

of the β -hairpin with the base of the A1 domain is unexpected and may account for the ability of VWD type 2B mutations to increase the binding affinity.

The A1 domain contains a compact α/β fold delimited by a single disulfide loop. In the free A1 domain, the sequence extending on the amino-terminal side of the disulfide bond lies against the α/β surface (8), and this amino-terminal extension is displaced when GpIb α binds (3). The amino-terminal extension is inhibitory: Recombinant A1 domains lacking this extension bind 5- to 10-fold more tightly to GpIb α (9). The structure of another high-affinity A1 domain mutant with the substitution I546V suggests that conformational changes are propagated from the mutation through the A1 domain to the GpIb α binding site at the top (10). Many VWD type 2B mutations, however, are within the amino-terminal extension or affect residues that it contacts, suggesting that the mutations mainly promote the displacement of the amino-terminal extension and facilitate binding of the GpIb α β -hairpin (3). The relative importance of these two mechanisms remains to be determined.

The mutations in GpIb α that cause platelet-type pseudo-VWD also stabilize the A1-GpIb α complex, but by a different mechanism. As determined by Huizinga *et al.* (3) and independently by Uff *et al.* (11), the β -switch loop is flexible and lacks defined secondary structure in uncomplexed GpIb α . Upon binding to the VWF A1 domain, the β -switch region forms a two-stranded antiparallel β sheet that aligns with the central β sheet of A1. Mutations in GpIb α that increase the affinity of this platelet glycoprotein for VWF A1 are located in the β -switch region and are predicted to stabilize the β sheet (3). Because VWD type 2B mutations and platelet-type pseudo-VWD mutations affect widely separated binding sites, one might expect them to be additive. Indeed, binding affinity was increased two- to threefold by either type of mutation singly, and fivefold when the mutations were combined (3).

The structure of the VWF A1-GpIb α complex challenges several widely held notions about how the proteins might interact. For example, the properties of chimeric GpIb α proteins suggest that LRRs 1 to 4 may be important for binding (12). Instead, the structure reveals that there is one contact with LRR 1 and multiple contacts with LRRs 5 to 8 at the opposite end of the GpIb α fragment. An anionic region closer to the carboxyl-terminal than the β -switch region contains three sulfated tyrosine residues (see the figure). Mutations that affect these tyrosine

residues reduce binding of GpIb α to VWF (13) and impair cell adhesion (14), suggesting that they might bind to VWF A1. However, this anionic region does not contact A1 in the complex, and Huizinga *et al.* could not show that deleting the anionic region reduced the binding affinity (3). Additional studies are needed to reconcile these observations.

The large structural changes associated with binding of GpIb α to VWF A1 are certain to breathe new life into studies of how protein conformation modulates platelet adhesion. The bidentate character of the complex suggests that binding (and dissociation) might develop in stages, so that a weak interaction at one site could evolve into a tight interaction involving both sites. If so, the present structure presumably represents the final embrace rather than the first molecular touch, which raises the question of whether certain conditions *in vivo* might help to tighten an initial low-affinity interaction between platelets and VWF. Perhaps required conformational changes are induced by surface binding of VWF or by fluid shear stress, or perhaps they simply develop in the course of binding. Many studies measuring the binding affinity of native VWF for GpIb α have obtained values for the dissociation constant

(K_d) of $>1 \mu\text{M}$, much higher than the 30 nM calculated by Huizinga *et al.* (3). This suggests that there are uncharacterized inhibitory interactions involving sites outside of the A1 and GpIb α domains represented in the crystal structure. The answers to such questions may be clinically relevant. For example, they may determine whether one or both of the identified binding interfaces between A1 and GpIb α could be useful targets for the prevention or treatment of thrombosis.

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PERSPECTIVES: GENETIC NETWORKS

Small Numbers of Big Molecules

Nina Fedoroff and Walter Fontana

B iologists tend to think deterministically. A case in point is their prolonged search for the "founder cells" of the slime mould *Dictyostelium*. These amoebae emit pulsatile cAMP signals under starvation conditions, mobilizing neighboring cells to surround them and form a motile multicellular slug that wanders off to form spores. Despite their efforts, biologists never could find the founder cells. The reason is that all *Dictyostelium* amoebae have the capacity to produce cAMP signals, and becoming a founder cell is a matter of chance—it's a stochastic process (1).

The issue of stochasticity—randomness or "noise," if you prefer—in living systems has been addressed theoretically (2–4). Two recent papers (5, 6), including

one by Elowitz and colleagues on page 1183 of this issue (6), provide direct and elegant experimental evidence that functional interactions among cellular macromolecules involved in gene expression are "noisy." Stochasticity arises because of the very small number of macromolecules involved in certain biological processes. Small numbers mean that both the randomness of molecular encounters and the fluctuations in the transitions between the conformational states of a macromolecule become noticeable.

The two new studies both measure the stochasticity of gene expression in bacteria using green fluorescent protein (GFP) reporter genes under the control of promoters regulated by the Lac repressor. Elowitz and colleagues (6) distinguish between different sources of noise, which they term "intrinsic" (inherent in the biochemical process of gene expression) and "extrinsic" (due to fluctuations in other cellular components required for gene expression).

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As they define it, intrinsic noise is the difference in gene expression that arises between two identical copies of a gene expressed under precisely the same conditions. Their experimental approximation of this ideal is two virtually identical Lac-repressible GFP reporter genes inserted in the *Escherichia coli* chromosome on opposite sides and roughly equidistant from the origin of replication. The genes encode the cyan and yellow forms of GFP, which can be quantified by monitoring fluorescence intensity at their respective emission peaks. Deviations from equal gene expression within cells are interpreted as evidence for the intrinsic stochasticity of gene expression. Extrinsic noise is operationally defined as the cell-to-cell variation in expression levels of each reporter gene, which the authors attribute to variations in the output of other proteins that affect GFP gene expression.

Although one could argue that the inside of a bacterial cell is not a well-stirred pot and that the distinction between extrinsic and intrinsic noise is not perfect, this may be as good as it gets. Elowitz *et al.* ask how noise varies with the transcription rate, which they alter both genetically and by varying the concentration of isopropyl β -D-thiogalactopyranoside (IPTG), a compound that inactivates the Lac repressor. They make the interesting observation that the relationship between transcription rate and noise is different for the intrinsic and extrinsic components. Not unexpectedly, intrinsic noise decreases monotonically as transcription is cranked up. Extrinsic noise, however, initially increases, peaks at intermediate transcription levels, and then declines at high IPTG concentrations. The simplest interpretation is that fluctuations in the pool of Lac repressor proteins have the greatest impact at intermediate levels of transcription: when neither the GFP gene promoters are fully occupied by repressors (no IPTG), nor the repressor population is fully inactivated (high IPTG). Extrinsic noise is the dominant component over most of the IPTG concentration range, implying that most of the noise in their experiments came from fluctuations in the number of Lac repressor proteins regulating expression of the GFP reporter genes.

In a complementary study, Ozbudak *et al.* (5) also use a bacterial system (in this case, *Bacillus subtilis*), but with only one

Lac-repressible GFP gene. Thus, the authors were restricted to measuring total noise (intrinsic plus extrinsic). They, too, adjusted the transcription rate by varying the concentration of IPTG, as well as by engineering mutations in the GFP gene's promoter. However, they found a weak positive correlation between total noise and transcriptional efficiency. Part of the discrepancy between these reports resides in a difference in how the two groups normalize the variance. One group's noise is the variance divided by the mean (5),



Is uncertainty the only thing that is certain? (13). Shown are two versions of the painting by George Seurat, *A Sunday Afternoon on the Island of La Grande Jatte*. (Top) The coarse-grained version represents the noise level affecting gene expression in the cell. (Bottom) The resolved version illustrates noise reduction and raises the question of whether different pictures (and if so, what number) could have been obtained by different noise suppression mechanisms. Far from being a nuisance, noise in living systems contributes to adaptation and evolution.

whereas the other's is variance divided by the square of the mean (6). But there are differences between the experimental systems as well, such as the bacterial background and the Lac repressor gene itself. Of particular interest in the Ozbudak *et al.* study is their finding that when the translational efficiency was varied by using point mutations that affect the ribosome binding site, there was a strong positive correlation between translational efficiency and noise. These experimental results support the group's theoretical predictions (4) and lead them to conclude that inefficient translation of the mRNA encoded by

an infrequently transcribed gene is an effective mechanism for decreasing fluctuations in the concentration of the protein. They suggest that the inefficient translation of regulatory genes, such as the bacteriophage λ *cI* gene, may have been selected precisely for this low-noise property.

Indeed, we generally think of noise as a nuisance, leading to errors and necessitating noise-suppression mechanisms (see the figure). von Neumann's 1956 paper (7) on how to make reliable organisms from unreliable components suggests that the answer is redundancy, using multiple copies of imperfect components. This notion is rather close to contemporary concepts of genetic redundancy, emerging from the awareness that genomes grow by duplication and that most genes are members of gene families (3). But it is increasingly apparent that this is not the only way that organisms achieve reliability, given that lethal mutations in yeast are just as likely to result from the elimination of duplicated genes as from the elimination of unique genes (8). So how do organisms manage—and perhaps even capitalize on—molecular noise? Becskei and Serrano provide experimental evidence that autorepression (repression of a gene by its protein product) is a genetic noise-reduction device (9). As noted, inefficient translation reduces fluctuations in proteins required at low concentrations. There is also increasing awareness of the ways in which the properties of cell signaling pathways, circadian clocks, and developmental switches minimize or amplify the effects of fluctuations. Elowitz *et al.* report that noise increases when the Lac repressor gene is on a multicopy plasmid (probably because of cell-to-cell variations in plasmid copy number) and when the *recA* gene is deleted, perhaps leading to transient copy number differences between different parts of the chromosome. Noise also increases when Lac repressor expression is rendered oscillatory by a simple feedback genetic circuit. Oscillatory processes are everywhere in living systems. The principles that impose coherence upon the behavior of collections of noisy oscillators, whether they are the cells of a *Dictyostelium* slug or those of a mammalian heart, are lively areas of investigation.

But fluctuations in living systems may be much more than just a nuisance. Living systems change constantly: Organisms develop, they make physiological adjustments to internal and external cues, and they age. What are the sources of this constant unfolding, adaptation, and change? The deterministic view of stable states (of a gene network, for example) requires exogenous agents of change to initiate transi-

tions, whether these transitions be developmental or adaptive. If stochasticity is a fact of life, states are by definition metastable, and fluctuations can cause transitions between them. We are comfortable with the notion that antigenic phase variation in bacteria is stochastic (10), but might not stochastic mechanisms equally underlie the well-orchestrated, seemingly deterministic progression of states we call organismal development? We recognize the importance of the stochastic genetic mechanisms that generate diversity in the immune system. When coupled to suitable feedback mechanisms, such as the clonal amplification of cells expressing a particular antigen, these constitute a powerful means of learning, of crafting appropriate responses to unforeseen situations. Will we discover an analogous role for stochastic gene activation?

Stochasticity is inherent in all biological processes and it can be argued that the proliferation of both noise and noise-reduction systems is a hallmark of organismal evolution. One of us (11) has sug-

gested that the reason it took so long to figure out that most genomes, including our own, are stuffed with transposons is because they "jump" from one chromosomal site to another so rarely that they are almost invisible genetically. Genome growth, dependent on the stochastic processes of gene duplication and transposition, may well have necessitated the prior invention of ways to suppress the inevitable countervailing deletions and genome scrambling caused by homologous recombination. Adrian Bird (12) has suggested that the inherent imprecision of gene regulation also sets an upper limit upon gene numbers. He has argued that the jump in gene numbers that accompanied the prokaryotic-eukaryotic transition was made possible by the accretion of multiple transcriptional noise-reduction mechanisms including chromatin, DNA methylation, the separation of the transcriptional from the translational apparatus, and the introduction of a complex quality control machinery into the production of mRNAs. And so the question is

this: To what extent is the seemingly inexorable increase in complexity that we call evolution driven by the counterpoint of noise and noise reduction, of chance and the necessity of inventing and accumulating mechanisms to render coherent its random gifts?

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PERSPECTIVES: PHYSICS

Resolving Physical Processes on the Attosecond Time Scale

Maciej Lewenstein

Many molecular and atomic processes occur on a femtosecond time scale ($1 \text{ fs} = 10^{-15} \text{ s}$), which can be probed with coherent laser excitation. Now physicists are reaching for the next frontier. On page 1144 of this issue, Kienberger *et al.* (1) lead the way toward resolving physical processes on the even faster attosecond scale ($1 \text{ as} = 10^{-18} \text{ s}$).

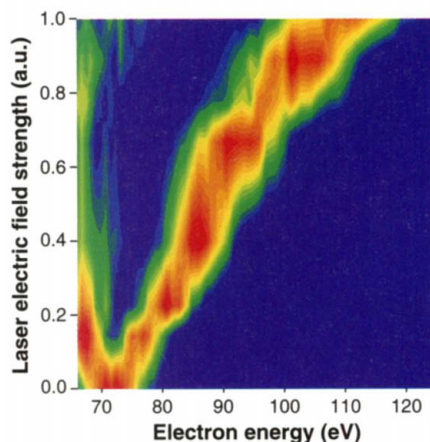
To achieve fast time resolution, the duration of the laser pulse must be controlled. With two short laser pulses, one can perform "pump-probe" experiments, in which the first pulse excites the system and the second pulse probes the resulting dynamics. For this method to work, the pulses must be shorter than the characteristic time scale of the dynamics in question.

A laser pulse cannot be shorter than the oscillation period of the electromagnetic field in the pulse. Hence, for a laser with a wavelength λ of $\sim 800 \text{ nm}$, the shortest pulse duration is ~ 4 to 5 fs . In time-resolved spectroscopy, such femtosecond pulses are used, for instance, to

monitor the dynamics of molecular reactions. However, tightly bound electrons in atoms and ions cannot be observed with femtosecond techniques, because their dynamics occur typically on the attosecond time scale.

To decrease the pulse duration beyond the femtosecond time scale requires coherent sources of ultraviolet and soft x-ray radiation. A method called high order harmonic generation (HHG) (2) offers a possibility of realizing this task. When an atomic gas is irradiated with an intense femtosecond pulse, the gas produces harmonics of the laser field. The frequencies of these harmonics are odd integers of the laser frequency (ω_L) and extend up to few hundred ω_L (3). They reach wavelengths of a few nanometers and periods in the attosecond regime.

In a macroscopic medium, HHG requires constructive interference (phase-matching) between the contributions of individual atoms. According to the "simple man's model" (4, 5), the HHG process occurs in three steps. First, the laser field causes an electron to tunnel to those regions in space where interactions with its nucleus are practically negligible. It then



How to steer electrons. The shift of this photoelectron energy spectrum toward higher energies with increasing laser field represents "steering" of the electronic wavepacket. [From (1)]

oscillates in the laser field as a free charge. If it comes back to the nucleus, it may recombine, emitting harmonics. This model has a solid quantum mechanical basis (6); in particular, it incorporates quantum mechanical interferences between various electronic trajectories.

Antoine *et al.* predicted (7) that the harmonics, generated in a macroscopic medium under phase-matching conditions, are locked in phase. Twice in a laser period T_L , groups of neighboring harmonics interfere constructively for a very short time interval, producing a train of attosecond pulses separated by $T_L/2$. The first indirect evidence that HHG generates at-

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