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Interview with Adrian Bird

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Adrian Bird is a molecular biologist focusing on the biology of the genome and genomic regulation. He graduated in biochemistry from the University of Sussex and obtained his PhD at Edinburgh University. Following postdoctoral experience at Yale University and the University of Zurich, he joined the Medical Research Council's Mammalian Genome Unit in Edinburgh. In 1987 he moved to Vienna to become a Senior Scientist at the Institute for Molecular Pathology. Since 1990, he holds the Buchanan Chair of Genetics at the University of Edinburgh.

Adrian Bird and his working group identified CpG islands in the vertebrate genome, i.e. genomic DNA that is full of CpG sequences that are not methylated and that became understood to be near promoters. He discovered proteins that read the DNA methylation signal to influence chromatin structure. Mutations in one of these proteins, MeCP2, cause the neurological disorder Rett syndrome, and he discovered that the resulting severe neurological phenotype is reversible.

Early career; research on gene amplification and methylation in Xenopus

UD: I understand that you graduated in biochemistry and later became a molecular biologist dealing with RNA. In the early 1970s, when you were a post-doc with Max Birnstiel at Zurich, you worked on ribosomal RNA genes and started to work on DNA methylation. That was at the same time that Aharon Razin worked on methylation in bacteria. Which organisms or cells were you working on when you started to work on methylation?

AB: I was working on frogs – *Xenopus laevis* – which is a South African clawed frog. And the reason was that it actually moved on from my interest in gene amplification to methylation in one step.

For my PhD I worked on gene amplification. At the time people didn't really know whether every cell in the body had the same genes or whether, if you wanted a lot of hemoglobin, you might amplify those genes and perhaps even throw away genes you didn't want. No one was sure of that because the tools for analysing the genome DNA were primitive back then.

Don Brown and Joe Gall in the States found that in frogs the oocytes amplified the ribosomal RNA genes; they basically took the genes out of the chromosome and made thousands and thousands of copies. This seemed like a paradigm for the way development might work, so I started to study that process. Gene amplification is an amazing phenomenon, but it has rather disappeared from view now because it's an exception; it's not actually a paradigm. A few years later it emerged that the genome doesn't really change very much with development. Immunoglobulin genes are another exception as they undergo rearrangement, but everything else stays solidly the same in every cell in our body.

While I was working on the frog egg gene amplification, it turned out – again from the Don Brown lab – that the amplified ribosomal RNA genes differ from the normal chromosomal copies with respect to DNA methylation. The methylated base is cytosine, which often acquires this chemical modification after DNA synthesis, provided it is part of the short DNA sequence CpG. Sequences other than CpG – for example CpC, CpA, CpT – don't usually get methylated, only CpG does. There is lots CpG methylation in chromosomal DNA, but the amplified ribosomal DNA is completely free of 5-methycytosine (5mC). In those days this could be detected because of a physical property, namely a different buoyant density when centrifuged at high speeds in a cesium chloride solution.

Knowing about this difference, I was able to interpret an experiment I did in Zurich when I was in Max Birnstiel's lab. Restriction enzymes as a way of mapping DNA had just come in, and Hamilton Smith, who won the Nobel Prize for finding that restriction enzymes could be used for mapping, appeared in Zurich for a sabbatical. The first thing he did was to make a restriction enzyme, so I tried it out on some samples of ribosomal genes that I had in the fridge. When I compared amplified with chromosomal ribosomal RNA genes after cutting with this restriction enzyme, the patterns turned out to be completely different even though they should have the same DNA sequence. The chromosomal stuff didn't get cut and the amplified stuff got minced into tiny little pieces. The explanation, which took a while to sink in, was that the difference was due to DNA methylation. The enzyme cut at a site with a CpG in it and methylation of the C stops the enzyme from cutting.

Because the difference in cutting pattern was due to DNA methylation, this result meant that one could use this restriction enzyme to map methylated sites. To demonstrate this, we compared the cleavage map for the methylated chromosomal genes, which told you which sites were blocked, with the map when no methylation was present, which told you where all the CpG sites actually were. That gave us the first map of methylated sites in the genome.

Before that, one knew that there was methylation in the genome, but not where it was. So this was a breakthrough in the field.

UD: And it was already known that methylation had some meaning in eukaryotes?

AB: No, not really. Nobody had any idea what it was for. The suggestion was made that it was heritable in a couple of influential theoretical papers, one from Arthur Riggs and the other one from Robin Holliday. A key point is that CpG sites occur in pairs on opposite strands of the DNA double helix. DNA replicates semi-conservatively; so a methyl group at CpG on the old strand won't be present at the corresponding CpG on the new strand. In theory, if the DNA methylating enzyme can look where the old strand methylation is and then put one in the same place on the new strand, then you've got a copying mechanism.

That intrigued me and I tried to do experiments to test it, but they didn't work for technical reasons. Actually the Cedar lab and Michael Wigler both did nice experiments and were able to confirm that this is how it works.

UD: Yes, Howard Cedar told me that.
Bacteria use methylation to protect themselves against restriction enzymes, I've read.

AB: That's right.

UD: But there is nothing like that in eukaryotes.

AB: Tim Bestor has championed the idea that methylation is used as a defense mechanism also in mammalian cells and he has suggested that actually transposons are held in check by DNA methylation. He has some evidence for that, but it's by no means perfect. In fact, I think this aspect is often overstated.

UD: It sounds so nice.

AB: Yes, that's actually a problem with this field – well, with science really – things that sound nice feel like they must be true, but they aren't always.

The discovery of CpG islands

UD: One of your major discoveries concerning the impact of genomic DNA on genomic regulation, is that of the CpG islands. I would like to ask you how this discovery came about. Was it also accidental or was it by means of induction, I mean, did you accumulate evidence that these islands exist, or -

AB: What happened was, we used the restriction enzymes, which cut very frequently in genomic DNA, on various organisms. It was a sort of phylogenetic exploration. We went to the local marine biology stations in St. Andrews and Millport on the west coast of Scotland – drove there in a Land Rover and picked up all these marine organisms. We didn't know what we were getting until we got back and looked them up. And then we made DNA from them and found that in most cases the DNA was quite well cut, suggesting widespread absence of genome methylation. But for vertebrate DNAs – frogs, birds, fish, mammals – the enzymes hardly cut at all; there was so much DNA methylation that all the sites were blocked.

A graduate student in my lab called David Cooper was working on this and we had the idea of end-labelling the fragments to detect trace amounts of unmethylated DNA. That meant that instead of seeing the *weight* of DNA that might go to the bottom, which is the way you normally look at it by gel electrophoresis, we saw the *number* of fragments. Suddenly he saw a big blob at the bottom of the gel that we hadn't been able to see before. It just looked like an artifact, really. But we pursued it, and it turned out that this was derived from GC-rich non-methylated domains that were really quite short. We cloned those sequences and showed that they were derived from the promoters of genes. Based on the rather minimal sampling of the genome, we had detected the CpG islands.

I should point out, though, that this was an idea whose time had come, because other people at roughly the same time drew related conclusions. They didn't follow through in the same way by showing that there was a CpG island fraction, but they looked at specific genes and found that the transcriptional start sites were full of CpGs that appeared to be non-methylated. Vertebrates have just a tiny fraction of unmethylated genome, and it is in the CpG islands.

So we were in the vanguard, but I wouldn't say that we were far ahead of the game, necessarily.

UD: You are very modest.

But is it possible to distinguish so clearly between these islands and other parts of the chromosome?

AB: Yes, it is. They are dramatically different.
First of all, in terms of methylation, the paradox is that there's no DNA methylation there despite a ten times higher density of methylatable sites (i.e. CpGs) compared to the rest of the genome.

UD: More sites of?

AB: More CpG sites. If you look at the density of CpGs in a CpG island, which is about a thousand base-pairs long, it's about 1 in every 10 bases, whereas in the rest of the genome, it's one in every hundred to two hundred bases. That's tenfold different. And I can tell you why. Two reasons – the base composition of the DNA of the CpG islands is rich in G and C. Most of the genome is 40% G plus C, which is the same as 60% A plus T, but in CpG islands it's the other way around. In fact, it's more extreme: 65% G plus C, so by chance alone you end up with more CpGs there. But that's not the biggest reason. The biggest reason is that methylation is mutagenic. You might not have heard that before.

UD: No.

AB: One of the most important biological, biomedical attributes of cytosine methylation in DNA is that it causes mutations. The reason is that cytosine is prone to deamination and loss of the amino group turns cytosine into thymine. I don't know if you want me to draw any chemicals on the board, probably not, but -

UD: I wouldn't mind. I studied it a long time ago.

AB: Okay, well I'll just say it first. It's a fascinating piece of biology. Water causes cytosine to deaminate so that you get uracil – it happens about 100 times per cell per day. Uracil is a natural base in RNA, but in DNA it would be a serious source of mutations. What has happened during evolution is that genomes have methylated the uracil so that now they can distinguish the deamination product of cytosine from a normal DNA base. So now when you deaminate methylated cytosine – it becomes a big problem, because instead of uracil, you get thymine. There is a whole machinery for removing uracil from DNA – repairing it.
But it will not touch thymine. The repair mechanisms for this change, though there are some, are very inefficient and, as a result, about a third of all the point mutations that give rise to human genetic diseases are at CpG. It's one of the most important biological features of DNA methylation. People tend to skate over it, but I think it's absolutely fascinating!

UD: It is fascinating - and devastating! That means that evolution kept a mechanism for so long that has these mutagenic effects.

AB: But evolution always has this cost-benefit component to it and presumably the benefit is bigger than the cost.

UD: It is fascinating. I agree.

AB: Yes, we've smeared a mutagen all over our genes, basically. It's a relatively mild mutagen; the mutation rate for methyl cytosine is approximately ten times higher than the mutation rate for any other base. Note that CpG islands have not been methylated for millions of years so they have not lost their CpGs. And that's the second reason why there are so many CpGs there.

UD: How is the activity of these promotor regions, which are lying close to these islands, regulated?

AB: Genes are regulated by proteins which usually have nothing to do with DNA methylation. They are sequence-specific DNA binding proteins or transcription factors. They are, if you like, the "smart" molecules in the cell as they can tell one bit of DNA from another by reading the base sequence. After all, that's the main thing that distinguishes one bit of the genome from the other. DNA methylation is not involved in short-term regulation of genes.

UD: Yes, Chaim Cedar told me the same. He said that most people misunderstand it. We'll come to that later.

AB: Yes, but how amazing that despite being told that for decades the misunderstanding persists. Once things get into the cultural germ line it's almost impossible to get them out again!

UD: A new kind of evolution, right?

AB: Yes, exactly!

The environment, cellular memory and methylation

UD: Coming to transcription factors, you have been working on the hypothesis that other short DNA sequence motifs are also recognized by sequence-specific binding proteins. This may impact on the chromatin structure and I found that very interesting. And it seems another example for what you have written several times of the intricate interaction between sequence-specific binding proteins and chromatin structure, which then -

AB: Yes, I think if you ask people about epigenetics, the first thing they talk about is the environment impacting on the way genes are expressed. And with epigenetics you also have the concept of heritability which offers a way in which you could get environmental information put into the genome and then transmit it.

But, in fact, the evidence suggests that the genome is heavily insulated from the environment, actually. It is not, sort of, waiting like a young bird in a nest for

environment input. To me, it seems that for a lot of aspects of genome management, like DNA methylation, the logic is internal to the cell. It's not dependent on the environment to give the instruction. And even in plants, where the long-term impact of the environment on gene regulation is best characterized, epigenetic changes are not adaptive, but seem to be random. The environment is not informing the genome. If the logic is internal, then the DNA sequence is likely to be impacting on the epigenome. And there's quite a lot of evidence that that's the case.

UD: I understand. But just to come back to these short DNA sequence motifs that are recognized by the specific proteins, are these proteins also transcription factors or repressors or are there different kinds of proteins?

AB: Yes, the trouble is those words are somewhat vague. So, there are proteins that bind to the CpG dinucleotide only if it's not methylated. Cfp1, for example, binds non-methylated CpG and recruits a histone methyl transferase that puts a so-called "active" mark onto the histones locally. So that is facilitating transcription, but is that a transcription factor? Sort of. But it's not recruiting RNA polymerase, it's just affecting the environment so that it becomes "promotor friendly".

UD: Yes, I see. I would like to bring up a topic which is also often related to DNA methylation, namely is cellular memory. Henikoff and Greally wrote that though cellular memory has often been claimed to be caused by methylation, there are only a few cases where this memory is attributable to it; in many organisms, methylation does not even exist. What is the cause of cellular memory?

AB: Yes, Drosophila doesn't have any methylation, nor does C. elegans. To me cellular memory is not as remarkable – it doesn't beg for an explanation – because when a cell divides, it's got its transcription factors there, and then it splits in half and afterwards each daughter cell has still got its transcription factors. So any positive feedback loop can re-establish the cell state. It's not a miracle that when something divides in half and both halves have the same constituents, that both continue to be the way they were before.

The same is not true of trans-generational memory, but we're not talking about that, we're talking about cells. Regarding the cellular memory conferred by DNA methylation, first of all it's a rather imperfect memory because by itself it's quite error-prone and after a few cell divisions is no longer what it was. So you may have to reinforce continuously – in which case it's not sufficient by itself to memorize cell state. But secondly, except in a few cases of transcriptional shutdown, it doesn't look as though most gene expression programs are remembered by DNA methylation at all. On top of that, DNA methylation doesn't

seem to be the key component that regulates gene expression, as we were saying before. So if it's not critically involved in regulating gene expression then what is being remembered?

UD: It's not the key, but Howard Cedar told me that once repression or activation of genes has occurred, it is kept that way because the genes are methylated or demethylated.

AB: I'm less convinced of that, because when a CpG island gets methylated you have very dense methylation and that then shuts down that gene. That is what happens on the inactive X, it's what happens on certain imprinted genes. There I would agree with Howard Cedar. But for globin genes and growth hormone and keratin and genes like that, they don't have CpG island promotors. And quite a lot of tissue-restricted, high expression genes characteristic of terminal differentiation states don't have CpG islands either. In these cases, the methylation density is low, and its influence on gene expression has been almost impossible to show convincingly. I would argue that DNA methylation density is an important parameter - if you don't have a high density, then the repressive effect of methylation is terribly weak.

UD: Density means -

AB: Density of methylated CpGs. Remember I said there is roughly one per hundred bases in the genome as a whole? That's miles apart. And the experiments have rarely shown that you can shut down a globin gene, or something like it, in a blood cell by a few methyl groups. It just doesn't have enough impact. CpG island genes, on the other hand, are shut down when they are methylated, but this rarely happens. The alpha-globin gene, for example, has a CpG island and is only expressed in blood cells, but in every other cell type you look in is still non-methylated. It doesn't get methylated when it gets shut down - that's not the way that gene is regulated. DNA methylation is not associated with all genes that are turned off.

UD: That's really interesting. I wasn't aware of it. They shut off because of the repressors?

AB: Yes, yes. Polycomb, for example, or absence of activators, and there are also lots of sequence-specific repressors that can be involved. DNA methylation is hardly ever found to be responsible for shutting down tissue-restricted genes to my knowledge. And in CpG island genes methylation is absent the whole time. Only on the inactive X, imprinted genes and a few other cases do you get reproducible methylation of CpG islands that clearly contributes to silencing.

UD: And it also stays the same way over mitosis?

AB: Yes. The sort of copying mechanism I talked about helps that, but there is some evidence that silencing has to be continuously reinforced. In other words, it's not the cells says "methylate this" and can then forget about it; if you forget about it, it may lose its methylation gradually.

X inactivation provides a classic example where "none of these epigenetic mechanisms is enough." There you have methylation of the DNA, you have late replication during S-phase, you have polycomb which is an independent repression mechanism, you have histone deacetylation, position in the nucleus, and all of these things matter. I think it's because making a switch in a liquid state is not very easy. We tend of think of cells as analogous to computers. But computers work with switches that are binary: 1 or 0. In liquid, however, when you've got chemical reactions going on, nothing is ever 0.

This means that cells have to indulge in tricks to make switches. And one of the ways they do it is, rather than spend huge amounts of effort making a perfect switch, they stack imperfect mechanisms one upon the other. So let's say you have a mechanism that is 90% efficient that silences a gene. That means that 10% of the time the gene is going to switch on by mistake. Now let's say you have an independent mechanism operating on the same process which is also 90% reliable; you have achieved 99% efficient silencing. If you have three such separate mechanisms, 99.9% etc. So by stacking inefficient mechanisms one on top of the other, you can get really, really good repression. DNA methylation is one of those mechanisms, but it's not all of them.

UD: So methylation is just part of a number of mechanisms that add to repression of activities. It's really what Francis Crick said, that Occam's razor should not be applied to biology. This is a good example.

AB: Well, I actually increasingly think of organisms as rather cumbersome bureaucracies! They are like human bureaucracies.

UD: Not a nice picture.

AB: Well, bureaucracies have a bad reputation, but actually they are jolly stable! Bureaucracies can be inefficient for ages and still continue, so biologically that's a good property!

UD: Our romanticism is gone!

AB: Well, I think biology is great at destroying romantic delusions!

UD: You are so right. Even Darwin understood that.

Epigenetics and inheritance

So now we come to epigenetics and inheritance, including the trans-generational one which is included in so many definitions of epigenetics which take for granted the characteristic of inheritance also through the germ line. You have criticized this definition in several instances pointing, for example, to the fact that chromatin marks are short-lived and methyl marks are not accurately replicated – what you said right now. What is your current view of the inheritance of epigenetic marks across the germ line?

AB: Across generations?

UD: Across generations.

AB: Well, first of all, there is an industry of labs producing evidence – or claiming to produce evidence – for trans-generational inheritance. It's something that is absolutely fascinating to people, and it is indeed fascinating. The idea that you, perish the thought, slap someone and as a consequence some memory of this goes into their germ line and is transmitted to the next generation; it's a very attractive idea in some ways. But I think much of the evidence is really weak. In certain situations, it clearly can happen, for example in immunity of worms to parasites – I think I wrote about this in that article in *Cell* – that if you become immune to a particular parasite, then transmitting your immunity to your offspring has a huge selective advantage.

The evidence that other things are transmitted is out there, but the effects are often either miniscule or dubious – there have been fraudulent experiments and some of them have been retracted, not all. I think there is a huge pull. The journals tend to love anything that smacks of transgenerational epigenetic inheritance, even if it is only detected in mutant organisms. For example, in fission yeast and C. elegans transgenerational inheritance of an epigenetic mark is reported, but only after the enzyme is mutated which normally removes this epigenetic mark. Press coverage tends to overlook this requirement, reinforcing still more the popular belief that the environment controls our genes. The idea obviously dates back to the nature/nurture controversy, which began in earnest in the 18th century. Locke and his followers claimed that individuals were all exactly the same – each a *tabula rasa* on which one could write information according to experience.

It’s almost as though humans fall into two categories. There are those who really like the idea that we are all the same and that any differences between us are due to experience. And then there are those who are willing to accept that there is a huge amount of genetic hard wiring about which one can do nothing. In this second case it’s your genetic inheritance that mostly determines the sort of person you become. Obviously the environment and the genetics interact, but where does the balance lie?

Coming back to the evidence for transgenerational epigenetics, I think much of it is weak, scientifically flaky, and based on a desire for a particular answer. I should stress that there are many other people working in the epigenetics area who are also deeply suspicious of much of that evidence. But despite this, it sometimes seems as though there is an unwritten pact not to express disbelief. Who knows, part of the reason you get your funding and your recognition may be because you’re in this field, which has these interesting connotations. I can feel it in myself sometimes when people say, “oh, epigenetics – that’s such a fascinating area! All that stuff about transgenerational inheritance.” And you don’t want to say, “Oh, I think that’s a load of twaddle”. Because it sounds like you are saying: “actually it’s rather more boring than you think”. And for that reason, the evangelical wing of epigenetics has not been dismantled as much as it might have been.

UD: It’s a little bit frightening, isn’t it? I mean -

AB: Science is done by humans, and humans are motivated primarily by self-interest of one sort or another.

UD: But somehow the idea of scientific truth has been prevalent all the time, more or less.

AB: It has.

UD: If something is hard-wired, it could be demonstrated.

AB: I think it’s not always that easy to demonstrate these things definitively. It's interesting that identical twins, oddly enough, were adopted as an emblem of epigenetics.

UD: Yes, I read this article.

AB: It’s extraordinary. They’re called identical twins because they’re very, very difficult to distinguish, but epigeneticists have focused only on the few differences – which actually could be caused by mutations. There’s quite a lot of evidence now for mutations causing diseases that arise after the fetus is formed. So if you

have two fetuses then they can easily acquire different mutations; it's obvious, really. Differences between identical twins could be because of that, or of course they could be due epigenetics; I don't know. But to me, identical twins are THE best evidence that the epigenome and the environment are of limited significance.

UD: In addition, the mechanisms of epigenetics, methylation and others - I think it has been shown by you and others that they all involve DNA sequence-specific proteins.

AB: Yes.

UD: But the people who claim the environment does it directly omit this crucial point.

AB: They often do. And I think it is reaching a crisis point in a way, because the idea that the environment dominates the genes (to put it simply) and that it is inherited for many generations is now out there. I believe it's in school curricula.

I had a questionnaire from a Swedish group asking how I thought epigenetics should be incorporated into the teaching of psychiatrists and psychologists and sociologists and school pupils, and I found it extraordinary; it was basically inviting an attack on the teaching of genetics. It was all yes/no, agree/disagree, but the questions were formulated in such a way that clearly they gave you the option of saying that genetics was vastly overrated and the environmental influence on the epigenome underlay all sorts of phenomena that had previously been considered to be relatively hard-wired. The way the argument is often presented is that people who believe in genetics are old-fashioned and not moving with the times. They are reactionaries and this new epigenetic revolution promises to liberate people from their genomes.

I, personally, feel quite uncomfortable that I haven't written more. And this business of having in the school curriculum that the mothers' diet during their pregnancy can impact not only their unborn child, which is perfectly reasonable since it's part of their body, but also future generations. I find it insidious. But it's almost accepted as true now.

UD: Putting such pressure on people, mothers and others, right?

AB: Yes, it is. And Lamarck is one thing, but as you well know, Lysenko is another.

UD: Yes, I have written about it.

AB: I know; I've seen some of your work.

UD: Yes, because Lamarckism is just the most idiotic thing to assume, because most of the cases where epigenetics is supposedly involved are detrimental, such as obesity.

AB: Exactly. Everything we know of in biology works to minimize or correct damage, and the idea that you would preserve it… it's a difficult time. There was an article on the front page of *The Guardian* maybe two years ago now about Holocaust survivors and the inheritance of the abuse in subsequent generations. It was just poor science, but it was treated seriously. And it wasn't just on the front page; it was the lead article in one of the main, serious broadsheets in the UK. I didn't do anything there. Other people did, but I was just depressed by it, honestly.

Humans tend to coalesce in groups with fixed ways of thinking about things. Science at its best, I think, is a series of tricks for allowing people to see the world the way it really is. Because left to ourselves, we are subjective and biased.

You know, Popper's ideas that you can only really falsify something are also vitally important and have almost been forgotten by people doing research. It's considered slightly old-fashioned to try to destroy your hypothesis, but it's what we should be doing all the time. I try to impress on people in my lab, "what's the most painful experiment you can do to see if your idea is really right?" Because we naturally avoid pain so often don't want to do the experiment that says that the hypothesis, for which you may be famous or on which your PhD thesis depends, is wrong. Bad science is decorating your favorite hypothesis with favorable data. And good science is doing your best to destroy it. But it requires a certain amount of courage. I've even seen written down that we shouldn't try to falsify hypotheses in biomedical research because it's bad for the morale of a student to find that they have it wrong.
Sorry, I'm digressing ridiculously!

UD: No, no, no! That's interesting. These developments are very disturbing. There is also the often cited example of the Dutch Hunger Winter. I have heard that the epidemiology is sound, but that there is no statistical evidence that the traits in question are caused by epigenetics.

AB: The trouble is I'm not a mathematician or a statistician, but clearly the effects are very small. In fact, Kevin Mitchell has criticized this work for "multiple testing" – that is scouring the data for all possible correlations until one turns out to be statistically significant, which is likely to happen by chance. There is another point which is overlooked. These people were probably in familial contact or living with each other. If you wanted to do a controlled experiment, you would

isolate the various generations and then ask what traits they inherit, but one obviously can't do that with humans. So you have to assume that living in a house where it is common knowledge that the father or grandfather starved during this terrible period has no relevance to any survival differences you see. It's far-fetched.

Epigenetics and neurobiology

UD: I want to move on to research you have been doing for a while, epigenetics and neurobiology. In the paper you wrote with Illingworth and others you question the assumption that DNA methylation causally impacts neural activity and learning. You wrote that autism spectrum disorders like Rett and Fragile X syndromes are caused by conventional mutations but involve downstream aberrations of the methylation system. What is the cause for this aberration?

AB: Rett syndrome is caused by mutations in a protein that reads DNA methylation. It binds to the methylated sites, and then it gently pushes down on transcription, and brains seem to need this. Why brains need this subtle effect on thousands of genes is unclear, but that's what this protein seems to do. DNA methylation doesn't change in its absence, but the protein that reads it is lost.

UD: But I remember you wrote that the syndromes involve downstream aberrations in the methylation system.

AB: No. I mean, since MeCP2 reads DNA methylation, it is part of the system, in a sense. But the methylation pattern doesn't change at all. This protein is a reader of DNA methylation, not a writer of DNA methylation. That's the distinction.

A case where there are important changes in methylation is Fragile X syndrome. But again, it is caused primarily by a mutation. So in both of these conditions, the DNA sequence changes and then epigenetics is involved downstream of that. In the case of Fragile X syndrome, what happens is you have a sequence, GGC GGC GGC, normally about 40 of these trinucleotide repeats, near the start of the Fragile X gene, FMR1. In affected individuals it goes from 40 to over 200 and when that happens you get Fragile X syndrome, which is the most common cause of intellectual disability in males. Now, you may say that has all to do with DNA sequence. But the downstream consequence is DNA methylation of that whole repeat region (it's got CpG sites every three base pairs). So suddenly you have extremely dense methylation, and that shuts down the gene. If you take away the methylation the gene works, so that the expansion is not a problem, it's the fact that the expansion causes DNA methylation which is the proximal cause of the disease.

UD: I see, and how is the methylation started again by -

AB: Nobody knows. Actually, if you put the same expanded GGC sequence into a mouse (because one tries to make models of all these disorders) it doesn't get methylated. Mice are not people, it seems! So DNA methylation aberrations can be involved in disease, but the key underlying cause is a change in DNA sequence, i.e. mutation.

UD: I've read about your discovery of the MeCP2 gene.

AB: Yes, terrible name! My fault.

UD: It binds to methylated DNA and it is defective in Rett syndrome – did you show this or somebody else?

AB: Somebody else. It was Huda Zoghbi. Her lab mapped the gene for Rett syndrome and found it was *MECP2*.

UD: How did you discover this mutation, and particularly the reversibility of this syndrome? That was amazing.

AB: About the finding of the protein, we were working on DNA methylation because what I initially wanted to do was find proteins that bound to CpG islands. To do this I had to make a piece of DNA. Nowadays you order an oligonucleotide and it comes next week, but in those days I had to find a company that would do it and it took them nearly six months. I had got them to make a sequence full of restriction enzyme sites that could either all be methylated or all be unmethylated. My hope was that we could find a special protein that bound to CpG islands but not the methylated control DNA. In that way I'd get some new factor peculiar to CpG islands. What I found was the opposite: things bound to the methylated sequence, the control, but nothing bound to the unmethylated CpG islands. So after a long period while this sunk in, we decided to go after those proteins that bound to the methylated DNA.

The first one we found was MeCP1, but we couldn't purify it because we were initially rubbish at protein purification, but when we got a bit better at it we purified MeCP2. It bound to methylated DNA, but what else did it do? It actually bound to a co-repressor that deacetylates histones and therefore inhibits transcription. That was all done before the discovery that this gene underlay the human disorder Rett syndrome.

When I heard about the link to Rett syndrome, we were making a mouse model, and it turned out to mimic Rett syndrome extremely well. The *MECP2* gene is on the X chromosome and male mice don't survive, just like human males with

these mutations. Human females acquire the symptoms at about 6 to 12 months and in mice it's about the same. Females are fine for a while and then they get chronic symptoms just like humans. So it's a very good model of human Rett syndrome.

The idea that Rett syndrome might be reversible comes from the fact that neurodegenerative disorders like Parkinson's or Alzheimer's or Huntington's, kill neurons. And once they're gone, they're gone. But in Rett syndrome and other autism spectrum disorders and in psychoses like schizophrenia, the neurons are still there. In Rett syndrome we know that they are morphologically a bit simpler, as though they are "underpowered" neurons.

But because they're still alive, the question arose: if you put back MeCP2 would a sick mouse get better? The interest of the experiments comes from an element of mythology in the field. The belief that brains, if they don't develop properly first time, can never go back and redevelop and consequently are "scarred". It may be a reflection of the way that most people tend to feel when they see someone with a serious neurological disorder. We don't think "if only we had a medicine to cure that." We rather think that nothing can be done; it's too late. In the neuroscience field that fits with the classical experiments of Hubel and Wiesel, who found that if they covered the eye of a kitten at a young age stage, the so-called critical period, then the eye's function could not be recovered again later. If you miss a critical period when the brain is being set up, that's it, you can't go back.

The common perception was that a disorder like Rett syndrome, which is seriously debilitating, would likewise be irreversible. In fact, what we found is that if you allow the mouse to develop without the protein and then switch it on – that is, you put back the protein — the mouse gets better.

To someone who is a boring molecular biologist, it's a machine with a piece missing and you put the piece back and it works again. But if you have a historical view of brain development, that's an extraordinary result. I have to say that makes it sound as though I was always expecting it to be reversible, but in fact I wasn't.

UD: It sounds really, really amazing.

UD: Do you know the cause of the diseases of the autism spectrum that predominantly occur in male individuals?

AB: You mean why is it males?

UD: Yes, and what causes them. Are they genetic and the genes simply haven't been found?

AB: They have now. Right now, we're going through an extraordinary period where cases of developmental delay are liable to be sequenced. Usually it's just the exons of the genes, but increasingly it's becoming sequencing of the whole genome to try to find mutations. What they study is trios, which is two parents and the affected offspring to look for new mutations that are present in the offspring but absent in the two parents. Rett syndrome is an example of a condition that is always caused by new mutations because neither males nor females with the mutation reproduce, essentially. Sequencing in the clinic is revealing hundreds of new mutations that cause "autism." And as a result autism, like cancer, is dissolving into loads of different disorders. So what clinicians classify as autism covers a multitude of different diseases with different genetic causes.

For a long time, the statement that "we can't find the genes that cause these disorders" was true, and in this kind of vacuum, epigenetic theories of autism flourished. But as the experiments got better and better, the scope for pure epigenetic explanations has reduced dramatically. Although many examples of autism still have not yet been explained by genetics, the number caused by mutations continues to grow. And who knows, when you sequence the whole genome instead of just the exome, it could be you'll get close to the lot, but we'll see. The other likely possibility is that special combinations of several different mutations are causal, making them more difficult to track down.

There seems to be a wing in neurobiology that attributes phenomena to DNA methylation; even people who believe that memory is encoded by DNA methylation. Some of the data is pretty flaky in my personal opinion. Neuroscience was in some respects a self-contained world, though this has changed. It is funny, because molecular biologists are traditionally outward looking and opportunistic with no particular set of beliefs - just trying to find out how things work. Neuroscientists are more likely to inherit a culture regarding the way things ought to be thought about and studied.

UD: And they are more closely related to philosophy.

AB: Yes, and there are different wings.

UD: Frank Lyko, an epigeneticist from Heidelberg -

AB: Yes, I know him.

UD: He told me that he made the observation that epigeneticists are split into two communities: developmental biologists and neurobiologists and that in particular the latter group has published many unsupported claims, especially regarding plasticity. Has this also been your observation?

AB: To be honest, I don't read a lot of those studies; I have been to a few talks that I don't find believable. So I agree with him to some extent, but developmental biologists are not totally without blame either.

UD: I agree with that. You wrote in one of your articles that your data suggests that DNA methylation is unexpectedly constant between brain regions and fits with the notion that many differences in DNA methylation in the brain are determined genetically.

AB: Yes, we were agreeing with other people who had said that for different reasons. To be honest, that study was quite low-resolution. To me, the impressive thing about it was that we saw more differences between the cortex of person A and the cortex of person B than we saw between the cortex of person A and the thalamus of person A and the hippocampus of person A. In other words, these were different regions with different functions in the brain, but their methylation was indistinguishable to us. The differences between different individuals – the same region in a different individual – were bigger. And that's because of DNA sequence polymorphisms almost certainly. So it was a relatively low-resolution study, but I suspect that people sometimes exaggerate differences between tissues when they look at DNA methylation.

DNA methylation patterns and gene expression; the linkage of DNA methylation and genetics

AB: If you look at the screenshot of where the methylation is in the genome, it's rather even distributed. CpGs have a probability of about 70% of being methylated, except for the CpG islands where methylation disappears. In a liver or in blood or skin or brain the pattern looks remarkably similar. So a striking feature of methylation is its constancy. But because we are more interested in differences than similarities, you don't get any plaudits for pointing out things that are the same. As a result, an outsider gets the impression that the DNA methylation patterns in different tissues vary enormously. But they don't!

UD: That is surprising! That's unlike what developmental biologists are saying.

AB: There are differences, but they're pretty subtle and you've got to look really hard to find them. That doesn't mean they're unimportant; they may well be important.

For example, in the brain DNA methylation over genes that are highly expressed drops and this probably matters. But one should always point out that the differences are against a background that is strikingly constant on average. Otherwise you get the view, and you still find it in the literature after many years, that DNA methylation switches gene expression patterns and I just don't think the evidence supports that.

UD: That is really interesting. So that means the DNA gene activities between these regions are more different than the methylation?

AB: Yes. That's exactly right! That statement is exactly correct.
Some years ago we looked at this in T cells. When you are challenged by some toxin or bacterium, then T helper cells turn into Th1 or Th2 [two classes of T helper cells that play an important role in the immune system] depending on what sort of battle they've got to fight. You can get these from mice and turn them into Th1 or Th2 in a dish. There are thousands of gene expression differences between Th1, Th2, and the T helper cells and almost no DNA methylation differences.

We were astonished by that, but others have seen the same. For example, muscle development, taking myoblasts and fusing cells to make a multi-cellular syncytium where all the nuclei are in one cytoplasm going on to make actin-myosin striations – virtually no changes in DNA methylation. The conclusion is that gene expression is not being regulated by DNA methylation, but so ingrained is the idea that it is, that it's almost impossible to displace.

UD: Where did you publish this?

AB: The title we gave it is slightly at odds with what I just said, but anyway it's Deaton et al, Genome Research 2011. Number of gene expression changes between two cell types of more than twofold, 2518. Number of CpG island methylation number of changes, 5. Number of non-CpG island methylation changes, 42. Those are the sorts of numbers. Our results indicated that DNA methylation changes play a relatively minor role in the late stages of differentiation. We don't rule out the possibility that there have been methylation changes earlier than that because we didn't look there. But we did look when the switches in gene expression are taking place and found there little happening. One could argue that the important changes were early, but then I would go back to the fact that there aren't that many changes anywhere.

UD: That is extremely interesting to me. Thank you for that.

To go on, you were at some time elected scientist of the month. I couldn't find when it was and where-

AB: It was Abcam, I think.

UD: Yes, but they didn't give any date. Not even the year. You made a statement that I found very convincing, namely, "instead of arguing about what the word 'epigenetics' means, scientists are now getting on with the job of deciphering how the genome actually works. This has to be a good thing. We are learning that epigenetics and genetics are inextricably linked." And that is what you had expressed in many papers, already in 2007.
Could you please summarize how they are linked?

AB: The two examples of Rett syndrome and Fragile X syndrome are cases where people refer to them sometimes as epigenetic diseases because they both involve DNA methylation. Rett syndrome involves a reader of the methylated sites and Fragile X syndrome involves massive methylation of a promotor region of a gene which leads to its shutdown. In that sense they are epigenetic. But they both, as I stressed before, are caused by mutations that change the DNA sequence. The primary change is the DNA sequence and the secondary consequence are the epigenetic changes. In the case of CpG islands, it's pretty clear that the chromatin structure, which I haven't mentioned before, is different there. There is, for example, methylation of histone H3 lysine 4. This is characteristic of CpG islands, and this is recruited by a protein that binds to non-methylated CpG sites, of which there are a lot in CpG islands - and very few elsewhere. This protein is going to CpG islands because of the DNA sequence; nothing to do with epigenetics. The protein is going there and recruiting the enzyme that methylates the histone. So the epigenome is being -

UD: The histones are methylated?

AB: Yes, histones are methylated, acetylated, ubiquinated, phosphorylated – they have lots and lots of chemical moieties on them, many of which are not terribly well understood.

Coming back to Cfp1, the DNA sequence at CpG islands is informing the structure of the epigenome by directing methylation to histones. So I see the epigenome as primarily the client of the DNA sequence. And if the epigenome changes, it's because developmental programmes put different mediator proteins in place that are adapting the epigenome based on the underlying DNA sequence. This is very different from the view that the environment is dictating the epigenome.

Ideas, testing, and Popper

UD: Have you always held this view or did you once believe that there was an epigenetic revolution?

AB: I've always been a bit of a skeptic. In fact, Robin Holliday used to chastise me for not being more enthusiastic and always pointing out the weaknesses in the argument that DNA methylation was the key to development.

UD: And Holliday was one of the main promoters of that, right?

AB: Yes, and he sometimes used to say, "that's enough about data, give me ideas."

UD: Ah, and that's where your famous saying comes from, to put it the other way around!

AB: Yes, and there's something to be said for ideas, obviously. Where would we be without them? But you've got to actually test them.

For me there is an enormous pleasure in showing that what everybody originally believed is wrong. Because that's the way that you know that you've done something new. It's a sort of assay for doing something new. Everybody believes A, and you show that it's in fact B. So the Rett reversal was one of those. But I think it's a nagging desire that I have, perhaps some flaw in my personality, that I actually like to explore the ways in which the assumptions that most people have are incorrect.

That doesn't mean I'm negative about things, but I'm always suspicious of things that sound a little bit too good to be true. There's an element of – it sounds unscientific – an instinct that what you feel is likely to be the way things have evolved and a difference between that and the way people sometimes attribute functions. This is all getting rather vague and waffley!

I have always been suspicious of the idea that DNA methylation controls gene expression. Actually it goes back, to a great extent, to an experiment we did in sea urchins during our phylogenetic phase -

UD: That was in the 1980s?

AB: 1979 we published it, I think. In that phase, when we were getting restriction enzymes and digesting DNA from every marine organism we could find, the sea urchin had big non-methylated domains and big methylated domains. We knew that there's a set of histone genes that are on in the early embryo and then they

get switched off - a dramatic change in expression. We also knew that in the germ cells, these genes were non-methylated. So the question became: is the switching off accompanied by DNA methylation? And the answer is no, they stay methylation-free.

There are other cases where genes would change their expression and not their methylation. It wasn't just an irrational desire to go against the status quo. It was also based on evidence. Just in case it comes across that I'm a bit of bigot in wanting to show people wrong, I have had several occasions in my career where things that I have really strongly believed, I have proved to be unequivocally wrong. That's obviously a negative experience, but it shows the scientific process has allowed you to show that you yourself are wrong. I quietly find that exhilarating.

UD: It is very impressive, and it's reminiscent of Popper.

AB: That was an absolutely brilliant observation of his. Have I read Popper exhaustively? I have not. But that's all you need to know; you can never really show something is true, you can only show that something is untrue. People are quite critical of Popper because actually discovery is messy. But nevertheless, if you want to know if something is true, you've got to try to falsify it. And I've known people avoid the experiment that could do that.

UD: But, of course, as a scientist you are not necessarily driven just to find that something is wrong, but you are driven to show that something is right.

AB: Exactly.

UD: But the question is, of course, how do you do it and what are you accepting as evidence?

AB: Yes, we mislead ourselves all the time.

UD: By the way, the more I understand how science advances or progresses or regresses, I realise that induction is a necessary prerequisite. You need to do inductive steps, you have to accumulate data, and only then you come up with your hypothesis.

AB: Yes!

UD: The hypothesis doesn't come from the sky, as Popper says. So it's a little bit weird.

AB: Yes, it's not as monolithic as he might have liked. But nevertheless he's arrived at a truth in there. And that's always valuable.

UD: I agree.

The importance of scientific rigor; the practice of top journals

UD: Another topic – the importance of scientific rigor. We have already discussed it. I don't know of any other scientist who has been emphasizing the importance of scientific rigor as much as you have been doing, especially in regard to revolutionary discoveries such as long non-coding DNA or DNA methylation. When did you become aware of the fact that there was a lot of rigor wanting in these fields?

AB: I'm not sure I would say I'm peculiar in going on about rigor. I don't know how many times I've said it in print, to be honest, but it's obvious that one needs to be rigorous. It is vitally important because of the complexity of biology, which means that the number of possible explanations for things is always larger than you could imagine, therefore you've got to look at your data as dispassionately as possible. It's got to be properly powered to be statistically reliable.

We have a culture in the literature at the moment where a few journals – they call them CNS, which stands for central nervous system but also is *Cell*, *Nature* and *Science* – dominate. If you publish in these journals in China, you get several thousand dollars bonus and that is symbolic of what they mean everywhere. We published a paper in *Nature* last year that will probably bring added income to our institution. In the UK there is a "research assessment exercise" whereby the amount of money a university gets depends on how well they do in research. Of course the panels are supposed to evaluate the content of the papers, but it's notoriously difficult to see past the fact that it was in *Nature* or *Science* or *Cell*.

These journals exert a stronger stranglehold over scientific merit, or perception of scientific merit, than is healthy, to be honest. And what it's got to do with rigor is that more papers from *Nature*, *Science* and *Cell* are retracted than any other journals. The reason is probably that your paper doesn't get in there unless you're saying something reasonably dramatic. Many of the advances in science are not life-changingly dramatic, but they can sometimes be made to seem it with a relatively small tweak. There is such pressure to publish in these journals that it's almost an invitation to cut corners.

There is a huge temptation, which I am sure many scientists would never submit to, to just fudge things slightly. Now that sounds as though what I'm saying is that people fake data. That's rare, but I do think some data is viewed through rose-

tinted spectacles. As a result, claims that are made in those journals can be exaggerated.

Oddly, on grant committees the word “incremental” is something of an insult. What they are often after is “game-changing” outcomes. It strikes me as odd because results that move us incrementally forward are a good thing. On the other hand, being able to plan in advance to make mind-bogglingly life-changing discoveries is something no one can promise. The whole point about surprising discoveries is that they’re a surprise!

A third point I wanted to make about this is that many of the biggest discoveries have been made not by scientists who said they were going to do something crazy, but by scientists who said they were going to do something that didn’t seem very interesting. CRISPR-Cas9 is a classic example. They wanted to study a bacterial immunity system. We already know of dozens of bacterial immunity systems. It was an apparently tedious project to look at yet another bug and try to find out how it becomes immune to invasion by viruses, phages etc. And yet it led to CRISPR-Cas9. Suddenly we can go in and change the genomes of animals, plants, humans, anything. And it came from research that didn’t seem wildly exciting, but likely to be terribly boring.

And there are other examples too. The mechanism of oxidative phosphorylation, which turned out to reveal this extraordinary molecular machine.

I don’t think we’re always very good at evaluating science. We should insure that the quality of those doing it is high. Rigor, which all of those studies had in common, is absolutely essential. I think of science as a cultural plant. You’ve got to be careful to keep it alive and healthy. Focusing only on top journals with big discoveries and slogans that make people feel excited, means you may miss a core part of it. So I’m not overly happy with the system the way it is, but I don’t think in history anybody has been totally happy with their system.

UD: There are articles that invoke epigenetics and other things against genetic determinism of development in order to advocate a new holism that embraces organism and environment in an inseparable union.

AB: There was a study of obesity in Scandinavia, which had these identical twins reared apart which concluded that the relation between birth weight and later development of subcutaneous fat in different types of twins did not show that intrauterine nutrition is of etiological importance.

UD: A concept that has been under repeated attack, not only by epigeneticists, is Crick's 'central dogma'. I am not talking about prions but about small or non-

coding RNAs. What is your opinion about the validity of this central dogma today?

AB: You mean DNA makes RNA makes proteins?

UD: Actually, he only said that information gets into proteins and not out. He did not say RNA - DNA and included the reverse transcriptase -

AB: To be honest, I haven't been exposed that much to people who say the central dogma is not correct, but I would find it fascinating if you could find a system whereby the amino acid sequence of a protein could revert back into a nucleic acid. I just don't think there is one. Crick's way of thinking is so powerful because he also focuses on the key point. He criticized holists for believing that a steam engine could not be fully explained by the properties of water and metal etc. And to be honest, Waddington, whose diagram of the ball rolling down a hill is in every epigenetics talk: he was something of a holist. He believed that biological systems are more than the sum of their parts. I am suspicious of claims like that. What exactly is more than the sum of its parts?

UD: That is interesting. First of all, Waddington – he proposed the epigenetic landscape. But it is tied to genes, shaped by -

AB: Yes, exactly! I have a correspondence between Crick and Waddington in which Crick says, "I've been wondering about this epigenetic landscape. In particular, my question is can one think of any model of development to which this diagram would <u>not</u> apply?!" And Waddington gets quite stroppy in response!

UD: Where did you find this correspondence?

AB: There's a forthcoming book by Ben Martynoga about Edinburgh genetics.

You know that Sydney Brenner said that going from the bottom up, you can be certain that the square of two has to be four. But the square root of four could either be plus two or minus two. So it's automatically uncertain when you come downwards. We had Sydney Brenner to kick off the systems biology department here; he was meant to open it. And the first sentence in his talk was, "you don't want to do systems biology." And then he gave that example. He was opening the systems biology department! He's a dyed-in-the-wool reductionist, so that was a beautiful moment.

Brenner was here a couple of years ago, but now I hear he's in Singapore and doesn't travel any more. He came on the train and we had to help him off with his oxygen supply because he has respiratory issues. And I thought, "Blimey, can he give this talk?" But he gave a talk with no slides, brought the house down, and

then he went for dinner and at 11:30 we had to urge him out of the restaurant still talking entertainingly! A failing body but the brain still very much there.

UD: Eric Davidson was in a similar state before he died. A failing body but a brilliant and sharp mind.

The role of "big data"

UD: Can we briefly talk about "big data" and biology? To what extent are you using what is called "big data" techniques, for example, in the discovery of Rett syndrome and its reversal?

AB: I would hesitate to call it “big data,” though it is quite data intensive. We do whole genome sequencing because it’s just a technology is useful. You can sequence all of the RNA in a cell very deeply; we do that. You need statistics and bioinformatics to interpret it; we do that. But I don’t think big data has impacted – well, I suppose it has impacted what we do in the sense that we now have to use computational methods to answer questions. But we use them just like any other technique.

UD: The way you use it, it’s just, as you said, another method and maybe a powerful one, but it is still part of a Popperian science of hypothesis-testing through experiments. But there are people who say that we don’t need that anymore; the data speak for themselves.

AB: I think that’s an insidious claim – it's justified as “hypothesis free” and therefore pure and not biased in any way. But if you look at what you learn, there is only a list at the end of it. And lists are not understanding. If you’re going to get understanding, you have to actually challenge in some way. So to me, the best way of viewing big data is as “hypothesis generating”. It only acquires true value once you’ve tested the hypotheses that it has generated. There is a danger that big data is seen as intrinsically good even if you don’t test any hypotheses.

UD: Correlation is enough.

AB: It could of course be that this is merely self-justification for a historical phase when we’re getting loads of information that in the future will turn out to be useful. When I was training, people were doing Cot curves. You probably don’t remember what they were. You couldn’t sequence DNA, so you used to denature and then allow the strands to come together. Eric Davidson was a major exponent of this - Britten and Davidson.

It was not terribly productive, and led to some interesting theories of development that turned out to be wrong. But the technology that arose as a result, re-association of nucleic acids, underpins hugely important technologies – e.g. PCR and, these days, CRISPR. So it was valuable, but not for the reason that people were doing it for at the time. It could be that big data is going to generate huge amounts of information that will turn out to be useful one day. I think the problem now is that, as Brenner said, we are "drowning in data but thirsting for knowledge." It's because no one has any idea of why biology is as complicated as it is. There are no theories powerful enough to explain why living things are the way they are.

And that comes down to that bureaucracy thing. I have some thoughts about that, but you probably don't want to hear them!

Selfish genes and biological complexity

UD: I would like to hear your thoughts.

AB: I am interested in the idea that there are more selfish genes than we currently appreciate. The selfish gene came on the scene with Dawkins and it was considered as heresy by many people, oversimplification. It was welcomed by some, Crick amongst them. But now it's been tamed by saying transposons are selfish and repetitive sequences and various other phenomena – imprinting and the T-complex. They are, if you like, the criminals in the genome. Everybody else is a team player working together for the one true goal, which is reproductive success of the species.

Imagine that that's <u>not</u> the case and actually genes are almost like humans (taking the criminal analogy probably unjustifiably far), even law-abiding citizens are motivated to a great extent by self-interest. So suppose there is a component of varying strength of self-interest in genes. Genes are basically scanning, through random mutation of course, for niches that allow them to stick around. The simplest example is the toxin/anti-toxin scenario. There are enzymes called editases that edit RNA. And there is also an essential gene that produces an RNA that's got a mistake in it. Without the editase you can't correct that mistake, and so you die. As a result, the species is stuck with this mutation and the antidote to this toxic change. There's another example of a gene in C. elegans that was thought to be essential for pharyngeal development but actually turned out to code for a detoxifying enzyme for another toxic gene. You remove both of them and everything is fine.

I'm just interested in the idea that genomes are just a bit like ecosystems. Genes are opportunistically searching for a way of operating within the genome so that they get to persist in succeeding generations.

For example, DNA methyltransferase is a candidate. It's essential in mammals, but does different things in other organisms. It's obviously a defence mechanism in bacteria; that probably is its primordial function. Some organisms have managed to get rid of it and in others it's acquired client proteins, like MeCP2 which reads DNA methylation. You can either view them as being life enhancing for the organisms that have them, or you could view them as having found a gambit that allows them to get into the system so that you can't get rid of them anymore. Crucially, the resulting complexity can accidentally become creative. Because complexity is intrinsically creative – that's the argument.

It's devoid of any quantification, and I'm sure the population geneticists will hate it. Brian Charlesworth is working across there, who is a rigorous quantitative evolutionary biologist. I have written some words down and one day I'm going to show it to him and you'll probably never hear of it again!

UD: I would like to hear if or when you have it published.

AB: The attraction of it is that it can explain biological complexity. Why would you make things more complicated than they need to be? The answer is that organisms are sort of over-engineered. An article by Doolittle in *Science* a few years ago discusses the possibility that organisms are over-engineered, but he doesn't use the selfish gene analogy. He does though allow that there is more than one level of selection. What this says is that there is one level of selection on the gene (which is the selfish gene idea really) and there is also selection on the organism, and a bit on the population too.

UD: Your ideas emphasize the role of chance. That is unacceptable to many, also to people in epigenetics. Everything should be purposeful. The environment acts on the organisms and they adapt to it in a purposeful manner.

AB: People don't like chance. I have encountered some autism charities dominated by people who believe that autism is caused by mercury contamination in vaccines and that this has been covered up by the pharmaceutical industry. In other words, it's not chance, its somebody's fault. Actually, most autism is caused by new mutations that arise in the germ line, but not everybody likes those sorts of accidental explanations for things.

UD: This is in part an explanation for the new age developments not only outside but also inside science.

AB: I know. Scientists are a cross-section of humanity. There are many that are extremely good, extremely rigorous, and extremely careful. And there are others.

UD: Historians of science have shown that there was a strong irrational holistic movement in biology at the beginning of the 20th century.

AB: Its disappointing that these things go in waves; if you see off one wave there's always another one coming in the future!

UD: In contrast, the era of molecular biology has really been a rigorous period in biology.

AB: I have to say, I got into science on the assumption that rationality and evidence-based judgement would take over the world because it was so obviously the best that humans could manage. But if you look at the world now, it clearly isn't the way things went.

UD: Thank you so much for sharing your thoughts and critical reflections with me.