

BIOCHEMISTRY

Curbing the excesses of low demand

Metabolic processes are regulated by the relative need for the end product, but this control mechanism may fail if demand is very low. A safety mechanism that copes with low demand has been discovered in bacteria. [SEE LETTER P.237](#)

ATHEL CORNISH-BOWDEN

In bacterial cells, the overproduction of metabolites is normally avoided by mechanisms that are similar in principle to control systems in engineering. In this issue, Reaves *et al.*¹ (page 237) report what happens in mutant bacteria that lack a supposedly essential control mechanism to prevent excessive production of pyrimidine nucleotides — metabolites that act as building blocks for the synthesis of genes, but which are potentially toxic if allowed to accumulate. Instead of observing the expected accumulation, the authors discovered a mechanism in which excess nucleotides are eliminated. In so doing, they identified a plausible role for an enzyme whose physiological function had hitherto been unknown*.

We all know that a kitchen sink is liable to overflow if the tap is left on with the plughole blocked. In most domestic sinks this danger is averted, at least partially, by having an overflow outlet near the top. But in more elaborate engineered systems, such as the domestic toilet, an overflow is avoided by means of negative feedback: as soon as the water in the tank reaches a certain level, the inflow is switched off. Bacterial metabolism is in many ways similar, in that feedback mechanisms prevent the potentially harmful build-up of metabolites. The great explosion of interest in biological regulatory mechanisms in the 1960s followed the realization that negative feedback in metabolism operates in the same way^{2,3} as in engineered systems, by allowing the output of an end product to match demand⁴ (Fig. 1a).

From an evolutionary perspective, what matters in metabolism is not so much the 'overflow' that occurs in the absence of biological feedback controls, but the build-up of compounds that arises when the requirement for a metabolite is too low to be handled adequately by feedback inhibition (Fig. 1b). This is difficult to test experimentally, but one can mimic the situation by studying mutant bacteria in which the feedback mechanism is suppressed. For example, mutants of *Escherichia coli* bacteria that are insensitive to feedback inhibition of the production of the amino

acid lysine have been studied⁵, and behaved as expected: the cells grew poorly, probably as a result of lysine accumulation. By contrast, Reaves *et al.* observed that the expected overflow does not occur in mutant *E. coli* lacking feedback inhibition of the first enzyme in the biosynthetic pathway leading to pyrimidine nucleotides. Furthermore, the authors saw little effect of this lack of inhibition on the levels of the pathway's end product, cytidine triphosphate (CTP), and they found that the growth rates of the bacteria were normal, except under energy-limiting conditions.

CTP is essential for the synthesis of genes. Although it is produced from aspartate, an amino acid, it is about as different in structure from aspartate as a metabolite can be. Enzymes are normally inhibited by molecules that resemble their substrates, and the discovery that the first step of CTP biosynthesis — the one that uses aspartate as a substrate — is inhibited by CTP was one of the factors that led to the recognition of feedback regulation in metabolism⁵.

Surprisingly, Reaves and co-workers observed that neither CTP nor the biosynthetic intermediates leading to it accumulated in the mutant bacteria that lacked feedback

regulation. So why not, and what happened to the excess CTP produced? The authors found that it was degraded and excreted in the form of uracil (an RNA base). They describe this mode of regulation as "directed overflow metabolism"; others have called it "catabolic demand"⁴. The degradation involved a phosphatase enzyme that is evolutionarily conserved, but whose function was previously unknown.

Metabolic regulation is most easily analysed in economic terms of supply and demand^{4,6}, especially given that the primary function of feedback inhibition is to regulate metabolite concentrations, rather than fluxes. Biological responses to the demand for metabolic end products are common in many systems, thus explaining the occurrence of cooperative feedback inhibition, such as that by CTP in pyrimidine biosynthesis. But the degradation of CTP to uracil observed by Reaves *et al.* is not a response to demand for uracil, because there is no particular demand for it. Instead, this degradation is a response to the excessive supply of nucleotides, and allows the concentrations of CTP and its biosynthetic precursors to be kept essentially constant when demand for CTP is low (Fig. 1c). Regulation according to supply occurs in biological detoxication pathways, and also in central metabolism. In particular, the mammalian liver does not phosphorylate glucose to form glycogen — the polymer used for energy storage in mammals — to satisfy its own demand for energy, but to maintain glucose homeostasis in other organs by maintaining a constant concentration of glucose in the blood⁷.

The CTP-production pathway is therefore an example of a system in which regulation by demand for end product occurs side-by-side with regulation by degradation and excretion of excess end products. Evolution cannot have generated and conserved the excretion mechanism purely to compensate for the artificial

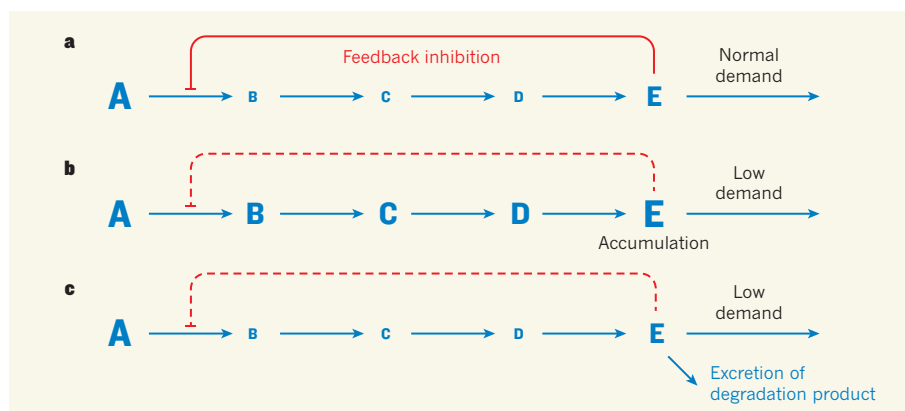


Figure 1 | Regulating metabolite production. **a**, In this general metabolic pathway, a starting material (A) is converted to an end product (E) via intermediates (B to D). When demand for E is normal, regulation of the pathway occurs by feedback inhibition of the first step — E inhibits the conversion of A to B. The sizes of the letters indicate the amount of each compound that accumulates. **b**, At very low demand, feedback inhibition is ineffective (broken line). Production of E is therefore excessive, and so it and the intermediates B to D accumulate. **c**, Reaves *et al.*¹ report that homeostasis of end products can be maintained when demand is very low if any excess is degraded and the resulting products are excreted. The authors discovered such a mechanism in the metabolic pathway that produces CTP, a pyrimidine nucleotide.

*This article and the paper under discussion¹ were published online on 31 July 2013.

deletion of feedback in experiments, so this pathway must represent a back-up strategy for physiological states in which demand falls below the finite range within which feedback is effective. If so, then we should expect to find examples of the same sort of behaviour in other pathways.

One other point will have occurred to alert readers. If excess production of pyrimidine nucleotides is overcome by converting the excess into uracil and excreting it, this implies that nutrients are not being used efficiently by the bacteria. That is why the mutant bacteria grew as well as normal ones when energy supplies were abundant, but somewhat more slowly when energy was limited. ■

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characteristics, including the relative lengthening of the thumb and multiple adaptations of the foot to support walking upright. These and other developmental details are evident early in embryonic development, and so the relevant regulatory regions of our genomes must be active at specific developmental times to drive the appropriate physiology.

Cotney and colleagues set out to map these regions in the human genome using comparative regulatory genomics — the recently developed technique of performing the same functional experiment in matched tissues of multiple species to gain insight into regulatory evolution³. What makes this study so significant is that the experiments were conducted on embryonic limb tissue, with careful matching of multiple developmental stages in samples from humans, macaques and mice. At each stage, the authors identified genomic locations at which the lysine amino-acid residue at position 27 of histone 3 proteins (histones are proteins around which DNA is wound) was modified by acetylation. This mark, referred to as H3K27ac, identifies⁴ active gene-regulatory regions, such as promoters and enhancers. H3K27ac markings at regions regulating the expression of *HOX* genes, which have a deeply evolutionarily conserved role in development, provided a natural control, and inspire confidence that the profiled tissues were indeed from the same developmental stage across the three species.

These maps of promoter and enhancer activity show the progression of regulatory innovation with evolution. The limb bud at human embryonic day 33 was the earliest stage profiled by Cotney *et al.*, and was also the stage at which the fewest promoter sequences (2%) showed H3K27ac marks in human samples but not at the related (derived from the same ancestor) sequence in macaque tissues — such comparisons were referred to as human-lineage gains. The latest developmental stage profiled

EVOLUTIONARY BIOLOGY

The handiwork of tinkering

A comparison of regulatory DNA sequences in humans, macaques and mice during embryonic limb development reveals thousands of sites of enhanced regulatory activity that are likely to have driven the evolution of our characteristic anatomy.

PAUL FLICEK

Understanding what makes us human is a long-standing research goal that has been made more accessible in the genomic era. It is clear that specific features of human anatomy are encoded in the genome, and the extent of gene sharing among mammals suggests that most of these features result from gene-regulatory changes that occurred at some point in the human lineage. Writing in *Cell*, Cotney *et al.*¹ have provided the first

comprehensive map of the genomic regions responsible for the unique morphology of human limbs. They also show that the vast majority of gains of regulatory activity in the human lineage occur in genomic regions that trace back to the base of the mammalian tree. These results demonstrate once again that, as Nobel-prizewinning biologist François Jacob described it², evolution is a tinkerer using the material at hand to create something workable.

Compared with other mammals and primates, humans have several specific limb

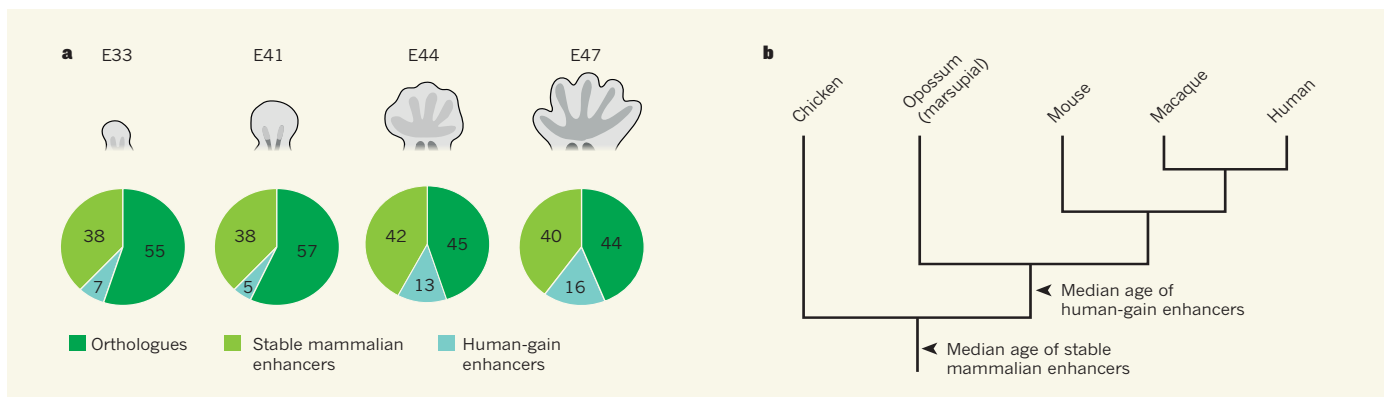


Figure 1 | The evolutionary origins of mammalian and human limb development. Cotney *et al.*¹ studied limb tissue from mice, macaques and humans at various stages of embryonic development (equivalent to human embryonic day 33 (E33), E41, E44 and E47) to identify active gene-regulatory sequences (enhancers and promoters). **a**, Their results show that, as development proceeds, the fraction of human enhancers that were active in human tissue only (human-gain enhancers) increased,

demonstrating the specific needs of human limb development. The other categories are enhancers that were active in all three species (stable mammalian enhancers) and enhancers that are present but not active in all three species (orthologues). **b**, The study also reveals that many of the sequences that are stably active in the three mammals are also present in non-mammalian vertebrate genomes, whereas the human-gain enhancers are, on average, younger.