Annals of R.S.C.B., ISSN:1583-6258, Vol. 17, Issue 2, 2013, Pages. 15 - 26
Received 08 September 2013; Accepted 15 December 2013.

Molecular Biology Application in Biomedicine and Toxicology

Dr. Manoj Kumar
Assistant professor, Department of Zoology
St. Columba's College, Hazaribag(Jharkhand)
Locatedr.manojkumar@hotmail.com

Abstract

The study of basic scientific concerns is only one area in which the techniques of molecular biology can be put to good advantage; they are adaptable to many areas that have substantial effects on people's daily life. Disease prevention and therapy are two areas where molecular biology has been put to use, the creation of novel protein products, as well as the engineering of animals and plants to show desired phenotypic traits. Molecular biology approaches are utilised extensively for these and other applications. These methods, on account of their extensive applicability, are rapidly becoming an ingrained component of our technologically advanced society; nonetheless, some people view this as an invasive practise. In spite of the fact that scientists have a reputation for being critical critics of testing data, they are astonishingly agnostic regarding the relative societal benefits of numerous uses of scientific inquiry. In this paper, the many applications of cell genetics in biomedicine and taxonomy will be discussed.

Keywords: Biomedicine,biology, science

INTRODUCTION

Molecular biology is the study of the mechanisms at the cellular & subcellular levels that are the basis for all life processes. Because all matter, both living and nonliving, is composed of chemicals, a molecular biologist is someone who studies these molecules & how they interact with one another to carry out the essential processes of life. Since all matter, both living and nonliving, is composed of chemicals, this field of study is called molecular biology. Even though molecular biology relies on its own set of processes, these approaches can readily be combined with those from genetics and biochemistry to generate new ones. Although molecular biology relies on its own set of procedures,

Molecular biology's reductionist approach has paid huge dividends in the study of biological phenomena. Now that the molecular processes that underlie illness situations have been sufficiently elucidated, molecular entities, diagnostic or therapeutic indicators, such as genes, proteins, or metabolites. The large number of molecular components that are entangled in intricate networks of linkages characterise biological systems as the most archetypal example of a "complex system."

Annals of R.S.C.B., ISSN:1583-6258, Vol. 17, Issue 2, 2013, Pages. 15 - 26
Received 08 September 2013; Accepted 15 December 2013.

Because of this, there are many features of them that cannot be explained by reductionism and can only be managed by adopting a systemic approach [1-4].

UNDERSTANDING THE CAUSE AND MECHANISMS OF DISEASE

The field of molecular biology has helped advance our knowledge of a wide range of disorders, include cancer and other forms of neoplasia. Genes are responsible for a substantial portion of the process that causes tumours to grow that are cancerous. Because neoplasia are diseases that are characterised by unchecked cell division, & because genes play an important role in regulating cell division, it implies that mutations in these genes can produce neoplasia. Neoplasia are diseases that are characterised by unregulated cell division. According to research conducted in the field of molecular biology [5], a common cause of cancerous deviations in the process of cell proliferation is the presence of mutations in genes that code for proteins or microRNAs related to cell division. It's no surprise that genetics play a big role in predisposing people to cancer & other neoplasias since a cell without a nucleus (and, consequently, without genes) does not develop into a neoplastic or malignant one. Red blood cells & platelets are both examples of adult blood cells in the body. There is no evidence to suggest that a mature RBC or platelet developing a neoplasia raises the likelihood of getting sick. These cells are unable to undergo neoplastic transformation because they are missing genes that are crucial for the process. Erythroleukemia, also known as Di Gugliemo's Disease, is caused by the neoplastic transformation of the erythroblast, which is an immature erythroid cell that ordinarily resides in the bone marrow and contains a nucleus and genes. In a similar manner, acute megakaryoblastic leukaemia manifests itself when a neoplastic change takes place in the megakaryocyte, which is a nucleated cell found in bone marrow and is responsible for the production of blood platelets. Genes that code for proteins involved in cell division can become mutated, which can lead to the development of tumours and other types of neoplasia. One of these is the transcription factor C-MYC, the expression of whose genes is altered in Burkitt's lymphoma. Another category of genes, known as JAK2 and ABL, are responsible for the encoding of enzymes that add phosphate groups to proteins and, as a result, activate proteins that are responsible for transmitting the mitotic signal.

NUCLEIC ACID PROBES

Nucleic acid probes are pieces of single-stranded nucleic acids that are used in molecular biology. These probes have the ability to bind to the sequences that correspond to them. The process known as hybridization is what makes it possible for probes to identify target molecules purely based on the primary structures of those target molecules. A detectable component is added by the label to

Annals of R.S.C.B., ISSN:1583-6258, Vol. 17, Issue 2, 2013, Pages. 15 - 26
Received 08 September 2013; Accepted 15 December 2013.

the target molecules so that they may be recognized and located (Figure 1).

DNA-DNA re-association and DNA-rRNA hybridization are two examples of the nucleic acid hybridization procedures traditionally used by bacterial taxonomists. At the time, these approaches were the only ones that were accessible, as nucleic acid sequencing was a time-consuming and expensive process. In the early 1960s, when the first hybridization experiments were carried out, taxonomists unanimously agreed that the methodology should be used [6,7].

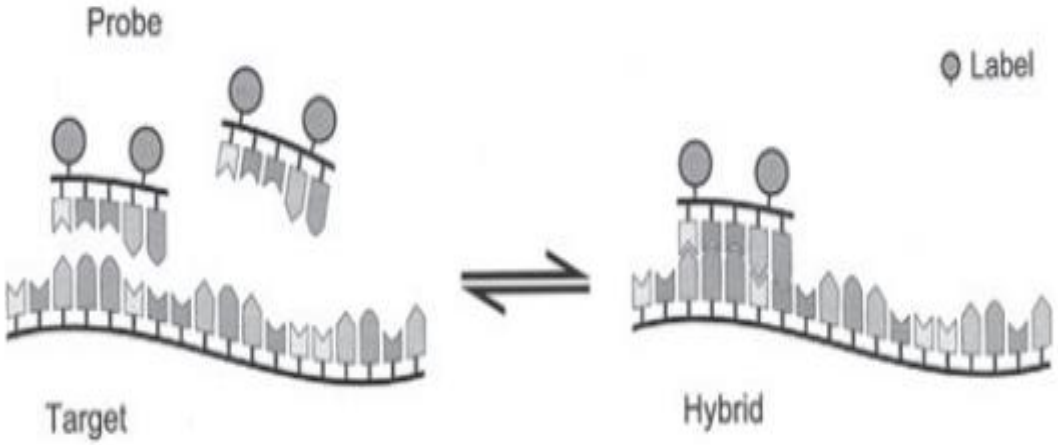

FIGURE 1. Specific hybridization of a nucleic acid probe to a target molecule

We can categorise probes into three broad categories: cDNA probes, cRNA probes, and synthetic oligonucleotide probes. The items are discussed because of their general applicability:

COMPLEMENTARY DNA (cDNA) PROBES

The cDNA copies that are used to create hybridization probes are obtained through enzymatic reverse transcription, duplicated, and afterwards cloned into a suitable vector. Hybridization probes have been created from these cDNA copies [8]. After a high-quality clone has been identified, it is then cloned onto a plasmid and then substantially multiplied in Escherichia coli. After that, following restriction endonuclease digestion of the plasmid, The cloned DNA is separated from vector sequence by gel electrophoresis and radio trying to label by nick translation [9]. Recent research has resulted in the manufacture of tagged DNA probes by employing agarose gel electrophoresis to purify restriction fragments, using the random primer, the Klenow fragment from DNA polymerase I, and doing so [10]. The radioactive DNA probes are then subjected to phenol treatment or column chromatography in order to eliminate any stray nucleotides that may have been

Annals of R.S.C.B., ISSN:1583-6258, Vol. 17, Issue 2, 2013, Pages. 15 - 26
Received 08 September 2013; Accepted 15 December 2013.

present. This process produces extremely radioactive DNA probes, which are perfect for hybridization because of their radioactivity. The application of these methods does have a few drawbacks, however, including the following: a) decreased probe availability due to reannealing of sense (current mRNA molecules) or After hybridization, strands that are the same (they have the same RNA sequence that binds to mRNA and stops translation b) the difficulty in doing melting (Tm) investigations due to probes of various lengths; c) the difficulty in performing [11]. The resolution of some of these challenges was made possible through the utilisation of M13 universal primers for the production of single-stranded DNA probes from recombinant templates inserted in M13 phages [12]. Poor transcription (only one transcript for every template molecule) and the chance that vector sequence transcripts will mix in are two of the limitations of this method, despite the fact that it is capable of producing single-stranded probes with a high level of specific activity [13].

COMPLEMENTARY RNA (cRNA) PROBES

Plasmids to defined Enzyme dna promoter regions (like those in phage T7 or phage Sp6) and a multiple cloning site (called a polylinker) next to the promoter have been made so that cDNA restriction fragments can be inserted into the gene promoter (8). After trying to grow the plasmid inside a host bacterium, purifying it, or cutting the spacer with such a restriction endonucleases that cuts away from the promotion company, To create a labelled probe, the replicated sequence is translated over and over with DNA-dependent Dna polymerase in the presence of radiolabelled nucleotides. Some vector transposable elements have a lot of cloning sites, and each one has a different promoter around it. This makes it possible to make antisense and sense RNA sections that match the mRNA target exactly. cRNA probes are better than cDNA tests even though (a) their single-stranded essence keeps them from reannealing, (b) their stability when hybridised to mRNA allows for more thorough washing after hybridization, (c) unhybridized probes can be broken down by RNase without affecting the cRNA-mRNA hybrids, or (d) their lengths are always short. But cRNA probes have a few problems, such as their susceptibility to RNases, the possibility that they need to be broken up with alkaline hydrolysis to work well in in situ hybridization studies, and the fact that some mRNA species have homologous regions that can hybridise to these regions, which can lead to unclear results (this also applies to cDNA probes). As an alternative way to get around these problems, synthesised oligonucleotide probes have indeed been made.

SYNTHETIC OLIGONUCLEOTIDE PROBES

If you know the sequence of a target mRNA, it's easy to make a probe. To match the target mRNA,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 17, Issue 2, 2013, Pages. 15 - 26
Received 08 September 2013; Accepted 15 December 2013.

the probe sequence has been written from 3' to 5'. The target sequence has been written from 5' to 3'. Probes are usually between 30 and 50 bases long that is stable at high temperatures and has 45 to 65% G+C. If the hybrid has a GC content of 45% or more, it is less stable at high temperatures, while a GC content of 60% or more makes it harder to label. From the other side, if all that is known is the order of amino acids, codon degeneracy would then make it harder to design a probe. A common method [14] is to choose segments of amino acid residues with the least amount of codon degeneracy (like methionine and tryptophan) and employ codons from databases that are unique to the mRNA. Deoxyinosine can be switched out for other deoxynucleotides (like A/T or G/T) at several ambiguous locations (like A/T or G/T) in a target gene to make it more stable [15], and the probe is made using an automated process. Once the probe is created, it can be labelled in a few different ways: 5' finished, 31 final, or primer extension. Since synthetic oligonucleotide units can't be marked with a specific activity like cRNA probes can, they can't be used to look into very rare mRNAs.

POLYMERASE CHAIN REACTION

Pcr technique is a method for quickly end up making a large number of duplicates of a small piece of DNA, that can then be started to look at in much more detail. In PCR, short pieces of synthetic DNA called "primers" are used to find the part of the genome that needs to be amplified. This is followed by many rounds of Target dna to make that segment bigger.

PCR is indeed a simple but sophisticated method that uses enzymes to make a specific piece of DNA from a complex pool of DNA bigger. Dr. Kary Mullis, who came up with the PCR assay, said that it "lets you pick the part of DNA you desire and obtain as much of it you want" (16). Source DNA for PCR could be taken from a wide range of tissues and organisms, such as peripheral circulation, skin, hairs, saliva, and microorganisms. Only a small amount of DNA is needed for PCR to start making enough copies for normal lab tests. This is why PCR is such a sensitive test.

A template DNA sample, DNA polymerase, nucleotides, and primers are required for a successful PCR process. DNA polymerase is also required. DNA polymerase is responsible for joining adjacent nucleotides in order to produce the product of a polymerase chain reaction (PCR). The nucleotides are made up of adenine, thymine, cytosine, but also guanine, which are the four bases which make up DNA (A, T, C, and G respectively). These are the components that DNA polymerase will use to fashion into the finished PCR product from the raw materials. Primer sequences are used to determine the target DNA sequence during the amplification process of DNA. Primers are very short stretches of DNA that have a sequence that is ideally matched with the DNA

Annals of R.S.C.B., ISSN:1583-6258, Vol. 17, Issue 2, 2013, Pages. 15 - 26
Received 08 September 2013; Accepted 15 December 2013.

that has to be amplified and identified. Primers come in the form of very short snippets of DNA. DNA polymerase can build its structure on top of these, which serve as a foundation.

In order to do DNA amplification, the components described above are mixed together in a test tube or 96-well plate, and then the contents of those containers are transferred to a machine where they are subjected to three distinct processes in a repetitive fashion. A thermal cycler is what it amounts to in its most basic form. It is necessary to place the PCR reaction mixture in its respective test tubes or plates before inserting them into the perforations of the heat block. The temperature of the block is raised and lowered by the machine in precise, accurate, and pre-programmed amounts [17]. When the temp of the reaction solution reaches or exceeds the melting point of the two complementary DNA strands that make up the target DNA, denaturation of the DNA molecule takes place. A reduction in temperature is necessary for the hybridization or annealing process, which is used by the appropriate primers to bind to the DNA segments that are the targets. Annealing will take place only in the event that the primers and the target DNA have the same sequence (e.g. A binding to G). When the temperature is increased one more time, DNA polymerase is able to add new nucleotides to the developing DNA strand, which extends the primers in the process (Figure 2). The quantity of DNA molecules that have been copied increases by a factor of two each time that this particular chain of three operations is repeated.

PCR is used more and more in therapeutic settings today. It can be used to find out if a baby has a genetic disease before birth, figure out the gender of a human embryo before in vitro fertilisation, find viral and bacterial infections quickly, type tissues to match organ donors but also recipients, and find genes that make people more likely to get autoimmune diseases [18,19]. The use of PCR to find the immune deficiency virus (HIV) genetic code has been especially helpful in situations where traditional test methods have ended in failure, For example, babies born to HIV-positive moms and people who don't have antibodies but are at an elevated danger for AIDS. By using 2 pairs of primers, one for each virus, but also primers that can bind to a sequence found in the genome sequences of any of the three viruses, they were able to do this, PCR can also be used to find two malware (HIV-1 and HTLV-I) that frequently spread at the same time. This can be done with primers that can find a series that is found in all viruses.

PCR could be effective in any situation where you need to look at DNA. Gene therapy, site-directed mellby, studying how proteins interact with DNA, sequencing or "footprinting" of DNA and RNA, measuring the levels of particular nucleic acid sequences and mRNA, and "footprinting" are all uses. The PCR method has helped the fields of archaeology, genetics, microbiology, forensic

Annals of R.S.C.B., ISSN:1583-6258, Vol. 17, Issue 2, 2013, Pages. 15 - 26
Received 08 September 2013; Accepted 15 December 2013.

science, or clinical laboratory science [20].

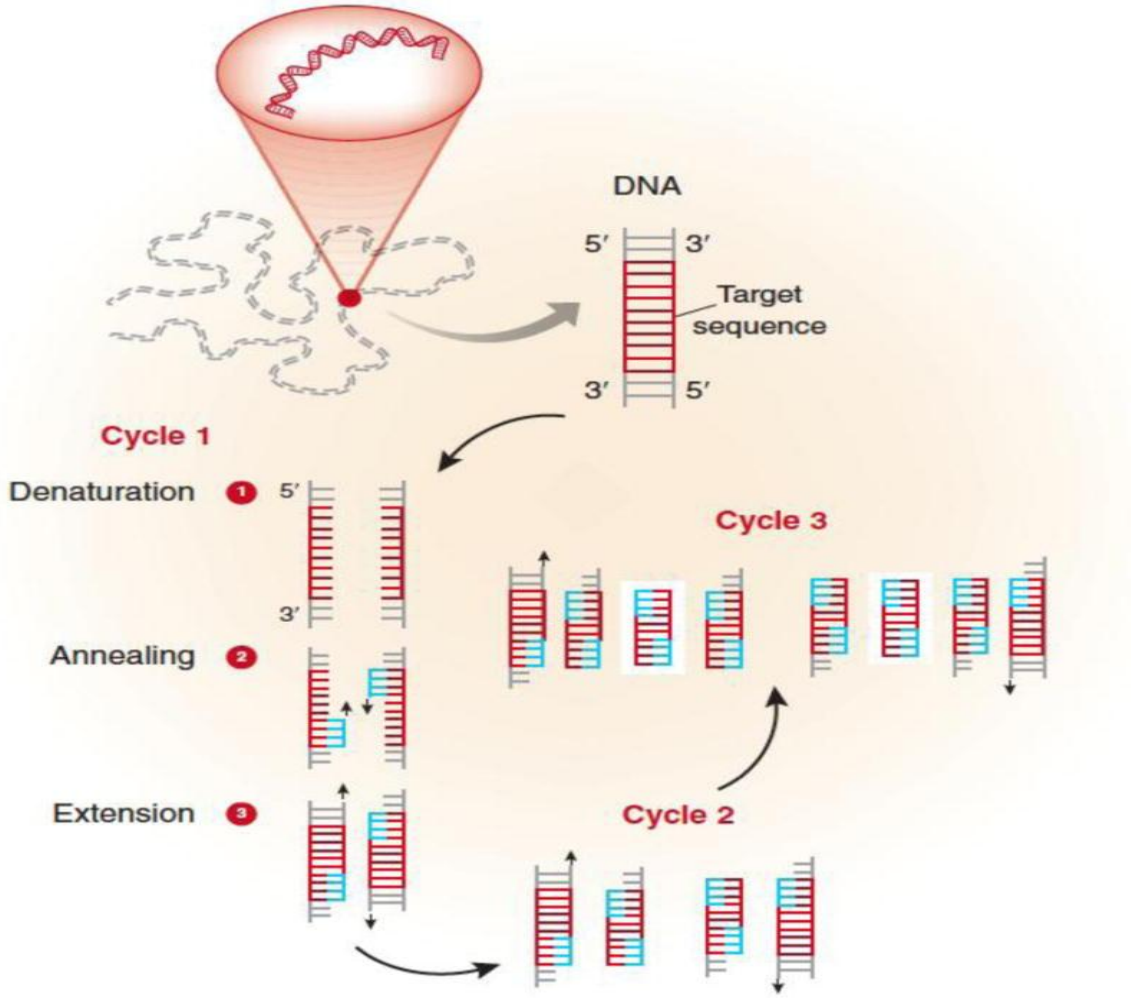

Fig. 2: Schematic presentation of the PCR principle

Use of Nucleic Acid Probes

Inside the cath lab, nucleic acid units have been used to find bacteria that grow very slowly, like Chlamydia trachomatis or Mycobacterium tuberculosis. Since these probes aren't automated, it's hard to do these procedures inside a regular healthcare setting, and you might need some expertise to figure out what the results mean. The discovery of DNA or RNA does not establish that an active infection is taking place in the body; rather, it merely demonstrates that the organism is present in the body. These tests have a hefty price tag, which means that they can only be carried out in small numbers and only under very specific conditions. It is intended, however, that as production numbers increase and processes are optimised, these limits will become less of a problem [21]. Nucleic acid probes have been shown to be useful in many situations, such as chromosomal

Annals of R.S.C.B., ISSN:1583-6258, Vol. 17, Issue 2, 2013, Pages. 15 - 26
Received 08 September 2013; Accepted 15 December 2013.

aberration assessment, in situ transcription, cloning, expression mapping, or growth factor analysis [22].

DNA FINGERPRINTS

RFLP and PCR analysis are commonly utilised in the course of conducting DNA fingerprint analyses. DNA fingerprint analyses are a method for characterising DNA. The majority of genes have relatively low levels of sequence variability in the portions of their chromosomes that code for proteins, but there is a great deal of variation in other parts of the chromosomes. There are thousands of copies of satellite DNA in every human cell, and each one carries a short nucleotide sequence element that is found in the human genome. Satellite DNA may be found in every human cell. There are many repeated imitations of a short segment that are often next to each other in certain places on mammalian chromosomes. However, the precise figure of repetitive copies can be very different, which can cause small changes in the size of the area that has the repeated component (Figure 3). It is conceivable for this repeating DNA to be incorporated in DNA pieces of slightly varied sizes, which can manifest in a variety of different persons. The DNA bands that are produced as a result of doing an analysis of these repetitive sequences employing RFLP or PCR techniques are often utilised in the process of verifying the identity of the individual whose DNA was collected.

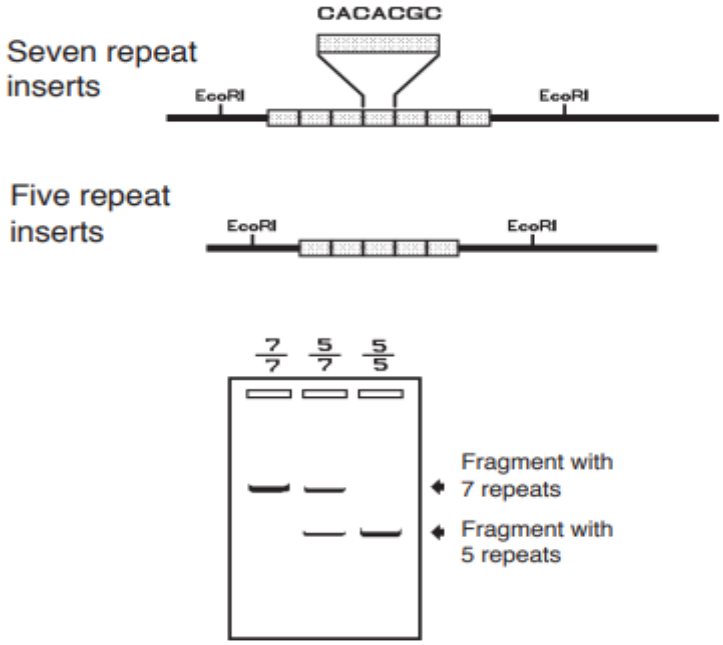

Fig. 3: Linearly repeated sequence elements like the sequence CACACGC can be present at different copy numbers in the same region of different chromosomes.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 17, Issue 2, 2013, Pages. 15 - 26
Received 08 September 2013; Accepted 15 December 2013.

CANCER

The most important use of molecular genetics for cancer early detection and prevention may be finding people who have genes that make them more likely to get cancer. Mutations in at least one oncogene (ret) and all tumour suppressor genes, as well as DNA repair genes like the homologous recombination genes that cause hereditary nonpolyposis colon cancer, can cause these inherited cancer risks. Through genetic testing, mutations in only certain genes can be found, allowing individuals who are at risk of getting a disease to be found before it happens.

In addition to affecting decisions about family planning, careful monitoring of high-risk people may help find cancer earlier and treat some types of cancer better. With a colonoscopy, for example, colon benign growths can be found and treated before they turn into cancer. Patients with familial polyposis, which is caused by mutations in the APC tumour suppressor that are passed down, usually making hundreds of adenomas within the first 20 years Because some of these polyps will turn into cancer, their colons are usually removed before this happens. Patients with genetic nonpolyposis colorectal cancer have fewer polyps as they get older and may benefit from regular colonoscopies and medicines that stop colon cancer from developing, such as non - steroidal anti-inflammatory drugs (NSAIDs) [24].

Direct heritage of tumours caused by mutations in gene sequences is rare. Only about 5% of all melanoma cases are caused by this. Hereditary nonpolyposis colorectal cancer is the most common inherited cancer risk. It causes about 15% of colorectal cancers with between 1% and 2% of all types of cancer in the U.S. Mutations inside the tumor-suppressor genes BRCA2 and BRCA1 also occur often and cause 5 to 10% of all cancer cases. Cancer-prone genes that haven't been found yet may cause a larger number of common malignant tumors like breast, colon, and lung cancers to grow. So, continuing to find genes that make people more likely to get cancer is a big deal with clear practical implications. People with these hereditary genetic variants could be told to stop being exposed to relevant carcinogens (like tobacco smoke inside the case of lung cancer) and closely watched to find cancers at earlier, easier-to-treat stages. The accurate identification of cancer-vulnerable individuals, if accompanied by proper cancer prevention and early detection strategies, could have a substantial effect on cancer mortality [25].

USE OF TRANSGENIC ANIMALS IN TOXICOLOGY

Specific genes can be used in a number of ways to learn about how chemicals affect the body. For example, a human gene that codes for an enzyme that breaks down drugs can be directly injected and its effect on toxic reactions can be studied. On the other hand, the effects of removing

Annals of R.S.C.B., ISSN:1583-6258, Vol. 17, Issue 2, 2013, Pages. 15 - 26
Received 08 September 2013; Accepted 15 December 2013.

individual genes from of the mouse genome could be judged by how toxic they are. Several dangerous chemicals change the way genes are expressed in target cells. This has the potential to look for different kinds of toxic responses to an insult. For this kind of research, the promotor of either a stress-regulated genotype can be fused to just a useful reporter gene, like lad and fluorescence dye proteins, but then put into the genome of such a useful test species. This reporter is made by cells that really are vulnerable to a toxic effects of a chemical agent injected into them. It can be found by using the right test method. This last method makes it easy to test a large variety of chemicals quickly for one‘s toxic potential and also gives information about how they affect different types of cells and tissues. Experiments to transgenic organisms can be hard, and caution is required to make sure that the results aren't affected by what's going on in the species as a whole. Due to the mouse's normal activity, putting in a specific human cyp450 gene may not change how a drug or poison is broken down in the body. As the toxicity of a chemical is determined by a number of processes, such as absorption of drugs, metabolism, detoxification, as well as repair, species-specific discrepancies between humans as well as the animal model could cause a toxic reaction with in animal study but no toxicity in humans [23].

CONCLUSION

The domains of medicine, genetics, and even toxicology have all benefited from molecular biology's methods. There are many clinical laboratory applications for nucleic acid probes, including in situ transcription, genetic recombination & mapping, detecting chromosomal abnormalities, and the identification of slow-growing organisms. The polymerase chain reaction-based amplification of ONA has had a profound impact on the domains of genes, evolution, organismal biology, and clinical and criminal medicine. The cloning and improved diagnosis of numerous disease genes were made possible by the discovery in DNA polymorphism. In conjunction with polymerase chain reaction, it accelerated the typing for DNA for use in law enforcement and civil cases. The effective human genome sequencing process also owes something to DNA polymorphism. The technology that the human genome project used has made it possible to find many genes that regulate all aspects of cellular homeostasis and evolution in a systematic way. Molecular biology has allowed for the identification of tumour suppressor genes in the study of cancer. Furthermore, it shown that cellular mutations caused by insufficient DNA repair predispose cells to cancer. The identification of two genes that code for a familial form for colon cancer in humans was made possible by fundamental research into the molecular mechanics of DNA repair in bacteria and fungi. Toxicology has benefited from molecular biology. DNA lesions serve as reliable dosimeters for assessing the effects of exposure to various physicochemical carcinogens and

mutagens. Molecular biology is also having an impact on how we extrapolate results from studies on one species to another, as well as how we extrapolate results from studies on high-level exposure to carcinogens to those exposed to extremely low levels. Because animals won't be subjected to the stress of being exposed to near-lethal doses of dangerous chemicals, The development of genetically engineered organisms vulnerable for cancer induction at natural levels of exposure to carcinogenic compounds, as is the case in many cases of traditional animal toxicity bioassays, may improve risk estimation.

REFERENCES

1. Nurse, P. (2003). Systems biology: understanding cells. *Nature*, *424*(6951), 883-883.
2. Kitano, H. (2002). Computational systems biology. *Nature*, *420*(6912), 206-210.
3. Mazzocchi, F. (2008). Complexity in biology: exceeding the limits of reductionism and determinism using complexity theory. *EMBO reports*, *9*(1), 10-14.
4. Regenmortel, M. H. V. (2004). Reductionism and complexity in molecular biology: Scientists now have the tools to unravel biological complexity and overcome the limitations of reductionism. *EMBO reports*, *5*(11), 1016-1020.
5. Croce, C. M. (2008). Oncogenes and cancer. *New England journal of medicine*, *358*(5), 502-511.
6. Marmur, J., & Lane, D. (1960). Strand separation and specific recombination in deoxyribonucleic acids: biological studies. *Proceedings of the National Academy of Sciences*, *46*(4), 453-461.
7. Hall, B. D., &Spiegelman, S. (1961). Sequence complementarity of T2-DNA and T2-specific RNA. *Proceedings of the National Academy of Sciences*, *47*(2), 137-146.
8. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., &Struhl, K. (1992). Short protocols in molecular biology. *New York*, *275*, 28764-28773.
9. Rigby, P. W., Dieckmann, M., Rhodes, C., & Berg, P. (1977). Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. *Journal of molecular biology*, *113*(1), 237-251.
10. Scheibe, R. J., Moeller-Runge, I., & Mueller, W. H. (1991). Retinoic acid induces the expression of alkaline phosphatase in P19 teratocarcinoma cells. *Journal of Biological Chemistry*, *266*(31), 21300-21305.
11. Chesselet, M. F. (1990). *In situ hybridization histochemistry*. CRC press.
12. Nien-tai, H., & Messing, J. (1982). The making of strand-specific M13 probes. *Gene*, *17*(3), 271-277.

13. Angerer, R. C., Cox, K. H., &Angerer, L. M. (1985). In situ hybridization to cellular RNAs. In *Genetic engineering: principles and methods* (pp. 43-65). Springer, Boston, MA.
14. Beaucage, S. L., &Iyer, R. P. (1992). Advances in the synthesis of oligonucleotides by the phosphoramidite approach. *Tetrahedron*, *48*(12), 2223-2311.
15. Manahan, S. E. (2002). *Toxicological chemistry and biochemistry*. CRC Press.
16. Mullis, K. B. (1990). The unusual origin of the polymerase chain reaction. *Scientific American*, *262*(4), 56-65.
17. Weier, H. U., & Gray, J. W. (1988). A programmable system to perform the polymerase chain reaction. *Dna*, *7*(6), 441-447.
18. Bhattacharya, S. (1996). The genesis, status and future of environmental toxicology. *Indian journal of experimental biology*, *34*(6), 491-495.
19. Eisenstein, B. I. (1990). The polymerase chain reaction: a new method of using molecular genetics for medical diagnosis. *New England Journal of Medicine*, *322*(3), 178-183.
20. Innis, M. A., Gelfand, D. H., Sninsky, J. J., & White, T. J. (Eds.). (2012). *PCR protocols: a guide to methods and applications*. Academic press.
21. Hildebrandt, F., & Igarashi, P. (Eds.). (2013). *Techniques in molecular medicine*. Springer Science & Business Media.
22. Josephy, P. D., &Mannervik, B. (2006). *Molecular toxicology*. Oxford University Press on Demand.
23. Wolf, C. R., & Henderson, C. J. (1998). Use of transgenic animals in understanding molecular mechanisms of toxicity. *Journal of pharmacy and pharmacology*, *50*(6), 567-574.
24. Bertram, J. S. (2000). The molecular biology of cancer. *Molecular aspects of medicine*, *21*(6), 167-223.
25. Gallinari, P., Marco, S. D., Jones, P., Pallaoro, M., &Steinkühler, C. (2007). HDACs, histone deacetylation and gene transcription: from molecular biology to cancer therapeutics. *Cell research*, *17*(3), 195-211.