saw a stimulatory effect at less than 500 nM (Sagot et al., 2002b). One interesting possibility is that the slowly elongating FH1-FH2-associated barbed ends may require the recruitment of profilin-actin complexes via the FH1 domain to maintain elongation rates.

Mammalian Formins such as mDia1 trigger the formation of thin actin fibers when expressed in an activated form (Tominaga et al., 2000). While it is overly simplistic to directly compare yeast actin cables and mammalian actin fibers, these two processes may share a common mechanism. Indeed, mammalian Formins contain well-conserved FH1 and FH2 domains. However, whether the mammalian Formins operate in the same manner as yeast Formins remains an open question to be addressed by future experiments.

In summary, the function of Formins is beginning to come into focus. They represent, along with Ena/VASP proteins, a novel class of actin binding proteins that can bind to the growing barbed end of actin filaments. This allows them to anchor growing actin filaments at particular locations in living cells and possibly change the dynamics of actin assembly. Combining this property with the ability to interact with microtubules puts them in a unique position to regulate cell polarity.

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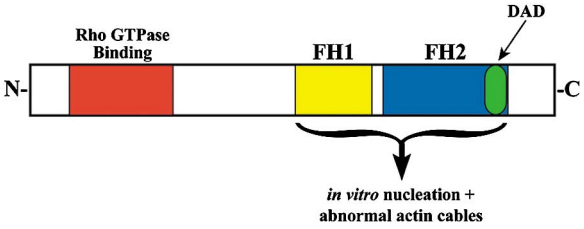


A Schematic Representation of the Formin Domain Structure

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# REV-ving Up the Clock

**Circadian rhythms are generated by a transcription/translation feedback loop consisting of two limbs, one positive and one negative. The nuclear orphan receptor, REV-ERBα, is identified as a molecular link coupling these two limbs.**

Circadian rhythms are daily cycles manifested in the physiology and behavior of virtually all organisms. Although these rhythms are endogenous, they are influenced by environmental factors such as light. In mammals, the central circadian pacemaker resides in the suprachiasmatic nucleus (SCN) of the hypothalamus, and is based upon the cyclic transcription and translation of core clock genes over a 24 hr period (Reppert and Weaver, 2001). Oscillations of clock gene RNA and protein levels form a feedback loop comprised of a positive limb and a negative limb. The positive limb, consisting of the transcription factors CLOCK and BMAL1, promotes transcription of the genes *Period* (*Per*) and *Cryptochrome* (*Cry*). PER and CRY form the negative limb by inhibiting the activity of CLOCK and BMAL1, and consequently their own transcription. PER and CRY protein turnover allows the loop to be reset. *Bmal1* transcript and protein levels also cycle, but with a phase opposite to that of PER and CRY. Transcription of *Bmal1* is self-inhibitory, and is possibly activated by PER2, one of three isoforms of PER; however, the mechanisms underlying *Bmal1* cycling are unknown. Recent work by Ueli Schibler's laboratory identifies REV-ERBα as a link between the positive and negative limbs of the feedback loop by establishing its role in cyclic *Bmal1* expression and by demonstrating its regulation by PER proteins (Preitner et al., 2002; see Figure).

REV-ERBα is a member of the ligand-activated nuclear receptor superfamily of transcription factors (McKenna and O'Malley, 2002). Because its activating ligand is unknown, REV-ERBα is called an "orphan" nuclear receptor. REV-ERBα acts as a transcriptional repressor and is implicated in the regulation of adipogenesis and metabolism. REV-ERBα first became linked to chronobiology when Schibler's laboratory demonstrated that its transcript level oscillates in mouse liver (Balsalobre et al., 1998). Importantly, all known circadian clock genes are expressed in, and cycle in, peripheral tissues. Expression of clock genes in the periphery may establish oscillators in individual organs that communicate with the central pacemaker to control local physiology.

In the current work, published in the July 26th issue of *Cell*, Schibler's group identified two binding sites for the REV-ERB subfamily of nuclear receptors in the *Bmal1* promoter. A complex that binds to these sites with a very striking daily oscillation was detected in liver

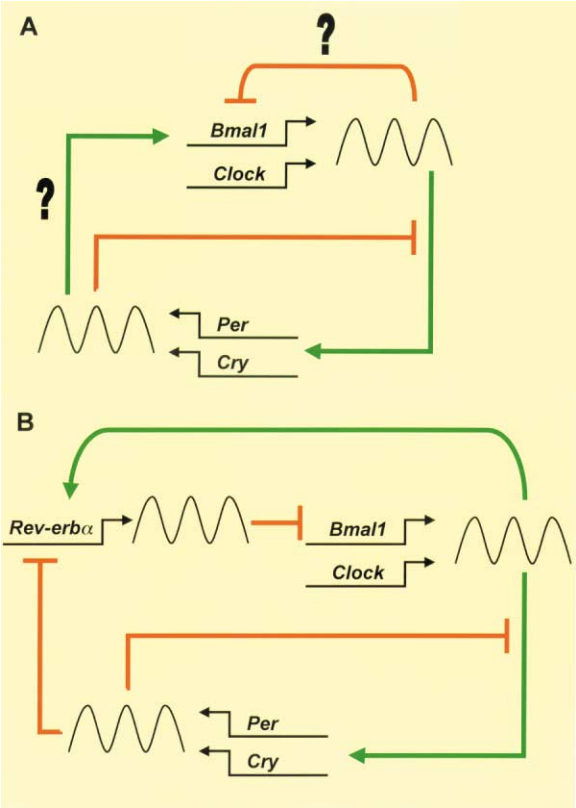
extracts. REV-ERBα was tentatively identified as the protein responsible for this oscillating complex because the phase of transcript and protein accumulation matched that of the complex. Notably, REV-ERBα accumulation is antiphasic to *Bmal1* accumulation, suggesting that REV-ERBα represses *Bmal1* transcription.

To examine REV-ERBα function in circadian rhythms, a knockout mouse model was created. The liver of the knockout animal displays nearly constant, high-level expression of *Bmal1*, strengthening the hypothesis that REV-ERBα represses *Bmal1* transcription. Additionally, *Clock* and *Cry1*, one of two *Cry* isoforms, do not cycle in the knockout mouse, suggesting that REV-ERBα also controls cyclic transcription of these genes. REV-ERBα does not control expression of every clock gene, however, because cyclic expression of both *Cry2* and *Per2* are unchanged in the knockout mouse. On a protein level, BMAL1 and CLOCK are high in knockout animals at all times of day, consistent with the constant high RNA level. However, CRY1 still oscillates despite the lack of mRNA cycling, indicating that transcript cycling is not necessary for protein cycling in all cases. This observation is similar to results found in insects (Yang and Sehgal, 2001).

Given the establishment of REV-ERBα as a negative regulator of *Bmal1*, and possibly *Clock*, a natural question is: how is circadian expression of *Rev-erb*α established? Since PER2 upregulates *Bmal1* expression, it may repress REV-ERBα activity to indirectly activate *Bmal1* transcription. To address this possibility, the authors examined *Rev-erb*α gene expression in *Per2* and *Per1/Per2* knockout mice. The peak of *Rev-erb*α gene expression cycling was considerably advanced in *Per2* mutant mice, and expression was constitutively high in double knockout animals. Taken together, these data suggest that the PER proteins are negative regulators of *Rev-erb*α. Additional evidence is presented that *Rev-erb*α is activated by the CLOCK/BMAL1 heterodimer. Thus, the negative effect of BMAL1 on its own transcription would be through upregulation of its own repressor (REV-ERBα).

All of the aforementioned molecular data involve the liver. However, *Rev-erb*α expression is also cyclic in the SCN, suggesting that it functions within the central pacemaker, and thus is likely to influence activity rhythms. With rodents, circadian activity is assayed by wheel running, and follows a repetitive cycle, or period, of approximately 24 hr. The *Rev-erb*α knockout mouse lacks cycling of *Bmal1* in the SCN. Surprisingly, though, the circadian phenotype of these animals is subtle. Knockout mice have shortened activity rhythm period lengths and a greater diversity in these lengths, suggesting that REV-ERBα is necessary for maintaining precision of the clock. Such a phenotype is in contrast to *Bmal1* knockout mice, which lack circadian activity rhythms altogether. These divergent effects suggest that expression of positive clock components is important for establishing the rhythm, and that oscillation of these components is necessary to maintain rhythm precision, especially in the face of environmental challenges. Consistent with this possibility, *Rev-erb*α deletion enhances phase shifting in response to a light pulse delivered in the latter half of the night.

The establishment of REV-ERBα as a molecular link between the positive and negative limbs of the molecular circadian clock is a great step forward in our understanding of molecular chronobiology. This discovery also raises intriguing questions regarding peripheral clock physiology. For example, deletion of *Rev-erb*α may have significant effects on liver physiology, especially with regard to adipogenesis and metabolism. Indeed, recent microarray studies have implicated entire metabolic pathways in the liver as being under circadian control (Akhtar et al., 2002; Panda et al., 2002; Storch et al., 2002). Moreover, evidence implicates other nuclear receptors as clock regulators, especially in the periphery. The retinoic acid receptor (RARα) and retinoid X receptor (RXRα) both affect circadian transcription in the peripheral vasculature in a ligand-dependent manner (McNamara et al., 2001). Moreover, glucocorticoids, ligands for another class of nuclear receptors, can reset the clock (Balsalobre et al., 2000). In the near future, nuclear receptors may emerge as the basis for tissue specificity in peripheral oscillators.




Rev-erbα Is a Link between the Positive and Negative Limbs of the Mammalian Transcription/Translation Circadian Feedback Loop

(A) A heterodimer of the basic helix-loop-helix transcription factors CLOCK and BMAL1 activates transcription of *Period* and *Cryptochrome*. PER and CRY inhibit the activity of CLOCK and BMAL1, thus establishing cyclic transcription and translation of *Per* and *Cry*, as indicated by the wavy line. *Bmal1*, and possibly *Clock*, are also cyclically transcribed and translated. *Bmal1* expression is both activated by PER and autoinhibited. How this occurs was unclear.

(B) REV-ERBα is a transcriptional repressor that inhibits *Bmal1* transcription. It is activated by BMAL1 and CLOCK and inhibited by PER (in particular PER2). This discovery explains the actions of BMAL1 and PER on *Bmal1* expression.

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# Coupled Zones of F-Actin and Microtubule Movement in Polarized Cells

**Interactions between the actin and microtubule cytoskeletons occur during cell polarization. Two papers in a recent issue of the *Journal of Cell Biology* use fluorescent speckle microscopy (FSM) to analyze the relationship between actin and microtubule movements in migrating epithelial cells and in polarizing neuronal growth cones.**

Cells are capable of polarizing in response to a variety of external cues and internal programs. While this ability depends on actin and microtubules, an intriguing question is how the two filament systems interact with each other during various types of cell polarization. The best-described physical interactions are those involving capture of microtubule ends at cortical sites and cotransport of cargo between polymer systems; there are also many intriguing examples of functional interactions, but their full mechanisms remain obscure.

Technical constraints have made it challenging to study the mechanistic details of microtubule and actin movements, together and in isolation. Both polymer systems are distributed throughout the volume of the cell in dense networks that undergo continuous, rapid turnover. This turnover is subject to regulation at many junctures, including nucleation, severing, polymerization and depolymerization, and transport. Both filaments can remodel their subunit position without gross changes at the light microscopic level (examples are poleward microtubule flux in the spindle or actin treadmilling). Abundant regulatory feedback loops and interdependences occur within each filament system, and probably between them, making linear, unidirectional scenarios seem overly simplistic.

The introduction of fluorescent speckle microscopy (FSM; Waterman-Storer and Salmon, 1997) helped immensely in imaging actin and microtubules. FSM uses low concentrations of fluorescent subunits to create fiduciary marks within the polymer that can be tracked relative to both ends, to other filaments, and to other structures. Unlike photoactivation and photobleaching, FSM does not require a laser, and the low concentrations of proteins or markers introduced are less perturbing than standard methods of live cell fluorescent imaging. FSM has been used to discriminate individual actin and microtubule filaments and to distinguish polymer dynamics from filament transport. In both of the studies discussed here (Salmon et al., 2002; Schaefer et al., 2002), the investigators have pushed the technological envelope of FSM, recording near simultaneous images of F-actin and microtubules and using a combination of processing algorithms (applied to confocal, deconvolved images in the first case) to enhance the final image. They used kymographs (compressed time-lapse montages) and on-line supplemental movies to illustrate the time dimension. (The movies are well worth viewing, and can be found linked to the papers at http://www.jcb.org).

## Actin-Microtubule Interactions during Cell Migration

The Waterman-Storer group used epithelial cells from an explant of newt lung, which migrate at a rate of ~100 μm/day, to ask whether actin and microtubule movements were directly coupled in migrating cells (Salmon et al., 2002). They described four zones of actin movements on the cell's ventral surface (the presumed source of traction) from the leading edge inward: the ruffling lamellipodium, the flat lamellum, the transition zone, and the cell body. Building on previous work (Waterman-Storer and Salmon, 1997), they described the retrograde flow of actin in the lamellipodium and lamellum, depolymerization and lack of movement in the convergence zone, and anterograde flow in the cell body. They then characterized the movements and dynamics of microtubules relative to the actin in these four zones, differentiating between microtubules that were parallel versus perpendicular to the lamella (perpendicular microtubules were thus parallel to the direction of actin flow). They found that perpendicular microtubules moved at the same rate and direction as actin in the cell body