clearly not sufficient because purified Hrr25 was unable to uncoat COPII vesicles *in vitro*. Full uncoating may be triggered by additional, as yet-to-be-determined signals. However, the degree to which vesicles uncoat before fusion takes place is currently unclear. Although more than 64% of the COPII vesicles were reported to retain their outer coats during tethering [1], these data could also mean that each vesicle loses approximately one-third of its coat. In principle, a partially uncoated COPII vesicle could retain enough Sec23 to allow TRAPPI binding and tethering, while leaving enough exposed membrane to allow pairing of SNARE complexes between the two membranes and fusion of the vesicle with the Golgi.

Partial uncoating would make it easier for TRAPPI to interact with Sec23 on opposing membranes during the homotypic fusion of mammalian COPII vesicles [5]. With both COPII vesicles fully coated, the distance between Sec23/24 complexes on each vesicle is 140 Å [9]. TRAPPI is a rod-shaped particle that measures 180 Å from end-to-end [10]. However, for TRAPPI to interact with Sec23 on different membranes via its two copies of Bet3 and still be able to bind and activate Ypt1, it would have to lie flat on the membrane [4], requiring that the two vesicles be less than 75 Å apart.

Now that many different types of vesicle coat are known to interact with tethers [6], it will be possible to test whether regulation by Hrr25 or other kinases is a general feature of coat–tether interactions. Interestingly, in the case of vesicles bearing the AP-3 adaptor complex, which are linked to the vacuole by the HOPS tethering complex, coat–tether interactions are regulated by the Hrr25-related kinase Yck3. However, instead of phosphorylating the coat to release the tether, Yck3 does the opposite, modifying the HOPS subunit Vps41 to expose the binding site for the AP-3 coat and promote tethering [11]. Although the details may differ, the work of Lord *et al.* [1] provides a new paradigm for the regulation of vesicle tethering and fusion that may apply to all transport steps: namely, that each organelle harbours a kinase that lies in wait for incoming vesicles, ready to cut them free of their tethers and release them from their coats.

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Animal Cooperation: Keeping a Clean(ing) Reputation

Cleaner wrasses are a model for the study of animal cooperation. Prospective clients can observe whether the cleaner works faithfully, and cleaners being watched remove just parasites while those that are not, nip the client for a tastier snack.

Russell D. Fernald

Why do unrelated individuals help one another? Numerous studies have found answers to this question in direct reciprocity — 'help me now and I'll help you later' — or mutualism — 'if you don't cooperate now, there will be a cost greater than if you helped'. Both of these mechanisms implicitly require some preparation for the future. But, can animals prepare for the future without a representation of it? Possibly such future 'preparation' resides in an improved self image. For example, helpers can increase the chance that bystanders will assist them later by increasing their image score (e.g., how that individual is viewed by a group). Among non-human animals, eavesdropping bystanders offer an opportunity for helpers to improve how they are viewed but this has been tough to document.

Cleaner fish have long been studied as a model system for animal cooperation. The marine cleaner wrasse, *Labroides dimidiatus*, removes ectoparasites from visiting reef fish clients. This is a mutualistic relationship because the client gets cleaned of ectoparasites and the cleaner gets a meal, the parasite. But there's a rub: cleaner fish prefer the client's tasty layer of mucus to its ectoparasites [1]. This means that there's a conflict of interest between cleaners and clients — clients want to be cleaned and cleaners prefer to eat mucus. These conflicting goals mean that clients want cleaners to do something they would rather not. However, cleaner fish service up to 2000 clients every day [2] and many of those encounters happen in the presence of observing bystanders, including future clients.

Does the presence of bystanders influence cleaner fish behavior? A paper by Ana Pinto, Redouan Bshary and colleagues [3] in this issue of *Current Biology* suggests that it does.

In humans, image scoring or reputation has been shown to be potentially important in understanding how cooperation might arise and persist in societies. The concept of 'indirect reciprocity' posits that a cooperating individual displays the image of a valuable community member to partners or collaborators [4]. For example, in a game in which players could repeatedly give and receive money from others but would never interact with the same individuals, players who were more generous to others in earlier interactions received more donations [5]. The key point in this experiment was that although individuals remained anonymous, a player's history of giving was displayed at each interaction. This knowledge would lead to indirect reciprocity that may have been important in the evolution of human social structures given the evidence that our ancestors probably lived in large networks of unrelated groups [6]. In addition to such anonymous reciprocity, there is recent evidence in humans about the effects of being watched or even cues of being watched on enhancing cooperation. For example, the effect of an image of a pair of eyes on voluntary contributions for drinks resulted in three times the collected amount as compared with a control image, suggesting that reputational concerns can influence human cooperative behavior [7].

But do animals evolutionarily distant from humans, such as cleaner fish, know (or care) who is watching? And how could we know? Previous field observations and some laboratory experiments support the idea that bystander clients prefer to invite inspections from cooperative cleaner fish and that cleaners are more cooperative in the presence of bystanders [8,9]. A key measure of cleaner–client cooperation is a jolt in the client's body — this involuntary twitch is a response to a cleaner fish nipping mucus rather than ectoparasites [10]. Pinto and colleagues [3] used two species of client fish and the cleaner fish *Labroides dimidiatus* (Figure 1). Cleaners were tested on clients with or without parasites. The cleaners chose to interact primarily with clients without ectoparasites and inspected clients with parasites less frequently when they were *not* being observed by bystanders. Introduction of a bystander led to an immediate increase in cooperation by the cleaner fish, decreasing the number of jolts during cleaning, implying that the cleaners spent more time removing ectoparasites rather than eating mucus. Moreover, bystanders also avoided cleaners that produced many jolts in their clients. As jolts did not result in interruption of the cleaning actions by chasing etc., it seems that observation of jolts alone was an adequate cue for bystanders. At cleaning stations in the natural habitat, potential clients could collect information about the cleaning behavior of particular cleaner fish just by observing jolts! But, do they count the number of jolts, or compare cleaners using some other metric? It is unknown how clients might tabulate 'jolt' data to decide what to do or whether they store this information for future visits to cleaning stations.

Figure 1. Scoring a cleaner image.

A cleaner wrasse (*Labroides dimidiatus*), cleans the gill cover of a coral rabbitfish (*Siganus corallinus*) client, while another looks on. Such interactions have the potential to inform potential clients about a cleaner's service — i.e. whether it faithfully removes parasites or 'cheats' by eating client mucus instead. In the presence of bystanders, cleaner fish have now been shown to clean more faithfully. (Photograph: Andy Lewis.)

Should we be surprised that cleaner fish take into account who is watching them? Perhaps not since recent studies have shown that other fish species are able to infer social rank and assess potential mates through observation alone [11,12]. Darwin puzzled over animals being clever and first suggested comparing the 'mental powers' of animals with those of humans [13]. The effort to understand cognitive abilities in animals has continued with the demonstration of human-like abilities in numerous species under a variety of conditions [14]. Successful experiments of this kind require exquisite knowledge of the social context and challenges of the species as well as careful behavioral criteria for testing. It seems likely that animal cooperation could arise and be sustained by social interactions in many species and that indirect reciprocity will likely to be a common explanation. In the future, we can expect neural and genomic analyses of how key social behaviors function, but for now we need to find good examples of animals behaving in unexpectedly clever ways.

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Apiology: Royal Secrets in the Queen's Fat Body

Royalactin, a component of royal jelly, induces queen differentiation in honeybees. Surprisingly, royalactin has a similar effect on growth in fruit flies, highlighting many unexpected features of growth regulation by the insect fat tissue.

Naoki Yamanaka and Michael B. O'Connor*

The eusocial honeybee, *Apis mellifera*, has two interdependent female castes: queen and worker. Queens are long-lived and specialized for reproduction, whereas workers are typically short-lived, reproductively inactive and specialized for nursing and foraging. It has long been known that a dietary signal, not an intrinsic genetic program, promotes queen differentiation: larvae nourished with royal jelly become queens. Royal jelly is a secretion from an organ named the hypopharyngeal gland of adult workers. When a colony needs a new queen, workers choose some young larvae and feed them with massive amounts of royal jelly, which turns the larvae into queens. So nurture is above nature, at least in the society of honeybees, although the exact factor in royal jelly responsible for queen differentiation has been elusive for many years.

In a recent issue of *Nature*, Kamakura reported the identification of a 57-kDa protein in royal jelly, called royalactin, as the queen-differentiating factor [1]. Supplementation of inactivated royal jelly with purified or recombinant royalactin fully restores its queen-inducing activity; larvae reared with royalactin-supplemented food show accelerated developmental progression and increased body and ovary size — all characteristics of queens. In contrast, supplementation with an equal amount of casein or a different royal jelly protein induces no response, arguing against the possibility that the effects of royalactin are simply nutritional. These results imply that royalactin is taken up by larvae in an unknown manner that circumvents normal digestion to exert its regulatory effects on honeybee development.

To elucidate the signaling pathway that mediates the caste-differentiating effect of royalactin, Kamakura turned to the fruit fly with its numerous genetic tools. Surprisingly, feeding *Drosophila* larvae with royalactin produces 'queen flies' with accelerated development, larger body size, increased fecundity and extended lifespan. Similar effects were obtained by overexpression of *royalactin* using the Gal4/UAS system, again suggesting that the effect of royalactin is not simply nutritional.

Taking advantage of *Drosophila* genetic tools, Kamakura successfully attributed the effects of royalactin specifically to activation of the epidermal growth factor receptor (EGFR) signaling pathway [2,3] in the fat body. The insect fat body is a functional counterpart of mammalian adipose tissue and liver, and growing evidence suggests that the fat body controls development and metabolism at the systemic level by secreting humoral factors (e.g., [4]). A wealth of data presented in the Kamakura paper [1] strongly indicates that royalactin somehow activates EGFR in the fat body, and downstream signaling cascades coordinate multiple responses throughout the body, most likely through the release of different secreted factors (Figure 1). Among these, activation of the PI3K/TOR/S6K pathway downstream of EGFR in the fat body leads to a signal that, either directly or indirectly, stimulates growth in other tissues. Interestingly, this signal is likely to be different from fat body-derived insulin [5,6] or any other fat body factors that control insulin/IGF signaling in other tissues [7–12], as it is effective even in insulin receptor mutant backgrounds and specifically increases cell size but not cell number, whereas insulin/IGF signaling affects both [13]. Therefore, although it is well known that S6K has a cell-autonomous effect on cell size [14], it also seems to have a non-cell-autonomous effect through regulation of a secretory factor that is again specialized for cell-size control.

Kamakura's study [1] suggests that there are at least two other uncharacterized signaling pathways downstream of EGFR in the fat body, each of which leads to activation of a distinct hormone biosynthetic pathway in a remote endocrine gland and therefore indirectly controls