

A FEW EXAMPLES OF NICE PAPERS IN WHICH BONA FIDE GENES ARE NOT DNA SEQUENCES

Silencing in *Ascobolus* (Rossignol and Faugeron 1994; Colot, Maloisel et al. 1996; Goyon, Barry et al. 1996; Goyon, Rossignol et al. 1996; Meyer 1996; Selker 1999) Colot, V., L. Maloisel, et al. (1996). "Interchromosomal transfer of epigenetic states in *Ascobolus*: transfer of DNA methylation is mechanistically related to homologous recombination." Cell **86**(6): 855-64.

The transfer of methylation between alleles represents a plausible epigenetic mutational mechanism to explain loss of imprinting in mammals and paramutation in plants. Here, we have exploited advantages unique to the fungus *Ascobolus immersus* to obtain direct experimental evidence that methylation transfer can occur between homologous chromosomes. A methylated allele and an unmethylated allele of the *Ascobolus* b2 spore color gene were brought together in individual meiotic cells. Frequent transfer of methylation to the unmethylated allele was observed. This transfer was polarized 5' to 3' along the b2 gene, as is gene conversion, and always accompanied the latter process when tested in the same cross. These and other observations strongly suggest that methylation transfer and recombination are mechanistically related.

Edskes, H. K. and R. B. Wickner (2000). "A protein required for prion generation: [URE3] induction requires the Ras-regulated Mks1 protein." Proc Natl Acad Sci U S A **97**(12): 6625-9.

Infectious proteins (prions) can arise de novo as well as by transmission from another individual. De novo prion generation is believed responsible for most cases of Creutzfeldt-Jakob disease and for initiating the mad cow disease epidemic. However, the cellular components needed for prion generation have not been identified in any system. The [URE3] prion of *Saccharomyces cerevisiae* is an infectious form of Ure2p, apparently a self-propagating amyloid. We now demonstrate a protein required for de novo prion generation. Mks1p negatively regulates Ure2p and is itself negatively regulated by the presence of ammonia and by the Ras-cAMP pathway. We find that in *mks1*Delta strains, de novo generation of the [URE3] prion is blocked, although [URE3] introduced from another strain is expressed and propagates stably. Ras2(Val19) increases cAMP production and also blocks [URE3] generation. These results emphasize the distinction between prion generation and propagation, and they show that cellular regulatory mechanisms can critically affect prion generation.

Fernandez-Bellot, E., E. Guillemet, et al. (2000). "The yeast prion [URE3] can be greatly induced by a functional mutated URE2 allele." Embo J **19**(13): 3215-3222.

The non-Mendelian element [URE3] of yeast is considered to be a prion form of the Ure2 protein. The [URE3] phenotype occurs at a frequency of 10^{-5} in haploid yeast strains, is reversible, and its frequency is increased by overexpressing the URE2 gene. We created a new mutant of the Ure2 protein, called H2p, which results in a 1000-fold increase in the rate of [URE3] occurrence. To date, only the overexpression of various C-terminal truncated mutants of Ure2p gives rise to a comparable level. The h2 allele is, thus, the first characterized URE2 allele that induces prion formation when expressed at a low level. By shuffling mutated and wild-type domains of URE2, we also created the first mutant Ure2 protein that is functional and induces prion formation. We demonstrate that the domains of URE2 function synergistically in cis to induce [URE3] formation, which highlights the importance of intramolecular interactions in Ure2p folding. Additionally, we show using a green fluorescent protein (GFP) fusion protein that the h2 allele exhibits numerous filiform structures that are not generated by the wild-type protein.

Fiering, S., E. Whitelaw, et al. (2000). "To be or not to be active: the stochastic nature of enhancer action." *Bioessays* **22**(4): 381-7.

Transcriptional enhancers are traditionally considered to regulate the rate at which a linked promoter transcribes mRNA, but recent experiments suggest a reevaluation of this model is necessary. Single-cell assays of transgenes reveal that enhancers increase the probability that a reporter gene will be active, but have little or no effect on the transcription rate once a gene has been activated. These results raise the question of how enhancers affect gene expression in their native contexts. A simple interpretation is that enhancers act in a stochastic fashion to increase the probability that a regulated gene will be transcribed; such a model is compatible with programs of cell differentiation in which multiple similar cells subject to similar environmental stimuli do not respond uniformly. Copyright 2000 John Wiley & Sons, Inc.

Garrick, D., S. Fiering, et al. (1998). "Repeat-induced gene silencing in mammals [see comments]." *Nat Genet* **18**(1): 56-9.

In both plants and *Drosophila melanogaster*, expression from a transgenic locus may be silenced when repeated transgene copies are arranged as a concatameric array. This repeat-induced gene silencing is frequently manifested as a decrease in the proportion of cells that express the transgene, resulting in a variegated pattern of expression. There is also some indication that, in transgenic mammals, the number of transgene copies within an array can exert a repressive influence on expression, with several mouse studies reporting a decrease in the level of expression per copy as copy number increases. However, because these studies compare different sites of transgene integration as well as arrays with different numbers of copies, the expression levels observed may be subject to varying position effects as well as the influence of the

multicopy array. Here we describe use of the lox/Cre system of site-specific recombination to generate transgenic mouse lines in which different numbers of a transgene are present at the same chromosomal location, thereby eliminating the contribution of position effects and allowing analysis of the effect of copy number alone on transgene silencing. Reduction in copy number results in a marked increase in expression of the transgene and is accompanied by decreased chromatin compaction and decreased methylation at the transgene locus. These findings establish that the presence of multiple homologous copies of a transgene within a concatameric array can have a repressive effect upon gene expression in mammalian systems.

Goyon, C., C. Barry, et al. (1996). "Methylation of DNA repeats of decreasing sizes in *Ascobolus immersus*." *Mol Cell Biol* **16**(6): 3054-65.

In *Ascobolus immersus*, DNA duplications are subject to the process of methylation induced premeiotically (MIP), which methylates the cytosine residues within the repeats and results in reversible gene silencing. The triggering of MIP requires pairing of the repeats, and its detection requires maintenance of the resulting methylation. MIP of kilobase-size duplications occurs frequently and leads to the methylation of all C residues in the repeats, including those belonging to non-CpG sequences. Using duplications of decreasing sizes, we observed that tandem repeats never escaped MIP when larger than 630 bp and showed a sudden and drastic drop in MIP frequencies when their sizes decreased from 630 to 317 bp. This contrasted with the progressive decrease of MIP frequencies observed with ectopic repeats, in which apparently the search for homology influences the MIP triggering efficiency. The minimal size actually required for a repeat to undergo detectable MIP was found to be close to 300 bp. Genomic sequencing and Southern hybridization analyses using restriction enzymes sensitive to C methylation showed a loss of methylation at non-CpG sites in short DNA segments, methylation being restricted to a limited number of CpG dinucleotides. Our data suggest the existence of two distinct mechanisms underlying methylation maintenance, one responsible for methylation at CpG sites and the other responsible for methylation at non-CpG sites.

Goyon, C., J. L. Rossignol, et al. (1996). "Native DNA repeats and methylation in *Ascobolus*." *Nucleic Acids Res* **24**(17): 3348-56.

We identified two classes of native dispersed DNA repeats in the *Ascobolus* genome. The first class consisted of several kilobase long, methylated repeats. These repeats, named Mars (methylated *Ascobolus* repeated sequences), fell in one family of LINE-like elements and in three families of LTR-containing retrotransposable elements. The methylation features of Mars elements were those expected if they were natural targets for the MIP (methylation induced premeiotically) previously discovered in *Ascobolus*. The second class consisted of short repeats, approximately 100 bp long,

corresponding to 5S rRNA and tRNA genes. As expected from their size, which was too small to allow MIP to occur, they were unmethylated, as were 26 kb of unique sequences tested. These observations are consistent with the hypothesis that MIP is targeted at natural DNA repeats and constitutes a defensive process against the detrimental consequences of the spreading of mobile elements throughout the genome. The 9 kb tandem repeats harbouring the 28S, 18S and 5.8S rRNA genes displayed methylation features suggesting that rDNA methylation proceeds through a process other than MIP.

Grewal, S. I. (2000). "Transcriptional silencing in fission yeast." *J Cell Physiol* **184**(3): 311-318.

In eukaryotes, epigenetic events govern diverse processes, ranging from gene expression to other aspects of global chromosome architecture essential for preserving the integrity of the genome. Transcriptional silencing at the mating-type locus, centromeres, and telomeres of the fission yeast is regulated by epigenetic mechanisms. Epigenetic states are inherited in cis during mitosis and, remarkably, even through meiosis. Several trans-acting genes that affect silencing are found to encode either chromatin proteins such as chromodomain proteins Swi6 and Clr4 or the factors that affect chromatin assembly, including histone deacetylase homologs Clr3 and Clr6. A recent study showed that Swi6 is involved in imprinting at the mating-type locus and contributes to the cellular memory responsible for maintenance of the silenced state. The "gene" in this instance thus comprises DNA plus the associated Swi6-containing protein complex. Copyright 2000 Wiley-Liss, Inc.

Kearns, M., J. Preis, et al. (2000). "Complex patterns of inheritance of an imprinted murine transgene suggest incomplete germline erasure." *Nucleic Acids Res* **28**(17): 3301-3309.

Here we report a transgenic mouse line that exhibits significant deviations from a classic pattern of parental imprinting. When the transgene is passed through the female germline, it is completely silenced in some offspring while in others expression is reduced. This variable expressivity does not appear to be the result of differences in the presence of unlinked modifiers. Female transmission of the transgene is associated with hypermethylation. The transgene is generally reactivated on passage through the male germline. Extended pedigrees reveal complex patterns of inheritance of the phenotype. The most likely explanation for this result is that the imprint is not completely erased and reset when passed through the germline of either sex. FISH analysis reveals that the transgene has integrated into chromosome 3 band E3, a region not known to carry imprinted genes, and the integration site shows no sign of allele-specific differential methylation. These findings, in conjunction with other recent work, raise the possibility that the introduction of foreign DNA into the mammalian genome, either through

retrotransposition or transgenesis, may be associated with parental imprinting that is not always erased and reset during meiosis.

Klar, A. J., A. V. Ivanova, et al. (1998). "Multiple epigenetic events regulate mating-type switching of fission yeast." *Novartis Found Symp* **214**: 87-99.

Two epigenetic events at *mat1*, one of which is DNA strand specific, are required to initiate recombination during mating-type switching. The third, a chromosomally borne imprinted event at the *mat2/3* interval regulates silencing and directionality of switching, and prohibits interchromosomal recombination. We speculate that the unit of inheritance in the *mat2/3* interval is both DNA plus its associated chromatin structure. Such a control is likely to be essential in maintaining particular states of gene expression during development.

Meyer, P. (1996). "Repeat-induced gene silencing: common mechanisms in plants and fungi." *Biol Chem Hoppe Seyler* **377**(2): 87-95.

One of the most surprising observations made in plant science in recent years is the inactivation of transgenes triggered by interactions between DNA repeats. In plants, we can differentiate between transcriptional silencing, most likely reflecting a regulation at the DNA level, and post-transcriptional silencing that affects steady state RNA levels. In the filamentous fungi *Ascobolus immersus* and *Neurospora crassa*, we find two premeiotic silencing processes that are also based on the interaction of repeated sequences. A common feature of transcriptional silencing in plants and premeiotic gene inactivation in filamentous fungi is that the repeated sequences undergo cytosine methylation. DNA methylation, which is either the cause or the consequence of gene silencing, can be associated with changes in chromatin structure. These structural changes are reminiscent of homology-based silencing mechanisms in *Drosophila*, an organism that lacks DNA methylation. Repeat-induced silencing may therefore reflect the activity of an endogenous mechanism, present in some species, which screens for homology and has significant implications for the organization and evolution of the genome.

Morgan, H. D., H. G. Sutherland, et al. (1999). "Epigenetic inheritance at the agouti locus in the mouse [see comments]." *Nat Genet* **23**(3): 314-8.

Epigenetic modifications have effects on phenotype, but they are generally considered to be cleared on passage through the germ line in mammals, so that only genetic traits are inherited. Here we describe the inheritance of an epigenetic modification at the agouti locus in mice. In viable yellow (*A(vy)/a*) mice, transcription originating in an intra-cisternal A particle (IAP) retrotransposon inserted upstream of the agouti gene (*A*) causes ectopic expression of agouti protein, resulting in yellow fur,

obesity, diabetes and increased susceptibility to tumours. The pleiotropic effects of ectopic agouti expression are presumably due to effects of the paracrine signal on other tissues. Avy mice display variable expressivity because they are epigenetic mosaics for activity of the retrotransposon: isogenic Avy mice have coats that vary in a continuous spectrum from full yellow, through variegated yellow/agouti, to full agouti (pseudoagouti). The distribution of phenotypes among offspring is related to the phenotype of the dam; when an A(vy) dam has the agouti phenotype, her offspring are more likely to be agouti. We demonstrate here that this maternal epigenetic effect is not the result of a maternally contributed environment. Rather, our data show that it results from incomplete erasure of an epigenetic modification when a silenced Avy allele is passed through the female germ line, with consequent inheritance of the epigenetic modification. Because retrotransposons are abundant in mammalian genomes, this type of inheritance may be common.

Nakayama, J., A. J. Klar, et al. (2000). "A chromodomain protein, Swi6, performs imprinting functions in fission yeast during mitosis and meiosis." *Cell* **101**(3): 307-17.

Inheritance of stable states of gene expression is essential for cellular differentiation. In fission yeast, an epigenetic imprint marking the mating-type (*mat2/3*) region contributes to inheritance of the silenced state, but the nature of the imprint is not known. We show that a chromodomain-containing Swi6 protein is a dosage-critical component involved in imprinting the *mat* locus. Transient overexpression of Swi6 alters the epigenetic imprint at the *mat2/3* region and heritably converts the expressed state to the silenced state. The establishment and maintenance of the imprint are tightly coupled to the recruitment and the persistence of Swi6 at the *mat2/3* region during mitosis as well as meiosis. Remarkably, Swi6 remains bound to the *mat2/3* interval throughout the cell cycle and itself seems to be a component of the imprint. Our analyses suggest that the unit of inheritance at the *mat2/3* locus comprises the DNA plus the associated Swi6 protein complex.

Novick, A. and M. Weiner (1957). "Enzyme induction as an all-or-none phenomenon." *Proc. Nat. Acad. Sci. usa* **43**: 553-566.

Rossignol, J. L. and G. Faugeron (1994). "Gene inactivation triggered by recognition between DNA repeats." *Experientia* **50**(3): 307-17.

This chapter focuses on phenomena of gene inactivation resulting from the presence of repeated gene copies within the genome of plants and fungi, and on their possible relationships to homologous DNA-DNA interactions. Emphasis is given to two related premeiotic processes: Methylation Induced Premeiotically (MIP) and Repeat-Induced Point mutation (RIP) which take place in the fungi *Ascobolus immersus* and

Neurospora crassa, respectively. The relationships between these processes and genetic recombination are discussed.

Selker, E. U. (1999). "Gene silencing: repeats that count." Cell **97**(2): 157-60.

Sparrer, H. E., A. Santoso, et al. (2000). "Evidence for the prion hypothesis: induction of the yeast [PSI⁺] factor by in vitro- converted Sup35 protein [see comments]." Science **289**(5479): 595-9.

Starting with purified, bacterially produced protein, we have created a [PSI(+)]-inducing agent based on an altered (prion) conformation of the yeast Sup35 protein. After converting Sup35p to its prion conformation in vitro, we introduced it into the cytoplasm of living yeast using a liposome transformation protocol. Introduction of substoichiometric quantities of converted Sup35p greatly increased the rate of appearance of the well-characterized epigenetic factor [PSI⁺], which results from self-propagating aggregates of cellular Sup35p. Thus, as predicted by the prion hypothesis, proteins can act as infectious agents by causing self-propagating conformational changes.

Sutherland, H. G., M. Kearns, et al. (2000). "Reactivation of heritably silenced gene expression in mice." Mamm Genome **11**(5): 347-55.

Epigenetic modifications that suppress gene activity in mammals are generally considered to be cleared in the germline, restoring totipotency of the genome. Here we report the germline inheritance of transcriptional silencing in mice, and reversion to activity after as many as three generations in the silent state. In a series of lines made with a LacZ transgene, one line exhibits variable expressivity: genotypically identical littermates have proportions of beta-Gal- positive erythrocytes that vary over at least four orders of magnitude, and in some offspring expression is completely silenced. The silent state of the transgene is inherited for multiple generations in the founder strain irrespective of the sex of the parent, implying maintenance of the epigenetic state through meiosis. Crosses of silenced mice with C57BL/6 mice result in reactivation of the transgene in approximately a third of F(1) littermates. The silencing involves a stochastic, all-or-none mechanism. Furthermore, silencing is transcriptional and correlates with methylation of the transgene as well as an inaccessible chromatin structure; these changes are reversed when expression is reactivated. This work supports the notion that silent genetic information in mammals can be inherited and later reactivated, and implies a mode of phenotypic inheritance that is less stable than Mendelian inheritance.

Tuite, M. F. (2000). "Cell biology. Sowing the protein seeds of prion propagation [comment]." Science **289**(5479): 556-7.

Wickner, R. B., K. L. Taylor, et al. (2000). "Portable prion domains [In Process Citation]." Curr Biol **10**(9): R335-7.

Self-propagating abnormal proteins, prions, have been identified in yeast; asparagine/glutamine-rich 'prion domains' within these proteins can inactivate the linked functional domains; new prion domains and reporters have been used to make 'synthetic prions', leading to discoveries of new natural prions.