Morfolino are molecules similar to **RNA or DNA** in that they comprise the same nitrogenous bases, although bound to morpholine rings instead of ribose or deoxyribose rings, and linked through **uncharged phosphorodiamidate groups** instead of anionic phosphates. They are typically **18–30 bases in length** and **bind to targeted RNA sequences by base pairing**. However, unlike siRNA, these **phosphorodiamidate morpholino oligomers (PMOs or Morpholinos)** do not result in degradation of their target RNA since their backbone structure is foreign and not recognized by cellular nucleases. Rather, Morpholinos act by "steric blocking": binding to a target subsequence within the RNA, thereby getting in the way of molecules that might otherwise interact with the RNA.

Mechanisms of action of antisense morpholino oligomers.

(a) **Translational blockage**. PMOs block the translation initiation complex binding sites on mRNA and prevent translation from occurring.

(b) **Modification of splicing/exon skipping**. PMOs block splice sites on pre-mRNA, prevent recognition of these sites by the spliceosome that in turn causes exon skipping.

(c) **miRNA maturation blockage and miRNA inactivation**. PMOs may block maturation enzyme cleavage sites (i.e., Drosha, Dicer) on pri- or pre-miRNA to prevent its maturation. PMOs may complementarily bind to mature miRNA and prevent it from binding to target mRNA.

(d) **Ribozymes.** PMOs may bind to enzymatically active RNAs (ribozymes), blocking their active sites and preventing them from cleaving their target mRNAs.

25-nucleotide morpholino (MmEx10) binds to the exon 10–lamin A splice donor site. In addition, using the same coordinates as were specified in the original human HGPS targeting experiments, another 25-nucleotide morpholino binds the c.1827C>T;p.Gly609Gly mutated sequence in the region of the Lmna transcript that corresponded to exon 11 (MmEx11), to test whether the combined administration of both morpholinos could be more effective at reducing progerin amounts than the separate administration of each individual oligonucleotide.

In an initial attempt to evaluate the effects of these morpholinos on splicing of the LmnaG609G allele, the authors transfected the oligonucleotide reagents **into a fibroblast cell line derived from a heterozygous mouse** that carried the c.1827C>T;p.Gly609Gly mutation and found that **both MmEx10 and MmEx11 morpholinos each reduced progerin amounts in a dose-dependent manner**;

Western (immuno) blot analysis of mouse G609G/+ fibroblasts (MF) treated with varying concentrations of morpholinos MmEx10 and MmEx11 (top panel). AG01972c HGPS fibroblasts (HF) treated with varying concentrations of morpholinos HsEx10 and HsEx11.

MmEx10 was more effective than MmEx11 at reducing progerin concentrations when the morpholinos were administered separately (30% reduction in 10 mM MmEx11-treated cells when compared to untreated cells; 40% reduction in 10 mM MmEx10-treated cells when compared to untreated cells; $P < 0.01$). When the two reagents were administered to cells at the same time (at a final concentration of 40 mM), progerin amounts were reduced to undetectable levels (Fig. 4B). We obtained similar results after using the human equivalents of the morpholinos in an HGPS fibroblast cell line (Fig. 4B). We also observed that progerin reduction mediated by administration of morpholinos correlated with **the correction of nuclear abnormalities in a cell line carrying the homozygous c.1827C>T;p.Gly609Gly mutation and in an HGPS fibroblast cell line**. Thus, both independent administration of each morpholino and their combined administration were able to reduce, in a dose-dependent manner, the percentage of cells with nuclear abnormalities to wild-type levels.

Lamins are intermediate filament proteins that **form a scaffold, termed nuclear lamina, at the nuclear periphery**. A small fraction of lamins also localize throughout the nucleoplasm. **Lamins bind to a growing number of nuclear protein complexes** and are implicated in both nuclear and cytoskeletal organization, mechanical stability, chromatin organization, gene regulation, genome stability, differentiation, and tissue-specific functions. The laminbased complexes

and their specific functions also provide insights into possible disease mechanisms for human laminopathies, ranging from muscular dystrophy to accelerated aging, as observed in Hutchinson–Gilford progeria and atypical Werner syndromes.

The domain organization of a lamin monomer showing the **N-terminal (head)** domain; the **central rod domain**, which is composed of four α-helices (1A, 1B, 2A, 2B); three linker regions (L1, L12, L2); and the **C-terminal (tail) domain**, which includes the nuclear localization signal (NLS), immunoglobulin (Ig) domain, and a CaaX motif (C, cysteine; a, aliphatic amino acid; X, any amino acid). The location of the six heptads (yellow) present in lamins, and absent in mammalian cytoplasmic intermediate filaments, is shown.

Ectopically expressed Drosophila B-type lamin (Dm0) fused to bacterial DNA argininemethyltransferase (DamID) in Drosophila Kc cells and analyzed the methyl groups added by the lamin fusion protein to adenine residues at GATC sequences in the genome . These **lamina-associated domains (LADs)** in the genome **are enriched in transcriptionally inactive genes and heterochromatic histone marks**; they replicate late and are mostly gene poor. These experiments added to the concept that the lamins associate primarily with heterochromatic genomic regions. This spatial organization of the genome in relation to lamins is conserved in mammalian cells. The human genome contains over 1,300 LADs ranging from 10 kb to 10 Mb in length, covering nearly 40% of the genome. The human LADs are characterized by low gene expression levels and a repressive chromatin environment and are flanked by insulator protein CTCF-binding sites.

Association of LADs with the nuclear lamina may also affect gene expression by preventing long-range interactions of promoters within LADs with their distant enhancers outside of LADs, through **binding of the insulator protein CTCF** at both ends of the LADs (116). In line with this model, **Hi-C chromosome conformation capture analysis reveals that interactions between regions located within LADS are more frequent than the interaction of a region within LADs with a region outside of LADs** (117). Furthermore, a recent study, which tested the expression of thousands of randomly integrated reporter genes, shows that integration of the transgene within LADs generally attenuates transcription, likely by reducing access to transcription factor–binding sites.

Lamins interact with a plethora of proteins in the INM and nucleoplasm. Initial proteomic analyses in rat liver identified ~80 nuclear envelope transmembrane proteins (NETs) (132), and subsequent studies showed that different tissues express different sets of NETs (133). This led to the hypothesis that **complexes of tissue-specific NETs with abundantly expressed lamins may define the tissue-specific functions of lamins (134), giving rise to tissue-specific phenotypes seen in lamin-linked diseases** (see the section titled Mutations in Lamins Cause Many Different Diseases, below). However, only a subset of NETs has so far been experimentally confirmed to interact with lamins (134, 135). Lamin-binding proteins that have been characterized contribute to nuclear architecture and mechanics as well as to chromatin organization and signaling.

Among the first identified mammalian laminbinding proteins of the INM are the **lamina-associated polypeptide 1 (LAP1)**, which also binds AAA+ ATPase torsin A in the perinuclear lumen and the LEM protein emerin in the INM. LAP1 regulates torsin A ATPase activity , and this interaction seems to be particularly important in neuronal cells because a torsin A mutant that exhibits stronger binding to LAP1 causes DYT1 dystonia, a disease of the central nervous system.

LBR is an INM protein with eight transmembrane domains and has sterol reductase activity. Mutations in this gene cause a Pelger-Huet anomaly, most likely linked to nuclear defects, and Greenberg skeletal dysplasia, linked to a deficiency in sterol reductase activity. Recent studies have revealed an essential role of LBR in tethering chromatin to the lamina and in epigenetic gene silencing.

SUN and KASH domain proteins represent a large family of proteins localized in the INM and ONM, respectively. They are evolutionarily conserved from yeast to human and often comprise many isoforms expressed in a tissue-specific manner. They form LINC complexes through interaction of trimeric SUN proteins with three KASH proteins in the perinuclear space. As SUN proteins bind lamins and different KASH proteins interact with the three main cytoskeletal components (microtubules, actin filaments, and microtubules), the LINC complex, which has a **plethora of functions in mechanotransduction** nuclear positioning, migration, and centrosome association , forms a direct physical link between the nucleoskeleton and the cytoskeleton. During meiosis, the C. elegans SUN-1–ZYG-12 LINC complex tethers chromosomes to the nuclear envelope, which is essential for the pairing of homologous chromosomes and subsequent crossover and Holliday junction formation. In mammalian meiosis,

SUN1 (154), possibly SUN2 (155), and KASH5 (156, 157) fulfill similar roles by tethering telomeres to the lamina. LEM proteins represent another evolutionarily conserved group of proteins in metazoan cells, characterized by the presence of the bihelical LEM motif, which mediates binding to the conserved chromosomal protein BAF.

The LEM protein family includes proteins of the INMas well as non-membrane-associated proteins, and many of them directly interact with A- and/or B-type lamins and/or require A-type lamins for their proper localization in the INM (reviewed in References 114 and 135).The number of different LEM proteins has increased during evolution, and many are also expressed as multiple isoforms. They fulfill partly unique and partly redundant functions. C. elegans has two LEM proteins in the INM (LEM-2 and emerin), and only a double knockdown of both proteins in embryos or adult worms causes severe/lethal defects in nuclear structure, chromatin organization, and mitosis, although the loss of function of one gene has only a mild phenotype. Mammalian cells have five LEM proteins in the INM (emerin, LAP2, LEMD1, MAN1, LEM2) (114). Loss of emerin expression in humans causes X-linked Emery–Dreifuss muscular dystrophy (EDMD) (164), but emerin-null mice have only a subtle phenotype (165). The LEM proteins in the INM, together with lamins, anchor heterochromatin to the lamina (see the section titled Lamins Regulate Chromatin Organization, above). They have several chromatin-interacting domains. The LEM domain, present in all LEM proteins, interacts with the DNA-associated protein BAF, and an additional LEM-like motif found at the N terminus of all LAP2 isoforms interacts directly with DNA. A winged helix domain present in the C terminus of MAN1 and LEM2 may also interact with DNA.

Lamins are major structural components of the nucleus. As such, they contribute to the biophysical and mechanical properties of the nucleus and the entire cell.

Role in differentiation.

Nucleocytoskeletal coupling of the lamina with the cytoskeleton via SUN domain proteins in the INM and nesprins in the outer nuclear membrane (ONM) (the LINC complexes) allows force transmission from the extracellular matrix (ECM) through cell adhesion complexes and the cytoskeleton into the nucleus and contributes to mechanosignal transduction. Exciting new studies indicate that this force transmission regulates the levels of lamins and, thus, biophysical properties of the nucleus; even more surprisingly, it affects cell fate and differentiation. This model suggests that the physical link between the lamina and the tissue

microenvironment transduces forces that cause deformation of the nuclear envelope and lamina, which, in turn, causes local distortion of chromatin and changes in transcription factor accessibility (72). In an outside-to-inside mechanosignaling, cells can sense increasing forces in a stiff microenvironment and respond by changing the biophysical properties of the nuclear lamina by changing lamin A conformation and phosphorylation and by inducing transcriptional upregulation of lamin A (64). The increase in lamin A expression induces an **inside-to-outside signaling by triggering the Yes-associated protein (YAP), serum response factor (SRF) and retinoic acid receptor (RAR) pathways**. These pathways affect the dynamics of the cytoskeleton and the composition of the ECM to achieve tension homeostasis (64). Thus, the amount of vertebrate lamin A is directly correlated to the ability of cells to undergo differentiation, whereby increased amounts of lamin A lead to differentiation into stiffer tissue, and reduced amounts of lamin A lead to softer tissue and increase the cells' ability to migrate. Similarly, in the hematopoietic system, high levels of lamin A promote erythrocyte differentiation and inhibit megakaryocyte differentiation . The lamin A- and actin-binding protein, emerin, contributes to the cellular mechanotransduction response. Both lamin A- and emerin-deficient cells show an attenuated activation of stress-induced gene expression, including the NF-κB pathway. These results can be interpreted in at least two ways: Lack of lamins and emerin primarily affect mechanosensing and force transmission, thereby impairing the activation of mechanical stress-induced signaling, or lamin and emerin deficiencymay lead to mechanotransduction-independent downstream changes in signaling. Recent observations showing that disruption of the LINC complex impairs force transmission, but does not affect mechanical stress-induced signaling (71), favor the second possibility.

How, then, does mechanotransduction-independent sensing work?

In an elegant study, Lammerding and coworkers (74) showed that the nuclear translocation of the mechanosensitive transcription factor megakaryoblastic leukemia 1 (MKL1) is impaired in lamin A-deficient cells, wherein emerin mislocalizes to the endoplasmic reticulum. They also found that mislocalization of emerin causes changes in actin dynamics and that ectopic expression of emerin in lamin A-deficient cells rescues actin dynamics, MKL1 nuclear localization, and signaling. These observations provide a novel mechanism for the effect of emerin on mechanosensitive signaling via the regulation of actin dynamics.

RNA splincing.

RNA splicing, which is the removal of introns followed by exon ligation, is a two-step biochemical process. **Sequential transesterification reactions are initiated by a nucleophilic attack of the 5′ splice site (5′ss) by the branch adenosine (branch point; BP) in the downstream intron resulting in the formation of an intron lariat intermediate with a 2′, 5′-phosphodiester linkage**. This is followed by a **5′ss-mediated attack on the 3′ss, leading to the removal of the intron lariat and the formation of the spliced RNA product** (see the figure, part a).

The difficult tasks of splice site identification and regulated splicing is accomplished principally by two exceptionally dynamic macromolecular machines, **the major (U2-dependent) and minor (U12-dependent) spliceosomes**. Each spliceosome contains five small nuclear ribonucleoprotein particles (snRNPs): U1, U2, U4, U5 and U6 snRNAs for the major spliceosome (which processes ~95.5% of all introns126); and U11, U12, U4atac, U5 and U6atac snRNAs for the minor spliceosome (see the figure, part b). Spliceosome recognition of consensus sequence elements at the 5′ss, 3′ss and BP sites is a crucial step in the splicing pathway, and is modulated by an array of cis-acting exonic and intronic splicing enhancers (ESEs and ISEs, respectively) and exonic and intronic splicing silencers (ESSs and ISSs, respectively), which are recognized by auxiliary splicing factors, including the Ser/Arg-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs). Although early studies indicated that U12-dependent introns initiated with AT and ended with AC, previously referred to as ATAC introns (this is also why the minor spliceosome snRNAs are named U4atac and U6atac), subsequent studies demonstrated that these terminal dinucleotides were not required126. In part b of the figure, the height of the residue corresponds to relative frequency of each nucleotide in each given position. U2 and U12 consensus sequence frequencies were obtained from the Splice Rack and U12 databases, respectively, and BP site data and probabilities were calculated with Pictogram (see further information). Ultimately, this intricate network of RNA and protein interactions results in the recruitment of spliceosomal components followed by snRNP remodelling, spliceosome activation, catalysis and generation of the spliced RNA product.

High-throughput RNA sequencing (RNA-seq) studies have suggested that alternative splicing is a routine activity in human cells with 90–95% of **human multi-exon genes producing transcripts that are alternatively spliced**. Alternative splicing adds another layer of complexity with multiple and developmentally regulated splicing patterns including the inclusion of alternative first and last exons (AFE and ALE, respectively), retained intron, cassette exon, mutually exclusive cassettes and alternative 5′ and 3′ splice sites. Cassette exon skipping is the most common alternative splicing event in humans but a recent study demonstrates that intron retention is also routine in mammals, occurring in nearly 75% of multi-exon genes, and is a co- or post-transcriptional mechanism designed to reduce transcript levels during development6,127. The interrelationship of a large array of cis-regulatory elements and trans-acting RNA-binding proteins (RBPs) suggests that alternative splicing is controlled by a 'splicing code' that could be useful as a predictive tool for cell-, and tissue-specific responses to developmental transitions and environmental changes.

Mis-splicing of a single gene results in different diseases.

Aberrant splicing of lamin A (LMNA) pre-mRNA is associated with multiple hereditary disorders. Normal exons are shown in blue, introns are shown as thick black lines, normal splicing is indicated by thin black lines, and disease-associated splicing is indicated in dotted lines or purple boxes (intron retention).

a | **Limb girdle muscular dystrophy type 1B (LGMD1B)** is caused by a G>C 5′ splice site (5′ss) mutation that results in intron 9 retention, a premature termination codon (PTC) and nonsense-mediated decay (NMD). **c.1608 + 5 indicates that the mutations occurs 5 nucleotides into the intron that follows coding position** (c) 1608. However, a lamin A/C protein truncated in intron 9 with a unique carboxy-terminal sequence may also be produced.

b | **In familial partial lipodystrophy type 2 (FPLD2), a G>C transversion mutation occurs in the exon 8 5′ss, leading to intron 8 retention**, NMD and potential translation of another truncated lamin A/C with a unique C-terminal region.

c | A common cause of **Hutchinson–Gilford progeria syndrome (HGPS) is a C>T transition in exon 11, which activates a cryptic 5′ss and results in a 150 nucleotide** deletion that is translated into the ageing-associated protein progerin.

d | For **LMNA-linked dilated cardiomyopathy (DCM),** an **alternative 3′ss is generated by an A>G mutation upstream of the normal exon 4 3′ ss** so that nine additional nucleotides are inserted in-frame between exons 3 and 4, resulting in a 3-amino-acid insertion in the resultant protein.

Major and minor spliceosome mutations.

The figure shows the splicing steps and core spliceosomal components of both the major (U2-dependent) and minor (U12-dependent) spliceosomes, including their interactions in the pre-spliceosomal complex (complex A) and spliceosome (complex C). Pre-mRNA processing factor 3 (PRPF3), PRPF4), PRPF6, PRPF8 and PRPF31 components of the U4/U6.U5 tri-small nuclear ribonucleoprotein (tri-snRNP) dysregulated in autosomal dominant retinitis pigmentosa (adRP) are shown. Also indicated is the SNRNP200 helicase, which is required at several dissociation steps in the spliceosomal cycle. Several PRPF components are common to both the U4/U6.U5 tri-snRNP and the U4atac/U6atac.U5 tri-snRNP complexes. Some mutations in the U4atac snRNA 5′ stem-loop found in microcephalic osteodysplastic primordial dwarfism type 1 (MOPD I) are highlighted in red. In addition, stress-induced upregulation of p38 mitogen-activated protein kinase (MAPK) leads to increased stability of U6atac (t1/2 <2 hours).

Co-transcriptional splicing factor recruitment and disease mutations.

Models for splicing factor and precursor RNA mutations and disease-associated mis-splicing.

a | Splicing factors recognize and bind to RNA polymerase II (RNA Pol II) transcripts in the nucleoplasm or directly at the carboxy-terminal domain (CTD) of RNA Pol II. These factors may contain RNA-binding motifs (such as RNA recognition motifs (RRMs) or zinc fingers (ZnFs)), as well as auxiliary domains composed of low complexity (LC) regions with prion-like domains in heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1), TDP-43 and FUS (LC regions shown as green, yellow or red lines for hnRNPA1, TDP-43 and FUS, respectively), or other regions that either mediate protein–protein interactions (in muscleblind-like (MBNL)) or function as flexible linkers between RRMs (in hnRNPH). Splicing factors might bind to single-stranded RNA (ssRNA) motifs or pre-formed RNA structures (for example,

G-quadruplexes), resulting in the formation of dynamic RNA–RNP complexes that are continuously remodelled by RNA helicases and protein–protein interactions before nuclear export.

b | Mutations (red star) in the LC regions of hnRNPA1, TDP-43 and FUS could cause mis-folding of RNA–RNP complexes and lead to abnormal splicing.

c | For diseases caused by microsatellite expansions, splicing factors such as MBNL, which recognize a motif within the repeated sequence, are sequestered by the repeat expansion (ssRNA, top; RNA hairpin, bottom), leading to loss-of-function and mis-splicing.

Therapeutic strategies.
Examples of therapies based on antisense oligonucleotide (ASO) and small molecule approaches.

a | **Duchenne muscular dystrophy** is often caused by chromosomal deletions (black triangle) that remove exons 48–50, resulting in a frameshift (blue rectangles, exons with intact codons; trapezoids, exons with incomplete codons) and loss of dystrophin protein. The red hexagon indicates the **premature stop codon** resulting from frameshifted exon 51. To prevent frameshifting, both **phosphorodiamidate morpholino oligomer (PMO) and 2'OMePS (2'O-methyl-phosphorothioate) ASOs (black semicircle) block an exon 51 exonic splicing enhancer (**ESE; green rectangle) and shift splicing to the in-frame exon 52.

b | **In spinal muscular atrophy**, survival of motor neuron 1 (SMN1), which produces the majority of SMN protein, is either deleted or inactivated by mutations, and the paralogous SMN2 expresses low levels of SMN due to a C>T transition (grey box) that suppresses exon 7 splicing. **ASO-10-27 targets an intronic splicing silencer** (ISS; red bar) and enhances exon 7 splicing to produce stable SMN protein.

c | In **myotonic dystrophy type 1**, CUG expansion (CUGexp) RNA (red hairpin) binds muscleblind-like (MBNL) proteins (green ovals) and causes mis-splicing of MBNL RNA targets. **Mutant MBNL–RNA complexes accumulate in the nucleus, and so ASO gapmers preferentially target mutant RNAs for degradation** (dotted red line). Alternatively, **small molecule compounds bind to mutant CUGexp RNA**, displace MBNL and rescue abnormal splicing. DMPK, DM protein kinase.