targeting following bacterial escape into the cytosol is not yet known.

Caspase-11 is now recognized as an important part of the inflammatory arsenal that can be either protective (3) or damaging depending on the physiological context of its activity (6, 7). Yet, the study of Aachoui et al. raises two major questions. What is the signal(s) that activate caspase-11? A “noncanonical inflammasome” has been proposed to activate caspase-11 (6), but no molecular players have yet been identified. And what are the substrates of caspase-11 that promote pyroptosis? These gaps in understanding of caspase-11 function will be important topics for future studies.

There is a dynamic interplay between caspases and bacterial pathogens. Some bacteria use caspases to promote their pathogenesis. For example, Listeria monocytogenes exploits caspase-7 to repair bacteria-induced damage to the plasma membrane to preserve its replicative niche (12). Similarly, S. typhimurium exploits caspase-3 to cleave and activate a virulence factor, SipA (13). By contrast, other pathogens inhibit inflammasome activation to attenuate the immune response. Bacterial factors from Mycobacterium tuberculosis and Yersinia pestis promote their virulence by inhibiting inflammasome activation, thereby dampening their host’s immune response (14, 15). In the case of Y. pestis, its type 3 secreted effector, YopM, sequesters caspase-1 into the nucleus where it can no longer interact with the inflammasome (14). It remains to be elucidated whether some cytosol-adapted pathogens, like L. monocytogenes and Shigella flexneri, have devised strategies to evade or exploit caspase-11–dependent pyroptosis.

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**BIOCHEMISTRY**

**Integrative Structural Biology**

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Integrative approaches using data from a wide variety of methods are yielding model structures of complex biological assemblies.

Biological assemblies and machines often elude structural characterization, hampering our understanding of how they function, how they evolved, and how they can be modulated. A number of macromolecular assemblies have been reconstructed over the years by piecemeal efforts, such as fitting high-resolution crystal structures of individual components into lower-resolution electron microscopy (EM) reconstructions of the entire complex (1). Although notable successes have been achieved in this way, ambiguous or conflicting models can still arise (2–4). Thus, structural and computational biologists have been looking for new ways to put all of the pieces back together. Sophisticated integrative approaches are being developed (5, 6) that combine information from different types of experiments, physical theories, and statistical analyses to compute structural models of multicomponent assemblies and complex biological systems.

In addition to the conventional biophysical techniques of x-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, EM, and small-angle x-ray scattering (SAXS), a growing number of experimental methods can also provide valuable infor-

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Complex structure solutions. Models of macromolecules and their complexes can be constructed by combining different types of information generated by various experimental and theoretical techniques (gray box). The data are converted into spatial restraints, which are combined into a scoring function that guides sampling algorithms to obtain a detailed structural model.

The model because the data were collected from crystal structures. Although neither model resolved all interactions, work by exhaustive calculations on February 27, 2013www.sciencemag.org Downloaded from www.sciencemag.org on February 27, 2013
extremely informative about the evolution and function of the 26S proteasome. At low resolution, chromatin has also been modeled through integrative approaches. In this way, Duan et al. constructed a three-dimensional model of the yeast genome (15), uncovering the topology and spatial relationships of different chromosomal elements. For this study, the restraints were garnered from cross-linking, restriction enzyme digestion, ligation, and deep sequencing, thereby revealing the three-dimensional structure of the genome at a level of detail not accessible to any conventional imaging method typically used to study assemblies of this size. These inferential, cellular-scale approaches enable comparison of normal and aberrant cells and may eventually serve an important diagnostic role in medicine.

As integrative methods evolve, structural models can then be revisited and new data incorporated, so that the model can be continuously improved and revised using the latest information (13). Integrative software tools should therefore be flexible enough to incorporate new data and/or restraints. The Protein Data Bank (www.pdb.org) is facilitating this process by acting as a curator for a variety of structural data from different methods as well as models based on these data.

Any experimental observation can in principle be converted into a restraint for building ever more complex models. The reach and impact of structural biology can thus be extended to a wider and more diverse audience. Using these new computational and bioinformatics approaches to collect and integrate diverse pieces of structural and experimental data, Humpty Dumpty can be put back together again.

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PLANT SCIENCE

Preservation of Recalcitrant Seeds
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Concerns about the rapid erosion of plant diversity have spawned a host of seed-banking initiatives (1). These repositories provide critical germ plasm needed to understand, maintain, and manage natural variation within and among species (2). However, numerous plant species and much of the humid tropics are underserved in these endeavors because of the perceived problem of seed recalcitrance (3). About 75 to 80% of angiosperm species (4, 5) produce orthodox seeds that can survive drying and prolonged storage at −20°C. By contrast, 5 to 10% of angiosperm species produce recalcitrant seeds that do not survive desiccation (3) and are killed in the freezer when ice crystals form. How can their preservation be ensured?

These seeds are called recalcitrant because they die rapidly when treated under the same genebanking conditions used to store orthodox seeds. Recalcitrant seeds, like most living organisms but unlike orthodox seeds, need water to survive. By nature, recalcitrant seeds are short-lived; they either germinate or are eaten by animals in the wild (3). “Intermediate” seeds, produced by 10 to 15% of angiosperm species (4), withstand sufficient dehydration to prevent formation of lethal ice; nevertheless, their seeds are short-lived in the freezer for unknown reasons (6).

The misperception that recalcitrant seeds cannot be stored arises from the assumption that there is only one storage strategy at our disposal—standard freezers—to manage the wide variation in storage physiology exhibited by plant seeds. In reality, long-term storage of recalcitrant and intermediate seeds (or seed parts) is possible with cryogenic technologies (7–9). Because cryogenic storage requires specialized infrastructure and personnel and is costly, most genebanks, even some of the newer ones, invest only in freezer storage.

Cryopreservation involves storage at ultralow temperature, often in liquid nitrogen (−196°C) (7–9). Rapidly advancing methods can be used to essentially stop water from freezing within recalcitrant seed cells and obviate lethal freezing damage (3, 7–9). The technology requires carefully dehydrating tissues and cooling them extremely rapidly (7). Small samples are required to obtain diffusion and heat transfer rates needed to prevent ice formation (7).

The problem is that recalcitrant seeds tend to be large compared with orthodox or intermediate seeds (see the figure) (5). In fact, most recalcitrant seeds are far too large to be rapidly dehydrated or cooled effectively when exposed to liquid nitrogen. The major breakthrough that led to successful cryopreservation of recalcitrant germ plasm was the ability to surgically dissect out the growing portion of the seed (termed the embryonic axis) and germinate it in vitro (10). Recovery may be enhanced by exposing embryonic axes to cryoprotectants (substances that protect against dehydration and freezing damage) (8, 9) and reducing stress-related free radical–mediated damage (11).

Cryogenic technology has been successfully refined for embryonic axes of a spectrum of temperate recalcitrant-seeded species (8). Intermediate seeds are smaller and drier and do not even require these exacting preparations. However, unmet challenges still limit successful cryopreservation of embryonic axes of some recalcitrant seeds from the tropics and subtropics (11). The seeds of these species have embryonic

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