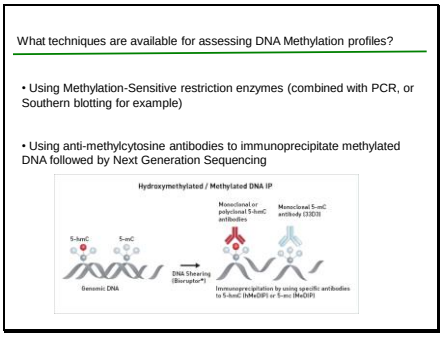
Slide 9



What techniques are available for assessing DNA Methylation profiles?
Various ones, differ in their level of resolution, compared to single base resolution.

Techniques that are valid but not high resolution is Using Methylation-Sensitive restriction enzymes (combined with PCR, or Southern blotting for example). Lots of the restriction enzymes are sensitive to the methylation status of cytosine, wont cut if the cytosine is methylated for example. So you can use techniques- the enzymes linked with techniques such as PCR or southern blotting.

Take genome, cut with particular enzyme that's methylation sensitive, use PCR primers that would flank that site and you're asking if you can amplify that region or not- if its cut you can't amplify, but if its not cut you can amplify. But you're limited to the restriction enzyme site, which is valid but you can't get a whole genome view.

Other techniques: have antibodies that will recognise methyl-cytosine and also the base introduced last week, the hydroxymethylcytosine. Have antibodies that will specifically recognise those modifications.

Take it, shear that randomly into smaller pieces of DNA and use the antibodies to pull down DNA fragments that have those specific marks. So once purified down-immunoprecipitated DNA. If you were interested in looking at single genes you can use it with PCR primers for the gene you're interested in- ask if its there or not.

Or you can use NGS, sequence all the DNA you've pulled down with the antibodies- which regions of the genome have been enriched with the antibodies. This is higher resolution than the first example using enzymes, but its not giving you single base resolution as you wouldn't know from that exactly which cytosine had been methylated and which had not.

You'd just know in that region theres cytosines that have that mark. So although lots of studies use this technique you're not getting single base resolution.

From powerpoint:
Next Generation sequencing: Illumina (or similar) high-throughput methods that are applicable to large and complex genomes such as those found in vertebrates and plants. Can also do standard Sanger sequencing on single (or few) targets.

A lot of these are HP1, they were looking for suppressors of this heterophenotype. HP1 is well studied in lots of organisms.

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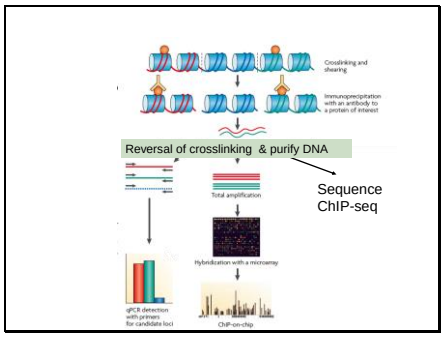

If we wanted to look at those histone marks either on a single region of a genome or over a whole genome, what techniques do we have available? We need to link the histones with a particular region of the genome- requires us to do chromatin immunoprecipitation.

Works really well with histones as they're in intimate contact with DNA. What will happen is we need to crosslink the histone with DNA- formaldehyde, then shear the DNA (to make it possible to do the immunoprecipitation) and we have antibodies against a lot of the histone marks.

We use these antibodies to immunoprecipitate your histone bound to DNA. And then we can use techniques to reverse the cross linking once we've immunoprecipitated. If looking for specific gene- would do quantitative PCR to say is the DNA associated with that particular mark or not. Controls required- not using the antibody or using it against something else.

If you were interested in the whole genome you would do a genome wide sequencing- ChIP seq, which areas of the genome are enriched for a particular mark.

Probably less common is also to take the DNA amplify it and do some hybridisation array so you can get a CHIP with all the regions of genome on it and do a hybridisation. But because sequencing is so cheap these days it's the favoured genome wide method.

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Histone modifications and Epigenetics

- Strong evidence that histone modifications affect gene expression
- Can patterns of histone modifications be maintained through cell division? i.e **are they truly epigenetic in nature?**

Read slide.
Still far from clear/heavily debated.

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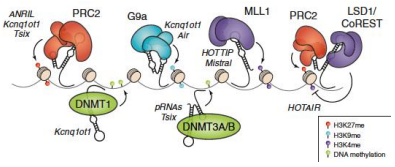
Long non-coding RNAs and directing epigenetic change
• can form extensive secondary structure
• can act as scaffolds for chromatin modifying complexes



Importance of extensive secodnary structure and ability of RNAs to recruit chromatin structure modifying protein.

This secondary structure that can be created: theres examples of different structures being recruited: the DNA methyl transferases that are going to come in and methylate specific residues, examples of repressive marks being put down recruiting repressive complexes

Examples: hot air where it actually recruits two different complexes, one that puts down repressive marks and one that blocks active marks- two pronged approach.

From powerpoint:
It is becoming increasingly apparent that eukaryotes code for many ncRNAs and certainly these long ncRNAs are very often found associated with epigenetic loci. The ability of RNAs to interact with both DNA and proteins and form extensive secondary structure makes them ideal molecules to recruit the enzymes and proteins that will trigger changes in chromatin structure

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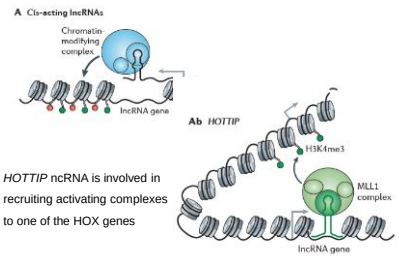
Long ncRNAs can act in cis



HOTTIP ncRNA is involved in recruiting activating complexes to one of the HOX genes

Come back to when will give these examples.

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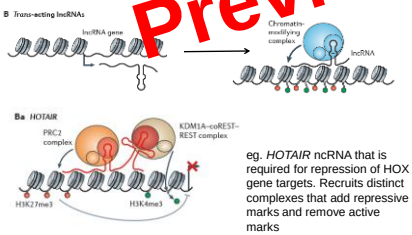
or long ncRNAs can act *in trans*



eg. *HOTAIR* ncRNA that is required for repression of HOX gene targets. Recruits distinct complexes that add repressive marks and remove active marks

We will revisit HOTTIP and HOTAIR ncRNAs in a later lecture

Slide 44

Learning Outcomes: L2

By the end of this lecture students should be able to:

• Provide an overview of the DNA methylation profiles of different organisms
• State what are CpG islands
• Describe current ideas regarding how histone modifications influence chromatin structure
• Discuss whether histone modifications are heritable
• Describe how non-coding RNAs can direct epigenetic change
• Understand the basis of techniques that can be used to assess epigenetic marks of single genes and whole genomes

Need to know when techniques should be applied, how to interpret outputs etc.

Lecture 2: Reading List

• Kouzarides, T. (2007) Chromatin Modifications and their function. Cell 128, 693-705
• Zentner & Henikoff (2013) Regulation of nucleosome dynamics by histone modifications. Nature structural & molecular biology. 20, 259-266
• Fatica & Bozzoni (2014) Long non-coding RNAs: new players in cell differentiation and development. Nature Reviews Genetics. 15, 7-21
• Sabin et al. (2013) Dogma Derailed: The Many influences of RNA on the Genome. Molecular Cell. 49, 783-794
• Deaton & Bird (2011) CpG islands and the regulation of transcription. Genes & Dev. 25, 1010-1022