

its lowest energy structures (A, B, or C). The resulting ensemble was then probed with two lasers: an IR laser irradiating the mixture during the expansion (~1 mm from the nozzle), and a UV laser further downstream (~4 mm from the nozzle). The UV absorption spectrum of the sample gives information about the relative populations of A, B, and C in the mixture. Each structure exhibits a unique spectrum (7). When the IR laser was turned on and tuned to a specific NH stretching vibration of a particular conformation (A, B, or C), the relative intensities of the three different bands in the UV spectrum changed, providing evidence for an IR-induced isomerization reaction.

It is not surprising that the relative populations of A, B, and C change when the ensemble is irradiated by IR light. IR-induced isomerization of a polyatomic molecule (nitrous acid, HONO, in a low-temperature matrix) was first observed more than 40 years ago (8). Real-time studies of protein folding often use IR pulses to initiate conformational change, either directly or indirectly (9), thereby mimicking the well-known behavior of proteins to denature at elevated temperature.

What is surprising about the results of Dian *et al.* (5) is that they are conformation specific. The substates (A, B, or C) populated after IR excitation are the same as in the absence of IR excitation. But the population ratios A:B:C before and after IR irradiation are different. Further, the experiments show that the population ratios depend uniquely on which conformation is excited, and on which NH stretching vibration is excited within a given conformation.

According to conventional wisdom, this kind of mode specificity should not exist in such a large molecule, at least not on the few-microsecond time scale of the experiment. NATMA has 102 normal modes of vibration. Some of these have relatively high frequencies, such as the NH stretching modes at ~3400 cm⁻¹ (the energy of IR excitation), but most have much lower frequencies of a few hundred cm⁻¹ or less. Hence, there is a high density of states at the energy of excitation. It is further expected that some couplings must exist between these modes. The energy deposited in the NH stretching mode of a particular structure should therefore be redistributed rapidly and irreversibly among all or most of the remaining modes, a process known as intramolecular vibrational relaxation (IVR).

As stated by Dian *et al.* (5), "given that the three conformers (A, B, and C) have a similar energy (within ~2 kJ/mol) and receive nearly identical amounts of energy in the vibrational excitation, one might have imagined that all transitions out of the three conformers would distribute their excited population similarly . . .

this is clearly not the case." Instead, the IR-induced dynamics is nonstatistical, an unprecedented result in such a large molecule (10).

Under the conditions of the experiment, collisions must play an important role in the dynamics. Selective collisional cooling of molecules with flexible side-chain conformations has been observed by several groups [see, for example (11)]. In their elegant "chemical timing" experiments, Parmenter and co-workers (12) arrested IVR in an electronically excited state of *p*-difluorobenzene by using an O₂ buffer gas to relax the molecules back to the ground state on short time scales. The first collisions after IR excitation of NATMA likely occur in less than a nanosecond. They may thus be similarly responsible for the observed nonstatistical behavior. More sophisticated ab initio calculations (13) will be required to identify the regions of the energy landscape to which access is blocked in such large molecules.

The results of Dian *et al.* (5) clearly show that there are distinguishable pathways on the energy landscape of NATMA, at least on some time scales. These pathways play a critical role in dictating the redistribution of populations under the conditions of the experiment. We do not know the extent to which this will be true in even larger molecules. But if such pathways exist and can be accessed, then we can look forward to obtaining ever more detailed information about biological processes at the molecular level.

IR lasers provide an entry point to previously unexplored regions of the energy landscape. Solvent-induced changes and the role

of water and other small molecules in influencing conformational choice can be probed by forming solvent-solute complexes in the supersonic jet (14). It may also be possible to determine the extent to which different pathways are connected and to probe the vibrational coordinates along which these connections are made. Such experiments would provide stringent tests of the "new view" that multiple routes dominate the dynamics of protein folding and other complex biological processes (15, 16). The future of biomolecules in the gas phase is bright.

References and Notes

1. J. R. Winkler, H. B. Gray, Eds., special issue on Protein Folding, *Acc. Chem. Res.* **31** (1998).
2. C. M. Dobson, A. Sali, M. Karplus, *Angew. Chem. Int. Ed.* **37**, 868 (1998).
3. M. R. Betancourt, D. Thirumalai, *J. Phys. Chem. B* **106**, 599 (2002).
4. R. Riek *et al.*, *Nature* **382**, 180 (1996).
5. B. C. Dian, A. Longarte, T. S. Zwier, *Science* **296**, 2369 (2002); published online 23 May 2002 (10.1126/science.1071563).
6. D. Evans, D. J. Wales, personal communication.
7. T. S. Zwier, *J. Phys. Chem. A* **105**, 8827 (2001).
8. J. D. Baldeschwieler, G. C. Pimentel, *J. Chem. Phys.* **33**, 1008 (1960).
9. I. K. Lednev, A. S. Karnoup, M. C. Sparrow, S. A. Asher, *J. Am. Chem. Soc.* **123**, 2388 (2001).
10. Nonstatistical behavior is frequently predicted (and observed) in the chemical reaction dynamics of small molecules. See (7) for a recent example.
11. P. D. Godfrey, R. D. Brown, F. M. Rogers, *J. Mol. Struct.* **376**, 65 (1996).
12. R. A. Coveleskie, D. A. Dolson, C. S. Parmenter, *J. Phys. Chem.* **89**, 645 (1985).
13. G. M. Florio, R. A. Christie, K. D. Jordan, T. S. Zwier, *J. Am. Chem. Soc.*, in press.
14. E. G. Robertson, J. P. Simons, *Phys. Chem. Chem. Phys.* **3**, 1 (2001).
15. R. L. Baldwin, *J. Biomol. NMR* **5**, 103 (1995).
16. K. A. Dill, H. S. Chan, *Nature Struct. Biol.* **4**, 11 (1997).
17. L. Sun, K. Song, W. L. Hase, *Science* **296**, 875 (2002).

PERSPECTIVES: EVOLUTION

Chaperones as Buffering Agents?

Thomas Mitchell-Olds and Charles A. Knight

Does evolution draw on existing genetic variation in animals and plants or must it wait around for new mutations to arise? Sixty years ago, Waddington argued that cryptic genetic variation is present for many traits, but that expression of these variants under normal environmental conditions is prevented by a process of "genetic buffering" (1). As Waddington demonstrated, stressful environmental conditions compromise the genetic buffering system, leading to the breakdown of normal development and enabling the expression of cryptic genetic variation as visible

phenotypic changes. Recently, heat shock proteins (HSPs), a type of molecular chaperone, have been implicated in the genetic buffering of the fruit fly *Drosophila*. Now, Queitsch *et al.* (2) report in a recent issue of *Nature* that the chaperone HSP90 provides genetic buffering in *Arabidopsis* and may contribute to the evolutionary adaptation of this plant.

HSPs are induced by high-temperature stress in organisms as diverse as bacteria, fungi, plants, and animals. These molecular chaperones prevent irreversible aggregation of denatured proteins after heat or other protein-denaturing stresses. They also bind to a range of client proteins that are crucial for regulating growth and development. The evolutionary conservation of the heat shock response, and

The authors are at the Max Planck Institute for Chemical Ecology, Winzerlaer Strasse 10, Jena 07745, Germany. E-mail: tmo@ice.mpg.de

the fact that expression of HSPs correlates with high-temperature stress suggests that accumulation of HSPs increases thermotolerance. This prediction is borne out by numerous studies in animal and plant cells. For example, *Arabidopsis* plants that overexpress HSP101 exhibit increased resistance to heat stress (3). Furthermore, after heat stress a low molecular weight HSP in plant chloroplasts stabilizes photosystem II proteins that produce oxygen, and also maintains electron transport in photosystem II (4). There are strong correlations between the heat shock response and survival (5), specific leaf area (6), and other ecologically important traits. However, without genetic or biochemical experiments, it is difficult to calculate the extent to which HSPs contribute to genetic buffering in plants.

In *Drosophila*, natural genetic variation and mutations induced by genetic engineering reveal that HSP70 is important for inducing thermotolerance. Transgenic flies that overexpress HSP70 are better able to resist heat stress (7). Natural genetic variants (polymorphisms) in wild fly populations can be selected for in the laboratory. For example, Bettencourt *et al.* (8) selected genetic variants of the HSP70 promoter in *Drosophila* that correlated with altered regulation of HSP70 gene expression. They found that different variants rose to a high frequency in five laboratory fruit fly populations that had been grown at different temperatures for 20 years. Intriguingly, these variants showed similar patterns of allele frequencies along a latitudinal transect of Australia with a wide variation in climate. Both studies suggest that environmental temperature stresses result in selection of particular HSP70 variants in both wild and laboratory fruit fly populations.

In both animal and plant cells, the HSP90 chaperone has a limited number of crucial client proteins that it keeps in a state of activation, including steroid hormone receptors, signaling kinases, and a variety of transcription factors (9). In the fruit fly, pharmacological blockade of HSP90 activity causes selective inactivation or degradation of these client proteins, resulting in developmental abnormalities and numerous morphological changes in many structures. Likewise, HSP90 inhibitors display antitumor activity, altering the expression of cell cycle genes (10). Given their direct interac-

tions with signal transduction proteins and other regulatory proteins, HSPs are predicted to influence physiological and morphological variations, and hence are good candidate genetic buffering molecules.

In a previous study in fruit flies, Rutherford and Lindquist (11) revealed that HSP90 buffers against expression of genetic variation by stabilizing the protein constituents of signal transduction pathways. When HSP90

activity was impaired either by mutation or by pharmacological blockade, a considerable number of phenotypic variants appeared. The authors selected several phenotypic variants in the HSP-compromised flies over a few generations. They found that the new morphological traits were expressed in flies even when HSP90 activity was restored to wild-type levels (an example of genetic assimilation). Furthermore, under high-temperature stress conditions, these same morphological variants appeared in wild-type fruit fly populations, presumably because HSP90 had been recruited to the heat shock response and was no longer available to buffer the regulatory pathways influencing these phenotypes. Rutherford and

Lindquist argued that the buffering capacity of HSP90 could contribute to evolution.

In their new work, Lindquist and co-workers (2) chose a completely different organism, *Arabidopsis*, in which to seek a parallel example of HSP90 buffering capacity with similar evolutionary implications. In contrast to animals, plant development is highly plastic and intimately connected with environmental cues. For several strains of *Arabidopsis*, pharmacological or heat-induced reductions in HSP90 activity produced a variety of morphological variants, such as those with altered color and presence of true leaves, shape and expansion of cotyledons, root morphology, and orientation of seedlings. These developmental abnormalities were strain-specific, suggesting that HSP90 does indeed buffer hidden genetic variation. Lindquist and colleagues propose that HSP90 regulates release of hidden genetic variation, generating morphological changes that may contribute to adaptive evolution. However, the evolutionary potential of newly revealed genetic variation may differ between *Drosophila* and *Arabidopsis*. HSP90 inactivation results in grossly abnormal morphologies and reduced survival in *Drosophila*, hence these cryptic genetic variants may not be evo-

lutionarily advantageous. In contrast, HSP90 blockade in *Arabidopsis* induced numerous morphological variants with good survival frequencies in the laboratory.

Although genetic and pharmacological manipulation of HSP90 in *Drosophila* and *Arabidopsis* in the laboratory unveils cryptic genetic variation, the crucial question is whether extant hidden genetic variation actually contributes to evolutionary change in the field. A recent study by Lauter and Doebley in maize (12) suggests that it may. These authors analyzed three morphological traits that distinguish maize from its wild ancestor, teosinte, but that are uniform within ancestral teosinte populations (see the figure). Quantitative trait locus (QTL) mapping of variations in a testcross teosinte population identified 22 QTLs controlling morphological traits that are normally invariant in teosinte populations. Thus, the evolution of modern maize may have drawn upon cryptic genetic variation that already existed in teosinte.

HSPs are excellent candidates for the genetic buffering system that Waddington predicted in the 1940s. The Lindquist laboratory (2, 11) has certainly demonstrated that temperature stress or HSP inhibition reveals potentially adaptive variation. But do HSPs actually buffer adaptive genetic variation in the field, and does release of this variation drive adaptation? To address these questions, evolutionary biologists will need to show that cryptic variation can be adaptive (as suggested by the work of Lauter and Doebley), and must also demonstrate that HSP90 is responsible for keeping this cryptic variation hidden. This will require experiments demonstrating that adaptive evolution differs between HSP-wild-type and HSP-compromised plant or fruit fly populations.

It is clear that HSPs are important for regulating gene expression and for inducing thermotolerance. But we have much more to do if we want to obtain convincing evidence that breakdown of HSP-mediated buffering is an important contributor to adaptive evolution in animals or plants.

References

1. C. H. Waddington, *Nature* **150**, 563 (1942).
2. C. Queitsch, T. A. Sangster, S. Lindquist, *Nature* **417**, 618 (2002); advanced online publication, 12 May 2002 (10.1038/nature749).
3. C. Queitsch, S. Hong, E. Vierling, S. Lindquist, *Plant Cell* **12**, 479 (2000).
4. S. A. Heckathorn, C. A. Downs, T. D. Sharkey, J. S. Coleman, *Plant Physiol.* **116**, 439 (1998).
5. S. K. Park, R. Chang, R. Shivaji, D. S. Luthe, *Plant Physiol.* **115**, 229 (1997).
6. C. A. Knight, D. D. Ackerly, *Am. J. Bot.* **88**, 411 (2001).
7. S. P. Roberts, M. E. Feder, *Funct. Ecol.* **14**, 353 (2000).
8. B. R. Bettencourt, I. Y. Kim, A. A. Hoffmann, M. E. Feder, *Evolution*, in press.
9. J. C. Young, I. Moarefi, F. U. Hartl, *J. Cell Biol.* **154**, 267 (2001).
10. L. Neckers, *Trends Mol. Med.* **8** (suppl.), 56 (2002).
11. S. L. Rutherford, S. Lindquist, *Nature* **396**, 336 (1998).
12. N. Lauter, J. Doebley, *Genetics* **160**, 333 (2002).



A cereal's ancestor. Teosinte (*Zea mays* ssp. *parviglumis*), the wild ancestor of modern maize, from the Balsa River region of Mexico.