<<小分子RNA介导的基因表达调控>>

图书基本信息

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内容概要

内源小分子RNA广泛存在于各种生物中，包括人类、小鼠、果蝇、蠕虫、真菌和细菌等。
microRNA作为一种细胞调控关键因子能够修饰基因的表达。
在高等真核生物中，microRNA甚至能调控约50%基因的表达。

本书汇集了众多科技工作者的前沿性工作，内容包括从细菌到人类等生物组织中microRNA调控途径的多样性。
除了阐述调控小分子RNA的生物合成机制及其加工过程，作者还探讨了这些途径的功能在寄主体内的重要性。

本书围绕小分子RNA这一新发现的调控分子，针对其参与调控的广度与创新性进行了阐述。
小分子RNA已经成为研究基因功能的强有力工具，并带来了一系列的重大发现，必将对增进基因功能与疾病治疗的理解带来革命性的改变。

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biologicalsense, such a definition is quite straightforward: an miRNA is simply a small,single-stranded regulatory RNA molecule that is generated from its precursor moleculesvia successive processing by Drosha and Dicer. It is much more difficult,however, to define practicable criteria that are readily testable on an experimentalor computational basis and that can unequivocally identify a candidate sequence asa genuine miRNA. Following the realization that miRNAs represent abundant moleculesexpressed in a wide variety of organisms, a consortium of researchers agreedon a set of criteria that have to be fulfilled before a candidate can be called a bonafide miRNA.1 According to these guidelines, it is necessary to provide evidence that(1) the candidate sequence is expressed as an appropriately sized RNA molecule inliving cells and, furthermore, does not stem from random degradation (Expressioncriteria), and (2) that the maturation of the candidate involves processing by Droshaand Dicer (Biogenesis criteria). The expression criteria are preferentially satisfied bydetection of a distinct band of approximately 22 nt on a Northern blot. Alternatively,the ability to detect the molecule in a library of cloned, size-selected RNAs is consideredsufficient evidence, especially if the library contains high copy numbers ofthe particular candidate sequence.To satisfy the biogenesis criteria, the guidelines by Ambros et al.1 call for experimentalproof of Dicer processing by demonstrating that increased levels of the precursoraccumulate in cells with decreased Dicer expression. In contrast, experimentalproof of Drosha processing is generally not required; instead, it is sufficient to showthat the putative precursor transcript has the capacity to adopt a secondary structurethat is likely to be amenable to Drosha processing. Of course, given the incompleteknowledge of the rules governing recognition of target mRNAs by Drosha, it is notknown what exactly makes a given RNA structure amenable to Drosha processing,and (as will be discussed later) this complicates the computational predictionof miRNA candidates considerably. Based on the characteristics of known miRNAprecursor structures, however, it is generally agreed that the minimal requirementsare (1) the adopted structure is a hairpin that does not contain many or large internalbulges, and (2) the mature miRNA is to be found within the stem (not the loop) partof the hairpin.Evolutionary conservation serves as a third biogenesis criterion: As miRNAsare often conserved in closely related (and sometimes even in distant) species,phylogenetic conservation of the miRNA sequence itself as well as its fold-backstructure is considered strong evidence that the candidate sequence represents agenuine miRNA. An ideal miRNA candidate would meet all of the preceding criteria;however, it is generally considered sufficient to provide convincing evidencefor at least one criterion out of the two categories. Indeed, because Dicer knockoutcells are not readily available for most organisms, and effective knockdown ofDicer is technically challenging, positive experimental proof of Dicer processingis rarely shown.1.3 A dvantages and Disadvantages of Experimentalversus Computational miRNA IdentificationThe “ traditional ” approach to identifying miRNAs consists of cloning of small RNAmoieties. Although several protocols for the efficient cloning of such molecules havebeen devised, they all rely on the common principle of ligating linkers to size-fractionatedRNAs, followed by cDNA synthesis and typically PCR amplification. Theobtained products are then either cloned (often after concatamerization to increasethe information obtained in a single-sequence read) and sequenced, or subjecteddirectly to massive parallel sequencing approaches (“ deep sequencing ”). Accordingto the guidelines described earlier, these candidates are then further evaluated toensure that the putative pre-miRNA sequence adopts an appropriate hairpin structurearound the candidate. If this is the case, the candidate can generally be considered abona fide miRNA, since the recovery of the clone from a small RNA library alreadysatisfies the expression criterion (nevertheless, Northern blots are often performed toallow for proper quantification of the miRNA).The cloning approach has been extremely successful, and although increasingnumbers of miRNAs are being identified via computational means, the majorityof confirmed miRNAs currently listed in the miRNA database (miRBase, http://microrna.sanger.ac.uk) still have been identified via this method. One of the greatadvantages of the cloning protocol is that it provides the precise sequence of themature miRNA molecule. Therefore, in contrast to hybridization-based methods,even closely related miRNAs that differ in only one nucleotide position can be distinguished.Also, the currently available computational prediction tools generally onlyallow identification of miRNA precursors but do not reliably predict the location ofDrosha and Dicer cleavage sites. In contrast, cloning identifies the precise 5 ′ and 3 ′ termini of the mature miRNA molecule.As it appears that nucleotides 2 to 8 of the miRNA (the so-called seed region)are especially important for target recognition, knowledge of the precise ends (andparticularly the 5′ terminus) is a distinct advantage if a computational predictionof target transcripts is to be performed. As might be expected, the frequency withwhich a given miRNA is cloned often is approximately equivalent to its abundance(although this frequency may also be affected by other factors; see the following text)and therefore provides a rough estimate of its expression levels. Thus, abundantlyexpressed miRNAs are usually readily identified. However, it can be challengingto achieve a saturated screen that also captures rare miRNAs. Furthermore, even ifsuch miRNAs are contained within the library, one can never be entirely certain thatenough clones have been sequenced to identify all of them.In addition to these constraints, the scope of a cloning screen is also limited byits source material; naturally, only miRNAs that are expressed in the cells fromwhich the RNA material was derived can be identified. Many miRNAs, however,are expressed in a tissue-dependent manner, or are only expressed at certain developmentalstages. This limitation can be partially overcome for relatively simple organisms,where the RNA can be prepared from whole animals (e.g., mixed larvae stagesand adults from worms or insects).In organisms with higher complexity such as vertebrates, however, the situation ismore difficult: RNA from different embryonic or adult tissues can be mixed, but thesensitivity of the screen will dramatically decrease with the complexity of the sourcematerial, and it is very unlikely that nonabundant miRNAs could be identified insuch screens. While these problems could be theoretically solved by massive screeningefforts, that is, performing separate screens with material prepared from everyindividual tissue at each developmental stage, the cloning approach also appearslimited in a more fundamental way. Several observations suggest that some miRNAsare more readily cloned than others owing to intrinsic properties such as sequencecomposition, the presence of certain nucleotides at their termini, or posttranscriptionalmodifications such as methylation or RNA editing.2?6Computational approaches to miRNA discovery are not subject to many of thelimitations that apply to the cloning method. Certainly, one of the biggest advantagesof computational miRNA identification is the universal scope of the analysis; as theprediction does not require experimental material, it can potentially discover all ofthe miRNAs encoded by a given organism, even those that are expressed only atvery low levels, in rare cells, or during brief periods of development. However, thisadvantage is partially annulled by the insufficient precision of the presently availablealgorithms: as the programs (to varying degrees) produce large numbers of falsepositivepredictions, experimental verification is still a necessity. Northern blotting isfrequently performed to investigate the expression of the computationally predictedcandidates, or the predicted sequences are amplified from small RNA libraries.These procedures are not particularly compatible with high-throughput screening,and since many computational methods produce large numbers of candidates, only asmall contingent of the predictions is usually subjected to experimental verification,whereas the majority remains untested. More importantly, the experimental validationmethods are subject to many of the same limitations that hamper the cloningapproach. Thus, even if an experimental verification is attempted and fails, it is oftenimpossible to decide whether the failure was due to a false-positive prediction, insufficientsensitivity of the experimental detection method, or lack of expression in thetested tissue or cell line.It is thus perhaps not surprising that the expression criterion has not been satisfiedfor most computationally predicted miRNA candidates. While some groups haveattempted to reconcile these difficulties by developing expression analysis tools thatare, for example, more sensitive or allow high-throughput screening, there is alsotremendous effort to increase the reliability of computational prediction methodssuch that experimental confirmation is becoming less important.1.4 Computational Prediction of miRNA sA plethora of computational approaches aimed at the prediction of miRNAs havebeen devised, and although nearly all of them use the evaluation of features that arethought to be characteristic for miRNAs in order to identify novel candidates, theyvary significantly in scope, complexity, and level of sophistication of the underlyingalgorithms. Some approaches strive to identify the totality of miRNAs encoded by agiven organism, whereas others aim to identify only miRNAs that represent closelyrelated ortho- or paralogs of those that are already known. Some programs investigatesome of the largest genomes, those of mammals, whereas others consider onlysome of the smallest, those of viruses.Despite these differences, most of the approaches function according to a commonscheme that might be abstracted as follows. First, a pool of input sequences(usually representing the complete genome of a given organism) is filtered in order tolimit the number of candidates that have to be evaluated by downstream algorithms.I will refer to this process as upstream filtering in the following. The filtered pool isthen subjected to a structure prediction. The obtained structures are then comparedto those of known pre-miRNAs, and a score calculation is performed, depending onthe degree of similarity. Finally, experimental validation is attempted, usually for aselection of the highest-scoring candidates.There are considerable differences in the degree to which structural features areinvestigated during the scoring step; sometimes the filter might simply ensure that thecandidate forms a hairpin structure, whereas in other cases it might investigate thecandidate ’ s structure down to the minutest detail. The level of sophistication, in largepart, will depend on the design of the upstream filter and the efficiency with whichthis filter preselects a set of candidates enriched for genuine miRNAs. For example,phylogenetic conservation is the most widely used upstream filter (and at least presently,it is also appears to be the most efficient). Indeed, if the sequence of a knownmature miRNA is perfectly conserved in a closely (or even distantly) related species,a relatively simple structural analysis that shows that the ability of the surroundingsequences to adopt a fold-back structure is conserved as well might suffice.In contrast, an ab initio prediction method in which the upstream filter is minimalwill require a much more detailed structural analysis during the downstream scoringstep. Thus, a highly efficient upstream filter requires a less elaborate downstreamstructure evaluation, and vice versa. The cloning method might be considered a specialcase of this scheme in which the upstream filtering is based on an experimentalprocedure; since this method produces only little background, the subsequent structuralinvestigation can be minimal.All of the available computational approaches are subject to the productionof false-positive (i.e., candidates that pass the filters but do not represent genuinemiRNAs) and false-negative predictions (i.e., bona fide miRNAs that are rejectedduring the upstream filtering or the downstream scoring step). The ratio with whichtrue-positive versus false-positive predictions are made will determine the algorithm ’ saccuracy, while the ratio of true-positive versus false-negative predictionswill determine its sensitivity. Such rates are frequently estimated in order to judge analgorithm ’ s performance.Estimating the rate of false-negative predictions is a relatively straightforwardprocess. Often, only a limited number of the contingent of known miRNAs is used toestablish the parameters of the filtering and scoring algorithms. The remaining miRNAsare then subjected to the prediction procedure, and the number of rejected versusretained miRNAs is determined. Alternatively, the full complement of miRNAsis repeatedly passed through the filters, and the method parameters are adjusteduntil an acceptable ratio between rejected and accepted miRNAs is achieved (whatexactly an acceptable ratio is will greatly vary with the overall design and scope ofthe method).The estimation of false-positive prediction rates is a more complicated matter:in order to measure such numbers with high reliability, one ideally wouldhave a set of sequences that assuredly does not contain any miRNAs at all, or aset in which all of the genuine miRNAs are known beforehand. In theory, sucha set can be created artificially from randomly generated sequences, or by shufflingnaturally occurring ones, but since biological sequences are nonrandom, sucha reference set would be hardly representative of the experimental sequence set.Alternatively, one might select genetic elements that have known functions andare thus unlikely to additionally represent miRNAs, but this would reduce thecomplexity of the reference set so drastically that the gained information would beclose to meaningless.In reality, the rate of false-positive predictions is often estimated on an experimentalbasis. For this purpose, a representative subset of the predictions (or all of

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