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Cephalopod Camouflage by Transparency

Mesopelagic Cephalopods Switch between Transparency and Pigmentation to Optimize Camouflage in the Deep

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Summary

Animals in the lower mesopelagic zone (600–1,000 m depth) of the oceans have converged on two major strategies for camouflage: transparency and red or black pigmentation [1]. Transparency conveys excellent camouflage under ambient light conditions, greatly reducing the conspicuousness of the animal's silhouette [1, 2]. Transparent tissues are seldom perfectly so, resulting in unavoidable internal light scattering [2]. Under directed light, such as that emitted from photophores thought to function as searchlights [3–8], the scattered light returning to a viewer will be brighter than the background, rendering the animal conspicuous [2, 4]. At depths where bioluminescence becomes the dominant source of light, most animals are pigmented red or black, thereby reflecting little light at wavelengths generally associated with photophore emissions and visual sensitivities [3, 9–14]. However, pigmented animals are susceptible to being detected via their silhouettes [5, 9–11]. Here we show evidence for rapid switching between transparency and pigmentation under changing optical conditions in two mesopelagic cephalopods, *Japetella heathi* and *Onychoteuthis banksii*. Reflectance measurements of *Japetella* show that transparent tissue reflects twice as much light as pigmented tissue under direct light. This is consistent with a dynamic strategy to optimize camouflage under ambient and searchlight conditions.

Results and Discussion

The animals of the mesopelagic realm hold a certain fascination for many, and perhaps none more so than for the visual ecologist. In this vast, three-dimensional wilderness, animals have evolved an impressive and diverse range of solutions to the problems associated with life where sunlight is low or nonexistent, food is scarce, and mates are hard to find. As well as great diversity, we also see shared solutions in the face of shared problems. Of these, convergences in camouflage strategies are maybe the most striking [1]. In the upper mesopelagic, virtually every phylum present has a transparent representative, whereas in deeper waters, this trend shifts to a majority of the taxa represented being pigmented deep red or black [2]. Mesopelagic animals that are confined to a camouflage strategy of either transparency or pigmentation risk being sighted by predators under either biological searchlights or downwelling light, respectively (summarized in Figure 1). Furthermore, the boundary between environments where one or the other strategy would be most useful is neither sharp nor fixed, changing with factors such as time of day, cloud cover, and turbidity [1, 9, 14]. Being able to switch between

strategies in response to specific threats or changing optical conditions would be highly advantageous to an animal seeking to survive in this unique environment (Figure 1C).

Japetella heathi (Berry, 1911) is a small mesopelagic bolitaenid octopus, obtaining a maximum mantle length (ML) of ~80 mm [15]. This genus is reported as having an ontogenetic change in vertical distribution, with mature individuals occurring in deep waters (800 m+) and juveniles more abundant in shallower waters (400–700 m) during daylight [16]. Having observed that *J. heathi* are able to vary their appearance between transparency and pigmentation via the expansion and contraction of the red chromatophores on the mantle and arms (Figure 2A), we were interested to learn whether the onset of pigmentation was triggered by the addition of a directed beam of light when maintained under ambient conditions, as predicted for maintaining optimal crypsis under ambient downwelling light and directed bioluminescence, respectively [9]. Undisturbed juvenile *J. heathi* in the transparent mode were tested under low ambient room light with the addition of directed blue light (450 nm peak emission 20 nm full-width at half maximum intensity (FWHM); Figure 3C). This led to a rapid and reversible expansion of the chromatophores (Figure 2B; see Movie S1 available online), escalating with each bout of light exposure and eventually eliciting an evasive response (retraction of the head into the mantle). Continued exposure beyond maximum chromatophore expansion led to a subsequent reversion to transparency, and continued exposure elicited no further chromatophore response (Figure 2B). Animals tested with red light (600 nm cut-on wavelength; Figure 3C) did not respond with significant body pattern changes or evasive behavior.

Benthic shallow-water cephalopods such as cuttlefish (*Sepia* spp.) show strong chromatophore responses to visual threats such as unfamiliar moving objects and overhead shadows (e.g., [17, 18]). To ensure that the response reported above was not a generalized visual stress response, we tested the response of transparent *J. heathi* under ambient room light with three stimuli: overhead shadows, passing objects, and tactile contact. Only the tactile stimulus had a significant effect on the expression of chromatophores, with an acute rapid expansion coupled with evasive behavior (retraction of the head into the mantle) (Figure 2C). We were, however, confident that the animals were able to see the visual stimuli because they could be observed tracking them with eye movements (Figure S1). These results suggest that the use of chromatophores in *J. heathi* is not a generalized response to visual threat; the deployment of chromatophores may reduce the visibility of the octopus to specific predators at risk of drawing attention from other predatory strategists (Figure 1). This risk would be limited by the transient pigmentation responses seen here. These observations are reminiscent of the predator-specific defense responses observed in other cephalopods [18].

We went on to test the chromatophore responses of another cephalopod species to directed light. *Onychoteuthis banksii* (Leach, 1817) is a medium-sized oegopsid squid, with adults reaching a mantle length of up to 140 mm [19]. *O. banksii* are most commonly sampled at epipelagic (between 150 m and

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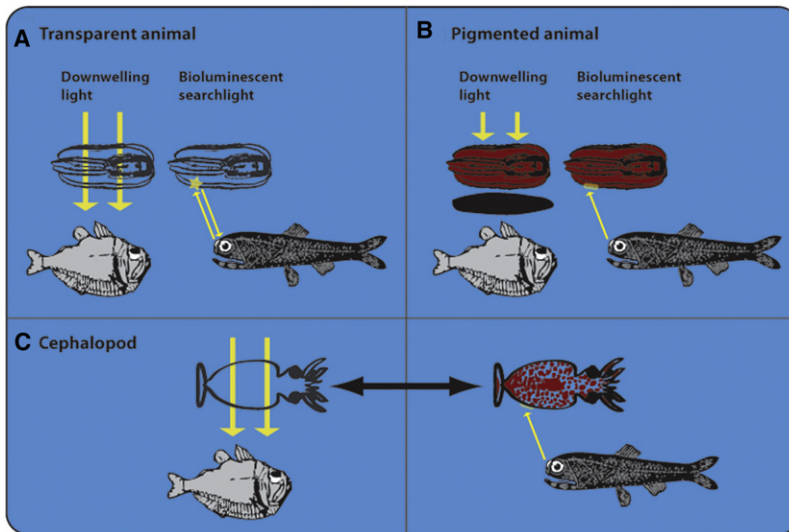


Figure 1. Cartoon Summary of the Strengths and Weaknesses of Transparency and Pigmentation as Camouflage Strategies in the Mesopelagic

In (A) and (B), hypothetical transparent and pigmented animals are viewed against the downwelling ambient light by *Argyropelecus*, a deep-sea hatchet fish with its characteristic upward-orientated eyes suited to the task, and under the biological searchlights of the myctophid *Diaphus*, known as the headlight fish.

(A) The prevalence of transparency in the upper mesopelagic can be explained as a close-to-perfect solution to the dilemma faced by pelagic organisms of having nowhere to hide, being effective from all viewing angles (body parts that cannot be made transparent, such as guts and eyes, are often mirrored or hidden via counter-illuminating photophores) [2]. Imperfections in tissue transparency result in unavoidable internal light scattering which, under diffuse light, has a minor effect on the animal's visibility. However, under directed light, such as that emitted from the photophores thought to function as biological "searchlights" possessed by predators (or indeed by any other close-by source of bioluminescence) [3–7], the scattered light returning to a viewer will be much brighter than the background, leaving the animal at risk of predation [2, 4].

(B) At greater depths, the ubiquity of transparency shifts to one of red or black pigmentation [9, 10], suggesting that another form of visual detection is at play. Minimum reflectance for measured pigmented deep-pelagic animals occurs at ~480–490 nm [12], coinciding with the wavelengths of most known bioluminescent emission peaks and visual pigment absorption maxima of a majority of deep-sea fish and invertebrate species measured [3, 11, 13, 14]. This will enable them to avoid detection under biological searchlights and other nearby sources of bioluminescence, but they will be susceptible to detection via their silhouettes under downwelling light, which is often orders of magnitude brighter than the upwelling light.

(C) This paper reports on findings from two species of cephalopod that suggest they switch between transparency and pigmentation to be optimally camouflaged under both ambient downwelling light and under directed bioluminescence. This switch can occur rapidly in both directions via the expansion and contraction of intradermal chromatophores, allowing predator-specific camouflage to be maintained.

the surface), but individuals have been sampled from over 800 m and as deep as 4,000 m [16]. The complex light organs possessed by these animals suggest that juveniles at least have a deeper water distribution [20]. We obtained three small (30 mm ML) juvenile *O. banksii* from a nighttime trawl to 200 m and noted that, like *J. heathi*, these squid were transparent when undisturbed under ambient room light. When individually tested with blue directed light (as described above; Figure 2C), *O. banksii* responded with rapid expansion of red chromatophores on the dorsal mantle surface (Figures 2A and 2B; Movie S2). Unlike *J. heathi*, the response did not appear to diminish over time, although the squid took some evasive action in attempting to jet away from the light beam. We also tested the reactions of the animals to red light (as described above; Figure 2C) and observed no significant chromatophore or behavioral response. As a control, we tested the reaction of the animals to the same movements involved in the presentation of the light-source but without switching the light on or off. No chromatophore or behavioral responses were observed.

To compare the reflectances of *Japetella heathi* in the transparent and pigmented modes in a behavioral context, we took measurements of reflectance from four live *J. heathi* using a spectrometer and reflectance probe positioned at an angle of 90° to simulate what an eye combined with a sub-ocular photophore would detect (Figure 3A; Experimental Procedures). We found that, under identical conditions, animals consistently reflected twice as much light when in the transparent mode compared with the pigmented mode (Figure 3B) over the wavelengths most relevant to most deep-sea visual systems (i.e., in the 450–500 nm range). Pigmented animals reflected more light of longer wavelengths (550–700 nm) when in the pigmented mode but here still at

a lower percentage than when in the transparent mode. Measurements of *J. heathi* in the pigmented mode are comparable to those obtained from a range of red and black mesopelagic fishes and invertebrates in a previous study [12], where reflectance measurements were taken at a 45° angle and so probably underestimating reflectance relative to searchlights. In keeping with our results, these measurements were generally <20% across all wavelengths and less than 5%–10% in the blue-green region relevant for deep-sea vision. Here we took reflectance measurements from a single mantle area forward of the gut to enable consistency between measurements taken in the two modes; we might expect to find a greater difference in reflectance over other body areas such as the gills and gut.

Remarkably little is known about patterns of chromatophore use in deep-water cephalopods (although see [21]) compared with their shallow-water relatives (e.g., [22–24]). Experimental evidence for the function of physical traits of mesopelagic animals is largely based on physiological studies, whereas behavioral data are rare [13]. The difficulty in obtaining, maintaining, and observing mesopelagic animals makes such studies challenging. Here, for the first time to our knowledge, we have carried out laboratory behavioral studies into chromatophore use in mesopelagic cephalopods and have attempted to do so in an ecologically relevant way. We conducted our shipboard behavioral experiments under ambient room light under the assumption that both *J. heathi* and *O. banksii* have a wide depth distribution and are therefore able to visually adapt to a wide range of light levels. Indeed, that *J. heathi* could be observed tracking moving objects and that both species reacted to changes in light levels suggests that they were adequately able to deal with the bright (relative to the mesopelagic) ambient light environment used.

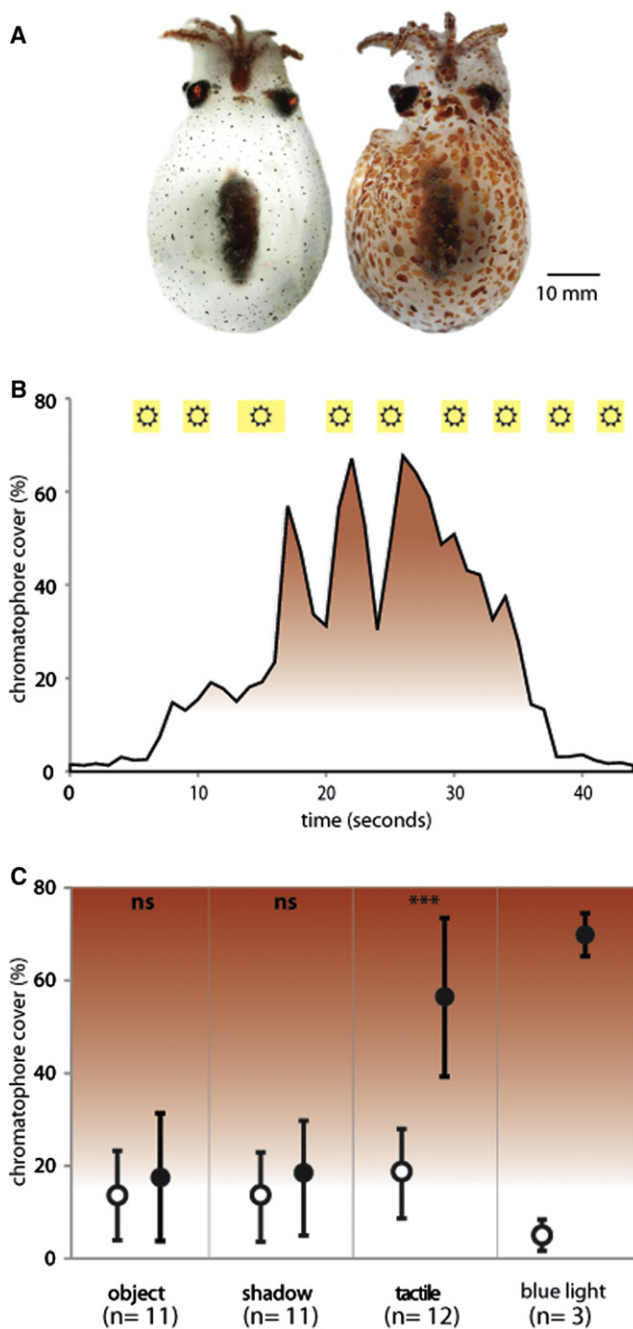


Figure 2. Results of Behavioral Experiments with *Japetella heathi*

(A) The same individual *J. heathi* octopus in transparent mode (left) and pigmented mode (right). Expansion of chromatophores was elicited by a tactile stimulus (a blunt needle pressed against arms). Photographs were taken within 5 s of each other.

(B) Responses of a single *J. heathi* to directed blue light. Yellow boxes and icon indicate onset and cessation of individual lighting “bouts,” consisting of a flashing blue light at one flash per second. Most bouts lasted for 3 s and therefore subjected the animal to three flashes. Chromatophores can be seen to expand seconds after initial exposure. After continued exposure, the animal ceased reacting to the light with chromatophore responses and instead displayed evasive behavior such as swimming away from the light source and retraction of the head into the mantle. See [Movie S1](#) for footage.

(C) Responses of *J. heathi* to four different stimuli. Objects passed in front of the animals and shadows passed overhead failed to evoke a significant increase in chromatophore expression, although the animals could be

seen to track the stimulus with eye movements. A tactile stimulus (touching the arms with a blunt needle) resulted in the rapid expansion of chromatophores and evasive behavior (retraction of the head into the mantle cavity). By comparison, directed blue light resulted in a rapid and strong expression of chromatophores (data obtained in a separate experiment). White circles = prestimulus, and black circles = poststimulus. Error bars show standard deviation from mean. P relates to paired t tests between pre- and poststimulus chromatophore cover. ns = not significant with $p > 0.05$; *** = significant with $p < 0.001$. No test for significance is included for blue light treatment because of the low n.

Experimental Procedures

Japetella heathi and *Onychoteuthis banksii* used in behavioral experiments were obtained from nighttime midwater trawls 100–500 m depth in the Peru-Chile Trench (between 8° S, 81° W and 28° S, 72° W) during a cruise on RV *Sonne* in September of 2010. Reflectance measurements were obtained from four *Japetella* caught in daytime midwater trawls during a cruise on RV *New Horizon* in the Sea of Cortez (26° N, 110° W) in July of 2011 at a depth of approximately 1,000 m.

Animals were kept in a darkened cold-room for several hours prior to commencement of experiments. For light response behavioral experiments, experimental tanks were held under low ambient room light (fluorescent bulbs). The light source used to test for responses was a single white light emitting diode (LED). Wavelengths were modified by the addition of Rosco Superlux gel filters (blue: 74, red: 26). The responses to light were recorded at 30 frames per second using macro digital video (Canon Ixus). Frames were extracted every second and converted to grayscale, and a threshold was applied to retain chromatophore information. This resulted in chromatophores being isolated black areas against a white background. The “count particles” function in ImageJ [25] was used to estimate the chromatophore coverage within the outline of the animal. Large pixel groups were excluded to remove the gut and eyes from the analysis. This ultimately led to an underestimation of total coverage, because chromatophores occurring over the gut area were not counted using this method. A further underestimation may have occurred because all images were thresholded to the same levels, determined under ambient light conditions.

For spectrometer measurements, individual animals were placed in a seawater-filled tank on a matte-black background. Because reflectance probes are susceptible to variation with angle and distance, we took care to ensure that these were minimized measurements. The tank used was of a small size and of limited water depth so as to restrict animal movement, and the probe was held in position with a rigid clamp. Reflectance measurements were calibrated against a standard (OO WS-1) placed in an identical tank under the same depth of seawater as the animal’s mantle surface. A reflectance probe was used with an Ocean Optics USB2000 spectrometer and PX-2 xenon light source. The reflectance probe contained seven 400 μm diameter optical fibers in a six-around-one arrangement. The six outer fibers were coupled to the light source and illuminated the specimen (Figure 4A). The central fiber collected the light reflected from the specimen and was coupled to the spectrometer. The end of the reflectance probe was always placed at a distance of 10 mm from the measured surface and held at an angle of 90° to the mantle surface of the animal using a rigid mount. Animals were left undisturbed until chromatophores were retracted and transparency obtained, at which point transparent mode measurements were taken, carefully moving the tank to enable measurements from five regions on the dorsal mantle surface forward of the gut. A blunt needle was then pressed on the arms to elicit a chromatophore response, while taking care to minimize overall disturbance to the animal, and then pigmented mode measurements were taken from the same regions.

seen to track the stimulus with eye movements. A tactile stimulus (touching the arms with a blunt needle) resulted in the rapid expansion of chromatophores and evasive behavior (retraction of the head into the mantle cavity). By comparison, directed blue light resulted in a rapid and strong expression of chromatophores (data obtained in a separate experiment). White circles = prestimulus, and black circles = poststimulus. Error bars show standard deviation from mean. P relates to paired t tests between pre- and poststimulus chromatophore cover. ns = not significant with $p > 0.05$; *** = significant with $p < 0.001$. No test for significance is included for blue light treatment because of the low n.

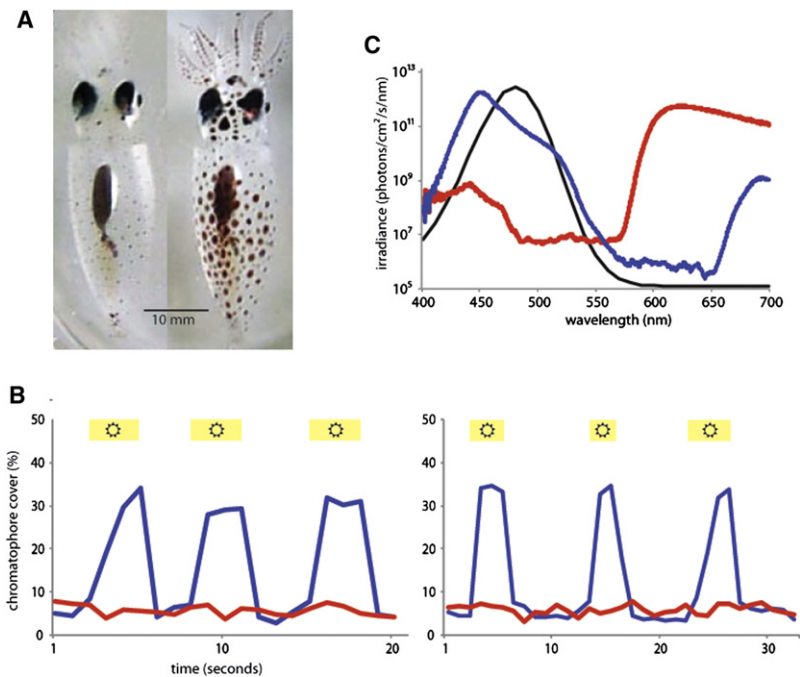


Figure 3. Results and Details of Behavioral Experiments with *Onychoteuthis banksii*

(A) Images of *O. banksii* taken from video footage showing (L) the chromatophores contracted and the animal transparent under ambient light conditions and (R) with the chromatophores expanded and the animal pigmented when subjected to directed light. See [Movie S2](#) for footage.

(B) Chromatophore responses of two individual *O. banksii* to three bouts of directed light. The blue line indicates response to blue light, and the red line indicates response to red light (see C). Yellow boxes with symbols show the onset and offset of each lighting bout. (C) Irradiance of lights used in experiments, blue line for blue filter with white LED (450 nm peak emission 20 nm full-width at half maximum intensity) and red line for red filter with white LED (600 nm cut-on). Black line shows a generalized sensitivity curve for a single visual pigment with a peak sensitivity at 480 nm typical of deep-sea vision [11] (y axis arbitrary).

Supplemental Information

Supplemental Information includes one figure and two movies and can be found with this article online at [doi:10.1016/j.cub.2011.10.014](https://doi.org/10.1016/j.cub.2011.10.014).

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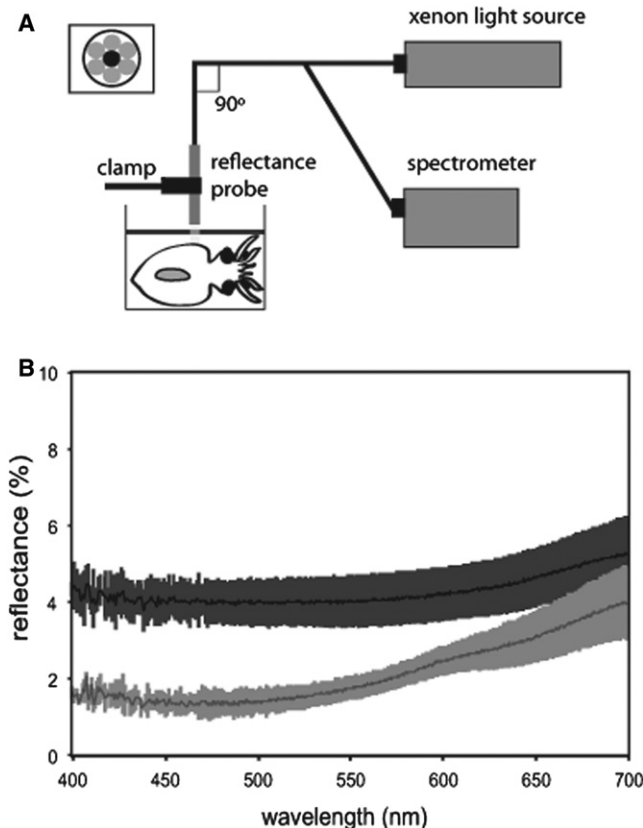


Figure 4. Reflectance Measurements and Data

(A) Setup for measuring reflectance from *Japetella* showing reflectance probe connected to light source and spectrometer. Inset shows arrangement

of six illuminant fibers surrounding a single measurement fiber. Measurements were taken underwater, from five positions on the mantle forward of the gut. The reflectance probe was positioned 90° relative to the skin and held at a constant angle and distance by a clamp. The animals were placed in a small tank in limited water depth to restrict movement.

(B) Measured reflectance from live *Japetella* skin. Black line shows reflectance from transparent form, with chromatophores contracted. Gray line shows reflectance from pigmented form, with chromatophores expanded. Means from five areas measured on four individuals (mantle length of ~50 mm). Dark and light shaded areas show standard deviation from means.

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strength of FilGAP to filamin A. Alternatively, the applied tension in the network could mechanically stretch filamin A (*in vitro* measurements of unfolding show that filamin A stretching occurs in a physiological force range [13]), exposing its cryptic integrin-binding sites and promoting the integrin–filamin A interaction (Figure 1C).

The findings of Ehrlicher *et al.* [3] provide insights into the complex issues of how matrix–cytoskeleton binding and actin dynamics are regulated by mechanical forces. They also support previous observations related to filamin A. For one, Ithychanda and Qin [14] recently demonstrated that filamin A has the potential to bind integrin at numerous cryptic sites along its length. The authors proposed, as have others, that filamin A mediates adhesion maturation by clustering integrins into larger adhesive structures. Recent work from our lab and others shows that cells depleted of filamins cannot generate stable levels of internal force (although peak forces are at control levels) and, as a result, adhesions do not mature [9], which is in agreement with the finding that internal strain increases integrin binding in actin networks crosslinked by filamin A [3]. Furthermore, because local application of forces causes inhibition of plasma-membrane-proximal Rac in a FilGAP-dependent manner [15], the finding that FilGAP binding is weakened by application of stress on the network also fits well with cell-based studies. Ehrlicher *et al.* [3] also suggest that regulation of FilGAP could be purely mechanical in nature, as FilGAP would be tightly bound to filamin A until force generation occurs, at which point the crosslinking angle of filamin A would increase, thereby weakening FilGAP binding and promoting its recruitment to the leading edge of the cell. An alternative explanation is that filamin A stretching results in conformational changes that weaken FilGAP binding without a crosslinking angle change. In any case, these results are important, for many cell activities require that the responses to mechanical strain be robust and include stabilization of matrix–cytoskeleton linkages and alterations of actin dynamics.

Filamin A is now added to the list of intracellular proteins that respond to strain by altering either binding

(talín), enzymatic (titin), or substrate (p130Cas) functions [16]. The biochemical complexity of focal adhesions, which can contain over 100 types of molecules [17] that are potentially mechanosensitive in their interactions [18], can be at times discouraging, and we often think of mechanotransduction as a tangled web of biochemical signaling. Ehrlicher *et al.* [3], however, have shown us that strain in the actin–filamin A network can simultaneously regulate both actin dynamics and adhesion of the actin cytoskeleton to the surrounding matrix. Further studies, however, are required to elucidate the extent of filamin A stretching during cell mechanotransduction and where filamin A activities fit into microenvironmental controls of cell stasis versus growth or differentiation.

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Marine Optics: Dark Disguise

Survival in the deep sea depends on seeing others without being seen yourself. A recent study examined two switchable camouflage strategies in cephalopods: transparency and dark pigmentation.

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A bucket of animals trawled up from the mid-waters of the deep ocean is an

extraordinary sight: a mix of silver and black fish, deep red shrimp and transparent jellies of many kinds. The key to why these creatures are so different from those from the shallows

is camouflage: the need to avoid being seen in an environment where there is nowhere to hide. With increasing depth in the ocean the light gets dimmer, by a factor of 10 for every 70 m in the clearest ocean water, with long wavelength light attenuated most strongly so that, eventually, the light left contains only blue wavelengths close to 480 nm. By a depth of 500 m in daylight the ambient light from above is like dim blue moonlight. By 700 m it is too dim for humans to see anything, and by 800 m fish vision fails too. However, the waters beyond this depth are not lightless, and the animals that live there do not, in general, lose their eyes. Most fish and crustaceans and many cephalopods make their own light, with luminescent structures of many kinds. In the waters below 500 m 70% of fish species and 65% of decapod crustaceans are bioluminescent [1]. It is in this dark and hostile environment that two forms of camouflage are particularly valuable: being transparent or being darkly pigmented. A study in this issue by Zylinski and Johnsen [2] shows that the camouflage strategy adopted by cephalopods depends on whether their potential predators hunt their prey using what is left of the daylight, or their own bioluminescence.

In the upper mesopelagic waters, from 200 m down to 600 m, vision using residual daylight is still possible, and camouflage needs to be precise and often elaborate. Predators often have 'tubular' eyes directed upwards, in order to spot potential prey against the down-welling light. Many animals disguise themselves against this kind of predation by being more or less transparent. Others, particularly fish, use a three-fold strategy, with dark pigment along the back, silvery sides, and photophores on the ventral surface. The dark pigment serves to prevent detection from above (Figure 1A, 1). The silvery sides render the fish invisible, because the light intensity reflected from a plane mirror has the same intensity as the light that would have passed through it [3]. This works because, away from the surface, the background brightness at any given angle to the vertical is the same for all azimuths (Figure 1B). The problem is that the sides of fish are not plane (although hatchet fish come close to this), but as Denton and Nicol [4] showed, the platelets that make up the reflectors are angled relative to the

surface profile, so that they stack up vertically relative to the sea surface (Figure 1A, 2). The one direction that cannot be disguised in this way is from directly below: the body of the fish inevitably produces an opaque silhouette, and the only way to disguise this is by producing a pattern of illumination that mimics the light that would have passed through the body, and this is done by using downward-directed photophores (Figure 1A, 3). To be valuable in camouflage, and not beacons that make the animal more visible, the emissions of these photophores need to match the down-welling light in brightness, spectrum and angular distribution, and to do this many have a highly sophisticated structure with partially silvered mirrors and coloured filters [1]. Some fish, decapod shrimps, euphausiids and squid are known to be able to regulate their light output over a thousand-fold range [5]. Many otherwise transparent animals have opaque organs, notably the eyes and gut, which would render them visible from below, and these structures are frequently silvered and equipped with photophores.

Luminescence is used for other purposes. Many smaller animals emit flashes to startle or distract predators. Some shrimp and squid emit luminous secretions which persist while the animal itself shoots away. In some fish and squid the pattern of photophores is different in males and females, suggesting (but not proving) a role in sexual communication. In other fish, luminous organs are used in predation. Angler fish use luminous lures to attract their prey. Other fish, notably the flashlight fishes (Anomalopidae), lantern fishes (Myctophidae) and dragonfishes (Stomiidae), have large luminous organs near the eye, which act as searchlights, illuminating potential prey in the water around them (Figure 1C, D). In the flashlight and lantern fishes, the light is blue (as is most mid-water bioluminescence), but the dragonfishes have both blue and red photophores, and, almost uniquely for deep-water fish, they have red-sensitive photoreceptors [6]. This gives them both a private channel of communication, and also a means of illuminating red prey, which by blue light would appear an invisible black.

At about 600 m, the light environment changes from one in which the main

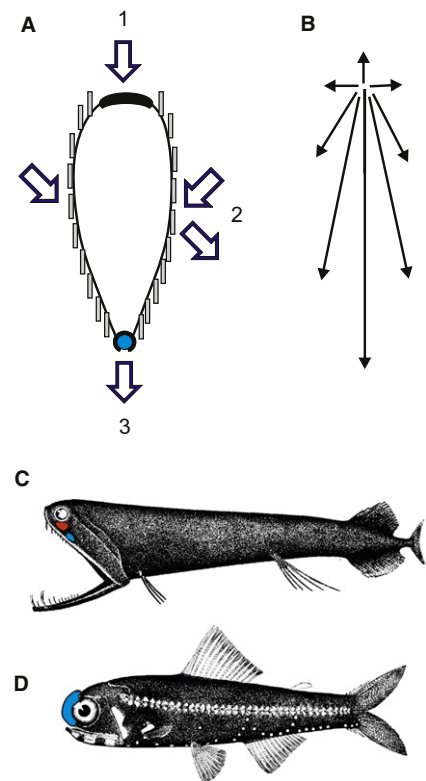


Figure 1. Camouflage and bioluminescent predation in the mesopelagic environment.

(A) Diagram of a section through an upper mesopelagic fish, showing three forms of camouflage. 1, Countershading from above. 2, Silvered sides with vertical reflecting platelets. 3, Downward-pointing counter-illumination from photophores. (B) Distribution of light around the vertical in the deep sea. For any direction relative to the vertical the intensity is the same for all azimuths. In reality the upwelling intensity is about 0.5% of the downwelling light. (C) The black dragonfish *Malacosteus niger*, with both red and blue sub-orbital photophores. (D) The lantern fish *Diaphus effulgens*, with paired frontal 'headlights'.

source of light is residual daylight to one where bioluminescence dominates. Silvering, valuable in the former conditions, is now a liability, since silvered animals become easily visible to fish with headlights. Only two strategies remain useful: being transparent and being black — or red if there are no dragonfish around. In their paper Zylinski and Johnsen [2] explore the relative merits of the two strategies. They show that in two mesopelagic cephalopods, a squid and an octopus, the default strategy is transparency, but when suddenly illuminated the animals rapidly extend their chromatophores and become a dull red-brown. There is a problem with the transparency strategy that does not

seem immediately obvious. Biological tissues, even if unpigmented, vary slightly in refractive index, which means that the interfaces between them, and the interface with the water outside, produce slight reflections. This reflected intensity is only a few percent at worst, and in diffuse residual daylight this merges with the background. But in a beam of light from a predator against a dark background, such reflections become visible. In these circumstances the overall reflectance can be halved by darkening the body. The ability to expand chromatophores on a time scale of about a second

from dot-like structures to comprehensive body covering is a strategy available to most cephalopods [7], and this is put to good use here to switch from one form of camouflage to a slightly better one.

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Centromere Clustering: Where Synapsis Begins

Centromeres congregate into a large cluster called the chromocenter during *Drosophila* oogenesis. Two recent studies now define a function and a genetic basis for this remarkable structure.

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The formation of haploid sperm and egg cells from diploid germ cells involves some extraordinary chromosome acrobatics. Most of these movements occur in the course of meiosis, a specialized cell division program, during which homologous chromosomes as well as sister chromatids are segregated from each other in successive events. In preparation for the meiotic divisions, cells undergo a series of transitions in nuclear organization, which serve to identify and pair homologous chromosomes. This is followed by the stabilization of chromosome pairing interactions, often in the context of a highly structured protein scaffold known as the synaptonemal complex (SC). In most organisms, including fungi, plants, mice and humans, homolog pairing initiates with telomeres clustering at the nuclear envelope. This chromosomal configuration is known as the bouquet because it lends a distinctive shape to the chromosome assembly [1]. Similarly, in worms, the tethering of special telomere-proximal chromosomal regions near the nuclear

envelope assists the pairing of homologues and SC formation (synapsis) [2]. By contrast, no bouquet stage is observed in *Drosophila*, which interestingly lack traditional telomeres. However, *Drosophila* oocytes have long been known to form another structure at this stage in meiosis called the chromocenter, which is composed of clustered centromeres [3,4]. Two studies from the Hawley and McKim laboratories [5,6], published in a recent issue of *Current Biology*, now reveal some intriguing functional parallels between the *Drosophila* chromocenter and the bouquet, and designate the chromocenter as the structure where synapsis first begins.

The two groups arrived at their shared conclusion that the chromocenter initiates synapsis from somewhat different starting points. Hawley and colleagues [5] investigated the genetic basis of centromere clustering, which they noted occurs very early during oogenesis, whereas McKim and colleagues [6] were establishing a time course of chromosome synapsis in *Drosophila* oocytes. Both groups analyzed C(3)G, a protein that forms part of the 'rungs' that connect homologous chromosomes in the context of the SC.

The key characterization made by both groups was that early in meiosis, C(3)G formed only a couple of foci on chromosomes, and these foci co-localized perfectly with centromere clusters (Figure 1A). Only at later stages did C(3)G also coat non-centromeric sites, suggesting that the centromeres act as the earliest sites of synapsis initiation in *Drosophila* oocytes. In some organisms, including worms and grasshoppers, synapsis only initiates at one or two sites along each chromosome, most commonly near the telomeres. This is not the case in *Drosophila*. As demonstrated in a careful analysis by McKim and colleagues, C(3)G forms clearly distinguishable patches along chromosomes rather than a single widening stretch, indicating that synapsis also initiates at interstitial chromosomal sites (Figures 1B–D). What defines these interstitial sites is unclear, but in budding yeast and mice, interstitial sites of synapsis are thought to be associated with sites of homolog identification.

Interestingly, many components of the SC are required for centromere clustering in the first place, suggesting that synapsis initiation and centromere clustering are tightly coupled. Analysis of mutations in SC components by Hawley and colleagues revealed that many exhibited strong defects in centromere clustering. Moreover, both groups showed that the meiotic chromosome cohesion protein ORD is essential for centromere clustering and the initial synapsis at centromeres. One interesting exception is the SC