Researchers develop technique to count messages made by single genes

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In a study in the advance online edition of *Nature Structural and Molecular Biology*, researchers from Albert Einstein College of Medicine describe a technique for looking more precisely at a fundamental step of a cell's life – a gene, DNA, being read into a message, mRNA. The technique could provide a window into the process by which genes are switched on inappropriately, causing disease.

The new technique provides a detailed look into processes that until now were proven but never visualized. The more detailed view of DNA being made into RNA in a single cell will help answer questions about how much of a gene is made over time and how much that level varies from cell to cell. Insight into how genes work at a more precise level, ultimately advances understanding of disease mechanisms that trigger cancer, for example, which arise when genes no longer work at their correct capacity or time.

"The classic textbook cartoon illustration of a single strand of DNA with little mRNA pieces coming off it can now be shown with real photographs," explained Daniel Zenklusen, Ph.D., an Einstein post-doctoral fellow and first author of the study. The technique was developed in the laboratory of Robert Singer, Ph.D., co-chair and professor of anatomy and structural biology at Einstein.

The new technology is a powerful refinement of fluorescent in-situ hybridization (FISH), developed in Dr. Singer's laboratory more than 26 years ago. FISH is now a widely used research tool to study gene

activation; that is how much a gene has been "turned on" in groups of cells. FISH is also used in genetic counseling to detect the presence of gene features that diagnose conditions including Down's syndrome or Prader-Willi syndrome.

Advances in fluorescence, microscopy and data analysis enabled the more powerful FISH application described in the paper. Until this work, FISH could only be used to look at genes or their messages that are present at very high levels and only in tissues, not at the smaller level of the cell. However, this it the first time that all the individual mRNA molecules within single cells can be counted.

Dr. Singer's "single RNA counting" technique has the potential to change some fundamental theories about how genes are regulated. As Dr. Singer explained, "our study using this new technique has already generated enough new ideas to keep students busy for the next 10 years."

One of the most important findings of this study was that "housekeeping" genes, which all cells need to survive, are not always expressed at a constant level. Variability, however, is restricted to a narrow range that seems to be characteristic for housekeeping genes. Combining single molecule measurement with mathematical modeling allowed the team to precisely determine how variability is controlled. This showed that unlike the findings of previous studies, housekeeping genes are not transcribed by transcriptional bursts but at a fairly constant rate. Bursting expression, however, is found for special classes for genes where higher variability might be an advantage for the cell. The next step is to see if this continuous/non-bursting theory of housekeeping gene control applies also to human cells. The work from Dr. Singer's group was performed in yeast cells.

Dr. Singer believes the approach of looking at biological processes in natural contexts (rather than in a test tube) at a single cell level reveals

details that can advance the field of cancer and other disease research. "Cancer derives from a single cell. So current microarray technologies that are used on a tissue-wide level and are based on "grinding up a tumor" may be a good first step at directing us where to focus, but they may need to be combined with newer techniques that provide the precision to home in on single cells," Dr. Singer said.

More information: The study, "Single-RNA Counting Reveals Alternative Modes of Gene Expression in Yeast," by Daniel Zenklusen, Daniel R. Larson and Robert H. Singer appears in the November 16, 2008 online edition of Nature Structural and Molecular Biology. www.nature.com/nsmb/journal/va … /ncurrent/index.html

Source: Albert Einstein College of Medicine