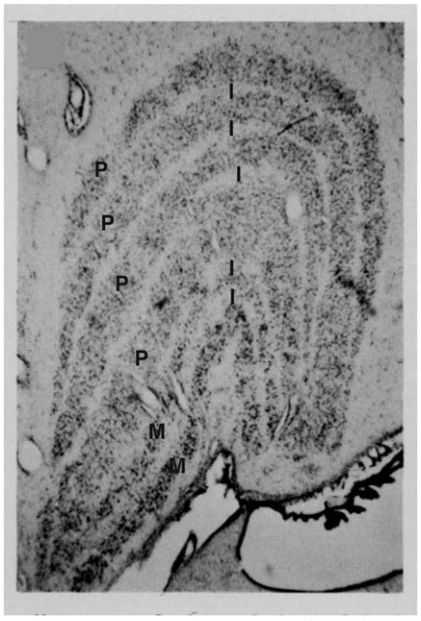

Figure 2. Nissl stained section through a normal macaque monkey's LGN.

P, parvocellular layers; M, magnocellular layers; I, interlaminar layers. Adapted with permission from [17].

stimuli in the field defect. But when the lateral geniculate nucleus was reversibly inactivated by the GABA agonist THIP — the effective region of which was visualised by concurrent structural magnetic imaging and by also injecting the magnetic resonance contrast-agent gadolinium — both the extrastriate activity and the behavioural discrimination were abolished.

As the GABA agonist does not affect the retinal axons passing beneath the LGN *en route* to the superior colliculus, the role of the latter and other mid-brain centres in blindsight has to be re-evaluated. Further steps are likely to include whether the pathway from eye to superior colliculus is indeed important but that the output from superior colliculus is routed via the interlaminar layers of the LGN. It will also be important to record from the amygdala, often implicated in affective blindsight [15,16] such as responses to emotional visual stimuli, in order to determine whether the amygdala has a privileged visual input that is independent of the LGN and might involve the collicular projections to the pulvinar nucleus and/or the medial dorsal thalamus. Lastly, as icing on the cake, it should be possible to demonstrate behaviourally that such monkeys are displaying blindsight when they respond. It seems that the resolution of a longstanding problem is at last in sight.

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Gene Silencing: Small RNAs Control RNA Polymerase II Elongation

Short interfering RNAs trigger histone silencing marks and stalling of RNA polymerase II at their genomic target sites through a mechanism termed transcriptional gene silencing (TGS). The Argonaute protein NRDE-3, along with NRDE-2, are needed for TGS in *C. elegans*. TGS also inhibits elongation and controls alternative splicing in mammalian cells.

Mariano Alló and Alberto R. Kornblihtt*

Three types of small, double-stranded RNAs play fundamental regulatory roles in eukaryotic cells. Although they share many functions, these RNAs differ in length, internal complementarity, protein partners and mechanisms of action. Whereas short interfering RNAs (siRNAs) and microRNAs (miRNAs) are 21–25 nucleotides long, PIWI-interacting RNAs (piRNAs) are 24–31 nucleotides long. The two strands of siRNAs and piRNAs are fully complementary, while a distinctive feature of miRNAs is the presence of internal mismatches. All three types are able to trigger degradation or translational arrest of mRNAs carrying target sequences in a cytoplasmic process known as post-transcriptional gene silencing (PTGS). PTGS elicited by transfection of siRNAs targeting specific mRNAs has become one of the most powerful tools to knockdown gene expression in mammalian cells and study gene function in a rapid, affordable and robust way. A second mechanism triggered by small RNAs, termed transcriptional gene silencing (TGS), is

much less known among the non-specialists but certainly not less important. Unlike PTGS, TGS is a nuclear mechanism that does not act on the mRNA molecule itself but at the gene level, by affecting the template chromatin structure in such a way that transcription is inhibited around the small RNA target site. TGS was discovered in plants, in which small RNAs produced from transgenes promote methylation of homologous endogenous DNA sequences and histone H3 lysine 9 (H3K9), silencing the expression of such sequences. The generation and maintenance of transcriptionally silent centromeric heterochromatin in unicellular eukaryotes like the yeast *Schizosaccharomyces pombe* and the ciliate *Tetrahymena* is also the result of TGS. Several reports confirm the existence of TGS mechanisms in *Drosophila* and *Caenorhabditis elegans* (for a comprehensive review see [1]). In all previous cases, TGS requires Dicer-like enzymes to generate the small RNAs and one or more types of Argonaute proteins: AGO4 in plants, the single AGOs of *S. pombe* and *Tetrahymena*, and AGO2 and PIWI in *Drosophila* and *C. elegans*. Another common feature is the need for base pairing between the small RNA strand (guide strand) interacting with the AGO-containing silencing complex and the nascent target RNA of the gene where the chromatin structure change is introduced.

TGS has also been reported to occur in mammalian cells, although evidence for an endogenous pathway is lacking. Exogenously applied siRNAs targeting promoter regions cause transcriptional inhibition in human cells via recruitment of chromatin modifying enzymes that create typical heterochromatin marks at the target sites: H3K9 dimethylation (H3K9me2), H3K27 trimethylation (H3K27me3), DNA methylation and histone deacetylation [2,3]. These effects require both AGO1 and AGO2 and the siRNA guide strand is supposed to base pair with RNA encoded by the promoter resulting from genome-wide pervasive transcription. Inspired by mammalian TGS, we recently showed that if, instead of targeting promoters, siRNAs are directed to sequences in the body of a gene, mapping at the intron downstream of an alternative exon, the siRNA leading strand base-pairs with the nascent pre-mRNA and subsequently generates a closed chromatin structure that, by creating a roadblock to RNA polymerase II (Pol II) elongation, affects alternative splicing [4]. The effect needs both AGO1 and AGO2 and is abolished or reduced by factors that favor an open chromatin structure or increase transcriptional elongation. The mechanism, named TGS-regulated alternative splicing (TGS-AS), involves the formation of facultative heterochromatin histone marks (H3K9me2 and H3K27me3) at the target site and downstream, plus the recruitment of the heterochromatin protein HP1. These chromatin changes affect the kinetic coupling mechanism between alternative splicing and transcription according to which the rate of transcription elongation determines the outcome of two competing splicing reactions that occur co-transcriptionally. Rapid, highly processive transcription favors exon skipping, whereas slower, less processive transcription favors the inclusion process [5,6].

A recent report published in Nature by the Kennedy group [7] elucidates

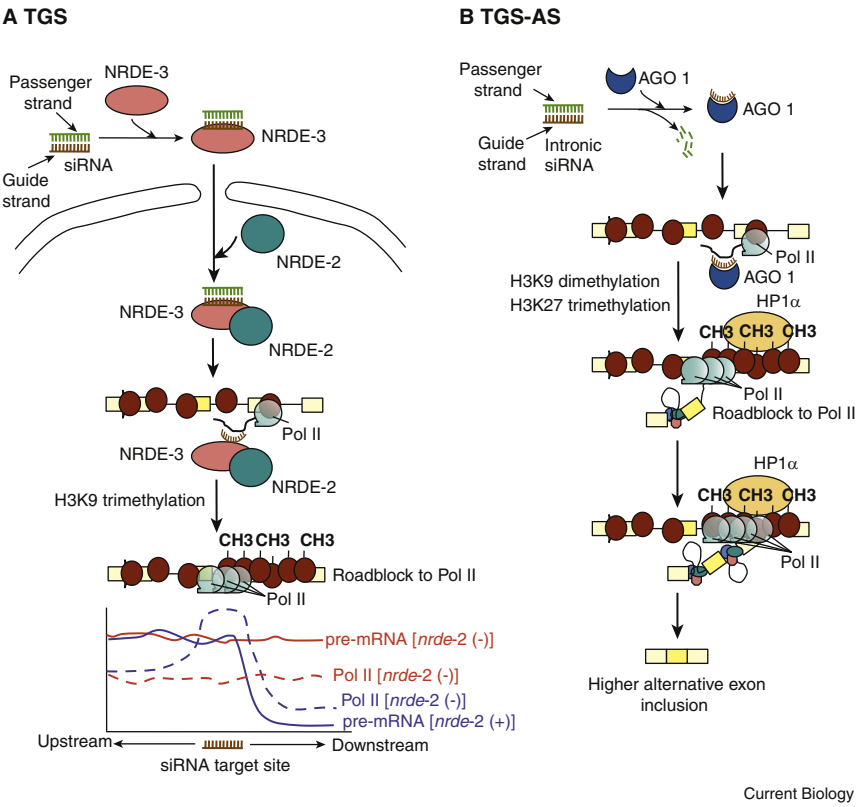

Figure 1. Transcriptional gene silencing (TGS) and TGS-regulated alternative splicing (TGS-AS).

(A) A model for TGS in *C. elegans*. The argonaute protein NRDE-3 transports siRNAs from the cytoplasm to the nucleus. Another argonaute protein, NEDE-2, is necessary for the base pairing between the siRNA guide strand and nascent RNA. This interaction promotes trimethylation of H3K9 (nucleosomes in brown) and subsequent stalling of RNA polymerase II (Pol II). The graph at the bottom illustrates the dramatic decrease in pre-mRNA abundance downstream of the siRNA target site upon siRNA action. This decrease is observed in wild-type (solid blue line) but not in *nrde*-2(-) worms (solid red line). Chromatin immunoprecipitation analysis reveals a peak for Pol II at the siRNA target region in wild-type (broken blue line) but not in *nrde*-2(-) worms (broken red line). Based on Guang *et al.* [7]. (B) A model for TGS-AS in human cells. Transfection with siRNAs targeting the intron mapping downstream of the alternative exon (dark yellow) promotes dimethylation and trimethylation of H3K9 and H3K27, respectively (nucleosomes in brown). Silencing is triggered by a silencing complex involving an siRNA guide strand and AGO1. HP1α is recruited and the resulting condensed chromatin structure generates roadblocks to Pol II elongation, causing higher inclusion of the alternative exon according to the kinetic coupling model. Based on Alló *et al.* [4].

the precise roles of two *C. elegans* Argonaute factors involved in TGS and, more importantly, confirms the importance of TGS in the control of transcriptional elongation. The *C. elegans* Argonaute protein NRDE-3 was known to transport siRNAs from the cytoplasm to the nucleus and therefore to be necessary for TGS. Guang *et al.* [7] identified a new protein, NRDE-2, which is also necessary for nuclear RNA interference (RNAi) (TGS). In fact, *nrde-2* mutant worms fail to both silence specific targets of exogenous siRNAs and produce the dramatic multivulva phenotype induced by RNAi targeting of the endogenous nuclear *lin-15b* RNA. NRDE-2 is a 130 kDa nuclear protein containing a conserved domain of unknown function (DUF) and two domains usually found in RNA processing factors: a serine/arginine-rich domain typical of SR proteins and a half-a-tetratricopeptide (HAT)-like domain. Most interesting, NRDE-2 has single orthologues in plants, *S. pombe*, *Drosophila* and mammals. The authors demonstrate that NRDE-2 functions downstream of NRDE-3 as NRDE-2 mutations do not affect siRNA transport to the nucleus, but this transport *per se* is not sufficient to trigger TGS. Within the nucleus, siRNA–NRDE-3–NRDE-2 complexes are recruited to nascent pre-mRNAs containing target sequences through base pairing with the siRNA guide strand. Several experiments suggest that NRDE-2 dependent TGS inhibits Pol II during elongation. Efficient silencing in *C. elegans* is dependent on the Rpb-7 subunit of Pol II, previously found to be necessary for siRNA-mediated heterochromatin formation in *S. pombe* [8]. As in TGS-AS, siRNAs complexed with NRDE-3 and NRDE-2 interact with unspliced mRNAs, thought to be processed co-transcriptionally. Furthermore, in an NRDE-2-dependent manner, siRNAs elicit H3K9 trimethylation at the target genomic sites and silencing of transcription, but only downstream of the siRNA target site, which was detected both by steady-state pre-mRNA quantification and run-on analysis. Finally, and most conclusively, chromatin immunoprecipitation experiments using anti-Pol II antibodies demonstrate that Pol II peaks at the siRNA target sites in *nrde-2*(+) but not in *nrde-2*(-) cells. The Pol II peak detected in *C. elegans* parallels the transcriptional roadblock postulated to explain the reduction in elongation observed in TGS-AS in human cells [4]. Figure 1 compares the mechanisms for TGS in *C. elegans* and TGS-AS in human cells.

These findings highlight the role of RNAi-mediated chromatin modification in the expression of genes not embedded in constitutive heterochromatin regions like pericentromeric chromatin or highly repetitive sequences. In euchromatic genes, siRNAs can create local, intragenic and restricted heterochromatin marks that would affect Pol II elongation. When transcription is inhibited at the initiation level, only the quantity of the resulting mRNA can be affected. Instead, inhibition of elongation not only affects the quantity but also the quality of the produced mRNA by influencing pre-mRNA processing events such as splicing.

Many questions remain unanswered. What is the function of the NERD-2 orthologue of mammalian cells? Is it necessary for mammalian TGS? Both the NERD-2-dependent TGS in *C. elegans* and mammalian TGS-AS were demonstrated for exogenous siRNAs. Are there endogenous small RNAs playing similar roles? Potential candidates are Piwi RNAs [9] and endogenous siRNAs (esiRNAs) [10], which have been shown to regulate gene expression in mammalian cells through histone modifications and DNA methylation; miRNAs, recently shown to trigger TGS in the moss *Physcomitrella patens* [11] and mammalian cells [12], and double-stranded RNAs resulting from bidirectional and overlapping transcription, and which trigger epigenetic silencing, are also good candidates [13]. Finally, the possibility that intragenic TGS may control alternative splicing in mammalian cells depending on the siRNA target site raises the converse question of whether NRDE-2-dependent TGS controls essential pre-mRNA processing mechanisms in *C. elegans* such as trans-splicing.

The results reported by Guang *et al.* [7] contribute to our evolving view of gene expression, from PTGS to TGS, from chromatin/transcription at promoters to the interior of genes, from initiation to elongation and from putative uniform distributions of Pol II molecules along a gene to a landscape of intragenic Pol II peaks representing barriers to elongation. Most importantly, a detailed knowledge of the mechanisms controlling intragenic chromatin modifications will have a significant impact on the emerging field concerned with the relationship between chromatin and splicing [14,15].

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Signal Transduction: Bacterial Thermometer Works by Measuring Membrane Thickness

Cells detect external chemical stimuli by directly binding a signaling molecule, but the strategies used by cells to detect and respond to non-chemical cues have been mysterious. Recent work suggests that a bacterial protein detects changes in environmental temperature by physically measuring membrane thickness.

Kumaran S. Ramamurthi

The ability to sense environmental conditions and mount an adaptive transcriptional response is conserved in all domains of life. Moreover, reversible protein phosphorylation is a widespread mechanistic strategy for the intracellular transduction of extracellular signals. In bacteria, a paradigmatic signal transduction mechanism (appropriately termed a 'two-component system') utilizes just two proteins [1]. The first is a transmembrane protein called a 'sensor kinase' that detects an environmental signal and transmits the information to the interior of the cell. Sensor kinases have two functional domains: a signal recognition domain (often extracellular) that may physically bind to a ligand, and an intracellular autokinase domain that contains a characteristic histidine residue [2]. Detection of an environmental signal activates the autokinase domain and results in phosphorylation of this histidine. The second component of the system is canonically a soluble transcription factor called a 'response regulator', which is activated when it accepts the phosphoryl group from its cognate sensor kinase. The response regulator then typically activates the transcription of appropriate genes that respond to the extracellular stress. In this model, chemical factors, such as salts, nutrients, and signaling peptides, may be detected by direct binding of these ligands to the sensor kinase, leading to its activation by autophosphorylation (Figure 1A). How, though, can a sensor kinase detect a more abstract, non-chemical environmental cue such as temperature?

In this issue, Cybulski *et al.* [3] report their studies on the *Bacillus subtilis* sensor kinase DesK, which is activated in response to reduced temperature [4]. Specifically, the authors wondered if there is a physical feature of the cell which changes in response to fluctuations in temperature that DesK may physically detect and exploit as an indirect measure for temperature. The plasma membrane of the bacterium was a key candidate for harboring this physical feature for two reasons. First, DesK is a polytopic membrane protein [4]. Second, previous studies had demonstrated that increasing the fluidity of the plasma membrane by increasing the incorporation of branched-chain fatty acids decreased the activation of DesK, suggesting that DesK directly responds to a physical property of the plasma membrane [5]. Now Cybulski *et al.* [3] propose a model in which DesK detects an increase in the *thickness* of the plasma membrane upon a drop in temperature, resulting in DesK activation.

The authors began by performing a systematic deletion analysis of the unwieldy five-pass integral membrane protein and discovered that deletion of just the first transmembrane region (TM1) abolished the ability of DesK to respond to lower temperature and resulted in a constitutively active protein, suggesting that TM1 harbored a temperature-sensing motif. Meanwhile, previous structural studies had indicated that the last transmembrane helix of DesK (TM5) is attached to the autokinase domain through a two helical coiled-coil motif that appeared to be critical for regulation of DesK activity [6]. The authors envisioned a model in which TM1 would detect a drop in temperature and transmit that information to TM5, which would then activate DesK. They therefore created a chimeric transmembrane region, consisting of amino-terminal residues of TM1 and carboxy-terminal residues of TM5, and fused it to the autokinase domain. Remarkably, this simplified DesK, harboring a single engineered transmembrane segment (the minimal thermo-sensor), worked almost as well as wild-type DesK harboring five membrane-spanning helices.

Curiously, the amino terminus of the minimal thermo-sensor contained a cluster of hydrophilic amino acids near the lipid–water interface. At high temperatures, when lipids are disordered and the bilayer is thinner, the authors reasoned that these residues could "float" on the membrane surface, like a buoy, while being tethered to the membrane itself by the hydrophobic residues of the transmembrane region (Figure 1B). In this conformation, the authors supposed that DesK autokinase activity would be low. A drop in temperature, conversely, would increase lipid ordering, resulting in a thicker plasma membrane. In this scenario, the authors predicted that these hydrophilic residues would become forcibly buried into the hydrophobic lipid bilayer, resulting in a conformational change that would activate the autokinase activity of DesK. To test this, the authors lengthened the transmembrane region of the thermo-sensor so that the 'buoy' was farther away from the surface of the membrane and would not be pulled into the bilayer when the membrane