Supercycling in DNA and chromatin
Nick Gilbert and James Allan

Supercycling is a fundamental property of DNA and chromatin. It is modulated by polymerase and topoisomerase activities and, through regulated constraint, by DNA/chromatin binding proteins. As a non-covalent and elusive topological modification, supercycling has proved intractable to research despite being a crucial regulator of nuclear structure and function. Recent studies have improved our understanding of the formation, regulation and organisation of supercycling domains in vivo, and reinforce the prospect that the propagation of supercycling can influence local and global chromatin structure. However, to further our understanding the development of new experimental tools and models are required to better dissect the mechanics of this key topological regulator.

Addresses
1 MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, The University of Edinburgh, Edinburgh EH4 2XU, UK
2 Institute of Cell Biology, The University of Edinburgh, Edinburgh EH9 3JR, UK

Corresponding authors: Gilbert, Nick (nick.gilbert@ed.ac.uk) and Allan, James (j.allan@ed.ac.uk)

Introduction
DNA is a dynamic molecule. In its relaxed state it adopts a right-handed helically coiled conformation, the detailed structure of which is dependent on the localised sequence. Winding DNA around its axis introduces supercoils increasing the free energy stored in the molecule; winding in the same direction as the helix introduces positive supercoiling whereas winding in the opposite direction generates negative supercoiling [1,2]. In addition to supercycling derived from changes in DNA twist, it is also a product of the coiling or bending of the helix in space, a parameter commonly termed writhe. twist and writhe are effectively inter-convertible and in a chromatin context it is useful to consider writhe as being of two types, constrained and unconstrained, the former resulting from direct physical wrapping around proteins or protein complexes. In prokaryotes, factors that package DNA, such as HU proteins, may control supercycling by binding to DNA and trapping the free energy of supercycling as writhe and subsequently releasing it through controlled dissociation [3,4]. Similarly in eukaryotes the regulated release of terminal DNA from a nucleosome, mediated by the acetylation of core histone tails, could release constrained writhe for conversion into negative supercycling. Although in vitro studies support this concept [5] its operation in vivo is elusive [6].

In prokaryotes and eukaryotes all activities that require DNA to be unwound (and rewound) are potent generators of supercycling. The classic example is the ‘twin supercoiled domain’ model where the elongating RNA polymerase, in unwinding the DNA, generates positive supercycling ahead and, in rewinding the DNA, generates negative supercycling in its wake [7,8] (Figure 1). The levels of supercycling produced in this process are prodigious, amounting to a positive and a negative supercoil for every 10 bp transcribed. Consequently the role of topoisomerases in releasing torsional stress is crucial if the template is to be maintained in a transcriptionally competent state. Genes that are negatively supercoiled are generally more efficiently transcribed [9,10] but topoisomerase inhibition studies [11,12,13,14] indicate that the accumulation of excessive positive or negative supercoiling will repress transcription. Therefore, there must be a regulated balance in the localised levels of supercycling through the concerted actions of polymerases [15] and topoisomerases [16,17].

Supercycling domains
When an activity supercoils DNA the torque generated is transmitted along the molecule. If the ends of the molecule are not fixed (or at least hindered), the supercycling will dissipate via the unhindered rotation of the helix. Therefore for supercycling to have a structural or functional influence on DNA or chromatin it must operate within a constrained environment where the energy is at least transiently trapped or restricted. For this reason it is anticipated that genomes need to be organised into supercycling domains with barriers that prevent the spread of topological stress.

In prokaryotes the Escherichia coli genome has a hierarchical organisation based on large structural macrodomains...
[3] with the Ter domain being subdivided into smaller, 35 kb domains via MatS/MatP interactions [18]. This organisation establishes a dynamic structural architecture enabling packaging without interfering with transcription or replication. The genome is also separately organised into about 500 independent ~10 kb supercoiling domains with demarcating barriers stochastically distributed and dynamically maintained [19,20]. However, as there are many more supercoiling domains than structural domains, the factors that define supercoiling boundaries must be distinct from those that characterise structural attachments.

Recent studies show that the eukaryotic genome is also organised into large (~1 Mb) loops, termed topologically associated domains (TADS) [21,22]. As these regions are invariant between cell types they appear to constitute a structural foundation to the genome and may not be directly relevant to functional activities such as transcription. The boundaries of TADS are enriched for CTCF binding sites. As some CTCF sites also recruit cohesion this suggests they may be involved in forming and maintaining chromosomal loops and potentially act as supercoiling boundary elements.

To understand the nature of eukaryotic supercoiling domains, psoralen binding has been used in combination with microarrays to map the distribution of DNA supercoils across entire genomes [23] or to particular chromosomal regions [24**,25**]. Psoralen preferentially intercalates into under-wound regions of the DNA helix and is fixed by long wave UV-light. To study supercoiling across large chromosomal domains in higher eukaryotes Naughton et al. [24**] used a biotin-tagged psoralen molecule (bTMP) and mapped the distribution of drug binding using microarrays (Figure 2a). Analysis of human chromosome 11 revealed this DNA is divided into a series of relatively large (~100 kb) underwound and overwound domains. These domains were relaxed by bleomycin treatment (introduces DNA nicks) indicating they were, topologically, a dynamic genomic feature. Most strikingly, the patterns of these domains were transcription and topoisomerase dependent implying they were established by the competing activities of these enzymes. Approximately 10% of supercoiling domain boundaries coincided with TAD boundaries (Figure 2b) suggesting that some of these structural interaction nodes could be barriers to the passage of supercoils. However, as supercoiling domains are approximately one tenth the size of TADS the factors that define the majority of boundaries must be distinct from those that demarcate structural domains.

In a similar approach Kouzine et al. [25**] also used psoralen to identify negatively supercoiled regions of the genome by isolating fragments of DNA resistant to denaturation due to psoralen cross-links. They focused on a subset of ENCODE promoters and showed that DNA supercoiling in these regions was restricted to relatively small foci (1.5 kb) centred upon transcription start sites. Supercoiling was dependent upon transcription with active genes being more negatively supercoiled than inactive genes. Inhibition of topoisomerases altered the pattern of DNA supercoiling and suggested that different topoisomerases might function separately on more highly and less highly transcribed genes.

Thus, as in bacteria, eukaryotic chromosomes appear to be organised into structural loops, overlaid with both large (100 kb) supercoiling domains and smaller supercoiling foci at transcription start sites (Figure 2c,d). The relationship between supercoiling domains and foci is not evident but domains may arise by supercoil diffusion from promoters. The mechanisms that constrain these domains are also unclear. Chromatin–chromatin interactions may act as supercoil diffusion barriers but the inherent drag, and therefore reduced rotation, caused by higher levels of chromatin organisation could in itself be sufficient to form the basis of supercoiling domains [26,27].
Figure 2

(a) Biotinylated-psoralen (bTMP) intercalates into the DNA helix preferentially at negatively supercoiled regions. bTMP binding, as a measure of DNA supercoiling, was mapped across a segment of chromosome 11 revealing the presence of underwound and overwound supercoiling domains (modified from Naughton et al. [24]). (b) The sequences defining supercoiling domain boundaries and topology boundaries [22] partially overlap but indicate that different factors must be responsible. (c) Organisation of prokaryotic genomes into large structural domains with architectural boundaries (orange spots) but subdivided into smaller supercoiling domains. (d) Eukaryotic genomes are similarly organised into large structural domains delimited by CTCF rich boundaries (orange spots) and divided into many smaller supercoiling domains.

Effects of supercoiling on DNA
RNA polymerase generates about seven DNA supercoils per second. If these are not efficiently removed the residual energy may influence DNA or chromatin structure locally [28], or, if the energy can be propagated along the fibre, at more distant sites. The capacity of negative supercoiling to unwind DNA and facilitate processes such as transcription [29,30] and replication and its ability to induce alternative DNA structures such as cruciform [31], G-quadruplexes and Z-DNA [32] have been noted.

To address how transcription-generated force might directly alter DNA structure in vivo, Kouzine et al. [33] used a tamoxifen-inducible Cre recombinase to excise a chromatin segment with its torsional stress trapped intact. As the segment, flanked by loxP sites, had been positioned on a plasmid between divergently transcribing promoters it was demonstrated that as transcription intensified the degree of negative supercoiling trapped within the excised segment increased. Using the c-myc FUSE element as a reporter they showed that supercoiling could propagate along the fibre, melt the FUSE element and promote the binding of ssDNA binding proteins (Figure 3a).

Although negative supercoiling promotes transcription initiation, supercoiling can also hinder polymerase elongation. To investigate how polymerase responds to different supercoiling environments Ma et al. [34], in a single-molecule approach, used an angular optical trap. RNA polymerase was immobilised on a slide whilst its DNA template, attached to a quartz cylinder, was held in the trap. Rotation and torque could be applied to and measured from the DNA by manipulation of the quartz bead whilst its height provided a measure of displacement. Upon transcription into a negatively supercoiled
template, the polymerase initially relaxed the DNA and then introduced positive supercoiling. As positive supercoiling accumulated ahead of the polymerase, it stalled. Thus, resisting torque slows RNA polymerase and increases its pause frequency.

In addition to facilitating the binding of polymerases or transcription factors, negative supercoiling can generate DNA substrates for more complex activities. In yeast, topoisomerase I inhibition promotes the formation of large ssDNA bubbles in highly expressed rRNA genes, which can be visualised by Miller spreads [12]. Parsa et al. [35] have shown that substantially smaller ssDNA patches located within active genes (Figure 3b) may be substrates for the mutagenic activity of activation-induced cytidine deaminase (AID). Employing an inducible gene in a hyper-negatively supercoiled E. coli strain they demonstrated that negative supercoiling increased ssDNA patch density compared to wild type and promoted a higher mutation rate. It will be interesting to know whether a similar effect is observed in eukaryotic cells where the DNA is packaged into chromatin and levels of supercoiling are probably buffered.

**Effects of supercoiling on chromatin**

In eukaryotic cells the effects of supercoiling have to be considered in the context of chromatin but unfortunately, we know very little about this situation. At the level of the ‘twin supercoil domain’ the scenario seems simplistic; positive supercoiling ahead of the polymerase will destabilize nucleosome structure and negative supercoiling behind will promote reassembly [36], actions that seem entirely consistent with the thermodynamic demands of transcription through a chromatin fibre. However, the many models that purport to explain the mechanics of how polymerase does in fact transcribe through a nucleosome reflects our ignorance of the details [37]. Things are no clearer at higher levels of chromatin structure. The idea that supercoiling might be generated at one site, say at a transcriptionally active gene, and then transmitted through the chromatin fibre to another location to create or remodel a domain or to influence a distant process, hinges on the concept that torsion can be transmitted along the fibre (Figure 4). Although we raised this issue, twenty-five years ago [38], the question essentially remains unanswered as the difficulty is multifaceted. We do not have a good understanding of the structure(s) that the higher-order chromatin fibre adopts, and yet this will undoubtedly constitute a profound influence upon the ability to transmit supercoiling. In addition, the composition and modification of the components of the fibre are also likely to affect its plasticity. Nucleosomes containing yeast histones are more sensitive to thermally induced torsional stress [39] than nucleosomes containing higher eukaryotic core histones suggesting, perhaps, a greater propensity for yeast chromatin to absorb rather than transmit negative supercoiling. In spite of these reservations pioneering single-molecule studies have attempted to provide an insight into this fundamental question. Using magnetic tweezers to introduce torsional stress into model chromatin fibres Bancaud et al. [40] found chromatin to be highly accommodating of supercoiling. To illustrate, they argued that supercoiling generated by transcribing 100 bp of DNA could be absorbed within a 10 kb chromatin fragment thereby diminishing the need for topoisoamerelaxation. Although such plasticity may not be typical of more condensed, native
DNA supercoiling also has a major role during DNA replication and the subsequent condensation and separation of replicated chromosomes. Positive supercoiling, generated in front of the DNA polymerase during replication (Figure 1b), is relaxed by topoisomerases I and II. However, when converging forks approach, relaxation of positive supercoiling is restricted and the build up of torsional stress causes swivelling of the replication complex required to complete replication [43**]. This causes intertwining of newly replicated DNA molecules behind the fork and the formation of precatenanes. Subsequently, most but not all catenanes are removed by topoisomerases II. On approaching mitosis the remaining catenations, or sister chromatid intertwinings are ‘identified’ by a process that involves an architectural change in chromatin structure, orchestrated by condensin-generated and mitotic spindle-dependant positive supercoiling [44]. This structural change then allows topoisomerase II to identify and resolve inter-chromosomal but not intra-chromosomal crossovers. Concomitantly, chromosome compaction starts during S-phase when condensin II is recruited to replicated regions [45]. Condensins introduce global positive writhe into the DNA/chromatin in vitro [46] and as a result changes in supercoiling energy are thought to co-dependently drive mitotic chromosome architecture [47] and resolution in vivo. Understanding how these processes are linked and determine the cytological chromosome structure will be key areas of future research.

Conclusions
A renewed interest in supercoiling research is clarifying how it influences nuclear processes and architecture. However, a lack of fundamental knowledge of the multi-layered structures of its substrate, the chromatin fibre, and given that supercoiling is such an inherently elusive topological force, will probably demand the development of new and innovative experimental approaches. The development of topologically constrained models of physiologically relevant chromatin fibres will enable studies of fibre stability, interplay between polymerases and topoisomerases and the propagation of supercoiling energy. Whilst minimally invasive probes are necessary to analyse chromatin structure and the distribution of supercoiling in vivo. With new approaches these will be exciting times for this area of research.

Acknowledgements
We would like to thank Sam Corless for crucial comments on the manuscript and Jon Baxter for his helpful insights into DNA supercoiling in transcription and replication. Research described in this review was supported by the Wellcome Trust and NG is now funded by the UK Medical Research Council.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Freeman LAL, Garrard WTW: DNA supercoiling in chromatin structure and gene expression. Crit Rev Eukaryot Gene Expr 1992, 2:165-209.
2. Sinden RR: DNA Structure and Function. Academic Press; 1994.
3. Travers AA, Muskhelishvili GO: Bacterial chromatin. Curr Opin Genet Develop 2005, 15:8-8.
4. Macvanin M, Adhya S: Biochimica et Biophysica Acta. BBA - Gene Regul Mech 2012, 1819:830-835.
5. Norton VG, Imai SS, Yau P, Bradbury EM: Histone acetylation reduces nucleosome core particle linking number change. Cell 1989, 57:449-457.
forms and remodels supercoiling domains unfolding large-scale chromatin structures. Nat Struct Mol Biol 2013, 20:387-395.

Using biotinylated-psoralen as a probe this paper demonstrates eukaryotic genomes are organised into under-wound and over-wound supercoiling domains. Domains are formed through polymerase and topoisomerase activity and appear to influence higher levels of chromsome organisation.

25. Kouzine F, Gupta A, Baranello L, Wojtowicz D, Ben-Aissa K, Liu J, Przytycka TM, Levens D: Transcription-dependent dynamic supercoiling is a short-range genomic force. Nat Struct Mol Biol 2013, 20:396-403.

This study uses psoralen as a probe for supercoiling, They demonstrate for a selection of ENCODE genes there are negative supercoiled foci associated with promoters in a transcription dependent manner.

26. Nelson P: Transport of torsional stress in DNA. Proc Natl Acad Sci U S A 1999, 96:14342-14347.

27. Stupina VA, Wang JC: DNA axial rotation and the merge of oppositely supercoiled DNA domains in Escherichia coli: effects of DNA bends. Proc Natl Acad Sci U S A 2004, 101:8608-8613.

28. Ljungman M, Hanawalt PC: Localized torsional tension in the DNA of human cells. Proc Natl Acad Sci U S A 1982, 79:6595-6599.

29. Mizutani M, Ohta T, Watanebe H, Handa H, Hirose S: Negative supercoiling of DNA facilitates an interaction between transcription factor IID and the fibroin gene promoter. Proc Natl Acad Sci U S A 1991, 88:718-722.

30. Schultz MC, Brill SJ, Ju Q, Sternglanz R, Reeder RH: Topoisomerasises and yeast rRNA transcription: negative supercoiling stimulates initiation and topoisomerase activity is required for elongation. Genes Dev 1992, 6:1332-1341.

31. Mizuuchi K, Mizuuchi M, Gellert M: Cruciform structures in palindromic DNA are favored by DNA supercoiling. J Mol Biol 1982, 156:229-243.

32. Lee M, Kim SH, Hong SC: Minute negative superhelicity is sufficient to induce the B-Z transition in the presence of low tension. Proc Natl Acad Sci 2010, 107:4965-4990.

33. Kouzine F, Sanford S, Elisha-Feil Z, Levens D: The functional response of upstream DNA to dynamic supercoiling in vivo. Nature 2001, 413:435-438.

34. Ma J, Bai L, Wang MD: Transcription under torsion. Science 2013, 340:1580-1583.

35. Using an elegant optical trap-based approach this study shows that on a single DNA template excess positive and negative supercoiling can inhibit and stall RNA polymerase.

36. Morse RH, Pederson DS, Dean A, Simpson RT: Yeast nucleosomes allow thermal untwisting of DNA. Nucleic Acids Res 1987, 15:10311-10330.

37. Bancal A, Conde e Silva N, Barbi M, Wagner G, Allemand J-F, Mozziocaccioni J, Lavelle C, Croquette V, Victor-J-M, Prunell A et al.: Structural plasticity of single chromatin fibers revealed by torsional manipulation [Internet]. Nat Struct Mol Biol 2006, 13:444-450.
41. Joshi RSaBP, Roca J: Positional dependence of transcriptional inhibition by DNA torsional stress in yeast chromosomes [Internet]. EMBO J 2010, 29:740-748.

42. Kegel A, Betts-Lindroos H, Kanno T, Jeppsson K, Ström L, Katou Y, Itoh T, Shirahige K, Sjögren C: Chromosome length influences replication-induced topological stress. Nature 2012, 471:392-396.

43. Baxter J, Aragon L: Physical linkages between sister chromatids and their removal during yeast chromosome segregation. Cold Spring Harbor Symp Quantitative Biol 2011, 75:389-394.

This focused paper shows that on approaching mitosis catenations that have escaped topoisomerase II resolution during replication are identified through a supercoiling dependent structural change mediated by condensin and dependent on the spindle. This change enables topoisomerase II to identify and resolve the remaining interchromosomal crossovers.

44. Baxter J, Sen N, Martinez VL, De Carandini MEM, Schwartzman JB, Diffley JFX, Aragon L: Positive supercoiling of mitotic DNA drives decatenation by topoisomerase II in eukaryotes. Science 2011, 331:1326-1332.

45. Ono T, Yamashita D, Hirano T: Condensin II initiates sister chromatid resolution during S phase. J Cell Biol 2013, 200:429-441.

46. Kimura K, Rybenkov VV, Crisona NJ, Hirano T: Cozzarelli NR: 13S condensin actively reconfigures DNA by introducing global positive writhe: implications for chromosome condensation. Cell 1999, 98:239-248.

47. Shintomi K, Hirano T: The relative ratio of condensin I to II determines chromosome shapes. Genes Dev 2011, 25:1464-1469.

48. Liu J, Kouzine F, Nie Z, Chung H-J, Elisha-Feil Z, Weber A, Zhao K, Levens D: The FUSE/FBP/FIR/TFIIH system is a molecular machine programming a pulse of c-myc expression. EMBO J 2006, 25:2119-2130.