely to play a critical role in camouflage. In the event of becoming exposed, the skin darkening response of the amphibian serves to camouflage the animal against the typically dark background. This response must be tightly regulated because underdarkening or overdarkening will cause the animal to become dangerously conspicuous. The melanophores of larval amphibians darken in response to light and α-MSH (Isoldi et al., 2005). These multiple pathways must "talk" to each other to produce the appropriate level of darkening. Future studies aim to establish whether the crosstalk described in this report is absent in the light-insensitive skin of postmetamorphic adults.

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