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New developments on an ancient front: pre-Cambrian evolution of animal life

To anybody not committed to the motto "bigger is better", the most spectacular episode in the history of life preserved in the fossil record has to do, not with dinosaurs, but with the appearance in the Cambrian era [beginning about 450 million years (My) ago] of an astounding variety of complex multicellular animal life. The Cambrian fauna included not only representatives of all the major modern phyla, but also an amazing array of taxa—from genera to phyla—which never made it beyond that early phase of metazoan evolutionary exuberance: only a small fraction of this Cambrian diversity survived into later times. The sudden appearance and elaboration of the Cambrian fauna, in the geologically brief span of a few tens of millions of years has frequently been described as the "Cambrian explosion". Cambrian animals sported a variety of skeletal structures such as shells, carapaces and sclerites, which lend themselves more readily to fossilization than soft-bodied animals.

Does the Cambrian explosion therefore represent the extremely rapid evolution of a diversity of entirely novel body plans? Or does it represent the acquisition of skeletal structures (and subsequent diversification) among different groups whose appearance in the fossil record was preceded by a protracted period of pre-Cambrian evolution, during which the different basic body plans represented among these groups had already evolved? In other words, how much of an evolutionary explosion really occurred during the Cambrian? The belief that such major evolutionary developments could not have occurred in so short a span of time encouraged the search for pre-Cambrian metazoan life, leading to the discovery of the Ediacaran fauna from the late Vendian period just preceding the Cambrian. However, the Ediacaran fauna are modest compared to Cambrian finds, and do not greatly extend the period of early metazoan evolution, the upper limit being about 580 My ago. There is no unambiguous fossil evidence in support of the evolution of complex metazoan body plans prior to this. Is this because none existed? Or is it because the prevailing geochemical and ecological conditions did not favour the preservation of soft-bodied and likely microscopic forms? Or is the fossil evidence somewhere out there, but we have not known what to look for and where?

Several papers appearing over the last year or so have shed new light on—or at least added fresh fuel to—the debate about early metazoan evolution. These represent three lines of argument and evidence:

(i) Comparisons of protein and rRNA sequences have been used to estimate the times at which different lineages diverged. However, the use of genes as molecular clocks in this way can be problematic; this is tellingly illustrated by the vastly different times that have been arrived at for the origin of metazoans by different groups. In an influential paper, Wray *et al* (1996) analysed rates of sequence evolution of 18S rRNA and seven protein-coding genes, to derive a metazoan origin between 1000 and 1200 My ago. This supported a time of origin about 500 My earlier than palaeontological estimates. In a recent paper, Ayala *et al* (1998) take issue with this estimate. Using a similar data set, but different statistical methods to eliminate biases arising from non-uniform rates of molecular evolution, they arrive at the more conservative figure of a metazoan origin some 670 My ago, which can be reconciled with the fossil record. Other papers published this year arrive at yet other figures, such as that of 830 My ago (Gu 1998). Back to square one?

(ii) Following an earlier report (Zhang and Pratt 1994) of fossil metazoan embryos from the middle Cambrian, two more recent papers (Bengtson and Zhao 1997; Xiao *et al* 1998) report even older fossil embryos. Bengtson and Zhao present beautifully detailed evidence on metazoan embryogenesis from early Cambrian deposits, including a reconstruction of an entire developmental

sequence running from early cleavage stages to newly hatched individuals. Xiao *et al* (1998) go even further back in time, with evidence of pre-Cambrian metazoan embryos from about 570 My ago, making these contenders for the oldest pre-Cambrian fossils to date. What makes these two papers significant, apart from the awe-inspiring detail visible in these fossil embryos, is the fact that it is at all possible to obtain well-preserved invertebrate fossil embryos. This points the way for one important approach to the further study of early metazoan life: fossil embryos will be able to provide invaluable information on the evolution of development—a central concern in the attempt to reconstruct early metazoan evolution. Furthermore, the direct mode of development inferred from two of these fossils (Bengtson and Zhao 1997) suggests that, contrary to a widely-held view, the evolution of major invertebrate phyla may not have needed to proceed through the agency of free-floating, plankton-feeding larval stages (Morris 1998), and may therefore not have required the longer period of pre-Cambrian evolutionary "preincubation" implied by this view.

(iii) A very recent paper (Seilacher *et al* 1998) should hold special interest for Indian readers, since it reports findings from Churhat in Madhya Pradesh, of collaborative work by an Indo-German team. Seilacher *et al* (1998) claim that the traces they found in the Churhat sandstone represent burrows formed by triploblastic, probably worm-like, animals tunnelling beneath bacterial mats. What makes this claim dramatic is that the Churhat sandstone has been dated at 1100 My, which would make this by far the oldest evidence of metazoan life. Not surprisingly, this claim is attracting critical scrutiny. In a commentary (Brasier 1998) on their paper, it has already been pointed out that the diameter of the burrows does not necessarily imply the activity of triploblastic animals; that it is very strange that no other evidence of metazoan life has been found over the huge time span of 500 My separating this date from the earliest subsequent metazoan fossils; and most importantly, that the dating of the Churhat sandstone is in doubt. Clearly, convincing evidence on the age of the Churhat sandstone will have to be forthcoming before the interpretation of Seilacher *et al* (1998) finds wide acceptance. Should it turn out to be supported by new information on the age of the Churhat sandstone, it would have profound implications for our view of the course of early metazoan evolution, and should lead to an intensive search for further fossil evidence from deep time. In the absence of such finds, we would be left with the intriguing possibility that multicellular animal life made one false start, and that it took another half a billion years or so before a successful second attempt.

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Red ants with green beards

In colonies of social insects such as ants, bees and wasps, only one or a small number of individuals function as fertile reproductive queens. The remaining female members of the colonies function as sterile workers who spend all or most of their lives assisting the queens to reproduce. Such altruistic behaviour on the part of the workers appears paradoxical from the point of view of the theory of natural selection because we expect genes responsible for altruistic behaviour to be rapidly eliminated. Hamilton (1964a,b) showed however that natural selection can favour the spread of altruistic alleles if altruism is directed preferentially towards close genetic relatives of the altruists. But how do animals recognize their relatives so that they can so direct their altruism? Although the publication of Hamilton's paper led to an explosion of studies on altruism, especially in the social insects, little attention was paid to the explicit question of recognition. Everyone assumed that relatives are recognized by the context in which they are encountered—ants that happen to be in the same nest must be close relatives.

A classic paper by Greenberg (1979) led to other studies demonstrating that social insects can recognize kin even outside the context of their nests. But it appeared that recognition operated via shared, environmentally acquired cues rather than because two individuals are genetically related (Gadagkar 1985; Fletcher and Michener 1987; Keller 1997). However, genes that can 'recognize each other' have long existed in the fertile minds of theoreticians. To quote Hamilton (1964a,b) "That genes could cause the perception of the presence of like genes in other individuals may sound improbable; at simplest we need to postulate something like a supergene affecting (i) some perceptible feature of the organism, (ii) the perception of that feature, and (iii) the social response consequent upon what was perceived." Dawkins (1976) drew widespread attention to these hypothetical genes with the words, "It is theoretically possible that a gene could arise which conferred an externally visible 'label', say a pale skin, or a green beard, or anything conspicuous, and also a tendency to be specially nice to bearers of that conspicuous label." Green beard genes have been discussed frequently in the literature but have generally been dismissed as unlikely for two kinds of reasons. One, to expect a single gene to confer a conspicuous label, make the bearers of this label recognize a similar label on other individuals and also make them behave differently towards such individuals seems unlikely. Second, even if a green beard gene did arise, it would soon go to fixation (Wade and Breeman 1994) so that everyone in the population would possess a green beard and we would then no longer recognize this as something special.

Now, Keller and Ross (1998) (see also Grafen 1998; Hurst and McVean 1998) have produced what appears to be the first experimental evidence for a green beard gene in polygynous colonies (colonies with many queens) of the so called red fire ant, *Solenopsis invicta.* This is an Argentinean ant that has been accidentally introduced into the United States and has gone on to become something of a pest. Keller and Ross (1998) were not really looking for a green beard gene; like many social insect researchers they were studying variable allozyme loci to determine genetic relatedness within and between ant colonies and populations. During this study they hit upon a locus, *Gp-9*, that has two alleles, *B* and *b*. Diploid individuals (all females,—queens and workers included are diploid) are expected to have one of the three genotypes, *BB*, *Bb*, *bb*. They found that *bb* individuals are very rare both among queens and workers, the reason being that they appear to die from intrinsic causes. *BB* individuals are found among workers and also among virgin (as yet non-reproductive) queens but are completely missing among reproductive queens. The heterozygotes *Bb* are however found both among queens and workers. Keller and Ross (1998) looked closely at the social dynamics of the colonies in an attempt to unravel the mystery of the missing *BB* queens. What they found was remarkable: all *BB* queens attempting to reproduce were killed, and they were killed primarily by workers of the genotype *Bb*. In other words, workers who possess at least one copy of the gene *b* kill reproductive queens that do not possess at least one copy of *b*. The recognition of *BB* queens appears to be due to a transferable odour cue because *Bb* workers involved in killing *BB* queens sometimes acquire the offending smell and themselves become victims of aggression by other *Bb* workers. Keller and Ross (1998) interpret *b* as a green beard gene itself, or as a gene that is closely linked to a green beard gene that

confers (i) an externally perceptible label (smell), (ii) the ability to recognize the presence and absence of this label on other individuals and (iii) the behavioural repertoire required to behave differently towards those who possess the label (not kill) and those that lack it (kill).

These findings raise many questions. Why should *Bb* workers kill only reproducing *BB* queens and not non reproductive ones? A plausible proximate answer to this question is that queens possess a smell that is correlated both with their reproductive activity and with their genotype. The 'ultimate' answer to this question is obvious differential long-term survival of the alleles *B* and *b* will be ensured by differential behaviour towards the reproducing queens (killing of non-reproductive individuals is, in this sense, of no consequence). Why were Keller and Ross (1998) successful at discovering a green beard gene (even if by accident) while others were not? Because of the fact that unlike what was predicted on theoretical grounds, this green beard gene has not gone to fixation. But why has it not gone to fixation? The answer, and thus the secret of Keller and Ross's success, seems to lie in the misfortune of the *bb* individuals who die prematurely. The only individuals who possess *b* and survive to carry on their crusade against *B* are the heterozygotes, who automatically also harbour a copy of *B*. Thus *B* can never be completely eliminated. It is because of this quirk that *b* has not gone to fixation and we still recognize it as a green beard allele and why Keller and Ross have succeeded where others have failed. This implies that hypotheses about genes programming individuals to recognize other individuals that carry the same gene, and to behave differently towards them, are not so far fetched after all. The findings underscore the point that the interplay between cooperation and conflict are to expected at all levels of biological organization (Gadagkar 1997) and that their investigation requires us to shed our traditional compartmentalization into biochemists, ecologists, evolutionary biologists and so on and combine bold theoretical speculations and meticulous empirical investigations.

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To b(gl) or not to b(gl): how cryptic are "cryptic" genes?

A striking feature that has emerged from the genome sequencing projects that have been completed so far is the extremely high coding percentage seen in all bacterial genomes. In the case of *Escherichia coli*, it is higher than 90% (in higher eukaryotes including humans, it can be as low as 2%). Despite running such a tight ship, many prokaryotic genomes carry genes that—it seems—are unable to function at all. Contrary to what one would expect on evolutionary grounds, these "cryptic genes" have nevertheless been retained as a part of the genetic repertoire of the organism (reviewed in Mukerji and Mahadevan 1997). Unlike the pseudogenes that are found in many eukaryotes, cryptic genes can be activated by a single mutational event, often the integration of an IS sequence within the promoter. This means that even though normally their promoters are silent, they have not been converted to pseudogenes by the accumulation of inactivating mutations over evolutionary time.

Several explanations have been put forward to account for their maintenance. One is that the genes are silent only under certain conditions (such as growth in the laboratory) but are expressed under specific "natural" conditions without the benefit of an activating mutation. A recent publication (Khan and Isaacson 1998) provides evidence in favour of this hypothesis in the case of the cryptic *bgl* operon of *E. coli* (which consists of a set of genes that participate in the uptake and catabolism of β-glucoside sugars such as salicin and arbutin).

Most wild type isolates of *E. coli* are unable to ferment arbutin or salicin because the *bgl* genes are silent. Khan and Isaacson (1998) cloned random fragments of *E. coli* genomic DNA upstream of the chloramphenicol acetyltransferase (CAT) reporter gene. A pathogenic strain of *E. coli* carrying such a library was then introduced into mice treated with chloramphenicol. They found that the bacterial cells that survived in the liver of the mouse carried DNA sequences from the *bgl* region, upstream of the CAT gene. The implication is that the *bgl* genes are expressed under pathogenic conditions in bacterial cells that infect the liver. This has been further substantiated by the amplification of *bgl*-specific RNA from the cells by RT-PCR.

These results support the idea that "cryptic" genes can in fact function under specific physiological conditions. The simplest explanation for their maintenance, then, is that positive selection acts on them when they are expressed because—at least in the environment of the mouse liver—they increase the fitness of the bacterial cells that harbour them. This explanation is consistent with a recent observation on yet another "cryptic" operon, the *cel* operon of *E. coli* (hitherto believed to have as its function the catabolism of cellobiose). It turns out that the *cel* operon is inducible in the presence of the disaccharide *N,N'*-diacetylchitobiose, a breakdown product of chitin, brought about by the action of bacterial chitinases (Keyhani and Roseman 1997). In other words, while cellobiose utilization requires mutational activation of the operon, chitobiose is a natural substrate for the system (likely to be found in the gut of insect-eating carnivores) and is catabolized by the products of the operon as it exists.

The results with the *bgl* genes are striking, but they also raise many questions. Firstly, it is not clear what the selective advantage *is* of expressing them during pathogenesis. As far as we can make out, the apparent function of the *bgl* system is to enable the cell to catabolize β-glucosides, and it is difficult to imagine how the bacterium might benefit by expressing the *bgl* genes during infection (there being no reason to suspect the existence of β-glucoside sugars in infected livers). One might hypothesise that the permease encoded by the *bglF* gene, normally responsible for transporting the glucoside substrate, is involved in transport of other (essential) molecules under conditions of stress. However, this explanation is not consistent with the observation of Khan and Isaacson (1998) that, when a strain of *E. coli* carrying a deletion of *bglF* was used in infection experiments, no appreciable difference in pathogenecity could be observed. The second question concerns the apparent breakdown of the tight regulation of the genes *in situ*. Optimal expression by *bgl* in laboratory cultures involves, in addition to activation of the silent promoter, regulation of anti-termination of transcription that is brought about by the presence of the β-glucoside substrate. How is the cell is able to overcome this regulatory hurdle? An unfortunate limitation

of the experiments of Khan and Isaacson (1998) is that under the conditions used by them the expression of the *bgl* genes cannot be quantitated. Therefore it is not possible to compare the levels of expression detected by them with the corresponding levels under fully induced expression in the laboratory (of a strain carrying an activated copy of the gene). Despite these caveats the observations are thought-provoking because they raise the possibility that "cryptic" genes are not, silent relics of an evolutionary past after all—whose retention in the genome is paradoxical—, but are instead an integral part of the genome.

The puzzle of cryptic genes in prokaryotes resembles that of genetic redundancy in higher organisms. Many genes in metazoans exhibit stage and tissue-specific patterns of expression during development but show no effect when mutationally inactivated—or, once again, so it seems (see Cooke *et al* 1997 for a review). The problem of maintaining a redundant gene against mutational pressure is akin to that of retaining a cryptic gene. A number of theories have been advanced to account for "redundancy. Among them is that cells maintain 'back-up' genes for important developmental functions. On the face of it, this does not make sense in terms of what we know about how evolution works. Another way of explaining redundancy would be to say that gene duplication is sometimes followed by the retention of both copies because one of them begins to acquire a new function without entirely losing the old one. Then, under certain conditions, the DNA sequence carrying the new function may be able to substitute for the original copy, thereby conveying an impression of redundancy. But if examined in the right environment, a null mutation at either locus ought to manifest a detectable (if weak) phenotype. Ponte *et al* (1998) have brilliantly substantiated this hypothesis in the case of the cell adhesion molecule csA in the haploid soil amoeba *Dictyostelium discoideum.* Under standard laboratory conditions of development on agar plates, csA-null mutants develop normally. But under conditions that partially mimic the natural 3-dimensional soil environment, not only is their development poor, their fitness is markedly lower than that of the wild-type. The moral is on the lines of Lewis Wolpert's suggestion, which was made in the context of putatively phenotypeless transgenic mice. Bacteria too should be taken out periodically from their culture flasks to a "test evening at the opera" in order to examine whether "cryptic" genes might be functional after all.

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Transgene silencing: New insights into an old puzzle

The use of transgenic organisms has become routine in biology in the last decade. The technology is now being put to practice for creating grains with increased protein content, fruits and vegetables with enhanced nutritional value and flowers with exotic colours. A common means of doing so is to try and overexpress the plant's own proteins. Often however, instead of producing large quantities of proteins, the manipulation leads to exactly the opposite result: it causes suppression of the host gene(s) as well as of copies inserted as transgenes ("co-suppression") and results in a strong reduction in both host and transgene steady-state mRNA levels. This could occur as a result of methylation of DNA sequences, particularly promoter elements, leading to a decrease in transcription. In a number of cases however, the (trans)genes are apparently transcribed at normal rates in the nucleus, indicating that the suppression is post-transcriptional. When the transgenes contain a cDNA derived from the genome of an RNA virus, plants carrying the transgene can be resistant to the virus (Lindbo *et al* 1993). Although the effect has been documented in several systems, the mechanism by which it occurs remains unclear.

One model invokes a threshold level of RNA accumulation, following which, all homologous RNA in the cytoplasm is degraded specifically (Dougherty and Parks 1995). Another proposes that ectopic pairing of homologous DNA is involved in the initiation of silencing (Baulcombe and English 1996). There is also evidence that co-suppression is not cell autonomous, and can be transmitted between cells—perhaps through the entire plant. Palauqui *et al* (1997) address questions about the existence of a silencing message and its propagation within the plant. The work involves a series of elegantly designed grafting experiments with tobacco plants that exhibit co-suppression of nitrate reductase (*Nia*) host genes and transgenes, which results in chlorosis (yellowing of leaves). The authors demonstrate that when a normal non-suppressed scion (upper vegetative tissue) is grafted on to a suppressed stock (lower vegetative tissues and the root system), co-suppression is induced in the scion. Transmission is gene specific and requires the presence of a transcriptionally active non suppressed transgene in the scion. Moreover, it can occur in the absence of the roots of suppressed stocks. Strikingly, the information which triggers the *de novo* co-suppression is mobile and can be transmitted through as much as 30 cm of a non transgenic interstock segment. The phenomenon has been termed 'systemic acquired silencing' (SAS) by analogy with systemic acquired resistance, a mechanism that offers plants broad resistance to pathogen attack. Palauqui *et al* (1997) suggest that accumulation of *Nia* mRNA is required for both spontaneous and graft induced silencing.

A more piece of recent work (Palauqui and Vaucheret 1998) shows that transgenic lines accumulating *Nia* mRNA above the level of wild-type plants, can undergo graft induced silencing even if they are unable to spontaneously trigger co-suppression. In addition, non-transgenic mutants that over accumulate host *Nia* mRNA on account of metabolic deregulation, also display graft induced silencing. This implies that whereas the presence of a transgene is necessary for the initiation step, it is dispensable for the RNA degradation step of silencing.

What is the identity of the agent that carries the signal for co-suppression? Increasing evidence favours that this may be at least in part, an RNA molecule derived from the suppressed gene or its transcripts. Likely candidates include bits of transcript produced during RNA degradation, prematurely terminated transcripts, and copy RNA (cRNA) molecules produced from sense transcripts by endogenous RNA dependent RNA polymerases. A ribonucleoprotein (RNP) complex composed of cRNA molecules and plant proteins could be responsible for transmitting the signal into surrounding cells through plasmodesmata, the intercellular channels that connect plant cells (Jorgensen *et al* 1998). This hypothesis is consistent with the finding that plasmodesmata can facilitate cell to cell trafficking of proteins and their transcripts, thereby regulating plant growth and development (Lucas *et al* 1995). The identity of the SAS signal however remains unknown. Its characterization could be vital in understanding plant pathogen interactions. More significantly, it could reflect the existence of a complex information network that forms a basis for precise processing and transmission of information, central to plant development and physiology.

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When three is not a crowd

Beginning from the birth of RNA to its death, RNA-protein interactions require, in many instances, specific recognition of the RNA target by a protein. Specific recognition underlies mRNA processing, chromosome-end duplication and many developmental events such as early patterning of the *Drosophila* embryo. Decisions on cell fate during early stages of development regulate the activity, stability and cellular localization of mRNAs. Several experimental strategies to detect RNA-protein interactions have been designed to assess *in vivo* and *in vitro* interactions. Recently a variation of the widely used two-hybrid transcriptional transactivation assay for detection of protein-protein interactions has been adapted to explore functional RNA-protein interactions *in vivo*. In this genetic assay, termed the three-hybrid assay, the budding yeast is used as the biological system, and the assay is based on interactions between two fusion proteins and a hybrid RNA, which are used to activate transcription of reporter genes (SenGupta *et al* 1996).

In one of the three yeast shuttle plasmids used in this assay, sequences for the well characterized DNA binding domain of the bacterial LexA protein are fused to the bacteriophage MS2 coat protein sequences. The latter domain binds with high affinity to a 17nt RNA stem-loop normally present in the phage RNA genome. This LexA-MS2 fusion protein (hybrid protein1) is expressed constitutively in yeast. A second yeast plasmid transcribes a bifunctional hybrid RNA containing sequences of the MS2 RNA that constitute binding sites for the coat protein and also the desired test (potential) RNA binding sites. The third yeast plasmid expresses a library of cDNAs or genomic sequences fused to the transcription activation domain of the Gal4 protein to generate hybrid protein2. All these plasmids are expressed in a yeast strain in whose genome the bacterial *lexA* operator sites (*lexAop*), controls the expression of two independent reporter genes—LacZ and the yeast HIS3 gene. Binding of the hybrid protein1 at *lexAop*, and the corollary binding of the hybrid RNA to hybrid protein1, positions the test RNA sequences in the proximity of the transcription start sites of reporter genes. Further, if the second hybrid protein contains cognate binding sites for the test RNA sequences, this will position hybrid protein2 with the transcription activation domain favourably situated to activate the transcription of the reporter genes (figure 1).

This exquisite system has been used effectively to understand an important developmental switch during sex determination of the nematode *Caenorhabditis elegans*. Regulatory sequences in the 3′ untranslated regions (3′UTR) of mRNA are often critical in development, and in *C. elegans* they have been shown to regulate blastomere identity, and later on germline cell fate and the life cycle (Anderson and Kimble 1997). In hermaphrodite worms the switch from spermatogenesis to oogenesis is controlled by the 3′UTR of the *FEM3* gene. *FEM3*-based repression is required to switch from spermatogenesis to oogenesis. Mutational analysis of *FEM3* revealed a minimal five nucleotide stretch in the 3′UTR of its mRNA which defines a binding site for a regulator. Overexpression of an RNA with these sequences maculinizes the worm consistent with titration of a repressor. A 37 nt region of the FEM3 RNA containing the potential binding site has been effectively used in a three hybrid assay to identify a regulator of *Fem3* and thus of the sperm/oocyte switch. The hybrid RNA, i.e., the bait for specific interacting proteins, contained a tandem duplication of the 3′UTR sequence in FEM3 RNA. In screening a *C. elegans* cDNA library by the yeast three hybrid assay the group utilized the sensitive genetic markers available in yeast to specifically identify interactions that were hybrid RNA dependent (Zhang *et al* 1997). The specificity of the RNA-protein interaction was verified by using mutant versions of the target RNA sequences. The interacting clones were used to identify full-length cDNA which defined two highly related genes encoding what have been termed Fem binding factors (FBF1 and 2). The striking feature of the derived amino acid sequences in these factors is the tandem repetition (8 in this case) of a highly conserved sequence with homologues in the animal and microbial kingdoms. That these repeats form the core RNA binding sequences in Fbf1 was revealed by testing deletion derivatives of Fbf1 for RNA binding to 3′UTR of Fem3. The repeats of Fbf1 are similar to those of the *Drosophila* protein encoded by the *PUMILIO* gene. Pumilio binds to *nanos* response elements in the 3′UTR of Hunchback (HB) mRNA, thereby causing translational repression and destabilization of HB RNA (Curtis *et al* 1995). Thus Pumilio together with Nanos impose on Hunchback its asymmetric expression, restricting its expression from the posterior of the embryo. FBP may also have partners in its function and the best candidates for these roles are the *mog* genes which are also required for the sperm/oocyte switch (Graham *et al* 1993). Both FBF and Pumilio define members of a large and evolutionarily wide-spread protein family which these authors now define as the Puf family. The proposed functions for these factors are as sequence specific RNA binding proteins. Eight repeat units of decapeptide sequence and conserved segments before the first repeat and after the last repeat constitute identifying motifs in other homologues. In this general modular structure these RNA binding sequences are similar to the other classes of RNA binding sequences for instance the RRM domains. The success of this search for RNA binding developmental regulators, suggests that similar elements in the 3′UTR of many other genes may be amenable to the three hybrid analysis.

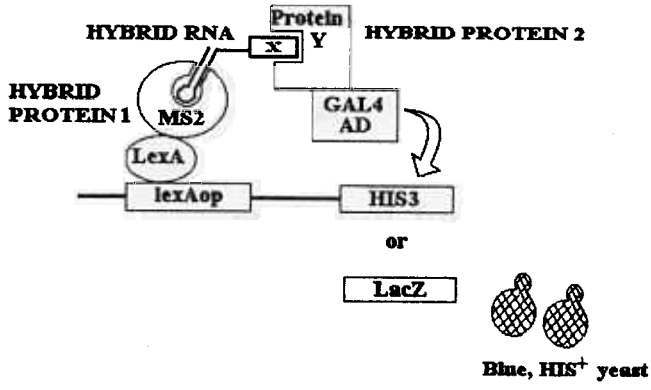

Figure 1. A schematic representation of the three hybrid assay [adapted from Zhang *et al* (1997)].

The bifunctional RNA bridges two hybrid proteins and contains a binding site for the MS2 protein and a test RNA sequence of interest: X. Bridging interactions transactivate the HIS3 and LacZ reporters, to give blue and histidine prototrophic yeast cells.

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Decisions, premonitions and hunches: Is there any rationality?

Rational thought and calculative decision-making abilities are evolutionary assets. In terms of how complex these can be, primates fall in a class quite apart from the rest of the fauna on earth. One may term these abilities as attributes of neural supremacy. Even though they can be labelled—not described—with relative ease, there are facets to them which escape definition. At times they end up being classified within the mystical realm—as premonition, sixth sense and even extra sensory perception. One might think that the serious business of day-to-day decision making is far too complex to be passed off so trivially. In fact, recent experiments suggest a hitherto unsuspected reliance of decision making on these 'extra' or 'emotional' elements.

Making a decision implies the execution of a choice subsequent to the successful completion of a series of events in the brain. These include (i) gathering of information on the situation, (ii) comparison of the current situation with previous experience (in an attempt to correlate long term repercussions of similar choices), (iii) computation of the choice that would reap the best benefit and (iv) initiation of appropriate motor activities concerned with the execution of the decision.

The first of the above processes, i.e., those concerned with the assimilation of information, have been extensively studied in the visual system of primates. The studies make use of a number of psychophysical assays like the direction discrimination task, the binocular rivalry task, visual search task, the delayed match-to-sample task etc. (Leon and Shadlen 1998). Many of these tasks reveal the animal's ability to pick out the information most relevant to the situation from a background of insignificant inputs. It is intriguing to speculate to what degree information-gathering and decision making run in parallel. The brain could encourage input from all available visual fields but choose to compute its decisions based largely on the dominant input. Or, it could focus all its information-gathering potential towards the dominant field. The latter would risk the loss of possible nuggets of useful information in ignored or discarded fields, while the former would require a continual weighting mechanism to define a dynamic dominance.

Studies on the visual cortex of monkeys using functional magnetic resonance imaging (fMRI) show that multiple stimuli in neighboring receptive fields compete with each other for functional access to the processing centers (Kastner *et al* 1998). The outcome of the competition seems to be biased by a weighting mechanism that is manifest in the animal's spatially directing its attention to a particular object. The authors argue on the basis of this that only a limited amount of what we see reaches the centers of consciousness. However, it is quite likely that the 'recessive' fields of information are not altogether discarded. The extent to which an explicit awareness of their existence impinges on the final decision is not easily estimated, but the activity of sensory centers suggests that this information is acquired. One might argue that it would be expected of an animal to demonstrate awareness (by the activity of effector circuits) of only a subset of what it is actually aware of (at higher levels of neural processing). Then it would be seen to tackle its most pressing concerns first, but its subsequent behaviour would be modulated on the basis of 'afterthoughts' that resulted from a rational computation of all the available data—dominant or not.

Perception of the situation and its relay to the centers of computation are not enough to ensure that a rational decision is made. In the early 90's Damasio and others noted that patients with

damages to the ventromedial prefrontal cortex are capable of normal recognition, memory and intelligence tasks but lack the ability to take rational ('wise') decisions. Such patients (from Damasio's index of 2000 brain damaged patients) appeared perfectly normal but went through their lives with a string of failed marriages, lost jobs and so on. The phenomenon was dissected via a mock gambling game using four decks of cards (Bechara *et al* 1997). The players (armed with fake bills worth 2000 dollars) were asked to turn the cards from either of the A, B, C or D decks, knowing that turning A or B would earn an immediate reward of $ 100 while C or D would yield only $ 50. Unknown to them, the researchers also introduced some penalty cards, which would (in the long run) harm a player who exclusively played from decks A and B. The players had no way of computing the penalty, nor did they know when the game would get over. In the course of the game, normal patients were gradually able to discriminate between the good and the bad decks and eventually pick cards preferentially from the C and D decks. Patients with bilateral damage to the ventromedial prefrontal cortex did not seem to make a discriminatory decision and continued to pick from the disadvantageous decks.

Bechara *et al* (1997) also found a correlate in the skin conductance response (SCR, a measure of changes in conductance due to sweating) of the participants during the course of the experiment. Soon after the penalties started coming at them, normal patients showed anticipatory variations in SCR as they reached towards bad decks. The Damasio laboratory repeated these experiments, interrupting them with questions aimed at revealing the amount of conscious thought which went into the decision to avoid bad decks and also timed the onset of anticipatory SCRs. In an initial ('pre-punishment') period all the decks were favoured equally and the participants generated no SCRs. However, by about card 10 (corresponding to the onset of losses due to penalties), normal players started demonstrating significant SCRs whenever they tended to approach the A or B decks. Interestingly, even by card 20 the participants had no idea how the game was progressing or which decks were risky. By card 50 most normal players mentioned a hunch that A and B were disadvantageous (but could not explain why), while by card 80, many (7/10) normal players could articulate the risk of choosing from the A and B decks. None of the brain-damaged patients displayed any anticipatory SCRs (towards A and B) nor modulated their choices towards decks C and D. Even after being able to conceptualize the danger of choosing the risky sets, such patients tended to pick from all four sets, revealing very poor judgmental abilities.

Although the findings were in tune with the expected defects associated with prefrontal lesions, the surprising revelation of the experiment was the onset of SCRs in normal people much before a logical deduction of risk could be made. This is suggestive of a distinct layer of neural awareness which not only computes consequences much ahead of the declared consciousness, but also initiates motor activity as soon as it derives an advantageous strategy. This could very well represent what is commonly known as intuition or premonition and might play a critical part in decision making. It is tempting to wonder whether there are layers of neural computation which work on data from seemingly irrelevant inputs at the same time as the brain is executing decisions based on the dominant fields. Such layers could possibly elucidate solutions which may not be immediately apparent, but are quite relevant to the executed behaviour.

A large body of work remains to be done before the finer points of such processes can be confidently discussed. But if anything, these findings should instruct us not to ignore our impulses—a seemingly irrational urge might well be rational but for reasons beyond our comprehension at that time.

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