Any advice for those starting careers in science? An academic career in science is amazing and rewarding, but also not for the faint hearted. Think carefully about why you want to pursue a career in science. I found my undergraduate degree fascinating and wanted to stay in biology, so a PhD seemed the natural next step. While my PhD was an amazing experience, during which I learnt a lot about research and myself, I regularly worried that I was doing it for the wrong reasons. In retrospect it was the right path for me, but isn't for everyone. It's hard to see students who find this out the hard way while working towards a PhD. Students starting a PhD now typically have a masters degree giving them a much better idea of what research involves 'warts and all', compared to students like me that went straight from undergraduate studies to a PhD.

Postdoctoral research is time to put into practice what you have learnt under the watchful eye of your graduate supervisor. I was fortunate to spend eight years (2002–2009) doing postdocs in two laboratories. I joined the first by applying for a position on a funded project. However, most of my postdoctoral research was the result of three grants written with Lars Chittka. For me these were more rewarding projects as they provided opportunities to identify the big questions I wanted to address and with whom I wanted to work. Whilst this is a higher risk career option, with no guarantees of funding, the potential rewards are significant.

How long should you be a postdoc? As contemporaries start securing faculty positions you start to worry you're getting left behind. However, the transition from postdoc to faculty member is associated with big challenges as you go from spending almost all your time on research to dividing time among multiple competing roles. Transitioning too soon can mean you haven't had time to establish your own research direction before you necessarily have to take a more strategic, supervisory view of research in your team. However, a lack of job security with serial short-term postdoc contracts was a major source of stress for me. Not knowing what I would be doing after each contract meant constant searching for positions and funding opportunities making it hard to make other important life decisions. In hindsight I feel a longer postdoc period was beneficial to my career as it allowed me more freedom to develop many of the research areas that I am still working on today.

What prompted moving to Canada? I've always been interested in exploring new places, perhaps one of the reasons I ended up in science exploring the boundaries of knowledge and understanding. Since working overseas in Mexico during my PhD, I have been keeping an eye open for other opportunities. My wife has family connections to North America and always thought she would live here at some stage. After our twins were born, we discussed the possibility of relocating for the right opportunity, and relatively soon afterwards the pollinator conservation chair at Guelph came up. From a professional perspective it was (and is) a fantastic, almost tailor-made, opportunity. For the family, the kids were still very young (pre-school age) and my wife was taking a break from her high-flying career in governmental science policy and was fully prepared to support me in making the inter-continental move. Overall it seemed too good an opportunity to pass up, although we do miss spending more time with family and friends in the UK. Compared to many couples with one or both people working in academia, my wife and I have been fortunate having only a few years of long-distance relationship. I know many couples that have endured years of international or even inter-continental commutes often resulting in either the relationship and/or academic career suffering or ending. As such, I feel tremendously fortunate the stars aligned producing the right opportunity and timing for both my career and family.

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Quick guide

Eyespots

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What are eyespots? Many animals possess circular markings that subjectively resemble vertebrate eyes. These often consist of concentric rings and are known as 'eyespots' (Figure 1). Eyespots are found in a number of taxa (insects, fish, amphibians and birds) and are, therefore, likely to have evolved a number of times independently. Animals can use eyespots to recognize individuals of their own species and to assess the quality of potential mates, but perhaps their most frequent function is to reduce the chance of being eaten by a predator. In some species, eyespots are positioned peripherally or on non-vital body parts, and predators direct their attacks towards the eyespots giving prey the opportunity to escape. In other species, eyespots intimidate or startle predators causing them to delay or forego their attacks. Whether predators are deterred because eyespots mimic the eyes of their own predators, or simply because the markings are conspicuous, is the subject of an ongoing debate.

Are predators deterred because eyespots are conspicuous? Predators have innate and learned aversions to conspicuously-coloured toxic prey, and may be deterred by eyespots simply because they are conspicuous. Several experiments on the survival of artificial palatable prey exposed to wild birds have shown that prey with large and more numerous eyespots survive better than those with smaller and less numerous eyespots; and prey with eye-like circular eyespots survive no better than those with other conspicuous markings (e.g. rectangles). However, laboratory experiments using live prey, or artificial prey with patterns based on those of live prey, have not always been able to replicate these findings.

Are predators deterred because eyespots mimic eyes? For many years, it was believed that predators were intimidated by eyespots because

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Figure 1. Animals with eyespots.
(A) Owl butterfly (*Caligo memnon*), photo: Thomas J. Hossie. (B) Lanternfly (*Fulgora laternaria*), photo: Thomas J Hossie. (C) Polyphemus moth (*Antheraea polyphemus*), photo: Michael Runtz. (D) Eyed elater (*Alaus oculatus*), photo: Michael Runtz. (E) Canadian tiger swallowtail (*Papilio canadensis*), photo: Thomas J Hossie. (F) Large four-eyed frog (*Pleurodema bufonina*), photo: Michael Webster.

they mistook them for the eyes of their own predators. But only recently have experiments provided some support for this idea: for example, the reflection of the sun in vertebrate eyes causes a glint (or spectral highlight) in the upper part of the eye. The eyespots of many species possess 'sparkle markings' that resemble spectral highlights, and predators are more deterred by eyespots when sparkle markings are found near the upper edge of the eyespot than when they are found in other (less realistic) positions. Also, bird predators are more wary of artificial caterpillars with eyespots that are positioned anteriorly (like the eyes of snakes) than those with centrally positioned eyespots. Birds are equally wary of images of butterflies with eyespots on their wings and images of a real predator's face; and are more wary of these than images of both butterflies without eyespots, and predators with their eyes closed. This indeed suggests that eyespots are mistaken for eyes. However, whose eyes they are mistaken for — those of predators or other species — remains unclear.

Why are predators fooled by eyespots? Although it has been argued that the mimicry hypothesis depends on complex cognitive misclassifications while the conspicuousness hypothesis does not, this argument implies that predators weigh up any given situation before deciding to attack or retreat. Given the fatal consequences of failing to act quickly, it seems much more likely that predators would evolve an innate aversion to objects that might represent a substantial threat. Indeed, naïve domestic chicks with no prior experience of dangerous vertebrates show a strong aversion to objects with eyespots compared to those without. Even rudimentary eyespots may be enough to exploit these unlearned aversions, as animals must necessarily respond quickly to imminent threats. However, wariness can be further enhanced by cues that increase the perception of risk (e.g. objects with large body size or predator-like postures).

So, why don't predators overcome their aversions and learn that these prey are harmless? In some laboratory studies they do, but in more natural settings predators can flee from prey that are perceived to be threatening, giving them little opportunity to learn that prey are bluffing. Intriguingly, even in some laboratory studies — notably cases where prey displays are multimodal (a sound coupled with an eyespot display, or eyespots coupled with a snake-like head) — predators are slow to learn, and quick to forget, that prey with eyespots are harmless. As long as there remains a reasonable chance that the prey is dangerous, predators are likely to benefit from being cautious: after all, a mistake could be fatal.

Why do experiments on eyespot function have mixed results? There appears to be some support for both the conspicuousness hypothesis and the eye mimicry hypothesis. So why do experiments have seemingly conflicting results? Predator eyes are often conspicuous, so eyespots may be perceived as both eyes and conspicuous marking simultaneously. Alternatively, some prey species may have eyespots that are perceived as eyes and others may have eyespots that are perceived as conspicuous markings; or different predatory species may be deterred for different reasons. However, there may be another explanation: the predictions arising from these hypotheses may not be as distinct as previously suggested. As eyes are a salient cue of predation risk, animals may rely on a simple salient feature (or sign stimulus) to identify them. When animals identify items using simple features, they often respond more strongly to items in which this feature is exaggerated (a supernormal stimulus), even if they look less like the original item overall (in this case eyes). Thus, even if more conspicuous but less eye-like eyespots deter predators more effectively, it does not follow that eyespots are not mistaken for eyes.

What do we know about the evolution and development of eyespots? Most of what we know about how eyespots develop comes from studies on the eyespots of butterflies. These elaborate markings are the result of the radial development of pigmented cells from each eyespot's centre. The outward expansion of developing cells appears to create a gradient of a 'morphogen', whose concentration determines the pigments produced within these cells, and generates the concentric rings of colour that characterize eyespots. As such, the initial occurrence of eyespots may have required less complex or co-ordinated signalling pathways than those needed to generate other patterns. This suggests that they could evolve quickly from simpler markings, which may explain why they have evolved independently in many taxa. Collectively, we are coming to understand not just why eyespots are selectively advantageous, but also how the eyespots are generated and where they might have originated, thereby providing a unified picture of adaptive morphological evolution at several levels of biological organization.

Where can I find out more?

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Primer

COP-coated vesicles

Natalia Gomez-Navarro and Elizabeth A. Miller*

Approximately one third of a cell's proteins are destined to function outside the cell's boundaries or while embedded within cellular membranes. Ensuring these proteins reach their diverse final destinations with temporal and spatial accuracy is essential for cellular physiology. In eukaryotes, a set of interconnected organelles form the secretory pathway, which encompasses the terrain that these proteins must navigate on their journey from their site of synthesis on the ribosome to their final destinations. Traffic of proteins within the secretory pathway is directed by cargo-bearing vesicles that transport proteins from one compartment to another. Key steps in vesicle-mediated trafficking include recruitment of specific cargo proteins, which must collect locally where a vesicle forms, and release of an appropriate cargo-containing vessel from the donor organelle (Figure 1). The newly formed vesicle can passively diffuse across the cytoplasm, or can catch a ride on the cytoskeleton to travel directionally. Once the vesicle arrives at its precise destination, the membrane of the carrier merges with the destination membrane to deliver its cargo.

Cytoplasmic proteins, called coat proteins, are responsible for both sorting cargo and sculpting the donor membrane to form a vesicle carrier. The first transport steps in the secretory pathway are mediated by vesicles generated by two distinct coat complexes (Figure 1). The coat protein II (COPII) complex mediates cargo recruitment and budding from the endoplasmic reticulum (ER), yielding vesicles that are destined for the Golgi (anterograde transport). The coat protein I (COPI) complex manages traffic from the Golgi back to the ER (retrograde transport), or between different compartments of the Golgi (intra-Golgi transport). Focusing on these two canonical coats, we will discuss the basic design principles that govern protein trafficking between the ER and Golgi and the physiological relevance of this process.

Donor and acceptor organelles in the early secretory pathway: ER, ERGIC and Golgi

Secretory proteins are translated by cytoplasmic ribosomes that engage with the ER membrane via a membrane-embedded translocation channel, through which the nascent polypeptide gains access to the ER lumen and/or membrane. The primary role of the ER is to provide an optimized environment for protein folding and maturation. Once properly folded, nascent secretory proteins accumulate at specific ER exit sites (ERES), which are discrete membrane domains coated with COPII coat proteins. These exit sites are relatively stable and act as a part of the ER quality control system because misfolded proteins and ER residents are largely excluded from these regions. It is at these sites that COPII vesicles destined for the Golgi apparatus are formed.

In plants and yeast, COPII vesicles likely fuse directly with the Golgi, which is located in close proximity to ERES. However, in mammalian cells, transport from the ER to the Golgi also includes a way-station — a series of membranes known as vesicular tubular clusters (VTCs) or the ER-Golgi intermediate compartment (ERGIC; Figure 1). These organelles are formed by the homotypic fusion of COPII vesicles to generate a structure that has a biochemical composition distinct from that of the ER or Golgi and that can alter its shape and size in response to environmental changes. The membrane clusters formed during fusion events rapidly recruit the COPI coat, which also marks this compartment. Ultimately, the ERGIC membranes move to the Golgi proper where they release their contents for onward transport. In addition to functioning as a carrier on the anterograde pathway, the ERGIC also plays a role in protein quality control by serving as a checkpoint compartment from which proteins can be retrieved back to the ER.

