there are still ten closely spaced columns at the central ring, but now only five have higher intensities indicating high TM occupancy, whereas the intensity of the remainder is closer to that of an Al column. This indicates that the broken symmetry at the central ring is due to chemical ordering, and that there are ten sites, but with different TM and Al occupancies. This is also inconsistent with the triangular arrangement proposed by Steinhardt *et al.*

There are significant differences between the structure model proposed by Steinhardt *et al.* and our atomic-resolution *Z*-contrast image. Although these do not invalidate the coverage picture, they do prevent our understanding the formation of quasicrystals.

We believe that the closely spaced column pairs in the central and outermost rings that were not predicted by the structure model of Steinhardt *et al.* are the key to understanding the formation of decagonal quasicrystals. They not only show that the structures of the decagonal quasicrystals and their crystalline approximants are more similar than Steinhardt *et al.* suppose, they also highlight the critical differences. On this basis, we have proposed a growth mechanism[5] that explains why these clusters prefer to overlap and follow the Gummelt coverage picture. Our growth model predicts that the overall structure will show ideal quasicrystal tiling, in the Gummelt coverage picture, when all clusters have strong chemical ordering in the central rings. If the clusters have no chemical ordering, the model predicts a random tiling. For real quasicrystals, their structure might be a mixture of both cases.

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1. Steinhardt, P. J. *et al. Nature* **396,** 55–57 (1998); correction *Nature* **399,** 84 (1999).
2. Gummelt, P. *Geometriae Dedicata* **62,** 1–17 (1996).
3. Steinhardt, P. J. & Jeong, H.-C. *Nature* **382,** 433–435 (1996).
4. Jeong, H.-C. & Steinhardt, P. J. *Phys. Rev. B* **55,** 3520–3532 (1997).
5. Yan, Y. & Pennycook, S. J. *Mater. Sci. Eng.* (in the press).

Steinhardt et al. *reply* — The purpose of our Letter[1] was to present experimental support for the quasi-unit cell picture of quasicrystals. This model proposes that the atomic structure can be reduced to a single repeating cluster satisfying certain 'overlap rules' (sharing of atoms by neighbouring clusters). We proposed that the quasicrystalline phase of AlNiCo can be decomposed into a repeating decagonal atom cluster (20 Å in radius). Yan and Pennycook do not refute the quasi-unit cell concept — they also propose a repeating cluster obeying the same

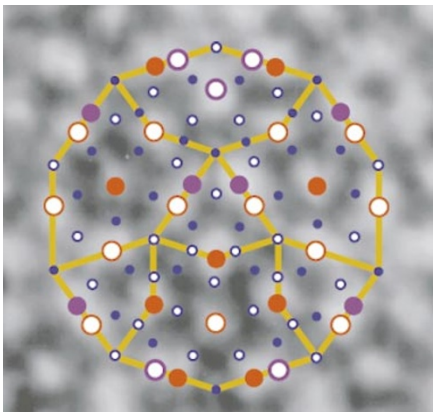

Figure 1 Improved decoration of the quasi-unit cell for AlNiCo compared to lattice image. Problematic TM sites in our earlier model[1] have been removed. The figure includes atoms added by overlap of neighbour clusters; these lead to the formation of neighbour TM column pairs, as seen near the centre. Large circles represent Ni (red) or Co (purple) and small circles represent Al. Solid circles represent $c=0$ and open circles represent $c=1/2$ along the periodic c-axis.

overlap rules. However, they propose a different atomic decoration for the repeating cluster that is ten-fold symmetric, whereas our decoration explicitly breaks ten-fold symmetry. This is important because our symmetry breaking corresponds precisely to the symmetry breaking of the overlap rules, and hence provides key evidence for the quasi-unit cell picture.

Yan and Pennycook's decoration is motivated by their impressive high-angle annular dark-field (HAADF) imaging, obtained with higher resolution than we had available. As they show, the imaging disagrees with the sites of four columns of transition metal (TM) atoms in our proposal (shown by arrows in their Fig. 1). However, we find that the problem can be resolved by a modest rearrangement of the previous decoration, switching 8 out of 100 atoms and retaining the broken ten-fold symmetry. The improved model in Fig. 1 has all the same qualitative properties as the original in ref. 1, matches the new HAADF (including Yan and Pennycook's Fig 2.) and even more recent high-resolution transmission electron microscopy (HRTEM) imaging, and has a density and stoichiometry that fits measured values to better than 2 per cent.

As more data become available (for example, from X-ray diffraction), further small refinements to our current best-fit decoration may be required, but the ten-fold symmetry breaking should remain as an essential property. The broken symmetry is necessary to explain three established features of AlNiCo: the broken symmetry consistently observed in through-focus HRTEM imaging of the clusters[2]; the broken symmetry found within the central ring of most clusters in HAADF imaging, such as our Fig. 1 and Yan and Pennycook's Fig. 2 (ref. 2) (the very rare, more symmetric rings, as shown in their Fig. 1, can be explained as defects; ref. 2 and M. Widom, personal communication); and the apparent quasiperiodic correlation in the broken symmetry direction on moving from cluster to cluster in HAADF images (see Fig. 1 of ref. 1), as is found for a configuration of overlapping decagons.

None of the features can be explained by symmetric clusters, even if chemical disorder is introduced to randomly break the ten-fold symmetry. M. Widom and coworkers (personal communication) have completed a total-energy-based prediction of the structure of AlNiCo, making no prior assumption about the existence of repeating 20-Å clusters. Yet decagonal clusters with broken ten-fold symmetry emerge as the lowest-energy configuration with nearly identical assignments of Al and TM positions, as in our improved model.

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1. Steinhardt, P. J. *et al. Nature* **396,** 55–57 (1998); correction *Nature* **399,** 84 (1999).
2. Abe, E. *et al.* http://xxx.lanl.gov/abs/cond-mat/9907160

Biological rhythms

Circadian clocks limited by noise

Circadian rhythms, which provide internal daily periodicity, are used by a wide range of organisms to anticipate daily changes in the environment[1]. It seems that these organisms generate circadian periodicity by similar biochemical networks within a single cell[2]. A model based on the common features of these biochemical networks shows that a circadian network can oscillate reliably in the presence of stochastic biochemical noise and when cellular conditions are altered. We propose that the ability to resist such perturbations imposes strict constraints on the oscillation mechanisms underlying circadian periodicity *in vivo*.

There is evidence that clock networks share common features in a wide range of organisms, from cyanobacteria to mammals[2]. For instance, all networks seem to include an interaction between two types of component (Fig. 1a): positive elements (or activators, such as KaiA in *Synechococcus*, Wc1-2 in *Neurospora*, Clc and Cyc in

Drosophila, and Clock and Bmal in mice) enhance the expression of negative elements (or repressors, such as KaiB and KaiC in *Synechococcus*, Frq in *Neurospora*, Tim and Per in *Drosophila*, and Tim and Per1,2 and 3 in mice).

The evidence indicates that the clock network is based predominantly on transcriptional regulation[2]. Although the contribution of post-transcriptional regulation to the oscillation mechanism is not yet clear[3], and it is too early to be sure that all the components and their interactions have been revealed in any single organism, we can already address the question of whether the common features of circadian networks are consequences of some underlying 'design principles'.

We can envisage many types of biochemical network that produce periodic oscillations[4,5] and that can be entrained to a 24-hour period by an external periodic stimulus, such as light or temperature. But there are additional constraints: for example, the periods of all autonomous circadian clocks must remain relatively constant over a wide temperature range, a property known as temperature compensation[1].

The ability to function reliably in the presence of internal noise may impose a further constraint on the oscillation mechanism. Internal noise in the operation of biochemical networks results from the stochastic nature of reaction events[6–8], and is particularly important when there are few molecules in the system, as is often the case in a cell[9]. These effects have sometimes been overlooked in previous analyses of circadian rhythms. For example, in a previously studied model that depends on a time-delayed negative feedback, reliable oscillations were found when reaction kinetics were approximated by continuous differential equations[10]. However, when the discrete nature of reaction events is taken into account, the oscillations persist but with periods and amplitudes that fluctuate widely in time (Fig. 1c). Noise resistance should therefore be considered in any postulated molecular mechanism of circadian rhythms.

We can illustrate this point with an example, based on existing data, of a class of biochemical networks that sustain reliable oscillations even in the presence of internal noise. In this class of biochemical network, the interactions between positive and negative regulatory elements lead to a hysteresis in the dependence of protein expression rates on the concentration of the negative element (see Fig. 1a,b). It is possible to formulate different versions of the model based on both transcriptional and post-transcriptional regulation (Fig. 1a).

The temporal evolution of the different components of the network is shown in Fig. 1d,e. We used a Monte Carlo algorithm, in which molecules participate in stochastic

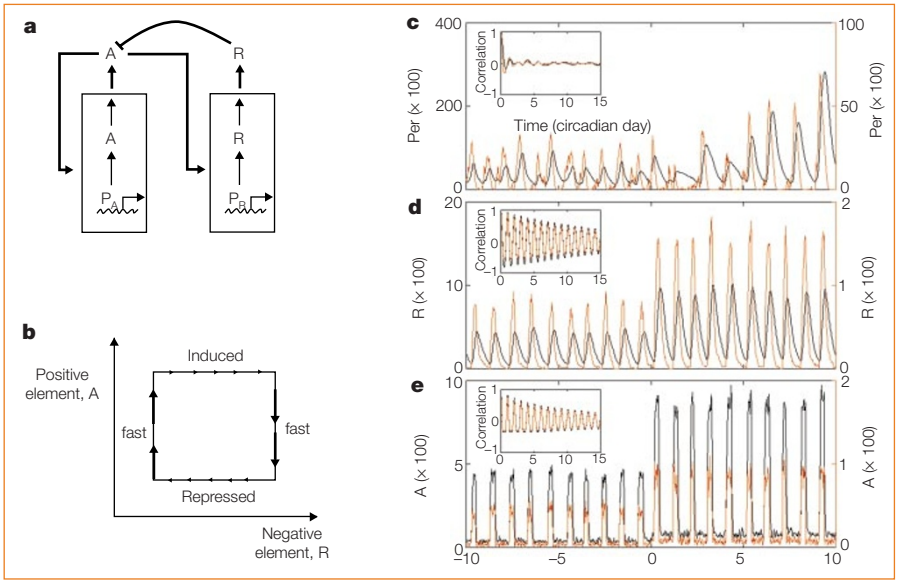

Figure 1 The hysteresis-based oscillation mechanism. **a,** A positive element, A, increases its own expression and that of a negative element, R. Strong binding of R to A inhibits A activity and so represses the expression of both elements by binding to the promoters P_A and P_R. **b,** The autoactivation of A results in a hysteresis: a bistable dependence of A concentration on R. Slow kinetics of R then leads to oscillations, which can be described as successive transitions between 'induced' and 'repressed' states. **c–e,** Monte Carlo simulations of models based on time delay (**c**) and hysteresis (**d,e**) for circadian oscillations. The time between two successive events is distributed exponentially around the usual mass-action reaction rate[6]. At time $t = 0$, all transcription rates were doubled. Black curves indicate proteins (left axis); orange curves, mRNA (right axis). Inset, temporal autocorrelation functions. Reliable oscillations result in temporal autocorrelations that persist for many periods. **c,** Model exhibiting reliable oscillation in the continuous limit[4]; we use the same parameter values as in Fig. 2 of ref. 4 (assuming that binding to the DNA promoter is diffusion limited, and that 1 nM corresponds to 1,000 molecules per cell). **d, e,** Simulation of the hysteresis-based oscillator. A is assumed to be a transcriptional activator. Further details are available from the authors.

reaction events[6]. The time between two successive occurrences of a specific reaction is exponentially distributed around its mean, which is given by the usual mass-action reaction rate (the same algorithm was used to simulate the noise effects for the time-delay oscillator in Fig. 1c). For a wide range of parameter values, the oscillations are reliable, with long time correlations (see insets in Fig. 1d,e) and small variations in period length, even with relatively small numbers of molecules. Reducing the transcription rate of the system depicted in Fig. 1d,e leads to oscillations in which average levels of messenger RNA are as low as 10 molecules per cell. In these systems, the standard deviation of the period remains less than 10%.

A further consideration is whether the circadian circuit can operate reliably within the cellular context. Global changes in transcription and translation rates may arise from variations in nutrition, growth conditions or temperature, and may affect the period of transcription or translation-based oscillators (Fig. 1c). In the hysteresis-based model, global transcription or translation rates have only small effects on the period, but changes in these rates alter the amplitude. The ability to maintain constant circadian periodicity despite global changes in the state of the cell[11] is probably necessary for the circadian clock to be successfully embedded within the cell.

It is not clear whether this hysteresis-based network is the mechanism underlying circadian oscillations. For instance, the regulation of positive and negative elements in the *Drosophila* clock might be more complex than this[12]. Oscillation mechanisms differ in their sensitivity to internal noise and changes in cellular conditions, however, and the ability to resist such uncertainties was probably one of the decisive factors in the evolution of circadian clocks and should be reflected in the underlying oscillation mechanism. Further studies of noise resistance may therefore help to uncover the design principles underlying circadian clocks.

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1. Edmund, L. N. *Cellular and Molecular Basis of Biological Clocks* (Springer, New York, 1988).
2. Dunlap, J. *Cell* **96,** 271–290 (1999).
3. Suri, V., Lanjuin, A. & Rosbash, M. *EMBO J.* **18,** 675–686 (1999).
4. Goldbeter, A. *Biochemical Oscillations and Cellular Rhythms* (Cambridge Univ. Press, 1995).
5. Murray, J. D. *Mathematical Biology* (Springer, Berlin, 1994).
6. Gillespie, D. T. *J. Phys. Chem.* **81,** 2340–2361 (1977).
7. Ko, M. S. *Bioessays* **14,** 341 (1992).
8. McAdams, H. N. & Arkin, A. *Trends. Genet.* **15,** 65–69 (1999).
9. Wodicka, L. *et al. Nature Biotechnol.* **15,** 1359–1367 (1997).
10. Leloup, J. C. & Goldbeter, A. *J. Biol. Rhythms* **13,** 70–87 (1998).
11. Kondo, T. *et al. Science* **275,** 224–227 (1997).
12. Glossop, N. R. J., Lyons, L. C. & Hardin, P. E. *Science* **286,** 766–768 (1999).