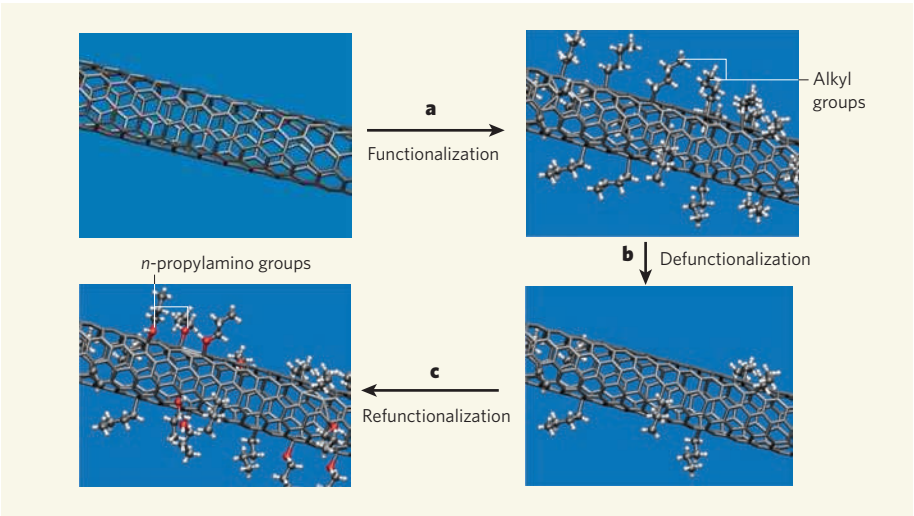

Figure 1 | Reversible modification of carbon nanotubes. Syrgiannis *et al.*[1] report a method for controlling the number and kind of chemical groups attached to a carbon nanotube (CNT). **a**, In the first step (the functionalization step), alkyl groups are attached to a CNT. **b**, When the resultant CNT is treated with lithium or sodium metal in liquid ammonia, some of the alkyl groups are removed (defunctionalization). **c**, Different chemical groups (*n*-propylamino groups) can then be attached to the CNT simply by adding *n*-propylamine to the reaction mixture, in a refunctionalization step. (Images courtesy of F. M. Toma and M. Melli.)

in liquid ammonia, the appended alkyl groups can be thinned out (Fig. 1b). Under these conditions, the metals donate electrons to the nanotubes; when the f-SWCNTs become negatively charged, they start to 'eject' the attached alkyl groups. Even more impressively, some of the alkyl groups could be replaced by *n*-propylamino groups simply by adding *n*-propylamine ($C_3H_7NH_2$) to the reaction mixture (Fig. 1c). In this way, f-SWCNTs containing two different chemical groups could be made.

The ability to attach a wide variety of chemical groups to CNTs opens up new possibilities for their use. For instance, electron-donor groups (such as porphyrins and phthalocyanines) have been covalently attached to SWCNTs. When these nanotubes are exposed to visible light, electrons are transferred from the donors to the SWCNTs[6], mimicking a crucial step in natural photosynthesis: the generation of charge separation to produce photocurrents.

A further example is that CNTs modified to become water soluble have been shown to cross cell membranes readily. In principle, if drug molecules can be attached to such CNTs, then CNTs could be used as drug-delivery vehicles[7]. This could be particularly useful for drugs that have a low bioavailability — those that fail to reach high concentrations in the body because they are extensively metabolized or inefficiently absorbed. The anticancer drugs doxorubicin, cisplatin and paclitaxel (Taxol) have all been attached to CNTs, for example. But it remains to be seen whether CNT-based drug delivery will work successfully and safely in clinical trials.

Neuroscience is another field that might benefit from chemically modified CNTs, which have attracted much interest recently as potential scaffolds to help establish connections between neurons. Part of this interest is because of the electrical properties of CNTs, which are reminiscent of those of small nerve fibres. By combining CNTs with neuronal cultures, it might be possible to make new hybrid materials, paving the way for potential applications in the field of tissue engineering[8].

Techniques for modifying CNTs with organic chemical groups have been in development for many years, but it is no exaggeration to say that the production of pure samples of functionalized CNTs that are all of the same size is still the grand challenge for the field. Achieving this goal will undoubtedly require contributions from the related fields of chemistry, physics and materials science. Any new approach that improves the chemical reactivity of CNTs, such as that reported by Syrgiannis *et al.*[1], is an important step towards complete control of the chemistry of CNTs. This work offers fresh opportunities for the modification of CNTs, provides a crucial tool for exploring how the properties of CNTs change in response to modification, and might ultimately facilitate the rational design of advanced materials. ■

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TRANSCRIPTION

Enhancers make non-coding RNA

Bing Ren

Genomes don't just encode protein-coding RNAs. They also give rise to various groups of RNAs that can regulate gene expression. Short RNAs that form from enhancer sequences might be one such class of regulatory RNA.

Enhancers are well known for their role in activating the transcription of target genes from a distance and, during animal development, they act in a time- and tissue-dependent way[1]. It is also becoming increasingly clear that variations in these genomic sequences contribute to human disease, as well as to the evolution of human-specific traits[2], and that they could be highly abundant in the mammalian genome[3]. But where exactly they are located in the genome and how they function are not well understood. On page 182 of this issue, Kim *et al.*[4] describe the identification of more than 12,000 neuronal enhancers in the mouse genome. They also show that these sequences are involved in regulating gene expression during neuronal activity. But most interestingly, the authors uncover a new class of non-coding RNA — enhancer RNA or eRNA.

Previous models of enhancer function emphasized the role of complexes of transcription factors that bind to these sequences and influence the transcription machinery located downstream at promoter sequences through protein–protein interactions and DNA looping[5]. But studies of several model genetic loci, such as the β-globin gene locus, have revealed[6] that the RNA polymerase enzyme is present at enhancers and that enhancers are transcribed into RNA. How widespread this phenomenon is in cells has been unclear. But as most of the human genome seems to be transcribed[7], enhancers might also give rise to RNA transcripts.

Kim *et al.*[4] now establish that a large number of enhancers do indeed produce RNAs. The authors set out to identify enhancer sequences involved in the activity-dependent regulation of gene expression in mouse cortical neurons

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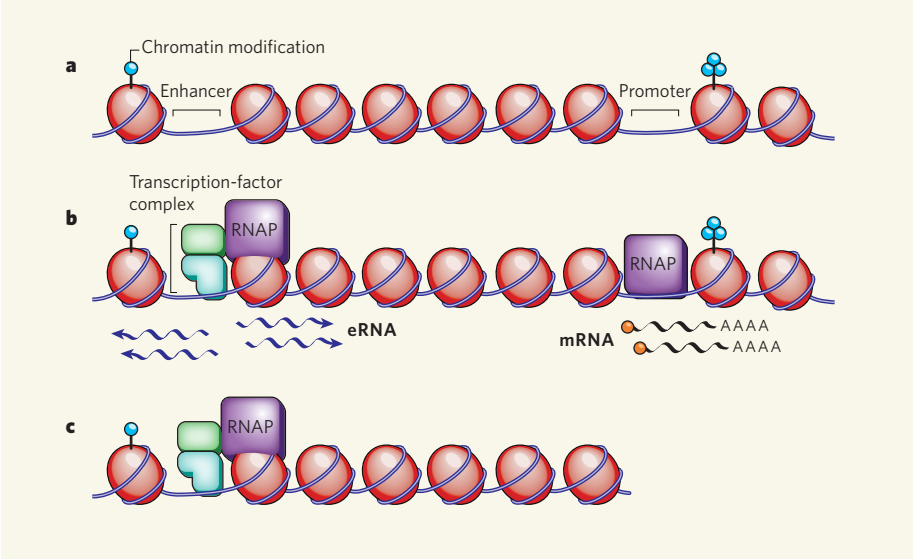

Figure 1 | Unsuspected sites of transcription. **a**, Kim *et al.*[4] find that, before treatment with KCl, neuronal enhancers and promoters are in an open configuration but produce no RNA transcripts, or only low levels of them. **b**, On activation by membrane depolarization following KCl treatment, transcription factors and RNA polymerase (RNAP) bind to enhancers, and enhancer RNA (eRNA) is made. Simultaneously, RNAP and transcription machinery also bind to promoters and initiate mRNA transcription. **c**, If the promoter is truncated, the enhancer still binds transcription factors and RNAP but can no longer make eRNA.

maintained in culture. They treated the neurons with high levels of potassium chloride (KCl), which caused the neuronal cell membrane to become depolarized — resulting in an influx of calcium ions and activation of several calcium-dependent signalling pathways, and eventually leading to the induction of genes regulated by neuronal activity. The authors then determined the enhancers involved by mapping where in the genome the enhancer-binding protein CBP had bound. To exclude any CBP-binding promoter sequences, the group further narrowed down their search to regions associated with an enhancer-specific modification to histone proteins, which together with DNA constitute chromatin[3] (Fig. 1). Kim *et al.* thereby defined more than 12,000 activity-dependent enhancers.

To understand how these enhancers activate their targets, the group investigated the binding of three sequence-specific transcription factors and RNA polymerase II throughout the genome. They found that RNA polymerase II binds not just at promoters, but also at nearly 25% of the identified enhancers. To find out whether the binding of the enzyme had an effect, Kim *et al.* analysed the RNA population in KCl-treated cells, and found transcripts at nearly every enhancer to which RNA polymerase II bound. These eRNAs, unlike messenger RNAs, lacked modification by polyadenylation, were generally short and non-coding, and were bidirectionally transcribed. What's more, eRNA levels were correlated with mRNA synthesis from nearby genes, and in the one case examined, eRNA transcription required the presence of the target promoter.

That only a subset of the enhancers identified by Kim and co-workers produce RNA suggests that eRNA synthesis depends on other factors besides CBP and chromatin modification. The involvement of RNA polymerase II is perhaps not surprising, but the binding of this enzyme is apparently insufficient for the transcription of eRNAs. Kim and colleagues show that eRNA transcription depends on the presence of an intact target promoter (Fig. 1c). In genetically engineered mice lacking the promoter for the *Arc* gene but carrying an intact enhancer upstream, RNA polymerase II still bound to the enhancer but no eRNA transcription was detected[4]. Further experiments are needed to establish why eRNA synthesis depends on the corresponding promoter, and whether other components of the transcription machinery — such as general transcription factors and the mediator complex — have a role.

Are eRNAs involved in enhancer function? There are several ways in which they might be. First, eRNA synthesis may be necessary for activating the corresponding promoter. This possibility is supported by indirect evidence[8] from the β-globin gene locus. When a 1-kilobase-long transcription termination sequence was inserted between the enhancer and the promoter sequences for the β-globin gene, transcription from the enhancer ended prematurely, and activation of the promoter by the enhancer was significantly reduced. This observation suggests that enhancer transcription may be necessary for activating promoters.

Another possibility, which Kim *et al.* propose, is that either eRNAs themselves or their transcription allow enhancers to adopt an open chromatin structure that in turn facilitates gene activation. Indeed, eRNAs may play an active part by interacting with other enhancer-associated proteins. This possibility could be verified by reducing the levels of eRNAs or mutating their sequence, because if they are essential, this should affect the transcriptional activation of targets by enhancers. But if enhancer transcription per se is important, eRNAs may be merely by-products with no particular functions of their own, and their specific sequence may be irrelevant to enhancer function. Future experiments, such as RNA interference (RNAi) or targeted mutagenesis, will help to determine which of these two potential mechanisms might occur.

Finally, another possibility offered by Kim and colleagues[4] is that, by acquiring biological functions that give the organism a competitive advantage, eRNAs are an evolutionary source of new genes. However, as these transcripts are not polyadenylated and so are unstable, there would probably be significant hurdles to overcome before eRNAs could turn into functional genes.

Because variations in enhancers have been implicated in human disease[2], modulating their function might emerge as a targeted strategy for preventing and treating certain illnesses. But manipulating enhancer function is much more difficult than interfering with gene function, for which RNAi has emerged as a powerful tool. There is currently no general approach to reducing the function of specific enhancers. But if eRNAs are indeed necessary for enhancers to activate the transcription of target genes, RNAi could potentially be used to inhibit an enhancer, offering an alternative route to targeted disruption of gene expression.

So, like all groundbreaking discoveries, the finding of eRNA transcription at enhancers poses more questions than it answers. On the one hand, eRNA adds to the growing family of non-coding RNAs, and may further explain the abundance of non-protein-coding RNAs transcribed from much of the human genome[7]. On the other hand, it is still unclear whether these bidirectionally transcribed, non-polyadenylated, short RNA species are functional. Scientists interested in the mechanisms of gene regulation must now contend with yet another player involved in the process. ■

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