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Connecting stalks in V-type ATPase

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Figure 1 Time course of the distal-proximal skin-temperature gradient (DPG). The gradient is shown from 14:00 to 7:30 for observations with the most negative DPG values between 22:30 and 24:00 (large vasoconstriction before lights out at 24:00; pink symbols), compared with the time course of the most positive DPG values (large vasodilation before lights out; green symbols) (mean $\pm$ s.e.m., $n = 18$; asterisk indicates significant differences between data points; $P < 0.05$, Bonferroni-adjusted least significant differences). The shaded area indicates the lights-out period. These two extreme patterns were selected after the mean of the three DPG values between 22:30 and lights out at 24:00 had been rank ordered out of 8 observations for each subject. Sleep onset latency (inset) is significantly shorter when subjects were most vasodilated (green bar) before lights out (triangle indicates significant differences; paired t -test, $P < 0.001$).

In a backward stepwise regression analysis among all predictor variables, only DPG contributed significantly to the model; that is, vasodilation of distal skin regions was the best predictor of the body's readiness for sleep (Fig. 1). Because interventions such as light or large carbohydrate-rich meals differentially manipulated the independent variables, the effect on the dependent variable showed that the link between distal vasodilation and the ability to fall asleep is functional, not just correlative.

The circadian clock prepares the thermoregulatory system for vasodilation to begin in the early evening as sleepiness increases, followed by a drop in core body temperature. Even lying down increases sleepiness by redistributing heat in the body from the core to the periphery⁷. Turning out the light is a complex cognitive and physiological signal that also leads to vasodilation⁴. There is a tight correlation between the timing of the endogenous increase in melatonin in the evening and vasodilation, an effect that is mimicked by pharmacological doses of melatonin^{4,7}. Before bedtime, then, many overlapping events orchestrate the thermoregulatory overtone.

We would predict that classical hypnotics⁸ and other sleep-inducing aids all cause dilation of distal blood vessels and heat loss before the onset of sleep. A hot-water bottle at the feet, while not acting on mechanisms in the central nervous system that underly the regulation of sleep, can rapidly induce vasodilation. The resulting heat loss is most efficient when the ambient temperature is cool⁹. Some sleep disorders (particularly those associated with ageing

and somatic illness¹⁰) may be secondary to an inability to vasodilate and prepare the body for sleep.

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Biological motors

Connecting stalks in V-type ATPase

In all organisms, adenosine triphosphate (ATP) provides metabolic energy for driving energy-dependent processes. It is synthesized and/or utilized by enzymes known as F-type and V-type ATPases, which are small rotary motors^{1,2}. Both types consist of a headpiece, F_1 or V_1 , respectively, which is connected by a stalk region to the membrane-bound part, F_0 or V_0 . Electron microscopic analysis of negatively stained particles has revealed a peripheral stalk, or stator, between V_1 and V_0 of the V-type (Na^+)ATPase of the thermophilic bacterium *Clostridium fervidus*^{3,4}, like that in F-type ATPases^{5,6}. We have analysed many more particles and now present a more complete structure of the V-type ATPase stator moiety.

A central stalk in the ATPase rotates within a ring of three α - and three β -subunits in F_1 (refs 7, 8), or three A and three B subunits in V_1 , in discrete steps of 120°. At the F_0/V_0 end, the central stalk is connected to a ring of c subunits in the membrane. These subunits rotate against other subunits of F_0 and V_0 , allowing ion translocation at the interface². A stator structure in the form of additional connections between F_1/V_1 and F_0/V_0 must presumably be present to prevent futile rotation of the $\alpha\beta_3$ and A3B3 headpiece².

We classified a set of 7,500 molecular projections of detergent-solubilized, negatively stained V_1V_0 , and found a few preferential orientations in which V_1 has either two or three lobes. In about 28% of all

views, there are three connections between V_1 and V_0 , but in the rest either one (33%) or both (39%) of the peripheral connections appear to be missing. The three connections are well separated in the bilobed views (Fig. 1a). In the classes with trilobed views, the left connection is less well resolved because it is closer to the central stalk and overlaps more with V_1 (Fig. 1b). The two peripheral connections are each attached to an oval-shaped area of density on top of V_1 . In some other classes (not shown), one of the oval densities is absent, which correlates with the loss of a peripheral connection. We conclude that the intact V-type ATPase has a central stalk and two stator connections (Fig. 1c, d). Likely candidates for the stators are the bacterial V-type subunits I, E and F, several copies of which are present.

The discovery of the second stator raises several points. First, although it has been suggested that there may be two stators⁹, this feature has not been observed before. This could be because the intact structure is easily damaged on preparation, which can be detected only by classifying large sets of projections. The two stators may be unique for V-type ATPases because, in the stalk region of F-type ATPases, only the b subunit is present in two copies, as a dimer¹⁰, participating in one stator. It is harder to visualize the stator(s) in F-type ATPase by electron microscopy because the stalk region is much shorter than in V-type ATPase⁸.

Second, there could be more than two stators: three would match the three-fold symmetrical ring of the six large subunits

Figure 1 Electron microscopy images of V_1V_0 in side view. **a**, **b**, Views obtained by classification: **a**, bilobed view³; **b**, trilobed view. **c**, **d**, Model of the arrangement of the stator moiety (green) and its attachment to the V_1 headpiece (yellow) by the two oval densities (red). The view in **d** shows a larger additional density (dark blue) of V_0 on the right side than that in **c**. The stator moiety is attached to this additional density, which represents the static part of V_0 . The view in **d** is obtained by rotating **c** about 30° backwards on the left. Scale bar, 100 Å.

in V_1 . There is no evidence for this, but the presence of another stator connection is a possibility if there is overlap in projection.

Finally, it is not clear why the ATPase motor has such a complicated stator moiety, with an overall U-shaped form that must avoid friction with the central rotating stalk, although it is likely to be related to its mechanical stability.

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Insect behaviour

Evolutionary origins of bee dances

Although bumble-bees are highly social insects, their foraging has been considered to be managed as an individual initiative^{1–4}, in which each bumble-bee visits flowers not only to collect food, but also to gather information about other potential food sources⁵. Here we show that bumble-bees instead use a primitive, but surprisingly efficient, recruitment system: by performing extended excitatory runs in the nest, a single successful forager can alert the entire foraging force of a bumble-bee colony. But in contrast to what happens in other social bees, such as honeybees, the recruits are not informed about the location of the food. Instead, the successful forager brings home the odour of the newly discovered food source, conveying to the recruits information about the species of flower. These findings about bumble-bee communication shed new light on the early evolutionary origins of the elaborate dance language of the honeybee.

To investigate whether bumble-bees (*Bombus terrestris*) can communicate information about the discovery of a food source, we connected a nest box with a bipartite flight arena. A single forager was allowed to

Figure 1 Recruitment in bumble-bees. **a**, The number of bees that leave the hive in a 5-minute period increases dramatically when one bee forages successfully (brown bars). **b**, Most bees choose the odour that is brought into the nest by a forager.

collect sucrose solution from an artificial flower in one half of the arena, whereas all other bees had access only to the other half of the arena, which did not contain food. This procedure ensured that interactions between bees could take place only in the nest, not at the food source. The number of bees entering the empty flight arena to search for food was counted during 12 periods of 1 hour each, when foragers were rewarded.

When compared with unrewarded control periods, the searching activity strongly increased when a single bee foraged ($P < 0.01$, Wilcoxon test; Fig. 1a). Successful foragers in the nest, instead of just emptying their crop load and continuing to forage, would typically spend several minutes running across the nest, frequently bumping into nestmates and occasionally buzzing their wings.

Odour-preference tests indicated that bees leaving the hive strongly preferred the odour that was brought home by the forager ($n = 90$, $P < 0.001$, χ^2 test; Fig. 1b). Because floral scents are species specific, the odour helps recruits to find the food source used by the successful forager. To test whether positional information is also conveyed, we trained three foragers to collect sucrose from a feeder positioned 100 m west of the hive in an open field. Recruits had the choice between that feeder and two additional feeders placed 100 m north and south of the nest. New recruits distributed themselves randomly at these three feeders ($P = 0.21$; χ^2 test). This failure of bumble-bees to recruit to specific points in space may explain why

their communication has previously been overlooked. Bumble-bees therefore have a recruitment system that is specific for a flower species but independent of its location. This system for transmitting information resembles the round dance used by honeybees when food is found in the immediate vicinity of the hive^{1,6}.

But why have bumble-bees not evolved a communication system that includes information about where to forage? Such a system can be very costly. Honeybee recruits may take more than an hour to decide where to go, even if only two different locations are advertised⁷. They also take a long time to find a food source after receiving information about its location⁸.

Bumble-bees live in small colonies, mainly in temperate habitats where floral food is less clumped than in the tropical habitats where the communication of honeybees and stingless bees evolved^{1,5,6}. The advantage of communicating location might therefore not offset the cost. It may be sufficient to specify flower species to recruits, who can then rely on their own memory or searching ability to find flowers but still know when and for what to forage.

Understanding the communication of bumble-bees is central to reconstructing the evolutionary origins of the honeybees' waggle dance, which is one of the most complex systems in animal communication. Such a reconstruction requires us to compare the honeybees' behaviour with that of their extant relatives, the bumble-bees and stingless bees, which have excitatory motor patterns that serve to recruit nestmates to food sources⁹. This behaviour may be derived from a social facilitation of activity at the nest entrance, which is widespread among social insects¹⁰. Bumble-bees also share with stingless bees⁹ and honeybees^{1,6} the ability to learn the floral odours brought back by returning foragers. The bumble-bee recruitment system might therefore resemble that of the last common ancestor of the eusocial bees.

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