The accumulation of a protein within a cell is determined by the rates of its synthesis and decay. Because only a minor fraction of all proteins actually executes rate-limiting functions, organisms are quite resilient to moderate changes in the concentrations of most proteins. However, some proteins must be regulated in a particularly precise manner, and this applies to components of the circadian clock, a biological device that regulates a range of physiological processes in many organisms, over a roughly 24-hour cycle. Two papers in a recent issue of Science, by Godinho et al. (1) and Busino et al. (2), and a recent study in Cell by Siepka et al. (3), exemplify the necessity of this precision by showing that mistimed degradation of two circadian clock proteins (cryptochromes) in the mouse causes their accumulation throughout the day. Their presence at the wrong time dampens the expression of other clock proteins and as a result, lengthens the period of the circadian cycle.

In mammals, most physiological processes such as sleep/wake cycles, heart rate, blood pressure, and metabolism oscillate in a daily cycle, influenced by the circadian clock (4). The rhythm-generating molecular circuitry in hypothalamic neurons and peripheral cells (3) relies on a negative-feedback loop involving the Cryptochrome (Cry1 and Cry2) and Period (Per1 and Per2) proteins. Cry and Per proteins are transcriptional repressors, and their expression is activated by a heterodimer containing the transcription factor Bmal1 and either of two other transcription factors, Clock or Npas2 (see the figure) (6). Once Per and Cry proteins reach critical concentrations, they form heterotypic complexes that bind to the Bmal1-Clock/Npas2 heterodimers and thereby annul their transcriptional activation potential. Consequently, Cry and Per transcription is reduced, Cry and Per protein accumulation falls below the concentrations required for autorepression, and a new cycle of Cry and Per expression can ensue. Although both Per and Cry proteins are dispensable in establishing the negative-feedback loop, the latter are the rate-limiting repressors of the molecular oscillator (7). Hence, the cyclic accumulation of Cry proteins must be controlled in a particularly rigorous manner.

Most short-lived proteins are degraded by the proteasome, a multisubunit molecular shredding machine. To be recognized by the proteasome, proteins must be tagged with multiple ubiquitin polypeptides on particular lysine residues. However, mammals express thousands of unstable proteins, and the question arises of how specificity of degradation by the proteasome is accomplished. This has now been solved for Cry proteins through biochemical and genetic experiments.

Busino et al. used mass spectrometry to identify Cry1 and Cry2 in a complex with Fbxl3, as revealed by coimmunoprecipitation of the proteins from cell lysates. Fbxl3 (which contains a motif called an F-box that mediates protein interactions) is a subunit of one of the more than 70 mammalian ubiquitin ligase complexes that recognizes targets for degradation by proteasomes. Specificity of the Fbxl3-Cry interaction was confirmed by showing that nine other F-box proteins did not associate with Cry proteins. Of these F-box proteins, only the overexpression of Fbxl3 reduced the stability of Cry2 in cultured cells. Perhaps more importantly, reduction of endogenous Fbxl3 messenger RNA (mRNA) by RNA interference (and the consequent decrease in Fbxl3 protein) abolished the cyclic expression of Cry and Per genes, presumably due to the continually high expression of the repressor proteins Cry1 and Cry2. Fbxl3 appears to influence clock gene expression specifically through its interaction with Cry proteins, because reducing Fbxl3 expression in mouse fibroblasts lacking Cry1 and Cry2 genes did not alter the constitutively high accumulation of Per1 and Per2 mRNAs in these cells.

By another approach, Godinho et al. and Siepka et al., used genetic screens in mice to search for mutations that affect circadian behavior. In both studies, mice were treated with a strong mutagen, and their offspring were examined for wheel-running activity in constant darkness (a condition in which the circadian oscillator is free-running). Whereas Godhino et al. analyzed animals for mutations that manifest themselves if only one of the two alleles is

Cry, no more. The mammalian circadian clock proteins Cry1 and Cry2 repress their own expression and that of the clock genes Per1 and Per2 in a negative-feedback loop. Once these clock genes reach a critical concentration, they form a complex that attenuates the transcription factors complex comprising Bmal1 (B) and Clock/Npas2 (CN). The negative-feedback loop drives robust circadian cycles only if Cry and Per mRNAs and proteins are short-lived. Fbxl3, a component of a specific ubiquitin ligase complex, participates in the proteasome-mediated decay of Cry proteins. Although Cry2 mRNA oscillates with weak amplitude, Cry2 protein displays robust oscillations.
affected (dominant, semi-dominant, or haploid-insufficient mutations), Siepka et al. also included recessive mutations (displaying phenotypes only when both alleles are mutated). Godinho et al. identified a mouse with a free-running circadian period length of ~24 hours, about 20 min longer than that of wild-type mice. This phenotype was called after hours (Afh), and positional cloning revealed Fbxl3 as the culprit gene for the deranged circadian locomotor activity. Sequencing identified a serine residue, rather than a cysteine residue, at position 358 in the mutated Fbxl3 protein. The peptide segment encompassing this mutated amino acid is involved in substrate recognition by Fbxl3. Indeed, Busino et al. found reduced affinity of mutated Fbxl3 for Cry proteins.

The importance of this evolutionarily conserved peptide segment is underscored by the study by Siepka et al. Again, the mutant phenotype, called overtime (Ovtm), was due to a mutation in Fbxl3. Sequencing revealed a mutation of an isoleucine to a threonine at position 364 of Fbxl3, six amino acids downstream of the residue change linked to the Afh phenotype. The Ovtm founder mouse was likely homozygous for the mutation, because it free-ran with a period of ~27 hours; mice homozygous for the Afh-associated mutation free-ran with a period of ~27 hours.

Despite the strong resemblance of the Afh and Ovtm phenotypes, however, Ovtm Fbxl3 bound to Cry only slightly less avidly than did wild-type Fbxl3 in cultured mouse cells. Moreover, the reduced abundance of Cry1 and Cry2 mRNA in the livers of Ovtm mice was not accompanied by equivalent changes in Cry1 and Cry2 protein accumulation. Nonetheless, the assignment of two independent mutations affecting circadian physiology to the same gene is unlikely to be a pure coincidence. Although it is difficult to reach statistical conclusions with the few circadian clock genes identified by “forward genetics” (using mutagenesis followed by screening to study gene function) (2, 3, 8, 9), the identification of Fbxl3 in two independent mouse mutant screens indicates that viable mutations affecting circadian clock functions are relatively rare in mammals.

Although groundwork for studying the regulation of Cry degradation has now been laid, two interrelated questions will have to be addressed. What signal triggers Fbxl3-Cry interaction? Is it a specific post-translational modification of Cry? The other question concerns the temporal regulation of Cry degradation rates. At least in liver, Cry2 protein accumulates with a markedly higher circadian amplitude than Cry2 mRNA (10). We do not yet know whether daily fluctuations in protein synthesis or decay rates account for this discrepancy. It may be that free Cry proteins are better substrates for Fbxl3-mediated degradation than Cry that is associated with Per proteins (see the figure). Now that we know that regulated protein destruction is essential to clock precision, deciphering its exact molecular mechanism is no longer a far cry away.

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GEOLOGY

Assessing Landslide Hazards

David K. Keefer and Matthew C. Larsen

O n 31 May 1970, a large earthquake shook the highest part of the Peruvian Andes. Millions of cubic meters of rock dislodged from a mountainside and initiated a rock avalanche that traveled more than 14 km in 3 min, burying a city and killing more than 25,000 people (1, 2). On 17 February 2006, a landslide of 15 million m$^3$ that initiated on a slope weakened by long-term tectonic activity buried more than 1100 people on Leyte Island in the Philippines (3).

Landslides such as these are a hazard in almost all countries, causing billions of dollars of damage and many casualties (4). Landslides also contribute to landscape evolution and erosion in mountainous regions (see the first figure). Here we discuss the latest strategies used to assess and mitigate landslide hazards.

The basic physics governing the initiation of landslides—the interactions among material strength, gravitational stress, external forces, and pore-fluid pressure—has been well understood for decades. The factors that govern whether landslide movements, once begun, will be catastrophic are less well understood. Nonetheless, much recent progress has been made in understanding those factors, as exemplified by basic research on fracture development in brittle materials (5) and on the properties of flowing material (6, 7).

Major causes of landslides are also well known, and these include rainfall, seismic shaking, human construction activities, landscape alteration, and natural processes of erosion that undermine slopes. Yet predicting just where and when a landslide will occur continues to be a complex proposition, because the properties of earth materials and slope conditions vary greatly over short distances, and the timing, location, and intensity of triggering events—such as storm precipitation or earthquake shaking—are difficult to forecast.

Two landslides at La Conchita in California illustrate the complexity of landslide occurrence and behavior. In 1995, a landslide consisting of a relatively coherent block of earth at La Conchita caused property damage but no fatalities. Ten years later, another landslide remobilized from the 1995 deposit, transformed rapidly into a highly fluid debris flow, and traveled downslope at a speed of 5 to 10 m/s, causing 10 fatalities (see the second figure) (8).

Current landslide hazard analyses and mitigation strategies tend to concentrate at one of two scales: intensive, site-specific analyses of individual slopes or landslide bodies, and regional-scale evaluations that seek to identify hazardous zones that are best avoided when construction is planned.