However, Monod *et al.*'s reasoning applies not only to the destruction of a symmetrical oligomer but also to its creation: Any change that can generate a favorable subunit contact in an oligomer with cyclic *n*-fold symmetry (C_n) will do so 2*n* times, leading to dimerization, if the contact is compatible with dihedral *n*-fold (D_n) symmetry. Contacts not compatible with such a symmetry can still promote subunit association but will generate an infinite helical fiber rather than a closed structure. A classical example is sickle-cell anemia, in which a point mutation in the β chain of hemoglobin causes the protein to polymerize.

In other systems, conformation changes rather than mutations may cause the formation of a fiber, and this can also lead to dimerization. The prion protein implicated in neurodegenerative diseases (such as bovine spongiform encephalopathy in cows and Creutzfeldt-Jakob disease in humans) undergoes both types of self-association under different conditions (*6*). Thus, fiber formation and dimerization are related, frequently observed, and possibly competing natural processes.

Grueninger *et al.* have now achieved both dimerization and fiber formation by site-directed mutagenesis, engineering new subunit contacts in five different bacterial proteins. They used the known crystal structure of these proteins to model assemblies with a two-fold symmetry and to select amino acid substitutions that introduce nonpolar side chains at the modeled interface. They then generated the mutations, purified the corresponding mutant proteins, and checked their molecular weight by size-exclusion chromatography and dynamic light scattering. They also crystallized several mutants and elucidated their structures to validate their solution data.

In the cases of 6-phospho-β-galactosidase and *O*-acetylserine sulfhydrylase, several mutant proteins showed various degrees of dimerization in solution but did not crystallize. In the cases of urocanase and L-rhamnulose-1-phosphate aldolase (Rua), substitution of one or a few residues resulted in complete dimerization, and crystal structures confirmed the presence of extensive new subunit interfaces involving the designed contacts. Urocanase is a C_2 dimer; substitution of three surface residues yields a tetramer with approximate D_2 symmetry. Rua is a C_4 tetramer; single substitutions convert it to two different octamers (one with the D_4 symmetry of Monod's eight-dice assembly, the other with a lower symmetry). A different Rua variant with three substitutions aggregates into fibers.

In another system described by Grueninger *et al.*—the bacterial MspA porin—dimerization results from a deletion. The porin (a membrane-bound C_8 octamer) becomes a soluble D_8 16-mer after the membrane-immersed part is deleted. A crystal structure confirms that the contact between octamers involves the large protein surface, mostly nonpolar, revealed by the deletion. Thus, the set of proteins prepared by Grueninger *et al*. shows a variety of responses to point mutations. Not all the engineered assemblies have the expected symmetry; some are only marginally stable, or they aggregate instead of dimerizing. But on the whole, the data indicate that it is relatively easy to convert a protein with *n* identical subunits into one with twice that number, at least for $n > 1$.

The successful dimerization of three bacterial proteins by Grueninger *et al.* has implications for the evolution of protein quaternary structures. The new subunit contacts created by mutation or deletion must show some specificity, because the mutant proteins do not aggregate in the crowded bacterial cytoplasm, where they fold and assemble correctly. How can specificity arise in the absence of natural selection?

The answer may be negative selection (*7*). In a cytoplasmic protein with *n* subunits and C_n symmetry, nonpolar side chains must be disallowed at any surface site where they could stabilize an assembly with 2*n* subunits and D_n symmetry. Mutations not compatible with that symmetry still promote aggregation, but a helical fiber lacks the multiple contacts that create cooperative effects in a D_n assembly. Thus, a single mutation may not suffice to stabilize a fiber, unless the protein is present at a very high concentration, such as hemoglobin in red blood cells.

References

1. K. U. Linderström-Lang, J. A. Schellman, in *The Enzymes*, P. D. Boyer, Ed. (Academic Press, New York, ed. 2, 1959), vol. 1, pp. 443–510.
2. J. Monod, J. P. Changeux, F. Jacob, *J. Mol. Biol.* **6**, 306 (1963).
3. J. Monod, J. Wyman, J. P. Changeux, *J. Mol. Biol.* **12**, 88 (1965).
4. D. Grueninger *et al.*, *Science* **318**, 206 (2008).
5. T. V. Borchert, R. Abagyan, R. Jaenicke, R. K. Wierenga, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 1515 (1994).
6. M. J. Bennett, M. R. Sawaya, D. Eisenberg, *Structure* **14**, 811 (2006).
7. D. B. Lukatsky, B. E. Shakhnovich, J. Mintseris, E. I. Shakhnovich. *J. Mol. Biol.* **365**, 1596 (2007).

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GEOPHYSICS

What Triggers Tremor?

Tidal forces and distant earthquakes can increase the low-frequency rumbling that occurs where tectonic plates collide.

Eliza Richardson and Chris Marone

An enduring mystery in geophysics is why the periodic stresses of tides do not commonly trigger earthquakes. If earthquakes simply represent reaching some threshold for the failure of rock, tidal forces should often trigger faults near failure by pushing them over the threshold. However, many studies show that this is not the case, except in special situations (*1, 2*).

Now, on page 186, Rubinstein *et al*. report that a seismic activity called nonvolcanic tremor is indeed triggered by lunar-solar tides (*3*) and, in some cases, as reported on page 173 by Gomberg *et al*., by distant earthquakes (*4*). Discovering how dynamic stresses trigger fault failure may provide important clues about the onset of earthquake rupture.

Earth tremor episodes involve quasi-continuous emissions of low-frequency seismic energy that last longer than ordinary earthquakes. Tremor is well known in volcanic settings, where it is associated with magma movement and fluid-cavity resonance (*5*, *6*). The recent discovery of tremor in nonvolcanic settings (*7*) has inspired multiple groundbreaking studies (*8–14*). An emerging question is how nonvolcanic tremor relates to aseismic (that is, radiating no measurable seismic energy) fault slip; they occur simultaneously and in predictable, periodic episodes in at least two locations where they have been studied extensively (*8*, *15*, *16*). The association of nonvolcanic tremor with fault slip suggests the tantalizing possibility that tremor could be used in earthquake forecasting, as it is in predicting volcano eruption (*5*).

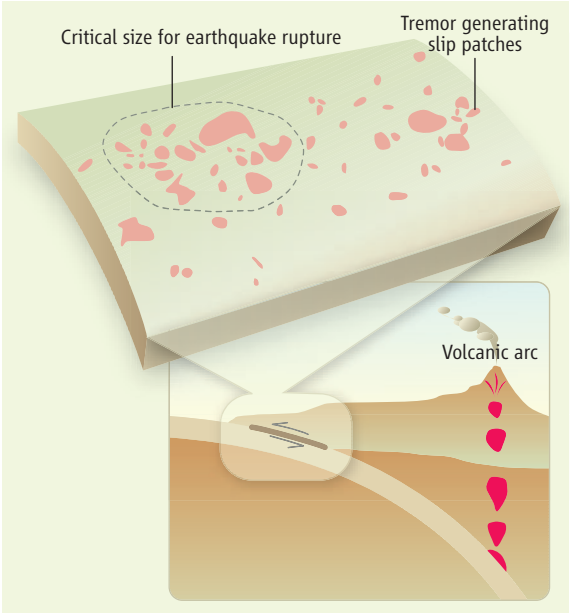
The authors are at the Istituto Nazionale di Geofisica e Vulcanologia, Rome, Italy, and the Department of Geosciences, Pennsylvania State University, University Park, PA 16802, USA. E-mail: eliza@geosc.psu.edu

Rubinstein *et al*. found a clear correlation between the dominant tidal forcing (at periods of 12.4 and 24 hours) and the amplitude of tremor along the Cascadia subduction zone offshore Vancouver Island, Canada. Tremor and aseismic fault slip recur periodically every 14 months in this area (*8*, *13*), and tidal stressing modulates tremor amplitude only during times of active tremor (*3*). This is an important point. The work of Rubinstein *et al.* (*3*) shows that tides are not sufficient to trigger tremor at arbitrary times during the cycle of repeated failure. In this sense, their result is actually quite similar to tidal triggering of volcanoes and ordinary earthquakes, when tides primarily trigger earthquakes during times of extreme activity, for example, just before or after large earthquakes (*1*, *2, 17*).

In contrast, a recent study (*14*) shows that the Cascadia tremor was triggered early in the failure cycle by the passage of surface waves from the magnitude 7.8 Denali, Alaska, earthquake on 3 November 2002. The Denali earthquake triggered the Cascadia tremor roughly 3 months before the predicted tremor episode in February 2003. Surface waves from Denali caused a spike in shear stress on the subduction interface at Cascadia (*14*), much higher than the stress from tides (*3*). This implies that tremor can be induced at arbitrary times during the seismic cycle as long as the perturbation amplitude is sufficient and its orientation is favorable to shear failure. Laboratory observations of triggered frictional stick-slip corroborate the idea that larger-amplitude perturbations are needed to trigger failure earlier in the seismic cycle (*18*). In addition, both laboratory experiments and earthquake studies suggest that a complex combination of amplitude and frequency of dynamic stressing determines the triggering threshold (*19*).

The mechanism of nonvolcanic tremor is poorly understood. Before the work of Gomberg *et al*., all studies except one (*9*) have observed tremor where tectonic plates are subducting. As a result, explanations have focused on fluid migration and thermal release of volatile elements in subduction zones. The discovery by Gomberg *et al.* that surface waves from the Denali earthquake triggered tremor in multiple locations along the North America-Pacific plate boundary in California suggests that these may not be the only viable mechanisms. Theoretical studies based on laboratory data suggest that transient, aseismic slip can arise spontaneously given the right fault rheology (*20*). This scenario is consistent with recent studies in Japan (*10–12*) suggesting that nonvolcanic tremor arises from shear failure on quasi-dynamic slip patches that radiate low-frequency seismic energy. Shelly *et al*. (*12*) show that tremor can be explained as a swarm of slow, low-frequency earthquakes. Thus, a central remaining question is: What causes slow fault slip? Special conditions appear to be necessary to limit acceleration during shear instability (*21*), yet seismic observations of slow and silent earthquakes indicate that this mode of deformation is widespread (*22, 23*).

Tremor may provide clues about the onset of earthquake rupture. All earthquakes are thought to nucleate as small patches of unstable slip, with some events becoming large earthquakes given the right combination of energy release and spatial homogeneity of fault strength. Tremor may represent slip on a series of subcritical fault patches that radiate low-frequency energy (see the figure), reflecting slip acceleration before reaching a critical size associated with fully unstable behavior and high-frequency seismic radiation. This idea is consistent with results from studies of small earthquakes, which document the acceleration of rupture velocity and the resulting initiation of high-frequency energy radiation associated with fast earthquake rupture (*24*, *25*).



Tides and temblors trigger tremor. Volcanic earth tremor is often generated by movement of magma. Nonvolcanic tremor may represent swarms of small slip patches (red shaded regions) that radiate only low-frequency energy. Rubinstein *et al.* and Gomberg *et al.* show that periodic stress caused by tides and remote seismic activity can increase nonvolcanic tremor. The patches may then coalesce (dashed line) to produce large earthquakes.

The studies by Rubinstein *et al.* (*3*) and Gomberg *et al*. (*4*) can guide future efforts to characterize nonvolcanic tremor, both in terms of observations and through laboratory experiments. For example, how widespread is the occurrence of tremor far from subduction zones? Is nonvolcanic tremor triggered by small earthquakes or vice-versa? What is the recipe for triggering tremor via stress transients (i.e., what roles do amplitude, frequency, time during the seismic cycle, or orientation of the remote perturbation play?)? How do tremor, earthquakes, low-frequency seismicity, and aseismic slip interact in time and space? Earth tremor represents one of a wide spectrum of behaviors ranging from aseismic fault creep to ordinary fast earthquakes. Future geodetic and seismic networks should help us learn much more about these phenomena.

References

1. M. F. Tolstoy, F. Vernon, J. Orcutt, F. Wyatt, *Geology* **30**, 503 (2002).
2. E. S. Cochran, J. E. Vidale, S. Tanaka, *Science* **306**, 1164 (2004).
3. J. L. Rubinstein, M. La Rocca, J. E. Vidale, K. C. Creager, A. G. Wech, *Science*, **319**, 186 (2008).
4. J. Gomberg *et al.*, *Science* **319**, 173 (2008).
5. S. R. McNutt, *Ann. Rev. Earth Planet. Sci.* **33**, 461 (2005).
6. L. Burlini *et al*., *Geology* **35**,183 (2007).
7. K. Obara, *Science* **296**, 1679 (2002).
8. G. Rogers, H. Dragert, *Science* **300**, 1942 (2003).
9. R. M. Nadeau, D. Dolenc, *Science* **307**, 389 (2005).
10. D. R. Shelly, G. C. Beroza, S. Ide, S. Nakamula, *Nature* **442**, 188 (2006).
11. S. Ide, D. R. Shelly, G. C. Beroza, *Geophys. Res. Lett.* **34**, 10.1029/2006GL028890 (2007).
12. D. R. Shelly, G. C. Beroza, S. Ide, *Nature* **446**, 305 (2007).
13. M. Brudzinski, R. M. Allen, *Geology*, **35**, 907 (2007).
14. J. L. Rubinstein *et al., Nature*, **448**, 579 (2007).
15. M. M. Miller, T. Melbourne, D. J. Johnson, W. Q. Sumner, *Science* **295**, 2423 (2002).
16. K. Obara, H. Hirose, F. Yamamizu, K. Kasahara, *Geophys. Res. Lett.* **31**, 10.1029/2004GL020848 (2004).
17. J. Kasahara, *Science* **297**, 348 (2002).
18. H. Savage, C. Marone, *J. Geophys. Res.* 112, B02301, 10.1029/2005JB004238 (2007).
19. R. M. Harrington, E. E. Brodsky, *Bull. Seismol. Soc. Am.* **96**, 871 (2006).
20. J. R. Rice, J.-C. Gu, *Pure Appl. Geophys.* **121**, 187 (1983).
21. M. Roy, C. Marone, *J. Geophys. Res.* **101**, 13919 (1996).
22. G. C. Beroza, T. Jordan, *J. Geophys. Res.* **95**, 2485 (1990).
23. J. J. McGuire, M. Boettcher, T. H. Jordan, *Nature* **434**, 457 (2005)
24. E. Richardson, T. H. Jordan, *Bull. Seismol. Soc. Am.* **92** , 1766 (2002).
25. R. E. Abercrombie, J. R. Rice, *Geophys. J. Int.* **162**, 406 (2005).

10.1126/science.1152877