

Igf2r transcription through the methylated island.

But loss of maternal methylation in region 2 could also be explained by the transcription-factor competition model. If the promoter is mutated, transcription complexes cannot bind there. So, more transcription complexes may bind to the antisense promoter, leading to demethylation and, subsequently, expression of the antisense transcript.

It is still not clear what attracts egg-specific methylation to region 2 (or any other 'imprinting boxes'). Can the short transgenes containing region 2, which Wutz *et al.* show cannot maintain methylation, attract it in the first place? If so, are the direct-repeat sequences that are found near 'imprinting boxes' necessary to attract germline-specific methylation, which apparently uses different machinery to embryonic *de novo* methylation?

Finally, the human *IGF2R* gene also has a differentially methylated region 2, but is monoallelically expressed in few people and/or only at early fetal stages¹⁰. Hence, it could be predicted that the antisense tran-

script exists in some human *IGF2R* alleles but not others, that its structure is polymorphic or, perhaps, that it has a different spatio-temporal regulation to the one that exists in the mouse. One thing is clear — imprinting has brought (and will continue to provide us with) interesting and surprising insights into the complex mechanisms of epigenetic gene regulation. □

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Particle physics

Panning for gold at the K stream

Frank Wilczek

In 1947, while studying cloud-chamber pictures of cosmic-ray showers, Rochester and Butler¹ found two pictures, among 50 exposures, that seemed to represent the decay of two unstable particles. The tracks were quite different from anything seen before, and were not anticipated by any theory. The researchers assumed that the pictures showed some kind of meson decay; they continued to make observations for two more years, but without further success.

This summer, almost exactly 50 years after those humble beginnings, a new milestone result in the study of K-meson decays was announced — an achievement that took several years of honing technique and gathering data, and which would have astonished Rochester and Butler. In collaboration with colleagues elsewhere in the United States and in Japan and Canada, scientists at Brookhaven National Laboratory have observed a decay mode of the positive K meson that occurs approximately once in ten billion decays; the details have just been published in *Physical Review Letters*². Specifically, the team concerned have for the first time identified an occurrence of $K^+ \rightarrow \pi^+ \nu \bar{\nu}$, the decay of a positively charged K into a positively charged pi meson, a neutrino and an antineutrino.

So what does it mean? Physics has been stuck with its remarkable success in producing a Standard Model of the interactions between the different fundamental particles; for over two decades, this theory has gone

from triumph to triumph, with no sign of any discrepancy with experiment (neutrino oscillations, if confirmed, would require a significant but only small extension of the Standard Model). This situation has become frustrating both to theorists, who would, for instance, like to see concrete evidence for the relevance of their exciting ideas about unification and supersymmetry, and of course to experimentalists, who grow weary of verifying old theories. A good way to look for something new, one that is complementary to the traditional push towards ever higher energies, is to look for rare processes at low energy. This approach provides a test of our theories, and a handle on new processes at high energies or short distances, that in the past has proved extremely informative.

There are quite definite rules for calculating the probability (or quantum-mechanical amplitude) of a process depicted in a Feynman diagram (Fig. 1), so the pictures exist at two levels: they are both portraits and recipes. Here the K^+ meson, made up of u (up) quark and s (strange) antiquark, fluctuates through a loop including heavy 'virtual' particles before materializing into a π (u quark and d (down) antiquark) and neutrinos. A W boson is necessarily involved, to change one type of quark into another, as is a Z boson, to couple to the neutrinos. Because the real versions of these particles are heavy, their virtual analogues arise only rarely as quantum fluctuations in the vacuum. That

is why the decay shown is very rare.

The orange particle, shown with a question mark in Fig. 1, can be either a u or a c (charmed) quark. Before 1970, it was considered that only the u quark would contribute, and the predicted value for this and related processes was much too large to be compatible with experiment. Then Glashow, Iliopoulos and Maiani³ proposed that an additional contribution from the c quark cancelled out most of the u quark's contribution. This suggestion was brilliantly confirmed by the experimental discovery of real c quarks in 1974. There is also a similar contribution with t (top) quarks, which (after u and c have mostly cancelled) actually dominates the predicted rate.

Looking at Fig. 1, one is struck by how many different, fundamental components of the Standard Model come into play in making this K-meson decay occur. Indeed, if we include gluons, shown as the squiggles in Fig. 1, to hold the quarks in the K and pi mesons together, they all do. It is quite amazing that this intricate description really works.

The big question, to be answered by experiments that are underway, is whether additional, unknown particles also contribute significantly. Just as the existence of Neptune, a planet beyond the then standard model of the Solar System, was inferred from its small but noticeable influence on the orbit of Uranus, the existence of heavy particles and phenomena beyond the Standard Model of fundamental physics might first be revealed by tiny effects on the behaviour of the known particles.

With just one event observed so far, however, no very definite conclusion can be drawn. The Standard Model predicts⁴ that

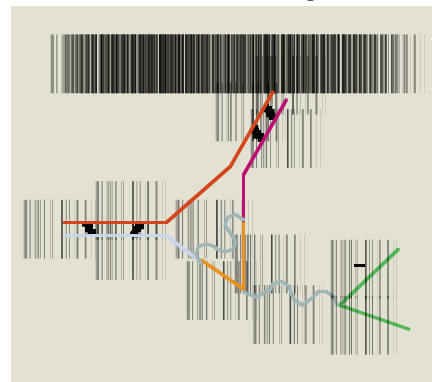


Figure 1 Virtual particles and the very rare case of K^+ -meson decay. In the language of quantum field theory, the decay of a particle is described as a series of events depicted in a Feynman diagram. This is the diagram describing a type of K-meson decay, resulting in a pi meson and neutrinos, which has just been observed experimentally for the first time². ν , neutrino; $\bar{\nu}$, antineutrino; Z and W, bosons; s, u, d, strange, up and down quarks. The particle shown in orange, with a query, can be a u, charmed or top quark. The squiggles holding the quarks in the K and pi meson together represent gluons.

$K^+ \rightarrow \pi^+ \nu \bar{\nu}$ occurs with a branching ratio $1.0 \pm 0.1 \times 10^{-10}$, whereas the one reported event corresponds to $4.2_{-3.5}^{+9.7} \times 10^{-10}$. If there is no physics beyond the Standard Model at work, the experimenters have been a little lucky to see an event so soon. And, of course, if the event is the first tangible hint of physics beyond the Standard Model, for example in reflecting the influence of supersymmetric particles, they will have been luckier still. Only half of the existing data have been analysed so far; the rest will be scrutinized over the next few months.

Beyond that, it will be essential to gather much more data, so that a decisive confrontation with the expectations of the Standard Model becomes possible. That confrontation is eagerly awaited. □

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G proteins

The arginine finger strikes again

Henry R. Bourne

The Ras protein and members of its family are G (guanine-nucleotide-binding) proteins, or GTPase enzymes, that play pivotal roles in transmitting regulatory signals between the cell surface and the nucleus. Ras itself, for instance, is involved in control of cell proliferation and differentiation. These proteins are turned on by binding GTP, and off when the GTP is hydrolysed to GDP by GTPase-activating proteins, or GAPs. But how does this crucial turn-off mechanism work?

Now we know, thanks to reports^{1–3} of the three-dimensional structures of Ras and two other GTPases, crystallized in the grips of different GAPs. The crystals reveal elegantly crafted examples of convergent evolution — three structurally distinct protein machines that use identical mechanisms to promote GTP hydrolysis. Details of the structures also point to hitherto unknown regulatory functions for GAP-related switches, and they show, at atomic resolution, why Ras mutations cause 25 per cent of human cancers.

The structures, respectively, are of the α -subunit, $G\alpha_{11}$, of a trimeric G protein in combination with RGS4 (a regulator of G-protein-signalling protein)¹; H-Ras with a catalytic fragment of RasGAP²; and, on page 758 of this issue³, RhoGAP in a complex with RhoA, a GTPase that controls remodelling of the actin cytoskeleton.

Efficiency of an enzyme reaction depends on the enzyme's ability to stabilize substrate atoms in a very specific arrangement, the transition state, which allows the reaction to proceed. The transition state's high energy makes it too unstable to be captured in a crystal structure. Instead, investigators crystallized GTPase–GAP complexes in a solution containing GDP and a combination of ions (Al^{3+} and F^-), which form a planar complex thought to mimic the atomic arrangement of GTP's γ -phosphate in the transition state (see box, overleaf).

The three GTP-hydrolysing machines form nearly identical webs of hydrogen

bonds that hold key substrate atoms in the configuration required for GTP hydrolysis (see box). In each complex, one protein (Ras, RhoA or $G\alpha_{11}$) shares with its counterparts in the other machines a common GTP-binding fold and stabilizes the transition state with the same set of identically positioned amino acids — with a single exception, an arginine residue whose guanidinium group interacts with the same substrate atoms in each transition state. Like fingers of different hands pointing at the same object, the arginines stick into the active site from different directions (see box).

The arginine fingers belong to three structurally different hands — RasGAP, RhoGAP and a built-in domain of $G\alpha_{11}$. In this last case, as in all $G\alpha$ proteins, the finger projects from a loop that connects the built-in extra domain to the GTP-binding fold. All three hands grasp GTPases that evolved from a common GTP-binding precursor and share the same 3D architecture. In contrast, convergent evolution reshaped three α -helical but otherwise unrelated protein folds to produce morphologically different hands able to point an arginine finger with equal precision. Despite their differing evolutionary origin, arginines pointed by RasGAP (see box; blue) and RhoGAP (cyan) occupy similar positions in the active sites of their respective targets. The built-in domain of $G\alpha_{11}$ points its finger (green) from almost the opposite direction; nonetheless, the position of its key guanidinium group is almost identical to those of RasGAP and RhoGAP.

In all three cases, amputation of the arginine finger creates a machine that hydrolyses GTP and turns off transmitted signals very slowly, as shown by the effects of missense mutations in the appropriate arginine codons of RasGAP⁴, RhoGAP⁵ and $G\alpha$ proteins^{6,7}. Indeed, such mutations in $G\alpha_s$, a $G\alpha$ structurally similar to $G\alpha_{11}$, cause endocrine tumours⁷ by triggering excessive synthesis of a second messenger, cyclic AMP.

When RasGAP is not available to deliver

its arginine finger to the active site in *trans*, Ras by itself hydrolyses only one GTP every 35 minutes. In contrast, the built-in arginine finger of $G\alpha$ proteins allows them to hydrolyse GTP more rapidly (at about 2–4 min^{-1})^{7,8}. In the presence of the appropriate partner — the right GAP or an RGS protein — Ras, RhoA and $G\alpha_{11}$ all hydrolyse GTP much faster, at between 10^2 and 10^3 min^{-1} .

This remarkable acceleration attests to a second functionally essential feature of GTP-hydrolysing machines, also created by convergent evolution: enhanced stability of other amino acids (not just the arginine finger) at the active site. The additional amino acids are located in two regions of the GTP-binding fold, switch 1 and switch 2. Their stability is critical: to steady the GTPase transition state, these residues must hold an attacking nucleophilic water molecule and the γ - and β -phosphates of GTP in precisely the right relation to one another. This stability is greatly increased in proteins gripped by RGS4 or the appropriate GAP, as shown by better-defined electron densities of switch 1 and switch 2 residues in crystals containing the partner proteins^{1–3}.

The stabilizing embraces of GAPs and RGS for their GTPase partners differ in detail, because the GAPs and RGS evolved from structurally different precursors. For instance, the side chain (see box; magenta) of a conserved glutamine residue in switch 2 must be in exactly the right place to position the attacking water molecule, or GTP will not be hydrolysed. Both RasGAP and RhoGAP use the 'knuckle' (main-chain carbonyl) of the arginine finger to stabilize the side chain of this glutamine^{2,3}. Because the knuckle is out of reach in $G\alpha_{11}$, RGS4 accomplishes the same end with a different amino acid¹.

Convergent structural evolution produces many opportunities to exploit these off switches in cell regulation. As we have seen, evolution engineered into trimeric G proteins an ability to switch signals off at two different rates. This was accomplished by supplying the arginine finger from a built-in $G\alpha$ domain and by assigning the other GAP function, stabilization of amino acids in the active site, to a separate hand — that is, an RGS protein (or in some cases to a downstream effector, as exemplified by phospholipase-C β ⁹, which turns off its regulator, $G\alpha_q$ -GTP). For the G_i -regulated K^+ channels that control brain synapses and heart rate, the accelerated turn-off rate provided by RGS proteins is physiologically essential. When a slower tempo would suffice, RGS proteins are presumably unnecessary — for instance, to shut off $G\alpha_s$ -mediated signals that promote conversion of liver glycogen into glucose.

More subtle variations may also furnish opportunities for regulation. Binding of