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DOI: 10.1016/j.cub.2005.05.001

ATP Synthesis: The World's Smallest Wind-Up Toy

ATP synthase contains two rotary motors coupled back-to-back: the protonmotive force-driven motor F_0 pushes the ATP-driven motor F_1 in reverse, causing it to synthesize ATP. Half of this process has now been reproduced *in vitro*, using tiny magnets instead of F_0 to drive the reverse rotation of a single F_1 molecule.

Richard M. Berry

F_0F_1 ATP synthase is a ubiquitous and highly conserved enzyme, found in the cytoplasmic membrane of prokaryotes and in the mitochondria of eukaryotes, which synthesizes most of the ATP used by most organisms on the planet. The rotary mechanism of this enzyme is well-established [1–5]. F_0 is a membrane-bound rotary motor driven by the protonmotive force, an electrochemical ion gradient across the membrane. F_1 is also a rotary motor, but driven by ATP hydrolysis [6–8]. In the complete F_0F_1 enzyme the rotors (γ and ε subunits in F_1 and the c-ring in F_0) and stators ($\alpha_3\beta_3\delta$ in F_1 and a and b subunits in F_0) of both motors are joined together, so that rotation of the two motors is directly coupled. The orientation of the motors is such that they are trying to rotate in opposite directions. Under normal physiological conditions F_0 is stronger: thus F_0 rotates forwards and drives F_1 in reverse, and the overall result is the synthesis of ATP driven by the protonmotive force (Figure 1A).

A great deal has been learned recently about the mechanism of F_1 operating in the forwards, ATP-hydrolysing direction by observing the rotation of various markers attached to the rotor [8]. The enzyme takes one 120° step for every ATP molecule hydrolyzed. This step is divided into sub-steps of 80° and 40°, triggered by ATP binding and by hydrolysis or product release, respectively [5,8]. However, much less is known about ATP synthesis.

In a recent study, Rondelez *et al.* [9] made accurate quantitative measurements of both ATP hydrolysis and synthesis coupled to rotation of single F_1 molecules in either direction. They were able to detect the presence or absence of a few thousand ATP molecules by following the rotation of a single F_1 molecule in a sealed microchamber. With F_1 running forwards and using up ATP in the chamber, measurements of the rate of slowing of rotation confirmed that one F_1 molecule hydrolyses three ATP molecules per revolution. This result had been inferred previously, but not directly demonstrated.

By pushing F_1 backwards to synthesize ATP and subsequently measuring the increased forwards rotation rate of the same molecule, Rondelez *et al.* [9] found that each F_1 molecule also synthesizes three ATP molecules per revolution, thus demonstrating that the enzyme is tightly coupled in both directions. They also confirmed the importance of the ε-subunit, which was necessary for coupling backwards rotation to synthesis but not for coupling hydrolysis to forwards rotation.

The most significant technical advance in the work of Rondelez *et al.* [9] is the confinement inside sealed chambers with volumes of a few cubic microns of single rotating F_1 molecules with magnets attached to the rotor. 600 revolutions of F_1 synthesize or hydrolyze 1800 molecules of ATP, producing a concentration change of 0.1 μM in such a small chamber. The corresponding change in the speed of rotation of

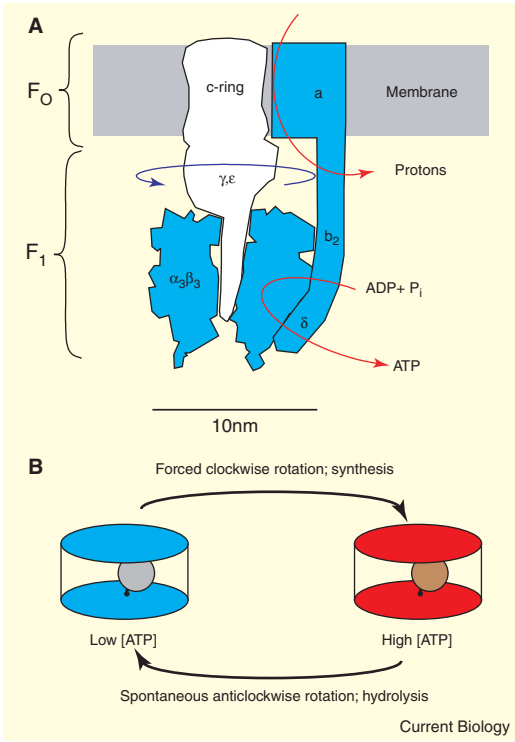

Figure 1. ATP synthesis driven naturally by F_0 or artificially by magnets.

(A) ATP synthase consists of two rotary motors connected back-to-back. Normally the proton-driven F_0 forces F_1 in reverse (clockwise) and ATP is synthesized in F_1. Isolated F_1 can also rotate forwards (anticlockwise) by hydrolysing ATP. The rotors and stators of both motors are connected to form one common rotor (white) and one common stator (blue). (B) Hydrolysis is detected by observing rotation of magnetic spheres attached to single molecules of F_1 immobilized inside sealed microscopic chambers. Synthesis is driven by backwards rotation of the magnets, and the ATP produced is detected by subsequent forwards rotation of the magnet when it is released.

F_1 is 0.5 revs sec^{-1}, easily measurable. These experiments represent an exquisite level of control over a biochemical process. By driving F_1 120° backwards, Rondelez *et al.* [9] are forcing the enzyme to synthesize one molecule of ATP which is confined in the chamber and subsequently hydrolysed in a 120° forwards step. The chamber can be opened and closed, returning the concentration to a default value for calibration of the ATP concentration and re-setting of the experiment.

A previous demonstration of ATP synthesis driven by magnets [5] used microchambers with more than 1000 times greater volume, containing many F_1 molecules. ATP was detected using luminescence of the luciferin–luciferase reaction, but the sensitivity of the experiments was too low to determine the ratio of ATP molecules synthesized per revolution.

Coupling between the rotors of F_0 and F_1 has previously been demonstrated by showing that ATP hydrolysis in F_1 drives rotation of membrane-bound c-rings in F_0 [10]. The requirement of the ε subunit for synthesis agrees with previous work [11], which indicated that ε is necessary for coupling *rotation* of the F_0 and F_1 motors but *not* for merely holding them together. However, Rondelez *et al.* [9] do not report the inhibitory effect of ε on ATP hydrolysis seen by previous authors [11–13]. The magnetic bead experiments differ from previous work in that they show slippage within F_1: the γ subunit is presumably rotating, being linked to the magnet by two bonds in parallel, but no ATP is being synthesized. Whereas previous results could be explained by postulating that ε controls the ability of the F_1 rotor to rotate relative to the F_1 stator, the new work [9] requires a decoupling between rotation of the γ subunit and ATP synthesis in the rest of F_1. This raises the question: where does the mechanism slip?

Another major challenge in this field is to understand the mechanism of ATP synthesis driven by rotation of F_0 in an energized lipid bilayer. The first steps have been taken by Diez *et al.* [4], who used a diffusion potential in lipid vesicles containing F_0F_1 to generate a protonmotive force to drive F_0, and fluorescence resonance energy transfer (FRET) to detect the resultant 120° steps of F_1. Stepping rotation corresponding to the elementary process of the proton-driven F_0 motor has not yet been observed, but the recent discovery of steps in nature's other ion-driven rotary motor, the bacterial flagellar motor (my group's submitted data), indicates that such steps may be observable.

If the technical advances reported by Rondelez *et al.* [9] can be combined with a system containing F_0F_1 in an energized lipid bilayer, the final piece in the story of this remarkable enzyme will be in place. Given the extraordinarily rapid progress in this field in recent years, perhaps the wait will not be too long!

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DOI: 10.1016/j.cub.2005.05.004

MicroRNAs: All Gone and Then What?

MicroRNAs are abundant gene regulatory factors whose function in animal development and homeostasis is poorly understood. A new study reports the genetic elimination of miRNA function on a full genomic scale and identifies a subfamily of miRNAs involved in brain morphogenesis.

Oliver Hobert

One of the perhaps biggest surprises in molecular biology in the past few years has been the discovery of a large number of previously completely overlooked regulatory molecules, termed miRNAs. miRNAs, ~22 nt long RNA molecules, regulate the expression of target genes by binding to their 3′UTR [1] (Figure 1).They were first identified more than 10 years ago in the nematode *Caenorhabditis elegans* [2], yet thought to be a worm specific oddity for the longest time. It was only the cloning of the second miRNA, *let-7*, that led the Ruvkun laboratory to note the conservation of miRNAs across phylogeny [3]. This in turn spurred intensive, genome-wide searches for miRNAs and current estimates of miRNA gene number range in the several hundreds for vertebrate genomes [4,5].

But what is it exactly that miRNAs do and how do they do it? The only clear theme that has emerged over the past few years is that they generally appear to repress gene expression [1,4]. But what cellular processes do miRNAs control? To date, there are still only four miRNAs — *lin-4, let-7, bantam* and *lsy-6* — whose physiological function has been elucidated *in vivo* and whose targets are known [2,3,6,7]. But speculation about the breadth of cellular processes in which animal miRNAs are involved have flourished over the past two years, mainly based on computational miRNA target prediction [8–10]. Yet, in contrast to plants, the usefulness of computational target prediction approaches has so far been limited in animals, which is illustrated by the striking lack of concordance of different target prediction algorithms. Nevertheless a common theme of all target predictions is that a large fraction of the genes in a given genome may be regulated by miRNAs. But how pervasive is miRNA function in reality?

This is where a new study by Alex Schier's lab [11] has provided fundamentally important new insights. Rather than eliminating a single miRNA, Giraldez *et al*. [11] eliminated *all* miRNAs by genetically removing the zebrafish gene coding for Dicer, an RNase required for miRNA processing [12] (Figure 1). A zebrafish *Dicer* mutant is not new *per se*; Plasterk and colleagues [13] had already reported the postembryonic lethality of *Dicer* knockout fish. However, their study was confounded by the fact that maternally supplied *Dicer* mRNA and/or protein from the heterozygous mothers of homozygous mutant embryos apparently allowed the generation of mature miRNAs during embryogenesis. This problem was now elegantly circumvented by using the germline replacement technique [14], which allows the study of homozygous mutant embryos devoid of both maternal and zygotic *Dicer* function. The observations of Giraldez *et al*. [11] on such maternal-zygotic *Dicer* mutant embryos are dramatic — not only because of the type of defect they observe, but also because of the type of defect they do not observe.

Given the vast abundance of predicted miRNA target genes, including genes involved in signaling and transcriptional control, maternal-zygotic *Dicer* null mutants displayed surprisingly normal axis and pattern formation [9]. Individual organs and multiple cell types were present and all anterior–posterior and dorsal–ventral patterning events examined do apparently not require miRNA function. Many of these initial patterning events are known to be under control of key signaling systems, such as Nodal, Hedgehog, Wnt, Notch, FGF, BMP and Retinoic acid [15]. As many of these pathways were predicted by *in silico* approaches to be targeted by miRNAs, the absence of any defects in these systems upon global removal of miRNAs is striking. Given the negative nature of this result, the authors showed that miRNA processing is indeed globally defective in *Dicer* mutants using a representative sample of many miRNA species. However, it can formally not be excluded that trace amounts of miRNAs are still being produced, for example by an unknown RNase other than Dicer.

In striking contrast to the lack of early patterning defects, *Dicer* mutants display severe defects in the morphogenesis of several distinct organ types [11]. In the nervous system, neurulation was severely affected, brain ventricles did not form properly, subregions of the brains were not appropriately demarcated and neuron position and axon projections were disrupted. Gross defects were also observed in cell arrangements during gastrulation, cardiovascular morphogenesis