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Visions & Reflections

Different strategies for color change

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All organisms exist in different colors and patterns depending on the unique distribution of various pigments, especially melanins, throughout the body surface. Pigmentation is regulated by genetic, environmental, and endocrine factors that control the amount, type, and distribution of melanin and other pigments in skin, hair, feathers, and eyes. In addition to its roles in photoprotection, camouflage and thermoregulation, melanin is an antioxidant and part of the innate immune system [1, 2]. The pigment is stored in melanosomes; tissue-specific organelles specialized in biosynthesis and storage of melanin and present in cells known as melanophores or melanocytes [3].

In many species of fish, amphibians, reptiles, and some invertebrates, melanosomes are highly motile, leading to visible changes in color of importance for both camouflage and communication (Fig. 1). The bidirectional translocations of melanosomes are fast and synchronized, especially in melanophores from fish, and regulated by hormones or direct innervation [4, 5]. Molecular motors carry melanosomes along the cytoskeleton to disperse them throughout the cell or to aggregate them in the cell center. During evolution the ability to rapidly aggregate and disperse melanosomes has changed, and mammals and birds are generally not using bidirectional translocations of pigment as a way of changing color (Fig. 1a). In an animal with fur or feathers movements of pigment within pigment cells would not show behind the insulation. Still, the melanosomes can be transported towards the tip of melanocytic dendrites where they are transported on actin filaments and donated to surrounding keratinocytes in the skin or to fur, hair, and feathers [6].

What is then the reason for slower melanosome aggregation and dispersion in amphibians than in fish, and what components are mammalian melanocytes lacking that make them incapable of regulated aggregation of these organelles? From a cell physiological perspective, the main molecular components are all there: cytoskeleton, molecular motors, hormones, receptors, and second messengers. Studies on pigmentation and pigment cells are generally performed on fish, frogs, mice, and humans, and these are the species that will be discussed in this review.

When it comes to speed and behavior of melanosomes there are differences between animal groups. Fast, long-range and bidirectional melanosome translocations are microtubule-dependent in both fish and frog melanophores [7, 8], but aggregation and dispersion are better synchronized in fish [9], and fish melanosomes move at a faster speed (see Table 1 for comparisons). Mammalian melanosomes move bi-

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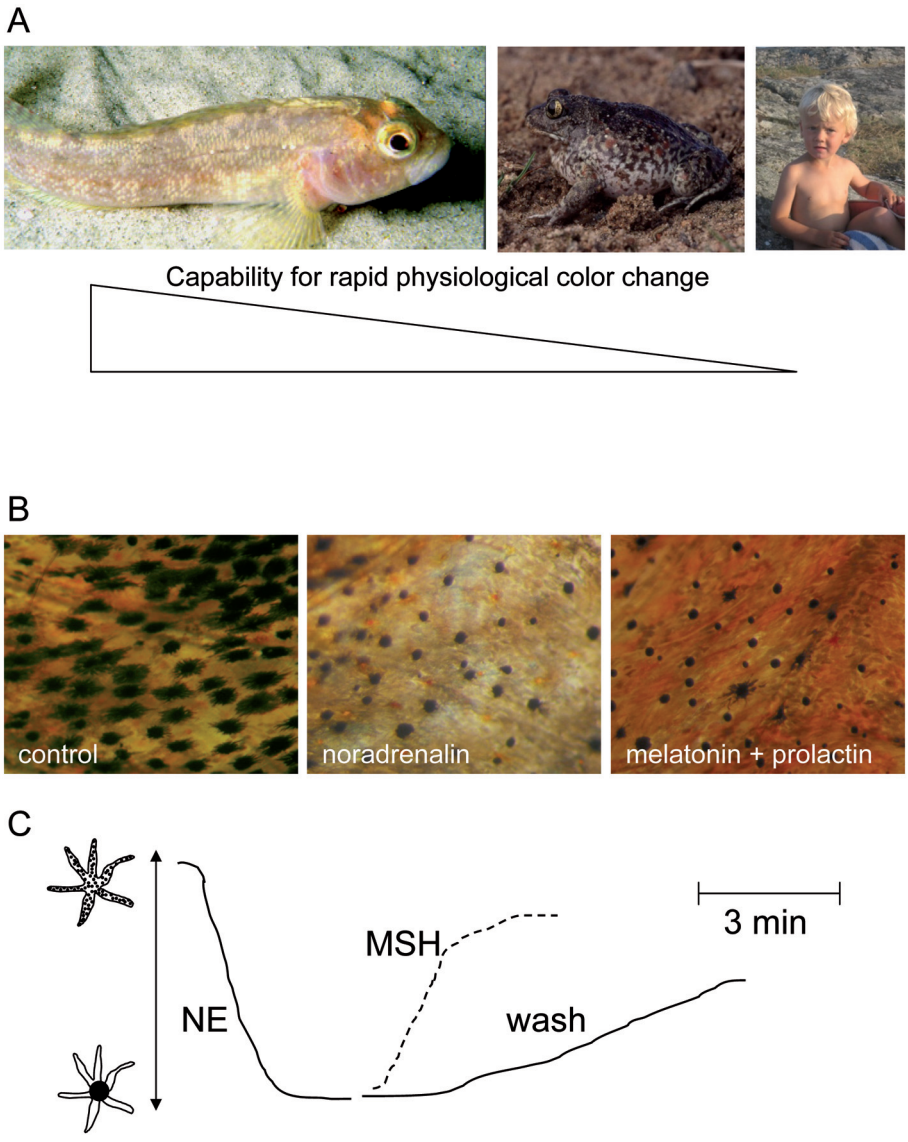

Figure 1. (*A*): The capability to change color fast (i. e. physiological color change) is well developed in fish, slower in amphibians, and regarded as absent in mammals. Fish *Grahamina capito* on light sand. Frog *Pelobates fuscus* on gravel (Image kindly provided by Dr Claes Andrén). Human on rock. (*B*): Skin biopsies from the two spotted goby normally display dispersed melanophores (control). In presence of noradrenalin, most chromatophore types aggregate their pigments. In the combination of prolactin and melatonin, the melanophores display aggregated pigments, whereas the red and yellow pigment cells display dispersed pigments. Data adapted from [36]. *(C):* Hormonal treatments of isolated fish melanophores results in a color change reaction within 3–10 minutes, as exemplified by data from the ice goby. Adapted from [37].

directionally as well, but the translocations are random and not synchronized [10].

The microtubule based minus-end directed motor cytoplasmic dynein is generally believed to be the driving force behind melanosome aggregation, and has been localized to melanosomes from fish [11], amphibians [12], and mammals [13]; although in mammals it is primarily present on immature melanosomes in early stages of the melanogenesis [14]. While dynein is present on the different melanosomes, it remains to be established whether the regulatory light and intermediate subunits of the motor are the same. Several studies have shown that dynein activity is regulated in fish and frog melanophores [9, 15–17], but it is not known whether dynein can be or is regulated in mammalian melanocytes. Indirect evidence shows that Rab GTPases may control recruitment of dynein to melanosomes in mammals [18].

Dispersion is more complex, since it involves both microtubules and actin filaments as well as their respective plus-end directed motors. Interestingly, data suggest that different organisms may use different motors for dispersion. Kinesins are typically involved in anterograde transport along microtubules, and in amphibian melanophores the kinesin involved in melanosome dispersion has been shown to be kinesin-II [12]. It is the only kinesin present in melanosome fractions, and inhibition of kinesin-II, but not conventional kinesin, inhibits dispersion in these cells. In contrast, conventional kinesin co-localizes with melanosomes in fish [19] and mammals [20]. The kinesin involved in dispersion is regulated in fish [9] but constantly active in amphibians [17], and it is not known whether it is regulated in mammals. Comprehensive biochemical and functional analysis are needed to establish which kinesins are being used

Table 1. Mechanisms behind color change in fish, frogs and mammals

	Fish	Frogs	Mammals
Darkening by			
Physiological color change			
by neurons	yes?	no	no
by hormones	yes	yes	no?
Ligthening by			
Physiological color change			
by neurons	yes	no	no
by hormones	yes	yes	no
Morphological color change			
by hormones (MSH etc.)	yes	yes	yes
by melanophore/			
melanocyte number	yes	yes	yes
by transfer	no	yes	yes
Intracellular components			
eumelanin	yes	yes	yes
pheomelanin	no	no	yes
melanosome size	300 nm	500 nm	500 nm
darkening by elevating cAMP/PKA	yes	yes	yes
lightening by lowering cAMP/PKA	yes	yes	unknown
melanosomal dynein	regulated	regulated	present, regulation unknown
melanosomal kinesin*	kinesin(s), regulated	kinesin-II, non-regulated	kinesin(s), regulation unknown
melanosomal myosin-V	regulated	regulated	regulated**
melanosome speed	0.5–1.5 μm/s	0.5 μm/s	0.7–1.0 μm/s***
melanosome behaviour	highly coordinated	less coordinated	non-coordinated

*"kinesin" represents kinesin-II and the unknown kinesin(s) involved in melanosome transport.
** via Rab27, which is present in the peripheral parts of the cell.
***1.8 μm/s in absence of myosin V.

for melanosome transport in the different animal groups, and whether kinesin diversity is a reason for differences in melanosome transport.

The importance of actin filaments and its myosin-V motor in melanosome dispersion differs significantly between animal groups. In mammalian melanocytes the main reason for relocating pigment to the dendritic tips is to put them in proper position for transfer to surrounding skin cells. To prevent pigment from returning to the cell center through microtubule-based transport, melanosomes are captured in the actin-rich domains in the cell periphery [10], resulting in a loss of repeated aggregation and dispersion. The melanosomes move from microtubules to actin filaments via myosin-V and its adaptor proteins Rab27a and melanophilin [21]. In amphibian melanophores, actin filaments are predominately required for proper dispersion and for keeping melanophores dispersed [8]. Substantially less myosin-V is bound to amphibian melanosomes during aggregation than dispersion [15], and this probably prevents the melanosomes from being irreversibly captured in the periphery. Myosin-V is present on fish melanosomes as well [9], but actin-based transport plays a less important role for the overall bidirectional pigment translocations in fish. Both aggregation and dispersion are faster when actin filaments are not present, and large amounts of actin are likely to interfere with melanosome movement [22]. Amphibian melanophores and mammalian melanocytes have larger melanosomes than fish, which results in a larger viscous drag in these cells. In mammalian melanocytes alternative splicing regulates binding of myosin-V to its cargoes [23, 24], but no such splicing has been reported in fish and amphibians. However, in these animal groups melanosomal myosin-V seems to be regulated via protein kinase A (PKA). PKA is linked to melanosomes via Rab32 in amphibians [25] and via melanophilin in fish [26]. The positioning of PKA on melanosomes ensures spatial specificity and implicates a role in motor regulation. Melanophilin contains two putative sites for phosphorylation by PKA, sites that are conserved in fish

and amphibians but not in mammals, providing an intriguing concept for fast regulation of pigment transport in fish and amphibians [26]. It is possible that the lack of PKA phosphorylation of melanophilin in mammals prevents myosin-V from detaching. Furthermore, the sequential binding of different effectors to properly attach myosin-V to mammalian melanosomes may add to the capture of pigment in the cell periphery [27].

The extracellular stimuli that trigger bidirectional pigment translocations can be neuronal (fish) or hormonal (fish and amphibians). Noradrenaline stimulates very fast pigment aggregation in fish melanophores, and the circadian hormone melatonin has been shown to either induce aggregation *per se* [28], or to potentiate noradrenaline-induced aggregation in these cells [29]. Binding of noradrenaline to α2-adrenoreceptors induces inhibition of adenylate cyclase followed by a decrease in cAMP and inactivation of PKA. It has also been shown that actin-dependent movements are down-regulated by noradrenaline and melatonin in fish, possibly via Ca^{2+}, which may explain why pigment translocations are faster in fish than in amphibians [9]. Amphibian melanophores lack neuronal regulation and depend on hormonal control only. Melatonin exerts its aggregating effect via binding to a mel1c-receptor, followed by inhibition of adenylate cyclase, a decrease in cAMP, and inactivation of PKA. Aggregation is, however, much slower in amphibians than in fish, possibly because amphibian melanosomes need to be released from the actomyosin system before aggregation can be initialized. In spite of the presence of melatonin receptors on mammalian melanocytes, the hormone does not induce aggregation of melanosomes in these cells. This may be due to limited amounts of the dynein motor, less efficient reduction of cAMP/PKA or that the pigment is captured by the actomyosin system due to lack of phosphorylation sites on melanophilin.

Pigment dispersion on the other hand can be initiated by melanocyte-stimulating hormone (α-MSH) in fish amphibians and mammals or, in the case of fish melanophores, by removing the aggregation stimuli. Intracellular cAMP levels are elevated in both cases. Comparative analysis indicated that cAMP elevation is primarily mediated by adenylate cyclase in fish, whereas amphibians depend more on phosphodiesterase activity [30]. This indicates that there may be differences in cAMP/PKA kinetics between animal groups. In mammalian melanocytes UV-radiation induces skin darkening. The first response can be rapid, due to oxidation of melanin or possibly mediated by melanosome dispersal in keratinocytes [31]. Long-term tanning is a result of increased local release of α-MSH from keratinocytes [32], followed by binding of α-MSH to receptors on melanocytes. α-MSH activates production of proteins involved in melanin-synthesis and cell proliferation as well as transfer of melanosomes into the extracellular space and surrounding keratinocytes [33]. Treating rat skin with cAMP agonists also results in skin darkening over time [34], but it is not known whether α-MSH and/or cAMP/PKA drive the kinesin-based long-distance transport of mammalian melanosomes from the cell center to the periphery.

Taken together, the present comparison between pigment transport in fish, amphibians, and mammals show that there is not one but several possible reasons why different animals use different strategies for color change. Comparative biochemistry, drug experiments, and making chimeras where frog or mouse melanosomes are introduced into fish melanophores and vice versa, may be potential experimental strategies to better reveal putative differences at the molecular and second messenger levels of melanosome transport. This will be needed to finally understand why aggregation and dispersion of melanosomes are slower in amphibians than in fish, and why mammalian melanocytes are incapable of fast synchronized aggregation of melanosomes. For a more comprehensive review on molecular differences in color change, see [35].

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