torque controls rotation of the visual stimulus; the fly is heated when a quadrant that contains one of the two shape types rotates into the front part of its visual field (with an identical shape simultaneously entering the rear quadrant). For classical conditioning, the visual stimulus was rotated at a constant rate, independent of the fly's behavior; heating was similarly paired with one of the two stimulus types.

Liu *et al.* [10] found that when neural transmission was disrupted in the adult fly, in a group of cells that included neurons of the central complex, pattern preference was not induced by operant conditioning. The gene *rutabaga*, previously studied in the context of olfactory learning, was found to be necessary for both operant and classical conditioning in this paradigm, as *rutabaga* mutants are incapable of forming a conditioned pattern preference, even though spontaneous pattern discrimination remained intact. Strikingly, expression of a constitutively active protein of the *rutabaga* pathway in neurons that include a subset of central complex neurons disrupted conditioned discrimination between one set of shapes, but not another. Conversely, rescue by expression of wild-type *rutabaga* in the same set of neurons in an otherwise *rutabaga* mutant animal was found to be sufficient to restore conditioned discrimination for the same set of shapes. Using a different driver to drive *rutabaga* rescue in a different set of neurons, which included a different subset of central complex neurons, the authors showed that conditioned discrimination of a different set of shapes could now be restored.

Taking these findings together, Liu *et al.* [10] concluded that the fly's memory traces for distinct visual features are stored in feature-specific circuits, rather than in a "common all-purpose memory center" (Figure 1). It is intriguing that the specificity of feature learning in the context of these experiments may be rendered by a pre-motor center of the fly's brain. It will be interesting to learn whether different elementary features thought to explain conditioned discrimination of different pattern types in the flight simulator — features such as center of gravity, area, or orientation — may be distinguished by subtly different behavioral strategies. To what extent is visual scene segmentation aided by active exploration?

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Eukaryotic Transcription: What Does It Mean for a Gene to Be 'on'?

Until recently, transcription could only be observed by measuring mRNA production of cell populations, thus obscuring the kinetics at the level of individual transcription events. A new study now shows that eukaryotic transcription, visualised in individual living cells, occurs in bursts — much as it does in prokaryotes.

Ido Golding and Edward C. Cox

When we say a gene is 'on', what do we mean? We usually measure RNA transcripts on large populations of cells, but what would we find if we could look at individual transcripts as they are being made? The simplest kinetics imaginable would be that each initiation event occurred as a simple Poisson process [1], whereby synthesis of individual mRNA molecules was initiated with a constant probability k as a function of time (Figure 1). Genes with high rates of transcription would then have high values for k, while repressed genes would have values close to zero. Thus, when we observe the total mRNA synthesis for a given gene in a population of cells, we would measure constant rates of RNA production [2], and this macroscopic rate would be equal to the microscopic probability per unit time k. This is the simplest model, but transcription dynamics can take on many other, more complex temporal patterns. Until recently, however, observations of transcriptional activity were limited to traditional methods, where mRNA levels were necessarily averaged over large cell populations, typically 10^8–10^9 cells in an experiment with bacteria. Individual events in single living cells could not be studied, and so we could not ask how

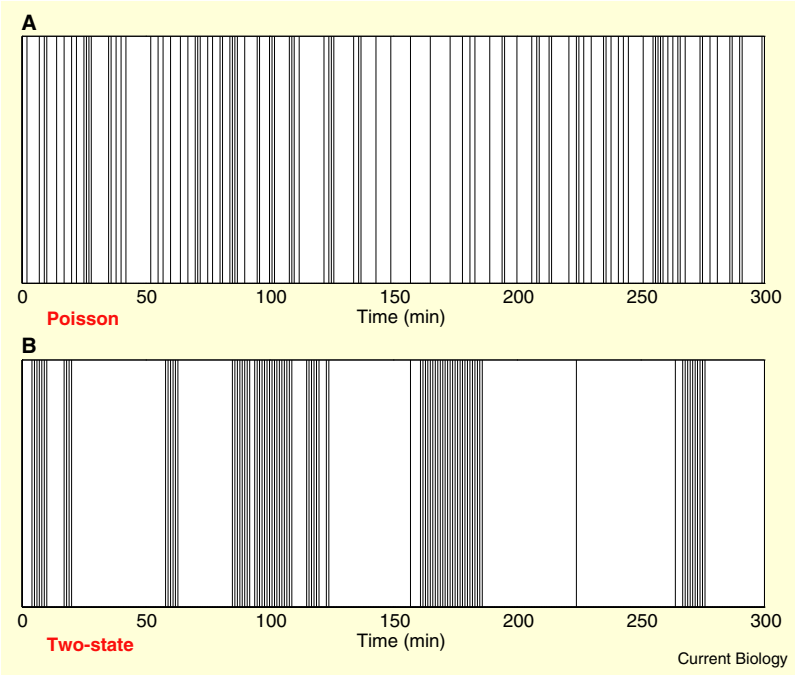

Figure 1. Possible kinetics of individual transcription events.

Typical time series created by Poisson and two-state processes. (A) The Poisson process is characterized by a single rate (or probability) of initiation of transcription, in this example k = 0.25 min^{-1}. (B) The two-state process is characterized by three parameters: The activation rate k_1 = 0.05 min^{-1}, the inactivation rate k_2 = 0.2 min^{-1}, and the events rate while in the active state, λ = 1 min^{-1} . The resulting two patterns have a similar number of total events (because $k = \lambda\, k_1 / k_2$). However, in the case of the two-state model, the events appear in a pulsatile manner, each pulse with an average on-time of about 5 minutes. The pulses are separated by an average off-time of about 20 minutes. The parameters were chosen to give similar kinetic values to the results of Chubb *et al.* [12]. See [11] for a discussion of these models.

they combine to give the behavior we observe in large populations.

This has now changed with the coming of age of a technique which allows for the detection and quantification of individual transcripts in live single cells in real time. This new approach was pioneered by the Singer laboratory [3], and has two essential features: The transcript of interest bears a hairpin repeat at the 3′- or 5′-end, which binds a GFP-tagged version of the phage MS2 coat protein [4], and the MS2–GFP fusion protein is expressed constitutively. When the hairpin-bearing sequence is transcribed, it very rapidly binds MS2–GFP and appears in the cell as an intense, well resolved spot. The fusion protein binds to its mRNA target with high specificity and affinity [5], and thus each mRNA molecule becomes decorated by a large number of fluorescent proteins with known stoichiometry [3,6–8].

A key feature of this system, which enables it to work in real time, is its independence from slow and highly stochastic cellular processes. The appearance of mRNA is detected by the rapid binding of pre-existing MS2–GFP molecules, and is thus not delayed by the kinetic bottlenecks of protein production, folding, and chromophore maturation, typical of other reporter systems [9]. What allows this approach to be turned into a real measurement system is the use of fluorescent imaging as a quantitative tool [10]. With the appropriate calibration, the number of mRNA molecules can be reliably estimated by measuring the intensity of localized target-bound MS2–GFP [11] .

In this issue of *Current Biology*, Chubb *et al.* [12] use this technique to reveal the temporal pattern of transcription for a developmental gene in individual cells of the slime mold *Dictyostelium discoideum*. They find a pulsatile form of transcription, with transcriptional bursts separated by periods of inactivity. This contrasts with the textbook picture we usually teach, according to which transcription proceeds smoothly and uniformly after induction. Chubb *et al.* [12] also find that transcriptional activity is spatially correlated between neighboring cells. This is perhaps not surprising, because *Dictyostelium* cells are known to signal to neighbors via cAMP. Surprisingly, however, the transcript levels in the population — the ensemble averages — appear to be determined by the number of cells that are active, rather than by the number of transcripts per cell, which was found to be relatively constant. Because Chubb *et al.* [12] estimate that each observed transcriptional event represented 10 or so transcripts, they could not distinguish between first and subsequent initiations.

Despite these complications, the statistics of the observed bursting events — an exponential distribution of pulse duration and inter-pulse-intervals — suggest that the underlying mechanism could be a simple two-state process of gene activation and inactivation, similar to the one recently described for *E. coli* [11]. There too the model that accounts best for transcriptional bursting is one in which two independent events are needed to account for the appearance of transcripts on a cell by cell basis: First, an induced gene can switch into an active state with constant probability as a function of time. Second, whilst in this state, it can at each moment produce an mRNA molecule with a constant probability, but it can also, with a constant probability, switch back to the inactive state [13–15]. The resulting time series is characterized by periods of transcriptional inactivity, interspersed with limited time windows of transcriptional activity (Figure 1).

What are the physiological mechanisms underlying these kinetics? Is the similarity between prokaryotes and eukaryotes evidence for similar processes at work? Many scenarios have been

offered as possibly leading to transcriptional bursting [11,13,15]. These include chromatin remodeling (for eukaryotes) as well as (for both kingdoms) the binding and unbinding of transcription factors, changes in DNA conformation and others. Whatever the details may turn out to be, studies of the kind reported by Chubb *et al.* [12] are beginning to give us a much clearer picture of how individual stochastic events add up to give us population results, a major goal of current work in systems biology. Elucidating the molecular mechanisms behind the newly found pattern of gene activity is a natural next step, and promises exciting new discoveries.

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Wiring Specificity: Axon–Dendrite Matching Refines the Olfactory Map

In *Drosophila*, about 50 classes of olfactory receptor neurons enter the brain where their axons form highly specific synapses with the dendrites of identified partner neurons. A recent study has shown that genetic manipulations that shift the position of one class of postsynaptic dendrites can cause an exact corresponding shift in the location of their partner axons.

Gregory S.X.E. Jefferis

The origins of wiring specificity in the brain have often been considered from the perspective of axon guidance — the presynaptic cell sends out an axon which grows over long distances, following molecular cues sniffed out by the growth cone. Of late, it has become more generally appreciated that dendrites are also capable of targeted growth (for example [1–3]) and can contribute equally to wiring specificity [4]. This implies that forming specific connections may often rely on active guidance by both axons and dendrites. If both axons and dendrites are involved in guidance, what kind of cues do they use so that partner axons and dendrites can find each other? Do they rely on common third party cues to navigate to exactly the same place or do the partner dendrites and axons recognise each other directly? A new study from Zhu *et al*. [5] provides the first experimental evidence that a shift in the position of postsynaptic dendrites can cause their partner axons to relocate.

Olfactory Development

The organisational logic of the olfactory system in *Drosophila* is very similar to that in mice: olfactory receptor neurons in the periphery typically express a single odorant receptor gene and send axons to specific glomeruli, the subdivisions of the first olfactory relay in the brain, the antennal lobe. Here, these olfactory receptor neuron axons form connections with projection neurons (similar to vertebrate mitral cells) whose dendrites innervate a single glomerulus. In a fruitfly, the ~50 glomeruli can be recognised by position and shape [6] and the molecular identity of the input to most of these glomeruli is known [7,8].

Work on the development of the olfactory system in mice and moths has mostly emphasised olfactory receptor neuron axons as organisers of the olfactory map (for example [9,10]). In *Drosophila*, genetic tools have revealed that projection neurons that connect to specific glomeruli are independently specified before connecting with their presynaptic partners [4]. Indeed, projection neuron dendrites form a coarse map in the developing antennal lobe before their axonal partners have even arrived [3].

These results have led to a three point model of olfactory development in *Drosophila* [3,11].