Topics

How vitamin B_{12} is synthesized.

Vitamin B_{12} is the largest, known vitamin, three times larger than all other vitamins synthesized exclusively by microorganism. It is also known as "red vitamin" having cobalt in the center of molecule and cyanocobalamin, which has a few related compounds like methylcobalamin and hydroxycobalamin, As one would expects from the complicated structure, many enzymes are involved in the synthetic pathway to form the last ligand of the vitamin, known as 5.6-dimethyl-benzimidazole (DMB).

Toga et al. reported an important gene and the product protein BluB in Nature 446: 449-453 (2007). BluB enzyme formed DMB by using oxygen to dismantle another vitamin co-factor, flavin mononucleotide, used by other enzymes as an electron shuttle. BluB has evolved into a closed box shape that traps the flavin, then uses oxygen to destroy and remold it into DMB. This is recognized as a surgical excision of a carbon from six-membered ring to generate a five membered ring. A similar "cannibalism" action is expected in the synthesis of vitamin H (Biotin) and unique structure and function of BluB suggest a new kind of enzymes called "flavin destructase" family.

The above study arose from finding a mutation of *bluB* gene of *Sinorhizobium meliloti* which can live inside of plant cells. The mutant is incapable of synthesizing B12. In humans, viamin B_{12} formed by intestinal organisms is absorbed from small intestine with aid of intrinsic factor secreted from stomach wall. The required amount is 3 ～ 5 μg/day and 5mg are stored in body. Thus, shortage of vitamin B_{12}, anemia, is scarcely observed, except in case of gastrectomy inducing a lack of intrinsic factor.

Genome switch

One of the biggest problems in biology is to probe evolution, especially production of new species. Vender and colleagues' work at the J. Vender Institute in Rockville, Maryland, U.S.A. was introduced in The Scientist. They replaced the genome of *Mycoplasma capricolum* with that of a closely related bacterium, *Mycoplasma mycoides*. Mycoplasma has a small genome (roughly one million base pair) and lacks cell walls, making it easier to insert bulky DNA molecules. They suspended *M. mycoides* cells in agarose to protect the soon-to-be-naked donor DNA from jostling and breakage, and incubated with enzymes to digest the other portion of cells.

After isolation of DNA, which is a critical step, the donor genomes was incubated with host bacteria in a buffer containing polyethylene glycol (PEG) to promote DNA transfer. The donor genome contained tetracycline-resistant gene to improve transfer of genome by antibiotic screening. Colonies that developed after three days were genetically identical to donor cells based on Southern blot analysis and carried surface antigens and cell proteins specific to *M. mycoides*.

They consider this the first step to create an organism which requires various accessory proteins and other components. Efficiency must be a concern because the procedure works one in 150,000 times in better experiments helped by selection by antibiotics. Applying this technique to higher organisms is an extremely long way off, due to the presence of various restriction enzymes to protect those organisms against invading DNA. They could not explain how the *M. capricolum* genomes disappeared after transplantation but speculate that after cell division, donor and recipient genomes separate into different daughter cells. Tetracyclin sensitive *M. capricolum* were culled. An other possibility is that donor genome might have a mechanism to cleave and destroy the recipient genome, as in case of eukaryotic

hybrid cells, heterokaryon and hybridoma cells.

Nevertheless, this is first evidence that a synthetic organism with minimal genome is engineered and further created, and modified organisms can make a contribution to our society through biofuels and other application, because modified cells have extraneous metabolic pasthways shifting energy away from chemical synthesis.

Hetero-karyon and Hybridoma

Fused cells containing two nuclei called hetero-karyons present chromosomes derived from both cells but as cell divisions are repeated their chromosome number decreases, and stabilize to a certain number depending on type of hetero-caryon.

A hetero-karyon is produced by suspending two types of cells in medium containing an inactivating virus (i.e. Sendai virus) or chemicals (i.e. polyethylene glycol) to alter the nature of cell membrane. Hetero-karyon represents a new phenomena. For example, fusion of chicken red blood cells with cultured cells induces an RNA synthesis in inactive nuclei and reactivates the replication of DNA. The first direct evidence that membrane proteins are able to move in the plane of the plasma membrane came from an experiment in which mouse cells and human cells were fused: although the mouse and human cell-surface proteins were initially confined to their own halves of the hetero-karyon plasma membrane, they quickly diffused and mixed over the entire surface of the cell. Such hybrid cells can be cloned to produce hybrid cell lines, but the cells tend to be unstable and randomly lose chromosomes. In human mouse cell hybrid lines, only one or a few human chromosomes are present. This has been used to identify human genes, for example, human chromosome 11 containing cells synthesize human insulin, indicating insulin gene is located on chromosome 11.

Antibody producing B cells has a limited life span; however, hybridization with tumor cells originated from B cells has given them an endless life, producing the same antibody. By cloning such cells called hybridoma one can produce a wide range of antibodies in quantity. Each of these are called monoclonal antibodies which are specific to a certain antigens and obtainable in large quantity in rather short period from stored stock lines.

Once monoclonal antibody is prepared, it can be used to identify the location of antigen and to study the nature of protein.

GM potato helps in feeding mono gastric animals

A variety of our foods are digest by secreting enzymes into the intestine but the presence of bacteria and their enzymes is also required. Mono gastric animals, like horses and pigs, and poultry need Xylanase to break down Xylan—that hampers the rate of digestion and the absorption of nutrients in those animals. Xylanase is currently commercially produced using microbial fermentation and added their feed. However, potato containing xylanase gene has been produced by Peilong Yang's group in China as an economical alternative. This potato can be directly fed to the animals. To produce the transgenic potato, the gene xynB from *Streptomyces* was introduced to the potato cultivar "Desiree". Xylanase activity was present at up to 5% of total soluble leaf protein and sufficiently high in the tuber . Both leaf and tuber extracts from transgenic potato are suitable for industrial or commercial purposes. Transgenic plants can supply known nutrient components, like Vitamin A, C, and E as well as immunological materials. Today, some commercial plants, such as corn , sugar cane and even rice are processed to produce fuel additive alcohol. In Japan, people are refusing to use and even try to carry out field tests of GM food, simply because those are not

natural. Now, the plants to be used for food can be use for money-making industrial commodityies

It is time to reconsider what is natural and face reality to have a more economical food supply.

No need to be identical even if the gene sets are identical.

It is known that monozygotic twins have an identical genetic trait and they are hard to be distinguished by other people except their mother at beginning. Later, their father and nearby keens can recognized their difference, followed by friends and neighbors as they grow. Investigating epigenetic profiles of 80sets of identical twins, age 3 to 74, Manel Esteller of the Spanish National Cancer Center in Madrid revealed unsurprisingly, older twins differ more in DNA methylation and histone acetylation the younger twins, onfirming that epigenetic differences correspond with increased phenotypic disparities. They are mapping twins with differnt penetrance for a particular disease, such as diabetes and autoimmune disease. By comparing the epigenomes of both twins, they are hoping to isolate genes that contribute to the development of these diseases. He wants to know whether or not the epigenetic changes he noted are heritable in human, as in mice. Also he is asking how stable are these marks and how long?
(From Proc. Natl. Acad. Sci. 102:10413-4.)

Metastasis genes from study of breast cancer.

Researchers at Memorial Sloan-Kettering Cancer Center found for breast cancer genes that were involved in tumor angiogenesis, the spread of tumor cells throughout the circulatory system, and invasion into lung parenchyma. The four genes tested are EREF, a growth factor gene; MMP1 and 2, two matrix remodeling proteinases; and COX2, which produces an inflammatory protein. These genes are a part of a group of 18 genes associated with breast cancer metastasis to the lung. It has been postulated that tumor cells interact with normal cells in the tumor microenvironment, but the study shows no evidence for this speculation. The genes produce proteins and molecules important for normal endothelial function, but also produce a substance which works for malignant purposes.

To understand the function of genes, they knocked down gene expression using a short hairpin RNA (shRNA) in mice targeting these specific genes. The genes had the strongest anti-metastasis effects when silenced in combination, rather than separately. When over-expressed together, they helped the primary tumor cell leaky and tortuous blood vessels spread into the circulatory system. By splicing four genes, all of these activities are suppressed. These four genes are already targets of FDA-approved drugs and shortens the process of going from mouse to patients. The drugs are following: GW6001 and celecoxib, which target the EGFR, MMP and cox2 genes, respectively. This study showed that the drugs appeared to stall primary tumor growth and the spread of the tumor cells into the circulatory system.

Celecoxib, also known as Celebrex, is currently prescribed as a treatment for arthritis. GM601 was tested for treatment of autoimmune encephalomyelitis. The list of genes suitable for targeting is increasing and makes a contribution to controlling diseases at various steps. (from URL, http://www.the-scientist.com.11th.april 2007).

Chromium alters gene expression of dairy cattle.

Chromium is one indispensable mineral for humans event though the required concentration is low. A shortage of chromium causes various metabolic abnormalities including glucose, fat and protein synthesis and breakdown. It is true in case of dairy cattle that they require chromium in their diet for good health and milk production.

McNamara's group in Washington State University has been working to identify genetic factors related to efficient milk production in which the adipose tissue play and active role in reproduction and lactation. One of their goals was to identify cows that gained neither too much nor too little weight during pregnancy and lactation. When cows store too much body fat, energy is diverted away from milk production and instead goes toward storing more fat. Yet, if a cow does not store enough fat or loses fat too fast, it's immunity will be impaired, causing insufficient production and leading to diseases like ketosis, milk fever and mastitis. Adipose tissue delivers several powerful hormones which control food intake, inflammation and immunity.

Chromium supplements increased feed intake and milk production in dairy cows through alteration of fat metabolism to increase the proper amount of fat and increase their immunity. This also prevented them from loosing too much fat. Researchers extracted RNAs from adipose samples and ran micro-array analyses to determine gene function. The study convincingly showed that chromium supplementation up-regulated a number of genes, causing them to become more active while others were down-regulated. This may be the first documentation to show that nutrigenomic activity in the body fat of dairy cows and lipolysis pathways which follow the breakdown and mobilization of fat tissue are a major contributor to milk production. It should be noted that the point of highest gene activity coincided with the point of greatest lipolysis. Five major genes were up-regulated during lactation, allowing the cow to utilize stored body fat for milk.
(URL: http://www.dairyherd.com/news)

Sleep is indispensable for brain function and immune response.

Sleep consists of REM (rapid eye movement) and non-REM periods and alters brain function at both neuron level and gene expression level. During non-REM, neuronal activity of brain stem decreases or partially stops, while that in cortical and frontal regions barely decrease. During non REM, cortical neurons next to each other fire with synchronous rhythm of low frequency wave, which may be related to regular respiration and heart-beat. While awake, neurons appear to fire independently using more energy than in REM and non REM periods. A group of brain cells at the base of brain stem stay most active during non-REM and are expected to function in induction of sleep and are called sleep inducers. If body temperature increases, these sleep inducer neurons will be activated. Thus, we feel sleepy after a hot bath or during a warm day.

The brain in REM sleep shows a similar pattern to that while awake in electric potential and consumption of energy. However, brain in REM sleep stops secretion of muscle stimulating transmitter except to the eye ball muscle. Thus, eye movement is observed during so-called REM sleep as well as the activity of viscera. For example, heart-beat and respiration are irregular as when awake, body temperature fluctuates accounting to outside temperature changes, and penis or clitoris enlarge independent from sexual dreaming.

There is a positive relation between sleep of 7 hours duration and a percent longevity. Thus, the hypothesis that sleep repairs the damages caused by radicals unavoidably produced from cellular activity during wakefulness and/or even just living. Damaged cells may be replaced by newly divided cells, however, neurons in brain do not produce as many cells as required, except in hippocampus working for learning and memory. It has been suggested that non-REM sleep with reduced metabolism plays a role in repairing damaged neurons. Further more, secretion of neurotransmitter monoamines, noradrenalin, serotonin, histamines is suppressed in REM and thus prevents depolarization of the receptor cells

which may lead to revival of transmission of stimuli. This leads to, not only increase of memory, concentration and creative function, as shown by scientific test, but also development and growth of brain and body.

Such phenomena are under the control of circadian rhythms and there is a report suggesting that infectious and autoimmune diseases may promote sleep by down regulating circadian gene expression. This in turn suggests that sleep increases immunity. (Proc. Natl. Acad. Sci. (USA) , On line July 16, 2007: The Scientist). The immune response to microbial infection activates pro-inflammatory molecules such as tumor necrosis factor alpha (TNFα). Previous work has suggested that TNF-α causes lethargy and fatigue in people with cancer, rheumatoid arthritis and sleep apnea. Study by Cavdini et al. in Switzerland showed TNF-α effects on expression of circadian clock genes. From an evolutionary point of view, increased sleep during illness may help animals to fight off infections or may keep sick animals from socializing to fight off infection to others. We often heard doctors say in case of cold or flu “Stay in bed, use aspirin and consume a lot of liquids” and many researchers believe that the way we sleep when we are sick facilitates recovery.

Advanced research mode in medical and biosciences.

Every clinical research needs cooperation from patients; and should give accurate information about advanced treatments, like gene therapy, regeneration treatment and data. To deliver such medical treatment, collaboration of researchers, doctors, research nurses, clinical research coordinators, data managers and various paramedics is indispensable. Unfortunately, our clinical research is far behind that of Europe or America, especially in the large scale studies of patients and handling of data, relative to our basic life science where many medical, doctors are involved. One method to reach the targets of clinical research from basic studies is ICR (Integrative Celerity Research). ICR aims to set the goal at early stage of basic and clinical research together and proceeds in cooperative fashion. Naturally, the view from many areas of social and humanity science should be included in ICR with the most advanced technology like genetic modification, micro-machines, nano-technology and immunological control. Up to now, greater discoveries in basic bioscience used be dependent on the bright ideas of a few geniuses, but it will change to a systematic and cooperative function from various specialists. The question is how people can combine their special talents toward the goal which is a must in collaboration.

Maintenance of brain.

Neurodegenerative disorders, Alzheimers, Parkinsons and frontotemporal demensias, common with advancing age are not always associated with age but result from the formation of filamentous aggregates, like amyloid, tau and synuclein. These aggregates quickly gum the normal physiology of neurons and become cytotoxic. An improved system to fold proteins and destroy unwanted proteins can be obtained from functional chaperone proteins, lysosomes and proteasomes to eliminate the accumulation of disease-causing proteins. However, this may cause neurological damage resulting in head trauma or stroke. Thus, a simple cell-for-cell replacement model of neurogenesis might provide a more elegant solution by waking up adult stem cells when cells die from some induction. A down side effect can be induction of cancer and connection of neurons, that must be in precise, can be overcompensated or slightly dysfunctional about neural crosstalk. Then, schizophrenia or seizure may occur.

Brain, out of function, may lead to deterioration and loss of body function, and heart attack that

comes from partial or total disability of body. Vision impairment even with corrective lenses comes from cataracts and other damage of aging and a life time exposure to incidental ultraviolet radiation.

The effect of calorie restriction on longevity in several animals including humans is well documented. Increasingly, evidence suggests that sensory systems such as smell may have be similarly effected. The life extending effects of dietary restriction in flies are partially reversed when flies are exposed to excess food odorants. Knocking out the odor receptor Or83b resulted in life extension that was largely independent of dietary intake. Perceptual systems may play a major role in informing the organism of its environment, and in doing so may trigge physiologic decisions that result in altered longevity.

Do genes and/or transcribing regions of chromosome move to a certain place in the nucleus? The answer came from collaboration.

Nuclear membrane for general genes and nucleoli for ribosomal genes have been suspected as the place where transcription occurs. When genes are activated, they move to designated position in nucleus, and then polymerases and other needed proteins congregate to such DNA sites. Recent collaborative study, between the laboratories of John T. Lis, the Barbara McClintock Professor of Molecular Biology and Genetics, and Watt W. Webb, professor of applied physics and the S.B.Eckert Professor in Engineering have used multiphoton microscopy, a technique developed by Webb that allows high-precision 3D images in living cells. They observed polytene chromosomes in the salivary gland of fruit flies that have many sets of genomes, instead of two sets as in usual somatic cells.

They activated heat shock genes, which areproduced to protect cells from high temperature, and watched them in real time as they began to be transcribed. They also tagged polymerase II (Pol II), working in messenger RNA production, with a fluorescent marker to track its movements within nucleus. They observed that the genes decondense and fill up with Pol II but they did not move anywhere. Instead of moving, they are covered by transcriptional machinery and start to function regardless of their position in nucleus. They bound fluorescence dye by in-situ hybridization, which allowed them to detect the location of specific DNA sequences along a chromosome in fixed cell. This also showed that genes did not move to a single site for transcription. Pol II is used repeatedly for transcription for a period, but the transcribing genes remain at the site.
(from Jour. Molec Cell, in Dec. 2008).

(Topics from Yasuo Hotta)