A

A →adenine

AA-platform Structural motif in RNA consisting of two adjacent *adenosine nucleobases in a coplanar arrangement.

aaRS →aminoacyl-tRNA synthetase

aa-tRNA →aminoacyl-tRNA

abasic site *Monomer residue within a contiguous nucleic acid strand (normally DNA) that does not carry a *nucleobase. Depending on the nucleobase lost (discernible in the case of *double-stranded DNA) an abasic site is either an apurinic or an apyrimidinic site. An abasic site arises from hydrolysis of a *glycosidic bond, which can either occur spontaneously or as the result of enzymatic catalysis. The latter reaction is carried out on a number of chemically damaged DNA residues and constitutes the first step of *base excision repair. With respect to the pentose moiety, abasic sites exist in equilibrium between an open-chain and two furanose forms (having β- or α-configuration). In DNA, the free aldehyde function of the open-chain form lends C/H acidity to carbon center C2′ that, in turn, facilitates base-induced strand cleavage on the 3′-side of the abasic site by β-elimination of a *phosphomonoester with the formation of a 2′,3′ double bond and vinylogous shift of C/H acidity to center C4′. In DNA, spontaneous base loss is promoted by low pH and single-stranded structure; it is faster for *purines than for *pyrimidines. Enzymes catalyzing hydrolysis of glycosidic bonds in DNA are called *DNA glycosylases (in connection with their substrate base, e.g. uracil DNA glycosylase). For historical reasons, abasic sites are also called AP-sites (for apurinic or apyrimidinic site). HANS-JOACHIM FRITZ

absorbance Characterized in spectroscopy as:

$$A = \log \frac{I_0}{I}$$

where A is the measured absorbance and equals the $\log_{10}$ of the intensity I_0 of the incident light at a given wavelength divided by the transmitted intensity I. Absorbance is often also referred to as *extinction or *optical density (→Beer–Lambert law). VALESKA DOMBOS

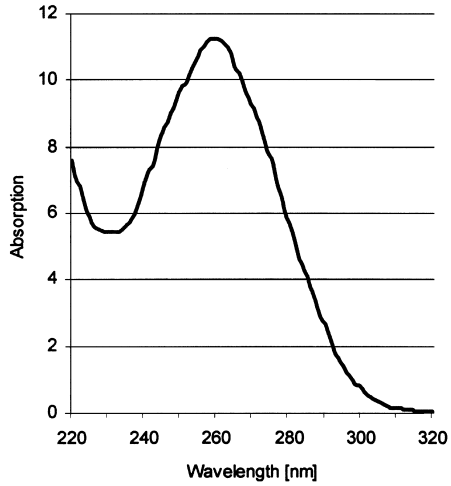
absorption Physical process of absorbing light. Nucleic acids show an absorption maximum near 260 nm. →absorbance, →absorption spectra of nucleobases. VALESKA DOMBOS

absorption coefficient (Synonym: extinction coefficient) →absorptivity

absorption spectra of nucleobases Used to determine the concentration of DNA and RNA in solution. The *nucleobases of DNA and RNA strands absorb ultraviolet light of 250–270 nm wavelength with the absorption maximum at 260 nm. According to the *Beer–Lambert law there is a linear relationship between the concentration and the absorption of light. The higher the *optical density, the higher the nucleic acid concentration of the sample. To determine the concentration of nucleic acid solutions, the sample is exposed to ultraviolet light at 260 nm and a photodetector measures the *absorbance. An example spectrum of a 41mer RNA is given below.

Nucleic Acids from A to Z: A Concise Encyclopedia. Edited by Sabine Müller

ISBN: 978-3-527-31211-5



Absorption spectrum of a 41mer RNA.

Knowing the *molar absorptivity of the sample, its concentration can be calculated. For an *optical density value of 1, there are some benchmarks corresponding to nucleic acids: (a) for *double-stranded DNA it is equivalent to a concentration of 50 $\mu g\,mL^{-1}$, (b) for single-stranded DNA to 37$\mu g\,mL^{-1}$ and (c) for RNA to 40 $\mu g\,mL^{-1}$. Furthermore, the spectrophotometric measurement allows the purity of nucleic acids to be determined. The absorption value at 230 nm reflects impurities such as urea, aromatic compounds or peptides, although nucleic acid itself shows little absorption at 230 nm due to the aromatic bases. Proteins have an absorption maximum at 280 nm. Therefore, the absorbance ratio 260/280 can be used to estimate the contamination of the nucleic acid sample with proteins. A ratio value greater than 1.9 for DNA or greater than 1.8 for RNA is considered an indicator of protein-free nucleic acid samples. BETTINA APPEL

absorptivity (Synonym: absorption coefficient, extinction coefficient) Proportionality constant $a(\lambda)$ of the *Beer–Lambert law. The absorptivity is wavelength dependent:

$$a(\lambda) = \frac{A}{cL}$$

where A is the *absorbance, L is the length of the light path and c is the concentration of the analyte. If the concentration is expressed on a molar basis, the absorptivity $a(\lambda)$ becomes *molar absorptivity ε, which is a fundamental molecular property in a given solvent at a particular temperature and pressure. In the literature, the *molar absorptivity is also referred to as the *molar absorption coefficient or *molar extinction coefficient. The unit given is usually $L\,mol^{-1}\,cm^{-1}$, but the SI unit is $m^2\,mol^{-1}$. VALESKA DOMBOS

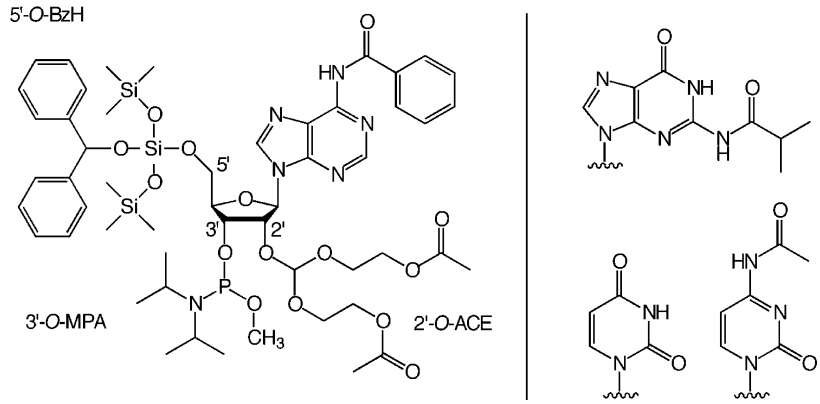
AC arm →anticodon arm, →transfer RNA

acceptor arm Amino acid accepting branch of the L-shaped three-dimensional structure of a *transfer RNA (tRNA) containing the conserved *CCA-tail at its 3′-end. It is formed by *coaxial stacking of the dihydrouridine (→DHU loop) and acceptor RNA helices of a tRNA cloverleaf. The *anticodon arm forms the second branch. STEFAN VÖRTLER

acceptor end Conserved single-stranded *CCA-tail of a *transfer RNA at the end of the *acceptor arm.

acceptor stem →acceptor arm

ACE method Allows for the chemical solid-phase synthesis of *oligoribonucleotides and is named after the *nucleoside 2′-hydroxyl protection – the bis(acetoxyethyloxy)methyl (ACE) group. First introduced in 1998, the method follows phosphoramidite chemistry (→phosphoramidite method) for the coupling of appropriately protected nucleoside building blocks and was developed under the aspect that mildly acidic aqueous conditions are ideal for the final 2′-*O*-deprotection of

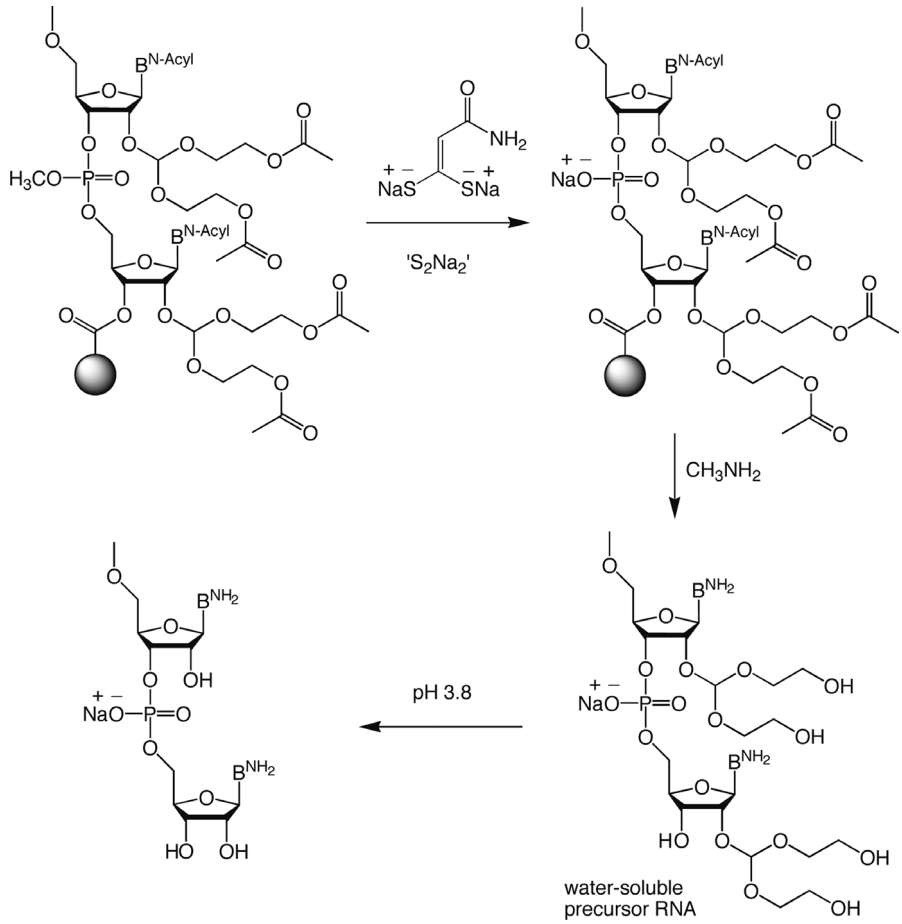


the synthesized RNA. Therefore, the protection strategy employs two orthogonal *protecting groups – a fluorine ion-labile silyl ether on the 5′-*O*-position and an acid-labile orthoester on the 2′-*O*-position. The 3′-*O*-position is derivatized with a (*N*,*N*-diisopropylamino)methoxyphosphinyl residue as the commonly used cyanoethoxyphosphinyl group is unstable in the presence of fluorine reagents required during strand elongation. The structures of the nucleoside phosphoramidite derivatives are depicted in the figure above.

Oligonucleotide assembly proceeds with coupling yields higher than 99% and with coupling speeds as fast as 2 nucleotides per minute. After the assembly, the phosphate methyl protecting groups are removed with disodium 2-carbamoyl-2-cyanoethylene-1,1-dithiolate trihydrate. This step may limit the kind of modifications that can be incorporated into oligoribonucleotides by the ACE method compared to the alternative *TOM or *TBDMS RNA synthesis methods where this treatment is not required. Then, basic conditions (40% aqueous $MeNH_2$) cause release from the solid support, along with the removal of the acyl protecting groups and, importantly, of the acetyl groups on the 2′-orthoesters. The resulting 2′-*O*-bis(2-hydroxyethyloxy)methyl orthoesters are 10 times more acid labile then prior to the removal of the acetyl groups. Consequently, very mild acidic conditions (pH 3.8, 30 min, 60°C) followed by a short treatment at pH 8.7 to effect hydrolysis of any intermediate 2′-*O*-formyl groups are sufficient for the final deprotection step.

The access to the 2′-*O*-bis(2-hydroxyethyloxy)methyl oligoribonucleotides is a major strength of the ACE method as these precursor RNAs are water soluble, can be analyzed by *HPLC and further purified if necessary. Of further significance is that the 2′-*O*-bis(2-hydroxyethyloxy)methyl protection interrupts *secondary structures, renders the RNA resistant to *nucleases and other forms of degradation, and therefore facilitates handling and long-term storage.

The 5′-*O*-silyl ethers and 2′-*O*-orthoesters have been structurally optimized to provide crude oligoribonucleotides of high sequence integrity, excellent purity and biological activity. Using commercial synthesizers (→DNA synthesizer), the ACE method enables routine preparation of oligoribonucleotides up to 60 bases in length, regardless of sequence or secondary



structure. The ACE method produces milligram to kilogram quantities of more than 90% pure RNA and has been successfully commercialized (Dharmacon Inc.).
RONALD MICURA

acidity of nucleosides and nucleotides The acid–base behavior of *nucleotides is a very significant physical characteristic. The pK_a value estimates the charge and the tautomeric structure (→tautomeric bases), the ability to donate or accept hydrogen bonds, and therefore the possibility for base pairing, and lastly also the structure of nucleic acids. All bases are uncharged in the physiological range (pH 5–9). The 2′,3′-diol in *ribose loses one proton only at pH higher then 12. In nucleotides, the remaining free hydroxyl group is deprotonated only above pH 15. The phosphate group loses one proton at pH 1 and the second proton at pH 7. Due to the amine–imine tautomerism, the character of the C–NH_2 bond has approximately 45% double-bond character. Therefore, the ring nitrogens are more readily protonated than the exocyclic amino groups. Due to the negative charge on the phosphorous residue, in nucleotides

pK_a values for bases in nucleosides and nucleotides

Base (site of protonation)		Nucleoside	3′-Nucleotide	5′-Nucleotide
Adenine	N1	3.52	3.70	3.88
Cytosine	N3	4.17	4.43	4.56
Guanine	N7	3.30	(3.5)	(3.6)
Guanine	N1	9.42	9.84	10.00
Thymine	N3	9.93	–	10.47
Uracil	N3	9.38	9.96	10.06

Data correspond to loss of a proton for $pK_a > 9$ and capture of a proton for $pK_a < 5$; calculated to 20 °C.

the ring nitrogens are more basic ($\Delta pk_a = +0.4$) and the ring N–Hs are less acidic ($\Delta pk_a = +0.6$) compared with the corresponding nucleosides. For dissociation constants, see table. BETTINA APPEL

activator *DNA-binding protein that increases the rate of *transcription by enhancing the interaction between *RNA polymerase and the *promoter.

active center The terms active center and *active site are often used as synonyms. However, active center may be a description for active site. For example, the active center of *group I introns is composed of elements P1, P3, P4, P6, P7, J4/5 and J8/7, but only a subset of *functional groups within these structural elements form the active site. The term *catalytic core, employed in the same context to describe the minimal architectural elements required for *catalysis, even goes beyond active center, as it additionally includes RNA elements that buttress the active center (e.g. comprising P4/5/6, P3/7/8/9 and P1/2/10 in group I introns). ROLAND K. HARTMANN, MARIO MÖRL, DAGMAR K. WILLKOMM

active conformation Folding state that predisposes a *catalytic RNA to immediate entry into its functional cycle. For reactions *in trans*, the RNA requires complexation with the substrate to achieve its active, catalytically competent conformation (→induced-fit model). As an example, the *RNase P RNA-binding module for the *CCA-tail of *precursor tRNAs becomes structured only upon substrate binding, which further entails a rearrangement of metal ions.

Three major factors contribute to the active conformation of RNA catalysts: peripheral structural elements of the RNA involved in *long-range tertiary contacts, metal ions and protein cofactors. In the case of the *hammerhead, it has been discovered only recently that natural variants form tertiary contacts between loop extensions in the two arms of their Y-shaped structure. These contacts seem to restrict the *RNA fold in a state that is equal or close to the catalytically active conformation. The important consequence is a substantially lower Mg^{2+} requirement for activity. To stabilize the *catalytic core and thus adopt an *active conformation, large *ribozymes utilize peripheral structural elements which engage in long-range tertiary interactions. In some *self-splicing introns, long-range interactions are reinforced by protein cofactors. RNase P RNA needs a small basic protein to stabilize local structure in the catalytic core in order to create high-affinity *binding sites for a set of Mg^{2+} ions

relevant to substrate binding and catalysis. The protein thereby lowers the Mg^{2+} requirement of the enzyme.

Ribozymes, as protein enzymes, traverse different conformational states during their functional cycle. For example, a large conformational change takes place when the guanosine cofactor of group I introns is replaced with the 3′-terminal omega G after the first *transesterification step. *X-ray analyses of the *hepatitis delta virus ribozyme in pre- and post-cleavage states have revealed a substantial conformational change that occurs after cleavage. This conformational switch can be described as a structural collapse of the product active site, during which the catalytic metal ion is expelled and the catalytic residue C75 is displaced such that the reverse reaction (→ligation) becomes unfavorable.

It is often unknown which fraction of a pool of ribozyme molecules populates a catalytically active conformation. Native polyacrylamide gels may give a first clue about different conformational states and whether the ribozyme population is heterogeneous in conformation.

ROLAND K. HARTMANN, MARIO MÖRL, DAGMAR K. WILLKOMM

active site Term employed for structural elements and functional groups of a *catalytic RNA, which (a) directly interact with or position the reactive *phosphodiester or phosphate, (b) are directly involved in chemistry or (c) that bind *ligands involved in the chemical step, such as catalytic metal ions. The active site may be more solvent exposed, as in the *hammerhead ribozyme, or deeply buried in the folded structure, as in the *hepatitis delta virus (HDV) ribozyme. Different modes of active site construction are found among *ribozymes, e.g. in the HDV ribozyme the active site is formed by a *nested double pseudoknot structure in which five helical segments form two parallel stacks, joined side-by-side by several strand crossovers. In the *hairpin ribozyme, the active site is created by docking of two irregular helices in a side-by-side manner, with the two helical stacks radiating from a perfectly base paired *four-way junction. *Group I introns fold into compact globular structures by assembly of three main domains (P4/5/6, P3/7/8/9 and P1/2/10), with large segments of the universally conserved *catalytic core buried from solvent. The *group I intron active site is created by juxtaposition of elements P1, P3, P4, P6, P7, J4/5 and J8/7.

ROLAND K. HARTMANN, MARIO MÖRL, DAGMAR K. WILLKOMM

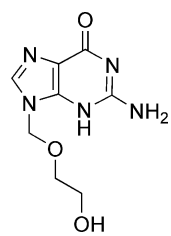
active site chemistry The natural *small nucleolytic ribozymes [*hammerhead, *Varkud satellite, *hepatitis delta virus (HDV) and *hairpin ribozyme] catalyze site-specific *phosphodiester hydrolysis with 5′-hydroxyls and 2′,3′-cyclic phosphates as cleavage products. They activate the 2′-hydroxyl at the scissile phosphodiester as the nucleophile. In contrast, the large natural ribozymes (*group I intron, group II intron *RNase P RNA) catalyze phosphodiester hydrolysis or phosphoryl-transfer reactions (→transesterification) that yield 5′-phosphates and 3′-hydroxyls as products. A common salient feature of their catalytic strategies is to prevent the 2′-hydroxyl at the reactive phosphodiester from acting as a nucleophile.

In the hammerhead example, a divalent metal ion is thought to form an *inner-sphere contact with the pro-*R*p nonbridging oxygen at the scissile phosphodiester. Linear dependence of the log of the reaction rate on pH has been interpreted to indicate that a hydroxide coordinated to the same metal ion deprotonates

the 2′-hydroxyl for nucleophilic attack. In the HDV genomic ribozyme, the N3 function of the catalytic key residue C75 acts as a general base and deprotonates the attacking 2′-hydroxyl. A hydrated metal ion bound nearby then serves as general acid by protonating the 5′-oxyanion leaving group. In the case of the hairpin ribozyme, there is no evidence for a catalytic role of divalent metal ions. Here, the N1 (possibly in its protonated form) and the 6-amino function of residue A38 provide electrostatic stabilization of the *transition state.

The large ribozymes utilize metal-ion-assisted catalysis, with a specific role for two or more magnesium ions, similar to protein phosphoryltransferases. Metal ions activate the nucleophile, stabilize the transition state or leaving group by direct coordination to non-bridging and bridging phosphate oxygens or facilitate proton transfer to the leaving oxygen.
ROLAND K. HARTMANN, MARIO MÖRL, DAGMAR K. WILLKOMM

acyclovir Analog of *guanine in which the nitrogen atom at position 9 is substituted by a hydroxy ethyl methyl ether. It is an antiviral drug, which is primarily used for treating herpes simplex virus. The viral thymidine kinase converts acyclovir selectively into a monophosphate and the monophosphorylated acyclovir is phosphorylated into the active triphosphate form (acyclovir-GTP) by the host cellular kinase.
BETTINA APPEL



adaptor hypothesis →transfer RNA, →wobble hypothesis

adenine One of the four naturally occurring *nucleobases in DNA and RNA.

adenine arabinoside →arabinonucleosides

adenine riboswitch Highly similar in its structure and function to the guanine riboswitch; therefore, both classes are usually referred to as *purine riboswitches.

adenine xyloside →xylonucleosides

adenosine Natural building block of RNA (→nucleosides).

adenosine diphosphate (ADP) →adenosine phosphates, →nucleoside phosphates

adenosine monophosphate (AMP) →adenosine phosphates, →nucleoside phosphates

adenosine phosphates Beyond their function as building blocks for nucleic acids, adenosine phosphates constitute the major form of energy storage in the cell. *ATP is an intermediate product of all cellular processes that produce chemical energy to be stored. Its biosynthesis occurs by phosphorylation of *ADP with the transferred phosphate coming from either high-energy phosphates or creatinine or other *nucleoside triphosphates (→nucleoside phosphates). Energy that is stored in ATP is used for the synthesis of macromolecules as well as for activation of different compounds and metabolites. Furthermore, in a number of cellular processes, adenosine phosphates serve the role as metabolic regulators, e.g. in glycolysis or in the Krebs cycle. In living organisms, *AMP, *ADP and *ATP appear in equilibrium with physiological concentrations of ADP and ATP of about 10^{-3} mol L^{-1}.
SABINE MÜLLER

adenosine triphosphate (ATP)
→adenosine phosphates, →nucleoside phosphates

A-DNA Right-handed double-stranded helix stabilized by *Watson–Crick base pairs. One of the several types of *secondary structures that DNA can adopt, depending on environmental conditions such as counterions and relative humidity. The A-form is favored in solutions that are relatively devoid of water. A-DNA has been well characterized in crystal structure. The reagents used to promote *crystallization of DNA tend to dehydrate it and this leads to a tendency for many DNAs to crystallize in the A-form. A-DNA is shorter and has a greater diameter than the *B-DNA. In A-DNA, 11 *nucleotide pairs complete one *helical turn. *Base pairs are tilted by 13–20° from the perpendicular. Moreover, they are shifted towards the outside of the helix, yielding the deep *major, but shallow *minor groove. The helix axis lies in the major groove, bypassing the bases. The key difference between A- and B-DNA is the *sugar puckering mode; the sugar units in A-DNA are all in the standard C3′-*endo* conformation. Individual residues in A-DNA-type fragments display uniform structural features. This contrasts with the B-DNA *double helix, where sequence-dependent structural modulations are observed. If the salt concentration of the environment is raised or the relative humidity lowered, the B–A transformation takes place. B–A transition occurs cooperatively, indicating the energy barrier between C2′-*endo* and C3′-*endo* sugar puckering. The transition from the B-form double helix to the A-form is essential for biological function, as shown by the existence of the A-form in many protein–DNA complexes. If *DNA–RNA hybrids are generated, they belong to the A-family and resist transformation into B-form (→DNA structures).
NINA DOLINNAYA

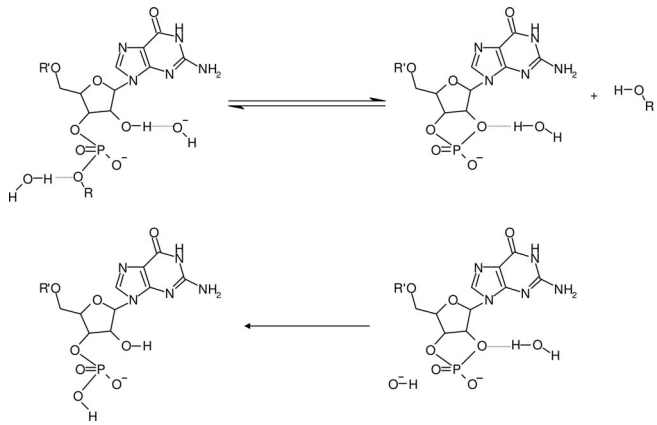
ADP →adenosine diphosphate, →adenosine phosphates, →nucleoside phosphates

AFM Abbreviation for atomic force microscopy (→scanning force microscopy).

A-form RNA Type of the double-helical structure of RNA if occurring as a *duplex. The *double helix of A-form RNA closely resembles the helical parameters defined for DNA of the A-family (→A-DNA).
SABINE MÜLLER

algorithmic self-assembly Generalization of crystal growth mechanisms that considers how a mixture of several molecular species can self-assemble to create complex forms. Molecules are modeled as Wang tiles, which are geometric shapes such as squares whose sides are labeled to indicate specific binding interactions. Given a seed structure, tiles may be added if they match on sufficiently many sides. Large, complex structures can be grown using a small number of tile types according to such rules. Wang tiles can, in principle, be created as properly designed proteins or other molecules, but to date experimental demonstrations have used DNA Wang tiles. Algorithmic crystals have been created that contain Sierpinski triangle patterns, binary counting and direct copying of information. Important questions include how to reduce error rates and how to nucleate growth on seed structures. →DNA nanotechnology.
ERIK WINFREE

alignment Arrangement of primary sequences of DNA, RNA or protein aiming at the identification of similarity regions as a putative consequence of functional, evolutionary or structural constraints. It is an integral part in many studies of molecular



biology and systematics to reveal homologous regions. There are two main types of alignment algorithms: local and global. Local algorithms like BLAST try to align only parts of sequences often avoiding gaps, whereas global algorithms like CLUSTAL try to align entire sequences and explicitly handle gaps. MAURO SANTOS

alkaline hydrolysis One remarkable difference between DNA and RNA is that unlike RNA, DNA is not degraded under alkaline conditions. This is due to the fact that RNA possesses a hydroxyl group at its 2′ carbon which can be activated by OH^- to trigger a nucleophilic attack on the phosphorous, which in turn leads to a *transesterification and ultimately breaks the RNA's *sugar–phosphate backbone. ULI HAHN

alkaline phosphatase Enzyme that cleaves phosphoric acid monoesters under alkaline conditions.

alkylating agent →DNA alkylating agent

alkyltransferase Enzyme supporting the transfer of an alkyl group (→methyltransferase).

allele One of two or more alternative forms of a *gene located at the corresponding site (locus) on *homologous chromosomes.

allosteric activation Induced change of the conformation of a biomolecule upon interaction with low-molecular-weight compounds. Allosteric effects play an important role in the regulation of enzymatic activity. Typically, the binding domain for the *ligand is located in a part of the molecule that is distant from the catalytic part. In the nucleic acids field, a number of *allosteric ribozymes have been constructed. Activity of these *ribozymes is coupled to the presence of a specific ligand that upon binding to the allosteric domain of the ribozyme regulates activity up (positive regulation) or down (negative regulation).

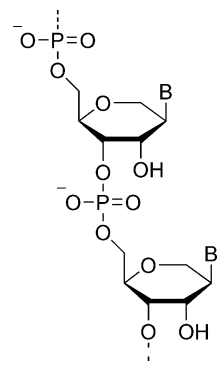
Allosteric activation concerns also the field of gene regulation at the level of *messenger RNA. Binding of metabolites to specific sites in the *5′-untranslated region of certain mRNAs can induce conformational changes that in turn lead to premature *transcription termination or *translation inhibition (→riboswitches). SABINE MÜLLER

allosteric ribozyme Also called *aptazyme, *ribozymes that can be regulated by an allosteric cofactor. An allosteric ribozyme is composed of a RNA motif that binds a specific *ligand with high affinity (→aptamer) and a catalytic RNA motif. Both motifs are connected via a bridge element or communication module that is capable of translating the binding event in the aptamer domain to the catalytic part and thus triggering activity. Allosteric ribozymes can be constructed by rational design, combining a pre-selected *aptamer with a known ribozyme structure. Many examples involve the *hammerhead ribozyme or the *hairpin ribozyme. Alternatively, allosteric ribozymes can be selected from a random *RNA library. In this case, a synthetic RNA library has to be screened for active species in the presence (or absence) of the specific ligand (→allosteric activation, →*in vitro* evolution of nucleic acids, →ribozyme). SABINE MÜLLER

allostery →allosteric activation, →allosteric ribozyme

alternative splicing Pathway to generate different mature *messenger RNA (mRNA) molecules from one *primary transcript. This mechanism was initially discovered in viruses, generally known to depend on very limited amounts of their own genetic material. Today, however, alternative splicing pathways have been identified in most eukaryotes. The possibility to generate more than one functional gene product from the same pre-mRNA is one of the most important features to explain the relatively low complexity of metazoan *genomes, e.g. the surprisingly small number of about 30 000 functional *genes in mammals. In addition to *transcriptional regulation, alternative splicing provides a second important basis for differential *gene expression during cell differentiation and developmental processes such as sex determination. Alternative splicing may not only result in a different selection of *exons from a *pre-mRNA molecule, but may also establish alternative initiation or termination sites for protein biosynthesis, i.e. *start and *stop codons. Although the precise mechanism of alternative splicing remains to be elucidated, it was found that exonic as well as intronic regulatory elements are involved. Those elements may act as either enhancers or silencers of splicing. At the protein level, the large family of *SR (serine/arginine-rich) proteins appears to be involved in splice site selection. In their function, SR proteins seem to be antagonists to the major *heterogeneous nuclear ribonucleoprotein (hnRNP) proteins. Alternative splicing may result in (a) exon skipping, (b) alternative 3′-splice site choice, (c) alternative 5′-splice site choice and (d) *intron retention. Although major class and minor class splice sites are incompatible with each other, a rare intron-within-an-intron architecture has been observed, meaning that a major class intron is flanked by two *atac splice sites and splicing occurs by one or the other pathway, alternatively. BERND-JOACHIM BENECKE

altritol nucleic acid (ANA) RNA analog with a phosphorylated D-altritol backbone. The nucleobase is attached at the 2-(*S*)-position of the carbohydrate moiety.



amber codon UAG *stop codon which can be decoded by a specific amber *suppressor transfer RNA. The artificial name "amber" (Am) was used to designate a mutation in a laboratory strain which turned out to be a *nonsense mutation. The name commemorates the work of one of the participating graduate students, Harris Bernstein (German: Bernstein = English: amber). STEFAN VÖRTLER

amber mutation Special case of a *nonsense mutation.

amino acid binding site Reaction center in *amioacyl-tRNA synthetases (aaRS) which is responsible for the specific binding and activation of one out of the 20 canonical proteinogenic amino acids. Pauling pointed out as early as 1954 that it is impossible to differentiate between structural isomers (Val and Ile) or amino acids which differ in as little as a methyl group (Thr and Ser or Cys) just on binding affinity. The energetics are far too similar, but aaRS must do exactly that. However, nature utilizes not one, but several reactions to achieve high selectivity by coupling them sequentially. (a) A double-sieve mechanism excludes larger or sterically non-fitting amino acids from binding, while smaller ones are disfavored by suboptimal binding energies. (b) An additional proofreading site tests either amino acid adenylates or already aminoacylated *transfer RNA (tRNA) and hydrolyses off any wrongly attached amino acid. These pre- and post-transfer mechanisms (equations 1 and 2, respectively) are known to operate at various degrees in different aaRS. A classic example is the differentiation of Val and Ile by IleRS:

Pre-transfer:

$$\text{Val-AMP} \cdot \text{IleRS} + \text{tRNA}^{\text{Ile}} \rightarrow \text{Val} + \text{AMP} + \text{IleRS} + \text{tRNA}^{\text{Ile}} \quad (1)$$

Post-transfer:

$$\text{Val-tRNA}^{\text{Ile}} \cdot \text{IleRS} \rightarrow \text{Val} + \text{tRNA}^{\text{Ile}} + \text{IleRS} \quad (2)$$

Considerable biotechnological interest concerns the activation and incorporation of unnatural amino acids into proteins by *genetic code expansion. To avoid any interference with normal *decoding, usually one of the three *stop codons will be reassigned to decode a *suppressor tRNA. The *ribosome during decoding does not control the nature of the tRNA with respect to the attached amino acid (→aminoacyl-tRNA synthetase, →translation). Major efforts were therefore concentrated to (a) engineer or evolve aaRS to recognize and activate novel amino acids, which is by nature very difficult as precise amino acid activation is a prime requisite of this enzyme class, and (b) develop novel tRNA which do not interfere with the natural aminoacylation systems of a given organism. Thus, the presence of any additional tRNA will not matter as long as it is not a substrate to endogenous aaRS. It works if yeast or archeal aaRS/tRNA pairs are used in a prokaryotic system as they mutually do not recognize the heterologous molecules. Several such orthogonal aminoacylation systems have been established by large-scale protein as well as cellular engineering techniques. The alternative strategy of chemical aminoacylation and supplementing translation systems with prepared aminoacyl-suppressor tRNA works, but suffers from the instability of the aminoacyl bond, limiting the efficiency as well as yield.

A direct amino acid-binding ability of RNA has been long speculated about and searched for with respect to the *RNA world, the *adaptor hypothesis and the origin of the *genetic code. Artificial *aptamers and *ribozymes with aminoacylation function posses such abilities, as well

as natural *riboswitches located in messenger RNA to regulate metabolism. Statistical analysis of known RNA-binding motifs with respect to the *codon assignment of the corresponding amino acids is an active field of investigation and could give clues to a stereochemical origin of the *genetic code: a particular *codon was assigned to a particular amino acid because it participated best in binding it. STEFAN VÖRTLER

aminoacylation The activation of amino acids for protein synthesis by esterifying the carboxy-terminus to the 2′- or 3′-hydroxyl of the terminal *ribose of a *transfer RNA (→aminoacyl-tRNA, →aminoacyl-tRNA synthetase).
STEFAN VÖRTLER

aminoacyl-tRNA Product of the *aminoacylation reaction catalyzed by *aminoacyl-tRNA synthetases which activate amino acids for *translation by esterifying them to the terminal *ribose of the corresponding *transfer RNA. The formation of a 2′- or 3′-hydroxylester depends on the class of the corresponding synthetase. Both are of high energy (around 30 kJ mol^{-1}) and reactivity, resulting in short half-lifes. Apart from hydrolysis and aminolysis involving the neighboring α-amino group, a *transesterification equilibrium between the 2′- and 3′-hydroxyls exists as all aminoacyl-tRNA incorporated by the *ribosome are acylated to the 3′-hydroxyl group. Stability of the esters increases dramatically once the α-amino group is involved in a bond, as in *peptidyl-tRNA, or sterically hindered and protected when bound to *elongation factor EF-Tu/EF1α. For this reason protein biosynthesis relies on the rapid binding of aminoacyl-tRNA to a sufficient pool of elongation factors, which are the most abundant proteins in the cytosol (5–10% of total protein).

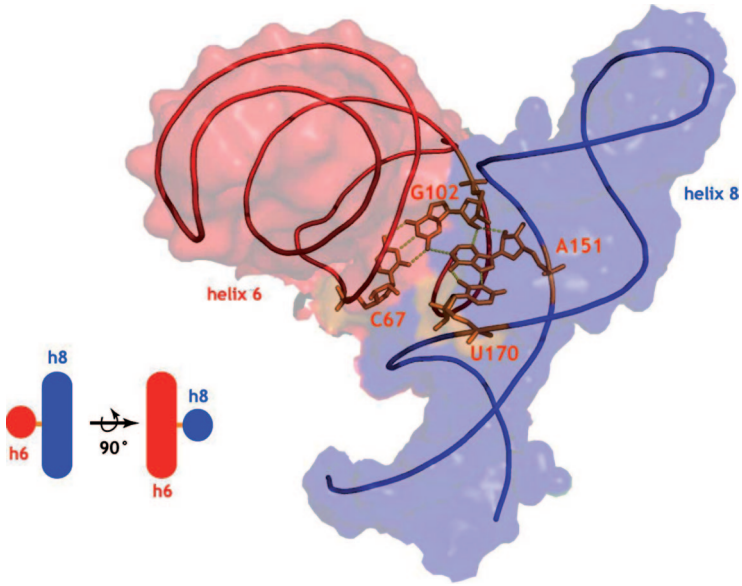
Activated amino acids are also precursors for porphyrin biosynthesis in plants and photosynthesizing bacteria (glutamate $tRNA^{Glu}$) or bacterial peptidoglycan synthesis (glycine $tRNA^{Gly}$). STEFAN VÖRTLER

aminoacyl-tRNA binding site (A-site)
Binding site for *aminoacyl-tRNA in the *ribosome (→translation).

aminoglycosides *Antibiotics that are often used to treat meningitis or mucoviscidosis. Aminoglycosides are not assimilated by the gut, so they need to be given intravenously and intramuscularly. The binding of aminoglycosides to the 30S *subunit of bacterial *ribosomes inhibits the *translocation of the *peptidyl-tRNA from the *A-site to the *P-site and may cause miscoding of *messenger RNA. This leads to inhibition of protein synthesis and to the cessation of bacterial growth.
BETTINA APPEL

2-aminopurine Fluorescent analog of *adenine. It forms a *base pair with *uracil that is isosteric to a natural AU base pair. Fluorescence of 2-aminopurine is high if the nucleobase is located in a highly flexible region of a molecule, but becomes quenched upon integration of the base analog into a more structured surrounding. Thus, 2-aminopurine has been used to follow structural dynamics of nucleic acids, in particular strand *association and *dissociation as well as tertiary folding (→fluorescence spectroscopy of nucleic acids). SABINE MÜLLER

A-minor motif One of the most interesting *tertiary structure motifs in RNA. It is, for example, found in the *hammerhead ribozyme, the P4–P6 domain of *group I intron and in the *ribosomal RNA. It describes a *nucleotide–nucleotide interaction involving interactions between



A-minor motif in the 16S ribosomal RNA. Structural representation of how a type II A-minor motif facilitates helix packing of the helices h8 (blue) and h6 (red); the nucleotides involved in the tertiary interaction are shown as sticks in orange, nucleotide A151 forms a type II A-minor motif by interacting with the base pair of G102–C67; A151 itself is additionally involved in a Hoogsteen type of interaction with U170.

*nucleobases and the *ribose moieties of the *nucleotide.

The motif is defined as a single-stranded *adenine that interacts via *hydrogen bonds and *van der Waals contacts in the *minor groove of a given *base pair (either AU or GC). The A-minor motif is classified into two types. Type I describes the interaction of the adenosine with both nucleotides involved in the base pair, whereas type II represents just the interaction with one out of the two base-pair-forming nucleotides. Boris Fürtig, Harald Schwalbe

AMP →adenosine monophosphate, →adenosine phosphates, →nucleoside phosphates

amplicon DNA products synthesized using *amplification techniques, such an amplified segment of a *gene or DNA is known as an amplicon. It could also be applied to a cloned, amplified DNA sequence, but is commonly used to describe sequences derived from the *polymerase chain reaction or the *ligase chain reaction. David Loakes

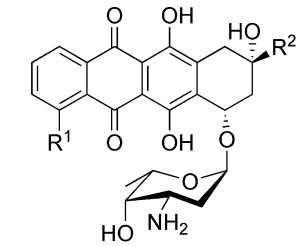
amplification Production of multiple copies of a DNA sequence starting with one or a few copies. The process of amplification can occur *_in vivo_* by the production of many copies of a *plasmid in a bacterial cell or in culture (→cloning) or *_in vitro_*, which is generally carried out through the *polymerase chain reaction (PCR). Amplification by most *polymerases (DNA and RNA) is usually linear (i.e. a single copy is produced during each round of replication), but occurs logarithmically during the PCR amplification of the target sequence. David Loakes

ANA →altritol nucleic acid

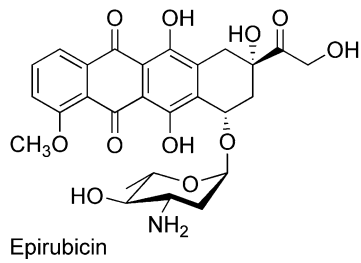
anchored primer *Genomic DNA and *messenger RNA (mRNA) contain significant regions of repeat sequences or polypurine/polypyrimidine regions, e.g. *poly(A) tails. Such regions are particularly difficult to accurately reproduce or sequence. Thus, an oligo(dT) primer on a poly(A) *template would have many *binding sites resulting in *primer slippage and extension products of varying length and *nucleotide composition as a direct result of mispriming. A method to fix a primer to such a sequence is the use of an anchored primer, which usually contains two specific *nucleotides at the 3′-end that are complementary to the *template sequence. For example, to anchor a primer to a poly(A) sequence which terminates with the nucleotide sequence CG the primer would be designed with GC at the 3′-end of the oligo(dT) region. It anchors the oligo(dT) portion of the primer directly at the junction between the poly(A) tail and the end of the *transcript, and anchors the oligo(dT) primer only to the subset of transcripts that are complementary at the dinucleotide position.

Anchored primers are of particular use in the differential display *polymerase chain reaction (DD-PCR), which is a method for identification of differentially expressed genes by comparative display of arbitrarily amplified *cDNA subsets. The essence of the differential display method is to use, for *reverse transcription, an anchored oligo(dT) primer that anneals to the beginning of a subpopulation of the poly(A) tails of mRNAs. The anchored oligo(dT) primers consist of 11–12 thymidines and two additional 3′-nucleotides to provide specificity. David Loakes

annealing of oligonucleotides Pairing of single-stranded RNA or DNA to a *complementary sequence by hydrogen bonding, forming a double-stranded *oligonucleotide. It is mainly used to describe the process of *primer binding to a DNA strand during the *polymerase chain reaction. Denise Strohbach



	R^1	R^2
Idarubicin	H	acetyl
Daunomycin	OCH_3	acetyl
Doxorubicin	OCH_3	2-hydroxyacetyl



Epirubicin

anthracyclines

anthracyclines Class of antibiotics used as chemotherapeutic agents to treat a wide range of cancers (e.g. leukemias, lung cancer). They inhibit *DNA replication due to the *intercalation with nucleolar DNA forming a highly stabilized complex. The aromatic D-ring interacts via the *major groove of DNA and the A-ring inserts into the *minor groove. The amino-sugar can make further *hydrogen bonds with the DNA, increased by the presence of water. Bettina Appel

antibiotics Compounds that hamper the growth of microorganisms. Several antibiotics interact with DNA or RNA and thus inhibit essential steps of *nucleic acid

metabolism (→anthracyclines, →ribosome interaction with antibiotics). SABINE MÜLLER

anticodon Single-stranded 3-nucleotide stretch (positions 34, 35 and 36) at the end of *anticodon arm of a L-shaped *transfer RNA. Rapid formation of a short duplex stretch with the corresponding *complementary *messenger RNA *codon in the ribosomal *A-site is the prerequisite for *decoding of the *genetic code during *translation. STEFAN VÖRTLER

anticodon arm One branch of the L-shaped three-dimensional structure of a *transfer RNA, which carries the *anticodon at its end ready to interact with *messenger RNA at the 30S *ribosomal subunit. It is formed by coaxial stacking of the double-stranded *TΨC and anticodon helices of a tRNA cloverleaf. The *acceptor arm forms the second branch. STEFAN VÖRTLER

anticodon loop Single-stranded loop region at the end of the *anticodon arm of a *transfer RNA containing the 3-nucleotide *anticodon. The loop contains 7 *nucleotides at positions 32–38 in the fringe being stacked to the double-helical stem and an invariant U33 inducing a *uridine turn in the *phosphate backbone. All of this leads to a pre-structured, accessible orientation of the anticodon bases ready to base pair with the mRNA. STEFAN VÖRTLER

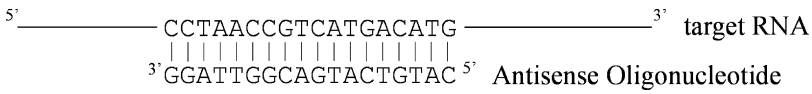
***anti* conformation** The *anti* conformation corresponds to a *glycosidic torsion angle κ in the range $180 \pm 90^\circ$. Due to steric hindrance, nucleosides and nucleotides are usually found in the *anti* conformation with $\kappa = -135 \pm 45^\circ$. →glycosidic torsion angle. KLAUS WEISZ

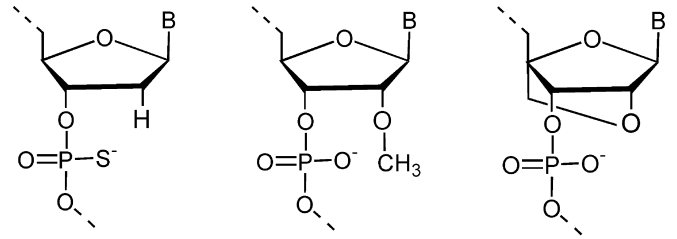
antiparallel orientation Mutual orientation of two chains in *double-stranded DNAs of the B-, A- and Z-families. In the case of antiparallel orientation of two DNA strands, their 5′,3′-*phosphodiester linkages run in opposite directions. NINA DOLINNAYA

antisense drug *antisense oligonucleotide used as a therapeutic agent in the treatment of various types of diseases, e.g. cancer or viral infections. The first antisense drug, vitravene, has been approved by the US Food and Drug Administration for the treatment of cytomegalovirus retinitis. IRENE DRUDE

antisense gene Sequence that is expressed as a RNA complementary to the *messenger RNA of a given *gene. Antisense genes efficiently knockdown the expression of endogenous genes and substantiate the *RNA interference knockdown strategies. MAURO SANTOS

antisense oligonucleotides (ASONs) DNA oligomers of 15–20 nucleotides in length that bind to a complementary target RNA by *Watson–Crick base pairing are referred to as ASONs. Their potential to inhibit *gene expression in a sequence-specific manner has been explored since the late 1970s. ASONs hybridize to their *target RNA and induce a cellular endonuclease, *RNase H, which recognizes the unusual *DNA–RNA hybrid and cleaves the RNA moiety of the *heteroduplex. As a consequence, the protein encoded by the targeted RNA is not synthesized. A second mechanism by which ASONs inhibit *translation is a steric blockade of the *ribosome.





Furthermore, ASONs can be directed against *splice sites to alter the splicing pattern of a *pre-mRNA.

Since natural DNA *oligonucleotides are rapidly degraded in biological fluids, ASONs have to be protected against nucleolytic degradation by the introduction of *modified nucleotides. The most widely used building blocks for ASONs are phosphorothioates in which one of the nonbridging oxygens is replaced by a sulfur atom. *Phosphorothioate oligonucleotides are stable and activate RNase H, but they have a comparably low target affinity and toxic side-effects have been observed due to unspecific binding to certain proteins. Therefore, a second generation of modified nucleotides has been developed containing alkyl groups at the 2′-position of the ribose (e.g. 2′-*O*-methyl RNA). In recent years hundreds of new modifications have been introduced to further improve the properties of ASONs (e.g. *locked nucleic acids). Chemical modifications of the *ribose usually result in a loss of the ability of ASONs to activate RNase H. To retain this activation, gapmers can be employed which contain modified nucleotides at both ends of the oligonucleotide for protection against exonucleases and DNA monomers or phosphorothioates in the center to induce RNase H cleavage of the target RNA. Another major challenge for *in vivo* applications of ASONs is their efficient delivery to the cells in the targeted tissue. JENS KURRECK

antisense RNA Cellular and viral *gene expression and the *transcription of *noncoding RNA give rise to transcribed RNAs that are essential for controlled biological processes in living cells. Among the commonly used *antisense strategies, antisense RNA is one out of various chemical forms of *nucleic acids. Those include long-chain nucleic acids that are usually RNA as well as short *antisense oligonucleotides both of which are complementary in nucleotide sequence with a given natural *transcript of above origin. Thus, antisense RNA represents one form of *antisense drugs that may be transcribed *in vitro* by *RNA polymerases such as the phage *T7 RNA polymerase prior to cellular delivery or it may be transcribed intracellularly in living cells. In the latter case, *antisense strands are transcribed by cellular polymerase from recombinant *antisense genes that have to be introduced into target cells and tissues by *gene transfer using strategies that are developed and exploited in the field of *gene therapy. Antisense genes may be under the control of constitutive *promoters giving rise to stable expression of antisense RNA. In the case of viral target RNA this concept has been termed “intracellular immunization”. For purposes of correcting aberrant cellular gene expression in a transient

fashion one also uses *inducible promoters. This method of transient target suppression by antisense RNA is commonly used in the fields of gene function analysis and target validation. GEORG SCZAKIEL

antisense strand Non-coding strand of *double-stranded DNA that serves as the *template for *messenger RNA synthesis during *transcription (→antisense gene).

antisense strategy *Messenger RNAs (mRNAs) transfer *genetic information (in sense orientation) from the *genomic DNA to the place of protein synthesis. *Oligonucleotides with a complementary sequence to a certain mRNA can be employed to bind to the targeted mRNA and prevent its *translation. These oligonucleotides can be viewed as being orientated in the antisense direction. Originally, *antisense oligonucleotides have been utilized for this purpose, but in a broader sense subsequently developed methods like *ribozyme technologies and *RNA interference can be considered to be antisense strategies as well. The term (post-transcriptional) *gene silencing is also frequently used to depict these applications.

Antisense agents hybridize to their target RNA by *Watson–Crick-type base pairing. Since a sequence of at least 16 nucleotides is statistically unique in the human genome, binding of oligonucleotides of this length can be expected to be highly specific. Antisense strategies therefore allow precise targeting even in cases in which low-molecular-weight compounds are not sufficiently specific due to structural similarities of closely related proteins.

Antisense strategies are widely employed techniques for functional genomics studies. Although the complete sequence of the human genome is now available, the precise function of many *genes and their encoded proteins is not yet known. Antisense strategies allow reverse genetic approaches to elucidate the role of genes by specifically inhibiting their expression. The resulting loss-of-function *phenotype can be analyzed to draw conclusions about the function of the lacking protein. Antisense strategies can be seen as a complementary approach to the generation of knock-out animals. They are, however, more straightforward, faster, cheaper and can even be employed in cases where the lack of the gene under investigation is lethal during embryonic development.

Furthermore, antisense strategies are promising approaches to treat diseases caused by the expression of deleterious genes. *Antisense oligonucleotides, *ribozymes and *small interfering RNAs have thus been tested in clinical trials to treat a broad range of diseases, including cancer, viral infections and inflammatory diseases. JENS KURRECK

antitermination Mechanism of *transcriptional control in bacteria in which *termination is prevented at a specific terminator site, allowing *RNA polymerase to express *downstream *genes. Transcription of *non-coding genes, such as *ribosomal RNA and *transfer RNA, which are mostly highly structured, constitutes a problem for the contiguous synthesis of RNAs. Here, RNA polymerase needs additional proteins, called antitermination factors (Nus proteins in phage λ or the ribosomal protein S10 in *Escherichia coli*) which prevent RNA polymerase from pausing. BEATRIX SÜß

A-platform →AA-platform

AP-site →abasic site

aptamers (Latin: *aptus* = "to fit") Aptamers are macromolecules composed of DNA,

RNA or modified nucleic acids (→modified DNA, →modified RNA) that bind tightly to a specific molecular target. Their properties are defined by their *nucleotide sequence and most aptamers are in a size range of 15–60 nucleotides. The chain of nucleotides forms intramolecular interactions that fold the molecule into a complex three-dimensional shape, which allows it to bind tightly against the surface of its target molecule. *Induced fit and adaptive binding play important roles in aptamer–target interaction. As a high diversity of molecular shapes exists within the universe of all possible nucleotide sequences, aptamers may be obtained for a wide array of molecular targets, including most proteins and many small molecules.

Aptamers are typically isolated by the *SELEX process (→aptamer selection). Aptamers can distinguish between closely related, but non-identical molecules, or between different functional or conformational states of the same macromolecule. In addition to high specificity, aptamers have very high affinities to their targets, typically in the picomolar to low nanomolar range. Although aptamers are chemically rather stable, they must be chemically modified for *in vivo* applications to reduce their sensitivity to enzymatic degradation or to improve their pharmacokinetics.

Aptamers hold tremendous potential as targeted molecular therapeutics. Furthermore, they are used for target validation, drug (small-molecule) screening, sensor development (→reporter ribozyme, →RNA sensor) and affinity separations.

In nature, aptamer domains have been discovered as components of *riboswitches.

The term aptamer is sometimes also used for high-affinity peptide ligands (peptide aptamers). ANDRES JÄSCHKE

aptamer selection Procedure for the selection of *aptamers and a variant of the *SELEX process. The selection procedure allows the isolation of aptamers on the basis of binding between a target and *nucleic acid molecules, and relies on standard molecular biology techniques. Steps of the selection cycle are (a) library preparation, (b) selection, (c) amplification and (d) aptamer isolation. In step (a), a large library (or *sequence pool) is synthesized. Each molecule in the library (typical complexity of up to 10^{15} different compounds) contains a unique nucleotide sequence that can, in principle, adopt a unique three-dimensional shape. Only very few of these molecules – the aptamers – present a shape that is complementary to the target molecule. The selection step is designed to find those molecules with the greatest affinity for the target of interest. The library is incubated with the target and the library members will either associate with the target or remain free in solution. Several methods exist to physically separate the aptamer target complexes from the unbound molecules in the mixture. The most common variants of the selection step are the use of immobilized or biotinylated targets, filter binding of RNA–protein complexes, or chromatographic/electrophoretic isolation. The unbound molecules are discarded, and the target-bound aptamers released and copied enzymatically to generate a new library of molecules that is enriched for those species that can bind the target. This enriched library is used to initiate a new cycle of selection and binding. Typically, after 5–15 cycles, the library is reduced from the 10^{15} unique sequences to a small number of tight-binding molecules. Individual members of this final pool are then isolated, their sequence determined, and their properties with respect to binding

affinity and specificity are measured and compared.

Aptamers have been isolated from RNA, DNA and modified nucleic acid libraries. Several variations of the selection technique exist. Aptamers with high affinity can be found for virtually any target, from small organic molecules to peptides, proteins, cell surfaces and tissue sections. Aptamers can be selected either manually or in an automated fashion. ANDRES JÄSCHKE

aptazyme *Ribozyme, the activity of which is controlled by an external *ligand (→allosteric ribozyme). In general, aptazymes consist of two domains – the *aptamer domain that binds specifically to a ligand (also called effector) and the ribozyme domain that catalyzes the cleavage reaction depending on this binding event. In this way the cleavage activity of a ribozyme can be manipulated in both directions: the binding event results either in structural stabilization or destabilization of the ribozyme domain and causes in up- or down-regulation of ribozyme activity.

*Oligonucleotides and *nucleotides, amino acids and proteins, small organic compounds, and even metal ions have been found to be potential ligands. A variety of nucleic acid-based *biosensors use the principle of allosterically switchable ribozymes. DENISE STROHBACH

apurinic site Special case of an *abasic site.

apyrimidinic site Special case of an *abasic site.

1-β-D-arabinofuranosyladenine
→arabinonucleosides

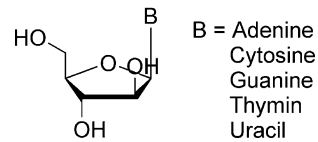
1-β-D-arabinofuranosylcytosine
→arabinonucleosides

1-β-D-arabinofuranosylguanine
→arabinonucleosides

1-β-D-arabinofuranosylthymine
→arabinonucleosides

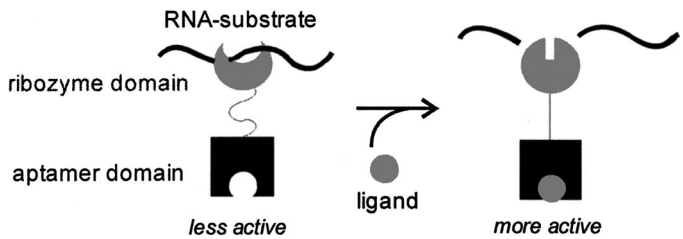
1-β-D-arabinofuranosyluracil
→arabino-nucleosides

arabinonucleosides Structural analogs of *ribonucleosides in which *ribose is replaced with *arabinose.



arabinose Pentose monosaccharide ($C_5H_{10}O_5$; 150,13 g mol^{-1}) that exists in two enantiomeric conformations: D(–)-arabinose and L(+)-arabinose.

arabinose operon (*ara*) Controls the utilization of L-arabinose in *Escherichia coli*. The three structural genes *araB*, *araA* and *araD* form the *araBAD* *operon, and encode enzymes that convert *arabinose to xylulose-5-phosphate, which can be metabolized via the pentose phosphate pathway.



aptazyme

The pBAD promoter is regulated by the arabinose-binding protein AraC. The *araC* *gene is divergently transcribed from the *araBAD* operon.

Depending on the intracellular arabinose concentration, AraC acts either as a *repressor or *activator. In the absence of arabinose, an AraC dimer binds simultaneously to two sites positioned 63 ($araI_1$) and 279 ($araO_2$) residues *upstream of the transcriptional start site of *araBAD*. The intervening DNA is looped and access of the *RNA polymerase to the pBAD promoter is blocked. AraC also is an autoregulator and prevents *transcription from its own *promoter by this mechanism.

In the presence of arabinose, binding of the sugar introduces a conformational change in AraC, converting it into an activator protein that binds to $araI_1$ (–63) and $araI_2$ (–43). Transcription of the *araBAD* operon requires additional binding of the cAMP–CRP (CAP) complex adjacent to AraC (at –92) to ensure that arabinose is used only if the better carbon source glucose is absent.

A series of expression vectors has been constructed on the basis of the pBAD promoter. *Plasmids containing the *araC* gene and the pBAD promoter followed by a *multiple cloning site can be used to clone a gene of interest. Expression of the plasmid-encoded gene in *E. coli* can be tightly controlled by the addition of glucose (repression) or arabinose (induction). FRANZ NARBERHAUS

arabinosides →arabinonucleosides

Argonaute Core component of *RISC, the effector complex of the *RNA interference pathway. In flies, two Argonaute homologs are present. Ago2 is associated with *small interfering RNAs in RISC and responsible for *messenger RNA (mRNA) cleavage, whereas Ago1 is associated with *micro-RNAs and responsible for inhibition of *translation of the targeted mRNA. NICOLAS PIGANEAU

artificial chromosome Linear DNA vector designed to be introduced in eukaryotic cells, and which is stably maintained in the nucleus by means of a *centromere and two *telomere-like termini. It is originally designed as a circular *shuttle vector that can be maintained and amplified in *Escherichia coli*. The vector is linearized using specific *restriction sites, and the region of interest or *insert (stretching from 100kb to 1Mb) is subsequently cloned and transfected into eukaryotic cells, where it is maintained as a nuclear *episome. GEMMA MARFANY, ROSER GONZÀLEZ-DUARTE

artificial nuclease Molecule that imitates the mechanism of enzymes that cleave RNA. These *nucleases can be metal complexes based on Cu^{2+}, Zn^{2+} and lanthanide ions. Moreover, constructs carrying imidazole moieties that are thought to simulate the two histidines in the *active site of *RNase A have been described. Artificial nucleases can also be *oligonucleotides themselves. An example for the latter agents are oligonucleotide-based artificial nucleases, which are *oligonucleotides (in many cases modified to prevent them from being degraded) carrying the cleaving agent covalently linked. →antisense strategy, →artificial ribozyme, →nuclease. JÖRN WOLF

artificial ribozyme *Ribozymes not known to exist in nature. While the currently known natural ribozymes catalyze only a narrow spectrum of chemical reactions (*phosphodiester hydrolysis, formation and *transesterification, and peptide bond formation), **in vitro* selection has successfully been used to isolate *catalysts for various other chemical

reactions, such as aminoacylation, *RNA polymerization, *N*-*glycosidic bond formation and cleavage, *pyrophosphate bond formation and cleavage, amide bond formation, *N*-alkylation, *S*-alkylation, porphyrin metalation, biphenyl isomerization, Diels–Alder reactions, Michael or Aldol reactions, and oxidations and reductions.

For the isolation of artificial ribozymes, two different variations of the *SELEX cycle have been developed. The first is the selection of *aptamers using *transition-state analogs as targets and the subsequent screening of the selected aptamers for *catalysis of the reaction that proceeds via the respective transition state. While such aptamers were frequently generated, only few of them displayed catalytic activity and rate acceleration was typically low. The second, more successful strategy is direct selection, which initially allowed only the isolation of RNA-modifying ribozymes. A *RNA library is mixed and incubated with a substance that might attach itself covalently to the RNA. RNA molecules that accelerate such a tagging reaction are then physically separated from the unmodified members of the RNA library, enzymatically amplified, and subjected to further rounds of selection and amplification. By tethering a second substrate to the RNA pool members, it has become possible to expand the scope of RNA catalysis to reactions between two small organic molecules, like the Diels–Alder and Aldol reactions.

While most artificial ribozymes are *single-turnover, self-modifying RNAs, some of them act as true enzymes with *multiple turnover and obey the laws of enzyme kinetics (→Michaelis–Menten kinetics, →Michaelis–Menten model). The catalytic repertoire of ribozymes can be enhanced by incorporating *modified nucleotides that contain additional functional groups. The catalytic versatility of artificial ribozymes provides support for the *RNA world hypothesis. ANDRES JÄSCHKE

A-site →aminoacyl-tRNA binding site

association For *nucleic acids the term association mostly refers to formation of *secondary or *tertiary structures, e.g. formation of a duplex upon *hybridization of two singe nucleic acid strands, or interaction of secondary structure elements such as loop–loop interactions or domain docking. Furthermore, any molecule binding to a nucleic acid associates to it.

A-stem *Acceptor stem–loop of a *transfer RNA

asymmetric base pair Any base interaction that does not have the regular (symmetric) *Watson–Crick geometry.

atac splicing Splicing of *introns marked by non-canonical splice site *consensus sequences. These "rare" introns have been found in a number of metazoan *genes. The splice site sequences mostly consist of AT (5′-splice site) and AC (3′-splice site) nucleotides, therefore "atac" introns. Since the U12-*small nuclear RNP (snRNP), instead of the U2-snRNP, is involved in removal of those rare introns, they have been designated as *U12-type introns. Together with three other rare *U-snRNAs (U11, U4atac and U6atac), U12-snRNA is a constituent of the *minor spliceosome. In vertebrates, the frequency of occurrence of the U12-type introns is about 0.2% relative to the major GT–AG introns, now often designated as U2-type introns. BERND-JOACHIM BENECKE

AT content The amount of adenosine–thymidine base pairs in double-stranded nucleic acids (→GC content).

atomic force microscopy →scanning force microscopy

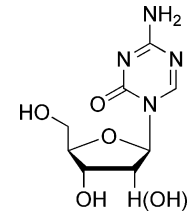
ATP →adenosine triphosphate, →adenosine phosphates, →nucleoside phosphates

attenuation Regulation of a wide variety of bacterial *operons by controlling *termination of *transcription at a site located before the first structural *gene. Classically, attenuation occurs when the transcribed RNA *upstream of an operon has the ability to fold into two mutually exclusive *RNA-fold structures, one which is termed an antiterminator and the other a terminator. If the terminator hairpin loop is allowed to fold, transcription is ultimately halted. Alternatively, if the antiterminator structure folds, the terminator is precluded from folding and transcription of the operon proceeds. The mechanisms that alternate between these two RNA folds (terminators and antiterminators) are quite diverse and can be mediated by direct RNA–ligand interaction (→riboswitches), by uncharged tRNA (→transcriptional attenuation) or stalled ribosomes (→translational attenuation). Regulation by *antitermination can be differentiated from attenuation by the fact that alteration of the transcription complex (rather than alternate RNA structures) decreases the efficiency of *downstream terminators (→translational control). Beatrix Süß

attenuator →attenuation

autosome *Chromosome that appears independent of the sex of a certain eukaryotic organism.

5-azacytidine (5-AC) *Cytidine analog that is used for studying *DNA methylation. If incorporated into DNA, 5-AC changes the methylation pattern. Due to the nitrogen atom at position 5 of the cytosine residue, enzymatic methyl transfer to this position is not possible. This inhibition of methyltransferases leads to undermethylation of the respective DNA and *gene activation, because many genes are turned off by cytosine methylation, particularly in *promoter sequences. Sabine Müller



azidothymidine (Zidovudine) Analog of *thymidine in which the hydroxyl group at C3′ is replaced by an azido group. Azidothymidine is an antiretroviral drug that inhibits the activity of *reverse transcriptase. The azido group increases the lipophilic character of azidothymidine, such that cell membranes can be crossed easily by diffusion. The effective form of azidothymidine is its 5′-triphosphate, which can be synthesized by cellular enzymes. Bettina Appel