Neuromol Med
DOI 10.1007/s12017-009-8063-4

ORIGINAL PAPER

Gene Dysregulation in Huntington's Disease: REST, MicroRNAs and Beyond

Rory Johnson · Noel J. Buckley

Received: 13 March 2009 / Accepted: 17 April 2009

Abstract Huntington's disease (HD) is an incurable, fatal neurodegenerative disorder that is caused by a polyglutamine expansion in the huntingtin (Htt) protein. Neuronal death in the striatum—the most obvious manifestation of the disease—is likely to result from widespread dysregulation of gene expression in various brain regions. To date, several potential mechanisms for this have been discovered, including one involving REST (RE1-Silencing Transcription Factor), a master regulator of neuronal genes. Recently, independent studies have demonstrated that post-transcriptional gene regulation by microRNAs is also disrupted in HD. Expression of key neuronal microRNAs—including mir-9/9*, mir-124 and mir-132—is repressed in the brains of human HD patients and mouse models. These changes occur downstream of REST, and are likely to result in major disruption of mRNA regulation and neuronal function. In this study we will discuss these findings and their implications for our understanding of HD. Using updated bioinformatic analysis, we predict 21 new candidate microRNAs in HD. We propose future strategies for unifying large-scale transcriptional and microRNA datasets with the aim of explaining HD aetiology. By way of example, we show how available genomic datasets can be integrated to provide independent, analytical validation for dysregulation of REST and microRNA mir-124 in HD. As a consequence, gene ontology analysis indicates that HD is characterised by a broad-based depression of neural genes in the caudate and motor cortex. Thus, we propose that a combination of REST, microRNAs and possibly other non-coding RNAs profoundly affect the neuronal transcriptome in HD.

Keywords Neurodegeneration · Huntington's disease · MicroRNA · Noncoding RNA · REST · NRSF

Abbreviations

HD	Huntington's disease
Htt	Huntingtin
mutHtt	Mutant huntingtin
BDNF	Brain-derived neurotrophic factor
miRNA	MicroRNA
REST	Repressor element 1-silencing transcription factor
RE1	Repressor element 1
NRSF	Neuron-restrictive silencing factor
NRSE	Neuron-restrictive silencing element
SCA	Spinocerebellar ataxia
SP1	Specificity protein 1
RISC	RNA-induced silencing complex
DRPLA	Dentatorubral-pallidoluysian atrophy
SBMA	Spinobulbar muscular atrophy
MRE	MicroRNA response element
CREB	cAMP response element binding
MeCP2	Methyl CpG binding protein 2
BACE1	β-site of APP cleaving enzyme
AD	Alzheimer's disease
ncRNA	Noncoding RNA
ChIP-Seq	Chromatin immunoprecipitation coupled to high-throughput sequencing

Both the authors are joint corresponding authors.

R. Johnson (✉)
Stem Cell and Developmental Biology Group, Genome Institute of Singapore, 60 Biopolis Street, Singapore 138672, Singapore
e-mail: johnsonrb@gis.a-star.edu.sg

N. J. Buckley (✉)
Centre for the Cellular Basis of Behaviour, Centre for Cell and Integrative Biology, King's College London, Institute of Psychiatry, London SE5 9NU, UK
e-mail: noel.buckley@iop.kcl.ac.uk

Published online: 21 May 2009

Introduction

Huntington's disease (HD) is a dominantly-inherited, incurable neurodegeneration affecting around seven in 10,000 people worldwide (Walker 2007). HD patients suffer from various neurological deficits—chorea, dementia, emotional dysfunction—that appear in adult life with a mean age of 35, and are ultimately fatal (Vonsattel and DiFiglia 1998). Neurodegeneration is highly selective: postmortem reveals greatest loss amongst the medium spiny neurons of the caudate and putamen (Ferrante et al. 1985) (in non-human mammals referred to as striatum), while cortex, thalamus and subthalamic nucleus become affected at later disease stages (Hedreen et al. 1991). Usually physical symptoms appear initially, followed by neurological and cognitive symptoms (Walker 2007). In 1993 the single causative mutation in the huntingtin (Htt) gene was shown to be a CAG expansion within the first coding exon, leading to a polyglutamine expansion in the Htt protein (Huntington's Disease Collaborative Research Group 1993). This places HD in the class of polyglutamine diseases alongside dentatorubral-pallidolyusian atrophy (DRPLA), spinobulbar muscular atrophy (SBMA) and various spinocerebellar ataxias, all of which are characterised by neurodegeneration. The polyglutamine expansion converts Htt from a neuroprotective to a neurotoxic protein (reviewed by Cattaneo et al. (2005)). Intriguingly, the age of disease onset is inversely correlated with the length of number of CAG repeats in the mutant Htt protein (mutHtt) (Vonsattel and DiFiglia 1998; Snell et al. 1993). The molecular mechanism by which a simple nucleotide expansion in this ubiquitous protein leads to selective neuronal death remains poorly understood. Nevertheless, development of effective therapeutics depends on our explaining this process.

Various molecular and physiological changes precede external symptoms, most notably profound changes in neuronal gene expression profiles (Augood et al. 1997). This transcriptional dysregulation is thus hypothesised to be a causative factor in HD neurodegeneration. Various laboratories have identified transcriptional regulatory pathways that are affected by the presence of mutHtt (reviewed by Cha (2007)). However, we now know that the disruption of normal gene regulation is more extensive than previously thought. Recently, two independent studies have uncovered a new pathway of gene regulation in HD—that involving post-transcriptional regulation of genes by the class of small noncoding RNAs, the microRNAs. It appears that a number of crucial neural-specific microRNAs are repressed in HD (Johnson et al. 2008b; Packer et al. 2008). Given the central role that microRNAs play in the nervous system, their loss is likely to have major effects on neuronal gene expression and function. In this article, we will review our current understanding of microRNA dysregulation in HD, and its likely contribution to disease progression. Using available datasets, we will demonstrate that this newly discovered pathway may explain a large proportion of gene changes observed in disease HD brains.

Transcriptional Dysregulation in HD

The tissues of HD sufferers display profound abnormalities in mRNA levels (Hodges et al. 2006). Early in situ hybridizations in the brains of HD patients demonstrated decreased levels of mRNAs encoding key proteins such as dopamine receptors D1 and D2 (*DRD1*, *DRD2*) (Augood et al. 1997) and NMDA receptor subunits NR1 and NR2B (*GRIN1*, *GRIN2B*) (Arzberger et al. 1997). More recently, microarray experiments have demonstrated that large number of mRNAs are both up- and down-regulated as a consequence of HD (Luthi-Carter et al. 2002; Luthi-Carter et al. 2000). This effect is not restricted to the brain, but has also been observed in the blood (Borovecki et al. 2005) (although these findings are controversial—see (Runne et al. 2007)). Within the brain, substructure-specific transcriptional profiles have been observed, with greatest number of altered mRNAs in the caudate and motor cortex (Hodges et al. 2006). A number of strategies have been employed to produce mouse HD models, including insertion of additional CAG repeats into the wild-type mouse locus (Wheeler et al. 1999), and overexpression of the mutant first exon of human Htt (Mangiarini et al. 1996). The latter, known as the R6/2 mouse, has been a widely used model that appears to recapitulate many of the disease symptoms. Encouragingly, the brains of R6/2 and other mice display broadly similar transcriptional profiles to those observed in human sufferers (Kuhn et al. 2007; Strand et al. 2007), suggesting that various transgenic modifications accurately model the disease state at the molecular level. For technical reasons, the consequent dysregulation of protein levels is challenging to observe, although a recent mass spectrometry study did observe protein dysregulation in the brains of R6/2 mice (Zabel et al. 2002).

Gene dysregulation in HD occurs before outward neurological symptoms (Augood et al. 1997), strongly suggesting that transcriptional dysregulation is an important causative factor in the disease. Clearly, understanding the cause of this gene dysregulation is a major aim in HD research, since such pathways represent promising therapeutic targets (Leone et al. 2008). To date, a diverse set of transcriptional dysregulation pathways have been identified in HD (reviewed by Cha 2007). These pathways either involve transcriptional regulation by mutHtt itself, or altered activity of transcriptional regulatory proteins which

interact with it. The Htt protein is capable of binding DNA and directly represses transcription of genes through recruitment of co-repressor complexes such as CtBP, mSin3A and NCoR (Benn et al. 2008; Steffan et al. 2000; Boutell et al. 1999; Kegel et al. 2002). This repression may be accentuated by mutant Htt (Kegel et al. 2002). Furthermore, Htt appears to physically interact with a number of important transcriptional regulatory proteins, TBP (Huang et al. 1998), TAFII130 (Dunah et al. 2002), Sp1 (Dunah et al. 2002), p53 (Steffan et al. 2000) and REST (RE1-Silencing Transcription Factor) (Zuccato et al. 2003). Some of these themselves contain polyglutamine repeats and physically interact with Htt or mutHTT (McCampbell et al. 2000; Nakamura et al. 2001). Between them, these proteins are capable of regulating many thousands of genes in multiple tissues. Paradoxically, while the HD state is characterised by a global upregulation of Sp1 protein (that antagonizes cell death in response to mutHtt), many important target genes display reduced Sp1 binding (Dunah et al. 2002; Chen-Plotkin et al. 2006; Qiu et al. 2006). Transcriptional dysregulation appears to be mediated by abnormal histone modifications at key genes in affected cells (Sadri-Vakili et al. 2007; Kim et al. 2008). In particular, downregulated genes are hypoacetylated, and it appears that restoration of these genes' promoter acetylation underlies the therapeutic effect of histone deacetylase inhibitors in HD models (Sadri-Vakili et al. 2007). Thus, multiple interconnected transcriptional and epigenetic pathways are affected in HD, and these represent obvious targets for development of therapeutics (Sadri-Vakili et al. 2007; Rigamonti et al. 2007).

REST in Huntington's Disease

Amongst the best understood molecular mechanism for HD neurodegeneration is that involving aberrant neural gene repression by the transcription factor REST. REST is an essential vertebrate transcriptional repressor that is highly expressed in the neural progenitors of the developing nervous system, where its down-regulation is necessary (and in some cases seems to be sufficient) for neuronal differentiation (Ballas et al. 2001; Chen et al. 1998; Greenway et al. 2007; Sun et al. 2005; Ooi and Wood 2007). REST can also be detected in some regions of the adult nervous system, particularly the hippocampus, where it is upregulated in response to seizure (Palm et al. 1998) and ischaemia (Calderone et al. 2003). REST represses a large cohort of neuron-specific genes, through specific recruitment to a long DNA regulatory motif, the repressor element 1 (RE1) (Chong et al. 1995; Schoenherr and Anderson 1995). Through its N- and C-terminal repression domains, REST nucleates the formation of a multisubunit complex consisting of histone-modifying and chromatin-remodelling activities that serve to repress gene transcription (reviewed by Ooi and Wood (2007)). A number of recent studies, both bioinformatic and experimental, have yielded detailed genomic maps of REST binding sites, giving us an unprecedented understanding of target genes for a single transcription factor (Bruce et al. 2004; Johnson et al. 2006; Mortazavi et al. 2006; Schoenherr et al. 1996).

While mutant Htt is expressed throughout the brain (and indeed throughout the body), certain neuronal populations appear particularly sensitive to it—in particular the medium spiny neurons of the striatum (Reiner et al. 1988). These neurons rely on brain derived neurotrophic factor (BDNF) transported from the cortex via corticostriatal afferents to survive (Altar et al. 1997). A long standing hypothesis is that striatal death in HD can be explained by loss of this trophic support (reviewed in (Zuccato and Cattaneo 2007)). What process disrupts BDNF production in cortical neurons? In 2001, the group of Elena Cattaneo were able to show that wild-type Htt protein is capable of activating transcription of the *BDNF* gene (Zuccato et al. 2001). Importantly, mutant Htt represses transcription of the *BDNF* gene, offering the causal link between the HD mutation, loss of cortical BDNF signalling and striatal death (Zuccato et al. 2001). This effect is mediated specifically by the second promoter of the complex *BDNF* gene (promoter II). Crucially, in a subsequent publication the group showed that Htt repression of *BDNF* gene transcription occurs specifically through an RE1 element in Promoter II, thus implicating REST in HD (Zuccato et al. 2003). Immunostaining and co-immunoprecipitation experiments indicate that Htt protein sequesters REST protein in the cytoplasm of wild-type neurons. This interaction is not direct; rather, Htt indirectly binds to REST through two intermediate proteins, dynactin p150^{glued} and RILP (REST/NRSF-interacting LIM domain protein) (Shimojo 2008). However, polyglutamine expansion appears to disrupt this interaction with RILP (Shimojo 2008), and expression of mutant Htt led to a redistribution of REST into the nucleus, resulting in transcriptional repression of *BDNF* (Zuccato et al. 2003). Interestingly, other promoters of the BDNF gene (promoters III and IV) are also repressed in the presence of mtHtt, presumably by REST-independent mechanisms (Zuccato et al. 2001).

More recently, it was shown that this effect is not confined to the *BDNF* gene. Other REST target genes are ubiquitously repressed in the presence of mutHtt (Zuccato et al. 2007). This raises the possibility that many of the 1000–2000 predicted REST target genes are repressed in HD neurons, and that HD is characterised by a large scale repression of the neuronal transcriptome. Below we will demonstrate that this is indeed the case, and that this pathway can explain a significant fraction of gene dysregulation in HD.

MicroRNAs in Neurodevelopment and Neurodegeneration

Since their original discovery in nematodes, thousands of microRNAs have been identified throughout metazoa (Griffiths-Jones 2004) (they have also recently been reported in a single-celled alga (Zhao et al. 2007)), and are considered to represent a major post-transcriptional gene regulatory mechanism. MicroRNAs probably regulate the majority of all mRNAs, and, since their recent discovery (circa 2001 (Ruvkun 2001)) have been implicated throughout the fields of development, physiology and human disease. MicroRNAs represent an elegant mechanism by which mRNAs are repressed or degraded in a sequence specific manner. They are transcribed by RNA Pol II and generally undergo two-step RNase-directed processing to yield a final single-stranded 21 nt RNA (Bartel 2003). This "mature" microRNA, is incorporated into the repressive RNA-induced silencing complex (RISC) and serves to target mRNAs via semi-complementary sequences called microRNA response elements (MRE) (Bartel 2009). The precise details of microRNA:mRNA recognition are not understood, and are likely to be a combination of complementary RNA base pairing and RNA secondary structure both within the MRE, and in adjacent mRNA regions (Bartel 2009). This makes accurate prediction of microRNA targets extremely challenging, despite various innovative bioinformatic approaches that have been attempted (Rajewsky and Socci 2004). The mRNAs that are targeted by this pathway are repressed by translational inhibition and/or degradation—how the choice is made between these two fates remains unclear (Filipowicz et al. 2008). In this way, microRNAs appear to be capable of repressing both large cohorts of genes within a given cell to maintain cell identity, or regulating specific individual genes in a particular pathway. MicroRNAs have been shown to be involved in most aspects of metazoan biology, including maintenance of stem cell pluripotency (Gangaraju and Lin 2009), embryonic patterning (Giraldez et al. 2005), development of the nervous system (Giraldez et al. 2005; Leucht et al. 2008), development of the cardiovasculature (Chen et al. 2006; Zhao et al. 2007), neuronal morphology (Schratt et al. 2006) and synaptic transmission (Vo et al. 2005)—and are emerging as key players in diverse human diseases including cancer (Lu et al. 2005; He et al. 2005), Tourette's syndrome (Abelson et al. 2005), cardiovascular disease (Care et al. 2007) and schizophrenia (Stark et al. 2008). However, in many cases the mechanism by which microRNA causes disease remains unclear—are a small number of target mRNAs important, or rather is the large scale protein repression sometimes the key factor?

MicroRNAs are essential gene regulators in the nervous system, both during development and patterning, and in the function of mature neurons (Kosik 2006). MicroRNA processing is necessary for neural development in zebrafish, where maternal zygotic knockout of Dicer results in impaired formation of neural tube, spinal column and retina (Giraldez et al. 2005). In mammals, a number of conserved microRNAs are induced during neural development and specifically expressed within the mature nervous system (Sempere et al. 2004; Smirnova et al. 2005; Krichevsky et al. 2003; Miska et al. 2004; Bak et al. 2008). Neural microRNAs mir-9/9* (9* indicates the 21 nt RNA that is processed from the opposite strand of the pre-miRNA hairpin, and which can sometimes be functional) and mir-124 (also known as mir-124a) modulate neurogenesis as embryonic stem cells are differentiated towards neurons in vitro (Krichevsky et al. 2006). A model neural-specific microRNA, mir-124 is highly and specifically expressed in the CNS of both mammals (Sempere et al. 2004) and flies (Aboobaker et al. 2005). A large number of non-neuronal mRNAs have mir-124 recognition sequences, and are capable of being degraded en masse in the presence of the microRNA (Lim et al. 2005). In developing neurons, mir-124 appears to facilitate the differentiation process by promoting neuron-specific splicing of mRNAs through repression of the splicing protein PTB1 (Makeyev et al. 2007). A separate study showed that the presence of mir-124 promotes neurite outgrowth in the P19 model of neural differentiation (Yu et al. 2008). Similarly, mir-9 is specifically expressed in neural cells (Sempere et al. 2004). In the developing mouse telencephalon, mir-9 expression is thought to control the differentiation of Cajal–Retzius neurons through repression of the *Foxg1* mRNA (Shibata et al. 2008). In zebrafish embryos, mir-9 defines the activity and maintenance of the midbrain–hindbrain boundary by repressing components of the Fgf signaling pathway (Leucht et al. 2008).

MicroRNAs also regulate gene expression in the mature nervous system—in particular at the synapse. For example, mir-132 is emerging as a critical component of the activity-dependent gene regulatory response in neurons. Localised to the pre-synaptic terminal, mir-132 is capable of repressing MeCP2, leading to activation of BDNF, which in turn functions as an activator of mir-132. In response to synaptic activity, mir-132 is transcriptionally induced by CREB (cAMP response element binding), a key positive regulator of dendrite growth (Vo et al. 2005; Wayman et al. 2008). It promotes dendrite growth through repression of the mRNA encoding p250GAP, a Rho GTPase activating protein (Vo et al. 2005). In contrast, another neural microRNA, mir-134 negatively regulates dendrite growth via repression of the mRNA encoding Limk1 (Schratt et al. 2006).

In light of their central role in the nervous system, it is not surprising that microRNA dysregulation is associated with diseases of this organ. Studies in a range of models consistently show that microRNAs—both individually and collectively—have neuroprotective functions. In fruit flies, the microRNA mir-8 prevents neurodegeneration through repression of atrophin (Karres et al. 2007). A separate study in flies showed that loss of microRNA processing exacerbates the toxicity of the polyQ mutant form of the protein SCA3 (Bilen et al. 2006). A similar effect is observed in mouse Purkinje neurons, where complete loss of microRNA processing by targeted removal of Dicer leads to ataxia and death (Schaefer et al. 2007). Elsewhere, other studies have implicated particular microRNAs whose loss results in neurodegeneration. In midbrain dopaminergic neurons, mir-133b regulates differentiation and function within a circuit including the transcription factor Pitx3 (Kim et al. 2007). Significant mir-133b downregulation is observed in human sufferers and mouse models of Parkinson's disease. Another microRNA, mir-107, is decreased in the brains of Alzheimer's disease (AD) sufferers, where it appears to regulate the mRNA encoding BACE1, the protease that degrades amyloid precursor protein (APP) during AD progression (Wang et al. 2008). A subsequent study showed that, in AD patients, the increase in BACE1 levels appears to result from a loss in expression of two upstream microRNAs, mir-29a and mir-29b-1 (Hebert et al. 2008). Furthermore APP itself may be a microRNA target—in cell lines, its levels are controlled by mir-106a and mir-520c (Patel et al. 2008). More generally, the expression of various microRNAs has been shown to change as a result of prion-induced neurodegeneration (Saba et al. 2008). Thus, loss of microRNA expression appears to be widely associated with neurodegeneration, and this pathway is an attractive target for therapeutic strategies.

Evidence for Dysregulation of MicroRNAs in Huntington's Disease

The role that microRNAs appear to play in the prevention of neurodegeneration, make them candidates in Huntington's disease. Two recent studies have demonstrated that the neural microRNA system is indeed perturbed in HD (Johnson et al. 2008; Packer et al. 2008). Expression of multiple neural microRNAs is decreased in HD neurons, resulting in derepression of downstream target mRNAs. These findings are important for two reasons: first, as we will demonstrate below, they help explain observed mRNA dysregulation in HD brains; second, they offer a new avenue for potential therapeutic intervention in HD.

An obvious strategy for finding candidate microRNAs in HD is to identify those that are targets of REST, since it has been shown that many of its target genes are dysregulated in HD (Zuccato et al. 2007). Conveniently, REST has very well understood genomic targeting (Johnson et al. 2007, 2008; Otto et al. 2007), and was amongst the first transcription factors to be shown to regulate microRNA transcription (Johnson et al. 2008; Conaco et al. 2006; Wu and Xie 2006). In fact, REST appears to be a major regulator of neural noncoding RNAs (Johnson et al. 2008, 2009; Conaco et al. 2006). Its targets include some of the best-characterised and most specifically neuronal microRNAs, including: mir-9/9*, mir-29a, mir-29b, mir-124 and mir-132. A comprehensive list of known and predicted REST target microRNAs can be found in Table 1. Two studies have recently examined the expression of predicted REST target microRNAs in mouse and human HD samples, and shown that the majority are indeed aberrantly repressed by REST in HD (Johnson et al. 2008; Packer et al. 2008). Our group employed a genome-wide map of predicted REST binding motifs to predict 14 putative target microRNAs, and tested their dysregulation in the cortex and hippocampus of R6/2 mice, as well as in the Brodman Area 4 of Vonsattel Grade 3-4 human postmortem brains (Johnson et al. 2008). Packer et al. (2008) predicted ten REST-target miRNAs based on analysis of a more recent experimental ChIP-sequencing dataset of REST binding sites, and validated their expression in human postmortem Brodman Area 4 from a range of disease grades. The latter list is almost a subset of the former; intriguingly, Packer et al. realised that both arms of the mir-9 precursor miRNA can be functional, and thus also tested mir-9* for dysregulation. On the whole, both studies showed that the majority of predicted REST target microRNAs are dysregulated in human HD samples, or mouse models of HD—underlining the validity of the target prediction process. However, in some cases the two studies reported strongly contrasting measurements of relative miRNA expression in HD compared to wild-type, suggesting that the experimental methodology, or perhaps heterogeneity of human postmortem samples, may have confounding effects on microRNA quantitation.

There is strong evidence for direct regulation of neural microRNAs by REST. Of five predicted REST-target microRNAs that could be detected in the neural cell-line *Hdh7/7*, four were demonstrably repressed by REST: mir-29a and mir-29b, as well as potent repression of mir-132 and mir-135b (Johnson et al. 2008). There is also complementary EMSA and ChIP data supporting direct genomic recruitment and DNA sequence recognition by REST at these microRNA genes (Johnson et al. 2008; Conaco et al. 2006). In another study, Conaco et al. (2006) showed that in mouse embryonic fibroblasts, REST binds to and

Table 1 Known microRNA targets of REST

MicroRNA	Publication	Evidence for regulation by REST	Implication in HD
mir-1-d	J	C	
mir-9	CJPW	C	Downregulated in HD (Packer et al. 2008)
mir-9*	P	C	Downregulated in HD (Packer et al. 2008)
mir-29a	JPW	R	Contradictory: Upregulated in HD, but downregulated in mouse cortex (Johnson et al. 2008)
mir-29b	JPW	R	Downregulated in HD (Packer et al. 2008)
mir-95	W	M	
mir-124	CJPW	C	Downregulated in HD (Packer et al. 2008) and mouse cortex/hippocampus (Johnson et al. 2008)
miR-132	CJPW	R	Contradictory: Downregulated in HD and mouse cortex/hippocampus (Johnson et al. 2008); Upregulated in HD (Packer et al. 2008)
mir-135b	JPW	R	Downregulated in mouse cortex (Johnson et al. 2008)
mir-139	JPW	C	
mir-153	W	M	
mir-203	J	C	
mir-204	J	C	Downregulated in mouse hippocampus (Johnson et al. 2008)
mir-212	JPW	C	
mir-218	PW	C	
mir-330	J	C	Upregulated in HD (Johnson et al. 2008)
mir-346	JW	C	
mir-455	W	M	

All known or predicted targets of REST from previous publications are included. Publications: C, Conaco et al. (2006); W, Wu and Xie (2006); J, Johnson et al. (2008); P, Packer et al. (2008). Evidence for regulation: C, chromatin immunoprecipitation; M, RE1 motif identification; T, transcriptional repression. Differential expression of microRNAs in human HD patients or R6/2 mouse model is shown where appropriate. Expression levels of microRNAs were measured by PCR-based methods in all cases

represses mir-9 and mir-124 genes, in addition to that of mir-132. Thus there is multiple consistent evidence that REST directly binds to and regulates transcription of neural microRNAs.

In our recent study, we screened candidate microRNAs in both mouse models of HD as well as in RNA extracted from the cortices of HD sufferers (Johnson et al. 2008). We profiled 14 candidate microRNAs in the cortex and hippocampus of R6/2 mice, of which six displayed statistically-significant differences compared to wild type littermates. Mir-124 and mir-132 were downregulated in both cortex and hippocampus of transgenic mice. Mir-204, about which little is known, was significantly downregulated in the hippocampus. Surprisingly, we found that the dysregulated microRNAs were not identical between the cortex and the hippocampus of these mice: for example, mir-29a was downregulated in the cortex but not the hippocampus, while mir-204 was not detected in the cortex but was detectable and downregulated in the hippocampus. Thus, the microRNA targets of REST-mediated dysregulation are likely to vary across brain regions and neuronal subtypes of affected individuals. Such tissue specificity may help explain the localised toxicity of mutHtt in the brains of human and mouse.

In their study, Packer et al. compared the levels of 10 processed microRNAs in the cortices (Brodman Area 4) of individuals with various grades of HD to unaffected individuals. A robust and progressive loss of mir-9, mir-9* and mir-29b was observed with increasing HD severity. While the level of mir-124 was significantly decreased, its level did not appear to correlate with disease progression. Surprisingly, a threefold *increase* was observed in HD for mir-132. The latter findings strongly contrast with our studies on both R6/2 transgenic mice (where mir-132 levels were decreased in both cortex and hippocampus), as well as in cortical samples (Brodman Area 4) from five HD-affected individuals (Vonsattel Grade 3-4), where mir-132 levels were observed to be significantly lower. A decrease in mir-132 levels is consistent with its being strongly repressed by REST in neural and non-neural cells, which was demonstrated in (Johnson et al. 2008; Conaco et al. 2006). Furthermore, the mRNA from *p250GAP*—a known target of mir-132 (Vo et al. 2005)—is upregulated in HD cortex, consistent with a decrease in mir-132. On balance, these

data argue that mir-132 is repressed in the presence of mtHtt. Other discrepancies were observed: while in our study we found no change in human patients, Packer et al. found mir-124 to be downregulated in HD (consistent with R6/2 mouse). Below we will present a bioinformatic analysis that supports this downregulation of mir-124 in human HD brain (Fig. 1). On the other hand, we found mir-29a to be strongly upregulated in HD, while Packer et al. found no significant change. These discrepancies are likely to result from the distinct methodologies employed to profile miRNA expression, as well as the ever present issue of human postmortem brain RNA quality and consistency. The Packer study employed a more sensitive Taqman assay to measure mature microRNA levels, while our study used SYBR green qPCR to measure pre-mir hairpin levels that may not accurately reflect the level of mature miRNA present. Indeed, as Packer et al. point out, it is possible that mir-132 undergoes extensive posttranscriptional regulation. These considerations highlight the importance of using multiple models and techniques for studies of this type, particularly given our reliance on relatively small cohorts of postmortem brains. Furthermore, as we will demonstrate below, bioinformatic analysis may be used as a powerful independent method for predicting dysregulated miRNAs.

MicroRNAs are regulatory molecules, and hence good confirmatory evidence for their change in expression comes from resultant alterations in the levels of their target genes. Fortunately, neural microRNAs such as mir-124 and mir-132 have amongst the best understood set of target mRNAs, based on experimental evidence rather than solely on bioinformatic prediction. As mentioned above, we observed significant downstream increases in 4/5 known target mRNAs of mir-124 (in the R6/2 mouse cortex), as well as the sole known target of mir-132 in human, *p250GAP* (Vo et al. 2005)—consistent with observed downregulation of those microRNAs. Indeed, the REST complex may also be itself a target of microRNAs. Feedback loops of this type are common throughout biology, enabling as they do complex regulatory behaviours. The REST cofactor SCP-1 is a target of mir-124 in the developing nervous system, indicating that REST can participate in mutually-repressive feedback mechanisms with neural microRNAs (Visvanathan et al. 2007). Using the TargetScan algorithm (Lewis et al. 2003), Packer et al. discovered predicted target sites for mir-9 in the 3′ UTR of the REST mRNA, while for mir-9* there is a predicted site in the 3′ UTR of CoREST. This is intriguing since it offers the possibility that the REST complex and mir-9/9* locus

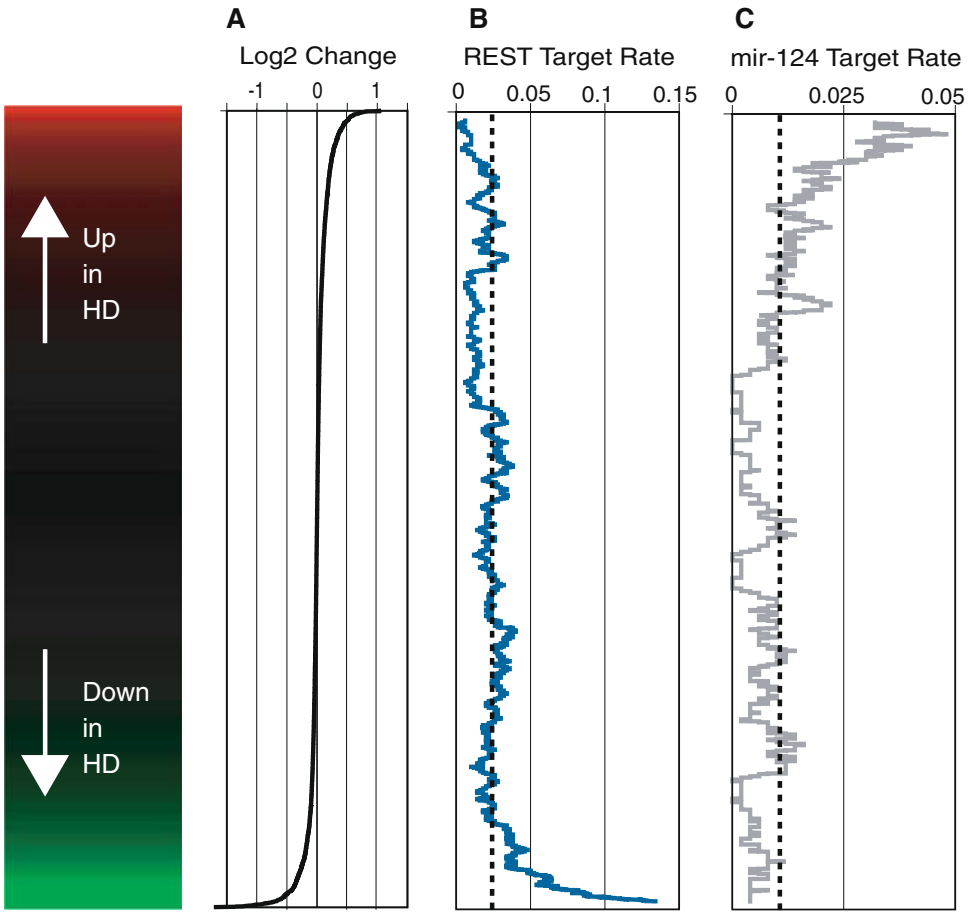

Fig. 1 (Color online) Disruption of transcriptional and post-transcriptional pathways can explain gene changes in HD. In order to assess the contribution of transcriptional and post-transcriptional regulation to mRNA changes in HD, we analysed mRNA data from a previous microarray experiment on the caudate of HD patients (Hodges et al. 2006). **a** The leftmost panels show genes ranked by fold change—red indicates those increased in HD brain, green decreased. **b** Targets of REST (defined as those having a REST binding site within 10 kb of transcription start site (Johnson et al. 2007)) or **c** mir-124 (identified experimentally (Lim et al. 2005)) were identified. The target gene density was plotted for a moving window of 100 genes across the ranked gene list. REST targets are clearly enriched amongst those genes most downregulated in HD caudate, while mir-124 targets are enriched amongst the most upregulated genes

mutually repress each other—in effect, a positive feedback relationship. Unfortunately this model remains to be validated: if correct, this regulation would be manifested in an increase of REST mRNA or protein in the HD brain—which has not been reported. There is some evidence to the contrary: we found no significant difference in REST mRNA levels in HD brain (Johnson et al. 2008), while Zuccato et al. (2003) showed that CoREST protein levels are unaffected by mutHtt. Therefore, it remains to be demonstrated whether this regulation of REST/CoREST by mir-9/9* actually occurs in human HD brain.

New MicroRNA Candidates in HD

The set of known microRNAs is still growing in human, due to ever deeper sequencing of small RNA libraries and improving bioinformatic methods (Friedlander et al. 2008; Landgraf et al. 2007). Furthermore, improvements in our predictions of genomic REST binding sites mean that our previous predictions of REST target genes are likely to be incomplete (Johnson et al. 2007). To discover new potential microRNA candidates in HD, we compared the latest human microRNA set to recent REST ChIP-Seq maps (Table 2). If we use a rather stringent definition that REST-target microRNA genes must reside within 20 kb of a REST binding site, then we predict 21 new target microRNAs. This list comprises 22 individual microRNA genes, targeted by 21 REST binding sites. Amongst these are brain-enriched microRNAs mir-129, mir-137, mir-184 and mir-7 (Bak et al. 2008). The latter is highly expressed in the nervous system and is encoded by three individual loci, of which two are targeted by REST. This is reminiscent of other known targets, mir-9 and mir-124, which are encoded by multiple genes that are each targeted by REST (Johnson et al. 2008; Conaco et al. 2006). Another target, mir-184 was recently shown to be expressed in neurons and upregulated in response to depolarization (Nomura et al. 2008). These microRNAs are now promising targets for dysregulation in HD. Experimental validation will be necessary to show whether these microRNAs are repressed by REST in HD.

A Role for Htt in the MicroRNA Repression Pathway

In addition to indirectly regulating the transcription of microRNA genes, Htt has a more direct role in post-transcriptional gene silencing by microRNAs. A recent seminal publication from Naoko Tanese's group (Savas et al. 2008)

Table 2 New predicted REST target microRNAs in human

MicroRNA	Chr	REST Binding Location (bp)	Distance (bp)	Comments
mir-146b	10	104169341	16919	
mir-1249	22	43991355	15790	Intron C22orf9
mir-1301	2	25419003	13909	Intron *DNMT3A*
mir-637	19	3922364	9854	Intron *DAPK3*
mir-602	9	139843034	9658	
mir-147	9	122054467	7318	
mir-1208	8	129226590	4955	
mir-129-2	11	43555156	4364	Cortex-specific (L)
mir-1179	15	86950133	2210	
mir-7-2	15	86950133	2180	Brain-specific (S); Hypothalamus, pituitary (B)
mir-1255a	4	102472775	2122	Intron *PPPC3A*
mir-137	1	98286437	1867	Brain-specific (S)
mir-1253	17	2600093	1514	
mir-184	15	77287671	913	Brain-specific (B)
mir-1224	3	185440974	363	Intron *DKFZp761K032*
mir-7-3	19	4721319	214	Brain-specific (S); Hypothalamus, pituitary (B). Intron *PGSF1*
mir-422a	15	61950057	1487	
mir-1257	20	59960627	9645	
mir-940	16	2271394	9772	
mir-375	2	219564903	12819	Pancreatic islet-specific (L)
mir-1267	13	106968779	17058	
mir-328	16	65776741	16919	Intron *ELMO3*

In order to find new candidate microRNA targets, we extracted all microRNA genes from the latest version of Mirbase (Griffiths-Jones 2004) (Release 12) within 20 kb of an experimentally-identified REST binding site (Johnson et al. 2007). Intronic microRNAs are all on the same strand as host gene, except for mir-328. Sources of microRNA expression data: B, Bak et al. (2008); L, Landgraf et al. (2007); S, Sempere et al. (2004)

has shown that Htt protein plays a major role in the microRNA silencing pathway by stabilizing the interaction of Ago2 with P-bodies (Processing bodies), both key components of the microRNA silencing pathway. In a mass spectrometry analysis of Htt immunoprecipitates, Savas et al. found proteins Argonaute 1 and 2 to be specifically bound. This was intriguing given that Ago proteins are responsible for microRNA-induced gene regulation (Meister et al. 2005). Ago proteins are capable of sequestering target mRNAs to cytoplasmic structures known as P-bodies. It appears that not only is Htt necessary for correct P-body formation, but that the PolyQ mutant protein inhibits their formation by around 50%. This has a major impact on the microRNA silencing pathway in affected cells: both exogenous and endogenous microRNA silencing was severely impaired in the presence of mutHtt. These findings suggest that microRNA processing as a whole may be impaired in HD. Since broad-based loss of microRNA processing has been found to cause degeneration of Purkinje neurons (Schaefer et al. 2007), it is possible that this loss of microRNA processing leads to HD neurodegeneration.

Integrating Biochemical and Genomic Data to Explain Gene Dysregulation in Huntington's Disease

Huntington's disease is remarkably well-studied from a genomic point of view. A wealth of high quality gene expression microarray data is available from both mouse models (Luthi-Carter et al. 2002) and large cohorts of human patients (Hodges et al. 2006). More recently, high-throughput sequencing technology has been coupled to chromatin immunoprecipitation, ("ChIP-Seq") enabling sensitive genome-wide mapping of transcription factor binding (Johnson et al. 2007). Indeed, REST was amongst the first factors to be studied in this way (Johnson et al. 2007). Undoubtedly these technologies will soon be applied to HD. In particular, the integration of diverse genome-wide datasets (e.g., transcription factor binding, mRNA expression, microRNA expression) will enable a more meaningful, holistic understanding of disease mechanisms underlying complex disorders such as HD. Existing hypotheses can be tested with statistical rigour, and new disease mechanisms can be predicted in silico and validated experimentally. In the following sections we present a simple pilot study on previously published datasets to demonstrate how such analysis can provide a robust, genome-wide validation of HD mechanisms.

Transcriptional Dysregulation by REST

First, we sought to address whether increased nuclear REST contributes to transcriptional dysregulation in HD, as proposed by Elena Cattaneo's group (Zuccato et al. 2003). While multiple lines of evidence exist to support increased levels of nuclear REST in HD, it is not clear whether this mechanism actually contributes substantially to transcriptional dysregulation observed in HD brains. To investigate this, we compared HD gene expression profiles with genomic maps of REST recruitment. If REST plays a major role in transcriptional dysregulation of HD, we would expect that genes observed to change in HD are enriched for REST target genes. More specifically, given that REST is a transcriptional repressor, we would expect that the association would only be observed for genes that are downregulated in HD. Upregulated genes should show no enrichment for REST binding above what is expected by chance. We used the microarray survey of gene expression in a large cohort of HD patients and matched controls that was carried out by Hodges et al. (2006), in conjunction with the recently published genome-wide map of 1946 REST binding sites as identified by ChIP-Seq (Johnson et al. 2007). We noted that amongst the REST target genes are some that have long been known to be downregulated in HD, including *DRD1*, *DRD2*, *GluR1* and *GluR2* (Cha 2007).

Hodges et al. (2006) measured gene expression changes in four brain regions in a large cohort of HD and control postmortem brain. We analysed data from the caudate, where the most extensive gene expression changes were observed (although similar conclusions were reached with motor cortex data (Brodman Area 4) where large mRNA changes also occur). Genes were ranked by their fold change in expression in HD brain (Fig. 1). We next calculated the proportion of genes that are REST targets, for every point in the list (Fig. 1). This analysis clearly shows that REST target genes are enriched amongst the genes whose expression decreases in HD. This effect is highly statistically significant ($P = 4 \times 10^{-14}$, Pearson chi-square test using continuity correction). Furthermore, it is clear that REST target genes are most enriched amongst the most highly repressed genes. Amongst genes whose expression *increases* in HD, the rate of REST target genes is the same as what would be expected by chance alone (indicated by the dashed line). These findings strongly support the Cattaneo group's model of increased REST repression of its target genes in HD brain (Zuccato et al. 2003).

Post-transcriptional Dysregulation by mir-124

Using a similar approach we sought to provide independent validation that expression of the microRNA mir-124 is repressed in HD brain, as was reported recently by Packer et al. (2008). If this model is correct, we would expect HD-upregulated mRNAs to be enriched for mir-124 targets. As before, we consulted the human HD microarray expression

data from human caudate (Hodges et al. 2006), this time in conjunction with a published set of 174 experimentally-determined mRNA targets of mir-124 (Lim et al. 2005). We found strong support for downregulation of mir-124 in both caudate (Fig. 1) and motor cortex (not shown) of HD patients: mir-124 target genes are highly and statistically significantly enriched amongst those that are upregulated in HD ($P = 2 \times 10^{-10}$). This enrichment is not observed for downregulated genes, nor for another microRNA, mir-1, which is not expressed in the brain (Johnson et al. 2008b) (data not shown). Consequently, the mRNA data strongly support the loss of mir-124 expression in HD, as suggested by Packer et al. (2008).

Altogether in the HD caudate, 5.2% of downregulated genes are targets of REST, and 2.2% of upregulated genes are targets of mir-124. These analyses are likely to have large false-negative rates, due to the fact that target relationships were curated from non-neural cell lines (REST in Jurkat T-cells (Johnson et al. 2007), mir-124 in HeLa cervical carcinoma (Lim et al. 2005)). We are hopeful that similar analyses involving other known HD pathways—both transcriptional (e.g., p53, Sp1) (Dunah et al. 2002; Chen-Plotkin et al. 2006; Qiu et al. 2006; Bae et al. 2005) and post-transcriptional (e.g., mir-132, mir-29) (Johnson et al. 2008; Packer et al. 2008)—will be carried out when appropriate experimental datasets become available. It may be possible to explain the majority of HD-associated gene expression changes using this approach.

Do Other Classes of Noncoding RNAs Play a Role in HD?

MicroRNAs are not the sole class of regulatory non-coding RNA. Recent and ongoing high-throughput transcriptomic and bioinformatic projects have identified tens of thousands of unannotated, noncoding RNAs—both polyadenylated and not (Carninci et al. 2005; Ravasi et al. 2006). These RNAs—referred to as lncRNAs (long non-coding RNAs) (Dinger et al. 2008) or macroRNAs (Ponjavic et al. 2007)—do not have well-defined structural or sequence properties, unlike the microRNAs—making them difficult to classify and preventing us from predicting their function based on recognisable sequence or structural domains. Clear evidence exists, however, that macroRNAs are functional: they are under demonstrable evolutionary selection (Ponjavic et al. 2007; Pollard et al. 2006) and often have highly tissue-specific expression profiles (Ravasi et al. 2006; Mercer et al. 2008). Furthermore, a growing number of individual macroRNA have been show to play important roles within the cell. Recent reports have identified macroRNAs that act as transcriptional cofactors (Lanz et al. 1999; Feng et al. 2006), epigenetic silencers (Rinn et al. 2007; Pandey et al. 2008), trafficking molecules (Willingham et al. 2005), antisense regulators of mRNAs (Tochitani and Hayashizaki 2008) and structural components in the nucleus (Clemson et al. 2009). They are also capable of affecting gene expression through the proximal promoter (Preker et al. 2008) and by interacting with the RNA Pol II complex (Mariner et al. 2008). Hence it has been proposed that macroRNAs play widespread but hitherto unappreciated roles in disease (Mehler and Mattick 2007), and evidence is emerging to this effect (Ji et al. 2003; Perez et al. 2008; Louro et al. 2007). There is also some evidence that macroRNAs are capable of rapid evolution, and may contribute to human-specific phenotypes (Lipovich et al. 2006; Varki and Altheide 2005). Thus, it is intriguing to speculate that such recently-evolved non-coding RNAs may play a role in the human-specific susceptibility to particular diseases, including Alzheimer's disease (Varki and Altheide 2005; Olson and Varki 2003), and could explain aspects of human diseases that are not recapitulated in mouse models. Indeed, exciting evidence for the role of noncoding RNA in neurodegenerative disease has recently emerged: a newly discovered antisense noncoding RNA, *BACE1-AS*, is capable of upregulating *BACE1* mRNA levels, and shows increased expression in AD patients (Faghihi et al. 2008).

Could macroRNAs be involved in HD? Certainly, they have several key features of HD candidate genes. Their transcription is highly regulated during differentiation (Dinger et al. 2008). Many are specifically expressed in the nervous system, and furthermore, a recent analysis of the Allen Brain Atlas identified macroRNAs that are specifically expressed in particular neuronal subpopulations or in specific structures within the mouse brain (Mercer et al. 2008). Most tellingly, their transcription can be regulated by REST: our group recently showed that REST is capable of regulating expression of neural macroRNAs, including *DGCR5*, which has been implicated in the neurodevelopmental disorder, DiGeorge Syndrome (Johnson et al. 2009). Therefore, macroRNAs, and particularly those with proximal REST binding sites, are likely to be dysregulated in HD. Such macroRNAs will not have been identified before the present because they are poorly represented amongst existing gene catalogues, and therefore not represented on commercial microarray platforms. The key challenge now is to identify these targets and isolate those which play essential roles in neural function, and hence whose loss contributes to HD progression. This will be possible in the near future, as macroRNA microarrays become available, in addition to sensitive unbiased sequencing-based transcriptomic methods such as RNA-Seq (Mortazavi et al. 2008; Farh et al. 2005). We anticipate that studies of this type, followed by appropriate loss-of-function screens in appropriate cell models, will yield new noncoding players in HD phenotype.

How Might Transcriptional and Post-transcriptional Dysregulation Contribute to HD Neurodegeneration?

The preceding sections demonstrate that mRNA and protein changes in HD are likely to result from disruption of both transcriptional and post-transcriptional regulatory pathways. The next challenge is to use this information to gain insight into HD progression.

Both REST and the neural microRNAs are capable of repressing large numbers of downstream target genes. MicroRNAs and their target genes tend to have inverse spatial expression patterns in the organism (Farh et al. 2005; Sood et al. 2006). Thus, neural microRNAs are inferred to target non-neural mRNAs—i.e., those which are generally expressed at higher levels outside the nervous system. This has been demonstrated in the case of mir-124 (Lim et al. 2005). Conversely, REST target genes are known to be highly enriched for those that are specifically expressed in the nervous system (Johnson et al. 2006; Mortazavi et al. 2006). Thus, the combined changes in REST and neural microRNAs in HD might be expected to lead to a general loss of neural-specific mRNAs, and an increase of non-neural mRNAs. We tested whether this was the case, by searching for enriched functional categories of genes amongst the upregulated and downregulated genes in the HD caudate using the Panther tool (www.pantherdb.org) (Fig. 2). This analysis demonstrated that there is a highly significant enrichment for various classes of neuronal ontology terms amongst downregulated genes, including "neuronal activities" ($P = 1 \times 10^{-53}$) and "synaptic transmission" ($P = 5 \times 10^{-35}$). Meanwhile, a large number of non-neuronal ontology classifications are enriched amongst genes which are upregulated in HD, including many related to the haematopoietic system ("immunity and defence", $P = 2 \times 10^{-20}$) and transcriptional regulation ("transcriptional regulation", $P = 2 \times 10^{-16}$). These data, consistent with a previous, substantial analysis by Strand et al. (2007), suggest that in HD neurons the transcriptome becomes fundamentally less neuronal. How this observation relates to neurodegeneration remains unclear. However, it may be possible that this phenotypic shift results in toxicity to affected neurons, as exemplified by the decrease in BDNF signalling previously described (Zuccato and Cattaneo 2007). Recently, mir-124 was shown to promote neural-specific splicing patterns, through post-transcriptional repression of the non-neuronal form of the splicing factor, PTB (Makeyev et al. 2007). Thus, the decrease in mir-124 levels is likely to promote the accumulation of non-neuronal splice isoforms in neurons. In summary, the disruption of transcriptional, splicing and translational regulatory pathways in HD neurons may, in combination, cause a pathological loss of the specifically neuronal nature of the transcriptome.

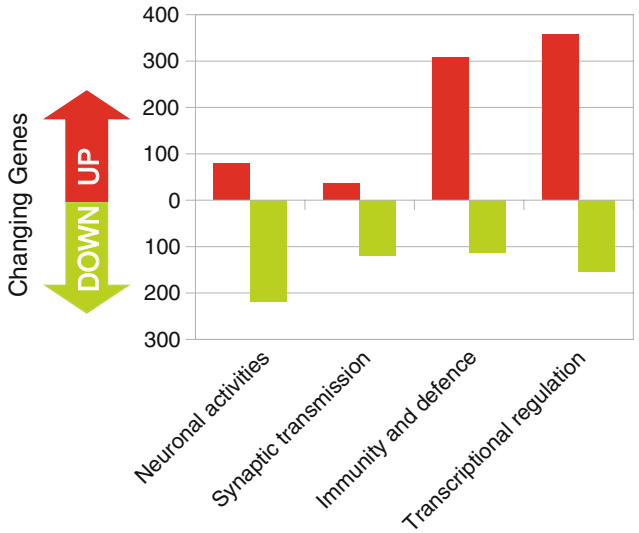

Fig. 2 (Color online) Loss of neuronal transcriptome in HD. The Figure shows the numbers of genes amongst upregulated (*red*) and downregulated (*green*) mRNAs within the indicated ontology categories. As before, we used gene expression data for caudate from the Hodges study (Hodges et al. 2006). Those genes that increase, or decrease in the HD brain were collected, and the numbers of genes with each gene ontology label within those sets were counted. Analysis was carried out using the Panther gene classification tool (www.pantherdb.org), and statistical significance cited in the text was Bonferroni corrected for multiple hypothesis testing

The observed microRNA changes in HD may also directly lead to alterations in neuronal function. Although our understanding remains incomplete, from what we know already it seems likely that microRNAs are key regulators of neuronal function. Specifically, mir-124 and mir-132 have both been shown to promote neurite growth. Thus, chronic downregulation of these molecules may lead to a loss of neuronal connectivity. We showed here that mir-184 is also a candidate target of REST, and hence may similarly be dysregulated in HD. Intriguingly, mir-184 was recently shown to be regulated in response to neuronal depolarization (Nomura et al. 2008). Thus, loss of mir-184 may also contribute to disruption of synaptic transmission.

Complex regulatory responses are enabled by the wiring of various kinds of feedback loops into gene networks. The available data suggest that some self-reinforcing feedback behaviours may exacerbate the gene dysregulation resulting from mutant Htt. One such is the previously-published feedback loop involving REST, mir-124 and the REST cofactor, CTDSP1 (also known as SCP1) (Visvanathan et al. 2007). *CTDSP1* mRNA is a target of mir-124. Thus, the loss of mir-124 in HD neurons may in turn promote the accumulation of CTDSP1 and hence reinforce REST repression of target genes (including mir-124) in a repressive positive feedback loop (Fig. 3). Similarly,

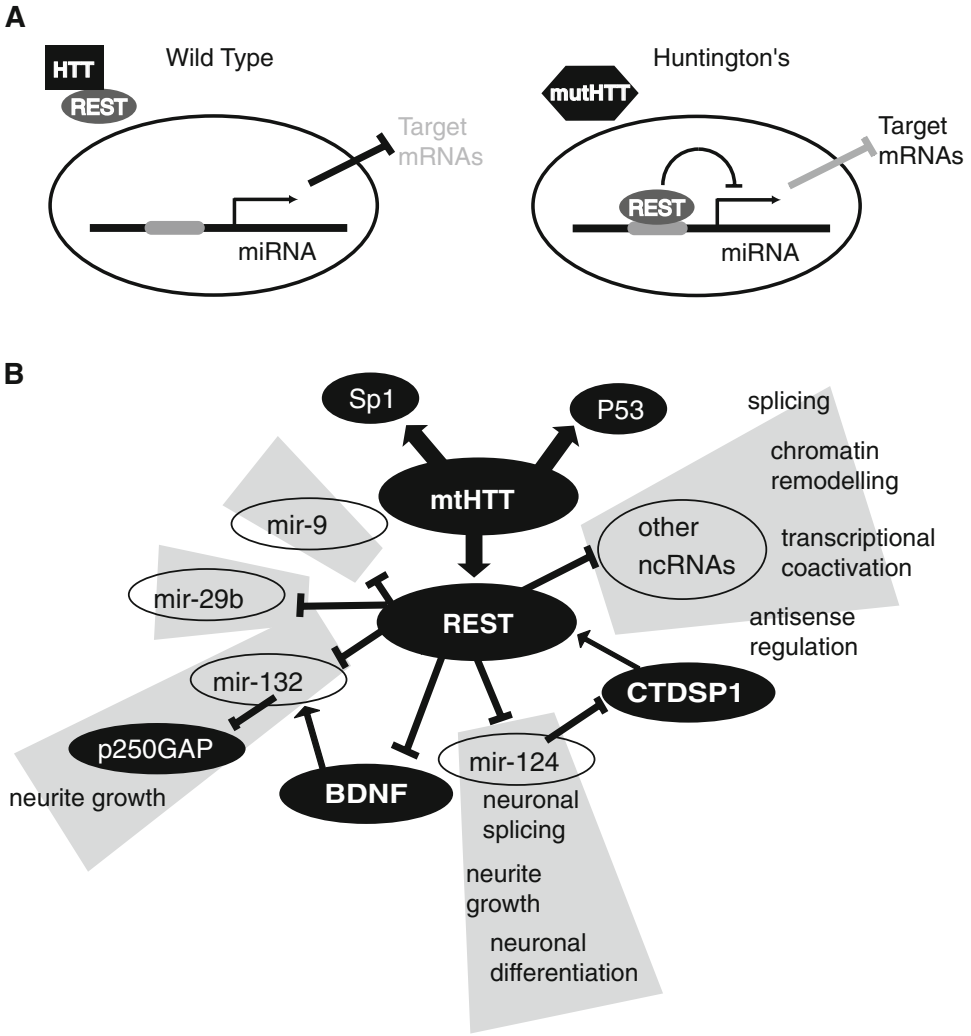

Fig. 3 Our current model of gene dysregulation in HD. **a** In wild type neurons, Htt sequesters REST in the cytoplasm. Neural microRNAs are transcribed at normal levels, leading to post-transcriptional repression of appropriate mRNAs. In neurons of Huntington's disease sufferers, mutHtt is unable to effectively bind REST (Zuccato et al. 2003). REST protein levels are elevated in the nucleus leading to transcriptional repression of neural microRNAs. Consequently, various non-neuronal mRNAs are derepressed post-transcriptionally. **b** A model of transcriptional and post-transcriptional gene regulatory disruption in HD. Proteins and RNAs are indicated by filled and unfilled ovals, respectively. Sp1 and p53 activities are affected by mutHtt (Dunah et al. 2002; Chen-Plotkin et al. 2006; Qiu et al. 2006; Bae et al. 2005), and are likely to have as-yet-unknown microRNA targets. Physiological processes that are likely to be downregulated in the HD state are indicated. Other REST target microRNAs, not included in the Figure, are likely to exist and participate in HD

feedback may accelerate the loss of mir-132 expression: while REST directly represses mir-132 transcription, REST also represses BDNF, a CREB-dependent activator of mir-132. Thus, REST may effectively repress mir-132 expression by two independent pathways, in effect a repressive feed-forward loop.

The majority of the data regarding microRNAs in HD is focussed on mechanisms downstream of REST. However, numerous publications have demonstrated that other regulatory disruptions take place in HD. For example, p53 function is upregulated in HD (Bae et al. 2005). Although little is presently known about the microRNA targets of p53 (apart from mir-34 (He et al. 2007)), such targets will represent additional HD candidates. Similar arguments apply to Sp1.

Finally, it is possible that neurodegeneration results not from downregulation of particular microRNAs per se, but rather that an overall reduction of the microRNA pathway is to blame. Compelling evidence suggests that the microRNA system is neuroprotective (Karres et al. 2007; Bilen et al. 2006; Schaefer et al. 2007; Kim et al. 2007). It is not clear what individual microRNAs mediate this effect. Given that mutant Htt disrupts the endogenous microRNA processing structures within the cell, it is possible that this disruption of the microRNA pathway contributes to Htt neurodegeneration.

Concluding Remarks

The discovery that noncoding RNAs such as microRNAs are dysregulated in HD provides an important new avenue for understanding this major disease. Given the large number that are expressed in the mammalian nervous system, we suspect that other noncoding RNAs—including macroRNAs—play a role in HD. As we have shown, newly discovered microRNAs can help explain the observed mRNA changes that accompany the disease state. As the molecular basis of HD becomes better understood, it will help focus attention on therapeutic strategies. Hopefully, this will also yield insight into enduring mysteries connected with HD: Why does age of onset negatively correlate with polyQ repeat length (Wheeler et al. 1999)? Why do carriers of mutant Htt alleles have greater reproductive fitness (Eskenazi et al. 2007)? Why does mutation in this ubiquitous protein lead to loss of such a specific neuronal subpopulation? We are hopeful that improved understanding of regulatory changes in HD brain, resulting from a fine grained mapping of gene expression patterns (including non-coding RNAs) throughout the brains of affected individuals, as well as large-scale integration of HD-related genomic datasets, will shed light on these questions.

Acknowledgements We wish to thank Chiara Zuccato (University of Milan), Andrew M. Thomson (Genome Institute of Singapore) and Lawrence W. Stanton (Genome Institute of Singapore) for advice, discussions and critical reading of the manuscript. RJ is a postdoctoral fellow funded by the Singapore Agency for Science, Technology and Research (A*STAR).

References

Abelson, J. F., Kwan, K. Y., O'Roak, B. J., Baek, D. Y., Stillman, A. A., et al. (2005). Sequence variants in SLITRK1 are associated with Tourette's syndrome. *Science, 310*, 317–320. doi:10.1126/science.1116502.

Aboobaker, A. A., Tomancak, P., Patel, N., Rubin, G. M., & Lai, E. C. (2005). Drosophila microRNAs exhibit diverse spatial expression patterns during embryonic development. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 18017–18022. doi:10.1073/pnas.0508823102.

Altar, C. A., Cai, N., Bliven, T., Juhasz, M., Conner, J. M., et al. (1997). Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature, 389*, 856–860. doi:10.1038/39885.

Arzberger, T., Krampfl, K., Leimgruber, S., & Weindl, A. (1997). Changes of NMDA receptor subunit (NR1, NR2B) and glutamate transporter (GLT1) mRNA expression in Huntington's disease—an in situ hybridization study. *Journal of Neuropathology and Experimental Neurology, 56*, 440–454. doi:10.1097/00005072-199704000-00013.

Augood, S. J., Faull, R. L., & Emson, P. C. (1997). Dopamine D1 and D2 receptor gene expression in the striatum in Huntington's disease. *Annals of Neurology, 42*, 215–221. doi:10.1002/ana.410420213.

Bae, B.-I., Xu, H., Igarashi, S., Fujimuro, M., Agrawal, N., et al. (2005). p53 mediates cellular dysfunction and behavioral abnormalities in huntington's disease. *Neuron, 47*, 29–41. doi:10.1016/j.neuron.2005.06.005.

Bak, M., Silahtaroglu, A., Moller, M., Christensen, M., Rath, M. F., et al. (2008). MicroRNA expression in the adult mouse central nervous system. *RNA, 14*, 432–444. doi:10.1261/rna.783108.

Ballas, N., Battaglioli, E., Atouf, F., Andres, M. E., Chenoweth, J., et al. (2001). Regulation of neuronal traits by a novel transcriptional complex. *Neuron, 31*, 353–365. doi:10.1016/S0896-6273(01)00371-3.

Bartel, D. P. (2003). MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell, 116*, 281–297. doi:10.1016/S0092-8674(04)00045-5.

Bartel, D. P. (2009). MicroRNAs: Target recognition and regulatory functions. *Cell, 136*, 215–233. doi:10.1016/j.cell.2009.01.002.

Benn, C. L., Sun, T., Sadri-Vakili, G., McFarland, K. N., DiRocco, D. P., et al. (2008). Huntingtin modulates transcription, occupies gene promoters in vivo, and binds directly to DNA in a polyglutamine-dependent manner. *Journal of Neuroscience, 28*, 10720–10733. doi:10.1523/JNEUROSCI.2126-08.2008.

Bilen, J., Liu, N., Burnett, B., Pittman, R., & Bonini, N. (2006). MicroRNA pathways modulate polyglutamine-induced neurodegeneration. *Molecular Cell, 24*, 157–163. doi:10.1016/j.molcel.2006.07.030.

Borovecki, F., Lovrecic, L., Zhou, J., Jeong, H., Then, F., et al. (2005). Genome-wide expression profiling of human blood reveals biomarkers for Huntington's disease. *PNAS, 102*, 11023–11028. doi:10.1073/pnas.0504921102.

Boutell, J. M., Thomas, P., Neal, J. W., Weston, V. J., Duce, J., et al. (1999). Aberrant interactions of transcriptional repressor proteins with the Huntington's disease gene product, huntingtin. *Human Molecular Genetics, 8*, 1647–1655. doi:10.1093/hmg/8.9.1647.

Bruce, A. W., Donaldson, I. J., Wood, I. C., Yerbury, S. A., Sadowski, M. I., et al. (2004). Genome-wide analysis of repressor element 1 silencing transcription factor/neuron-restrictive silencing factor (REST/NRSF) target genes. *PNAS, 101*, 10458–10463. doi:10.1073/pnas.0401827101.

Calderone, A., Jover, T., Noh, K.-M., Tanaka, H., Yokota, H., et al. (2003). Ischemic insults derepress the gene silencer REST in neurons destined to die. *Journal of Neuroscience, 23*, 2112–2121.

Care, A., Catalucci, D., Felicetti, F., Bonci, D., Addario, A., et al. (2007). MicroRNA-133 controls cardiac hypertrophy. *Nature Medicine, 13*, 613–618. doi:10.1038/nm1582.

Carninci, P., Kasukawa, T., Katayama, S., Gough, J., Frith, M. C., et al. (2005). The transcriptional landscape of the mammalian genome. *Science, 309*, 1559–1563. doi:10.1126/science.1112014.

Cattaneo, E., Zuccato, C., & Tartari, M. (2005). Normal huntingtin function: an alternative approach to Huntington's disease. *Nature Reviews Neuroscience, 6*, 919–930. doi:10.1038/nrn1806.

Cha, J. H. (2007). Transcriptional signatures in Huntington's disease. *Progress in Neurobiology, 83*, 228–248. doi:10.1016/j.pneurobio.2007.03.004.

Chen, J.-F., Mandel, E. M., Thomson, J. M., Wu, Q., Callis, T. E., et al. (2006). The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nature Genetics, 38*, 228–233. doi:10.1038/ng1725.

Chen, Z.-F., Paquette, A. J., & Anderson, D. J. (1998). NRSF/REST is required in vivo for repression of multiple neuronal target genes during embryogenesis. *Nature Genetics, 20*, 136–142. doi:10.1038/2431.

Chen-Plotkin, A. S., Sadri-Vakili, G., Yohrling, G. J., Braveman, M. W., Benn, C. L., et al. (2006). Decreased association of the transcription factor Sp1 with genes downregulated in Huntington's disease. *Neurobiology of Disease, 22*, 233–241. doi:10.1016/j.nbd.2005.11.001.

Chong, J., Tapia-Ramirez, J., Kim, S., Toledo-Aral, J., Zheng, Y., et al. (1995). REST: A mammalian silencer protein that restricts sodium channel gene expression to neurons. *Cell, 80*, 949–957. doi:10.1016/0092-8674(95)90298-8.

Clemson, C. M., Hutchinson, J. N., Sara, S. A., Ensminger, A. W., Fox, A. H., et al. (2009). An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. *Molecular Cell, 33*(6), 717–726.

Conaco, C., Otto, S., Han, J.-J., & Mandel, G. (2006). Reciprocal actions of REST and a microRNA promote neuronal identity. *PNAS, 103*, 2422–2427. doi:10.1073/pnas.0511041103.

Dinger, M. E., Amaral, P. P., Mercer, T. R., Pang, K. C., Bruce, S. J., et al. (2008). Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. *Genome Research, 18*, 1433–1445. doi:10.1101/gr.078378.108.

Dunah, A. W., Jeong, H., Griffin, A., Kim, Y. M., Standaert, D. G., et al. (2002). Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. *Science, 296*, 2238–2243. doi:10.1126/science.1072613.

Eskenazi, B. R., Wilson-Rich, N. S., & Starks, P. T. (2007). A Darwinian approach to Huntington's disease: Subtle health benefits of a neurological disorder. *Medical Hypotheses, 69*, 1183–1189. doi:10.1016/j.mehy.2007.02.046.

Faghihi, M. A., Modarresi, F., Khalil, A. M., Wood, D. E., Sahagan, B. G., et al. (2008). Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of beta-secretase. *Nature Medicine, 14*, 723–730. doi:10.1038/nm1784.

Farh, K. K., Grimson, A., Jan, C., Lewis, B. P., Johnston, W. K., et al. (2005). The widespread impact of mammalian microRNAs on mRNA repression and evolution. *Science, 310*, 1817–1821. doi:10.1126/science.1121158.

Feng, J., Bi, C., Clark, B. S., Mady, R., Shah, P., et al. (2006). The Evf-2 noncoding RNA is transcribed from the Dlx-5/6 ultraconserved region and functions as a Dlx-2 transcriptional coactivator. *Genes and Development, 20*, 1470–1484. doi:10.1101/gad.1416106.

Ferrante, R. J., Kowall, N. W., Beal, M. F., Richardson, E. P., Jr., Bird, E. D., et al. (1985). Selective sparing of a class of striatal neurons in Huntington's disease. *Science, 230*, 561–563. doi:10.1126/science.2931802.

Filipowicz, W., Bhattacharyya, S. N., & Sonenberg, N. (2008). Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? *Nature Reviews. Genetics, 9*, 102–114. doi:10.1038/nrg2290.

Friedlander, M. R., Chen, W., Adamidi, C., Maaskola, J., Einspanier, R., et al. (2008). Discovering microRNAs from deep sequencing data using miRDeep. *Nature Biotechnology, 26*, 407–415. doi:10.1038/nbt1394.

Gangaraju, V. K., & Lin, H. (2009). MicroRNAs: Key regulators of stem cells. *Nature Reviews. Molecular Cell Biology, 10*, 116–125. doi:10.1038/nrm2621.

Giraldez, A. J., Cinalli, R. M., Glasner, M. E., Enright, A. J., Thomson, J. M., et al. (2005). MicroRNAs regulate brain morphogenesis in zebrafish. *Science, 308*, 833–838. doi:10.1126/science.1109020.

Greenway, D. J., Street, M., Jeffries, A., & Buckley, N. J. (2007). RE1 silencing transcription factor maintains a repressive chromatin environment in embryonic hippocampal neural stem cells. *Stem Cells, 25*, 354–363. doi:10.1634/stemcells.2006-0207.

Griffiths-Jones, S. (2004). The microRNA registry. *Nucleic Acids Research, 32*, D109–D111. doi:10.1093/nar/gkh023.

He, L., He, X., Lim, L. P., de Stanchina, E., Xuan, Z., et al. (2007). A microRNA component of the p53 tumour suppressor network. *Nature, 447*, 1130–1134. doi:10.1038/nature05939.

He, L., Thomson, J. M., Hemann, M. T., Hernando-Monge, E., Mu, D., et al. (2005). A microRNA polycistron as a potential human oncogene. *Nature, 435*, 828–833. doi:10.1038/nature03552.

Hebert, S. S., Horre, K., Nicolai, L., Papadopoulou, A. S., Mandemakers, W., et al. (2008). Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 6415–6420. doi:10.1073/pnas.0710263105.

Hedreen, J. C., Peyser, C. E., Folstein, S. E., & Ross, C. A. (1991). Neuronal loss in layers V and VI of cerebral cortex in Huntington's disease. *Neuroscience Letters, 133*, 257–261. doi:10.1016/0304-3940(91)90583-F.

Hodges, A., Strand, A. D., Aragaki, A. K., Kuhn, A., Sengstag, T., et al. (2006). Regional and cellular gene expression changes in human Huntington's disease brain. *Human Molecular Genetics, 15*, 965–977. doi:10.1093/hmg/ddl013.

Huang, C. C., Faber, P. W., Persichetti, F., Mittal, V., Vonsattel, J. P., et al. (1998). Amyloid formation by mutant huntingtin: Threshold, progressivity and recruitment of normal polyglutamine proteins. *Somatic Cell and Molecular Genetics, 24*, 217–233. doi:10.1023/B:SCAM.0000007124.19463.e5.

Huntington's Disease Collaborative Research Group. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell, 72*, 971–983. doi:10.1016/0092-8674(93)90585-E.

Ji, P., Diederichs, S., Wang, W., Boing, S., Metzger, R., et al. (2003). MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. *Oncogene, 22*, 8031–8041. doi:10.1038/sj.onc.1206928.

Johnson, R., Gamblin, R. J., Ooi, L., Bruce, A. W., Donaldson, I. J., et al. (2006). Identification of the REST regulon reveals extensive transposable element-mediated binding site duplication. *Nucleic Acids Research, 34*, 3862–3877. doi:10.1093/nar/gkl525.

Johnson, D., Mortazavi, A., Myers, R., & Wold, B. (2007). Genome-wide mapping of in vivo protein-DNA interactions. *Science, 316*, 1497–1502. doi:10.1126/science.1141319.

Johnson, R., Teh, C. H., Jia, H., Vanisri, R. R., Pandey, T., et al. (2009). Regulation of neural macroRNAs by the transcriptional repressor REST. *RNA, 15*, 85–96. doi:10.1261/rna.1127009.

Johnson, R., Teh, C. H.-L., Kunarso, G., Wong, K. Y., Srinivasan, G., et al. (2008a). REST regulates distinct transcriptional networks in embryonic and neural stem cells. *PLoS Biology, 6*, e256. doi:10.1371/journal.pbio.0060256.

Johnson, R., Zuccato, C., Belyaev, N. D., Guest, D. J., Cattaneo, E., et al. (2008b). A microRNA-based gene dysregulation pathway in Huntington's disease. *Neurobiology of Disease, 29*, 438–445. doi:10.1016/j.nbd.2007.11.001.

Karres, J. S., Hilgers, V., Carrera, I., Treisman, J., & Cohen, S. M. (2007). The conserved microRNA miR-8 tunes atrophin levels to prevent neurodegeneration in Drosophila. *Cell, 131*, 136–145. doi:10.1016/j.cell.2007.09.020.

Kegel, K. B., Meloni, A. R., Yi, Y., Kim, Y. J., Doyle, E., et al. (2002). Huntingtin is present in the nucleus, interacts with the transcriptional corepressor C-terminal binding protein, and represses transcription. *Journal of Biological Chemistry, 277*, 7466–7476. doi:10.1074/jbc.M103946200.

Kim, M. O., Chawla, P., Overland, R. P., Xia, E., Sadri-Vakili, G., et al. (2008). Altered histone monoubiquitylation mediated by mutant huntingtin induces transcriptional dysregulation. *Journal of Neuroscience, 28*, 3947–3957. doi:10.1523/JNEUROSCI.5667-07.2008.

Kim, J., Inoue, K., Ishii, J., Vanti, W. B., Voronov, S. V., et al. (2007). A MicroRNA feedback circuit in midbrain dopamine neurons. *Science, 317*, 1220–1224. doi:10.1126/science.1140481.

Kosik, K. S. (2006). The neuronal microRNA system. *Nature Reviews Neuroscience, 7*, 911–920. doi:10.1038/nrn2037.

Krichevsky, A. M., King, K. S., Donahue, C. P., Khrapko, K., & Kosik, K. S. (2003). A microRNA array reveals extensive regulation of microRNAs during brain development. *RNA, 9*, 1274–1281. doi:10.1261/rna.5980303.

Krichevsky, A. M., Sonntag, K. C., Isacson, O., & Kosik, K. S. (2006). Specific microRNAs modulate embryonic stem cell-derived neurogenesis. *Stem Cells, 24*, 857–864. doi:10.1634/stemcells.2005-0441.

Kuhn, A., Goldstein, D. R., Hodges, A., Strand, A. D., Sengstag, T., et al. (2007). Mutant huntingtin's effects on striatal gene expression in mice recapitulate changes observed in human Huntington's disease brain and do not differ with mutant huntingtin length or wild-type huntingtin dosage. *Human Molecular Genetics, 16*, 1845–1861. doi:10.1093/hmg/ddm133.

Landgraf, P., Rusu, M., Sheridan, R., Sewer, A., Iovino, N., et al. (2007). A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell, 129*, 1401–1414. doi:10.1016/j.cell.2007.04.040.

Lanz, R., McKenna, N., Onate, S., Albrecht, U., Wong, J., et al. (1999). A steroid receptor coactivator, SRA, functions as an RNA and is present in an SRC-1 complex. *Cell, 97*, 17–27. doi:10.1016/S0092-8674(00)80711-4.

Leone, S., Mutti, C., Kazantsev, A., Sturlese, M., Moro, S., et al. (2008). SAR and QSAR study on 2-aminothiazole derivatives, modulators of transcriptional repression in Huntington's disease. *Bioorganic & Medicinal Chemistry, 16*, 5695–5703. doi:10.1016/j.bmc.2008.03.067.

Leucht, C., Stigloher, C., Wizenmann, A., Klafke, R., Folchert, A., et al. (2008). MicroRNA-9 directs late organizer activity of the midbrain–hindbrain boundary. *Nature Neuroscience, 11*, 641–648. doi:10.1038/nn.2115.

Lewis, B. P., Shih, I. H., Jones-Rhoades, M. W., Bartel, D. P., & Burge, C. B. (2003). Prediction of mammalian microRNA targets. *Cell, 115*, 787–798. doi:10.1016/S0092-8674(03)01018-3.

Lim, L. P., Lau, N. C., Garrett-Engele, P., Grimson, A., Schelter, J. M., et al. (2005). Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature, 433*, 769–773. doi:10.1038/nature03315.

Lipovich, L., Vanisri, R. R., Kong, S. L., Lin, C. Y., & Liu, E. T. (2006). Primate-specific endogenous cis-antisense transcription in the human 5q31 protocadherin gene cluster. *Journal of Molecular Evolution, 62*, 73–88. doi:10.1007/s00239-005-0041-3.

Louro, R., Nakaya, H. I., Amaral, P. P., Festa, F., Sogayar, M. C., et al. (2007). Androgen responsive intronic non-coding RNAs. *BMC Biology, 5*, 4. doi:10.1186/1741-7007-5-4.

Lu, J., Getz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., et al. (2005). MicroRNA expression profiles classify human cancers. *Nature, 435*, 834–838. doi:10.1038/nature03702.

Luthi-Carter, R., Hanson, S. A., Strand, A. D., Bergstrom, D. A., Chun, W., et al. (2002). Dysregulation of gene expression in the R6/2 model of polyglutamine disease: parallel changes in muscle and brain. *Human Molecular Genetics, 11*, 1911–1926. doi:10.1093/hmg/11.17.1911.

Luthi-Carter, R., Strand, A., Peters, N. L., Solano, S. M., Hollingsworth, Z. R., et al. (2000). Decreased expression of striatal signaling genes in a mouse model of Huntington's disease. *Human Molecular Genetics, 9*, 1259–1271. doi:10.1093/hmg/9.9.1259.

Makeyev, E. V., Zhang, J., Carrasco, M. A., & Maniatis, T. (2007). The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. *Molecular Cell, 27*, 435–448. doi:10.1016/j.molcel.2007.07.015.

Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., et al. (1996). Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell, 87*, 493–506. doi:10.1016/S0092-8674(00)81369-0.

Mariner, P. D., Walters, R. D., Espinoza, C. A., Drullinger, L. F., Wagner, S. D., et al. (2008). Human Alu RNA is a modular transacting repressor of mRNA transcription during heat shock. *Molecular Cell, 29*, 499–509. doi:10.1016/j.molcel.2007.12.013.

McCampbell, A., Taylor, J. P., Taye, A. A., Robitschek, J., Li, M., et al. (2000). CREB-binding protein sequestration by expanded polyglutamine. *Human Molecular Genetics, 9*, 2197–2202. doi:10.1093/hmg/9.14.2197.

Mehler, M. F., & Mattick, J. S. (2007). Noncoding RNAs and RNA editing in brain development, functional diversification, and neurological disease. *Physiological Reviews, 87*, 799–823. doi:10.1152/physrev.00036.2006.

Meister, G., Landthaler, M., Peters, L., Chen, P. Y., Urlaub, H., et al. (2005). Identification of novel argonaute-associated proteins. *Current Biology, 15*, 2149–2155. doi:10.1016/j.cub.2005.10.048.

Mercer, T. R., Dinger, M. E., Sunkin, S. M., Mehler, M. F., & Mattick, J. S. (2008). Specific expression of long noncoding RNAs in the mouse brain. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 716–721. doi:10.1073/pnas.0706729105.

Miska, E. A., Alvarez-Saavedra, E., Townsend, M., Yoshii, A., Sestan, N., et al. (2004). Microarray analysis of microRNA expression in the developing mammalian brain. *Genome Biology, 5*, R68. doi:10.1186/gb-2004-5-9-r68.

Mortazavi, A., Thompson, E. C. L., Garcia, S. T., Myers, R. M., & Wold, B. (2006). Comparative genomics modeling of the NRSF/REST repressor network: From single conserved sites to genome-wide repertoire. *Genome Research, 16*, 1208–1221. doi:10.1101/gr.4997306.

Mortazavi, A., Williams, B. A., McCue, K., Schaeffer, L., & Wold, B. (2008). Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature Methods, 5*, 621–628. doi:10.1038/nmeth.1226.

Nakamura, K., Jeong, S. Y., Uchihara, T., Anno, M., Nagashima, K., et al. (2001). SCA17, a novel autosomal dominant cerebellar ataxia caused by an expanded polyglutamine in TATA-binding protein. *Human Molecular Genetics, 10*, 1441–1448. doi:10.1093/hmg/10.14.1441.

Nomura, T., Kimura, M., Horii, T., Morita, S., Soejima, H., et al. (2008). MeCP2-dependent repression of an imprinted miR-184 released by depolarization. *Human Molecular Genetics, 17*, 1192–1199. doi:10.1093/hmg/ddn011.

Olson, M. V., & Varki, A. (2003). Sequencing the chimpanzee genome: insights into human evolution and disease. *Nature Review. Genetics, 4*, 20–28. doi:10.1038/nrg981.

Ooi, L., & Wood, I. C. (2007). Chromatin crosstalk in development and disease: lessons from REST. *Nature Reviews Genetics, 8*, 544–554. doi:10.1038/nrg2100.

Otto, S. J., McCorkle, S. R., Hover, J., Conaco, C., Han, J.-J., et al. (2007). A new binding motif for the transcriptional repressor rest uncovers large gene networks devoted to neuronal functions. *Journal of Neuroscience, 27*, 6729–6739. doi:10.1523/JNEUROSCI.0091-07.2007.

Packer, A. N., Xing, Y., Harper, S. Q., Jones, L., & Davidson, B. L. (2008). The bifunctional microRNA miR-9/miR-9* regulates REST and CoREST and is downregulated in Huntington's disease. *Journal of Neuroscience, 28*, 14341–14346. doi:10.1523/JNEUROSCI.2390-08.2008.

Palm, K., Belluardo, N., Metsis, M., & To, Timmusk. (1998). Neuronal expression of zinc finger transcription factor REST/NRSF/XBR gene. *Journal of Neuroscience, 18*, 1280–1296.

Pandey, R. R., Mondal, T., Mohammad, F., Enroth, S., Redrup, L., et al. (2008). Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. *Molecular Cell, 32*, 232–246. doi:10.1016/j.molcel.2008.08.022.

Patel, N., Hoang, D., Miller, N., Ansaloni, S., Huang, Q., et al. (2008). MicroRNAs can regulate human APP levels. *Molecular Neurodegeneration, 3*, 10. doi:10.1186/1750-1326-3-10.

Perez, D. S., Hoage, T. R., Pritchett, J. R., Ducharme-Smith, A. L., Halling, M. L., et al. (2008). Long, abundantly expressed noncoding transcripts are altered in cancer. *Human Molecular Genetics, 17*, 642–655. doi:10.1093/hmg/ddm336.

Pollard, K. S., Salama, S. R., Lambert, N., Lambot, M.-A., Coppens, S., et al. (2006). An RNA gene expressed during cortical development evolved rapidly in humans. *Nature, 443*, 167–172. doi:10.1038/nature05113.

Ponjavic, J., Ponting, C. P., & Lunter, G. (2007). Functionality or transcriptional noise? Evidence for selection within long noncoding RNAs. *Genome Research, 17*, 556–565. doi:10.1101/gr.6036807.

Preker, P., Nielsen, J., Kammler, S., Lykke-Andersen, S., Christensen, M. S., et al. (2008). RNA exosome depletion reveals transcription upstream of active human promoters. *Science, 322*, 1851–1854. doi:10.1126/science.1164096.

Qiu, Z., Norflus, F., Singh, B., Swindell, M. K., Buzescu, R., et al. (2006). Sp1 is up-regulated in cellular and transgenic models of Huntington disease, and its reduction is neuroprotective. *Journal of Biological Chemistry, 281*, 16672–16680. doi:10.1074/jbc.M511648200.

Rajewsky, N., & Socci, N. D. (2004). Computational identification of microRNA targets. *Developmental Biology, 267*, 529–535. doi:10.1016/j.ydbio.2003.12.003.

Ravasi, T., Suzuki, H., Pang, K. C., Katayama, S., Furuno, M., et al. (2006). Experimental validation of the regulated expression of large numbers of non-coding RNAs from the mouse genome. *Genome Research, 16*, 11–19. doi:10.1101/gr.4200206.

Reiner, A., Albin, R. L., Anderson, K. D., D'Amato, C. J., Penney, J. B., et al. (1988). Differential loss of striatal projection neurons in Huntington disease. *Proceedings of the National Academy of Sciences of the United States of America, 85*, 5733–5737. doi:10.1073/pnas.85.15.5733.

Rigamonti, D., Bolognini, D., Mutti, C., Zuccato, C., Tartari, M., et al. (2007). Loss of huntingtin function complemented by small molecules acting as repressor element 1/neuron restrictive silencer element silencer modulators. *Journal of Biological Chemistry, 282*, 24554–24562. doi:10.1074/jbc.M609885200.

Rinn, J., Kertesz, M., Wang, J., Squazzo, S., Xu, X., et al. (2007). Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell, 127*, 1311–1323. doi:10.1016/j.cell.2007.05.022.

Runne, H., Kuhn, A., Wild, E. J., Pratyaksha, W., Kristiansen, M., et al. (2007). Analysis of potential transcriptomic biomarkers for Huntington's disease in peripheral blood. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 14424–14429. doi:10.1073/pnas.0703652104.

Ruvkun, G. (2001). Molecular biology. Glimpses of a tiny RNA world. *Science, 294*, 797–799. doi:10.1126/science.1066315.

Saba, R., Goodman, C. D., Huzarewich, R. L., Robertson, C., & Booth, S. A. (2008). A miRNA signature of prion induced neurodegeneration. *PLoS ONE, 3*, e3652. doi:10.1371/journal.pone.0003652.

Sadri-Vakili, G., Bouzou, B., Benn, C. L., Kim, M. O., Chawla, P., et al. (2007). Histones associated with downregulated genes are hypo-acetylated in Huntington's disease models. *Human Molecular Genetics, 16*, 1293–1306. doi:10.1093/hmg/ddm078.

Savas, J. N., Makusky, A., Ottosen, S., Baillat, D., Then, F., et al. (2008). Huntington's disease protein contributes to RNA-mediated gene silencing through association with Argonaute and P bodies. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 10820–10825. doi:10.1073/pnas.0800658105.

Schaefer, A., O'Carroll, D., Tan, C. L., Hillman, D., Sugimori, M., et al. (2007). Cerebellar neurodegeneration in the absence of microRNAs. *Journal of Experimental Medicine, 204*, 1553–1558. doi:10.1084/jem.20070823.

Schoenherr, C., & Anderson, D. (1995). The neuron-restrictive silencer factor (NRSF): A coordinate repressor of multiple neuron-specific genes. *Science, 5202*, 1360–1363. doi:10.1126/science.7871435.

Schoenherr, C. J., Paquette, A. J., & Anderson, D. J. (1996). Identification of potential target genes for the neuron-restrictive silencer factor. *Proceedings of the National Academy of Sciences, 93*, 9881–9886. doi:10.1073/pnas.93.18.9881.

Schratt, G. M., Tuebing, F., Nigh, E. A., Kane, C. G., Sabatini, M. E., et al. (2006). A brain-specific microRNA regulates dendritic spine development. *Nature, 439*, 283–289. doi:10.1038/nature04367.

Sempere, L. F., Freemantle, S., Pitha-Rowe, I., Moss, E., Dmitrovsky, E., et al. (2004). Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. *Genome Biology, 5*, R13. doi:10.1186/gb-2004-5-3-r13.

Shibata, M., Kurokawa, D., Nakao, H., Ohmura, T., & Aizawa, S. (2008). MicroRNA-9 modulates Cajal–Retzius cell differentiation by suppressing Foxg1 expression in mouse medial pallium. *Journal of Neuroscience, 28*, 10415–10421. doi:10.1523/JNEUROSCI.3219-08.2008.

Shimojo, M. (2008). Huntingtin regulates RE1-silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) nuclear trafficking indirectly through a complex with REST/NRSF-interacting LIM domain protein (RILP) and dynactin p150 Glued. *Journal of Biological Chemistry, 283*, 34880–34886. doi:10.1074/jbc.M804183200.

Smirnova, L., Grafe, A., Seiler, A., Schumacher, S., Nitsch, R., et al. (2005). Regulation of miRNA expression during neural cell specification. *European Journal of Neuroscience, 21*, 1469–1477. doi:10.1111/j.1460-9568.2005.03978.x.

Snell, R. G., MacMillan, J. C., Cheadle, J. P., Fenton, I., Lazarou, L. P., et al. (1993). Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. *Nature Genetics, 4*, 393–397. doi:10.1038/ng0893-393.

Sood, P., Krek, A., Zavolan, M., Macino, G., & Rajewsky, N. (2006). Cell-type-specific signatures of microRNAs on target mRNA expression. *PNAS, 103*, 2746–2751. doi:10.1073/pnas.0511045103.

Stark, K. L., Xu, B., Bagchi, A., Lai, W. S., Liu, H., et al. (2008). Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. *Nature Genetics, 40*, 751–760. doi:10.1038/ng.138.

Steffan, J. S., Kazantsev, A., Spasic-Boskovic, O., Greenwald, M., Zhu, Y.-Z., et al. (2000). The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *PNAS, 97*, 6763–6768. doi:10.1073/pnas.100110097.

Strand, A. D., Baquet, Z. C., Aragaki, A. K., Holmans, P., Yang, L., et al. (2007). Expression profiling of Huntington's disease models suggests that brain-derived neurotrophic factor depletion plays a major role in striatal degeneration. *Journal of Neuroscience, 27*, 11758–11768. doi:10.1523/JNEUROSCI.2461-07.2007.

Sun, Y.-M., Greenway, D. J., Johnson, R., Street, M., Belyaev, N. D., et al. (2005). Distinct profiles of REST interactions with its target genes at different stages of neuronal development.

Molecular Biology of the Cell, 16, 5630–5638. doi:10.1091/mbc.E05-07-0687.

Tochitani, S., & Hayashizaki, Y. (2008). Nkx2.2 antisense RNA overexpression enhanced oligodendrocytic differentiation. *Biochemical and Biophysical Research Communications, 372*, 691–696. doi:10.1016/j.bbrc.2008.05.127.

Varki, A., & Altheide, T. K. (2005). Comparing the human and chimpanzee genomes: searching for needles in a haystack. *Genome Research, 15*, 1746–1758. doi:10.1101/gr.3737405.

Visvanathan, J., Lee, S., Lee, B., Lee, J. W., & Lee, S. K. (2007). The microRNA miR-124 antagonizes the anti-neural REST/SCP1 pathway during embryonic CNS development. *Genes and Development, 21*, 744–749. doi:10.1101/gad.1519107.

Vo, N., Klein, M. E., Varlamova, O., Keller, D. M., Yamamoto, T., et al. (2005). From the cover: A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. *PNAS, 102*, 16426–16431. doi:10.1073/pnas.0508448102.

Vonsattel, J. P., & DiFiglia, M. (1998). Huntington disease. *Journal of Neuropathology and Experimental Neurology, 57*, 369–384. doi:10.1097/00005072-199805000-00001.

Walker, F. O. (2007). Huntington's disease. *Lancet, 369*, 218–228. doi:10.1016/S0140-6736(07)60111-1.

Wang, W. X., Rajeev, B. W., Stromberg, A. J., Ren, N., Tang, G., et al. (2008). The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1. *Journal of Neuroscience, 28*, 1213–1223. doi:10.1523/JNEUROSCI.5065-07.2008.

Wayman, G. A., Davare, M., Ando, H., Fortin, D., Varlamova, O., et al. (2008). An activity-regulated microRNA controls dendritic plasticity by down-regulating p250GAP. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 9093–9098. doi:10.1073/pnas.0803072105.

Wheeler, V. C., Auerbach, W., White, J. K., Srinidhi, J., Auerbach, A., et al. (1999). Length-dependent gametic CAG repeat instability in the Huntington's disease knock-in mouse. *Human Molecular Genetics, 8*, 115–122. doi:10.1093/hmg/8.1.115.

Willingham, A., Orth, A., Batalov, S., Peters, E., Wen, B., et al. (2005). A strategy for probing the function of noncoding RNAs finds a repressor of NFAT. *Science, 309*, 1570–1573. doi:10.1126/science.1115901.

Wu, J., & Xie, X. (2006). Comparative sequence analysis reveals an intricate network among REST, CREB and miRNA in mediating neuronal gene expression. *Genome Biology, 7*, R85. doi:10.1186/gb-2006-7-9-r85.

Yu, J. Y., Chung, K. H., Deo, M., Thompson, R. C., & Turner, D. L. (2008). MicroRNA miR-124 regulates neurite outgrowth during neuronal differentiation. *Experimental Cell Research, 314*, 2618–2633. doi:10.1016/j.yexcr.2008.06.002.

Zabel, C., Chamrad, D. C., Priller, J., Woodman, B., Meyer, H. E., et al. (2002). Alterations in the mouse and human proteome caused by Huntington's disease. *Molecular & Cellular Proteomics, 1*, 366–375. doi:10.1074/mcp.M200016-MCP200.

Zhao, T., Li, G., Mi, S., Li, S., Hannon, G. J., et al. (2007a). A complex system of small RNAs in the unicellular green alga *Chlamydomonas reinhardtii*. *Genes and Development, 21*, 1190–1203. doi:10.1101/gad.1543507.

Zhao, Y., Ransom, J. F., Li, A., Vedantham, V., von Drehle, M., et al. (2007b). Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. *Cell, 129*, 303–317. doi:10.1016/j.cell.2007.03.030.

Zuccato, C., Belyaev, N., Conforti, P., Ooi, L., Tartari, M., et al. (2007). Widespread disruption of repressor element-1 silencing transcription factor/neuron-restrictive silencer factor occupancy at its target genes in huntington's disease. *Journal of Neuroscience, 27*, 6972–6983. doi:10.1523/JNEUROSCI.4278-06.2007.

Zuccato, C., & Cattaneo, E. (2007). Role of brain-derived neurotrophic factor in Huntington's disease. *Progress in Neurobiology, 81*, 294–330. doi:10.1016/j.pneurobio.2007.01.003.

Zuccato, C., Ciammola, A., Rigamonti, D., Leavitt, B. R., Goffredo, D., et al. (2001). Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science, 293*, 493–498. doi:10.1126/science.1059581.

Zuccato, C., Tartari, M., Crotti, A., Goffredo, D., Valenza, M., et al. (2003). Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nature Genetics, 35*, 76–83. doi:10.1038/ng1219.