

### Triclosan and fatty acid synthesis in *Plasmodium falciparum*: new weapon for an old enemy

With statistics portraying a mind-boggling 500 million clinical cases and 2.7 million deaths annually (World Health Organization 1997), malaria continues to be a bane of the tropical world and a nightmare to public health policy makers. Armed with the ability of acquiring resistance to almost all drugs that have been used against them, malarial parasites – especially *Plasmodium falciparum* – continue to defy human efforts to control malaria, let alone eradicate it. Attempts to control malaria have followed two routes: one, by targeting the vector, the *Anopheles* mosquito; and the other, by targeting the parasite. The rage that fuelled attempts to design anti-malarial vaccines having calmed down, we can now think of, and work towards, a more logical step – identifying essential steps in the metabolic pathways of the parasite and trying to inhibit them with new drugs. This strategy makes sense when viewed in the light of the fact that the *P. falciparum* genome is being sequenced. When deciphered, the resulting wealth of data will go a long way towards helping us to understand the biochemistry of the parasite.

*P. falciparum* is a member of the protistan phylum Apicomplexa (mostly parasites, the Apicomplexa are named after a characteristic apical complex of microtubules within the cell). A curious feature of the apicomplexan parasites is that these single celled organisms harbour within themselves a plastid – otherwise better known as an organelle found in plants and algae and classified into leucoplasts, chromoplasts and chloroplasts. The association, symbiotic today, is thought to have arisen via the engulfment of a cyanobacterium-like prokaryotic cell by an ancestor of the parasite (McFadden and Roos 1998). In the course of evolution the parasite has come to depend on the plastid for certain essential functions to such a degree that inhibitors of plastid biosynthetic pathways may act as potent anti-malarials. Since the fundamental nature of metabolism is different between plastids and animal cells, anti-plastid drugs are highly attractive candidates for chemotherapy: they open up the possibility that the parasite can be selectively killed without affecting the host. Fatty acid biosynthesis is of special interest because here the malarial parasite cannot do without its commensal plastid. Fatty acids are the building blocks of lipids and a major component of biological membranes; they play essential roles in energy storage, growth and development. Apicomplexans are surrounded by a structure known as the parasitophorous vacuole, whose membrane is made up of lipids partly derived from the host cell and partly from the parasite. In other words, fatty acids may be essential for the very existence of the parasite within its host.

The biosynthesis of fatty acids has been well studied in bacteria and plant chloroplasts. The relevant enzymes are organized in two strikingly distinct ways within living systems. Fungi, mammals and some mycobacteria accomplish fatty acid synthesis by multifunctional proteins in which each reaction is catalyzed by a distinct region within one large molecule. The successive synthetic steps are under the control of specific domains: in effect, there is a single polypeptide with the capability of seven enzymes (Smith 1994). Plants and bacteria use a different strategy involving the type II or ‘dissociated’ fatty acid synthases. Best characterized in *Escherichia coli*, in the case of type II synthases each of the individual condensation and dehydration reactions necessary for fatty acid synthesis is carried out by a separate enzyme. Unlike bacteria, which have fatty acids of chain lengths C-14 and C-16, parasite fatty acids are predominantly of chain lengths C-10, C-12 and C-14. The final, and in conventional terms, ‘rate-limiting’, step of elongation is catalyzed by the enzyme enoyl-ACP reductase (FabI). Triclosan (2,4,4'-Trichloro-2'-hydroxydiphenyl ether), a commonly used bactericide, was recently shown to target enoyl-ACP reductase in bacterial systems (McMurray *et al* 1998; Heath *et al* 1998, 1999).

Surolia and Surolia (2001) have exploited this finding to study the effectiveness of Triclosan as an inhibitor of the same enzyme in the malarial parasite.

They quantified the anti-plasmodium activity of Triclosan by using the uptake of [<sup>3</sup>H]hypoxanthine as an index of growth. The drug (IC<sub>50</sub>–0.7 μM) was found to lower the growth rate by 50%, with young trophozoites – the vegetative stage of the parasite – being especially susceptible. The target of the drug appears to be fatty acid synthesis. When studied in terms of the incorporation of 1,2-[<sup>14</sup>C]acetate [and also, in contrast to earlier reports (Holz 1977; Haldar *et al* 1985; Matesanz *et al* 1999), of [<sup>14</sup>C]malonyl-CoA] into fatty acids, 2 μM Triclosan inhibited the metabolism of both metabolites by 50%. In comparison, Chloroquine, the commonly used anti-malarial drug, had no effect on fatty acid synthesis at 100 μM. Work with partially purified extracts showed that enoyl-ACP reductase activity was dependent more on NADH than NADPH. Indeed, at high concentrations, NADH in the assay system could arrest the inhibitory effect of Triclosan. On 12% SDS-PAGE, affinity-purified enzyme showed a band of 34 kDa. The N-terminal sequence resembled that found in the enzyme from the plant *Brassica napus*. Thus the gene encoding FabI, although encoded by the genome of the parasite, finds its functional destination in the plastid – conceivably after it is translated in the host cytoplasm; this hypothesis remains to be established. Reverse Transcriptase-PCR with primers for the known *P. falciparum* gene sequence (picked up from the database) showed the expected 1.3 kb band. The cloned gene from the PCR product gave the same sequence of the putative *FabI* gene as the one reported in the database. Finally, mice infected by *Plasmodium berghei* were treated with Triclosan in order to monitor its efficacy as an anti-malarial *in vivo*. A single subcutaneous injection of 3.0 mg of the drug per kg body weight inhibited parasitemia by 75% within 24 h of administration, and at a dose of 38 mg/kg, completely cleared the parasite from circulation. No side effects were observed even after injecting 40 mg/kg of Triclosan.

The highlights of the study may be summarized as follows. (i) Fatty acid synthesis has been demonstrated in *P. falciparum* for the first time. It is likely that earlier attempts were unsuccessful because of the small amounts of [<sup>14</sup>C]acetate used, unsynchronized cultures with low parasitemia and inappropriate incubation periods. (ii) The pattern of fatty acid synthesis is distinct from that found in most bacterial systems. (iii) The incorporation of [<sup>14</sup>C]malonyl CoA in a cell-free fatty acid synthesis system is inhibited by Triclosan; the inhibition can be prevented by an excess of the reduced cofactor NADH. Thus, Triclosan inhibits the growth of *P. falciparum* by inhibiting *de novo* fatty acid synthesis. (iv) The similarity of enoyl-ACP reductase, a key enzyme of the pathway, to the corresponding enzyme from the plant *Brassica napus*, hints at the plastid-origins, not only of this enzyme, but of the pathway of fatty acid synthesis. (v) The most important observation, of course, is the demonstration of the potent anti-malarial activity of Triclosan in the mouse model.

Although Chloroquine has been the most frequently used antimalarial for the past 50 years, its mode of action remains enigmatic, precluding rational design of more potent analogues (McConkey *et al* 1977; Waller *et al* 1998; Jomaa *et al* 1999). Besides, resistance to chloroquine is becoming increasingly prevalent in *falciparum* malaria. What are the chances that Triclosan resistance too might become a problem in the future? Obviously, one cannot say. What is worrisome, however, is that many pathogenic bacteria not only do not contain FabI – the enoyl-ACP reductase that is sensitive to Triclosan –, but possess a Triclosan-resistant enzyme, FabK, that can catalyze the same reaction (Heath and Rock 2000). Heath and Rock went on to demonstrate that when a Triclosan-sensitive strain of *E. coli* was transformed with a plasmid that expresses FabK constitutively, the minimum inhibitory concentration of the drug was raised by four orders of magnitude. Be that as it may, among the set of newly reported drugs that target the malarial parasite, Triclosan is by far the most potent. The fact that humans have been exposed to Triclosan in the form of mouth-washes, after-shave lotions, toothpaste, soaps, detergents and childrens' toys reduces the apprehension that accompanies any new drug being introduced for human use. But clinical trials are still awaited.

## References

- Haldar K, Ferguson M A J and Cross G A M 1985 Acylation of a *Plasmodium falciparum* merozoite surface antigen via sn-1,2-diacyl glycerol; *J. Biol. Chem.* **260** 4969–4974  
Heath R J and Rock C O 2000 A triclosan-resistant bacterial enzyme; *Nature (London)* **406** 145–146

- Heath R J, Rubin J R, Holland D R, Zhang E, Snow M E and Rock C O 1999 Mechanism of Triclosan inhibition of bacterial fatty acid synthesis; *J. Biol. Chem.* **274** 11110–11114
- Heath R J, Yu Y-T, Shapiro M A, Olson E and Rock C O 1998 Broad spectrum antimicrobial biocides target the FabI component of fatty acid synthesis; *J. Biol. Chem.* **273** 30316–30320
- Holz G G Jr 1977 Lipids and the malarial parasite; *Bull. W.H.O.* **55** 237–248
- Jomaa H., Wiesner J, Sanderbrand S, Altincicek B, Weidemeyer C, Hintz M, Türbachova I, Eberl M, Zeidler J, Lichtenthaler H K, Soldati D and Beck E 1999 Inhibitors of nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs; *Science* **285** 1573–1576
- Matesanz F, Duran-Chica I. and Alcina A 1999 The cloning and expression of pfacs1, a *Plasmodium falciparum* fatty acid coenzyme A synthetase-1 targeted to the host erythrocyte cytoplasm; *J. Mol. Biol.* **291** 59–70
- McConkey G A, Rogers J M and McCutchan T F 1977 Inhibition of *Plasmodium falciparum* protein synthesis. Targeting the plastid-like organelle with thiostrepton; *J. Biol. Chem.* **272** 2046–2049
- McFadden, G I. and Roos, D S 1999 Apicomplexan plastids as drug targets; *Trends Microbiol.* **7** 328–333
- McMurray L M, Oethinger M and Levy S B 1998 Triclosan targets lipid synthesis; *Nature (London)* **394** 531–532
- Smith S 1994 The animal fatty acid synthase: one gene, one polypeptide, seven enzymes; *FASEB J.* **8** 1248–1259
- Surolia N and Surolia A 2001 Triclosan offers protection against blood stages of malaria by inhibiting enoyl ACP-reductase of *Plasmodium falciparum*; *Nature Med.* **7** 167–173
- Waller R F, Keeling P J, Donald R G K, Striepen B, Handman E, Lang-Unnasch N, Cowman A F, Besra G S, Roos D S and McFadden G I 1998 Nuclear-encoded proteins target to the plastid in *Toxoplasma gondii* and *Plasmodium falciparum*; *Proc. Natl. Acad. Sci. USA* **95** 12352–12357
- World Health Organization 1997 World malaria situation in 1994; *Weekly Epidemiol. Rec.* **72** 269–274

GURPUR PRAVEEN BHAT

NAMITA SUROLIA

*Molecular Biology and Genetics Unit,*

*Jawaharlal Nehru Centre for Advanced Scientific Research,*

*Jakkur,*

*Bangalore 560 064, India*

*(Email, surolia@jncasr.ac.in)*

## **Retinitis pigmentosa: mutations in a receptor tyrosine kinase gene, *MERTK***

Retinitis pigmentosa (RP) is a heterogeneous genetic disorder of the eyes. RP is characterized by abnormalities of photoreceptors (rods and cones) or the retinal pigment epithelium (RPE) leading to progressive loss of vision. It starts with the night blindness or defective dark adaptation in patients followed by constriction of the peripheral visual fields. Eventually, late in the course of the disease, the patients lose their central vision. The diagnosis of RP is made when a patient has rod dysfunction as measured by dark adaptation or electroretinogram (ERG) monitoring, progressive loss in photoreceptor function and loss of peripheral vision with bilateral involvement. In the early stage of the disease, the fundus (the bottom of eyes), appears normal. As the disease progresses, the fundus shows arteriolar narrowing, fine dust-like intraretinal pigmentation, and loss of pigment from the RPE. The retina in a RP patient at an advance stage of the disease is characterized by the presence of intraretinal and pre-retinal clumps of black melanin pigments appearing like bone spicules, markedly attenuated retinal vessels, loss of RPE and paleness of the optic nerve (Pagon 1993).

Electroretinography determines the functional status of the photoreceptors. Electroretinography is performed by using a double electrode contact lens placed on the cornea. After light stimulation of the retina, an electrical potential arises which represents a composite response of millions of retinal cells, and the electrical output is amplified and displayed on an oscilloscope. Rod function is reflected by

responses obtained under dark-adapted conditions whereas cone function is reflected by responses observed under light-adapted conditions. The two types of responses can be separated from each other, and the type and the extent of rod and/or cone involvement in the disease can be assessed. RP patients in an advance stage of the disease have no detectable rod and cone responses whereas young RP patients show early and severe impairment of pure rod responses (Pagon 1993).

RP is classified into non-syndromic (simple) and syndromic categories. In the non-syndromic category, the disease manifests only in the eyes, whereas in the syndromic category, other organs are also affected. A good example of syndromic RP is Usher syndrome where patients in addition to RP have hearing impairment. Non-syndromic RP can be familial or sporadic. Non-syndromic RP can be inherited as an autosomal dominant (AD), autosomal recessive (AR), X-linked recessive (XLR) or rare digenic (RDG) traits. Syndromic RP can also be caused by mutations in the mitochondrial genome.

The population incidence of RP ranges from 1 in 3,500 to 1 in 4,000 in the western countries (Pagon 1993; Dryja and Li 1995). Sporadic RP (sRP) represents 40–50% of all RP cases. RP is known to be present in all ethnic groups and races. Autosomal dominant RP (adRP), autosomal recessive RP (arRP) and X-linked recessive RP (XLRP) represent 15–25%, 5–20% and 5–15% of all RP cases, respectively (Pagon 1993; Dryja and Li 1995). Linkage analysis and other mapping techniques have revealed 34 loci for all forms of RP. There are 12 known loci for adRP located on chromosome 1q13-q23 (RP18), 3q21-q24, 6p21.2-cen (RP7), 7p15-p13 (RP9), 7q31.3 (RP10), 8q11-q13 (RP1), 11q13, 14q11.2 (RP27), 17p13.3 (RP13), 17q22 (RP17), 19q13.3 and 19q13.4 (RP11). Of 12 known loci for adRP, genes for only six loci are known so far. Rhodopsin (RHO), peripherin (RDS), RP1 protein, retinal outer segment membrane protein 1 (ROM1), neural retina luciferase zipper transcription factor (NRL) and cone-rod otx-like photoreceptor homeobox transcription factor (CRX or CORD2) are mutated in RP cases mapped to loci on chromosome 3q21-q24, 6p21.2-cen, 8q11-q13, 11q13, 14q11.2 and 19q13.3, respectively.

There are 16 known loci for arRP located on chromosome 1p31 (RP20), 1p21-p22 (RP19), 1q31-q32.1 (RP12), 2p11-p15 (RP28), 2q31-q33 (RP26), 2q37.1, 3q21-q24, 4p16.3, 4p12-cen, 5q31.2-q34, 6p21.3 (RP14), 6cen-q15 (RP25), 10q23, 15q22, 15q26, and 16p12.1-p12.3. Of these loci, genes for 11 loci have been cloned and characterized for mutations so far. Retinal pigment epithelium-specific 65 kDa protein (RPE65), ATP-binding cassette transporter-retinal (ABCR), crumbs homologue 1 (CRB1), arrestin (SAG), rod cGMP phosphodiesterase *b*-subunit (PDE6B), rod cGMP gated channel *a*-subunit (CNGA1), cGMP phosphodiesterase *a*-subunit (PDE6A), tubby-like protein 1 (TULP1), RPE-retinal G protein-coupled receptor (RGR) and cellular retinaldehyde-binding protein (RLBP1) are mutated in RP cases mapped to loci on chromosome 1p31, 1p21-p22, 1q31-q32.1, 2q37.1, 4p16.3, 4p12-cen, 5q31.2-q34, 6p21.3, 10q23 and 15q26, respectively. Interestingly, mutations in rhodopsin gene cause adRP as well as arRP mapped to chromosome 3q21-q34. Further, mutations in RPE65 gene also cause Leber congenital amaurosis (LCA). LCA is an autosomal recessive retinal dystrophy which is characterized by total blindness or greatly impaired vision at birth or within few weeks of life.

Of six known loci for XLRP, genes for only two loci are known. Mutations in retinitis pigmentosa GTPase regulator (RPGR) and novel protein with similarity to cofactor C genes cause XLRP in cases mapped to chromosome Xp21.1 (RP3) and Xp11.3 (RP2), respectively. Genes responsible for RP cases mapped to chromosome Xp22.13-p22.11 (RP15), Xp21.3-p21.2 (RP6), Xq22 (RP23) and Xq26-q27 (RP24) are yet to be identified. Mutations in RPGR and RP2 genes are the most common causes of XLRP, representing 25–30% and 10–15% of the XLRP cases, respectively. Digenic RP is caused by mutations in the heterozygous condition in both peripherin and ROM1 genes (Dryja *et al* 1997).

As 40–50% of all RP cases are sporadic, finding the genetic cause for these cases is a difficult task because no linkage analysis is possible. Recently, Gal *et al* (2000) have used a different and ingenious approach to find mutations in sRP cases. It is known that the mutation in a receptor tyrosine kinase gene, *Mertk*, in the Royal College of Surgeons (RCS) rat (D'Cruz *et al* 2000) causes defective phagocytosis of photoreceptor outer segments by the retinal pigment epithelium and retinal degeneration (D'Cruz *et al* 2000; also see Gal *et al* 2000). The human orthologue of the rat *Mertk* gene, *MERTK*, is located on chromosome 2q14.1 (Weier *et al* 1999). Gal *et al* (2000) determined the genomic structure of the *MERTK* gene and examined each of its 19 coding exons for mutations including the splice sites in 328 DNA samples from individuals with various retinal dystrophies and detected three different mutations in three individuals with RP. Their finding is the first evidence that a defect in the RPE

phagocytosis pathway could lead to human retinal disease. They found a homozygous 5-bp deletion in exon 15 (2070delAGGAC) in a 45-year-old man who is the product of a consanguineous marriage and had onset of night blindness and poor vision in early childhood and currently has only central vision. The second patient has a homozygous A → G transition in the intron 10 splice acceptor site (IVS10-2A → G) in a 34-year-old woman whose normal parents are not related. She had onset of night blindness and poor vision in early childhood and currently has only central vision. Only her father was heterozygous for the splice-site mutation, suggesting that she received two copies of the same mutation from her father and has paternal uniparental isodisomy for chromosome 2; this was confirmed by microsatellite analysis. As expected, microsatellite markers from other chromosomes showed biallelic inheritance in her. The third patient, who is a 21-year-old woman and had poor vision as a child and night blindness at age 12, has a heterozygous premature termination codon (R651X). Taken together, the work of Gal *et al* (2000) demonstrated a new arRP locus at 2q14.1 using a non-conventional method. It is possible that the third patient has a second mutation in *MERTK*, most likely in the intronic segments as they failed to find mutation by direct sequencing of exons 1–19. Conversely, it is possible that both homozygous as well heterozygous mutations in *MERTK* cause RP as has been observed in the rhodopsin gene. Further, mutations in the *MERTK* gene in RP patients and a mutation in the retinal dystrophic RCS rat demonstrate that *MERTK* is essential for the proper function of the retina, and a defect in the phagocytosis of the photoreceptor outer segments by the RPE results in retinal degeneration in a subset of RP patients.

### References

- D'Cruz P M, Yasumura D, Weir J, Mathes M T, Abderrachim H, LaVail M M and Vollrath D 2000 Mutation of the receptor tyrosine kinase gene *Mertk* in the retinal dystrophic RCS rat; *Hum. Mol. Genet.* **9** 645–652
- Dryja T P, Hahn L B, Kajiwaara K and Berson E L 1997 Dominant and digenic mutations in the peripherin/RDS and ROM1 genes in retinitis pigmentosa; *Invest. Ophthalmol. Vis. Sci.* **38** 1972–1982
- Dryja T P and Li T 1995 Molecular genetics of retinitis pigmentosa; *Hum. Mol. Genet.* **4** 1739–1743
- Gal A, Yun L, Thompson D A, Weir J, Orth U, Jacobson S G, Apfelstedt-Sylla E and Vollrath D 2000 Mutations in *MERTK*, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa; *Nature Genet.* **26** 270–271
- Pagon R A 1993 Syndromic retinal dystrophy; in *Retinitis pigmentosa: Present knowledge and outlook* (ed.) E Rinaldi (Napoli: Liviana Medicina) pp 151–166
- Weier H U, Fung J and Lersch R A 1999 Assignment of protooncogene *MERTK* (a.k.a.c-mer) to human chromosome 2q14.1 by *in situ* hybridization; *Cytogenet. Cell Genet.* **84** 91–92

ARUN KUMAR  
*Department of Molecular Reproduction,  
Development and Genetics,  
Indian Institute of Science,  
Bangalore 560 012, India  
(Email, karun@hamsadvani.serc.iisc.ernet.in)*

## Getting the beat

Probably the most important characteristic that sets us apart from everyone else in our common, billion-year-old, highly branched evolutionary tree is language (Pinker 1994). Language allows us to communicate across the boundaries of space and time and, according to some, is also the source of what is termed ‘consciousness’. The problem of language acquisition was not fully appreciated till the Chomskyan revolution in the 1950s. Chomsky pointed out that language learning was a problem because of (i) the impoverished nature of linguistic inputs to a child and (ii) the powerful, almost infinite creative power of the language learnt. Given the ability of learners to generate previously unheard sentences, all of them satisfying the non-trivial constraint of being grammatically correct, Chomsky pointed out that language acquisition cannot be explained in terms of the simpler description of the behaviourists, i.e., in terms of stimulus–response routines (Chomsky 1980).

Chomsky constructed (and continues to do so) an elaborate and compelling theory of language. An important part of the theory, what came to be called Universal Grammar (meaning that in a deep sense, all languages have the same grammatical structure), is its *innateness*. In effect, Chomsky said that Universal Grammar was written in our genes. By saying that language is innate, Chomskyans do not mean that we “know” the words and the grammar from birth. What they mean – and the exact meaning has been evolving ever since the theory was first propounded – is that there exists in the minds of babies a certain Language Module, which is some sort of a genetically-based neural blueprint or module waiting to encounter a real language in the world. The ‘blueprint’ is specifically designed to assimilate one or more languages and provide the means for using it. Today language learning is looked upon as an example of a broader idea, namely, that much of cognition is based on analogous modules, but that is another story. As an aside, it might appear that a genetically-based language module implies that language capability must have evolved by natural selection. But on this point Chomsky remains skeptical (see Pinker 1994).

How might the language module inside a baby figure out the grammar and the words? One would think that learning just the words should not be too difficult a task. Perhaps it could even be automated. All that would be required is to record someone’s voice and run the acquired speech waveform through the appropriate algorithms to discover all the words embedded in the waveform. (A speech waveform, like any sound waveform, looks like a squiggly line going along a time axis, and represents the amplitude of the vibration of your eardrum at every instant in time over the duration of the sound.) Only, it turns out that this ‘segmentation task’ is, computationally, a fiendishly difficult task. When we speak, we run words together in such a manner that there is no efficient algorithm today that can reliably pick out word-like entities from a speech waveform when the words are unknown. And, unless you believe that somehow the words are hardwired into a baby’s brain, this is exactly the task that the language module seems to perform, seemingly effortlessly. Over the years, several laboratories have shown that babies are exquisitely sensitive to several statistical properties of the sounds they hear and these features could aid the child in solving the segmentation task.

But our speech is not just words, it is also the way we speak them. We stress some portions and not others, our tone rises sometimes and sometimes it falls, some parts are said quickly while others are slowed down. These rhythmic and intonational properties are referred to as the *prosody* of the language, and it is similar to the beat or the tempo in music. Prosody is very different in different languages. In Indian languages words tend to be unstressed. In Italian most words receive the stress on the penultimate syllable; in French it is the ultimate syllable that receives the stress. Babies are sensitive to these cues, and could use these in addition to the statistical cues to segment the speech streams that assail them. Babies have been shown to be able to discriminate the rhythm of their own language (and languages with similar rhythms) by the time they are four days old. Their response is not merely to the statistical nature of the sounds produced; they do not show this sensitivity when speech to which they respond best is run backwards.

Those who believe that the language module is something unique to humans would say that these sensitivities should be specific to humans. But researchers have shown the need for caution by demonstrating that certain simple properties of the way we perceive speech such as the categorical perception of phonemes, that were once thought to be exclusively human, are also shared by other

animals like Chinchillas and even by quail and starlings (though these studies have been dogged with methodological and philosophical controversy). Phonemes are the basic sounds used in a language, like /pa/ and /ba/. English, for example, has about 44 phonemes. Categorical perception is when one automatically tends to ignore differences between acoustically different instances of the same phoneme. But could it also be the case for more complex stimuli like the overall rhythmic pattern in a segment of speech that other animals too share this analysis?

The groups of Jacques Mehler, studying language in babies (and adults) at CNRS, Paris and Marc Hauser, working on primate cognition at Harvard University, ran experiments in parallel on human newborns and cotton-top tamarin monkeys to address this question (Ramus *et al* 2000; Werker and Vouloumanos 2000). The experiment consisted of sentences in Dutch and Japanese, two languages with very different prosodic structures. The experiments used a paradigm, referred to as habituation-dishabituation, which allows one to see if two stimuli are perceived to be similar or different. It works like this: you show a baby (or a monkey) the first stimulus, and the baby responds in a measurable way because it senses something novel in its environment. After a few repetitions of stimulus #1, the baby gets tired (habituated) and stops responding. Now, if the experimenter switches to stimulus #2, and if the baby perceives it as being different, it will once again start responding; it would have become dishabituated. On the other hand, if the experimenter were to switch to stimulus of category #1, or if the baby did not perceive any difference between stimulus No. 1 and stimulus No. 2, it would continue being bored and not respond to the stimuli.

The basic result from this experiment was that both the babies as well as the monkeys were able to distinguish the Dutch sentences from the Japanese ones when the speech segments had been corrected to differ only in their prosody. Furthermore, this discrimination was evident only when the speech segments were played forwards; when they were played backwards, the monkeys as well as the babies failed to discriminate the two. Importantly, this is among the few reports in which the animals were not extensively pretrained. In fact the experiment relied on nothing more than the curiosity of the monkeys. This should be contrasted with, the experiments with quail. Several thousands of trials are required to get the quail to reliably discriminate between two phonemes (like /pa/ and /ba/).

But why did the workers choose to investigate prosody? As noted before, prosody can be useful in segmenting the speech stream. In addition, preliminary research seems to indicate that prosodic features in the speech might be related to the nature of the underlying grammar itself. If this were to be true, than an early cue like prosody might already be setting switches in the baby's language organ, telling it what grammatical forms it should expect, and which to reject. In sum, these experiments give us clear insight into the supposed uniqueness of the human 'language module'. As the authors note, the precise nature and extent of the language module is mainly an empirical matter. One can now begin to investigate the extent to which the language module is truly a uniquely human entity, and how much of it is a constraint imposed by our evolutionary history.

## References

- Chomsky N 1980 *Rules and representations* (New York: Columbia Univ. Press)  
 Pinker S 1994 *The language instinct* (New York: William-Morrow)  
 Ramus F, Hauser M D, Miller C, Morris D and Mehler J 2000 Language discrimination by human newborns and by cotton-top tamarin monkeys; *Science* **288** 349–351  
 Werker J F and Vouloumanos A 2000 Who's got rhythm?; *Science* **288** 280–281

MOHINISH SHUKLA  
 Cognitive Neuroscience Sector,  
 International School for Advanced Studies (SISSA),  
 via Beirut 9,  
 34014 Trieste, Italy  
 (Email, shukla@sissa.it)