dependent on upward movements of groundwater? Are they therefore periodically decoupled from climatic control? If this is so, then the use of bog stratigraphy as a climatic proxy[6] in such regions must be brought into question. Is the hydraulic conductivity of the catotelm peats in these continental raised bogs higher than in those from oceanic regions? If so, what are the structural differences in these peats? And can drought bring groundwater mineral supply within the reach of the roots of surface vegetation? Such reversals of the normal successional development towards low nutrient status and acidity have been previously recorded from the Minnesota area[7]. We can no longer assume that elevation automatically results in ombrotrophy and perhaps we need to redefine the term 'raised bog'. □

Peter D. Moore is in the Division of Life Sciences, King's College, Campden Hill Road, London W8 7AH, UK.

1. Glaser, P., Siegel, D. I., Romanowicz, E. A. & Shen, Y. P. *J. Ecol.* **85,** 3–16 (1997).
2. Godwin, H. *Fenland: Its Ancient Past and Uncertain Future* (Cambridge Univ. Press, 1978).
3. Moore, P. D. & Bellamy, D. J. *Peatlands* (Elek Science, London, 1973).
4. Ingram, H. A. P. *Nature* **297,** 300–303 (1982).
5. Ivanov, K. E. *Water Movement in Mirelands* (Academic, London, 1981).
6. Barber, K. E., Chambers, F. M., Maddy, D., Stoneman, R. & Brew, J. S. *Holocene* **4,** 198–205 (1994).
7. Glaser, P. H., Janssens, J. A. & Siegel, D. I. *J. Ecol.* **78,** 1021–1048 (1990).

Cell biology

The importance of being unfolded

Kevin W. Plaxco and Michael Groß

Dogma has it that the biologically significant state of every protein is the unique and well-defined native structure. Considering the folding of a protein from an unfolded conformation to its native state (U→N), much research has been done on the 'N' form, as well as on what is represented by the arrow, yet surprisingly little attention has been paid to the unfolded state. Developments in nuclear magnetic resonance and computer modelling techniques have made it feasible to start filling the gap and to investigate exactly how unfolded and random the 'U' form is. And it now emerges that dogma may be wrong in depriving unfolded proteins of any biological importance.

The strongest case for biological activity in an unfolded protein is presented by Daughdrill *et al.*[1] in this month's issue of *Nature Structural Biology*. The protein in question — the transcriptional regulator FlgM from *Salmonella typhimurium* — controls the supply of building blocks for the synthesis of flagella (Fig. 1). Flagella are the external filaments that are spun around like a propeller when bacteria swim[2], and each flagellar filament is attached through a flexible hook to a basal body, which anchors it in the cell membrane. Flagellar filaments contain an estimated eight per cent of the total cell protein, and their construction is controlled by a tightly regulated hierarchy of more than 50 genes.

For some time, researchers were puzzled by the observation that mutations in any of the three-dozen genes that are required for building the basal body and hook would induce FlgM to suppress the synthesis of the proteins required to form the filament. Bacteria build flagella very much like one would build a tower without having a crane: they transport the building blocks through the hollow centre of the flagellum to the end of the growing filament. The mystery of the regulation by FlgM was solved by the remarkable finding that it is exported through this same channel, as long as open hooks and incomplete flagella are available[3]. When all of the flagella are completed, the export channels are closed and FlgM starts to accumulate inside the cell, where it can regulate the expression of other genes by binding to the flagella-specific transcriptional activator, σ^{28}.

By studying this molecular-recognition event using multidimensional NMR, Daughdrill *et al.*[1] have now found that unliganded FlgM is largely unfolded under physiological conditions. Only on binding to σ^{28} does the carboxy-terminal half of FlgM attain a folded conformation, however, the first 40 amino acids remain unstructured in both the bound and free states.

The observation of an unstructured protein *in vitro* might have been dismissed as being due to an inability to recreate the cytoplasmic environment. But in this case, there is a plausible reason for FlgM to remain unfolded *in vivo*. The inner diameter of a bacterial flagellum is about 2.5 nm, so a globular protein with the molecular weight of FlgM — which could be as big as 3 nm in diameter when folded — might not fit through the export channel. In contrast, an unfolded polypeptide chain could be threaded through the channel more easily. So, the as-yet unknown export mechanism could be the evolutionary cause that precludes FlgM from being like other proteins and having a folded native state.

It is not clear how far these findings can be generalized, but certain other proteins show a similar behaviour, suggesting that FlgM is not the exception that proves the rule. The cell-adhesion proteins from various Gram-positive bacteria[4,5] (known as MSCRAMMS, for 'microbial surface components recognizing adhesive matrix molecules') and the cyclin-dependent kinase inhibitor p21$^{waf/cip1/sid1}$ (ref. 6) have been shown, both by far-ultraviolet circular dichroism and by NMR spectroscopy, to be unstructured under plausibly physiological conditions. Like FlgM, these proteins fold to form a tightly bound complex in the presence of ligand.

Another potential example is the SH3 domain of the *Drosophila* signal-transduction protein, Drk. NMR spectroscopy indicates that within a population of Drk molecules, a considerable proportion is unfolded under physiological conditions[7]. The rate of inter-

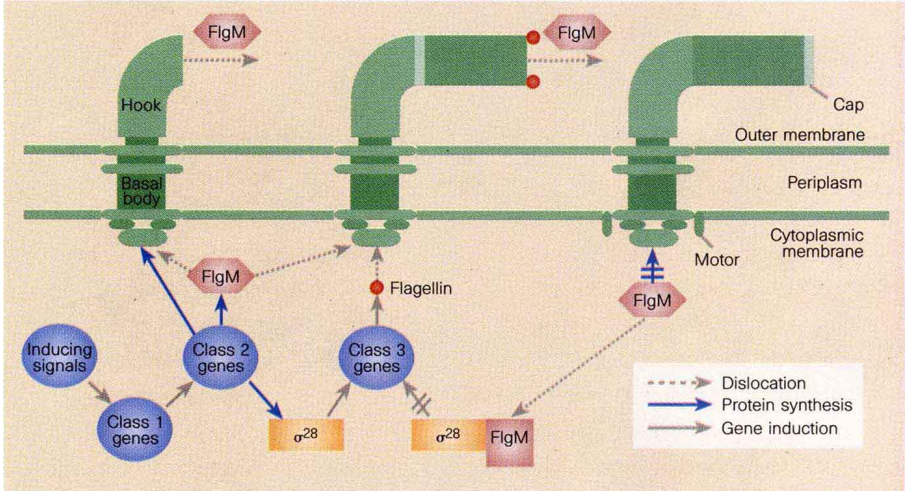

Figure 1 **The morphogenesis of bacterial flagella is controlled by a tightly regulated set of more than 50 genes, organized in a hierarchical array of three classes. Class 1 genes are the primary targets of environmental signals. Their protein products induce the transcription of class 2 genes, which code for the basal body and hook proteins, the regulatory protein FlgM and the flagella-specific sigma factor, σ^{28}. The latter induces the transcription of class 3 genes, which code for the building blocks of the flagellar filament. Class 3 genes can be suppressed by mutations in any of the 35 class 2 genes because the class 2-encoded suppressor, FlgM, is exported from the cell through a channel in the basal body and hook structure[3]. If this export fails, FlgM accumulates in the cell and suppresses class 3 genes by binding to σ^{28}. Daughdrill *et al.*[1] have now shown that FlgM is unfolded in its active state, potentially because of the requirement to fit through the export channel.**

conversion between the unfolded and the folded forms has also been monitored[8]: with a folding time-constant of around one second, unfolded protein molecules can presumably reorganize themselves to bind a target peptide rapidly enough that peptide binding and signal transduction are not impeded.

If the biological activity of unfolded proteins turns out to be a widespread phenomenon, how do these proteins escape degradation and aggregation in the cell? It is quite possible that unfolded yet active proteins can only occur on the cell surface, or in roles where they are found only at low concentrations in the cell — as with FlgM, which is either exported or bound to σ^{28}.

The awakened interest in the unfolded state corresponds with a 'new view' of protein folding[9]. This replaces the models of discrete folding pathways by multipathway 'energy landscapes' and defined intermediates by a distribution of partially folded conformations. Studies of unfolded proteins, both by NMR and by Monte Carlo simulations, have converged on the view that unfolded proteins sample backbone conformations that are remarkably similar to those found in fully folded proteins[10]. This presumably biases the conformational search that is undertaken by the polypeptide chain during folding, and is one of the mechanisms by which folding can avoid the astronomically long times that the Levinthal paradox predicts for a random search mechanism.

New evidence for the biological relevance of unfolded states has also arisen from studies of pathogenic proteins. Unfolded conformations seem to be critically involved in the transformation from normal to pathogenic forms of the prion, and of proteins that are implicated in amyloidosis-related diseases such as Alzheimer's[11,12]. We anticipate that future developments in this field, together with the insights gained from the 'new view' perspective, will lead to a more widespread appreciation of the idea that proteins are dynamic systems, sensitive to evolutionary modulation even in their unfolded conformations. □

Kevin W. Plaxco and Michael Groß are at the Oxford Centre for Molecular Sciences, New Chemistry Laboratory, South Parks Road, Oxford OX1 3QT, UK.

1. Daughdrill, G. W., Chadsey, M. S., Karlinsey, J. E., Hughes, K. T. & Dahlquist, F. W. *Nature Struct. Biol.* **4,** 285–291 (1997).
2. Schuster, S. C. & Khan, S. *Annu. Rev. Biophys. Biomol. Struct.* **23,** 509–539 (1994).
3. Hughes, K. T., Gillen, K. L., Semon, M. J. & Karlinsey, J. E. *Science* **262,** 1277–1280 (1993).
4. House-Pompeo, K., Xu, Y., Joh, D., Speziale, P. & Höök, M. *J. Biol. Chem.* **271,** 1379–1384 (1996).
5. Penkett, C. J. *et al. J. Mol. Biol.* (submitted).
6. Kriwacki, R. W., Hengst, L., Tennant, L., Reed, S. I. & Wright, P. E. *Proc. Natl Acad. Sci. USA* **93,** 11504–11509 (1996).
7. Zhang, O. & Forman-Kay, J. D. *Biochemistry* **34,** 6784–6794 (1995).
8. Farrow, N. A., Zhang, O. W., Forman-Kay, J. D. & Kay, L. E. *Biochemistry* **34,** 868–878 (1995).
9. Dill, K. A. & Chan, H. S. *Nature Struct. Biol.* **4,** 10–19 (1997).
10. Smith, L. J., Fiebig, K. M., Schwalbe, H. & Dobson, C. M. *Fold. Des.* **1,** R95–R106 (1996).
11. Booth, D. R. *et al. Nature* **385,** 787–793 (1997).
12. Funahashi, J., Ogasahara, K., Yamagata, Y. & Yutani, K. *J. Biochem.* **120,** 1216–1223 (1996).

Climate change

A greener north

Inez Fung

For whatever reasons, the period since 1980 has been the warmest in the past 200 years. Every gardener can speculate about the fate of his garden in response to the warming, but the bigger question is whether the biosphere has responded in some larger way to the climate perturbation. Sustained long-term observations of photosynthesis are rare. Furthermore, the biosphere is notoriously heterogeneous. It is very difficult to extrapolate from field measurements at a few sites to behaviour over a large region.

On page 698 of this issue[1], Myneni *et al.* present satellite evidence that, on average, the biosphere between 45° N and 70° N has been enjoying increased photosynthesis between 1981 and 1991. The authors suggest that temperature increases in the spring time have also brought a longer growing season to the high latitudes. The evidence presented by Myneni *et al.* is the first direct observation of the biosphere that photosynthesis has increased on such a broad scale for such a long time. The satellite observations are extremely provocative and, the authors argue, reveal specific areas where changes have occurred.

Remote sensing of photosynthetic activity is based on how plants use or dispose of solar radiation at different wavelengths. Green leaves absorb more than 85% of the incoming solar energy in the visible part of the spectrum and under 40% of the energy in the near-infrared: hence there is a large difference between the reflectivities at these wavelengths. By contrast, the reflectivities at these wavelengths are comparable for bare soils. A commonly used parameter for monitoring photosynthetic activity is the normalized difference vegetation index (NDVI): the difference between the reflectivities at the two wavelength bands normalized by the sum. A high NDVI is indicative of vigorous photosynthetic activity.

Satellite observations provide a global view of the land surface in days to weeks, depending on the days without clouds to obscure the view. The pioneering work of Tucker and his co-workers[2,3] has demonstrated the usefulness of the NDVI for deducing vegetation classes on a continental scale and for monitoring photosynthetic activity on a global scale. These studies employed satellite observations for a single year or a few years, and centred on the spatial and seasonal information contained in the NDVI distributions.

Looking for long-term changes in the satellite data is a challenge. The instruments were designed for weather applications in which the time scale of interest is days. The 11-year NDVI time series have been constructed from data from three radiometers on board three different polar-orbiting meteorological satellites. The performance of the radiometers diminishes with time, and at different rates for different wavelength channels. Furthermore there is the annoying interference by the intervening atmosphere. By selecting the maximum NDVI value over a period of 10 or 15 days, one hopes to have avoided clouds, which, being reflective in the visible spectrum, would yield a low NDVI. Also, because of the satellite viewing and solar geometries, there are differing amounts of atmosphere between the sensor and the surface; more importantly, the abundance of other atmospheric constituents (aerosols, for example) that interact with the radiation is not uniform in space or time. These must be removed before a clean signature of the surface can be obtained.

Myneni *et al.*[1] have taken a bold new step forward in extracting a secular trend from the NDVI information. They used two NDVI datasets, independently derived from the same raw satellite data. Each dataset includes a *post facto* correction for instrument calibration and atmospheric effects. As the authors point out, the correction is not complete, but they are confident that the NDVI trend at high latitudes is robust. For similar reasons, they have refrained from interpreting the trends in the tropics.

Should we believe in the NDVI trend? There are no 'ground-truth' measurements of photosynthesis at northern high latitudes over the same period, and so the accuracy of the trend cannot be established unambiguously. Nonetheless, the consistency between the two independently processed NDVI datasets inspires some confidence. For corroboration, the authors present Keeling and colleagues' finding[4] of an increasing trend in the amplitude of the atmospheric CO_2 annual cycle at Point Barrow, Alaska.

Photosynthesis removes CO_2 from the atmosphere. Carbon is returned to the atmosphere through respiration of microbes decomposing dead organic matter. The different timing in the photosynthesis and respiration gives rise to an annu-