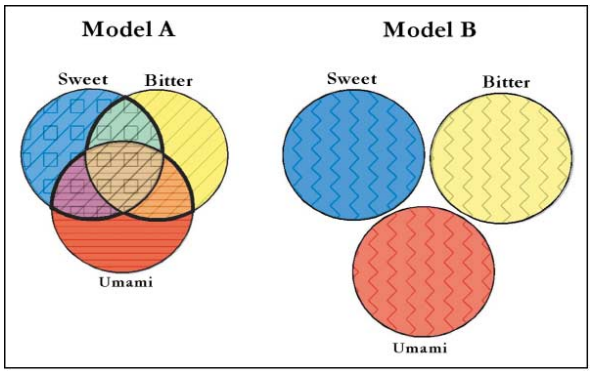

Models of Taste Perception

Model A favors a large fraction of broadly tuned taste receptor cells (TRC), where many cells can recognize bitter compounds, sugars, and amino acids and, hence, express multiple receptor types (area outlined with bold line). Moreover, this model favors multiple signaling pathways, even within a taste modality, such as sweet taste (indicated by different fill patterns). Model B proposes that each TRC is dedicated to a single taste quality converging on a common signaling cascade. Circles represent TRC populations expressing one receptor type (sweet = T1R2/T1R3, umami = T1R1/T1R3, bitter = many T2Rs).

KO mice in which *PLCβ2* function was restored in a subpopulation of TRCs (those expressing T2Rs). These mice, when challenged with numerous substrates, show complete lack of sweet and umami taste perception, but have bitter taste sensation restored to wild-type levels, an observation that is consistent with a separation of taste modalities (model B) at the single cell level and argues clearly against broadly tuned TRCs (model A).

This paper has hopefully put to rest some of the contentious issues of the past in taste signal transduction, although some of the details remain to be elucidated. Availability of transynaptic markers and promoters for specific TRC types can now be employed to approach the processing of taste perception in the brain from a molecular genetic perspective. However, even some basic issues remain puzzling. For example, α-gustducin is expressed only in some (sweet/umami) T1R-expressing cells. Yet, surprisingly, a significant reduction in sweet taste perception has been observed in α-gustducin KO mice (Wong et al., 1996). Another open question is whether sour (H^+) and/or salty (Na^+) stimuli, probably mediated by ion channels, are also recognized by TRPM5-/PLCβ2-expressing TRCs, or whether the remaining 50% of cells mediate these taste qualities. To borrow a phrase from the Zhang et al. paper, those issues are "simply a matter of taste"—at least for now.

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Circadian Clocks: A Tale of Two Feedback Loops

Circadian clocks in a wide range of organisms are thought to consist of two interdependent transcriptional feedback loops. In *Drosophila*, the first loop has been well characterized and controls rhythmic *period* expression. In this issue of *Cell*, Cyran et al. (2003) define a role for a transcriptional activator and a repressor in the second feedback loop.

Negative feedback loops typically maintain steady-state levels of gene expression. In contrast, circadian feedback loops are dynamic, even under constant environmental conditions, driving rhythmic gene expression. In *Neurospora*, *Drosophila*, and the mouse, circadian clocks are composed of two interdependent transcriptional feedback loops (Glossop et al., 1999; Shearman et al., 2000). In *Drosophila*, the first feedback loop controls daily oscillations of *period* (*per*) and *timeless* (*tim*) transcripts (Figure). In the *per*/*tim* loop, the CLOCK (CLK)/CYCLE (CYC) heterodimeric transcription factor activates *per* and *tim* transcription (Allada et al., 2001). Transcription and mRNA levels peak around dusk. PER and TIM accumulate and then directly inhibit CLK/CYC. *per* and *tim* transcript levels return to trough levels around dawn. A similar feedback loop with, some subtle differences, also exists in mammals.

A distinct feedback loop controls the rhythmic expression of *Clock* (*Clk*) RNA (reviewed in Allada et al., 2001). *Clk* RNA oscillations are roughly antiphase to those of *per* and *tim*, with peak levels in the early day. Various data have led to the conclusion that the effects of *per* and *tim* on *Clk* RNA require wild-type *Clk* and *cyc* (Glossop et al., 1999). The simplest model was that CLK/CYC activates a transcriptional repressor of *Clk*. Two groups, one in this issue of *Cell* (Cyran et al., 2003), and the other in *Neuron* (Glossop et al., 2003), provide evidence that this repressor is the basic leucine zipper (bZip) transcription factor *vrille* (*vri*).

Cyran et al. and Glossop et al. provide several lines

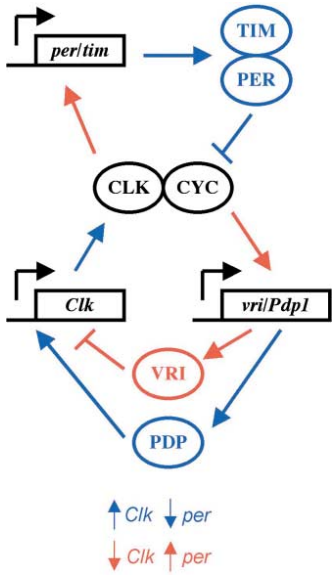
of evidence that *vri* is a repressor of *Clk* transcription. Previous work has demonstrated that loss of one *vri* copy shortens period, while *vri* overexpression lengthens or abolishes rhythms (Blau and Young, 1999). *vri* is directly activated by CLK/CYC (Blau and Young, 1999; McDonald and Rosbash, 2001). Cyran et al. and Glossop et al. observe rhythmic *vri* protein (VRI) expression that is roughly antiphase to that of *Clk* RNA. Loss of one *vri* copy increases *Clk* expression, and *vri* overexpression suppresses *Clk* levels by reducing *Clk* promoter activity. The effects of VRI on *Clk* are likely direct. VRI specifically binds *Clk* promoter elements in vitro. *vri*-induced repression does not require other circadian clock genes and occurs relatively rapidly after transient induction of *vri* in vivo.

The study by Cyran et al. provides an additional level of complexity to the second feedback loop with the identification of a *Clk* activator, *PAR domain protein 1* (*Pdp1*). Like VRI, several mammalian members of the bZip family are rhythmically expressed: activators that contain a PAR (*p*roline and *a*cidic *r*ich) domain such as DBP, HLF, and TEF, and a repressor, E4BP4, that like VRI, lacks a PAR domain (Mitsui et al., 2001). Previous studies had implicated a *Drosophila* bZip PAR protein, *PAR domain protein 1* (*Pdp1*), in circadian function as *Pdp1* is rhythmically expressed and is directly activated by CLK/CYC (McDonald and Rosbash, 2001).

Cyran et al. (2003) demonstrate that a specific *Pdp1* isoform, *Pdp1*ϵ, functions in behavioral and molecular oscillations. Molecular rhythms are largely absent in homozygous mutant larvae, at least in constant darkness (*Pdp1* mutants are not viable as adults). These effects on rhythmicity appear to be due to a failure to activate *Clk*. *Pdp1* mutants display reductions in *Clk* RNA levels. PDP1ϵ binds elements within the *Clk* promoter in vitro and activates transcription from the *Clk* promoter in S2 cells.

Taken together, the data indicate that CLK simultaneously activates an activator (*Pdp1*) and a repressor (*vri*) of its own transcription. How does this result in coherent rhythmic *Clk* expression? A potential answer is that VRI accumulates earlier (peaking around dusk) and disappears earlier than PDP1ϵ, temporally separating *Clk* repression and activation (Cyran et al., 2003). Cyran et al. further examine the interaction between VRI and PDP1 genetically and biochemically. Loss of one copy of *Pdp1* and overexpression of *vri* synergistically lengthen *period*. Cell culture experiments demonstrate that VRI antagonizes PDP1 activation of the *Clk* promoter and that both VRI and PDP1ϵ can bind the same *Clk* promoter site in vitro. A similar antagonistic relationship exists between E4BP4 and PAR domain activators (Mitsui et al., 2001). Competition between VRI and PDP1ϵ for *Clk* promoter sites may therefore determine *Clk* transcription rates (Figure).

The presence of interdependent feedback loops in diverse organisms from *Neurospora* to mice, suggests an adaptive importance to this mechanistic arrangement. In mammals, the *cyc* homolog *Bmal1* is rhythmically expressed to form a second feedback loop (Shearman et al., 2000). The orphan nuclear receptor REV-ERBα plays an analogous role to VRI, repressing *Bmal1* transcription by binding ROR elements (ROREs) within the *Bmal1* promoter (Preitner et al., 2002). ROREs



Proposed Model for Interdependent Feedback Loops in the *Drosophila* Circadian Clock

Red indicates processes/molecules that predominate when *per* is high and *Clk* is low, typically around dusk. At dusk, CLK/CYC activates *per*, *tim*, *vri*, and *Pdp1*, leading to maximal *per* levels. VRI accumulates prior to PDP1 and represses *Clk*. Blue indicates processes/molecules that predominate when *per* RNA levels are low and *Clk* RNA levels are high, usually around dawn. PER and TIM accumulation repress CLK/CYC activation, resulting in low *per* levels. PDP1 accumulates and VRI disappears to allow activation of *Clk*. Transition between the states (blue and red) appears to depend on the accumulation and degradation of PER and TIM proteins as well as the competition between the *Clk* repressor, VRI, and activator, PDP1.

are binding sites for REV-ERB and ROR orphan nuclear receptors. Although the mammalian VRI homolog E4BP4 is rhythmically expressed, its in vivo function in the second feedback loop has yet to be determined (Mitsui et al., 2001). Nonetheless, early indications are that the second feedback loops in flies and mice may not use homologous genes.

The identification of a rhythmically expressed activator in *Drosophila* makes a specific prediction for such a factor in the second feedback loop of mammals. Given the findings of Cyran et al., a closer examination of rhythmically expressed bZip PAR activators may be necessary to tease out their function in the second feedback loop. Another study has implicated a rhythmically expressed member of the ROR family as a potential *Bmal1* activator (Preitner et al., 2002).

Studies in flies and mice suggest that an intact second feedback loop may not be necessary for molecular or behavioral rhythms (Kim et al., 2002; Preitner et al., 2002). If so, what is the function of the second feedback loop? One possibility is that the second feedback loop is dispensable in the luxurious constant environmental conditions supplied in the laboratory but absolutely necessary for robust high-amplitude rhythms required for survival in natural environments. The finding of period defects in *vri* and *Pdp1* mutants is consistent with a role in the pacemaker (Cyran et al., 2003). Variable periods present in *Rev-erb*α knockouts also support the "ro-

bustness" hypothesis (Preitner et al., 2002). A second possible function for the second feedback loop is to transduce signals from a variety of environmental inputs to the first feedback loop (Preitner et al., 2002). A third possibility is that the second feedback loop controls the expression of time-specific circadian outputs. Several DNA microarray studies have identified dozens of genes under clock control with distinct temporal profiles (for example, McDonald and Rosbash, 2001). The second feedback loop may even be important for turning on rhythmic gene expression in development. *Drosophila* genetics should continue to be important in determining the function of these interdependent feedback loops in circadian systems.

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Better Chemistry for Better Survival, through Regulation

In this issue of *Cell*, Chabes et al. (2003) report on new aspects of the regulation of yeast ribonucleotide reductase, the mechanism by which dNTP levels are increased following DNA damage, and the consequences of the metabolic changes.

The maintenance of a cell's genome is a biological imperative. When DNA is damaged in a eukaryotic cell, the cell cycle is arrested, and the enzymatic resources needed for DNA repair are transcriptionally induced or otherwise activated. The regulated genes include many encoding DNA repair enzymes, others involved in cell cycle control, and the subunits of ribonucleotide reductase. As reported by Chabes et al. in this issue of *Cell*, ribonucleotide reductase lies at the center of an elaborately regulated pathway that supplies not only the enzymes required for DNA repair, but also the metabolic precursors required by those enzymes.

Ribonucleotide reductase (RNR) has been surprising and delighting researchers for decades (Jordan and Reichard, 1998). In the absence of a reasonable abiotic pathway to deoxyribonucleotides, the enzyme has evolutionary importance as a prerequisite for the transition from RNA to DNA worlds. The reaction itself, the replacement of a carbon-linked hydroxyl group with hydrogen, had no precedent in synthetic organic chemistry when the enzyme was first discovered. RNR was also the first enzyme found to make use of free radical chemistry. Even the regulation of the enzyme provided some new lessons, as not only the activity is regulated, but also the specificity of the enzyme.

There are three well-characterized classes of ribonucleotide reductases, each utilizing a similar chemical strategy but exhibiting little sequence similarity and relying on different cofactors. Reichard (Jordan and Reichard, 1998) has argued that the class III enzymes, found in some anaerobically growing bacteria, are the closest to the RNR progenitor. Oxygen destroys the stable glycyl radical in class III enzymes. The appearance of oxygen in the atmosphere then led to the divergent evolution of class II enzymes, which are not affected by oxygen, and class I enzymes, which require oxygen. There are two variations of class I, called Ia and Ib. Similarities in reaction mechanism, regulation, and overall structure have helped bolster the common origin view (Jordan and Reichard, 1998; Stubbe, 2000). Many bacteria encode multiple ribonucleotide reductases, often from different classes. Eukaryotes generally possess class Ia enzymes, and yeast relies on a somewhat idiosyncratic version of the class Ia family (Chabes et al., 2000; Voegtli et al., 2001). Class Ia ribonucleotide reductases generally have an $\alpha_2\beta_2$ architecture. The active site, as well as all regulatory sites, is located in the large α subunit. Yeast has two genes encoding α subunits (*RNR1* and *RNR3*) and two encoding the smaller β subunits (*RNR2* and *RNR4*). All but *RNR3* appear to be essential. The small subunits may function as a heterodimer (Chabes et al., 2000; Voegtli et al., 2001).

The fundamental task of ribonucleotide reductases in cellular metabolism is to provide deoxynucleotides to support DNA synthesis. The same RNR active site accommodates all four ribonucleoside diphosphate substrates. However, the specificity at any given moment depends on the concentrations of different dNTPs and ATP, which all interact at the same allosteric binding site, called the specificity site. A complex regulatory scheme in which enzyme active site specificity depends on the nucleotide bound at the specificity site was laid out by Thelander and Reichard in 1979 (Thelander and Reichard, 1979). The scheme is designed to balance the concentrations of the various dNTPs as needed for replication. The same specificity modulation is observed with all classes of ribonucleotide reductases. In addition to this regulation of specificity, class Ia and class III enzymes have a second allosteric site, called the activity site. Binding of ATP or dATP to this site leads to enhancement or inhibition, respectively, of overall enzyme activity. In the yeast RNR, the inhibition by dATP is somewhat relaxed (observed at higher dATP concentra-