dient with decay length $\lambda = 20.2$ μm, whereas, in these in vivo conditions, Wg made a short-range gradient with $\lambda = 5.8 \pm 2.04$ μm (Figs. 3I and 4A). Which kinetic parameter could account for this difference? Because Dpp and Wg have inherently different properties—Wg is a lipid-modified molecule (*20*); Dpp is not (*21*)—they are likely to display different mechanisms and kinetics of spreading through the epithelium.

The shorter decay length of the Wingless gradient was due to a higher degradation rate of GFP-Wingless, by a factor of 5, and to a lesser extent its smaller diffusion coefficient (Fig. 4). Although the Gal4 driver was the same in the Dpp and Wg experiments, the production rate of Wg was about seven times that of Dpp, which implied that their maturation and secretion were controlled differently. In addition, while 62% of the Dpp molecules were immobile, the Wg pool was almost fully mobile at 25°C ($\psi = 9.2 \pm 13\%$), although, unlike Dpp, a significant immobile fraction appeared at higher experimental temperatures (Fig. 3, K and N). The different immobile fractions of Dpp and Wg at 25°C validated the specificity of the Dpp immobile fraction. Thus, the immobile fraction was not an artifact of incomplete recovery in sick cells. Finally, in contrast to Dpp, Wg transport and degradation were independent of Dynamin endocytosis (Fig. 3, K to O). Indeed, Wg movement has been suggested to be Dynamin-independent (*6*, *22*). In addition, expression of dominant-negative Dynamin and/or long-term thermosensitive *shibire* block caused an extension of the gradient in the wing (*5*, *22*, *23*), which was attributed to decreased degradation (*5*, *22*). Our FRAP approach studying the results of an acute block suggests that endocytosis is not required for Wg transport and degradation or, alternatively, that endocytosis of Wg is Dynamin-independent.

Altogether, the GFP-Wingless FRAP experiments (i) validated our FRAP assay and shibire-rescue experiment; (ii) indicated that different morphogen gradients can be generated by independently fine-tuning D, k, v, and ψ; and (iii) showed that different morphogens may use different mechanisms of transport and cellular machineries (e.g., Dynamin-dependent versus Dynamin-independent transport) to achieve the formation of morphogen gradients.

References and Notes

1. T. Y. Belenkaya *et al.*, *Cell* **119**, 231 (2004).
2. E. V. Entchev, A. Schwabedissen, M. González-Gaitán, *Cell* **103**, 981 (2000).
3. K. Kruse, P. Pantazis, T. Bollenbach, F. Jülicher, M. González-Gaitán, *Development* **131**, 4843 (2004).
4. A. D. Lander, Q. Nie, F. Y. Wan, *Dev. Cell* **2**, 785 (2002).
5. E. Piddini, F. Marshall, L. Dubois, E. Hirst, J.-P. Vincent, *Development* **132**, 5479 (2005).
6. M. Strigini, S. M. Cohen, *Curr. Biol.* **10**, 293 (2000).
7. A. A. Teleman, S. M. Cohen, *Cell* **103**, 971 (2000).
8. K. Basler, G. Struhl, *Trends Genet.* **10**, 187 (1994).
9. J. Lippincott-Schwartz, N. Altan-Bonnet, G. H. Patterson, *Nat. Cell Biol.* **5** (suppl.), S7 (2003).
10. G. Carrero, D. McDonald, E. Crawford, G. de Vries, M. J. Hendzel, *Methods* **29**, 14 (2003).
11. A. Charpilienne *et al.*, *J. Biol. Chem.* **276**, 29361 (2001).
12. D. Axelrod, D. E. Koppel, J. Schlessinger, E. Elson, W. W. Webb, *Biophys. J.* **16**, 1055 (1976).
13. J. Ellenberg, J. Lippincott-Schwartz, *Methods* **19**, 362 (1999).
14. R. Swaminathan, C. P. Hoang, A. S. Verkman, *Biophys. J.* **72**, 1900 (1997).
15. G.-H. Baeg, E. M. Selva, R. M. Goodman, R. Dasgupta, N. Perrimon, *Dev. Biol.* **276**, 89 (2004).
16. C. Han, D. Yan, T. Y. Belenkaya, X. Lin, *Development* **132**, 667 (2005).
17. T. Lecuit, S. M. Cohen, *Development* **125**, 4901 (1998).
18. J. Klingauf, E. T. Kavalali, R. W. Tsien, *Nature* **394**, 581 (1998).
19. S. Pfeiffer, S. Ricardo, J.-B. Manneville, C. Alexandre, J.-P. Vincent, *Curr. Biol.* **12**, 957 (2002).
20. K. Willert *et al.*, *Nature* **423**, 448 (2003).
21. J. Groppe *et al.*, *J. Biol. Chem.* **273**, 29052 (1998).
22. E. S. Seto, H. J. Bellen, *J. Cell Biol.* **173**, 95 (2006).
23. A. F. Rives, K. M. Rochlin, M. Wehrli, S. L. Schwartz, S. DiNardo, *Dev. Biol.* **293**, 268 (2006).
24. We thank J.-P. Vincent and A. Charpilienne for reagents, C. P. Heisenberg and E. Entchev for critical reading of the manuscript, as well as all members of the M.G.-G. and F.J. groups. This work was supported by the Max Planck Society, Deutsche Forchungsgemeinschaft, Volkswagen Stiftung, the European Union, and the Human Frontier Science Program.

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A "Silent" Polymorphism in the *MDR*1 Gene Changes Substrate Specificity

Chava Kimchi-Sarfaty,*† Jung Mi Oh,†‡ In-Wha Kim, Zuben E. Sauna, Anna Maria Calcagno, Suresh V. Ambudkar, Michael M. Gottesman†

Synonymous single-nucleotide polymorphisms (SNPs) do not produce altered coding sequences, and therefore they are not expected to change the function of the protein in which they occur. We report that a synonymous SNP in the *Multidrug Resistance* 1 (*MDR*1) gene, part of a haplotype previously linked to altered function of the *MDR*1 gene product P-glycoprotein (P-gp), nonetheless results in P-gp with altered drug and inhibitor interactions. Similar mRNA and protein levels, but altered conformations, were found for wild-type and polymorphic P-gp. We hypothesize that the presence of a rare codon, marked by the synonymous polymorphism, affects the timing of cotranslational folding and insertion of P-gp into the membrane, thereby altering the structure of substrate and inhibitor interaction sites.

The *MDR*1 gene product, the adenosine triphosphate (ATP)–binding cassette (ABC) transporter ABCB1 or P-gp, is an ATP-driven efflux pump contributing to the pharmacokinetics of drugs that are P-gp substrates and to the multidrug resistance of cancer cells (*1*, *2*). To date, more than 50 single-nucleotide polymorphisms (SNPs) have been reported for *MDR*1 (www.ncbi.nlm.nih.gov/SNP/GeneGt.cgi?geneID=5243). One of these, a synonymous SNP in exon 26 (C3435T), was sometimes found to be associated with altered P-gp activity (*3*–*6*) and, when it appears in a haplotype, with reduced functionality (*7*). This association may be explained in different ways. Perhaps it is because C3435T is in linkage disequilibrium with other common functional nonsynonymous polymorphisms such as G2677T. In fact, the C1236T (a synonymous SNP), G2677T, and C3435T polymorphisms are part of a common haplotype (*8*, *9*). Another possible explanation is that allele-specific differences in mRNA folding could influence splicing, processing, or translational control and regulation (*10*, *11*). A third possibility is that the effect of the C3435T polymorphism on the levels of cell surface P-gp activity or its function is rather modest or drug-specific. Finally, numerous environmental factors are known to affect the expression and phenotypic activity of P-gp (*12*).

To determine whether the C3435T polymorphism actually does affect P-gp activity, we expressed wild-type and polymorphic P-gps in HeLa cells with the use of a transient expression system (*13*). The same experiments were carried out on BSC-1 (epithelial cells of African green monkey kidney origin), Vero-76 (monkey kidney cells), and 12E1 (CEM human cells) cell lines (*14*), with similar results, indicating that this phenomenon is not specific to HeLa cells.

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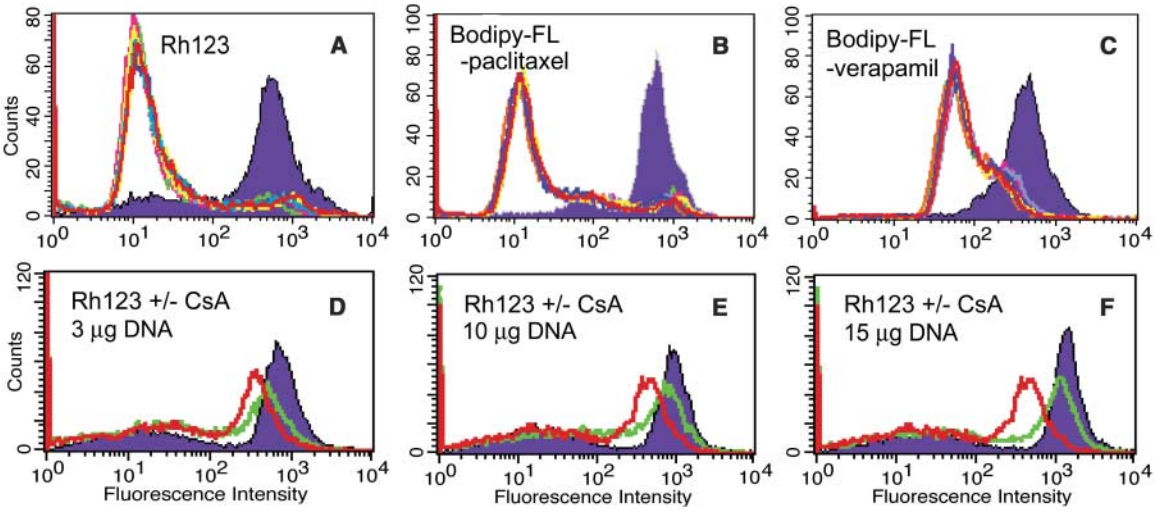

Fig. 1. Drug transport function of wild-type MDR1 and seven MDR1 SNPs and haplotypes. The drug efflux from vaccinia virus infected/transfected HeLa cells was determined by FACS analysis. Cells were transfected with pTM1 (control; purple), pTM1-*MDR*1 (wild-type P-gp; green), C1236T (pink), G2677T (lavender), C3435T (orange), C1236T-G2677T (blue), C1236T-C3435T (yellow), G2677T-C3435T (light blue), and C1236T-G2677T-C3435T (red). (**A**) 0.5 μM Rh123; (**B**) 0.1 μM bodipy-FL-paclitaxel; (**C**) 0.5 μM bodipy-FL-verapamil. (**D** to **F**) Effect of plasmid DNA concentration during infection/transfection on Rh123 efflux (0.5 μM) in the presence of an inhibitor, 10 μM CsA; infected/transfected DNA, (D) 3 μg, (E) 10 μg, (F) 15 μg.

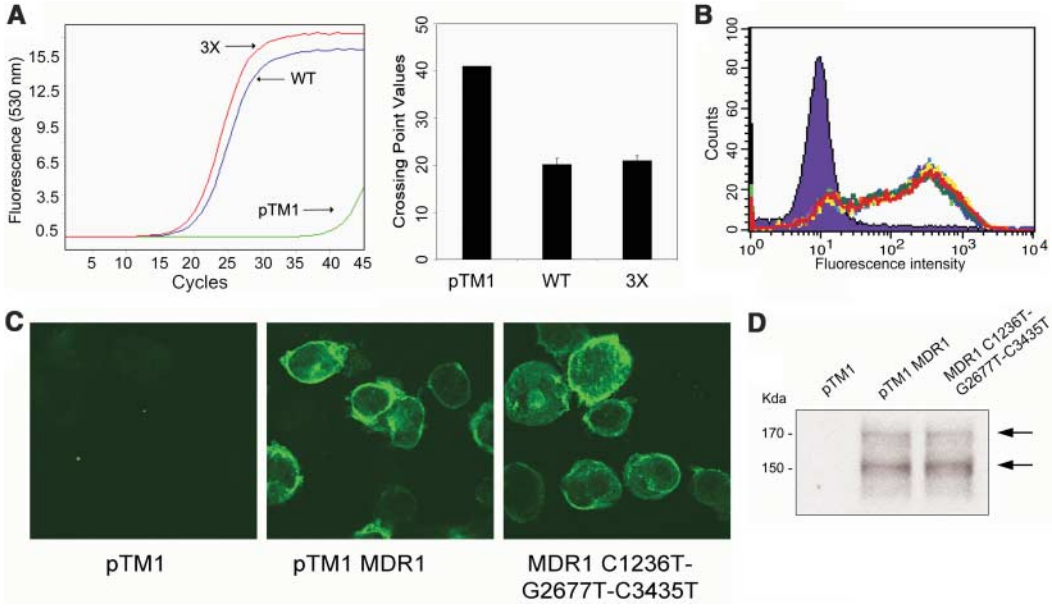

Fig. 2. mRNA levels and P-gp expression in the vaccinia expression system. (**A**) Analysis of pTM1 only, wild-type *MDR*1, and the haplotype C1236T-G2677T-C3435T (3X) with real-time quantitative RT-PCR. Crossing-point values for the graph on the left are plotted in the histogram. (**B**) Assessment of cell surface expression, using MRK16 mAb of all nine constructs as described in Fig. 1. (**C**) Confocal assessment of MDR1 expression, using MRK16 mAb with fluorescein isothiocyanate–conjugated secondary antibody of pTM1 (control; left panel), pTM1-MDR1 (wild-type P-gp; middle panel), and C1236T-G2677T-C3435T (right panel). (**D**) Immunoblot analysis of pTM1 only, wild-type MDR1, and the haplotype C1236T-G2677T-C3435T (2 μg protein/lane) with C219 mAb (*14*). The mature fully glycosylated (~170 kD) and immature P-gp bands (~150 kD) are marked by arrows (*19*).

Assays for P-gp's transport function with the fluorescent substrates Rhodamine 123 (Rh123), bodipy-FL-paclitaxel, bodipy-FL-verapamil, daunorubicin, bodipy-FL-vinblastine, and calcein-AM (*14*, *15*) were performed on HeLa cells expressing the MDR1 wild-type; polymorphisms at C1236T, G2677T, or C3435T; and haplotypes consisting of these polymorphic variant combinations: C1236T-G2677T, C1236T-C3435T, G2677T-C3435T, and C1236T-G2677T-C3435T. The functions of P-gp for all single-polymorphism plasmids as well as for wild-type MDR1, as measured by intracellular accumulation or by efflux of fluorescent compounds, were not distinguishable under standard conditions (*14*). HeLa cells expressing double- and triple-haplotype mutants also revealed results similar to those for the single mutants (Fig. 1, A to C). However, the P-gp inhibitors cyclosporin A (CsA) and verapamil (fig. S1) were less effective against all the substrates in cells expressing the double or triple haplotypes carrying C3435T relative to the wild type, the SNPs, or the haplotype that does not carry C3435T. Thus, it is not the presence of the nonsynonymous polymorphism G2677T that results in the phenotype, but rather the presence of C3435T in combination with one or two of the other polymorphisms.

We next tested to see whether these differences correlated with the concentrations of transduced plasmid DNA. The expression and function of all transduced cells were measured by fluorescence-activated cell sorting (FACS) with MRK16 monoclonal antibody (mAb) staining and by Rh123 in the presence of CsA, respectively (*14*). The differences in inhibition by CsA and Rh123 between the cells expressing wild-type MDR1 and the haplotype C1236T-G2677T-C3435T were more distinct as the concentration of the DNA increased (Fig. 1, D to F). These data suggest that the differences were more pronounced at higher levels of mRNA where more P-gp was being translated in the cells. The expression levels of P-gp from the vaccinia infection/transfection system and cells of normal human adrenal glands were found to be comparable (fig. S2).

Figure S1, C to E, shows that the haplotypes including C3435T had altered susceptibility to verapamil, but not to rapamycin (fig. S1F) (*14*). When the cells were incubated with the inhibitors before adding the fluorescent substrates, as opposed to simultaneous incubation with the drugs, the same pattern was observed. Bodipy-FL-verapamil, wild-type P-gp, and the haplotype (C1236T-G2677T-C3435T) exhibited different

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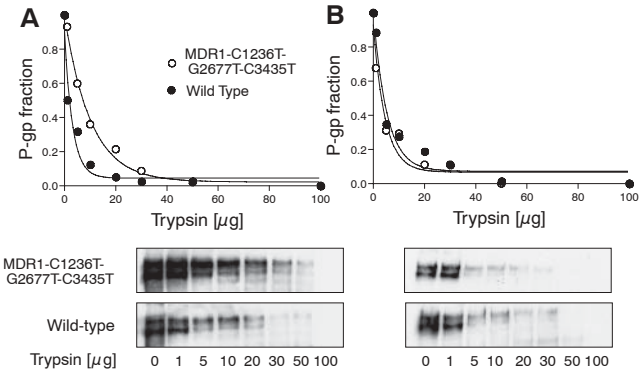

Fig. 3. Determining the sensitivity of wild-type and the haplotype C1236T-G2677T-C3435T P-gp to trypsin. Crude membranes prepared from vTF7-3 infected/transfected HeLa cells expressing wild-type MDR1 or the haplotype C1236T-G2677T-C3435T were treated with increasing concentrations of trypsin and the disappearance of the P-gp band was quantified as described above. (**A**) Experiment performed in the absence of verapamil; IC_{50} = 2.1 μg (wild type), 7.1 μg (C1236T-G2677T-C3435T). The mature (170 kD) and immature (150 kD) P-gp bands were also analyzed separately; IC_{50} = 0.68 μg (wild-type immature), 2.9 μg (haplotype immature), 2.8 μg (wild-type mature), 10.8 μg (haplotype mature). (**B**) Same experiment in the presence of 30 μM verapamil; IC_{50} = 3.7 μg (wild type), 3.3 μg (C1236T-G2677T-C3435T). Values for the mature and immature P-gp bands: IC_{50} = 2.5 μg (wild-type immature), 2.5 μg (haplotype immature), 3.6 μg (wild-type mature), 3.2 μg (haplotype mature). Immunoblots with C219 mAb are shown at the bottom.

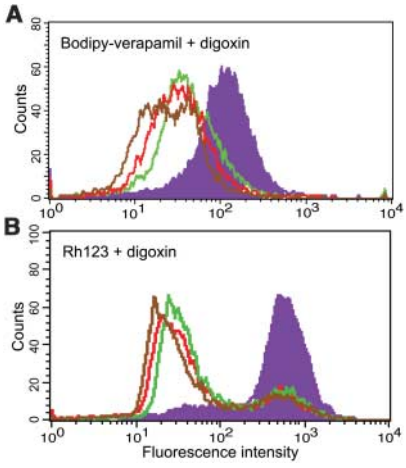

Fig. 4. Drug transport function of wild-type and two MDR1 haplotypes. The drug efflux of vaccinia infected/transfected HeLa cells was determined by FACS analysis (*14*). Cells were transfected with pTM1 (control; purple), MDR1, (wild-type P-gp; green), C1236T-G2677T-C3435T (red), and C1236T-G2677T-C3435A (brown). (**A**) 0.5 μM bodipy-FL-verapamil in the presence of 500 μM digoxin; (**B**) 0.5 μM Rh123 in the presence of 150 μM digoxin.

accumulations in a concentration-dependent manner, suggesting a change in affinity (fig. S3).

Synonymous SNPs or mutations can cause inactivation of the native splicing donor site, which results in a premature stop codon (*16*) or exon skipping, yielding a shorter mRNA. A previous report indicated that the polymorphism C3435T resulted in decreased levels of mRNA expression (*17*). We therefore compared mRNA levels (*14*) in the wild-type and haplotype (C1236T-G2677T-C3435T) with the use of real-time quantitative reverse transcription polymerase chain reaction (RT-PCR), which revealed equivalent mRNA levels (Fig. 2A). Western blots using C219 mAb showed comparable total cell expression of P-gp, and the infected/transfected cells expressed the same levels of P-gp as determined by FACS assays and immunohistochemical staining with MRK16 mAb (Fig. 2, B and C). This result was reproduced in different cell lines including BSC-1, Vero-76, and 12E1 (fig. S4). The complete amino acid sequence of MDR1 haplotype C1236T-G2677T-C3435T protein was identical to the predicted sequence.

We hypothesized that a conformation difference between wild-type and haplotype P-gp might explain these results. Indeed, UIC2 (*14*), a conformation-sensitive mAb, alone or in combination with CsA or vinblastine at 37°C, revealed pronounced differences in binding consistent with altered conformations in the haplotype (fig. S5) (*18*). To determine whether there are subtle differences in the folding of wild-type and haplotype P-gp, we compared their relative susceptibility to trypsin. Figure 3 shows the disappearance of the P-gp band as a function of trypsin concentration. The concentration required for 50% degradation (IC_{50}, here expressed as μg trypsin) was greater for haplotype P-gp than for wild-type P-gp by a factor of about 3.4; this result implies that the two have slightly different tertiary structures. Both wild-type and haplotype P-gps had comparable IC_{50} (μg trypsin) values in the presence of verapamil, which suggests that the altered conformation can be corrected by drug interaction with P-gp. The immature, core-glycosylated form of P-gp (150-kD band) was more sensitive to trypsin than the mature, glycosylated form, consistent with (*19*), by a factor of 5. However, the ratios (wild-type:haplotype) of the IC_{50} (μg trypsin) values were comparable for the mature and immature bands (3.86 μg versus 4.4 μg). Thus, it is unlikely that altered glycosylation is responsible for the functional differences observed.

The use of rare codons appears to influence the translation rate, which in turn affects protein folding (*20*–*25*), with the third base in the codon having the largest effect (*26*). We hypothesize that as the cell produces more P-gp (Fig. 1, D to F), the role of codon usage may become more critical when certain tRNA species become depleted. The codon usage for the SNP at position 12/1236 with GGC changed to GGT (both encode Gly) changes from 34% [relative synonymous codon usage (RSCU), 22.4] to 16% (RSCU, 10.8). The SNP at position 21/2677 that changes GCT (Ala) to TCT (Ser) also uses a less common codon (26% to 18%; RSCU values change from 18.5 to 15.1). The SNP at position 26/3435 that changes the codon from ATC (Ile) to ATT (Ile) reduces the codon usage from 47% to 35% (RSCU values change from 20.9 to 15.8). Clusters of rare codon usage (table S1) occur both upstream and downstream of each of these SNPs. Codon usage rates are similar in humans and monkeys, which explains the similarity in the results with all transduced cells (*27*).

To test whether codon usage compromises P-gp function, we introduced C3435A (isoleucine codon usage for ATA is 18%, RSCU 7.4) to produce the haplotype C1236T-G2677T-C3435A. Functional assays using bodipy-verapamil or Rh123 in the presence of digoxin (Fig. 4, A and B) showed even larger decreases in inhibitor effects between this haplotype and the common haplotype C1236T-G2677T-C3435T. Moreover, use of Rh123 in the presence of fexofenadine revealed median fluorescence of 26.9 for the wild type, 24.3 for C1236T-G2677T-C3435T, and 20.3 for C1236T-G2677T-C3435A. The median fluorescence in the presence of paclitaxel and fexofenadine was 38.2 for the wild type, 28.6 for C1236T-G2677T-C3435T, and 22.9 for C1236T-G2677T-C3435A.

The amino acid sequence of proteins is generally believed to determine protein expression, folding, and function; mutations that alter the primary structure of a protein can affect these properties. The important question addressed by this study is the role of silent mutations (i.e., those that do not affect amino acid sequence) in protein folding and function. Recent theoretical studies have suggested that codon usage is not random, and experimental studies in prokaryotes suggest that this may be so (*28*). Here we show that a silent mutation in a complex, mammalian membrane transport protein alters the substrate specificity. We hypothesize that when frequent codons are changed to rare codons in a cluster of infrequently used codons, the timing of cotranslational folding is affected (*29*) and may result in altered function. This finding may be clinically important. For example, mutations in the *MRP6* (*ABCC6*) gene cause the disease pseudoxanthoma elasticum, but missense and nonsense mutations are found in only about 60% of cases (*30*), raising the possibility that mutations that do not change coding sequence may contribute to disease by a similar mechanism.

References and Notes

1. M. M. Gottesman, T. Fojo, S. E. Bates, *Nat. Rev. Cancer* **2**, 48 (2002).
2. C. Cordon-Cardo *et al.*, *J. Histochem. Cytochem.* **38**, 1277 (1990).

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3. S. Hoffmeyer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 3473 (2000).
4. S. Drescher *et al.*, *Br. J. Clin. Pharmacol.* **53**, 526 (2002).
5. M. Goto *et al.*, *Pharmacogenetics* **12**, 451 (2002).
6. S. V. Ambudkar, C. Kimchi-Sarfaty, E. Z. Sauna, M. M. Gottesman, *Oncogene* **22**, 7468 (2003).
7. H. N. Salama, Z. Yang, T. Bui, R. J. Y. Ho, *J. Pharm. Sci.* **95**, 2293 (2006).
8. K. Tang *et al.*, *Pharmacogenetics* **12**, 437 (2002).
9. M. Horinouchi *et al.*, *Pharm. Res.* **19**, 1581 (2002).
10. L. X. Shen, J. P. Basilion, V. P. Stanton Jr., *Proc. Natl. Acad. Sci. U.S.A.* **96**, 7871 (1999).
11. L. Frittitta *et al.*, *Diabetes* **50**, 1952 (2001).
12. M. D. Perloff, L. L. von Moltke, E. Stormer, R. I. Shader, D. J. Greenblatt, *Br. J. Pharmacol.* **134**, 1601 (2001).
13. J. J. Gribar, M. Ramachandra, C. A. Hrycyna, S. Dey, S. V. Ambudkar, *J. Membr. Biol.* **173**, 203 (2000).
14. See supporting material on *Science* Online.
15. C. Kimchi-Sarfaty, J. J. Gribar, M. M. Gottesman, *Mol. Pharmacol.* **62**, 1 (2002).
16. H. T. Thi Tran *et al.*, *Mol. Genet. Metab.* **85**, 213 (2005).
17. D. Wang, A. D. Johnson, A. C. Papp, D. L. Kroetz, W. Sadee, *Pharmacogenet. Genomics* **15**, 693 (2005).
18. A. Ruth, W. D. Stein, E. Rose, I. B. Roninson, *Biochemistry* **40**, 4332 (2001).
19. T. W. Loo, D. M. Clarke, *J. Biol. Chem.* **273**, 14671 (1998).
20. I. Z. Siemion, P. J. Siemion, *Biosystems* **33**, 139 (1994).
21. S. Brunak, J. Engelbrecht, *Proteins* **25**, 237 (1996).
22. A. A. Adzhubei, I. A. Adzhubei, I. A. Krasheninnikov, S. Neidle, *FEBS Lett.* **399**, 78 (1996).
23. S. K. Gupta, S. Majumdar, T. K. Bhattacharya, T. C. Ghosh, *Biochem. Biophys. Res. Commun.* **269**, 692 (2000).
24. P. Cortazzo *et al.*, *Biochem. Biophys. Res. Commun.* **293**, 537 (2002).
25. A. A. Komar, T. Lesnik, C. Reiss, *FEBS Lett.* **462**, 387 (1999).
26. T. Xie, D. Ding, *FEBS Lett.* **434**, 93 (1998).
27. P. M. Sharp, W. H. Li, *Nucleic Acids Res.* **15**, 1281 (1987).
28. F. Supek, K. Vlahovicek, *BMC Bioinformatics* **6**, 182 (2005).
29. V. Anthony, W. R. Skach, *Curr. Protein Pept. Sci.* **3**, 485 (2002).
30. L. Bercovitch, P. Terry, *J. Am. Acad. Dermatol.* **51**, S13 (2004).

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Imaging of Germinal Center Selection Events During Affinity Maturation

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The germinal center (GC) is an important site for the generation and selection of B cells bearing high-affinity antibodies, yet GC cell migration and interaction dynamics have not been directly observed. Using two-photon microscopy of mouse lymph nodes, we revealed that GC B cells are highly motile and extend long cell processes. They transited between GC dark and light zones and divided in both regions, although these B cells resided for only several hours in the light zone where antigen is displayed. GC B cells formed few stable contacts with GC T cells despite frequent encounters, and T cells were seen to carry dead B cell blebs. On the basis of these observations, we propose a model in which competition for T cell help plays a more dominant role in the selection of GC B cells than previously appreciated.

Germinal centers (GC) represent critical sites within organized lymphoid tissues in which B cell responses to antigen are amplified and refined in specificity. A classical model of GC function holds that B cells in the dark zone undergo rapid rounds of proliferation and somatic hypermutation of their antibody genes, followed by exit from the cell cycle and movement to the light zone, where the B cells undergo selection based on the affinity of their surface antibody for antigen (*1*–*5*). The selection process is thought to involve competition between GC B cells for capture of antigen in the form of immune complexes displayed on the processes of follicular dendritic cells (FDCs) (*1*, *3*, *5*, *6*). However, recent experimental evidence and computer simulations have contradicted aspects of this classical model (*3*, *7*–*9*),

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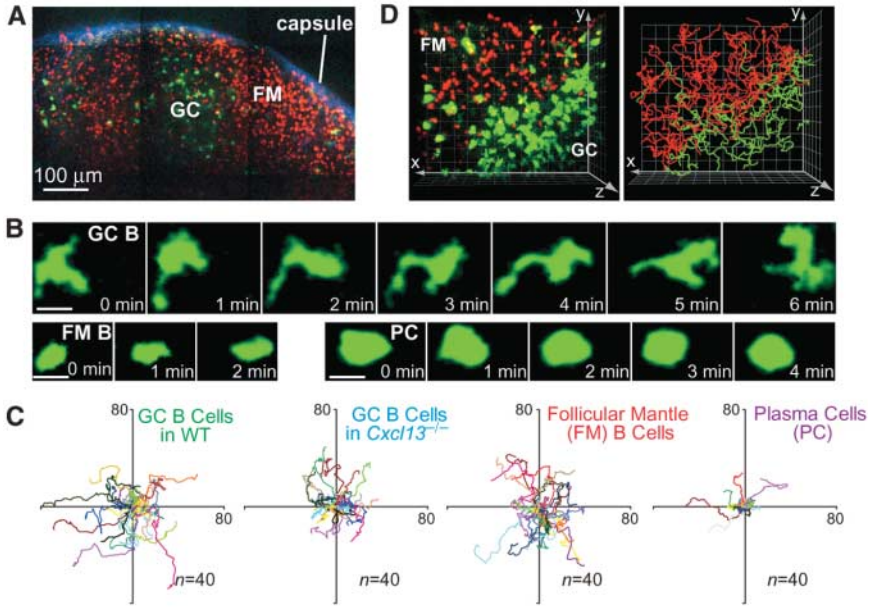

Fig. 1. Dynamics and motility of GC B cells compared with follicular mantle (FM) B cells and plasma cells (PC). (**A**) An 18-μm maximum intensity *z*-projection from two-photon microscopy image stacks of a GC and FM in an intact LN. A time-lapse recording corresponding to the center of this region is shown in movie S1. (**B**) Representative time-lapse images from two-photon microscopy showing the morphology of a GC B cell, FM B cell, and PC. The FM B cells in this experiment were naïve GFP$^+$ cells that were also labeled with CMTMR (*10*), and only the GFP channel is shown in the images. Scale bars, 10 μm. (**C**) Superimposed 15-min tracks of 40 randomly selected cells of each indicated type in the *xy* plane, setting the starting coordinates to the origin. Units are in micrometers. WT, wild type. Each color represents one cell's path. (**D**) (Left) Maximum-intensity projection of FM (red) and GC (green) B cells. (Right) Tracks of FM (red) and GC (green) B cells. The gridlines are separated by 20 μm.

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ERRATUM

Post date 30 November 2007

Reports: "A 'silent' polymorphism in the *MDR*1 gene changes substrate specificity" by C. Kimchi-Sarfaty *et al.* (26 Jan. 2007, p. 525). Based on an inquiry from Jack Kornblatt, the authors wish to clarify that the protein sequence was obtained from a detailed mass spectrometric study performed at the Harvard Microchemistry Facility (HMF) by microcapillary reverse-phase HPLC nano-electrospray tandem mass spectrometry. HMF performed both chymotryptic and pronase digestions of the protein. In all, 82 peptides (representing 37% of the Pgp sequence by amino acid count) were identified and sequenced (see Supporting Online Material at www.sciencemag.org/cgi/content/full/318/5855/1382/DC1). Each of these sequences was identical to the sequence of haplotype P-glycoprotein. Moreover, several different peptides encoded by the synonymous SNP (3435C>T), which is the key polymorphism linked to the functional change in Pgp, were sequenced and found to be unchanged. In addition, the analysis of codon usage, Table S1 in the original Supporting Online Material (see www.sciencemag.org/cgi/data/1135308/DC1/1) contains for each codon around the three polymorphisms the frequency of this codon per 1000 codons in the human genome instead of RSCU values as stated in the text. These values were obtained from the codon usage Web site at (http://www.kazusa.or.jp/codon/cgibin/showcodon.cgi?species=Homo+sapiens+%5Bgbpri%5D). Figure 1, panels D to F, shows results in the presence of cyclosporin A (+CsA) not (+/CsA) as indicated in the body of the figure. This is correctly stated in the legend. These clarifications do not affect the conclusions of the paper.

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LETTERS

edited by Jennifer Sills

Global and Local Conservation Priorities

IN THEIR POLICY FORUM, "GLOBALIZATION OF CONSERVATION: A view from the South" (10 August, p. 755), J. P. Rodríguez *et al*. claim that international nongovernmental organizations (INGOs) promote conservation from the top down, through global biodiversity priority-setting, rather than from the bottom up, by supporting local groups or building local capacity in areas of high biological importance. They also liken INGOs to transnational corporations. We respectfully disagree with both assertions.

Perhaps the best example of support from an INGO to community-based conservation is World Wildlife Fund's (WWF's) program in Namibia, where 50 communal conservancies operating over 119,000 km^2 allow indigenous groups to directly benefit from increased control over wildlife resources. Rather than top-down, this initiative devolves user rights to groups previously marginalized under apartheid. Another example is Qualilea Island, Quirimbas National Park, Mozambique, the largest marine reserve on the east coast of Africa, which was established with WWF support to local communities to better manage local fisheries (see photo).

Conservation organizations are not faceless transnational corporations; rather, they are led by passionate defenders of the natural world who are devoted to helping local communities and building local conservation capacity. Three outstanding examples draw from the work of Henri Nsanjama, Mingma Norbu Sherpa, and Chandra Gurung (*1*, *2*). During his lifetime, Nsanjama was Head of the African College of Wildlife Management before joining WWF, and in his tenure as Vice President for Africa he did more to build local capacity among African professionals than virtually any of his contemporaries. Mingma Sherpa and Chandra Gurung were world-renowned conservationists who helped create the first locally managed conservation area in Asia, in the Annapurna region. In September 2006, they and 22 others—including some of the region's leading conservationists—perished in a helicopter crash after successfully handing over the largest community-run nature conservation area, Kanchenjunga, to a local management committee. The memories of Henri, Mingma, and Chandra inspire all conservationists committed to supporting local conservation efforts.

Community empowerment. A local fisherman sets up a buoy marking a boundary of a community-enforced total protection zone near Rolas Island in the northern part of Quirimbas National Park.

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References

1. E. Dinerstein, P. Yonzon, *Conserv. Biol*. **21**, 278 (2007).
2. E. Dinerstein, *Pachyderm* **41**, 107 (2007).

IN THE POLICY FORUM "GLOBALIZATION OF conservation: A view from the South" (10 August, p. 755), J. P. Rodríguez and colleagues argue that large international nongovernmental organizations (INGOs) set the global conservation agenda by using tools to define worldwide priorities of conservation. As a result, they assert, INGOs increase their own fundraising capacity, investments in biodiversity conservation by local governments decline, and local NGOs (LNGOs) are forced out of the market. Thus, they compare INGOs to transnational corporations.

Current experience in Brazil is otherwise. First, the executed budget of the Brazilian Ministry for Environment has doubled between 1999 and 2006 (*1*), which parallels the range of increase in expenditures (shown by Rodríguez *et al*.) for global actions fostered by Conservation International (CI) and World Wildlife Fund (WWF)—INGOs with strong Brazilian branches.

Second, the argument that conservation training is insufficiently supported by INGOs does not hold true in Brazil. Graduate training, which has recently been credited with boosting Brazilian scientific productivity (*2*), is traditionally fostered by governmental agencies. However, INGOs and LNGOs now occupy a central role in graduate training on biodiversity sciences, with no niche overlap. Funding for field courses, research projects, and infrastructure is provided by both INGOs (such as CI and WWF) and LNGOs (such as Fundação Biodiversitas, Fundação O Boticário, and Instituto Internacional de Educação do Brasil) (*3*–*6*). The LNGO Instituto de Pesquisas Ecológicas (IPE) will soon pioneer a professional masters program focused on conservation (*7*). CI is providing funds, grants, and personnel to a graduate program on tropical biodiversity at the Federal University of Amapá in partnership with federal and local governments (*8*). Both IPE and CI are promoting high-quality training to conservationists and academics.

I agree with Rodríguez and colleagues' idea that conservation leadership ought to be decentralized and integrated into local conditions. In Brazil, however, this is a governmental issue, as defined by the federal constitution.

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There is plenty of room for both INGOs and LNGOs to help Brazil reach higher scientific standards in biodiversity sciences and to bridge scientific knowledge and decision-making (*6*).

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References

1. Ministério do Meio Ambiente, "Relatório de Gestão 2003:2006—Política Ambiental Integrada para o Desenvolvimento Sustentável" (Brasília, Brazil, 2007); www.mma.gov.br/estruturas/ascom_boletins/_arquivos/07032007_relatoriodegestao2003_2006.pdf [in Portuguese].
2. W. Glänzel *et al.*, *Scientometrics* **67**, 67 (2006).
3. http://ecologia.icb.ufmg.br/~ecmvs/exportar/manual_aluno.htm [in Portuguese].
4. Postgraduate Program in Ecology and Conservation, Universidade Federal de Mato Grosso; www.dbi.ufms.br/mstecopan/ [in Portuguese].
5. Postgraduate Program in Ecology and Conservation, Universidade Federal do Paraná; www.bio.ufpr.br/ecologia/ [in Portuguese].
6. Instituto Internacional de Educação do Brasil; www.iieb.org.br/ [in Portuguese].
7. F. R. Scarano, *Sci. Agric.* **64**, 439 (2007).
8. Postgraduate Program in Tropical Biodiversity; www.unifap.br/ppgbio/historico.htm [in Portuguese].

IN THEIR POLICY FORUM, "GLOBALIZATION OF conservation: A view from the South" (10 August, p. 755), J. P. Rodríguez *et al*. call upon Southern scholars to promote self-governing local institutions, enhance human capacity, and secure local participation in conservation. These goals cannot be met without certain prerequisites.

In order for the participation of local people and institutions to flourish, it is necessary to build local community capital (*1*), improve local governance (*2*), and enhance equity in benefit and burden sharing. Only 6% of biological scientists live in the South, which is home to more than 85% of the world's biodiversity (*3*); without bolstering human capital, our efforts in achieving these goals might be counterproductive. Moreover, in light of the cuts in university scholarships and the small percentage of the INGOs' budgets that is devoted to building capacity in developing countries, regional-level efforts are needed to generate scholarship. To improve training efficiency, online training and in-country or on-site trainings should be encouraged.

The South is home to some renowned universities and highly competent scientists. Other countries should send scholars to those universities, and regional scientists should mentor university students. More interaction between southern countries is crucial to achieving self-sustainability goals, but short- and long-term alliances between northern and southern countries can still reduce redundancy and improve efficiency. Let's work together to translate the slogan "think globally, act locally" into action.

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References

1. J. N. Pretty, *Science* **302**, 1912 (2003).
2. T. Dietz, E. Ostrom, P. C. Stern, *Science* **302**, 1907 (2003).
3. J. Sarukhán, R. Dirzo, in *Encyclopaedia of Biodiversity*, S. A. Levin, Ed. (Academic Press, San Diego, CA, 2001), vol. 1, pp. 419–436.

J. P. RODRÍGUEZ *ET AL*. (POLICY FORUM, 10 August, p. 755) highlight the need for increased funding and training for local conservation institutions to achieve biodiversity conservation on the ground—a conclusion with which we emphatically agree. They also claim that global conservation prioritization templates are equivalent to top-down development plans, but do not acknowledge that one purpose of global priority-setting is channeling globally flexible resources to the regions that need it most (*1*).

Conservation International (CI) and other global conservation organizations act as conduits for financial support, capacity-building, and technical assistance within countries. Over the past 5 years, CI has provided more than $100 million in funding to more than 1000 partners. Arguably, these resources come from sources that would not have been available without global conservation NGO action.

Global conservation priorities also serve as a scientific blueprint for governments to adapt and link local and national priorities with global ones. Madagascar provides one such powerful example: President Marc Ravalomanana's government has used CI's Hot Spots concept as a way to engage the international community in supporting a plan to triple the country's protected area coverage. Such national leadership combined with local implementation capacity has led to the creation of a national conservation trust fund that now has a capitalized value of over U.S. $30 million.

We agree with Rodríguez *et al*. that "solutions must integrate extremely diverse natural, socioeconomic, and cultural systems and usually require a sense of community ownership," which is why CI supports local groups in developing responses they deem appropriate. Approaches often support existing organizations in building others at scales below them. For example, CI's Critical Ecosystem Partnership Fund provides funds to the Liberia Conservation Action Fund, which then makes grants to small NGOs.

A fundamental pillar of our business model is to create partnerships for more lasting and

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powerful conservation results. These partnerships include a long-term component of capacity-building and learning so that CI can eventually divest from an area when local leadership is strong. Often, these solutions mean that CI funds organizations and programs well outside of universities or the biological sciences, believing instead that the "strong local institutions and individuals" that Rodríguez *et al*. call for must span civil society. It is precisely these strengthened sectors and partnerships, from local to global scales, that are needed to bring about conservation successes.

RUSSELL A. MITTERMEIER, CLAUDE GASCON, LEON RAJAOBELINA, JATNA SUPRIATNA, JOSE MARIA CARDOSO DA SILVA, CARLOS MANUEL RODRIGUEZ, LU ZHI, KATRINA BRANDON
Conservation International, Arlington, VA 22202, USA.

Reference

1. T. M. Brooks *et al*., *Science* **313**, 58 (2006).

Response

THE LETTERS BY DINERSTEIN, SCARANO, AND Chaudhary focus on the capacity-building elements of our article, rather than on our critique of branding strategies of international non-governmental organizations (INGOs). While we are grateful to the authors for providing cases of successful capacity-building, it remains unclear whether this is a prevalent trend among biodiversity conservation INGOs or governmental organizations worldwide. Investment in local capacity has not been a funding priority (*1*), even though the existing cadre of conservation professionals is substantially below the level required for biodiversity-rich countries (*2*, *3*). Additionally, strengthening local capacity is not identified in the mission statements of major INGOs (*4*), nor is it systematically assessed. Philanthropic organizations that fund these INGOs have not prioritized building local capacity, which may partially explain the reluctance of the INGOs to embrace this as a primary goal. Many institutions in developed countries rely on individual donations and endowments to cover core operating costs, sources rarely available in the developing world. Therefore, local organizations often depend on international funds for their projects, which are frequently tied to priorities set by the INGOs.

As Dinerstein and Scarano demonstrate, there is anecdotal evidence of local capacity-building. We recommend a sector-wide, systematic evaluation of investments in strengthening local scientific and institutional capacity for conservation as a basis for developing indicators to guide improvements. Commonly tracked variables in conservation, such as number of hectares protected, deforestation rates, species population trends, legislation passed, and policies changed, are not as useful for this purpose as metrics that track investment in capacity-building. We further recommend that donors create incentives so that grants given to INGOs are implemented directly by local organizations; fund more training at local universities, as suggested by Chaudhary; help local organizations raise funds for their home priorities; and provide management assistance. Better yet, developed country donors could mount a major fundraising effort and provide endowments for core support to local organizations that meet and maintain performance standards; the funds would be distributed based on conservation needs worldwide.

As the examples of Dinerstein and Scarano underscore, INGOs employ many dedicated and talented conservation scientists. Scarano

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shows that Brazil is among the vanguard of countries assigning high priority to building their conservation science sectors, but it is an outlier (along with Mexico) in Latin America. As of 2005, of the 40 formal programs offering conservation biology courses in the region, 67% were registered in either Brazilian or Mexican universities (*2*). Had the tragic event mentioned by Dinerstein occurred in a developed country, it would have remained an immense human tragedy, but the impact on conservation efforts would have been short-lasting; numerous qualified professionals would be available to follow in the tracks of those who died. This is not the case in Nepal or in the vast majority of the developing world.

Mittermeier *et al*. agree that large INGO branding strategies are useful for fundraising, but they offer no scientific evidence to support the prioritizing templates used in the brands. By devoting the large sums they raise to areas selected on grounds that are not the product of scientific consensus (such as Hot Spots) (*5*), they exclude many regions of high biodiversity importance (*6*). Moreover, if only Hot Spots are protected in a sea of development, they will not survive intact in the face of climate change and invasive species, including pathogens. Finally, we are pleased that Mittermeier *et al*. agree with us on the importance of local leadership and capacity-building; we believe that strong local leadership merits major, long-term investment.

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References and Notes

1. G. Castro, I. Locker, "Mapping conservation investments: An assessment of biodiversity funding in Latin America and the Caribbean" (Biodiversity Support Program, Washington, DC, 2000).
2. J. P. Rodríguez, J. A. Simonetti, A. Premoli, M. A. Marini, *Conserv. Biol*. **19**, 969 (2005).
3. J. P. Rodríguez, K. M. Rodríguez-Clark, M. A. Oliveira-Miranda, T. Good, A. Grajal, *Conserv. Biol*. **20**, 1340 (2006).
4. This includes mission statements of World Wildlife Fund (www.worldwildlife.org/about/), The Nature Conservancy (www.nature.org/aboutus/?src=t5), Wildlife Conservation Society (www.wcs.org/sw-our_mission), and Conservation International (http://web.conservation.org/xp/CIWEB/about/).
5. G. M. Mace *et al*., *Nature* **405**, 393 (2000).
6. G. Ceballos, P. R. Ehrlich, *Science* **296**, 904 (2002).

CORRECTIONS AND CLARIFICATIONS

Reports: "A 'silent' polymorphism in the *MDR*1 gene changes substrate specificity" by C. Kimchi-Sarfaty *et al.* (26 January, p. 525). Based on an inquiry from Jack Kornblatt, the authors wish to clarify that the protein sequence was obtained from a detailed mass spectrometric study performed at the Harvard Microchemistry Facility (HMF) by microcapillary reverse-phase HPLC nano-electrospray tandem mass spectrometry. HMF performed both chymotryptic

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TECHNICAL COMMENT ABSTRACTS

Comment on "Decagonal and Quasi-Crystalline Tilings in Medieval Islamic Architecture"

Emil Makovicky

Lu and Steinhardt (Reports, 23 February 2007, p. 1106) claimed the discovery of a large, potentially quasi-crystalline Islamic tiling in the Darb-i Imam shrine but regard the earlier Maragha tiling, previously described as quasiperiodic, as a small isolated motif. We demonstrate that the Darb-i Imam pattern is periodic and that the quasi-crystalline discs superimposed on its lattice are derivatives of the Maragha pattern.

Full text at www.sciencemag.org/cgi/content/full/318/5855/1383a

Response to Comment on "Decagonal and Quasi-Crystalline Tilings in Medieval Islamic Architecture"

Peter J. Lu and Paul J. Steinhardt

Our study showed that both Gunbad-i Kabud and Darb-i Imam tessellations belong to a sequence of Islamic tilings that resolve into a common set of girih tiles, so local similarities are expected. However, historically accurate reconstructions show that Darb-i Imam is unique, the only known example that does not repeat periodically and that displays a self-similar transformation enabling its continuation ad infinitum to a perfect quasi-crystalline pattern.

Full text at www.sciencemag.org/cgi/content/full/318/5855/1383b

Letters to the Editor

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