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CRF Prize Lecture

Professor Joan Steitz
Yale University

24 and 26 May 2004

PRE-mRNA SPLICING: THE TIE THAT BINDS

"The title of my talk emphasises that pre-mRNA splicing is in fact a very central process in the pathway of gene expression.

Let me go on to the next slide. I would like to start with a diagram that I have taken from a review article that was written by Tom Maniatis and Robin Reed several years ago. What it shows is all the molecular connections that had been uncovered at the time between splicing and other steps in gene expression. You see that splicing not only is connected to other steps in RNA processing like capping and polyadenylation, but it also talks to the various stages of transcription.

We have known for a long time that splicing is necessary for export of the messenger RNA to the cytoplasm. That makes sense because, of course, you need to remove the introns before you send the mRNA out to be translated by the ribosome. A relatively recent realisation is that proteins that associate with the messenger RNA during the process or after the process of splicing are also very important for things that happen to it once it gets to the cytoplasm. Specifically, they play a role in surveying the message to see if it has any mistakes in it, and they appear to play a role in localising messages in particular parts of the cell for their translation. Just recently we have realised that even the efficiency of translation is linked somehow to the fact that introns have been removed from the messenger RNA in the nucleus. So since all of these things involve RNA-protein interactions, what we are really thinking about is interactions in the remodelling of RNA-protein interactions in a dynamic way, as we proceed through the pathway of gene expression.

I would like to start out with a couple of slides that illustrate the diversity of RNPs in the universe. Here is an RNP. These slides were taken in India a number of years ago, and this RNP is clearly very large and very utilitarian; you can think of it as being like a ribosome. There we also saw other RNPs. This one is smaller, much more exotic, but clearly the same sort of functional theme. We also saw small RNPs but more modern-looking and streamlined. In fact, this one dates from about the time that molecular biologists began to appreciate that there were a lot of different kinds of small RNPs in cells doing various things in gene expression.

My talk today is going to be divided into four parts. I would like to start out by indulging in history, and going back about 25 years to the time when we stumbled across the fact that there were these things called snRNPs (small nuclear ribonucleoproteins) in cells and that they were involved in the process of splicing. As you will see when I tell you the story, it is a very typical story in science in that there is a lot of serendipity involved. But it is also an atypical story in biomedical science in that it is not at all a bench to bedside story. It is more a bedside to bench story. It is a case, an unusual case, where tools provided by clinical medicine, namely autoantibodies from lupus patients, provided a way of beginning to dissect what was going on in the basic biology of higher cells.

After I talk about that, I want to go on and mention briefly two surprises connected with mRNA splicing that have happened subsequent to making the connection between snRNPs and splicing. At the end, I will tell you a current story from the lab about investigations that illustrate the previous slide, where you saw the many connections between splicing and other steps in gene expression.

Let me begin by saying a bit more about lupus. Systemic lupus erythematosus is perhaps the best known of a group of diseases that could be categorised as rheumatic diseases or autoimmune diseases. Other diseases in this group are things like scleroderma, mixed connective tissue disease, dermatomyositis, polymyositis, and some kinds of rheumatism. These diseases are not uncommon in the US. They afflict about one in a thousand people, and I think the numbers are pretty much the same in the UK. They are more common in women than men, and they are more common in blacks than in whites.

What all these diseases have in common is that in the sera of patients are circulating autoantibodies, antibodies against one's own cellular components. To start out here very simply, everybody knows that the immune system makes protein molecules called antibodies, which are designed to defend us against foreign invaders like bacteria, viruses and sometimes cancer cells—which can have on their surfaces things that appear foreign.

Our immune systems learn very early in life, and this is currently being worked out, how to discriminate between self and foreign. Sometimes this discrimination goes awry and people begin to make antibodies against their own cellular components, autoantibodies. If you have autoantibodies, the problems, the diseases, the pathogenesis are not caused by the autoantibodies getting into cells in high enough numbers to interfere with the normal functioning of their cellular targets, but rather because cells are lysing and dumping their contents into the blood stream. What then form are immune complexes; these build up and cause various problems. For instance, when they lodge in the fine capillaries they cause the red rash from which lupus got its name, or they can lodge in your hair follicles and make your hair fall out, or in your joints and give you joint problems. They affect all the internal organs and cause inflammatory responses. So these are really systemic diseases, and they are not nice diseases.

One of the most interesting aspects of autoimmunity is that the cellular components that tend to be targeted, and this isn't understood at all, are components of cells which are very abundant and very highly con-

served, namely the components which are involved in the central dogma of molecular biology. What we are looking at here is bacterial DNA being transcribed to make RNA, and ribosomes attaching to translate the RNA into protein. Many lupus patients, the majority, make antibodies against DNA, anti-DNA antibodies. Some make antibodies against ribosomes, and many make antibodies against snRNPs. But snRNPs, small nuclear ribonucleoproteins, aren't in this picture because this is a picture of the central dogma in bacteria, not in higher cells. SnRNPs are specific to higher cells.

At this point I need to go back, to tell you about snRNPs, to the fact that as a graduate student, as a postdoc and as a beginning faculty member at Yale, I worked on RNA structure and function, but all in bacteria and its phages. When our first sabbatical leave from Yale was coming up in 1976/77, it was a good time to start thinking about something new. A lot of people at that time were turning their attention from working things out in bacteria to thinking about how they happened in higher cells. Everybody's presumption was that the basic features of gene expression were going to be the same, just more complicated in higher cells. As you all know, this has turned out not to be the case.

I decided I would like to jump on the bandwagon, but I also didn't want to stray too far from RNA. The problem I decided to investigate is illustrated here. Again we are looking at DNA, this time from a eukaryotic organism. The RNA transcripts are decorated not with ribosomes, because this is all happening in the nucleus and ribosomes are in the cytoplasm, but rather with RNA-binding proteins. The curious fact had emerged that in higher cells there was a huge turnover, a huge wastage, of RNA. A lot of the RNA that was synthesised in the nucleus simply got decayed and maybe only in the order of 10% of it ever made it out to the cytoplasm to become messenger RNA. I thought if the nuclear RNA gets decorated with proteins and a little bit more was known about these proteins, maybe the proteins that bind are deciding which of the RNAs survive and which of the RNAs get decayed. I decided it would be awfully nice to have antibodies against these RNA-binding proteins. We were in the lab of Klaus Weber and Mary Osborn in Göttingen, where they are good at making antibodies. So I spent seven months isolating these proteins, injecting them into various animals trying to get antibodies that could be used as tools to study this phenomenon. But I completely failed because these proteins are very highly conserved and very non-immunogenic. Thus, I ended up doing something else for the rest of my sabbatical.

Of course, 1977 was the year that evidence came together from labs in all different parts of the world that told us that our genes are quite different from the genes in bacteria. Namely, they are interrupted by introns, bits of apparent junk in the genes. In order for expression to occur, the RNA that is made has to get spliced, removing these junk regions. So, that did a lot towards explaining the huge RNA wastage, because the introns are usually much larger than the exons. On the other hand, it raised the question of what could be the cellular machinery that would very precisely remove the introns and join the exons back together so that the message would be able to be read and read absolutely in frame so that it could be translated into proteins.

When I returned to Yale in the fall of 1977, since we were an RNA lab, everybody was very excited about working on splicing. However, I admit we were not too clear about what we should do and where we should go. Then, the first bit of serendipity came in January of 1978, when a new issue of the journal *Nature* arrived, and at the back was an article with an obscure title. I have underlined the salient sentence here that says: "Patients with MCTD have high titres of antibody to nuclear ribonucleoprotein (RNP) which also gives a nuclear speckled pattern on cell substrates in direct immunofluorescence." The reason that this caught my eye is because when I was trying to make antibodies and was failing, several people had said that they had heard of some diseases where patients made antibodies against something that was nuclear and had RNA and protein in it. But at that time I didn't know any clinicians to ask about how I could get such patient antibodies.

When the article arrived, I had a new MD/PHD student in the lab named Michael Lerner. He had just been to all his medical school courses. I asked Michael, "Do you know anybody here at Yale that might have patients with MCTD?" He said, "Sure, I'll go and see Hardin." Hardin turned out to be John Hardin, head of the Rheumatology Section in the Department of Medicine. Michael went across the street and that very afternoon came back with a couple of vials of sera from patients with lupus and other related diseases. We began to work with them.

I want to inject here a rather sobering thought. What we did was possible 25 years ago. But if that happened today, before you can even think about working with human materials, you have to fill out all sorts of forms and go through all sorts of committees. We probably never would have proceeded, but we did at that time.

We started working with the autoantibodies and very quickly found out they were not directed against the large RNPs that I had tried to make antibodies against previously. However, since the antibodies were targeted against something that was small, was very abundant, and was very highly conserved, we decided to keep working. It was very frustrating. For about a year Michael kept trying to fractionate the antigen and it kept disappearing. We later realised that RNase was chewing up the RNA component.

The second piece of serendipity was when Joan Brugge came to Yale to give a seminar and talked about a new reagent called Pansorbin that had just come on the market. It was basically a preparation of *Staphylococcus aureus* cell walls, which has a protein in it called protein A, which binds to the constant region of antibodies. She was using Pansorbin with S35-labelled extracts of virus-infected cells to which there were antibodies to pull out the immune complexes and examine what proteins were there. So what we decided to do, as you see fractionated here, was to label Hela cells with P32, which labels all small RNAs from tRNA size (about 70 nucleotides) up to the size of U2, less than 200 nucleotides, and use the same trick to try to pull out these complexes. In this lane what you see is Michael's own serum; happily he didn't have

any autoantibodies which precipitated any RNA-protein complexes.

But with the various patient sera that we had accumulated, we saw very distinct patterns. When we looked at the RNA molecules, we realised that one was a molecule called U1 and one was a molecule called U2, which had previously been characterised as small nuclear RNAs in the labs of Busch and Weinberg and Penman. There were three other RNAs that we named U4, U5 and U6 in this lane. This is the RNP pattern, precipitating U1 RNA, that was mentioned in the *Nature* article. We knew from the medical literature that Sm was a related and overlapping autoantibody specificity.

You can do the same experiment by labelling cells with S35 to look at the proteins. There was lots of evidence that the antibodies were directed not against the RNAs themselves but against proteins that bound to the RNAs. I am not going to show you the data but instead a cartoon that gives our conclusions. Namely, there were some proteins in common between the particles containing the U1 small nuclear RNA and the U2, and then there were some proteins that were specific to each of these particles. We then called these particles small nuclear ribonucleoproteins, snRNPs, or "snurps" for short.

If you possess antibodies against something and you want to try to figure out what its function might be, there are several things you can do. We immediately realised that the sequence at the five prime end of the U1 RNA was complementary to the five prime ends of introns, whose sequences were beginning to be determined. So we suspected that perhaps that at least this particle might be involved in splicing. You can also use antibodies to localise things in cells. Here we see an autoantibody against ribosomes, showing that they occupy the cytoplasm and the nucleolus, which is the locus of ribosome biogenesis in cells. With the anti-RNP serum directed against the U1 particle, you see the converse pattern. It tells you that the U1 particles are in the nucleoplasm, which, of course, is where the chromatin is and where pre-mRNA is being made and spliced, but not in the cytoplasm nor in the nucleoli.

We also did a number of experiments where we put pre-mRNA substrates into an extract and allowed snRNPs to bind and asked by protection methods where did they bind. Were able to deduce that U1 indeed bound to five prime splice sites and U2 bound to what was at that time becoming recognised as the branch site, a very important locus (I'll get to that in a moment) for the splicing reaction.

One of the nice things we were able to rationalise even just at the time that *in vitro* splicing systems became available, was that the minimum size of introns was dictated purely by putting the different snRNPs on the pre-messenger RNA. This slide shows how much of an intron is protected from digestion by the binding of each of the snRNPs. If you add them up it comes to about 65 nucleotides, which is in fact the smallest size of our introns. Making an intron even smaller means you can't get all the snRNPs on. So, obviously, you can't splice out an intron that is smaller.

What I have told you so far is that gene expression in higher cells, in contrast to bacteria, involves a whole other class of RNAs, the so-called snRNAs. They are part of the snRNPs that assemble to make up the spliceosomes. They are, of course, necessary for the exact removal of introns from the pre-messenger RNA before you send the message out to the cytoplasm to be translated.

The next slide is a picture of the people in my lab at that time. This is Michael Lerner, whom I particularly wanted to point out. On the day this picture was taken, we were being visited by my colleague Sid Altman of catalytic RNA fame. That was because for a while we thought maybe the U2 snRNP might be his RNase P activity in higher cells. We had no idea initially what U2 was doing. It later turned out, of course, to be involved in splicing.

So let me flash forward a bit. Today we have beautiful pictures of the spliceosome in action. This is from Ann Beyer's lab in Virginia. We see here *Drosophila* chromatin being transcribed into RNA molecules and particles building up at the five prime and three prime splice sites. Here is an assembled spliceosome with the intron looped out. Thus, what one pictures from test tube experiments is in fact happening when visualised in the electron microscope.

Work from many different labs achieved *in vitro* splicing reactions, from which we put together a picture of what is happening during the spliceosome cycle. It starts with recognition of the three prime splice site by protein factors. There are probably on the order of 100 different protein factors involved in splicing, in addition to the proteins which are already tightly bound to the snRNAs. After the U1 and U2 particles bind, the U4/U5/U6 snRNP joins. It is a tri-snRNP. The next step is the nucleophilic attack of the two prime hydroxyl of the branch-site A residue on the five prime splice site to form the lariat intermediate. Then, in the second step, the two exons are ligated, and the intron is degraded and the snRNPs recycled.

I want to point out that by the time of the first step, already the U1 and U4 snRNPs are less tightly associated. Thus, the focus is on the U2,U5 and U6 particles as perhaps being part of the catalytic machinery of the spliceosome. Also, I want to emphasise that ATP hydrolysis is needed for both steps of splicing. This is not because the phosphates enter either the intermediates or the products, but because energy is needed for all the dynamic changes that take place during the process of splicing and probably also for the fidelity of the splicing process.

What is going on catalytically hasn't been proven, but everyone in the field suspects that a spliceosome conducts RNA catalysis assisted by proteins. Part of that comes from looking in a number of different organisms at the structures of the snRNAs that are at the core of the spliceosome. Although the snRNAs can vary in length and in secondary structure, each one has a short region that is almost absolutely conserved from yeast to man. Those regions have been localised as being right where the action is in the spliceosome during either the first or the second step. For instance, I have already mentioned that the five prime end of the intron base pairs with the five prime end of U1 snRNA, which is conserved from yeast to man. There is a region in U2 that base pairs with the branch site, bulging out the branch-site A residue. That is also very highly

conserved. A loop in U5 is also very highly conserved. It has been shown in a number of experiments that it helps align the two exons for the second ligation step of the reaction. Finally, there is a sequence in U6 that is very highly conserved that replaces the five prime end of U1 at the five prime splice site before the catalytic steps occur.

U6 is the most highly conserved snRNA, contributing to the belief that it is part of the catalytic core. U4, which you see paired to U6, is a sort of chaperone that brings U6 into the spliceosome. It is then released and U6 refolds, this time associating with U2.

The remarkable thing about the spliceosome I have described to you is its versatility. It can splice introns of greatly different lengths—huge ones as well as small ones. It somehow manages to find the right splice sites in the pre-mRNA sequence. Also, it can splice pre-mRNAs that have many different exons and introns. It manages to do this in an orderly fashion and also manages at different times of development or in different states of differentiation to combine the exons in different ways to give rise to alternative splicing. This is one of the great current challenges in splicing—understanding how the spliceosome, under some conditions, ignores the existence of an exon and splices in a different way to give rise to a different protein isoform.

Given this versatility, it turned out that it was a huge surprise—at least to me—that our cells and the cells of most other higher eukaryotes contain a second spliceosome. The second splicosome is in much lower abundance. It is absolutely necessary to remove a subset of our introns. I want to tell you a little bit about it at this point.

The evidence that there might be another spliceosome first began to emerge in the early 1990s when databases were accumulating to the extent that people could line up the boundaries between exons and introns and look at the consensus sequences. What was realised that there were a few genes that instead of starting with the nearly invariant GU and ending with AG, like most introns do, appeared to start with AU and end with AC. At the DNA level this is ATAC, and so they were called ATAC introns for short. I list the first ones to be recognised here, and you can see that there is no particular theme holding their genes together. There would be just one of these introns in a gene; not very many of them, just a few. The ATAC intron wouldn't be either the first intron or the last intron, or the biggest intron or the smallest intron. Just one of the introns would have these peculiar consensus sequences.

It was Rick Padgett at the Cleveland Clinic who first pointed out that low abundance snRNPs of the same class as the splicing snRNPs might be involved. These had been discovered by a graduate student in my lab, Karen Montzka Wassarman, several years prior. She had called them U11 and U12, which turned out to be prescient because U11 is the U1 analogue and U12 is the U2 analogue. She could have named them the other way around, in which case it would have been hopelessly confusing. Padgett pointed out that like U1 base pairs to a five prime splice site and U2 base pairs to the branch site, there are sequences in U11 that could potentially base pair with the different ATAC five prime consensus sequence and in U12 that could potentially pair with the different branch point sequence.

After that, a very talented postdoc in my lab, Woan-Yuh Tarn, managed to put together an *in vitro* splicing system that would in fact splice an intron containing the minor intron consensus sequences. She was able to show that U12 associates with the branch point by several means and that U11 is also part of the second spliceosome.

At first our thinking was that since U6 was believed to be part of the catalytic machinery, and U4/5/6 were sort of a core particle, maybe you just used U11 and U12 to recognise the intron ends and then pulled in the core machinery. When Woan-Yuh tried to look for U4, U5 and U6 in the second spliceosome, she didn't find U4 or U6. It turned out that in fact there are low abundance counterparts. Here again we are talking about 1/100th of the amount. Two additional RNAs called U4atac and U6atac again can base pair with each other. U4atac brings U6atac into the spliceosome, thus contributing a corresponding function to the second spliceosome.

One of the very interesting things about U6atac from human cells is that its sequence is more divergent from the regular U6 than the yeast and human U6s are from each other. This is quite divergent, but you can draw the same sorts of structures. What then turned out to be the case was that one snRNP is in fact utilised by both spliceosomes. That is the U5 snRNP, but somehow the minor class introns are recognised by four distinct snRNPs. Many of the protein components of the two spliceosomes turn out to be the same. This has been established in Reinhard Luhrmann's lab. It is still a mystery as to why the two systems don't get mixed up, with the minor spliceosome on the order of 1/100th the abundance of the major class snRNPs.

One of the most pleasing things about the minor spliceosome has been what it tells us about models for the catalytic core of the spliceosome. Here you see a picture, derived mostly from work in Christine Guthrie's lab—genetic suppression experiments—suggesting that U6 and U2 come together to form an elbow-like structure. Nearby are sequences that base pair with the five prime splice site and with the branch site. In this way, the branch-site A residue is juxtaposed for attack on the five prime splice site during the first step of splicing. Although only some of the sequences are conserved, the fact that you can draw the same types of structures lends credence to the idea that the model might be more or less correct.

We now know that minor class splicing is not determined by the terminal intron di-nucleotides. More often they are GU and AG, just like major class introns. Instead, it is the longer sequences at the five prime end and the branch site that are determining. We also know that these introns and the second spliceosome must be at least a billion years old because they are found both in higher plants and in us. About one in three hundred of our introns are of this second sort. There are some current eukaryotic species, the yeasts and worms, that don't have these introns. The phylogentic tree suggests that they might have had them at one point, and then lost them over evolutionary time.

So the biggest question (I just want to leave you with this about the second spliceosome) is where did it come from. Here Phil Sharp and his colleagues have proposed an idea that I actually quite like: maybe there was a progenitor spliceosome that then diverged in separate lineages into what we now know as the major spliceosome and the minor spliceosome. At some later point the two came together, fused and mixed. Because the requirements for the minor spliceosome are more definitive, what has been happening since was that we have converted many minor class introns into major class introns to be used by a spliceosome that is more flexible. That then could acccount for what we see in current-day genomes.

Next you must ask, why do minor class introns continue to exist with their corresponding spliceosome. What we do know, because of experiments done by Abhi Patel and an undergraduate in the lab, is that these introns are spliced more slowly. You can increase protein production by exchanging the splice-site sequences and thereby switching the spliceosome the cell uses. Therefore, potentially minor class introns could be regulatory for the splicing process and for the pre-mRNAs in which they occur. We don't yet know.

Let me go on at this point and talk very briefly about small nucleolar RNPs, or snoRNPs. This will be an introduction to the last little story I want to tell you about the connections between splicing and other steps in gene expression. We need to go on an excursion to the nucleolus. What you see here is just a part of the nucleus. The nucleolus is not membrane bound but rather where the repeated genes encoding ribosomal RNA are collected together and transcribed by RNA polymerase one into precursor rRNA molecules. Processing then happens in the fibrillar component, and in this larger granular component of nucleolus, the ribosomal proteins that have been made in the cytoplasm come in and assemble together with the newly synthesised ribosomal RNAs. The subunits then go back out to the cytoplasm to make proteins.

One way of looking at the nucleolus is by where it's not. That is what you see here: again we are looking at where the splicing snRNPs are and you see them in the nucleoplasm and also the Cajal bodies that Angus Lamond has done so much lovely work on. They are not in the nucleoli. The same cells are here stained with anti-fibrillarin antibodies. Fibrillarin is an abundant protein component of the fibrillar parts of the nucleolus. As you shall see in a moment, fibrillarin turns out to be a nucleolar (sno)RNP protein. Thus, you see the nucleoli and also slightly the Cajal bodies.

The main business in the nucleolus, as I have already mentioned, is to make a pre-ribosomal RNA. About half of the sequences in the pre-rRNA get thrown away in the process of releasing the mature RNAs, which are found in ribosomes. One of the things that happens very early to the pre-rRNA is that a number of nucleotide modifications are introduced. As you see here, they are introduced not into the spacer regions that get thrown away, but only into the parts of the pre-rRNA that are going to become mature molecules.

As indicated, there are two sorts of modifications: two prime O-methyl groups and pseudo U groups. You'll see the latter in just a moment. It turns out that for each and every one of these modifictions, the position is guided by a small RNA—a small nucleolar RNA—in the form of an RNP. Because there are so many of these, there is in fact a huge machinery in our nucleoli that is designed to put in these rRNA modifications. We are still struggling with what the modifications actually do for the ribosome, but the machinery is large.

There are two classes of small nucleolar RNAs (snoRNAs) and small RNPs (snoRNPs): one for introducing two prime O-methyl groups and one for the pseudo U groups. I am going to be telling you a bit more about the former class. These small RNAs have conserved sequence boxes called C and D, and internal copies that are similar called C prime and D prime. What happens is that the ribosomal RNA can base pair, usually perfectly, for 10–20 nucleotides upstream of either Box D or Box D prime. If you count 5 base pairs along this helix, that is where the methyl group is introduced into the ribosomal RNA. The two prime O-methylase turns out to be fibrillarin, which I already mentioned. It is an autoantigen that is often the target of autoantibodies in scleroderma patients.

The most remarkable thing, however, about snoRNAs is how they are encoded in our genomes. It turns out that they are not in their own transcription units. Rather, they are encoded within introns, usually of protein-coding genes, as you see here. Instead of introns being all junk, there are some fragments of introns that are in fact released, go to the nucleolus with bound proteins and have a second life. They do something in the nucleolus, namely guide the modification of ribosomal RNA. You see here that the intron is spliced out, is de-branched, and exonucleases chew from both ends as the snoRNA assembles together with proteins. At the time this slide was made (it is an old slide), we didn't know what the snoRNA function was. We now know that U15 is one of the RNAs that guides two prime O-methylation of a particular site in the large ribosomal RNA.

Most of the snoRNA genes, as I mentioned, hide in the introns of protein-coding genes. Interestingly, they tend to be genes for ribosomal proteins, as you just saw, for translation factors, or for nucleolar proteins—all things that have something to do with protein synthesis or the biogenesis of the protein synthesis apparatus. That makes sense.

But there are some very unusual genes in our genomes where almost every single intron has a snoRNA in it. When these genes are spliced, the spliced RNA doesn't have a long open-reading frame. It doesn't appear to be translated, but just very rapidly degraded. So these are sort of inside-out genes, where it's the intron pieces that are long lived, and splicing seems only to be a device for releasing the introns so that they can be processed into snoRNAs. As far as we know the spliced exons don't have any function in cells except to turn over.

With that I would like to move to the last part of my talk, where I want to talk about one particular connection between splicing and another step in gene expression that we have been working on recently. That is the assembly of snoRNAs with proteins into snoRNPs. Since the snoRNA sequences reside within introns, one might expect that this assembly process would be coupled to splicing. What I will be telling

you about is the work of Tetsuro Hirose, who was a Human Frontiers-supported postdoc in the lab and recently went back to Japan, and Mei-Di Shu, a technician who worked with him. I will be telling you about the splicing-dependent assembly of intron-encoded Box C/D snoRNPs. There is also a small component of splicing-independent assembly that I shall mention.

First, I need to tell you a little bit more about the protein composition of the particles in the Box C/D snoRNP class. I already mentioned fibrillarin. Cross-linking experiments were done by a graduate student, Niamh Cahill, who introduced 4-thioU residues into each of the U positions in the conserved boxes, which were believed to bind proteins. From her work, we think there are probably two molecules of fibrillarin, which makes sense because there are potentially two sites that guide two prime O-methylation. There are two proteins, Nop56 and Nop58, which are related, but non-interchangeable, that bind to different distinct places. Both Niamh's work and the work of Lara Szewczak, a postdoc who used nucleotide analogue interference mapping to study RNA functional groups required for the assembly of snoRNP particles, came up with evidence that the 15.5 kDa protein is binding to the terminal stem. That conclusion was also reached by several other labs, notably Nick Watkins in Reinhard Luhrmann's lab.

I also need to tell you a little bit about the terminal stem structure, which some of you will know a lot about as it has been worked on in David Lilley's lab. It is believed that the terminal stem forms a new RNA motif, a newly-recognised RNA motif, called a kink turn. We have crystallographic data on what this RNA motif looks like from structures of the ribosomal RNA in the ribosome and also from Luhrmann's lab, where the 15.5kDa protein has been co-crystallised with a piece of the U4 snRNA that forms this structure.

What's seen in these structures are two base-paired regions and then two sheared GA base pairs, which extrude a nucleotide that makes very close contact with the 15.5 kDa protein. Although we don't have structural evidence for the Box C/D snoRNAs, they do have the potential for forming the sheared GA base pairs. Lara's evidence indicates that this particular U residue assumes a very unusual geometry within the structure.

The question that I posed is, when do the snoRNP proteins, and particularly the 15.5 kDa protein, initiate the assembly of the snoRNP particle? Does it occur before the intron is spliced? Does it occur at some particular step during the splicing process? Does it occur after the intron has been released and de-branched? What we did know is that assembly has to occur before the exonucleases go to work because, at least for most of the snoRNP proteins, if they are not present the snoRNA disappears. It just gets completely chewed up.

The first indication that Tetsuro Hirose had that the two processes of assembly and splicing might be mechanistically linked came when he examined about 60 sequences of introns containing snoRNAs from the human genome and plotted where the snoRNA sat within the intron relative to the five prime and three prime splice sites. What you see here is that they very much prefer to sit about 70-80 nucleotides upstream from the three prime splice site. The distance from the five prime splice site is much more variable. This suggested that there was something important in the positioning.

What Tets first did were deletion experiments to look at snoRNA production in transfected cells. He concluded that there was no specific sequence required but that it was the distance between the snoRNA and the branch-point A residue that was critical—not the distance between the snoRNA and the three prime splice site. That made sense in terms of what we know about splicing. If you try moving the snoRNA closer to the branch point, you simply don't see it ever being released and assembled. If you move it farther away, the efficiency of synthesis simply just drops off. On the other hand, the snoRNA doesn't have any effect on the splicing process. Again, all these data indicated some sort of synergy between the splicing process and snoRNP assembly.

To look at this further, Tets set up an *in vitro* system where he could use a splicing substrate with an intron and snoRNA within the intron. He got both the splicing, the snoRNA trimming process and assembly to work in a test tube. Here we see the pre-mRNA and the lariat product, the spliced intron product, and the intermediates— the two-thirds lariat and the excised five prime exon. Here we see that the snoRNA has extended sequences at its three prime end and gets trimmed down to the mature-sized snoRNA. Again the requirements for Boxes C and D and for the correct spacing were all apparent in the *in vitro* system, as in the *in vivo* system.

One of the really nice things about lots of work having been done on *in vitro* splicing is that at this point we know much about the various stages of spliceosome assembly and function. We also have tools with which we can block the process at each of the various stages. As illustrated here, one of the ways of doing this is to use short RNAs, two prime O-methyl oligonucleotides, that interact with a snRNP RNA and block its action at a specific step. For instance, one oligonucleotide blocks the attachment of the U2 snRNP to the branch point. Another blocks the replacement of the U1 snRNP by the U6 snRNP. Yet another blocks the rearrangements that take place when the U2 and the U6 RNAs come together to form the catalytic core. Finally, it turns out that if you replace the AG at the three prime splice site, you block splicing after the first step and before the second step.

The idea then was to block the spliceosome at these various stages and ask when do we see that the snoRNP proteins have assembled. Are they assembling at a particular step? I am going to show you just one piece of data in this slide from such an experiment. What we are looking at here is immunoprecipitation with anti-fibrillarin antibodies. In an unblocked reaction, you can see association of fibrillarin with several different intermediates. But if you block between the first and second step of splicing, you see build-up of the two-thirds lariat intermediate. It is precipitated by anti-fibrillarin, antibodies against one of the snoRNP proteins. If the boxes in the snoRNA sequence are disrupted, you don't see that, so the results correspond to the features you would expect.

Knowing that the two-thirds lariat is in fact already binding snoRNP proteins, you can ask whether something about the architecture of this particular intermediate is important. Or, does the lariat have to be generated during the splicing reaction in order to get the snoRNP proteins assembled? What I am going to show you in my next slide is an experiment where Tets ran a splicing reaction, cut out the two-thirds lariat intermediate, and simply put it back into a splicing reaction. He asked whether it would pick up snoRNP proteins. Here we are looking at a different substrate, so things are running differently and we are using a tagged 15.5 kDa protein to do the immunoprecipitation. What you see is that if you throw the two-thirds lariat intermediate back into the reaction, it is not precipitated by association of the 15.5 kDa protein. However, if the lariat is generated during the course of the reaction, it is. This says something active is happening during the splicing process.

So, the conclusions to this point are that assembly of the snoRNP (and there are lots of data that I haven't shown you) does seem to occur at a particular step in the splicing reaction. If the snoRNA is too close to the branch site, assembly can't occur properly. Thus, you never get the snoRNP assembled and released, again arguing that there is synergy. How does it occur?

One possibility is that the spliceosome serves as a chaperone and helps the formation of the kink turn so that the 15.5 kDa protein can recognise it and seed the assembly of the rest of the snoRNP proteins. Other possibilities are that there are direct protein interactions between the 15.5 kDa protein and something in the spliceosome. There are actually two versions of this possibility. One is that something interacts and actually deposits the 15.5 kDa protein on the snoRNA at this particular stage of spliceosome function. Another possibility is that only at a particular stage of splicing is the intron cleared of non-specific RNA-binding proteins that prevent the snoRNP proteins from getting on. Only at that point do the sequences at the termini become available for the binding of the 15.5 kDa protein.

At the end, I want to quickly mention an alternative mode of snoRNP assembly. If I have convinced you that splicing is necessary for snoRNP assembly, some of you will have noticed in the graph I showed you earlier that there are some snoRNAs which sit very far away from the three prime splice site. So how do they get assembled? What Tets realised when he looked at one of our favourite multi-snoRNA host genes was that the snoRNAs that sit at the optimal distance have short terminal stems. In contrast, for the one that is far away, he could at least draw a longer stem in the vicinity. That also seemed to be the case for other snoRNAs which are located far from the three prime splice site of their host introns. In order to test this hypothesis, what one wants to do is to destabilise the stems. Various mutants were made that progressively destabilise the stem. One also wants to be able to move the snoRNA from its distant position to the optimal position and then ask what happens. Here are some *in vivo* experiments. What you see is that in the distant position, as you lower the stability of the stem, the efficiency of production of the snoRNA drops off. If the snoRNA is instead in the close position, the optimal position, the stability of the stem doesn't matter too much until it gets to be quite unstable. We think there are other reasons why this particular snoRNA is not well expressed.

Finally, the "nail in the coffin" experiment to ask whether this idea is right or not, is to take a snoRNA that is in the optimal position and move it far away. You expect to see its efficiency of production drop, but then by adding a stem you expect to recover it. The final bit of data shows that is in fact what happens. If you move the snoRNA to a distant position, its efficiency of synthesis drops way down, whereas if you include a long stem in the flanking sequences, you can up the efficiency of production to a pretty good level.

What I have told you would suggest that for most snoRNAs that are located at an optimal distance, there really is a mechanistic link between splicing of the host intron and the assembly of the snoRNP. For those that are located far away, having an external stem that perhaps helps the kink turn to form so that the 15.5 kDa protein can bind, enables assembly. A somewhat more colourful version of the story is shown here, with splicing-dependent assembly occurring at the C1 stage of the spliceosome reaction, and independent assembly occurring earlier. I would like to point out that at about the time we found this, there came a beautiful paper from Angus Lamond's lab which talked about the unusual trafficking of the 15.5 kDa protein to the nucleolus. Namely, it goes through the nucleus by transiting through speckles. Getting to the nucleolus moreover was dependent on RNA polymerase two transcription. Of course, this all fits very nicely with the idea that the 15.5 kDa protein is getting on to the snoRNA co-transcriptionally and co-splicing. It then moves from the speckles, where the snoRNP has been mostly assembled, to the nucleolus for the snoRNP to function. I am looking forward to discussing more of this with Angus tomorrow.

To give credit for the data that I have shown you, I again would like to thank Tetsuro Hirose and Mei-Di Shu and Human Frontiers, as well as HHMI and NIH. I would like again to return to the slide I started with, for there are lots more challenges here. There are many more interactions that need to be understood on the molecular level. A lot of fascinating biology is going to come out of such studies. What I find so remarkable is that a process that doesn't even exist in bacteria, the splicing process, has become so central to the whole gene expression pathway in our cells. I will leave you with that idea, and remind you that we started a long time ago with patients who had autoantibodies with various specificities against proteins that interact with small RNAs to form RNPs in cells.

I want to end by saying thank you not just to the people that I mentioned specifically, but to all the wonderful students and postdocs that I have had in the lab over the years. They were the ones that made this story possible. Here are a few of them that turned up at a recent Halloween party. Each one of them is a different snRNP, and you see they are connected with exons. They have five prime caps and three prime polyA tails. The whole story is there. I thank you for your attention."

The Bruce Preller Prize Lecture

Professor Sir Keith O'Nions FRS

Director General of Research Councils, Office of Science and Technology

6 September 2004

The Threat of Terrorism: The Place of Science

Sir Keith O'Nions is one of the most distinguished and eminent geoscientists that the UK has produced. He is internationally recognised as one of the most important figures in geochemistry of the past 30 years, leading and contributing seminal research that has had a major impact on our understanding of the Earth. His research area can be broadly summarised as the development of isotopic windows on Earth and Planetary processes. His innovative research in this field has been at the heart of the major conceptual and technical advances in isotope geochemistry and its application to understanding the origin and evolution of the Earth and evaluating the nature and time scales of processes operating both within and on it.

Sir Keith pioneered the use of the Sm-Nd isotopic system to quantify the rates of formation of the continents and timing and scales of depletion of the mantle. He integrated this with other isotopic systems to provide rigorous constraints on the nature and scales of chemical variability and cycling in the crust-mantle system of the Earth.

More recently, and using novel material and instrumental methods, he has distinguished isotopic reservoirs in the oceans and ocean current systems and so traced the history of key oceanic circulation patterns. With Sir Ron Oxburgh he developed quantitative methods for determining the flux of He from the Earth, applying this not only to assessment of the rates of crust production at mid ocean ridges and recycling at convergent zones but also to evaluating the energy resource potential of hot rocks at depth.

Sir Keith has also made telling contributions to Scottish geology through his long-term research programme on the age and crustal history of the ancient Lewisian gneisses of NW Scotland.

In his role as the Chief Scientific Adviser to the Ministry of Defence from 2000 to 2004, he turned his attention to the science of nuclear warheads. In this role he drove the development of simulations and models that ensure the functional quality of the weapons stockpile without the need to verify them by underground testing - an essential element of the Comprehensive Nuclear Test Ban Treaty.

His role with the MOD and involvement with the science of nuclear detection and weapons monitoring, as well as his deep understanding of isotopic, chemical and physical processes in the Earth, inevitably led to his strong role in the scientific assessment and analysis of the threats of terrorism, the topic of his talk today.

Sir Keith has now moved from the MOD to take up his new and critical position as the Director General of the Research Councils of the UK. In this pre-eminent position with the Office of Science and Technology he will play a major role in guiding, facilitating and promoting high-quality research in the UK.

This citation can provide but a flavour of the scope, breadth and importance of the scientific research produced by Sir Keith O'Nions. Through his many high-level administrative, committee, advisory and chairperson roles he is also one of the most influential and important figures in UK Earth Sciences. The RSE is proud to award Sir Keith O'Nions the Bruce Preller Prize Lectureship in the field of Earth Sciences.

"Over the five years that I've been in defence I've inevitably had to take an interest in areas of science beyond the ones I know - to become a jack of all trades and master of none. The lack of my depth in a number of areas will become exceedingly evident to specialists very quickly in this talk.

Since 9/11, inevitably one has thought about terrorism and the role of science. So I took this as an opportunity to put a few observations together. No answers, but a few observations.

I give you a couple of health warnings at the beginning. It's a subject in which the dividing line between things that are too sensitive to talk about and things that ought to be spoken about is difficult to find. But this is on the light side of the fence I can assure you.

The other thing is any views expressed here should not be construed as UK Government policy or anyone else's policy. They are my personal observations, which are collated for this lecture and for your benefit, if not my own.

Let me say something at the beginning about science in the UK. The first point I want to make is that the UK and many other governments have nowadays a very clear view as to why they support science. Take science in quotes, I mean science, technology and the broader research base - they have very clear outcomes for investing in science and research. And these, quite simply, are economic benefits to the societies and countries in which we live, and public good. And my job at the moment in the Office of Science and Technology, in effect is to work that interface and to make the arguments to Ministers and the Treasury of the economic and public good benefits of science and enable distribution of funds to the scientific community, transparently, following the criteria of scientific excellence and world class research. These are not incompatible objectives. Within that, public good obviously includes national security and that is the area that I am moving towards.

The standing of the UK in science and research in general is world class, by any yardstick that you invent. We are exceedingly fortunate in that regard. We have a very open and receptive research tradition. In many areas we are second only to the United States in pure output of excellent research and we rarely fall below number three in the world in others, so we have a very very strong tradition.

With regard to the threats of terrorism which the events of the last days [Russian school kidnappings - September 2004] have made us all too aware of, I must say it's one of the saddest things that I have ever experienced but I feel, and hope you will concur with me, that a very high quality world class research base is a prerequisite for dealing with the enormously complex problems of security.

To move on then, advances in science and technology are taking place at enormous speed and a much greater proportion of work today takes place in a global context. Because so much of our research is global, research carried out in universities and institutes here in Scotland can be made available and disseminated around the globe very quickly with modern information systems. By the same token, research carried out elsewhere in the world is available here in Scotland to appropriately skilled people. Yet at the same time it does offer terrorists new capabilities in communications in rapid access to information and indeed new weapons.

I will concentrate quite significantly on the potential threat of new weapons, or the weaponisation of technical advances. Noting in rather a sober manner that in the case of the awful events of the recent past in Russia or of 9/11, that the technical advances that have given the greatest impetus or ability to terrorists have probably been communications and information. The ability to communicate - the technology that we use to do very effective work, I think, has given great impetus to this, yet probably we are a little bit more preoccupied with the potential for new weapons. But I will be talking about potential weaponisation of new technology.

The next point is that these rapid advances in science and technology are not so strongly dependent on pure government research as they were. Nor do governments really have the ability to control the dissemination of technology. Rather they are driven by global enterprise and one should say, encouragingly, with great global benefit. But some perceive us to be in an era, living in an era, moving to an era, of much elevated societal risk from the rapidity of technical advance. and are concerned at our possible inability to cope with it in a regulatory sense and mitigate the risk. Sir Martin Rees, the Astronomer Royal has written on this topic.

Inevitably we have to balance the reality of that against the huge good and necessity for these advances.

The terrorist exploits dissimilar values to our own. Employs strategies that we would not contemplate; organisations and capabilities that have the habit of taking us by enormous surprise regardless of how much deep thinking we do; capitalises on perceived weaknesses in our society, our structures and the way we go about business. And gains often massive and disproportionate advantage from that asymmetric approach.

I will be talking quite specifically about what in shorthand is called the CBRN (Chemical Biological Radio Nuclear) threat. The principal reason is that there is really quite a focus on that threat and a much greater awareness of the potential of that threat following 9/11. Albeit that, as I've said, probably advances in communications provide the greatest capability gained so far. We have had, and continue to have, a real focus on the threat of the "weaponisation" if you like of CBRN. And this, coupled with rapid scientific progress in biological and biotechnology areas is sufficient to cause many people to focus on this point.

I'd like to say a little bit about this area in some of the observations that I make, but I don't want give out the message that I necessarily view this as a threat that is vastly greater than other sorts of threats or opportunities that terrorists might seize.

Going back in time, biological attacks and biological terrorism don't appear to be terribly new. Mankind has been pretty dastardly over the years and probably the earliest record of a biological attack took place at the siege of Kaffa (Camp Constantinople) in 1346 when Mongols catapulted remains of plague victims into the city and started a plague epidemic - pretty low-tech.

The current list of perceived CB threats will be well known to you all and includes things that have been used in a threatening manner in the recent past - the chemical agents, mustard gas which has been known for a very long time, Sarin gas, which was produced in the 2nd world war - the substance used in the Tokyo Underground, various others and the biological agents : Anthrax, of course; plague, nothing new there; Smallpox, we've all read about this. And the list is, of course, much longer but these are ones that are relatively easy to talk about. The reason why these appear to be so threatening, not only to the general public and governments, but also to most scientists, is the scientific and technical backdrop - the pace of change - the sheer rate that technical and scientific change is taking place.

In the biological area, mapping of entire genomes, and in particular the human genome, is probably the biggest scientific advance in the last 50 years. Genomes of all sorts of organisms are now extremely well known leading to increasing knowledge, mostly to the good of our health and so on, but admittedly offering opportunities elsewhere.

The technical rate of progress rings alarm bells. Can we cope? Will we be able to regain enough control to keep ahead of those that want to use these advances in a malign way?

So today where are we? Science and Technology is advancing rapidly. Results are available globally with increasing speed - it's a sort of self-sustaining system. The further we go, the easier it is to distribute the information. This is probably most evident in biomedical research where probably also levels of concern are highest. Without question, there is potential for misuse. There is particular concern over weapons of mass destruction - whether they be chemical, biological, or radiological. And this rate does raise certain questions which have yet to be clearly answered.

These are questions of

scientific regulation

- Is it a good thing?
- Is it a bad thing?
- Is it sensible?
- Is it naive? and

accountability

- whether researchers or research institutions or funders or the DGs of research councils, should or shouldn't be accountable in some way for the research that's being supported.

as well as the ethical issues.

These are questions that are still not fully answered, which are I think of importance to the scientific community.

I said I was going to stick around the biological area, not to give it a heightened importance in terms of a threat, because I don't necessarily believe it is, but it is certainly an area where we have a very high level of attention. And it's an area where the excellence of our research in the UK gives us a very good ability to predict, understand and mitigate.

So to the current threat. Where do you start? We have to start with intelligence and answers. Without these - nothing. There's no point putting a vast research effort into some potential pathogen if there's no evidence anybody knows how to make it, use it, or deploy it. So intelligence is where you start, and you will see this is the point of all aspects of terrorism. How do we meet those threats that intelligence today would say are realistic threats now, or within a reasonable timescale?

Let me give you an example of the process involved in the biological detection of pathogens - most of this research has really been done in defence, primarily with the thought of protecting deployed forces, but it's also applied more generally and I think it gives you the flavour for how biological detection of pathogens, both in the urban environment and also in the military environment takes place.

In principle a pathogen could be distributed in an area of conflict or indeed, closer to home, from a ship, plane or truck, to produce an aerosol cloud. These are things that military people have worried about for many decades and this is mostly where this research comes from.

Basically you ask a series of questions - a hierarchy of questions. In essence this means making or obtaining some sample of the atmosphere within a region of interest. In a technical sense the first question is "do the particles have the correct physical characteristics to be biological?". The non-specific question.

After collection of airborne particles, which are then put into an aqueous suspension, it becomes possible to ask the generic question, "are they biological". And then, through laboratory analysis, comes the specific question "is this biological material, x. y or z?". This is important in any environment, not just a military environment. Because it's rather like an earthquake. If somebody can tell us there's going to be an earthquake in Edinburgh next Wednesday we may be prepared to lock up and move. People will change their behaviour if they're confident of the predictions. But you've got to be pretty confident about a prediction of a biological material before you leave your home or shut up your windows, or find the recent booklet from the UK Government.

Using surface plasma resonance spectroscopy in the laboratory, we can identify pathogens linked to specific antibodies and obtain a quantitated estimate of the density.

The challenge in this area is in avoiding false alarms. Again it's like the earthquake. There's no point saying "well we think there's a 10% chance of something nasty". False alarms have got to be very low. We've got to have a very high level of confidence. The aim is obviously to move to unattended, real-time operations - if it takes you three months in the lab to analyse it, it's not terribly useful. And the further challenge is to try and develop technologies for stand-off detection and also for detecting the unknown. At the moment it's easy to say that it could be this, could be that - but what if someone makes something we haven't seen before?

Vaccination is an immensely important area. And the key to all this, as an observer rather than a practitioner, is in the progressive and improved understanding of the immune system overall in the human body - and particularly the role of dendritic cells. They have various functions. But one of them is to latch onto bacteria and other things. 1) they can destroy them and 2) they can, if you like, educate other cells - t cells and b cells - by imposing a memory into the system that this stuff is bad and if you see it, go get it.

And the vaccine, of course, is stimulating the host defences to enable the host to react. But the rate at which vaccination against these horrid things is developing is sort of in concert with the huge progress that's being made in understanding of the immune system. There are people here in the audience that will really be quite expert on these sort of things.

In terms of vaccines and storing vaccines, the challenges are clearly to obtain fewer doses. Those of you who remember anything about the Gulf conflict in 1991 will know that all participating troops had very large numbers of vaccines for this, that, and the other - quite large numbers of doses. Although there's no proof that there were adverse effects, there is still a debate as to whether some of the gulf illnesses were related to that.

So fewer side effects and rapid immunity are requirements. It's not much use if it's going to be six months before we develop immunity. And also simultaneous protection against several pathogens. Progress is very substantial in this area within the UK, to the point that improved plague vaccines, 100 times more effective than previous generations have now been developed.

The challenges here could eventually be in trying to vaccinate against something which is unknown, some engineered species, and whether a vaccine could be developed for that. As we enter the future, some of the concerns individuals have had is the potential for generating a micro organism from scratch, or indeed enhancing the virulence or survivability in the atmosphere of existing organisms. That's really all I wanted to say on that subject, only to highlight that if one

casts one's mind over some of the more recent terrorist activities they have been extraordinary low-technology in terms of weapons, and as I say, more capability has probably been gained from the ability to communicate, plan, acquire information, collate it, and so on.

Scientists face some new challenges as a result of rapid advances and increasing potential for misuse. There has been some debate, mainly in the US over whether there should be some imposed regulation and accountability, be that institutional or individual.

Another question is whether self regulation and awareness of researchers should be the appropriate way forward. You may remember, not too long ago, a joint declaration in both the journals *Science* and *Nature* on the need for awareness at institutional and individual levels of the potential for misuse.

Something we have seen in recent years, is a much greater demand from governments for advice from scientists on a wide range of issues, whether it be foot and mouth, whether it be GM, whether it be BSC, whether it be terrorism itself. Advice that Governments can view as independent and which can inform its policy and so on.

My sense is that that sort of demand will remain very high, and I can say that from personal experience, having been a researcher for most of my life, and one of those people who really felt that my independent view should never be questioned. Once you move into Government circles you really do lose that independence. Governments become very dependent upon societies such as the Royal Society of Edinburgh, the Royal Society of London, the professional societies, for an independent view. I think the demand for this will increase and I think it is much in our interests to engage fully and actively in that. I think that one of the most important things that scientists can do, is to have an expectation during their career, probably at an earlier stage in their career than my generation, to engage, to give up a bit of time to advise on regulation early in the event.

Just to finish up on biology. I think it unreasonable to take a scaremongering approach, but what can we do. I think the key things are :

- intelligence as the key to drive the protection efforts, stay ahead, know more, understand what is going on, and that is where we have a huge advantage in the UK. We have a very literate scientific and technical community.
- focus of research spending, not at the blue skies level, not necessarily at the level I am talking about with research councils, but focus spending into security against these threats. If you believe in what intelligence tells you, go at them and focus research effort there.

Returning to the debate as to whether there should be codes of conduct and accountability in terms of limiting the proliferation of progress in biotechnology. In my judgement, laissez-faire - No. I think we have very responsible research councils, universities and individuals, I think probably some slight reinforcement of that responsibility is important, but I don't think it is possible to prevent the dispersal of these technologies and what to a researcher in a lab may be quite harmless, may be alarming technology ten years down the road. So I think we have to be completely reasonable as to what limitations should be imposed on academic freedom.

I want to look at one last example before finishing off - Missile Defence. This is something which has oddly gone out of the news, I just want to use it as an example of a very determined response to a perceived threat from what might be best described as rogue states, rather than individual terrorists, but there is a fair comparison between serious terrorist organisations and a rogue state.

At the beginning of next month, the system of intercept missiles in California and Alaska comes on stream. I thought I would spend a moment on this, because technically it is really quite mind-boggling.

Missile launch trajectories can be described in three phases. The boost phase, lasting a couple of minutes, a 10-20 minute mid-course phase which takes place outside the atmosphere, and a short landing phase.

Were the US to intercept anything, it would be in the so-called mid-course phase where it is well outside the atmosphere. With both projectiles travelling at about 5KM per second, when these two things collide, or if they collide, with opposing velocity of 10KM a second, the amount of kinetic energy which is disposed of, is absolutely huge and everything is vapourised. That is called the kinetic energy kill-bill - you can see where I did my last year. In technical terms, for the ballistic experts in the audience, it is basically firing your gun or a rifle and someone firing one over there and getting the two bullets to collide and disintegrate.

Japan is also investing and moving in that direction, and we sit there and go "My God, a bit expensive". I mention that because the cost of the US system is the size of our defence budget.

But America is wealthy, feels that this threat is real, demonstrably has the technology to mitigate that threat to a reasonable level, and is prepared to invest very heavily in that. I think in some ways it this is an expression of how seriously the US has been affected and feels affected by these raw terrorist rogue state threats. A nation with enormous technical capacity and belief in what technology can do, scores a very determined response.

Let me sum up. The first concluding statement I would make is the one that I started with.. The key is an excellent science base that can better understand, predict and respond to the broad spectrum of these threats. A very strong scientific and technical base is a prerequisite.

In terms of scientific regulation - could we regulate, should we regulate? In defence and security research there are clear regulations. Elsewhere responsible behaviour is entirely preferable. Participation of active scientists in informing policy and regulation, is increasingly important, and I sense that young people coming along fully understand that and are fully prepared to do that.

What are the risks to society from very rapid technical advances? There is concern that the recognition of risk and regulations cannot keep up with the rapidity of a technical advance, and that this leads to heightened risk with catastrophic outcomes I think it is essential that the UK remains ahead of the curve, and that we

understand better, mitigate and regulate before the event rather than after, but actually in this rapid technical and scientific advance, the benefits, in my view, still massively outweigh the risks and threats that I described earlier.

That is all I have to say.

The Henry Duncan Prize Lecture

Professor Duncan MacMillan,
The Talbot Rice Gallery, The University of Edinburgh

4 October 2004

Scotland and the Origins of Modern Art

In 1990, as part of its Purchase of Rooms Appeal, the Society received a donation from the Trustee Savings Bank (Scotland), from which Council created a Prize Lectureship named after the Reverend Henry Duncan, founder of the first Trustee Savings Bank. It is awarded triennially to a scholar of any nationality for work of international repute in Scottish Studies. Past recipients have included Professor Tom Devine FBA FRSE, University Research Professor in Scottish History & Director, AHRB Centre for Irish & Scottish Studies, University of Aberdeen, and Professor David McCrone FRSE, Professor of Sociology, University of Edinburgh.

Duncan Macmillan, recently retired from a long and distinguished career in the Department of Fine Art, University of Edinburgh, is the pre-eminent authority on the history of Scottish painting from the Renaissance period to the present day. Through his various books and innumerable articles, he has played a central and pioneering role in bringing Scottish painting to a wider audience, not just within Scotland, but in Britain as a whole and internationally. By raising the general awareness of the centrality of painting to the whole of history of Scottish culture, Professor Macmillan has also provided the inspiration for a whole new generation of scholars in the field.

"Cézanne is universally regarded as the father of modern art. So what do we see when we look at such classic images as his Man Smoking a Pipe, or Woman with a Coffee Pot? We see certainty and uncertainty, the monumental and the provisional, all somehow combined. These are images that convince us intuitively of the solidity and grandeur of what we see, yet which we cannot capture intellectually. Indeed in human terms they are enigmatic. The sitters are present, but we cannot reach them. Their image seems permanent, yet somehow it is not fixed, but is part of a world that is in flux; indeed in all Cézanne's mature pictures we seem to see an ongoing process, not a state. Nor can we locate pictures like these as portraits. Their human content is important and they are certainly not still-lifes, but nor do they belong in any of the traditional genres of painting whose disappearance was a major part of the new modernism. Instead, like the still-lifes which were such a large and significant part of Cezanne's output, these pictures seem somehow to represent an epitome of the complex and elusive phenomena that make up our visual comprehension of the world.

So how did Cézanne get to this point? What was he trying to say to us? I cannot claim to give a whole answer, but I do believe that hitherto people have looked for it in the wrong place. I don't mean simply that they should have been looking in Scotland and have not done so, though Scotland is my main subject today. It is more that the place of modern art in the wider history of western thought has been generally misunderstood and the failure to appreciate the Scottish part of the story has contributed to that misunderstanding. I believe Cézanne is part of the central intellectual and imaginative project that has shaped the modern West; what we have to call the empirical project, the attempt to understand the world around us by investigation and description. This is the intellectual adventure that has given us modern science and technology, but, I will argue, it has also given us modern art. Looked at that way it is clear that this adventure did not begin somewhere in the late nineteenth century, but much further back. In the perspective suggested by the history of empiricism, too, it is beyond argument that the Scottish Enlightenment was a key episode in this story. You cannot fully understand the place of the history of art in this without the Scottish chapter that has hitherto been missing and it is my purpose today to provide that part of the narrative.

So after starting with Cézanne, I will go right back to the beginning and return from there by way of the Enlightenment to that moment at the birth of modernism, a century ago, where I began. This is a drawing of a pelican done from the life by an unknown artist some time around 1620. It is part of a huge collection of some nine thousand similar drawings by many different artists that form the Paper Museum of Cassiano del Pozzo. Cassiano was, like Galileo, a member of the Accademia dei Lincei in Rome. The first great scientific academy of the modern world, its name translates as the Academy of the Lynxes and the lynx is famous for the sharpness of its vision. The academy's name therefore stresses the importance of sight, of direct observation as one of the first principles of modern science and at its very beginning. It is in keeping that these drawings, precise visual records, formed the first ever attempt at an encyclopaedia, an empirical description of everything in the known world based on actual observation. It was a moment when the objectives of art and science were indistinguishable and is a witness therefore to the place of art at the heart of the intellectual revolution that was just beginning.

The Paper Museum could never have succeeded, but before that could even have become apparent, something else intervened to change the course of history. Rembrandt painted the *Blinding of Samson* around 1637 as a gift to his patron, the scientifically minded Constantin Huygens. But the picture does in my view represent only its overt subject from the Bible. The importance of sight itself could not be more plainly stated than in this gruesome image and I believe the real subject of this otherwise unexplained gift was the metaphorical (and later also actual) blinding of Galileo,

with his telescope the most sharp-eyed of all the lynxes, when in 1633 he was forced by the Papal Inquisition under threat of torture and imprisonment to retract his view that the earth was not the centre of the solar system.

It was a view that Galileo had ascertained empirically and so when he was forced to retract, Papal authority overruled the new empirical science. The Italian Renaissance ended abruptly and from that time forward the attempt to understand the world empirically fell to the northern Protestant societies which of course included Scotland. Rembrandt was claiming for painting a central place in this succession and artists did remain very much part of this endeavour in Holland especially. You see very clearly with Vermeer's painting called the *Little Street in Delft* how they pursued understanding through description and visual investigation. In Delft, too, Vermeer worked alongside such pioneers of optics as van Leeuwenhoek, inventor of the microscope, and there is a clear analogy between his art and the work of his scientific friends and colleagues.

Nevertheless, if you look more closely at Vermeer's work, and indeed if you consider the story that Tracy Chevalier has woven around his painting of the *Girl with a Pearl Earring*, you realise how he makes clear that all observation, however objective it may seem, is nevertheless inescapably coloured by our psychology. This is even more apparent if you consider Rembrandt's self-portraits. He was as fully conversant as Vermeer with the new science of optics and clearly from such pictures we can see that the painters had already begun to understand something that is central to my whole topic: that quite simply the ambition of objective description on which empirical science is based is deeply and permanently flawed. In his later self-portraits especially, Rembrandt looks at himself and asks, how can I be at once both subject and object? How can I describe objectively what I am inseparably part of? Faced with this paradox, scientists have generally had to ignore the uncomfortable questions it raises in order to maintain the fiction of objectivity on which their discipline depends. It is a variation of the literary idea of suspension of disbelief. From the start artists knew no such constraint. Art could ask such questions. It is there that art and science began to diverge to the point where their common pursuit was no longer recognisable. It does not mean that it does not exist, however, far from it.

Undeterred by such sceptical speculation, in England Locke and Newton formulated more fully than ever before the principles on which empirical knowledge is based. In doing so they helped lay the foundations of the Scottish Enlightenment, but it is also important to remember here how close Scotland and Holland were at this time. It should be no surprise therefore that it was in Scotland that Rembrandt and Vermeer found their first intellectual heirs and while the Scottish philosophers accepted the central principles of empiricism, that all understanding stems and can only stem from experience, examining these principles Hume came to the same conclusion as Rembrandt. He asked the same question: how can we describe a world of which we are part? Or as he himself put it, 'the difficulty is how far we are ourselves the objects of our senses.' He then concludes devastatingly, "it is absurd to imagine we can ever distinguish betwixt ourselves and external objects."

From this he goes on to argue that even when we look within ourselves, we find we are no more than "a bundle of different perceptions which succeed each other with an inconceivable rapidity and are in perpetual flux and movement." If even our own identity is an elusive and uncertain thing, how can we hope to be certain of anything else at all? What Hume did, as George Davie so eloquently puts it, was to uncover, "the scandal of the basic epistemological contradictions that made nonsense of all the high claims about the Age of Reason."

That is a key observation in this whole story. It is here, I believe, that modern art finds its text. It is founded on a paradox and the search for a way to resolve it and thus to succeed in the ambition to describe the world as we experience it. Art and science still have a common goal. It is only that the artists are free to recognise its elusive complexity. The results they have produced naturally reflect this.

The continuing community of purpose is apparent here where we see Hume's sceptical views reflected directly in the wonderful portrait of him painted by his close friend Allan Ramsay, the second one that he painted dating from 1764. In it we already glimpse something of Cézanne's position; how certainty and uncertainty must somehow coexist; and as part of this, the enigma of otherness even when we are dealing with those closest to us. And if Ramsay looks forward to Cézanne, he also looks back quite deliberately to Rembrandt, pointedly making the link for us between Hume and his greatest predecessor. You see that in the lighting of Hume's portrait. You see it even more explicitly in Ramsay's companion painting of Rousseau, painted to hang beside Hume's portrait, either as a commission from Hume, or as a gift to his friend from the artist; it is not clear which. Hume gives conflicting accounts, but the fact that he does describe it at one point as a gift does suggest the intimacy and significance of the commission.

In Rousseau's pose, position and in the way his face is lit against the darkness, Ramsay quotes directly from Rembrandt's *Self-portrait with a Hat*, now in Washington. In doing so, I believe, he was taking this argument even further in the direction I am now following. When Rousseau sat for this portrait in the spring of 1766, he had just started writing his *Confessions*. The book is a great self-portrait, the literary successor to Rembrandt's epic of self-examination and, after Rembrandt, the first modern exploration of the nature of self. The book must have been the subject of conversation when he was sitting for Ramsay who spoke good French. At the very least Ramsay's portrait suggests that he knew what Rousseau was writing, for in his picture the painter deliberately equates Rousseau with Rembrandt and thus, implicitly, his exploration of self, and with it the dilemma of the subject/object division at the heart of empiricism on which Hume was so eloquent, with Rembrandt's own exploration of self and his contemplation of the subject as object.

In portraits like these, in the way that Ramsay paints, lightly, suggestively and never definitely, but coaxing the image out of the shadows, identity is held in place in our perception only by imaginative hints as the

painter seeks a way of describing the uncertainty of our knowledge, indeed of our identity; but Ramsay also suggests, by this very technique of suggestion, how, above all with those around us, we overcome that uncertainty through imagination.

Here Ramsay also parallels directly, and no doubt consciously, one of Hume's key contributions to this debate, the argument that it is the imagination alone which allows us to hold together our fragmented perceptions and turn them into sense. It is here therefore that the term that has become definitive of the nature and purpose of art actually enters the language of art. But the imagination is also more than just a useful tool in making sense of the world. In the philosophy of moral sense as Hume developed it, it is the active agent of our moral natures, the key to the relationships on which society hinges; and Enlightenment thought was above all else social in its frame of reference.

Human nature was Hume's study and it was also Ramsay's and so you can see in his painting how art is still the peer and companion of empirical thought, even at its most penetrating. Ramsay's portrait of Margaret Lindsay is the epitome of this, of the imagination as the link between people, the only thing that can resolve that enigma of otherness, the agent even of love itself, for that is what we see in Margaret Lindsay's face turned towards her husband as he enters, interrupting her arranging flowers. His familiar presence is reflected in her gaze which is open and without any social barrier visible in it. And in Ramsay's later, red chalk drawing of his wife looking down, apparently unaware that he is drawing her, we also see him contemplating something else that I am sure he had discussed with Hume and which I will return to, the fragmentary nature of actual perception: how little we need to see in order to understand what we are seeing; how much we imagine in fact, and how above all this is true in our response to the human face; how vision itself is psychological.

Here Ramsay is not only working alongside Hume, with whom at just this moment he founded the Select Society here in Edinburgh, but also their mutual friend, Adam Smith. Smith extended Hume's interpretation of moral sense to argue that imagination, and, through imagination, sympathy, is the basis of society itself. Gavin Hamilton was a contemporary of Adam Smith at Glasgow University and a fellow pupil of Francis Hutcheson, first champion of the philosophy of moral sense and so of the argument that morality itself is a product not of reason, but of feeling.

The realisation that morality itself is psychological, not rational was the crucial breach in the integrity of the idea that empiricism and thus reason could describe, understand and also explain all phenomena. We should remember that in the essay that set out this argument, *An Inquiry into the Origins of our Ideas of Beauty and Virtue* (1725) Hutcheson already explicitly makes the connection with art.

It is with Gavin Hamilton's six paintings from the Iliad that we see the idea of the role of the imagination expressed for the first time directly in painting. It is implicit in Ramsay's work. For Hamilton and also his followers, however, its presence in a painting was a goal. It was a quality to be cultivated. And in his pursuit of that goal, we encounter another of the definitive ideas of modern art: the primacy of the imagination. But with it also came too another definitive idea: the superiority of the primitive and its appropriateness as a model for artists in search of the kind of imaginative authenticity that was essential to the proper working of our moral sense. It is the its claim to be specially equipped in the pursuit of that vital authenticity which allowed art first to claim the special privilege which it is still granted in the modern world.

Hamilton turned to Homer for inspiration because Homer seemed to be a witness of the earliest history of mankind when human society was young and still unspoiled. He did this in a context shaped not only by Francis Hutcheson, but also by Thomas Blackwell who in his essay on Homer (1735) pioneered the idea of the imaginative and therefore the moral superiority of the primitive, of the original state, long before Rousseau was to do take up the same discussion. It was consistent with the idea of moral sense that Blackwell should see Homer as the recorder of an actual, pre-classical state of mankind when, because the human imagination was not yet cluttered with preconception and prejudice, humanity enjoyed far greater imaginative freedom and transparency than in decadent later times. In consequence, he argued, there was much greater moral clarity. The beginning of history was a time when, as he put it so poetically, "So unaffected and simple were the manners of those times the folds and windings of the human breast lay open to the eye; nor were people ashamed to avow passions and inclinations, which were entirely void of art and design."

Blackwell's revolutionary view of Homer as pre-classical, therefore in the proper sense primitive and that his poetry was in consequence superior and more authentic to anything that came afterwards, is echoed by Adam Ferguson: "The artless song of the savage, the heroic legend of the bard have sometimes a magnificent beauty which no change of language can improve and no refinement of the critic reform." You can also match this sentiment very closely in André Breton's first Surrealist Manifesto, incidentally, in case you think I am imagining continuities that do not exist.

As doyen of the painters in Rome, Gavin Hamilton was also the leader of an international community of artists and both his art and his conversation, for he kept an open studio, were the vehicle for the wider transmission of these Enlightenment ideas. His circle included some who are recognised as the pioneers of modern art and others who do not yet enjoy that recognition. David's painting of the *Oath of the Horatii*, for instance, is universally seen as one of the first icons of modern art, but its model was Gavin Hamilton's painting of the *Death of Lucretia*. In that picture Lucretia is the heroine. Inspired by her self-sacrifice, her men folk turned to overthrow the Tarquins and establish the Roman Republic. Progress, moral progress, not technical progress, but improvement, the amelioration of society and its progression from the state of barbarism dominated by the masculine warrior code, depended on the actions of a woman undertaken in defence of the virtues of love, hospitality and individual dignity; the virtues in short of a world governed by true feeling.

This, when it is put alongside other works by both Hamilton and David which demonstrate his considerable debt to the older painter, gives a quite different meaning to David's picture from the conventional one of heroic masculinity heralding the Revolution; a meaning that was also retrospectively imposed on Hamilton's picture by analogy with what David's was believed to stand for. For years Hamilton's painting was called the *Oath of Brutus*. Art historians demoted Lucretia, the woman, in favour of Brutus, the man, though in fact he is not the protagonist, but only an agent in the action inspired by her.

The next act in the drama in David's picture was the slaughter, first of the rival Curatii, and then of the Horatii's own sister Camilla who is seen collapsed in despair with the other women in the picture. This is not just feminine weakness. It is real terror. Camilla had been rash enough to fall in love outside the clan. David's subject is actually a brutal honour killing. There is no reason to suppose that he did not intend us to see it as barbaric. Indeed a contemporary commented on the cruelty of the subject. David's real point, therefore, is the opposite of the meaning usually given and is once again the superiority of feminine feeling, of intuition, over brutal masculine violence and the need for feeling to prevail if society is to advance out of barbarism.

But in the work of the Scottish painters in Hamilton's circle, the modern idea of the primacy of the imagination and the place of the primitive as its model were even more directly expressed. Alexander Runciman's *Origin of Painting* indeed has the primitive, the original, the first state as its actual subject. Derived from Pliny, though rather fancifully, it tells the story of how the very first painting was created when a girl traced the outline of her lover's shadow on the wall as he slept. The shadow is nature's own drawing, art in its elemental, natural form. But continuing the previous point about sensibility, the first artist is also a woman and her hand is guided by Cupid, by love.

Runciman not only went to Pliny, however. His actual model was what he and his contemporaries perceived as 'primitive art' in exactly the same way as Gauguin, Matisse and Picasso were to regard the art of the Pacific and of Africa nearly a century and a half later. Greek vase painting seemed at the time to be an art contemporary with Homer. This was a mistake we now know, but it was a reasonable one in the state of contemporary understanding and it allowed these vases to be seen as also literally primitive, an artistic witness from the first state of mankind. These artists had access to these vases, or at least to the south Italian form of this art, through the collection formed by Sir William Hamilton in Naples, published with a commentary by a rather doubtful connoisseur called d'Hancarville, in a magnificent series of volumes in the early 1770s. One particular small vase decorated with the head of a girl in profile was identified, with great imaginative freedom and a singular disregard for historical fact, as the work of Debutades, the Corinthian potter to whose daughter Pliny attributes this historic action. Runciman models the strange and distinctive profile of the girl in his painting on that of the girl on this vase.

Ossian, when first published by James McPherson in the 1760s, was also perceived as primitive, a voice speaking to us from the first natural condition of mankind. Ossian also the additional cachet of belonging to the non-classical world and specifically to Scotland's own non-classical past, remembering that the Scots piqued themselves on never having been part of the Roma empire. In a drawing done in Rome in 1771 Runciman represents Ossian and his music as at one with the wind in the trees. Here he is the author of spontaneous, natural poetry. Just as he was described by Hugh Blair as shooting wild and free, as Runciman represents him, Ossian is already the model of the artist as the embodiment of spontaneity and natural freedom, unfettered by rules, a model that has endured to this day. Indeed sometimes now it looks as though that is all we can look for in our artists. Ossian's music is seen as wild and untutored as the waterfall that is associated with him, the Falls of Bran near Blair Castle painted by Runciman's contemporary Charles Steuart. A belvedere still stands over the falls that was designed to capture their natural music as though it was Ossian's song.

This part of the story crosses over to more familiar territory when we look at Runciman's contemporary in Rome and also in Hamilton's circle, David Allan. Allan collaborated with Burns through George Thomson to illustrate the songs that Burns collected and composed. Burns also recognised in Allan's work the qualities of primitive, unspoiled simplicity that he found in these songs. Even more striking, however, in this quest for the defining concerns of modernity, indeed of modernism, is Allan's Preface to his illustrations of the Gentle Shepherd, Allan Ramsay's father's pastoral play, famous for the naturalness with which it was held to record the lives and loves of unspoiled country people. In that text, published in 1788 and dedicated to Gavin Hamilton, not only does Allan claim to have followed Ramsay's example and recorded the actual places and people about whom he wrote, and therefore that his own art is equally naturalistic, but that his own naturalism also mirrors their simplicity, their lack of sophistication, their naivety even. He makes the remarkable claim that his own command of the new technique of aquatint that he used in his illustrations manifestly lacks skill, but that that lack is a virtue. It was not his intention, he says, to produce expensive smooth engravings, but expressive and characteristic designs. In other words he disclaims skill in favour of expression. That is a very modern attitude indeed, the deliberate assumption of untutored naivety.

Thus far we have not yet reached the end of the eighteenth century in this story, but from Hume and the philosophy of moral sense we already have in place some of the key ideas of modern art: the primacy of the imagination, the importance of spontaneity, the disregard of rules, of skill even, the imitation of the primitive and the cultivation of naivety. These are present in the work of these painters and through them are also already being transmitted to their continental contemporaries and most notably as we have seen they are reflected in one of the recognised icons of the early history of modern art, David's *Oath of the Horatii*. They are also seen even more directly, in fact, in the work of one of David's pupils and Ingres's

contemporary, Paul Duqueylar. Not only is Ossian the subject of his enormous picture now in Aix en Provence, but simplicity and even naivety are adopted as a virtue by a painter who actually called himself Primitive. For Duqueylar seems to have been the leader of a shadowy group of David's pupils who called themselves Les Primitifs.

It may seem that in pursuing these ideas I have strayed from the epistemological questions with which I began, but I will now return by a route that I think shows how closely these things are connected. David Allan's *Penny Wedding* is a scene of rustic simplicity. A penny wedding was an exercise in cooperative living. It represents a world where property is held in common and so is not divisive. It is a world recognisably akin to one that Burns often invokes where the poor are happy and carefree and the rich are miserable, weighed down with the cares of possession.

The key is harmony and that is represented in Allan's picture by the dance and the musicians who lead it. The same image appears in an apparently very different guise in Raeburn's wonderful portrait of Neil Gow, the greatest fiddle player of his age. Gow was untutored. Nominally therefore he was like Ossian, a naive, natural musician. Raeburn captures that brilliantly as he conveys to us how Gow is turned inward to find the music within himself, it is literally original, and he externalises it for us and for the dancers. He is represented as alone, but he is social too. He is surely not playing for himself, but is leading the dance. He is an epitome of the artist and I think this is how Raeburn wanted us to see, not just Neil Gow, but also himself. He identifies himself with Gow. Raeburn painted directly and spontaneously. He did not draw or prepare for his portraits. He may have learnt this from Alexander Runciman too, but more than that if we look at the detail of how he painted in his double portrait of Sir John and Lady Clerk of Penicuik for instance, or his tremendous portrait of Lord Newton, we can imagine how he saw an analogy between his direct and vivid brushwork and the bow of Gow's fiddle.

Gow's music was strong and simple, never flashy, so is Raeburn's art. But more than that, Raeburn's approach brings us back to those questions of epistemics and the role in them of intuition, a quality in both Gow's music and Raeburn's painting. Raeburn too is a social artist. He simplifies in order to emulate our actual social vision, the way we read a face intuitively, broadly and without analysis.

After Hume, the key discussion of these questions was in the philosophy of Thomas Reid. Reid was held to have answered Hume's scepticism, his view of the uncertainty of all knowledge, with his philosophy of common sense; with the argument that Hume had missed the point; we do not understand the world of experience intellectually, but intuitively. Intuition is the key and through intuition the external world impacts directly on our senses. There is no intermediary intellectual stage between experience and knowledge. This is how Raeburn describes the faces of his sitters. Thus just as Neil Gow is an intuitive musician, Raeburn is an intuitive painter.

Indeed all along intuition has been implicit in this argument. What we have seen in the work of the artists I have been looking at is a search for a way to liberate the imagination from the intellect in order to operate more intuitively and therefore with a purer moral understanding.

You can now see Hamilton's argument about the role of the feminine as also about the role of intuition and a century and a half later, when Henri Bergson took up this discussion in a way that again had a direct bearing on painting, he described intuition as specifically feminine. Thus Reid joins up the ends here to bring intuition back into the argument about epistemics, the nature of knowledge.

In his new epistemics Reid recognised that knowledge must have a physiological dimension. There must be a direct medium of exchange between the mind and the external world. Thus he changed completely our understanding of the nature of the mind. But what concerns us first of all here is his explanation of perception. In this he is also radical and his radicalism bears directly on painting because painting is the analogy that he constantly uses to explain how he believes we arrive at our perceptions of the world. He provides a vivid account of the subjectivity of vision; how psychological it is; how it is not a mechanical process of transmitters and receivers, but an intuitive process in which we select what we need from sensations; how it is a language of signs; how they are incoherent and meaningless in themselves, but are the raw material from which the mind constructs perceptions. The painter's position in this process of selection, he says, is what sets him or her apart. His business is with the signs, with the incoherent sensations on which perception is based, not what they signify, nor the perceptions themselves. These are the result of our intuitive interpretation of those signs. Reid constantly reiterates this distinction, between the sign and what it signifies. This is how he puts it: "I cannot therefore entertain the hope of being intelligible to readers who have not by... practice acquired the habit of distinguishing the appearance of objects to the eye from the judgement that we form of their colour, distance, magnitude and figure. The only profession in life wherein it is necessary to make that distinction is painting. The painter hath occasion for an abstraction with regard to visible objects somewhat similar to that which we here require; and this indeed is the most difficult part of his art. For it is evident if he could fix the visible appearance of objects without confounding it with the thing signified by that appearance, it would be as easy for him to paint from the life ... as it is to paint from a copy".

Surely Raeburn's art echoes Reid's view of how painting works? There is no question that he was familiar with these ideas. He not only painted Reid's portrait, but he was also a close friend of Reid's principal interpreter, Dugald Stewart. The first volume of Stewart's *Elements of the Human Mind* published in 1804 has perception as its subject. Nor were these ideas abstruse. Philosophy was the dominant discipline, the matter of ordinary conversation. Hume and Dugald Stewart's monuments together dominate Edinburgh still. In the detail of Raeburn's *Lord Newton* you can see how the image is made up of the painter's unmodified record of the raw material of perception. Ideas have no part in it. We reconstruct the meaning from the painter's account of his retinal sensations just as if they were our own.

Not only did Reid dismiss ideas from painting, and they had been its principal justification since the Renaissance, he had by this time also already located it as a wholly psychological phenomenon. Far from resolving the subject/object dilemma, he pushed art firmly towards the subjective, where it has remained ever since. He has made it the sum of two subjectivities, ours and the painter's, and there is no certainty between them. This is already recognisably the modern position.

Pursuing that, let us stay with that idea of subjectivity for a moment. Reid's epistemics have two sides. Perception is the product of the external world acting directly on the mind. Expression is its compliment, the product of the mind acting directly on the body and so becoming apparent in the external world. Expression - facial expression and body language - is also the medium of social exchange. It is one of the principal means by which we understand each other. Society is a psychological construct and its proper working depends on such exchanges.

Charles Bell was Reid's interpreter here and his investigation of the nature of the nervous system was a direct response to the question formulated by Reid and reiterated by Dugald Stewart: that the answer to these epistemological questions must be physiological. Bell provided the physiological answer to this question, but the study of expression was part of the way he reached it. He himself was trained to draw by David Allan and in 1806 he published his *Anatomy of Expression for Artists*. Wilkie shared Bell's interest and indeed contributed to his book. It is a reminder that art and science are still proceeding in close partnership at his point in the Enlightenment.

In the directness of his portrait of Mr and Mrs Chalmers-Bethune and their daughter Isabella, you can see already how psychological Wilkie's painting is, how vividly, through his account of expression, it records his own subjective experience of a situation. Indeed he is visibly present in the gaze of father and daughter; and he did this in 1804 when he was only nineteen. In the little girl Isabella, we see also the innocent eye, the natural untutored critic, intuitive if you will, who clearly warmly admires the artist.

In the same year Wilkie painted *Pitlessie Fair*. It is a rumbustuous picture, but I only want to dwell on one aspect of it, that it is a picture of Wilkie's home village. It is local and autobiographical. Again it locates his art in his own personal experience. What is new about that? Surely that is where we expect painting to find its locus? It was not so before this. Here it owes something to Burns and to Archibald Alison (as did Wordsworth). It is also an idea that Wilkie passed on to his friend Constable who thereafter based his art on his own countryside and his own formative experiences. It represents a crucial step towards the modern position that art is and only can be a matter of personal and inescapably subjective experience. There is no place for generalisation.

These are also ideas that Wilkie shared with Walter Scott and Scott's vision of history itself as personal, subjective and local had a European influence. Scott had a huge reputation in France where his vision of history had enormous appeal in the reconstruction of the country's self-image in the decades after the Napoleonic wars. But if Scott's influence was important in French painting, he could hardly provide a direct model. Wilkie could however and he did. Among French painters he was equally celebrated. His example helped guide them in the vital shift that took place in the late 1820s and thirties away from the primacy of history painting towards an informal art based on a subjective, psychological vision in which, for progressive painters at least, the classical genres broke down. This was discussed explicitly at the time. Amedée Pichot, for instance, dismissed the official tradition of academic art as 'l'art ministeriel', favouring instead an art that was informal and popular in which he explicitly recognised the importance of the example of the Scots painters.

The key picture in this process was Wilkie's painting of the *Chelsea Pensioners reading the Despatch of the Victory of Waterloo*. It was as well known in its time as *Guernica* was in the twentieth century and was so widely imitated that it is hard to see now just how radical it was. The scene is the breaking of the news of Waterloo with the publication of the Gazette Extraordinary on the morning of 22 June. That date is not the date of the battle and so suggests how it is really the subjective dimension of time that is Wilkie's subject, not the apparently objective facts of history. In order to capture the sense of time, Wilkie spent a lot of effort getting the daylight right in his painting. It pins down the moment. The place is specific too. The ultimate measure of time is the sun and the daylight in the picture sets the time of day. History, however grand, takes place under the common light of day. Time does not differentiate. History has no special dispensation. It can claim no privilege.

In the picture neither the time nor the place so carefully represented are in fact those of the actual event. The scene is actually taking place four days after the battle had been fought and won. History is elusive. This great event has no permanent presence. Here in this picture, ostensibly a history painting, history is already remote in time as well as place. It only exists as narrative and here Wilkie gives us a cheeky double take, for of course what he portrays is literally a narrative, a picture of someone reading a story. So much for grand events, this is all that history can ever be, an old man reading a newspaper. There is a similar bit of anti-history in Bonington's *Quentin Durward at Liège*. His most ambitious picture, it was painted when Bonington and Delacroix were sharing a studio. Wilkie's inspiration was behind the picture just as much as Walter Scott's.

History dissolves as it happens into the infinite multiplicity of individual experiences, once again, all those bundles or collections "of different perceptions which succeed each other with an inconceivable rapidity and are in a perpetual flux and movement." And so the specific time and place in Wilkie's picture are extended into the detailed identification of individuals, who are, nevertheless, not great men and women, just ordinary people.

The change in the status of history painting that Wilkie achieved shaped the art of the nineteenth century and this was not only in true in Britain, but also in France. There the reputation of the Chelsea Pensioners began even before it was completed. Géricault saw it when it was still in Wilkie's studio. He praised the picture in

glowing terms, but picked out one particular figure for attention. "I shall mention to you only the one figure that seemed the most perfect to me, and whose pose and expression bring tears to the eye however one might resist. It is the wife of a soldier who, thinking only of her husband, scans the list of the dead with an unquiet, haggard eye ... Your imagination will tell you what her distraught face expresses". What he describes, at the very centre of the painting, is one anonymous woman's anxiety, her personal, individual drama. He was right. This figure above all tells us that the narrative can only ever be a compound of multifarious, subjective individual experiences and they are all ultimately unknowable. The great have no priority over the small. There is no grand design. Wilkie takes history, real, actual unfolding history and then turns history painting on its head by gently, but undeniably feeding into it all the uncertainties and subjectivities that Hume had recognised will ultimately confound any proposal to describe the world objectively.

Géricault was predisposed to admire Wilkie because his own great painting of *The Raft of Medusa* was already directly inspired by Charles Bell. It is in fact a response to a key passage in Bell's *Anatomy of Expression*.

It is only when the enthusiasm of an artist is strong enough to counteract his repugnance to scenes in themselves harsh and unpleasant, when he is careful to seek all occasions of storing his mind with images of human passion and suffering, when he philosophically studies the mind and affections as well as the body and features of man that he can truly deserve the name of a painter. I should otherwise be inclined to class him with those physicians who, being educated to a profession the most interesting, turn aside to grasp emoluments by gaudy accomplishments rather than by the severe and unpleasant prosecution of science.

Like Wilkie, Bell had an enormous reputation in France. His first biographer was Amedée Pichot, Scott's first translator into French, and whom I have already quoted on the subject of 'l'art ministeriel'.

There is also much more evidence than I have time for here of Wilkie's direct impact on Delacroix's art. For the moment it is enough to note that the same passage from Bell, quoted above, also inspired Delacroix in the *Massacre at Chios*. So now two more of the icons of the early history of modern art are located in the exchange between France and Scotland which I have been tracing. But that exchange is reflected even more directly in this picture. It is usually given a special status in this story because, it is held, in it we see for the first time a scientific account of aerial perspective, but that brings us back to Thomas Reid.

In France after the fall of Napoleon, like Scott, Reid played a central part in the imaginative and intellectual reconstruction of the country and was seen as doing so at the time. He was regarded almost with reverence and his philosophy was taught first at the Sorbonne in 1814 by P. P. Royer Collard. This teaching was then continued by Victor Cousin. In 1818 Delacroix wrote in a letter to a friend: 'I should be very glad too if we could once again attend the opening of Cousin's course. Then in his *Journal* in May 1823 he noted: "I decided to paint scenes from the Massacre at Scio. I go to see Cousin tomorrow". Many years later in 1855, remembering this period in his early life and confirming Cousin's importance to him, he wrote: "When I left College, I too wanted to know everything; I thought I was becoming a philosopher with Cousin".

Here is just one example of how close a bearing what Reid wrote had on painting. It is a passage on the subject of aerial perspective, the quality that is held to be so important in Delacroix's painting of the *Massacre at Chios*, the painting that here he himself associates with Cousin's teaching: "In an apple tree which stands at the distance of about twelve feet, covered with flowers, I can perceive the figure and the colour of the leaves and petals; pieces of branches, some larger, others smaller, peeping through the intervals of the leaves - some of them enlightened by the sun's rays, others shaded; and some openings of the sky are perceived through the whole. When I gradually remove from the tree, the appearance, even to colour, changes every minute. First the smaller parts, then the larger, are gradually confounded and mixed. The colours of leaves, petals, branches and sky, are gradually diluted into each other, and the colour of the whole becomes more and more uniform. This change of appearance, corresponding to the several distances, marks the distance more exactly than if the whole object had been of one colour".

So by 1824 to add to the catalogue of elements of modernity that had already appeared in Scotland and thence in France, we can now add the overturning of the academic hierarchy of the genres and the dethroning of history painting to replace it with a personal, intuitive and subjective vision of the world, but also crucially the appearance in French art of a kind of scientific naturalism, a phrase that was closely echoed in contemporary French accounts of Reid's philosophy, described by Royer-Collard as both scientific and naturalistic.

But the story does not end there. Perhaps the most celebrated moment in the emergence of the new, modern painting in France was the exhibition in 1849 of Courbet's great painting, *L'Aprés Midi à Ornans*. It should be no surprise now to find that it is a picture that is intimately linked into this story. It is not simply that Courbet's composition is based directly on Wilkie's *The Cottar's Saturday Night*. But in the picture Courbet makes exactly the same connection as Raeburn had done between the spontaneity and informality of his own painting and the music of the fiddle player. As a painter he even claimed to be untutored and thus himself naive. More than that, like Wilkie, he raises a personal, local (and non-metropolitan) iconography to the level of high art. In doing so he proclaims the primacy of the subjective and intuitive. Indeed, painting *L'Atelier du Peintre*, the largest self-portrait in history, perhaps Courbet is already suggesting that the only answer to the dilemma of trying to find and describe an objective reality is, in spite of Hume, the big 'I am', the naked ego.

Courbet's approach was followed by Manet and the Impressionists, but not, I believe, without a further and unexpected intervention from Scottish epistemics. In 1842 Cousin, as in effect Minister of Education, reformed the Baccalauréat making philosophy compulsory and in doing so laid down a syllabus which had at its heart the writings of Thomas Reid. A student

edition was produced and so, if it was indeed still on the syllabus, when they were at school Monet and Manet and all the rest had to read Reid's discussion of perception. As we have seen, he made it extraordinarily vivid and relevant to painters. I believe this may be part of the reason why there seems to be such a close affinity between Manet and Raeburn for instance. Both also owed much to Velasquez and it may be a coincidence, therefore. Nevertheless it is striking that we also find at just this moment, above all with Monet, the emergence of a scientific approach to the description of perception in painting that does match very closely what we read about the processes of perception in Reid: how it is the painter's business to put down the unmodified sensations, leaving the viewer to reconstruct perceptions from them.

This is not the whole story of course, but it is a part of it which I believe has not been told before and which seems to locate this whole thrilling episode in the history of painting in the much wider story of the history of Western thought and the exploration of the nature of knowledge.

And so that brings us back to Cézanne. It may again be coincidence and it is certainly not simply cause and effect, but I think his painting does also fit into this interpretation of events. He cultivated a rather illiterate image of himself, but I was intrigued to find that at the Lycée in Aix, far from being the backward boy sent to do carpentry and to join the art class because incapable of more literary pursuits, he won all the prizes. He was a scholar and so as philosophy was compulsory, presumably he too may have studied Thomas Reid. It certainly would seem that there is at least an affinity between this kind of description by Reid and what Cézanne actually does in his painting.

The visible appearance of things in my room varies almost every hour according as the day is clear or cloudy, as the sun is in the east, or south or west, and as my eye is in one part of the room or another: but I never think of these variations otherwise than as signs of morning, noon, or night, of a clear or a cloudy sky. A book or a chair has a different appearance to the eye, in every different distance and position of the body of which it's visible or perspective appearance is a sign and an indication.

It is the painter's job to describe and make sense of all this. The rest of us need not trouble our heads with it. But to add into that complex the recognition of the indivisibility of time from all the rest of our subjectivity and perhaps it does become possible to understand Cézanne's intellectual position as shaped, if not directly by Thomas Reid, at least by a far longer debate than conventional art history can offer. His approach is intuitive, informal and direct. The image is held together by the imagination. These are all the things that had entered painting over the last century and a half, or even longer, for it aligns him not only with Hume, but with Rembrandt before him. The reason why he is rightly regarded the father of modern art is perhaps that with him we reach the watershed, the dividing of the streams, or indeed he is a mountain peak high above the watershed. Certainly he looks both ways. He summarises and draws to a conclusion so much that has gone before and in doing so he opens the way to the future.

And so, before I conclude, what happened next? Does the apparently radical departure that we see in the subsequent emergence of Modernism invalidate this whole argument? Initially at least things continue as before, Cubism in its purest form can be seen as a direct response, not only to Cézanne, but also to Bergson's account of time and space. You might also say Bergson himself began with Reid. An early publication was on Common Sense, *Le Bon Sens et les Etudes Classiques*. He also certainly set intuition at the centre of his own philosophy, and indeed the fragmented vision that Picasso offers us could equally easily illustrate Hume's account of the fragmentary nature of perception and of the subjective self at its heart.

But it was Nietzsche who cut the Gordian knot of the empirical paradox. If we can never resolve the question of the indivisibility of subject and object in our attempt to describe experience, then abandon the search and celebrate the purely subjective, the individual will, for its own sake.

It is no longer a matter of the imagination struggling to make sense of the fragments of experience. All that is needed is an act of will. Picasso made this the starting point for a career of nearly inexhaustible creativity: the artist as net creator is remade in a divine image. It was unsustainable, even by Picasso himself, however. His own sexual impotence was the constant a theme of his old age. His art was so personal and subjective, the artistic urge and the sexual urge were conflated.

Nevertheless, in an action that has been more widely imitated than any other in the century since he made it, Duchamp demonstrated the power of Nietzche's idea. Art is an exercise of the will. It is simply what you say it is. No doubt it was intended to mark the end, the abandonment of the ambition to describe experience that had driven the evolution of progressive art for three centuries. In spite of Duchamp you can see the art of the twentieth century as a search for new goals and new agendas. In this search artists were liberated by the new freedom. Nevertheless the old goals were not abruptly abandoned. Surrealism which proved to be one of the most fertile movements of the mid-century took its text from the eighteenth century as I suggested earlier. Indeed if you consider Freud an empirical scientist, Surrealism could be seen as a renewal of an old partnership. It is only in the last few decades that Duchamp's position has become the dominant one, but it is nevertheless ultimately sterile. It is little more than an aphorism. Leading everywhere, it leads nowhere. It offers no goal. But happily if you consider our contemporaries, such as Eduardo Paolozzi or Ian Hamilton Finlay, you see that the attempt to make sense of the world, to find order in it for us, is still their inspiration. Indeed it is their theme. And imagination is still the key, a point that brings the artists back alongside the scientists. More than ever we need them to work together. On his great sculpture outside the Royal Bank of Scotland's offices at the Gyle in Edinburgh, and which appropriately he called *The Wealth of Nations*, Paolozzi has inscribed a quotation from Einstein that is effectively a summary of Hume: "Knowledge is wonderful, but imagination is even better". And so the story goes on."