

THE CELLULAR HULLABALOO

The inner life of a cell is noisy. **Helen Pearson** discovers how the resulting randomness makes life more challenging — and richer.

Sunney Xie's eight-year-old twins have a lot in common — starting with their genome. But they have different fingerprints, footprints and very different personalities. Any father might wonder how two apparently identical cells could turn into daughters fairly easily distinguished, at least by a parental eye. For a father who is also a biochemist at Harvard University in Boston, Massachusetts, that question is not just one for idle musing. "It's my biggest mystery," Xie says.

Two years ago, Xie's interest in 'identical' cells that act differently led him to produce the first comparisons of individual cells producing proteins one by one^{1,2}. Although each of the cells studied under his cutting-edge fluorescent microscope produced the proteins he was looking for in bursts of up to 20 at a time, the sizes of the bursts and rhythm of their timing changed from cell to genetically identical cell. Xie wonders whether some such stochastic difference might have propelled the two identical cells that were to become his children down divergent biological paths.

In text books, molecular biology seems to leave little room for chance and random fluctuations. Cells are seen as exquisite machines, their component molecules fitting together to form mechanisms as neatly as any blind watchmaker could require. Genetic information is treated as software for this hardware to run, and the ways in which chance might make the software run differently in different cellular machines are seen as sources of error.

But in the past few years, some researchers have been exploring a far more haphazard picture of what goes on in the cell. Using techniques that allow one cell to be compared with another, they have found identical genes in identical cells doing wildly different things. Now researchers are trying to understand whether this variation or 'noise' actually matters. How hard do cells work to suppress it, what mechanisms do they have for tolerating it? A study from 2006 suggests that the workings of cells get noisier and noisier as they get older, and that this might contribute to the ageing body's decline³. "People are fascinated by how we do what we do despite this noise," says James Collins who studies stochastic processes at Boston University. "That's what everyone is ultimately trying to get at."

Noise in cells is inevitable. It traces back to the fact that each cell carries only one or two working copies of each gene. The DNA in which the genetic information sits is constantly shifting in both shape and structure, while jiggling proteins attach themselves to it and fall off again. At a given moment, the same piece of DNA may be bound by an activating protein in one cell, and not in another. In the first, a gene will be switched on, triggering the transcription of messenger RNA (mRNA) molecules and their subsequent translation into proteins. In the second, it will not, for the time being. There was no plan — it was all dependent on tiny changes in the configuration of the DNA, or the proximity of specific molecules. "You can never get away from the fact that it's all probability. Every event is up to chance," says Johan Paulsson, a systems biologist at Harvard Medical School. It is this that makes the noise in the cell so pervasive, and so interesting to those who wonder how cells control themselves. It is not just that the turning on and off of genes is noisy; the systems that regulate that turning on and off are themselves ineradicably noisy too. "Every chemical process is a constant battle between randomness and correction," says Paulsson.

An awareness of the part that chance might play has ebbed and flowed over the history of molecular biology, although many current

researchers are "stupefyingly unaware" of the issue, according to Roger Brent, a molecular biologist at the Molecular Sciences Institute in Berkeley, California. The main difference between that earlier thought and today's interest, he says, is new tools that provide "all kinds of abilities to ask questions about single cells".

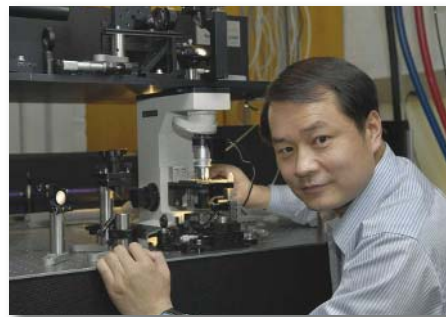
A million states

An early example of these tools is a 2002 study by Robert Singer at the Albert Einstein College of Medicine in New York. Singer's team used the relatively recently developed technology of fluorescent tagging to make four different coloured probes that bound themselves in different combinations to the mRNA transcripts of eleven different genes⁴. Each mRNA was thus given a different colour 'barcode'. The team showed that cells in a genetically homogeneous sample tended to each switch on a different combination of genes at different times. "No two cells seemed to be doing the same thing at the same time," Singer says. "Each cell could be in one of a million different expression states."

The amount of mRNA and protein produced by a population of cells typically forms a classic bell-shaped curve about the mean. The noisiness of a gene's expression is reflected in the width of the relevant bell curve. Some genes are quiet, with neatly peaked curves. Loud ones have their curves spread out. In the noisiest cases, the lowest performers may be making almost an order of magnitude less protein than the most productive cells.

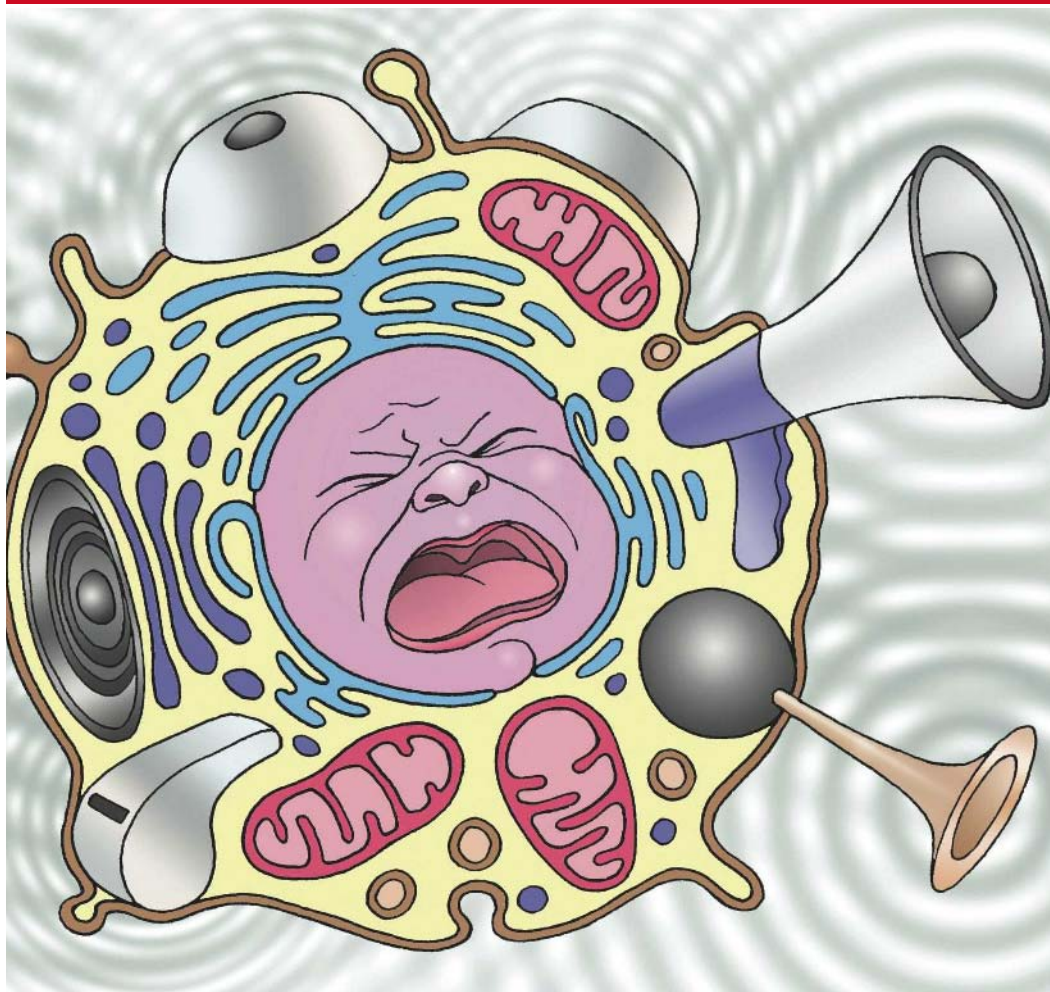
Simply doing away with all this noise is not an issue. In collaboration with Glenn Vinnicombe, an engineer at the University of Cambridge, UK, Paulsson has carried out theoretical work showing that noise abatement can take a huge amount of effort.

The pair modelled bacteria with varying numbers of DNA rings called plasmids. The plasmids control their own copy number by making repressor proteins that block plasmid replication. The more fluctuation there is in



Sunney Xie has found that a random event can trigger one cell (yellow, below) to choose a different fate from that of its identical neighbours.





elderly people balance, and has applied to patent the technology.

In 2006, Collins's team described engineering mutations into the control region of a gene that confers antibiotic resistance to create two strains of the yeast *Saccharomyces cerevisiae*, one with noisier expression of the gene, one with something more steady. Faced with a lethal antibiotic, the noisier strain survived better⁵. This result supports the idea that noise is a form of 'bet hedging' for cells: a population is more likely to survive in a changing environment if its members are noisy because some are likely to be making the quantity of a protein best suited to that situation. "A system that is covering more possibilities has a greater chance of survival in unpredictable settings," says Collins.

Two-colour eyes

Another situation in which cells may have used the stochasticity of gene expression to their advantage is to create a scattered pattern of photoreceptors in the developing retina of the fruitfly *Drosophila*. Here, some 30% of cell clusters called ommatidia include cells that are sensitive to blue light and 70% include those sensitive to green light. Claude Desplan at New York University and his colleagues showed that cells in the developing retina randomly switch on the *spineless* gene, making a transcription factor that controls other genes' activity⁶.

Once on, *spineless* triggers a feedback loop that ensures the cell making it, and the one next door, together form a green-light sensitive ommatidium; those that do not turn on *spineless* make a blue-sensitive ommatidium (see picture, overleaf). Desplan says that evolution has taken advantage of small and random asymmetries between cells to ensure that the photoreceptors are spread across the retina. "Stochasticity makes life very difficult but it must have an evolutionary advantage," he says.

Other researchers are not yet convinced that noise has such biological value. "You can always make up a story of how cell-to-cell variation could be an advantage," says Brent. Noise could present a major obstacle when information is at stake — for example when a cell is measuring conditions outside its walls and needs to transmit that information faithfully to the nucleus. When yeast cells receive an external signal in the form of a pheromone, Brent has shown that their responses (such as recruiting signalling proteins to the cell membrane) are precisely calibrated to the strength of the signal, thus ensuring that they switch on pheromone-response genes in proportion to the pheromone's strength. But in a mutant strain, the signal and response are no longer perfectly aligned, and the cell's pheromone

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the number of repressors, the more fluctuation there will be in the number of plasmids: thus noise will tend to amplify itself. At the same time there is a very strong incentive for the plasmids to clamp down on the noise by producing more repressor proteins — because the more wildly the plasmid number swings, the greater its chance of that number dropping to zero. Selection will favour plasmids that avoid going extinct in this way.

Turning down the volume

According to Paulsson and Vinnicombe's unpublished model, a cell must produce enormous numbers of repressor proteins in order to control plasmid number noise — producing, for example, 160,000 repressors per cell division to make it possible to keep four plasmids to within a 5% standard deviation. Only by manufacturing so many repressors can the cell ensure that the inhibitor levels provide a faithful read-out of the underlying plasmid levels, so that there are enough around to control plasmid number. (Real versions of this type of plasmid use some of the strongest promoters found in nature to turn out many thousands of inhibitors per cell cycle.) Paulsson's theoretical work has convinced him that only the most critical cellular processes are worth the large investment it takes to keep noise under control. "If you

want to do a good job you have to spend a huge amount of energy," he says. "If you want to do a half-decent job you pay much less, but it's still surprisingly expensive."

If removing the noise is not feasible, cells need to make a trade-off between the effort that is required to lower the noise levels and the effort that's required to live with them. The default noise level would thus be, almost by definition, the loudest that's bearable — any higher and the system breaks down. "I think in lots of cases the noise we see is simply the best the cell can do," says Paulsson. "People ask how come an organism works so well. Perhaps it doesn't work so well. Perhaps organisms without these fluctuations would outcompete us."

On that analysis, noise is simply something to be put up with. But another school of thought suggests that it might be more than that. It may be that for some purposes cells have learned to like it noisy. Rather than using energy to minimize all sources of noise or insulate all systems from its effects, the cells may be using the noise to their own ends.

"People are fascinated by how we do what we do despite this noise."

— James Collins

Collins is one of those who sees advantages to noise. His studies over the past 14 years have shown that mechanical and electrical noise applied to neurons can help them respond to weak incoming signals, because they are already partially excited. He also showed, on the same basis, that vibrating insoles can help

response becomes more unpredictable. “Noise is actively worked around,” says Brent, which suggests that evolutionary pressure to ensure signals are transmitted in noise-resistant ways must be strong.

Cellular noise could also pose a problem in the development of an embryo, when cells have to act together to produce precisely timed and coordinated events. “How does a cell control that and make sure it doesn’t take a wrong exit at the wrong time? That’s a very interesting question,” says Alexander van Oudenaarden, a physicist at the Massachusetts Institute of Technology in Cambridge. Working with *Caenorhabditis elegans* embryos, van Oudenaarden is examining the type of developmental mutations that are puzzling because they cause an obvious defect in some animals, but leave others intact. His idea is that fluctuations in noisy genes could explain this behaviour, nudging only some embryos down the mutant developmental pathway. A similar phenomenon might explain why some people with high-risk mutations that predispose them to breast cancer or another disease actually escape unscathed.

The chance of ageing

If noise-abatement is important in suppressing disease, its breakdown may have particular ramifications at the end of our lifespan. Jan Vijg of the Buck Institute for Age Research in Novato, California, measured the expression level of 15 genes in individual muscle cells plucked from the hearts of young, six-month-old mice and elderly 27-month olds³. The variation in gene expression was around 2.5 fold larger in older cells when compared with younger ones, showing that biological noise increased with ageing.

Vijg suggests that heart cells accumulate wear and tear to their DNA, and that this upsets the systems the cells normally use to keep noise reined in, a phenomenon that may contribute to the gradual decrease in heart function with age. “There is a lot of evidence that ageing has this stochastic component,” he says. If it is true that ageing interferes with the control of almost all genes, then drugs or treatments that can reverse the ageing process may be unrealistic, Vijg says. “It will be very difficult to intervene in a basic process of life that you can’t get rid of.”

Vijg and other researchers now wonder whether there are genes and proteins the main function of which is in controlling or suppressing biological noise. Chaperones, proteins that help other proteins fold into the correct shape, are one candidate. They might help compensate



The pattern of photoreceptors in the eyes of the fruitfly depend on random activation of the *spineless* gene.

for volatile protein concentrations by stalling the folding process until all of a system’s components are present in the numbers needed to form a working piece of machinery.

Whereas some are working on the controls over noise, others are looking at its causes. In one of the first such experiments, van Oudenaarden studied genetically identical bacteria making a green fluorescent protein⁷. His group engineered mutated strains in which either transcription of DNA to RNA or translation of RNA to protein was altered — and showed that the noisiness in protein output could be changed systematically.

This and other studies have led to the idea that most noise stems from the rarer events during transcription — typically the infrequent activation of a gene or the slow, fitful production of mRNA molecules. If only five mRNAs for a given protein are present in each cell, then the creation or destruction of one or two extra can produce large variations relative to the mean. If, by contrast, a cell contains 500 copies of an mRNA molecule then the addition of a few extra makes little difference. Michael Elowitz, one of the leaders in the field at the California Institute of Technology in Pasadena, makes the comparison to raindrops falling on a roof. When rain is light, the numbers hitting each roof tile can vary dramatically; but when rain is heavier, those fluctuations are washed away. This means that you need a lot of Paulsson’s repressors around to stop these variations in number from having a drastic effect.

Experiments such as van Oudenaarden’s

have left some researchers wanting more. The total concentration of protein in one cell is itself the result of many separate stochastic moments, separate events in which mRNA and then protein were made one by one. Some scientists wanted to eavesdrop on this process. “We realized we needed to go one step higher in resolution,” says Ido Golding, a physicist at the University of Illinois in Urbana-Champaign. This has been a technological challenge, because fluorescent cameras are usually only sensitive enough to detect the signal coming from 20 or 30 fluorescent proteins in one spot — and the signals can be blurred by diffusion of the protein.

Random bursts

In 2005, Golding described a way around this resolution problem, working at the time in the lab of Edward Cox at Princeton University, New Jersey. The researchers introduced a repetitive motif into one *Escherichia coli* mRNA molecule so that each molecule was bound by 50–100 copies of green fluorescent protein⁸. As each mRNA molecule was produced, enough glowing proteins latched onto it to see it in real time.

As they watched, the gene suddenly switched on and produced an intense burst of mRNAs rather than, as most had thought, constant, steady production. The group calculated that this gene would be off for an average of 37 minutes and then on for 6 minutes, during which time it would produce anywhere from one to eight RNA molecules. The bursts may be caused by random changes in the conformation of DNA that permit transcription, or the random binding and detaching of proteins

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that activate or repress transcription. And it is these bursts, which vary both in length and in the numbers of molecules produced, that eventually produce much of the wide variation in protein levels between cells.

According to Singer, only certain genes may be prone to bursts of activity. Other genes may be permanently accessible for transcription, which proceeds at a more leisurely and continuous pace. For these 'housekeeping' genes, involved in running mitochondria, say, or cellular transport, it may be too risky for cells to leave production to random bursts. For others, such as those involved in responding to a rapidly and unexpectedly changing environment, it may be too risky not to.

Xie found echoes of burst activity in his studies of proteins^{1,2}, where each burst corresponds to the brief lifespan of a single mRNA before it decays. He was able to visualize single proteins because he studied some of those which are anchored in the cell membrane. Being secured in place, their fluorescent signals are not blurred by diffusion. And in the last two years, Xie has edged a little closer to understanding how his two genetically identical daughters became who they are. He has shown how the stochastic behaviour of a single molecule can drive two genetically identical cells to adopt different fates.

Xie's team studies bursts in the production of LacY, a membrane channel through which the sugar lactose enters into *E. coli*. When lactose is present at low levels, a repressor protein binds DNA and prevents production of the channel and other proteins involved in lactose metabolism. When lactose levels are high and enough

has entered the cell, the repressor detaches and the cell switches into a state where it can metabolize and use the sugar. This classic system of gene regulation was first studied by Francois Jacob and Jacques Monod in the 1960s.

Xie's graduate student Paul Choi tracked the channel proteins and the repressor. He found that, now and then, the repressor protein drops off the DNA entirely and takes a minute or so to latch back on. This allows production of mRNA followed by a massive burst of 200–300 channel proteins. Lactose floods into the cell, preventing the repressor from re-binding and triggering lactose metabolism.

So a chance event — a single repressor protein dropping off DNA — thrusts the cell into an entirely different state, or phenotype. And by chance, only a proportion of cells in a population will trigger this switch. This may work to the bacteria's advantage when lactose concentrations are too low to support the entire population of cells, because only some will flip into a state where they can take up and make use of the sugar. "Stochasticity of a single molecule solely determines a life-changing decision of the cell," Xie says. "I don't think it's a stretch to think that these single molecule events will determine more complex phenotypes as well."

After Xie published his protein-bursting papers in *Science* and *Nature* in 2006, he says he received a surprise phone call from a programme manager at the Bill & Melinda Gates

Foundation, in Seattle, Washington, asking him to think about whether his work could somehow help in their fight against tuberculosis. So Xie thought — and he is now embarking on a project to investigate whether stochastic events such as those he has observed in *E. coli* might explain why, from a population of genetically identical bacteria, only some will become extremely drug resistant.

Stochastic events can turn two genetically identical bacteria into ones that behave differently. Can the same really be said of Xie's genetically identical twins? They are, after all, far more complicated organisms than the unicellular types under his microscope. "In very simple cells we have shown for sure that a single stochastic event can lead to different phenotypes," Xie says. For his daughters, "I can't say for sure it's due to that".

Helen Pearson is Nature's biology features editor.

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