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NuRD and SIN3

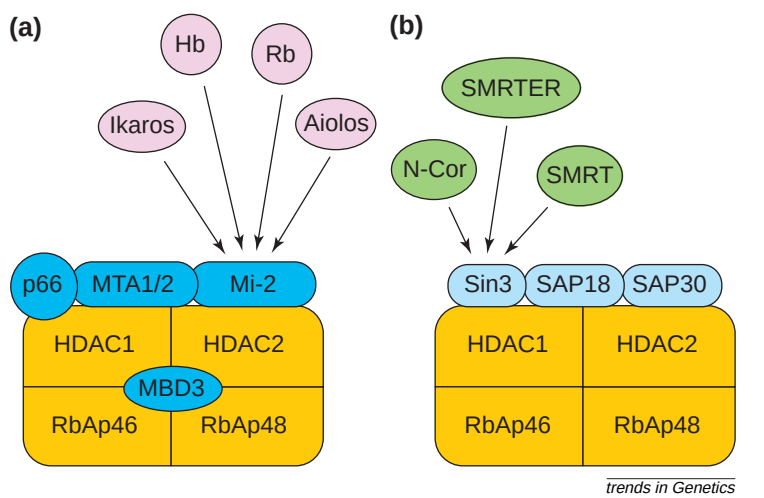
histone deacetylase complexes in development

Transcription repression mediated through histone deacetylase (HDAC) complexes is widespread, and mechanisms by which HDAC complexes act have been revealed by extensive studies *in vitro* and in cell culture. However, until recently, little has been known about the developmental roles of histone deacetylation. Mutants now exist for a number of members of the two major HDAC complexes (NuRD and SIN3) and some associated proteins. The emerging picture is that these complexes have specific functions in development, rather than being required for most cellular processes.

During the development of an organism, a wide variety of cell fate decisions are taken based on specific inheritance or cell interactions. Ultimately, most of these decisions are carried out by sequence-specific transcription factors, through activation or repression of gene expression. Clearly, alterations of gene expression must occur in the context of chromatin, but until recently, only a few developmental roles for changes in chromatin structure were known. Over the past few years, a series of results has been helping to explain how chromatin regulation contributes to making developmental decisions.

It has long been known that activation and repression of gene expression correlate with the acetylation state of histones. In general, acetylated histones are correlated with more open chromatin and active gene expression, whereas deacetylated histones correlate with closed chromatin and repressed gene expression. Early work using histone deacetylase inhibitors showed that they disrupted normal development, indicating that the acetylation state of histones is developmentally important (e.g. see Ref. 1). Recently, enzymes that carry out histone acetylation (histone acetyltransferases, or HATs) and deacetylation (histone deacetylases, or HDACs) have been identified (reviewed in Ref. 2). These function in several different large multiprotein complexes that are associated with sequence-specific DNA-binding proteins, which are thought to target the complexes to specific genes, leading to local chromatin modification. Many studies have been concerned with the biochemical activities of these complexes and have been conducted *in vitro* or in cell culture. However, recent *in vivo* work in several systems has provided some functional information during development. A good review of developmental roles of HATs has been published recently[3]. Here, I review developmental functions of the two major histone deacetylation complexes, NuRD (for nucleosome remodelling and histone deacetylation) and SIN3 and their associated proteins, focusing primarily on cases where mutants have been studied.

FIGURE 1. NuRD and SIN3 complexes



Yellow boxes indicate core components shared between **(a)** NuRD and **(b)** SIN3. The components that are specific for NuRD and SIN3 are in dark blue and light blue, respectively. The positions of components are not meant to show physical interactions between subunits in the complexes. Arrows indicate identified physical interactions with proteins associated with NuRD or SIN3 that are discussed in the text.

Below, I briefly summarize the composition and biochemical properties of these complexes.

The NuRD complex[4–8] (also known as Mi-2) is approximately 2 MDa in size and in mammalian cells comprises at least seven polypeptides (Fig. 1 and Table 1; reviewed in Ref. 9). The histone deacetylases HDAC1 and HDAC2 and two histone-binding proteins (RbAp46 and RbAp48) are also found in the SIN3 complex. In addition to histone-deacetylase activity, the NuRD complex has ATP-dependent nucleosome-remodelling activity because it contains Mi-2/CHD family proteins, which have a chromodomain, a DNA helicase/ATPase

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TABLE 1. Biochemical functions of NuRD and SIN3 complex members and associated proteins

Component	Function	Refs
NuRD complex		4–8
HDAC1, HDAC2	Histone deacetylases	
RbAp46, RbAp48	Histone binding proteins	
Mi-2α (CHD3), Mi-2β (CHD4)	ATP dependent nucleosome remodelling	
MTA1, MTA2	Unknown	
MBD3	Scaffold?	
p66[a]	Unknown	
Proteins associated with NuRD or its components		
Hb	Zinc finger DNA binding	28
Ikaros	Zinc finger DNA binding	50
Aiolos	Zinc finger DNA binding	50
MBD2	Methyl CpG binding	5
SIN3 complex		13–17
HDAC1, HDAC2	Histone deacetylases	
RbAp46, RbAp48	Histone binding proteins	
Sin3	Scaffold?	
SAP18	Unknown	
SAP30	Targeting SIN3 complex to N-CoR associated repressors?	
Proteins associated with SIN3 or its components		
N-CoR	Co-repressor	18
SMRT	Co-repressor	19
SMRTER	Co-repressor	53
EcR	DNA binding nuclear hormone receptor	53
Ikaros	Zinc finger DNA binding	51
Aiolos	Zinc finger DNA binding	51
MeCP2	Methyl CpG binding	26

Only those components discusssed in this review are listed; see Ref. 9 for a list of additional associated proteins.
[a]p66 has so far only been found in the *Xenopus* Mi-2–NuRD complex[7].

domain of the SWI/SNF family, and PHD fingers. Another distinguishing feature of NuRD is the inclusion of MTA1 or the similar protein MTA2. MTA1 was originally identified as being overexpressed in metastatic carcinomas[10]; it has a number of defined domains[11], including a zinc finger and a SANT domain[12] (a region similar to the DNA binding domain of myb), but its biochemical function is not known. NuRD further contains MBD3 (similar to methyl CpG-binding domain proteins). In *Xenopus*, p66 of unknown function is also found in NuRD[7].

The SIN3 complex[13–15] shares four core proteins with NuRD (HDAC1, HDAC2, RbAp46 and RbAp48) and additionally contains Sin3, SAP18 and SAP30 (Table 1). The functions of these latter proteins are not clear, but Sin3 is proposed to act as a scaffold for the complex, and SAP30 might couple the complex to particular repressors[16,17]. The SIN3 complex has been extensively studied with respect to its involvement in repression by nuclear hormone receptors such as the retinoic acid and thyroid hormone receptors, which can activate or repress transcription, depending on the presence of ligand. Two co-repressors, N-CoR (Ref. 18) and SMRT (Ref. 19), facilitate transcriptional repression by physically linking the SIN3 complex with receptors that are not bound to a ligand[20,21]. Interestingly, like MTA1, N-CoR and SMRT both contain SANT domains. The function of this domain is not known, but it is also found in proteins of the SWI/SNF and ADA chromatin-modifying complexes, suggesting that it has a role in chromatin regulation[12]. Further information on the biochemistry of NuRD and SIN3 can be found in recent reviews[9,22,23].

The NuRD complex has been identified in mammalian and *Xenopus* cells, and SIN3 in mammalian cells and yeast. However, subunits of the NuRD and SIN3 complexes have also been found in *Drosophila*, *Caenorhabditis elegans* and *Arabidopsis*, suggesting that the complexes exist widely in animals and plants. Below, I describe known developmental functions for genes encoding NuRD or SIN3 members, or associated proteins in a range of different organisms (Table 2). As histone deacetylation appears to be a widely used repression mechanism, it might be expected that these two major HDAC complexes would each be needed for most cellular functions. However, surprisingly, the cases studied show that NuRD and SIN3 complexes have specific developmental roles, rather than being required for general cellular functions. It is possible that other, as yet uncharacterized HDAC complexes have more general roles; besides HDAC1 and HDAC2, at least four other HDACs have been identified (reviewed in Ref. 9). NuRD and SIN3 complexes appear to be needed for a range of developmental processes, at different times and places during development, therefore mutations in components have pleiotropic defects that can be difficult to interpret. For this reason, in most of the work described below, links to NuRD and SIN3 have come about from studying a developmental process, rather than by studying developmental functions for these complexes.

NuRD and SIN3 involvement in methylation

Besides interacting with sequence-specific transcription factors, NuRD and SIN3 might also play a role in transcriptional repression by DNA methylation. The methyl CpG-binding protein MeCP2 has been shown to recruit the SIN3 complex[24]. MeCP2 is important for human development: recently, it was found that mutations in MeCP2 are a cause of Rett syndrome, an X-linked progressive neurodevelopmental disorder that is a major cause of mental retardation in females[25]. Therefore, one possibility is that lack of repression of methylated genes might be involved in Rett syndrome.

The NuRD complex also appears to have links with methylation in vertebrate cells, but data on this is not yet clear. The *Xenopus* NuRD component MBD3 shows a strong preference for methylated DNA *in vitro*, whereas mammalian MBD3 shows only a weak preference[26], and the mammalian NuRD complex does not bind to methylated DNA (Ref. 5). However, another protein with a methyl CpG-binding domain, MBD2, which can interact with mammalian NuRD *in vitro*, does bind to methylated DNA *in vitro* and *in vivo*. This suggests that MBD2 might recruit NuRD to methylated DNA (Ref. 5). Recruitment by DNA methylation is clearly not the only mechanism by which NuRD and SIN3 act, as animals that lack DNA methylation (e.g. *Drosophila* and *C. elegans*) have genes that encode subunits of both complexes.

NuRD and repression by Polycomb group proteins

In *Drosophila* and mouse, Polycomb group (PcG) proteins maintain the repression of Hox gene expression[27]. They exist in complexes and are found associated with particular regions of DNA that show silencing activity, but their mechanism of action has been unclear until now. Recent data suggest that one mechanism by which PcG repression occurs could be through histone deacetylation via the NuRD complex[28].

In flies, the sequence-specific DNA-binding protein Hunchback (Hb) initiates repression of Hox genes and PcG proteins maintain this repression. A two-hybrid screen to find proteins that might aid repression by Hb

identified dMi-2, which is similar to Mi-2 of the NuRD complex[28]. *dMi-2* mutants arrest as first or second instar larvae with no obvious defects, which might be due to the abundant maternal *dMi-2* mRNA produced. Embryos that lack this maternal mRNA could not be made because dMi-2 is essential for the development of germ cells. However, further genetic analyses of *dMi-2* mutants proved informative: double mutants between *dMi-2* and *hb* showed increased derepression of the Hox gene *Ubx* over *hb* mutants alone. A similar interaction was observed in double mutants between *dMi-2* and *PcG* genes, including *posterior sex combs* and *polycomb*. Therefore, dMi-2 appears to function with Hb and PcG proteins in Hox repression. One possibility is that Hb might recruit NuRD by interaction with dMi-2, which would modify chromatin to allow binding of PcG proteins, or perhaps NuRD could directly recruit PcG proteins.

Further support for an interaction between PcG proteins and histone deacetylases comes from a study of the mouse EED protein (homologue of the *Drosophila PcG* gene *extra sex combs*), a component of one of two distinct mammalian PcG complexes identified[29,30]. EED and the EED-containing complex physically interact with histone deacetylases, whereas the other complex (containing the human Polycomb homologue HPC2) does not[30]. Therefore, histone deacetylases might mediate repression by some, but not all, PcG proteins. Consistent with this idea, repression by Xpc1 (a *Xenopus* Polycomb homologue) is independent of histone deacetylase[31], and HDACs do not copurify with PRC1, a *Drosophila* PcG complex containing the Polycomb protein[32]. This contradicts the suggestion of Kehle *et al*.[28] that dMi-2 (and therefore a histone deacetylase complex) might be involved in repression by Polycomb. However, the genetic interaction seen could have been indirect and caused by the inhibition of two pathways in parallel, one involving histone deacetylase and one not.

NuRD, Ras and patterning in *C. elegans*

In *C. elegans*, studies of histone deacetylase complex members have been greatly facilitated by the use of the RNA interference (RNAi) technique. Introduction of double-stranded RNA corresponding to a gene of interest into worms will inactivate that gene by targeting the endogenous mRNA for degradation[33,34]. Initially, it was found that the histone deacetylase gene *hda-1* and the RbAp46/48 homologues *rba-1* and *rba-2* (the latter now known to be *lin-53*) are essential for embryogenesis[35]. *hda-1* was shown to antagonize the activity of the histone acetyltransferase-encoding gene *cbp-1*, perhaps through inhibition of differentiation, but the study did not define clear developmental roles for these HDAC complex proteins. Subsequent work has shown that NuRD complex members are important for embryonic patterning, Hox expression and function, Wnt signalling, and antagonizing the Ras pathway during vulval development .

Three groups identified mutations in *egl-27*, one of two *C. elegans* homologues of the NuRD component MTA1, and characterized different aspects of the mutant phenotype[11,36,37]. These studies showed that EGL-27 has numerous roles in patterning during development. For example, in *egl-27* mutants, anterior–posterior patterning of ventral ectodermal cell fates is abnormal[37]. The defects are caused by posteriorly shifted Hox expression domains and by unregulated Hox protein function: the Hox protein MAB-5 is inappropriately active, and a combinatorial interaction between MAB-5 and another Hox protein, LIN-39, does not occur in the absence of EGL-27. Therefore, proper activity of these homeodomain transcription factors requires EGL-27. As EGL-27 is likely to function as part of a *C. elegans* NuRD complex, NuRD might be needed to carry out Hox directed patterning generally. Another analysis of *egl-27* mutants revealed defects in two processes controlled by wnt signalling[36]: the polarity of an asymmetric cell division and the migration of the QL neuroblast are incorrect (this latter defect is at least partly due to lack of expression of the Hox gene *mab-5*). Finally, *egl-27* and a second *C. elegans* MTA1 homologue, *egr-1*, were shown to be partially redundant in overall embryonic body patterning[11]. Embryos lacking EGL-27 and EGR-1 are very disorganized but have normal cell divisions and tissue differentiation, suggesting a specific role in body patterning. A similar phenotype is obtained after inactivating the histone deacetylase gene *hda-1* by RNAi (Ref. 35; F. Solari and J. Ahringer, unpublished), supporting the idea that EGL-27 and EGR-1 functions are carried out through a histone deacetylase complex. In *Drosophila*, the HDAC gene *Rpd3* is important for segmentation of the embryo, suggesting that, as in *C. elegans*, HDAC complexes are important for embryonic patterning[38].

Further work on *egr-1* and an earlier study of *hda-1* and an RbAb46/48 homologue *lin-53* revealed a function in antagonizing Ras signalling in the *C. elegans* vulva[39,40]. Ras signalling induces vulval development in certain ventral ectodermal cells in *C. elegans*[41]. Two groups of functionally

TABLE 2. Developmental functions for NuRD and SIN3 complex members and associated proteins

Gene	Organism	Developmental role	Refs
HDAC			
Rpd3	*Drosophila*	Embryonic segmentation	38
		Groucho-mediated transcriptional repression	61
hda-1	*C. elegans*	Embryonic viability	35
		Vulval development	40
RbAp46/48			
lin-53	*C. elegans*	Embryonic viability	35
		Vulval development	39, 40
rba-1	*C. elegans*	Embryonic viability	35
		Vulval development	40
Mi-2			
dMi-2	*Drosophila*	Hox repression, larval and germ cell viability	28
chd-3	*C. elegans*	Vulval development	40
chd-4	*C. elegans*	Vulval development	40
PKL	*Arabidopsis*	Repression of embryo and meristem genes	46, 47
MTA1/MTA2			
egl-27	*C. elegans*	Embryonic patterning	11
		Hox regulation	37
		wnt signalling	36
		Vulval development	40
egr-1	*C. elegans*	Embryonic patterning	11
		Hox regulation	37
		Vulval development	40
Sin3			
dSin3A	*Drosophila*	Embryonic viability	56
		Ecdysone receptor mediated repression	53
Transcription factors			
hb	*Drosophila*	Hox gene repression	62
Ikaros	Mouse	Haemopoiesis	49
Aiolos	Mouse	Haemopoiesis	49
EcR	*Drosophila*	Moulting and morphogenesis	55
Methyl CpG binding			
MeCP2	Human	Neurodevelopment (cause of Rett syndrome)	25

redundant genes (called synMuvA and synMuvB genes) help prevent the Ras pathway from inducing inappropriate vulval development adjacent to the normal vulva[42]. Studies of NuRD complex members (*egr-1*, *egl-27*, *hda-1*, *rba-1*, *lin-53*, *chd-3* and *chd-4*; the latter two are homologues of Mi-2) found that they functioned to inhibit Ras-induced vulval development through the synMuv pathways[39,40]. A large interaction study confirmed physical association between some of these *C. elegans* NuRD proteins[43]. The results suggest that the NuRD complex is needed for repression of vulval development genes that are activated by Ras signalling.

By looking at the phenotypes caused by lack of different NuRD members, it was found that *egr-1* behaved differently from the other NuRD members. Whereas *hda-1*, *rba-1*, *lin-53*, *chd-3* and *chd-4* function within the synMuvA and the synMuvB pathways, EGR-1 functions only in the synMuvA pathway[40]. This suggests that the NuRD component MTA1 (EGR-1) might act as an adaptor to the complex rather than being an integral part of it. A protein that functions only in the synMuvB pathway, LIN-35, is a homologue of the Retinoblastoma (Rb) protein[39], a transcriptional co-repressor[44]. Rb physically interacts with histone deacetylases[45], raising the possibility that LIN-35 (Rb) could bring NuRD to DNA. The data suggest that the synMuv pathways might function redundantly to regulate or recruit the NuRD complex. Further work on this system should provide a better understanding of the tissue-specific recruitment of NuRD.

A NuRD member in *Arabidopsis*

The NuRD complex probably also exists in plants, as the *PICKLE* (*PKL*, aka *GYMNOS*) gene of *Arabidopsis* was recently shown by two groups to encode an Mi-2 homologue[46,47]. An earlier study of *pkl* mutants showed that they inappropriately express embryonic differentiation characteristics during post-embryonic development[48]. For example, LEC1, a seed-specific transcription factor that promotes embryonic identity, is derepressed post-embryonically in *pkl* mutants[47], as are seed-storage protein and storage lipid-deposition genes[48]. Therefore, PKL is necessary to repress embryonic development to allow the transition to post-embryonic development.

A second group isolated *pkl* mutants as genetic enhancers of *crabs claw* (*crc*) mutants[46]. On their own, the *pkl* mutants displayed pleiotropic defects, but a general characteristic is the delayed maturation of a number of different tissue types. This phenotype is consistent with a role for PKL in repressing meristematic genes, similar to that proposed for PKL in repressing embryonic genes. Together, the studies suggest that the transition to a determined cell type at two different stages of *Arabidopsis* development requires repression of genes that promote a more pluripotent character. A plausible mechanism for repression of embryonic and meristematic genes by PKL is via histone deacetylation by the NuRD complex

HDACs, Ikaros and Aiolos in mouse haemopoiesis

A role for HDACs in haemopoiesis was recently revealed through study of the mouse proteins Ikaros and Aiolos. These two proteins are related zinc-finger DNA-binding factors that are necessary for the development of the haemopoietic system in the mouse[49]. In their absence, mice are immunodeficient and display B- and T-cell lymphomas. Although no target genes are known for Ikaros and Aiolos, they associate with HDAC complexes, suggesting that they have a repressive function.

In one study, the Ikaros and Aiolos proteins were found to interact directly with the mouse Mi2α protein; in T cells, they are associated with a large complex that is likely to be NuRD (Ref. 50). The finding that Mi-2 proteins interact with sequence-specific DNA-binding proteins, both in mouse cells (Ikaros and Aiolos) and in *Drosophila* (Hb; see above), suggests that they might generally link NuRD to transcription factors.

Immunolocalization studies support the idea that Ikaros and Aiolos function in transcriptional repression[50]. In resting T cells, Ikaros, Aiolos, HDAC1 and Mi2α all have a diffuse nuclear localization pattern. However, upon T-cell activation, they associate with toroidal structures around heterochromatin. Localization of HDAC1 and Mi2α to regions of heterochromatin is lost in Ikaros-deficient T cells, suggesting that Ikaros recruits HDAC1 and Mi2α to these structures. As gene expression is generally inhibited in heterochromatin, these localization studies indicate that Ikaros and Aiolos probably have a repressive function in T-cell development.

In another study, Ikaros and Aiolos were found to associate with the SIN3 complex and to physically interact with Sin3 isoforms[51]. Interaction of Ikaros and Aiolos with SIN3 and NuRD suggests that they might act in different ways on two different sets of genes. One possibility is that the association with NuRD (which has a nucleosome-remodelling activity) could cause histone deacetylation in inaccessible regions, whereas the SIN3 complex might repress active genes.

Further complexity is added by the finding that Ikaros is also associated with a SWI/SNF complex (thought to function in activation of gene expression)[50]. The association with two different repressive complexes (NuRD and SIN3), as well as with an activating complex, indicates that Ikaros and Aiolos probably have a number of different activities in gene regulation. The identification of target genes and further regulators will be needed to help define gene-specific activities.

SIN3 and repression by nuclear hormone receptors

A wealth of biochemical data has established that unliganded nuclear hormone receptors repress transcription through interaction with co-repressors such as N-CoR and SMRT, which associate with the SIN3 complex (reviewed in Ref. 52). A recent study of the *Drosophila* Ecdysone receptor (EcR) has provided evidence for this type of regulation *in vivo*[53]. EcR is a nuclear hormone receptor that functions as a heterodimer with the *Drosophila* retinoic X receptor (RXR) homologue ultraspiracle (USP)[54]. The EcR–USP heterodimer is a key regulator in moulting and metamorphosis, and acts as either a transcriptional activator or repressor, depending on the presence of the hormone ecdysone. Mutations in EcR are lethal and cause developmental defects[55]. *In vitro*, EcR was found to associate with mammalian SMRT, and this is necessary for its repressive activity[53]. The protein encoded by a mutant allele of EcR (A483T) cannot mediate repression and, importantly, does not interact with SMRT, suggesting this EcR mutant fails in repression because it cannot associate with the SIN3 complex.

This work was satisfyingly brought back to *Drosophila* with the results of a two-hybrid screen for proteins that

interacted with EcR (Ref. 53). Here, a *Drosophila* homologue of SMRT and N-CoR (named SMRTER) was isolated. SMRTER also interacts directly with *Drosophila* Sin3 (dSin3A). Genetic interactions between *dSin3A* mutants and those in *EcR* provided additional evidence supporting the idea that the SIN3 complex is necessary for repression by EcR. Therefore, the mechanism by which EcR mediates transcriptional repression in *Drosophila* is probably the same as that occurring in mammals: the SMRTER co-repressor links the nuclear hormone receptor EcR with the SIN3 complex. Besides acting with EcR, dSin3A clearly has additional roles in *Drosophila* development. *dSin3A* mutants are embryonic lethal[56] and were also isolated as genetic enhancers of a mutant in the *sina* gene, which aids transmission of Ras signalling in the eye[57].

Nuclear hormone receptors and HDACs have also been implicated in human leukaemia[58]. Translocations of the retinoic acid receptor (RAR) with promyelocytic leukaemia (PML) or promyelocytic leukaemia zinc finger (PLZF) result in acute promyelocytic leukaemia (APL). Histone deacetylase inhibitors improve the response of APL cell lines to retinoids, indicating that the RAR translocations mediate leukaemogenesis through recruitment of an HDAC complex. This study suggests that pharmacological manipulation of HDAC or co-repressor activity might be a useful approach in disease treatment.

Conclusions and future prospects

Given the finding that NuRD and SIN3 have wide developmental roles, those studying transcriptional repressors involved in development should keep an eye out for their involvement. It might emerge that most developmental decisions involving transcriptional repressors require an HDAC complex, either NuRD, SIN3, or a different complex. It is almost certain that other HDAC complexes exist: in addition to HDAC1 and HDAC2 found in these complexes, at least four additional histone deacetylases (HDAC3–6) are known (reviewed in Ref. 9), and recent work on HDAC4 indicates a link to myogenesis[59,60].

These initial studies should act as a springboard for further *in vivo* research that will identify additional functions for NuRD and SIN3. Many more adaptors and even new core components and complexes are likely to be found biochemically, and their study will probably reveal that the picture is far more complex than is now apparent. In the future, one of the most important goals will be the identification of target genes and additional transcription factors that function with HDAC complexes, as their study should allow the understanding of how, where and when NuRD and SIN3 act.

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New uses for new haplotypes

the human Y chromosome, disease and selection

Recent discoveries of many new genes have made it clear that there is more to the human Y chromosome than a heap of evolutionary debris, hooked up to a sequence that happens to endow its bearer with testes. Coupled with the recent development of new polymorphic markers on the Y, making it the best-characterized haplotypic system in the genome, this gives us new opportunities to assess its role in disease and selection, through association studies with phenotypes such as infertility and cancers. However, the peculiar genetics of this bizarre chromosome means that we should interpret such studies particularly cautiously.

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After we are born, the first thing anyone wants to know about us is our sex – boy or girl? Unlike the other phenotypic variation among humans, this most fundamental of differences is conferred by the presence or absence of a chromosome: the Y chromosome determines maleness through the action of a single gene, *SRY* (*sex-determining region Y*)[1]. For many years it was thought that there were very few other Y-chromosomal genes, although genetic evidence had long suggested that the Y also bears loci that are important in spermatogenesis[2]. Recent developments have now revealed a large number of additional genes (Ref. 3; Fig. 1), including some that play roles in fundamental cellular processes[3,4]. These findings raise two important and connected questions. First, what is the role of the Y in disease? If differential susceptibilities to important disorders exist, then this will have implications for aetiologies, screening programmes and possibly treatment. Second, to what extent is natural selection acting on the Y chromosome? This question is becoming of increasing importance as greater emphasis is placed on the Y chromosome as a tool for the study of human evolution: if selection, as well as neutral processes such as genetic drift and population history, influences the distribution of Y-chromosomal variation in human populations, then this must be taken into account.

The recent advances in our knowledge about Y-chromosomal genes have been mirrored by a massive increase in the number of available polymorphic markers (e.g. Ref. 5), and we now possess the molecular tools to study Y-chromosomal diversity in fine detail (Box 1; Fig. 2), and so to address these questions. While most of the polymorphisms themselves are probably neutral, we can use them to look for associations with particular phenotypes and, conversely, to ask if phenotypes are influencing the distributions of polymorphisms within populations. The first studies of these kinds have now been published but, because of the peculiar genetics of the Y chromosome, they are not as easy to interpret as the more usual autosomal association studies, and they need critical evaluation. The purpose of this article is to review evidence for a role for the Y chromosome in disease and selection, and to propose ways in which its influence can be assessed.

The Y chromosome, human evolution and selection

Non-recombining segments of the genome, such as the Y chromosome excluding the pseudoautosomal regions[6] (Fig. 1), have advantages in human evolutionary studies – the absence of the reshuffling effect of recombination