tissue-specific membrane trafficking pathway for GLUT4. Specifically, CHC22 copurifies with proteins important for sorting GLUT4 out of endosomes or the trans-Golgi network and into storage vesicles in human muscle cells. CHC22 colocalizes with GLUT4 in these vesicles, and its depletion results in the apparent loss of these vesicles. Conversely, expression of the human gene encoding CHC22 in mice resulted in abnormal glucose homeostasis. The number of GLUT4 storage vesicles appears to be increased. GLUT4 was poorly mobilized by insulin, as seen in humans with type 2 diabetes (see the figure).

The findings by Vassilopoulos *et al.* highlight the possibility that altered vesicle trafficking may contribute to diabetes pathophysiology, independent of impaired insulin signaling (*8*). CHC22-coated storage vesicles harboring GLUT4 may not be targeted by the insulin signal. In cultured adipocytes, for example, only about two-thirds of GLUT4 storage vesicles are translocated by maximal insulin stimulation (*5*, *9*). Why is the remainder of this pool inaccessible? Is the fraction of GLUT4 that can be translocated reduced in diabetes? Alternatively, GLUT4 may accumulate within storage vesicles because of deficient insulin signaling. Yet in the mice engineered to express human CHC22, activation of Akt, an enzyme that functions in one signaling pathway that affects GLUT4 translocation, was increased. Other signaling pathways have been implicated in both GLUT4 translocation and diabetes pathophysiology, and these may be important to mobilize vesicles formed by CHC22 (*10*–*12*).

Muscle contraction causes translocation of GLUT4 to enhance glucose uptake, similar to insulin, and it will be interesting to learn if the vesicles that are mobilized also have CHC22 coats. CHC22 appears to participate in trafficking GLUT4 storage vesicles in adipose tissue as well as in muscle. An interesting possibility is that GLUT4 storage vesicles shed their clathrin coats in response to insulin. Finally, it will be important to learn if obesity alters the abundance of CHC22 or of associated proteins, in either muscle or adipose tissue, to curtail insulin action.

Most studies on GLUT4 trafficking have used rodent models, in which CHC17 forms the GLUT4 storage vesicles (*13*, *14*), so it's not clear how CHC22 functions differently. For example, why does the diabetes-like phenotype of mice that express CHC22 become apparent with age; would a high-fat diet exacerbate the defect? A detailed analysis of the metabolic phenotype in these mice, possibly by infusions of insulin and a glucose tracer, should provide some answers.

The work of Vassilopoulos *et al.* highlights the differences between humans and mice by providing the first molecular identification of human-specific GLUT4 sorting. The exact role CHC22 may play in human diabetes remains uncertain. However, this work reminds investigators studying diabetes pathogenesis that it is important to think outside the vesicle.

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CELL BIOLOGY

It's the DNA That Counts

A simple genetic circuit that counts molecular events may be further developed to program complex cell behaviors.

Christina D. Smolke

Natural biological systems have evolved genetic programs that control complex activities through the coordinated processing of signals received from their environments. The engineering of synthetic biological systems to perform programmed information processing and computational functions has remained a challenge. Counters represent one class of information-processing systems and can be used to trigger events in response to a series of detected signals that are integrated and processed over time. Engineered biological counters would enable many applications, such as regulating cell death after a specified number of cell division cycles, controlling cell differentiation in response to temporal cues, noninvasive monitoring of aging, and recording the frequency of environmental events. On page 1199 in this issue, Friedland *et al.* (*1*) report an important step toward the construction of genetically encoded counters.

Counters can be assembled from a variety of simpler functions, such as signal detection and processing (the ability to respond to an input signal), time delay (the ability to integrate signals and trigger events after a delay from the initial detection event), and memory (the ability to remember and track earlier detection events). Genetic circuits that encode these basic operations have been demonstrated, including systems that use both protein-based transcriptional regulators (*2*–*4*) and RNA-based posttranscriptional regulators (*5*–*7*). For example, time delays have been encoded in cascades of transcription (the production of RNA from corresponding DNA) that trigger the production (or repression) of a protein (via translation of the corresponding RNA) upon the detection of an initial signal. This event subsequently activates the production (or repression) of the next protein. A linear sequence of such events, in the form of repeating modules, can be designed in which the last module triggers the production of the desired protein output (*8*, *9*).

Transcriptional cascades introduce delays in triggering the final protein output through the latency associated with expressing each intermediate protein in the series. Such cascading systems exhibit other properties including signal amplification, signal filtering, sensitivity to detecting the signal, and modulation of variation across cell populations (*8*). Other genetic mechanisms for introducing time delays in protein production are based on increasing the time associated with RNA processing steps (*10*).

Memory has been encoded in genetic circuits by means of feedback loops that lock a system in one state following a signal that sets that state. For example, a circuit based on the incorporation of two mutually inhibitory tran-

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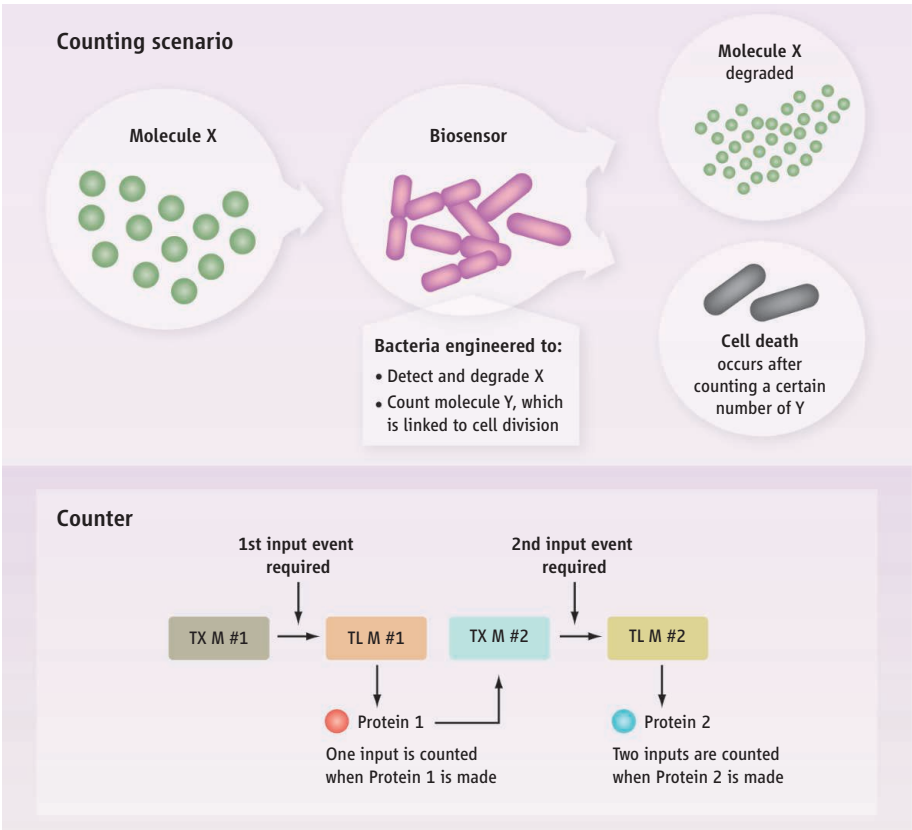

Why count, and how? (**Top**) In the hypothetical counting scenario shown, bacteria can be engineered to sense molecule X and degrade it (such as in bioremediation). At the same time, as a safety mechanism, the bacteria are engineered to count molecule Y, which is associated with cell division. After counting a certain number of molecule Y, the bacteria execute a cell death function. (**Bottom**) An example of a simple genetic counter that counts up to two, based on a conditional transcriptional cascade. The cascade includes transcription modules (TX M) and translation modules (TL M).

scriptional negative-feedback loops acted as a genetic toggle switch, in which transient input signals moved the system between two states (*11*). Each state was associated with the production of a protein that inhibited the system from moving into the other state unless the appropriate input signal was applied. In a different memory circuit, based on an autoregulatory transcriptional positive-feedback loop (*12*), a transient input signal triggered the production of a protein that subsequently activated its own production, such that the system remained in the activated state after removal of the signal.

Early attempts to build heritable memory systems that do not require the sustained production of proteins were based on enzymatic mechanisms that allow the system state to be written directly into the structure of DNA (*13*). To date, these systems have been developed with recombinases, enzymes that can invert, insert, or remove specified DNA sequences and thus dynamically rewrite genetic programs in response to specified signals. For example, a genetic circuit based on two overlapping inversion systems encoded multiple output states depending on the order in which recombinases processed the DNA (*14*).

Friedland *et al.* take important steps to build biological counters by integrating these functional operations. The authors propose two different circuit architectures for encoding counters that trigger the expression of a desired protein following the processing of two or three input signal pulses. Each counter combines a time-delay operation (triggered by the initial detection event—setting the "1" state) with conditional regulation linked to the immediate detection of subsequent signals (allowing the counting of additional detection events "2" and "3"). In one system, the time delay is encoded through a transcriptional cascade operation. Conditional regulation is achieved through an RNA molecule that is expressed only when the input signal is present. The expression of this RNA is under the control of a protein-based transcriptional regulator and is required for the expression of the protein output from the transcriptional cascade modules. Because of the delay associated with protein accumulation from each transcription module and difference in decay rates between the protein and RNA components, the RNA regulator decays before a sufficient amount of the protein output from the previous module has accumulated to trigger activation of the next module. This resets the conditional regulation operation and allows counting of input pulses (see the figure).

In their second system, the time-delay operation is encoded within a heritable memory cascade. Each memory module encodes a protein that flips a segment of DNA within that module to turn off its own expression and primes the next module to be activated by the input signal by correctly orienting a protein-based transcriptional regulatory element. The time required for each memory module to prime the next module is longer than the signal pulse length such that counting is achieved. By incorporating memory modules, this second architecture allows signals to be integrated over longer time frames, as the priming of the module is "hard-coded" into the DNA. In addition, this architecture could allow identical or different input signals to be counted via the choice of each module's conditional regulatory element.

Friedland *et al.* provide examples from which to build more sophisticated counters. An important next step will be to develop circuits that can report on intermediate states, in addition to the final state. Another critical counter property will require the development of circuits that allow counters to distinguish between continuous and transient signals. Given the broad applications of counters that operate inside living cells, the continued development of next-generation genetically encoded counters will be of critical importance to synthetic biology (*15*).

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