Docket #: S97-059

Development of a Regulatable Retroviral-Vector System for Identification of Untranslated Regions of mRNAs

There are two aspects to this invention, the RetroTet-ART vectors themselves, and the use of those vectors to identify novel regulatory elements (untranslated regions, or UTR's).

Continuous regulation is required to maintain a given cell state or allow it to change in response to the environment. Studies of the mechanisms underlying such regulation have often been hindered by the inability ot control gene expression at will. Among the inducible systems available for regulating gene expression in eukaryotes, the tetracycline (tet) regulatable system has distinct advantages. It is highly specific, non-toxic and non-eukaryotic, and consequently does not have pleiotropic effcts on host cell genes. Previously this system also had drawbacks, as it did not extinguish gene expression completely, precluding the study of toxic or growth-inhibitory gene products. This technology is the development of a facile reversible tetracycline-inducible retroviral system (designated RetroTet-ART) in which activators and repressors together are expressed in cells. Gene expression can now be actively repressed in the absence of tet and induced in the presence of tet, as Stanford inventors have engineered distinct dimerization domains that allow co-expression of homodimeric tet-regulated transactivators and transrepressors in the same cells, without the formation of non-functional heterodimers. Using this system, it has been shown that growth arrest by the cell cycle inhibitor p16 is reversible and dependent on its continuous expression.

The identification of novel regulatory elements required for the correct expression of genes is an essential step in understanding and controlling their function. The UTRs

are known to regulate gene expression in disease states, such as cancer, cardiovascular disease, and myotonic dystrophy. To aid in the study of FUNCTIONAL GENOMICS, a new modular tetracycline inducible retroviral system has been developed which contains multiple modifications of the SIN-RetroTet vector making it particularly suitable for the study of non-protein coding RNA sequences, such as the UTRs of mRNAs that are often ignored. UTRs are known sensors of hypoxia and growth factor deprivation, stresses common to tumors and vascular compromise (ischemia). A few UTRs are known to play a major role in regulating gene expression in these environments. Yet the number of UTRs studied is relatively low due to the lack of tools. This system is rapid and allows the regulated expression of a reporter gene fused with the UTR sequences in populations of thousands of independently infected cells within one week. Using this new vector system, a number of mammalian UTRs are being analyzed and can be rapidly screened for their potential role in localization, stability, translation and growth control. These functions are critical to gene regulation in disease states (Myotonic Dystrophy is a heritable 3'UTR disease), cancer (some UTRs are tumor suppressors), and for upregulation of genes in hypoxic environments.

These regulatory sequences that control gene expression in tumors and in cardiovascular comprised tissues are generally not well studied, characterized or patented. There is a paucity of 3'UTR sequences in the data base. This technology allows the rapid identification of novel regulatory DNA sequences with a role in cardiovascular disease and cancer.

Applications

- Identification and characterization of regulatory-sequences in 5'UTRs and 3'UTRs that play a role in stability, translation, and localization of mRNAs.
- Regulated expression of cDNAs encoding toxic molecules which at low levels could adversely affect cell growth or differentiation, thus requiring tight control.
- Regulated expression and characterization of untranslated RNA's or transcripts lacking an open reading frame (e.g. H19, ribozymes, antisense RNAs) that can directly or indirectly affect cell proliferation and/or differentiation.
- Controlled expression of therapeutic genes for gene therapy. Regulatable and tissue-specific expression of RNAs (e.g. ribozymes, and antisense RNAs) to specifically interfere with transcription of oncogenic gene products involved in cancer.

Advantages

- Only system for studying the function of untranslated RNAs with minimal extraneous sequences.
- Fast and efficient.
- Can be introduced into large populations of cells.
- Can be regulated (turned on and off or expressed to different levels)
- Modular - can be studied with and without other gene-specific sequences
- Can readily be used to find interacting proteins that provide targets for drugs

Publications

- Rossi FMV, Guicherit OM, Spicher A, Kringstein AM, Fatyol K, Blakely BT, and Blau HM, Tetracycline regulatable factors with distinct dimerization domains allow reversible growth inhibition by p16. Nature Genet., 20, 389-393 (1998).
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- Kringstein AM, Rossi FMV, Hofmann A, and Blau HM, Graded transcriptional response to different concentrations of a single transactivator. Proc. Natl. Acad. Sci., 95, 13670-13675 (1998).

Patents

- Published Application: WO9842854

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