

# A Human Cytome Project – an idea – Update 17 Dec. 2005

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A Human Cytome Project – an idea

<http://ourworld.compuserve.com/homepages/pvosta/humcyt.htm>

By Peter Van Osta, MD

Hi,

As the on–line version of my article on the Human Cytome Project and the application of cytomics in medicine and drug discovery (pharmaceutical research) evolves, I put the updated version in this newsgroup for reference. The original "question" on a Human Cytome Project was posted in bionet.cellbiol on Monday 1 December 2003.

## Vision

Attaining new insight in the biology of man, shaping medicine, biotechnology, drug discovery and development into a premier precision tool of the future, for the improvement of disease diagnosis, disease outcome prediction and curing the diseases of mankind.

## Introduction

This article is dedicated to the patients hoping and waiting for new treatments of unmet medical needs and the improvement of existing diagnostic techniques and therapies. It is also dedicated to all the scientists working in basic and applied research, working hard to deliver these new drugs and treatments. A lot of hard work has been done, but a lot more is needed for the future. Improving medicine, drug discovery and development is not a simple endeavour. Although this article is critical about the (evolution of the) overall process it does not want to diminish the individual contributions of scientists and physicians over the years which save and improve the lives of many people. Let us look at the present with the future in mind.

The completion of the Human Genome Project (HGP) holds many promises for the understanding of the genetics of man and the involvement of genes in human diseases. However the use of this information has to be viewed from another perspective as is currently being done, if we want to use this knowledge to

improve medicine more efficiently. Predicting the dynamics of the cell and its fate in diseases from the genome upwards is likely to fail due to the complexity of metabolic processing and environmental influences on the cellular metabolism and the phenotype of the entire organism. Going "from genes to health" improvement requires an understanding of disease processes beyond the boundaries of genomics and proteomics. We need a better understanding of cellular physiology and beyond. The "Book Of Life" is a novel, not a dictionary.

The clinical reality of disease processes extends beyond the present-day disease models and the (current) boundaries of scientific development. When we close the doors of our labs behind us and as physicians are confronted with the clinical reality of diseases in the outside world, our disease models fail all too often, as we can witness in the diagnosis and treatment of complex diseases. This is also painfully obvious in the dramatically high attrition rates during clinical development of new drugs.

When the endpoint of research is not only an experiment in a laboratory, but to have an impact on the clinical reality of everyday pathological processes, we fail to deliver in more than 80 to 90 percent of all drugs being developed. Reality extends beyond the frontiers of science. Outside the boundaries of scientific knowledge, significant parts of (biological/clinical) reality remain un-explained for and not well understood.

Drug discovery and development has to come up with drugs which can stand the test of clinical reality, but is being squeezed between the failing (theoretical) disease models and the demands for success of pharmaceutical companies and society. Applied research has to provide the step stones to cross the river from basic theoretical disease models to clinical reality, ideally without getting our feet wet or drowning before we reach the other side of the river.

How do we close the gap from model to clinic and find new directions for research? The functional correlation between genome structure and clinically expressed disease is too low to lead to functional predictions from the genome and even proteome level upwards, without taking into account the spatial and temporal dynamics of cells, organs and organisms. Pathological processes have to be viewed from another organizational level of biology in order to capture the dynamics of in-vivo processes involved in diseases.

The current bottom-up view on genomic and proteomic research suffers from a correlation and prediction deficit in relation to the entire organism. The genome and proteome are the omega of biological research, not the alpha of drug discovery or disease treatment. From disease to gene we may find a link, but turning around and go back to develop a treatment for the clinical disease fails in many cases. We may find that a gene or genes may be part of a disease process, but we cannot explain the entire disease process from the genome level alone. A gene may be involved in a disease, but the entire disease process is not contained within the gene. To discover the involvement of a gene or protein in a disease, does not predict the

potential for successful development of a treatment for the clinical disease entity as such.

In-vivo variation is not an artefact of life, but a fact of life. The extraction of the appropriate attributes of a biological process in health and/or disease requires capturing the spatial and temporal dynamics of its manifestations at multiple scales and dimensions of biological organization. Disease entities express themselves in a space-time continuum in which their physical and chemical attributes evolve in a highly dynamic way. Capturing the appropriate features and disease describing parameters from the background noise of their surrounding processes and structures is more difficult than finding a needle in a haystack.

On Monday 1 December 2003 I posted a message about the idea of a Human Cytome Project (HCP) to the bionet.cellbiol newsgroup (Van Osta P, 2003). It seems that it was the right moment to ask the question, as there were already ideas emerging on the role of the cell as the final arbiter in the production of metabolic products and also the concept of predictive medicine by cytomics (Valet G, 2003).

The idea of a Human Cytome Project is already being discussed at scientific conferences (FOM 2004, ISLH 2004, ISAC XXII, EWGCCA 2004 .). At Focus on Microscopy (FOM) in Philadelphia on Wednesday afternoon, 7 April 2004, the idea of a Human Cytome Project was for the first time discussed at a scientific meeting. A round table discussion was held at the European Microscopy Congress (EMC). The next major conference with workshops on the Human Cytome Project (HCP) and cytomics is the ISAC XXIII 2006 Conference in Quebec, Canada.

Already articles start to appear on the idea (Valet G, 2004; Valet G, 2004b; Valet G, 2004c; Valet G, 2005a; Valet G, 2005b).

As the idea of a Human Cytome Project seems to have generated some interest in the scientific community, I decided to put the original message and question on my personal website for reference, so here it is.

Overview of related articles on this website

The original posting of the idea can be found on this webpage.

Personal interest and background

Scientific background about the idea can be found this webpage

The potential impact on the efficiency of drug discovery and development

A proposal of how to explore the human cytome

A concept for a software framework for exploring the human cytome

References

Monday, 1 December 2003 10:57:46 +0100

Hi,

I was wondering if there is already something going on to set up a sort of "Human Cytome Project"? In my opinion the hardware and most of the software seems to be available to set up such a project? For the cellular level, light–microscopy based reader technology would be very interesting to use?

Studying and mapping the genome, transcriptome and proteome at the organizational level of the cell for various cell types and organ models could provide us with a lot of information of what actually goes on in organisms in the spatio–spectro–temporal space?

I have been thinking (working) about a concept which could provide the basic framework for exploring and managing this cellular level of biological organization research on a large scale, but I would like to know if there is already some thought/work going on in the direction of setting up an initiative such as a "Human Cytome Project" ?

This is just an idea, so I am really interested to hear if there is something in it, or even if it is not worth while what I just wrote.

Best regards,

Peter Van Osta.

Why a Human Cytome Project?

Human Genome Project

".. Nearly two centuries ago, in this room, on this floor, Thomas Jefferson and a trusted aide spread out a magnificent map ... the map was the product of his courageous expedition across the American frontier, all the way to the Pacific ... Today, the world is joining us here in the East Room to behold a map of even greater significance. We are here to celebrate the completion of the first survey of the entire human genome. Without a doubt, this is the most important, most wondrous map ever produced by humankind..."  
President of the USA, Bill Clinton, June 20, 2000

The Human Genome Project (Lander ES, 2003; Venter JC, 2003) has set a new milestone in medicine and the understanding of human biology (Guttmacher, A., 2002; Guttmacher, A., 2003). Since its conception in 1986, it has answered many questions, but it has also left us with more questions to answer and it opened new horizons for exploration (Dulbecco R., 1986; Collins F., 2003). The results of the Human Genome Project lead to a first estimate that there are only about 34,000 genes in the human genome and by the end of 2003 the number was reduced to some 25,000 genes (Claverie J.–M., 2001; Wright F. A., 2001; Pennisi E., 2003). Now at the end of 2004 the euchromatic sequence of the human genome is complete, the number of genes is estimated to be about 20,000 to 25,000 (Collins FS, 2004).

The *Caenorhabditis* (*C. elegans*) genome is comprised of over 18,000 genes. The fruit fly (*D. melanogaster*) genome consists of about 13,000 genes and as such it has fewer genes than *C. elegans*, although as an organism it is far more complex. Gene number alone does not predict functional complexity. Although there is much more variation in the sizes of the genomes, this is not reflected in the number of genes.

The functional uncoupling of the dynamics of cellular function to its genomic gene-count came as a shock. The complexity and diversity of organisms is not reflected in the structural complexity of their genomes alone, but to a large extent it is hidden in the dynamics of gene expression and cellular processing. As there is no linear relation between the complexity of an organism and the physical structure of its genome, there is also no one-on-one relation between the phenotype of an organism and its genome. Relatively small differences between organisms, such as man and chimpanzee do result in large functional differences in gene processing and functional expression.

The structural relatedness of the human and chimpanzee genome, does not explain the large difference in brain function for which gene expression profiles in the brain are a better predictive instrument (Caceres M, 2003; Fortna A, 2004; Uddin M, 2004). Functional differences between chimpanzee and man are more outspoken in the brain than in other organs. Gene expression differences are more related to cerebral physiology and function in humans than gene sequences. Epigenetic phenomena within individual cells and differential processing in different cell types have more predictive power than the piecemeal and one-dimensional gene sequence approach, when applied on complex structures such as the brain (Wilson KE, 2004).

>From single gene and genome to the entire cell

Now we are starting to use the information coming out of the Human Genome Project, people start to understand that the dynamics of the cell and its fate in disease processes cannot simply be explained from its individual genes, genome or its proteome. Although all cells in the human body share the same genome, there is considerable heterogeneity in their phenotype and dynamics. Structural information alone or information from too low an organizational level cannot sufficiently predict higher-order phenomena as it does not sufficiently take into account interactions at higher organizational levels and influences from outside the low-level organizational unit. Cells have come up with compensation mechanisms to maintain their structural and functional integrity in the face of perturbations and uncertainty (Stelling J, 2004). Organisms are capable of buffering genetic variation (Hartman JL 4th, 2001). Genetic buffering mechanisms modify the genotype-phenotype relationship by concealing the effects of genetic and environmental variation on phenotype (Rutherford SL., 2000).

So if the structure of the genome alone cannot explain the differences between species, disease processes and the dynamics of the cell, where does our functional complexity and interspecies differences come from? How do we

continue in the post-genome era to study the dynamics of the cell and entire organisms? How are genes related to the function of an organism and where do we lose track? These questions are not of academic importance alone, but their answers have a significant impact on the diagnosis and treatment of (complex) diseases, drug discovery and development.

Let us take a walk from gene to protein and take a closer look at "The Central Dogma of Molecular Biology", which I personally prefer to call an axiom instead of a dogma. Science should only have axioms and leave dogmas to religion.

#### Associating genes with diseases

In order to start studying the contribution of a certain gene to a disease we must first find the gene(s) which might play a role in a given disease. The strength of the association must be detectable by the method being applied, which in complex gene-disease relationships has to find the association on a background of significant functional and phenotypical noise, such as in multifactorial diseases like diabetes (Doria A., 2000). Variation in the phenotypical expression of many quantitative traits (length, weight .) is due to the simultaneous segregation of multiple quantitative trait loci (QTL) as well as environmental influences. Genetic dissection of complex traits and quantitative trait loci is a complex process (Darvasi A., 1998; Darvasi A, 2002). A mono-factorial approach is likely to fail in a multifactorial process of pathogenesis (Templeton AR., 1998).

Giving a gene its place in a disease process is not a trivial endeavour and it is complicated by both technological and methodological difficulties. Association studies offer a potentially powerful approach to identify genetic variants that influence disease processes (Lohmueller KE, 2003; Roeder K, 2005). The density of Single Nucleotide Polymorphisms (SNP) makes them a popular target for studying gene-disease associations. However it is not only the density alone which counts, but also the information content of a given polymorphism (Bader JS. 2001; Ohashi J, 2001; Byng MC, 2003; Chapman JM, 2003; Garner C, 2003).

False positive correlations of genetic markers with disease are reported due to a flawed statistical analysis (Nurminen M., 1997; Edland SD, 2004; Wacholder S, 2004). In microarray experiments defining the appropriate sample size to find differentially expressed genes is an important issue (Wang SJ, 2004). In complex diseases in which not only multiple genes and the dynamics of gene products play a role, associating particular genes with a disease entity is even more difficult than in so-called monogenic diseases (Carey G., 1994; Long AD, 1999). Proper subgroup analyses in a randomised controlled trial (RCT) require careful design (Brookes ST, 2001).

Turning a gene-disease association into determining its role in the actual causation of a disease process is even further away from finding and establishing a positive correlation (Templeton AR., 1998).

>From genome sequence to gene activity

The genome sequence alone does not allow us to predict the functional impact of sequence variations as epigenetic modulation influences functional gene expression. Epigenetic modulation of gene function is a cause of non-Mendelian inheritance patterns and variability in the expression and penetrance of a disease. Even transmission of an identical gene sequence is not a guarantee for identical gene expression as the (in)-activation of a gene by epigenetic modulation occurs differently when a gene is of paternal or maternal origin. Where (in what cells or tissues) and when (at what stage of development or under what conditions) genes are expressed is a highly dynamic process. The repression of gene activity and the maintenance of the repressed state are fundamental requirements of cell differentiation, ordered embryonic development and tissue integrity (Czermin B, 2003). These spatial and temporal gene expression patterns can be assembled into "localizome" maps (Dupuy D, 2004).

Epigenetic modulation of gene expression is heritable during cell division but is not contained within the DNA sequence itself (Reik W, 2001; Bjornsson HT, 2004; Kelly TL, 2004; Chong S, 2004). Epigenetic modulation is one of the problems encountered when cloning, as the cloning process differs in its epigenetic regulation of (embryonic) gene expression (Mann M, 2002).

This differential inactivation of genes from maternal and paternal origin even leads to functional X-chromosome mosaicism in women as their cells at random inactivate one of their X chromosomes. X-inactivation occurs early in embryonic development and all cells subsequent inherit a different functional X chromosome. The inactivated X chromosome can be seen in a microscope as a Barr body in the interphase nuclei of female mammals. Differential activation of genes creates a functional chimera.

Chemical modification by methylation of cytosine residues is a major regulator of mammalian genome function and plays an important role in the intra-uterine development of an organism and the regulation of gene expression (Urnov FD, 2001). Tissue specific imprinting in genes leads to differential gene expression in different tissues (Weinstein LS, 2001). Aberrant DNA methylation has been implicated in the pathogenesis of a number of diseases associated with aging, including cancer and cardiovascular and neurological diseases (Walter J, 2003; Jiang YH, 2004; Macaluso M, 2004). A dietary component such as folic acid is a key component of DNA methylation during in utero development, disease development and aging (McKay JA, 2004). Genes and environment interact and this might play a critical role in the pathogenesis and inheritance of complex diseases (Vercelli D, 2004).

Transcriptional regulation in eukaryotes involves structurally and functionally distinct nuclear RNA polymerases, corresponding general initiation factors, gene-specific (DNA-binding) regulatory factors, and a variety of coregulatory factors that act either through chromatin modifications or more directly to facilitate formation and function of the preinitiation complex (Roeder RG., 2005).

The gene expression flow from mRNA to tRNA is not a smooth unregulated process in itself. Cells use RNA-induced silencing complexes (RISCs) programmed with small interfering RNA (siRNA) to knock down target RNA levels (Wassenegger M, 1994; Robb GB, 2005). RNAi is used by Eukaryotes for sequence-specific, post-transcriptional gene silencing (Cullen BR., 2004; Scherr M, 2003). RNA silencing genes play a role in DNA methylation (Chan SW, 2004). This mechanism adds another feedback loop onto the multiple layers of gene expression regulating mechanisms.

The correlation of even a gene sequence to the first steps in its expression does not show a one-on-one relation to the gene sequence itself. Modulators and regulators of transcription and translation are showing a highly dynamic process regulation mechanism. Cells use several mechanisms to create functional flexibility from (relative) structural (genome sequence) rigidity. The genome is a repository of our genetic potential, but only a part of it is active at different spatial and temporal locations during our lifetime. It is not only important to know what we can do within the limitations of our genomic boundaries, but also how we deal with this potential in spatial and temporal patterns during our lives. We do not deploy the full potential of our genome at every moment of our life and in all our cells in the same way. Although all our cells share the same genome, they are highly diverse in their structure and function, not only are they spatially differentiated but also temporally. The relation of gene structure to its function is a bidirectional process of which our understanding of the impact of different modulators is still not sufficient to create highly correlating disease models.

>From gene to protein, a bumpy road

A eukaryote, such as Homo sapiens, has no one-on-one relation to its genes. The dynamics of gene expression is regulated by hypo-, iso- and epigenetic operators. The gene may be the structural unit of inheritance, but the protein domain is the functional unit of metabolism.

When we talk about protein structure, the primary structure refers to the amino acid sequence in a protein (1D). The primary structure is most closely related to mRNA and as such the gene sequence and gene structure from which the protein originates. The terms secondary and tertiary structure refer to the 3D conformation of a protein chain. Secondary structure refers to the interactions of the backbone chain (alpha helical, beta sheet, etc.). Tertiary structure refers to interactions of the side chains. Quaternary structure refers to the interaction between separate chains in a multi-chain protein (4D). The combined shape of the secondary and tertiary structure and the quaternary structure is referred to as the conformation of the protein. With increasing dimensionality, the relation between a higher order organization of protein structure and its gene relaxes as other physical and chemical influences play an increasingly important role in its physical and functional integrity.

In a mature enzyme, only a relatively small number of its amino-acids interact with a ligand, the majority of amino-acids help to create the

appropriate 3D and even 4D structures required for its in-vivo functionality. Structural proteins and enzymes may show interactions over larger parts of their molecular surface to form functional homo- or hetero-polymers in their quaternary structure. From a single gene to a protein, we have to deal with the dynamics of gene expression regulation and mRNA formation (promoters, cis- and trans-regulation, transcription, splicing). We have to deal with the interaction of tRNA with mRNA in the translation of an mRNA sequence into a protein sequence and post-processing of the protein sequence into a functional 3D and 4D structure (Wobble, sequence processing, protein folding and interaction).

A structural similarity at the genome level does not lead to functional similarity, due to epigenetic regulation (Eckhardt F., 2004). Sequence variation, due to mutations does not bleed through to the protein level one-on one. Basic mechanisms act as powerful uncouplers of gene structure from protein function. Mutations in the DNA and errors during transcription of the DNA-sequence into mRNA are not linear predictive for the structure and function of the protein resulting from the translation of the DNA-sequence into the protein-sequence, due to the degeneration of the genetic code. The deleterious effects of sequence variations are up to a certain extent suppressed by the Wobble-mechanism used in base-pairing in translating mRNA to protein (Crick F, 1966).

Protein sequence =  $k \times$  gene sequence

In this formula, 'k' is always smaller than one for most amino acids built into a protein, due to mechanisms such as splicing variation, Wobble mechanism.

In eukaryotes, a relatively simple genome compared to their functional and structural complexity can be used, because of the existence of introns and exons. An exon in general defines a functional domain and these domains are rearranged to create a more complex proteome than the genome it is derived from. Constitutive and alternative splicing of genes is dynamically regulated at the moment of transcription and pre-mRNA splicing by cis- and trans-acting factors (Kornblihtt AR, 2004; Sharp PA, 1988). Before the completion of the Human Genome Project was finished it was expected that man would need about 100,000 genes to explain the structural and functional complexity of our species. This number has collapsed to about 25,000 genes and is about four times (75 percent) lower than expected (Collins FS, 2004). The functional differences between species are more related to differential processing, due to different up- and down regulation of genes in different cell types and organs.

The use of different promoters and splicing variants is used to tune protein and enzyme structure and function in different cell locations and organs (Ayoubi TA, 1996, Masure S, 1999; Nogues G, 2003, Yeo G, 2004). Promoter variation and differential splicing allows for spatiotemporal differentiation in protein expression, while the organism does not have to manage an explosion in genomic size and sequence-complexity. This mechanism helps to uncouple the protein from the rigidity of the gene sequence in

order to allow for functional variation while restricting structural variation at the genome level (Nadal–Ginard B, 1991). Functional differentiation in gene expression allows for a better adaptability to changing conditions, without the need for fast–paced changes in gene structure.

Protein folding of a linear amino–acid sequence into a 3D protein also acts as a functional uncoupler of gene sequence to protein function. Changes in the physical and chemical environment of the protein may change the shape and alter the conformation of a protein. By putting a protein in a different physical and chemical environment which will change the ability of the van der Waals, hydrogen, ionic and covalent bonds which hold the protein together in its particular conformation, it is possible to cause the molecule to unfold by breaking those bonds and make it change or even lose its function (denaturation). 3D and 4D protein folding is a complex process. Even today the protein folding problem remains one of the most basic unsolved problems in computational biology. Predicting protein folding from the gene upwards ignores the influence of the post–translational modification (PTM) and the influence of the in–vivo physico–chemical environment of the protein. Proteoglycans and glycoproteins are not derived from a gene sequence as such, but their structure is the result of extensive post–translational modification. Cell membranes contain phospholipids, which are not encoded by DNA as such, but they result from metabolic processing and nutritional components.

While the protein–sequence at the moment of translation is related to the gene–sequence, the final structure and function of an enzyme is in addition defined by post–translational modification (PTM) and its physico–chemical environment (Kukuruzinska MA, 1998; Uversky VN, 2003; Schramm A, 2003; Seddon AM, 2004). Studying protein folding is a computational complex process and still the focus of intensive research (Murzin A. G., 1995; Orengo, C.A., 1997; Dietmann S, 2001; Day R, 2003; Harrison A, 2003; Pearl F, 2005). Epicellular regulation of protein glycosylation also plays an important role in the dynamics of protein activity (Medvedova L, 2004).

The majority of proteins are subjected to a multitude of post–translational modifications. Post–translational modification involves cleaving, attaching chemical groups (prosthetic groups), internal cross–linking (disulfide bonds). Already more than hundred different types of PTM are known, which act as functional uncouplers of protein structure from the gene sequence (Hoogland C, 2004). A protein precursor may be differently processed in different cell types and, in addition, diseased cells may process a given precursor abnormally (Dockray GJ., 1987; Poly WJ., 1997; Rehfeld JF., 1990; Rehfeld JF, 2003). Post–translational protein modifications finely tune the cellular functions of each protein and play an important role in cellular signaling, growth and transformation (Parekh RB, 1997; Seo J, 2004).

In a functional protein only a very few specific residues are actually responsible for enzyme activity, while the fold is much more closely related to ligand type (Martin AC, 1998). The effect of an amino–acid change on protein structure and function depends on the location of the amino–acid in

the 3D structure, its physico–chemical properties and the physico–chemical environment it is being processed and used. Amino–acids which are distant neighbours in the protein sequence can become close neighbours in the 3D structure of the protein and as such a protein sequence variation is only a weak determinant of the function of a mature protein.

Proteins do not operate in void, but they depend from other proteins and molecules for their function. Proteins build complex cell signaling networks (CSNs) in which the functional outcome cannot be predicted from each individual protein alone (Berg EL, 2005; Eungdamrong NJ, 2004; Lengeler JW., 2000).

By just going from DNA–sequence to 3D protein structure, the relation between genome sequence and the functional status of a cell begins to fade. By taking this relation even further from gene to organism, we lose additional predictive power. How will be able to design models that will allow us to predict the functional outcome of a disease, when we use a fuzzy model to start with? Powerful uncouplers of the structural relation of even a protein to the gene it is primarily derived from, do not allow us to draw hard conclusions about impact on the functional status of an organism from the gene and genome sequence.

>From proteome to cell

Eukaryotic cells are highly compartmentalized; proteins do not exist in the cell as in a homogeneous fluid, but in different compartments of the cell, each with a different physico–chemical environment. The 3D and 4D structure of a protein and its functionality is highly dependent from the in–vivo physico–chemical environment of the protein. Cellular structure and metabolism is organized and differentiated in both space and time.

Studying proteins without taking into account their spatial and temporal organization in a cell, ignores the complexity and dynamics of protein expression and interaction in a cell. Studying proteins in–vivo reveals more about their function and dynamics (Chen, X., 2002; Hesse J, 2002; Pimpl P, 2002; Viallet PM, 2003; Murphy R. F., 2004). Without information about the relation between cellular structure and function, a lot of information is lost. A 2D protein–profile may show the entire protein content of a cell, but we lose all information about the intracellular spatial and temporal distribution of these proteins.

Eukaryotic cells are highly spatially differentiated structures. Proteins involved in trans–membrane trafficking, require a membrane to do their work and cannot do their work outside this specific physico–chemical environment. A protein has to reach the appropriate physico–chemical environment in the cell in order to do its work properly (Graham TR., 2004). Studying a protein outside its in–vivo physico–chemical context leads to a loss of correlation with its in–vivo dynamics.

There are three main cellular compartments in a eukaryotic cell, the nucleus, cytoplasm and the cell membrane. The nucleus itself is a highly

organized 3D structure with highly spatial and temporal differentiated DNA– and RNA–processing machinery (Lamond AI, 2003; Politz, J., 2003; Pombo, A., 2003; Iborra F, 2003; Spector DL., 2003; Cremer T, 2004). Both transcription and splicing of the mRNA message are carried out in the nucleus (Sleeman JE., 2004). The distribution of eu– and heterochromatin changes throughout the cell cycle, chromosomes and spindles appear during cell division. The dynamics of gene transcription is visible in the chromatin condensation patterns in the nucleus (Craig JM., 2005; Lippman Z, 2004). The nuclear envelope separates transcription and DNA replication in the nucleus from the site of protein synthesis in the cytoplasm (Rodriguez MS, 2004).

The cytoplasm itself contains several organelles, smooth and rough endoplasmic reticulum (SER and RER), ribosomes, the Golgi apparatus, mitochondria, lysosomes and the cell membrane. Each organelle deals with a different set of processes necessary for cell development and maintenance. The membranes of organelles are highly dynamic structures which undergo profound changes during the life cycle of a cell (Ellenberg, J. 1997; Zaal, K. J. M., 1999). The endoplasmic reticulum (ER) is a multifunctional signalling organelle that controls a wide range of spatially and temporally differentiated cellular processes (Berridge MJ., 2002).

The structural compartmentalisation of the intracellular environment allows for a functional differentiation and provides a process flow management mechanism. The membrane structure and the mitochondrial membrane potentials (MMP) of mitochondria play an important role in their function. (Zhang H, 2001; Pham N.A, 2004). Microtubules play an important role in cellular function and their organization and dynamics are being studied by microscopy based techniques (De Mey J., 1981; De Brabander M., 1986; Geuens G, 1986; De Brabander M, 1989; Geerts H., 1991; Olson KR, 1999).

The dynamics of intracellular ion–fluxes such as for calcium ( $Ca^{2+}$ ) is organized in a highly dynamic and spatial and temporal complex pattern. Ions are themselves not encoded by the genome, but play an important role in cellular function. The intra– and extra–cellular dynamics of ions (concentration, flux) interact with a spatial and temporally regulated pattern for protein expression and differential protein activity. The complexity of intracellular calcium–signaling extends beyond the mere expression profiles of genes encoding the proteins involved in calcium–dynamics (Berridge MJ., 1981; Bootman MD, 2002; Cancela JM, 2002; Berridge MJ., 2003; Berridge MJ, 2003b). For their proper function and survival cells have to manage  $Ca^{2+}$  concentration and flux in space, time and amplitude (Bootman MD, 2001). Calcium is involved in the delicate process of spatially and temporally organization of cellular communication (Berridge MJ., 2004).

As an example of spatial compartmentalisation in the cell, hydrolytic lysosomal enzymes require a specific physical and chemical environment to do their work, which inside the cell only exists inside the lysosomes (De Duve C, 1955). The boundary membrane of the lysosome keeps the hydrolytic enzymes away from the rest of the cytoplasm and so controls what will be digested (De Duve C., 1966).

The cell membrane separates the interior of the cell from its environment, but is a highly dynamic structure (Kenworthy, A. K., 1998; Varma, R., 1998). The appropriate spatial and temporal dynamics of the cell membrane are vital for the survival of the cell. The cell membrane provides the physical boundaries in which the cell can maintain a highly dynamic physical and chemical environment. Cell-to-cell communication is dynamically managed at the level of the cell membrane (Nohe A, 2004).

Proteins do their work in spatially different cellular environments and with different spatial and temporal patterns. A protein can be mobile in one cellular compartment and immobile in another (Ellenberg J., 1997). Co-expressed proteins may in reality never interact with each other because they do their work in separate cellular compartments. The substrates of proteins may migrate through different cellular compartments in order to be subjected to a highly dynamic interplay of enzymatic processes. Proteins which do their work in the same cellular compartment may only be expressed at different stages during the life cycle of a cell. Spatial and temporal protein localization information can help us to find entries into eukaryotic protein function (Kumar A, 2002).

An important temporal differentiation of cellular processes occurs during the cell cycle. The different stages in the cell cycle each depend on the spatial and temporal expression of multiple proteins. The passage of the cell through the cell cycle is controlled by proteins in the cytoplasmic compartment, such as different Cyclins, Cyclin-dependent kinases (Cdks) and the Anaphase-Promoting Complex (APC). First there is the G1 phase (growth and preparation of the chromosomes for replication). Secondly the cell enters the S phase (synthesis of DNA and centrosomes) and finally the G2 phase which prepares the cell for the actual mitosis (M). The mitosis itself consist of a spatial and temporal sequence of events, called the prophase (mitotic spindle), prometaphase (kinetochore), metaphase (metaphase plate), anaphase (breakdown of cohesins) and telophase where a nuclear envelope reforms around each cluster of chromosomes and these return to their more extended form.

However our understanding of the cell cycle is still far from complete. The regulation of the cell cycle by G1 cell cycle regulatory genes is more complex than we thought (Pagano M, 2004).

Cells also operate in a temporal pattern based on internal and external clocks. Cellular events must be organized in the time dimension as well as in the space dimension for many proteins to perform their cellular functions effectively (Okamura H., 2004). Circadian molecular clocks regulate protein dynamics in temporal patterns (Crosthwaite SK., 2004; Hardin PE., 2004; Harms E, 2004; Hastings MH, 2004; Ikeda M, 2004; Rudic RD, 2004; Schwartz WJ, 2004; Shu Y, 2004; Takahashi JS., 2004). In mammals there exists a central circadian pacemaker which resides in the hypothalamic suprachiasmatic nucleus (SCN), but circadian oscillators also exist in peripheral tissues (Yagita K, 2001).

We need to study and understand the intracellular in-vivo dynamics of protein metabolism and its spatial and temporal organization in different cell types. We need to study intracellular protein ecology, not just ex-vivo protein interactions or building a protein catalogue of only scalar dimensions. The spatial and temporal patterns of intracellular protein dynamics are an important factor in health and disease.

The dynamics of cellular function

Taxonomy is the science of organism classification and refers to either a hierarchical classification of things, or the principles underlying the classification. Today the emphasis of biological research is on classifying genes, proteins in large catalogues, instead of studying the spatial and temporal dynamics of cellular processes in vivo. The global analysis of cellular proteins or proteomics is now a key area of research which is developing in the post-genome era (Chambers G, 2000; Ideker T., 2001; Aitchison J.D, 2003). Proteins show functional grouping into modules which can be grouped into elegant schemes (Hartwell, L.H., 1999; Segal, E., 2003).

In-vivo however the spatial and temporal distribution and interaction of proteins with other proteins, substrates, etc., adds another layer of complexity which is not taken into account by functional studies alone. Expression studies, no matter how we group them, do not reveal the intracellular spatial and temporal distribution of proteins and the functional outcome of their metabolic activity (spatial and temporal substrate trafficking) in various cellular compartments. Studying proteins only from a functional point of view ignores the impact of their intracellular spatial and temporal dynamics. Molecular taxonomy or systems biology (genomics, proteomics) will not provide us with the functional answers we need to know.

Systems biology studies biological systems systematically and extensively and in the end tries to formulate mathematical models that describe the structure of the system (Ideker T., 2001; Klapa MI, 2003; Rives A.W, 2003). However the level of biological integration which is being studied, genes, proteins, pathways is still too far away from pathological reality to allow for the development of highly predictive and highly correlating disease models in relation to clinical reality. The end-point of present day systems biology only takes into account infra-cellular dynamics and loses track when iso- and epi-cellular phenomena interfere with the dynamics of the model. Studying the physics and chemistry of protein interactions cannot ignore the spatial and temporal dynamics of cellular processes. We study nature "horizontally", e.g. the genome or proteome, while the flux in nature goes "vertically", e.g. from genome to proteome. The focus of our systems research is perpendicular to the flow of events in nature. The resultant vector which signifies our understanding of nature is aligned with the way we work, not with the true flow of events in nature.

The cell is at the crossroads of life itself, being the lowest order functional unit operating in a functional complete way. It is the basic object of nature. As such the cell is for life what the atom is for physics,

the smallest biological level of organization, operating as a functional unit. Dysfunctional cells by whatever cause, either gene malfunction, infection, nutritional or environmental problems will eventually cause the entire organism to lose its functional integrity. The dynamics of cellular systems allow for the adaptation of the cell to a wide variety of conditions and challenges, a relatively uniform physical structure combined with a web of interacting dynamic processes leads to the multitude of cells which we see in living organisms. In a living organism there is no such thing as an average cell type from a functional point of view. Cells are functionally highly diverse in both spatial and temporal dimensions.

The stochastic variation of cellular processing at the molecular level is another cause of functional uncoupling of the cytome from the genome and adds to the variability in functional behavior between cells (McAdams H.H., 1999; Raser J.M., 2004). Structural research alone underestimates the complexity of dynamic processes as it does not capture sufficiently the dynamic complexity of the cell. The dynamic interaction of processes in multiple pathways is the centerpiece of cellular life, not the individual components or even individual enzymatic reactions in the cell. There is no monotonic sequence of causation from genome structure to cellular dynamics.

Cellular function can be compared to a symphony in which multiple "instruments" contribute to a complex, but in a healthy state harmonic, "sound".

#### Genes and the dynamics of disease processes

The challenges faced by the medical world today are no less today than the ones we faced a century ago. The spectrum of diseases may have changed through time, as degenerative diseases and cancer play an increasing role in modern society. On the other side an old enemy is back on the rise, how much we thought that infectious diseases were a thing of the past; they are back and with a new and frightening face.

Our increase in the knowledge of the involvement of our genes and large scale proteomics in disease processes has not lead to an increase in the productivity of pharmaceutical research (Drews J., 2000; Huber, L.A., 2003; Lansbury PT Jr., 2004). The gap between the gene and the functional outcome of a disease is too wide to bridge it from one direction only (Workman P., 2001). Much thought has gone into finding a way how the knowledge coming out of genomics and proteomics could revolutionize drug discovery, such as for drug target discovery (Lindsay MA., 2003). The target of a drug molecule may be a protein, but the target of disease therapy is the entire cell and by extension the cell population of an organism. Every drug and its target may be part of a disease therapy, but the therapy is not restricted to the drug and its target. Every target is part of a therapy, but not every therapy is confined to a traditional drug target.

In the case of diseases where we have already found a genetic basis, this does not always allow us to create a model for the disease process. To discover the involvement of a gene in a disease process does not tell us

anything about its place and relative importance in the multiple and multilevel elements involved in the causation of a disease, such as genes, nutrition, infectious agents and the environment. To discover a causative element is not the same as understanding and predicting its dynamic involvement in a disease process. What we do know is that all causation has to pass through cells, as they constitute the "quanta" of the organism itself.

Many diseases of clinical importance have heterogeneous mechanisms which lead to the disease and only in a subpopulation the diseases can be traced back to a single gene. In most cases a multiplicity of mechanisms contributes to the diseases process. Genetic information has a high predictive value in only a minority of cases.

Non-coding sequences, inter-gene and epigenetic interactions have a significant impact on the prediction of the age of occurrence, severity, and long-term prognosis of diseases (El-Osta A., 2004, Perkins DO, 2004).

The importance of the dynamics of the cell and its involvement in pathological processes and current therapeutic efforts also requires a better understanding of its function and phenotype in its relation to pathological processes in diseases, such as in cancer, Alzheimer disease and infectious diseases, such as AIDS, tuberculosis (TBC), influenza (flu), etc.

Trying to predict a disease process from the genome (proteome) upwards, is like trying to solve a higher order polynomial while omitting the majority of elements and expecting that the equation will work:

e.g.: Disease process =  $a \times x + b$

Instead of using a higher order multi-dimensional model, closer to in-vivo functional dynamics in which a matrix or web of causation and consequences interacts in a high-dimensional space-time continuum:

e.g.: Disease process =  $a \times u_n + b \times v_o + c \times w_p + d \times y_q + e \times z_r$

In addition, each parameter which is being used in an equation is in itself the result of an underlying or "overlying" dynamic process. Each layer of organization can be fed into higher or lower order levels of organization as there is always a cross-influence in both directions. It is a matter of expanding or collapsing the set of parameters and taking into account or ignoring underlying "modifying" influences. Reducing the complexity allows for a better understanding of a simplified model, but has a decreased match to the complexity and dynamics of biological reality. When we create a model, we should not regard it as a one-on-one substitute for reality which we capture only partially into our model.

Infectious diseases

Infectious diseases still pose a significant threat to the health and well being of (modern) society. After years of relative neglect, nations are

increasingly aware of the present and future threats of infectious diseases and are even setting up new agencies, such as the European Centre for Disease Prevention and Control (ECDC) or expand the role of existing organizations, such as the Centers for Disease Control and Prevention (CDC). Beside their political and economical impact on society, how do we deal with infectious diseases in science?

In infectious diseases the environment, in this case the infectious agents, interacts in a complex way with the host defense system of which much remains to be explored. We must be aware of the fact that the golden era of antibiotics is already behind us as many infectious agents (e.g. TBC, MRSA and other bacterial diseases) are showing an increasing resistance against most classes of antibiotics which are available today (Davies J, 1994). We have succeeded in less than a century to destroy our best weapons against infectious diseases, due to misuse of antibiotics both by physicians and their patients. Only the elderly remember the days when mortality due to infections was a major cause of premature death, but the moment is approaching when this nightmare will return. Emerging infectious diseases (EIDs) and re-emerging infectious diseases challenge our defenses (Ranga S, 1997; Fauci AS., 2004; Morens DM, 2004).

Viral diseases (e.g. AIDS, influenza) are even harder to fight as they use the cellular machinery of the body itself to reproduce. We need to study the pathological process in cells in more detail and in a different way, in order to have a chance to succeed in the new therapeutic challenges ahead of us. Viruses, under selective pressure of modern antiviral drugs are also showing increasing resistance to treatment. We are running out of time in our battle against infectious diseases and a systematic approach will only give us the answers when it will be too late. We are not setting the agenda, but the diseases are taking the lead.

Due to modern technology, the time to respond to a new infectious challenge is being reduced. In modern times, diseases take planes too, which makes it even harder to fight them by classical isolation or quarantine. Airplanes may be safe to travel with, compared to other transport systems, but they can cause secondary mortality by transporting pathogens over large distances at a speed unknown to previous generations, which gives a new meaning to airborne infections (Gerard E, 2002; Van Herck K, 2004; Blair JE, 2004). Infectious diseases may initially go unnoticed in underdeveloped areas of the world (e.g. Ebola virus Lassa fever, Marburg virus), but as soon as they board a plane, it is modern technology which will give them free access to the world (Clayton AJ, 1979; Gillen PB, 1999). A relatively long incubation time combined with a high mortality rate will allow a disease to spread widely and cause a pandemic, before we even can start a treatment program. If an unknown disease causes such a pandemic, we may run out of time before we can find a cure as we first have to develop a diagnostic tool. A recent example which is a model of what can happen was the Severe Acute Respiratory Syndrome or SARS (Peiris, J.S.M. 2003, Berger A, 2004; Heymann DL, 2004; Tambyah PA, 2004).

Robert Koch presented his work on Tuberculosis on 24 March 1882 before the

members of the Berlin Physiological Society, which meant a breakthrough in the understanding of this terrible disease (Winkle S, 1997, pp. 137–141). Now after more than 100 years of research and drug development, TB is on the rise again. In the war against infections such as Tuberculosis, there are no easy wins. We may win a fight but for the majority of pathogens we can only reach a status quo, but never completely win the war. Variability by mutating is a powerful weapon against our drug treatments and pathogens use it to their great advantage.

We must keep our defenses up to date and changing in order to outsmart our bacterial and viral enemies. New antibiotics are not found within the human genome. Penicillin was discovered by accident and many important antibiotics were found at the most unlikely places (Fleming, A, 1929). No hypothesis or model can be formulated to find the unexpected, but we have to find new antibiotics as bacteria are closing in on us and some of our worst enemies are even winning the race.

Scientists are waiting with fear for the next influenza pandemic which will hit us some day (Gust ID, 2001; Capua I, 2004). Scientists are trying to understand the lethal potential of the deadliest influenza epidemic of all times, which occurred after the first World–War. Soon the virus which caused the influenza pandemic, called the 'Spanish flu' will re–emerge out of the test tubes of the laboratory. Recent outbreaks of avian flu have given us a preview of what can happen and evidence is increasing that the possibilities for spreading avian influenza A virus (H5 or H7 subtype) are worse than previously was assumed (Koopmans M, 2004; Kuiken T, 2004).

New pathogens can have a devastating effect on a human population. Examples of what can happen when a new infectious agent hits a population with little or no immunological "experience" with a (re–)introduced pathogen, can be found in the histories of indigenous people confronted with infectious diseases introduced by European colonization as in Australia and Tasmania. Within 100 years of European colonization the total population of full–blood Aboriginal people in Tasmania became extinct. Introduced infectious diseases killed many more Aborigines than did direct conflict. Infectious diseases such as smallpox, measles, and influenza were major killers and even chickenpox was deadly as the Aborigines had no immunological history even with chickenpox. Of the 90 percent of the Aboriginal population that died out as a result of European contact, it is estimated that around 80 or 90 percent of the deaths were the result of disease.

Most people have no idea of the role smallpox played in the destruction of an entire civilization after it was brought to America by the conquistadores. About 50 to 90 percent of the Native American population died of smallpox and the speed at which people died is beyond our imagination (McMichael AJ, 2004; Winkle S., 1997, pp. 855–861). A mortality of 50 percent for a new disease, for which we have no immunity, could kill half of the population of a country or an entire continent. Western society now has to fear the introduction of new pathogens from distant places and when the disease has the right pathological profile; it will spread extensively into the population before it is being diagnosed (e.g. AIDS).

Re-emerging infectious diseases are a global problem with a local impact. It is an unpleasant thought that this time we will face the fate of the indigenous people during European colonization. In modern times we not only have to fear the accidental spreading of infectious diseases, but bio-terrorism will challenge our defenses sooner or later (Broussard LA, 2001, Gottschalk R, 2004).

Finding the infectious agent for a new and unknown disease requires something else than sequencing a genome as this approach only works when we have the time to do the sequencing while the pathogen takes its course. Analyzing the genome sequence of a new infectious agent can only start after it has been isolated by more traditional means (Berger A, 2004). Once we know the new pathogen, we can use its genome sequence to develop rapid diagnostic tools, based on PCR, but in order to do this we must first isolate it from the patient. Developing a therapy after this, takes much longer and the genome sequence itself without additional functional information is not enough. Only after Koch's postulates had been fulfilled, the WHO officially declared on 16 April 2003 that a previously unknown coronavirus was the cause of SARS.

Modifying the disease progression requires an interaction with the actual disease process which extends beyond understanding the genome structure of the pathogen. Focusing more on the dynamics of the interaction of cellular systems with pathogens and using tools for functional research of the disease process at the cellular level (and beyond) will hopefully allow us to respond in time when we are faced with an unknown pathogen.

When we do not already have an antibiotic, antiviral drug or vaccine at hand at the moment a new disease hits us, either by accident or on purpose in biological warfare or bioterrorism, we are in serious (and lethal) trouble. In this case the only thing left is the medieval solution of quarantaining the infected people, which only works if we are able to contain them before they spread over a country or even the planet (e.g. Ebola, SARS or HIV).

Although all cells in the human body may share the same genome, there is a high spatial and temporal differentiation in gene expression and metabolic dynamics in different cell types and organs. In HIV, it is the CD4 lymphocytes which express the receptors by which the virus can enter the cell (Fauci AS, 1996). A hepatocyte may share its entire genome with a CD4 lymphocyte, but it does not express the proteins encoded by the gene which allows the virus to enter the cell. The progress of a HIV infection is also a highly dynamic process of interaction between the host and the virus (Wei, X., 1995). The observation of differences in disease progress leads to the discovery of a genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CCR5 structural gene (Dean M, 1996). The emerging picture on infectious diseases is one of highly polygenic patterns, with occasional major genes, along with significant inter-population heterogeneity (Frodsham AJ, 2004). The complex interactions and regulation of the Interleukin-1 (IL-1) family of proteins is just one of the issues in elucidating the dynamics of the human immune system (Laurincova B., 2000).

Clinical observations lead to genetic conclusions, but the way back to clinical treatment of diseases is a long and winding road for which the gene sequence or protein structure does not provide us with all the necessary information about the dynamics of the disease process. Studying the cellular dynamics of disease processes provides us with one of the step stones from gene to clinic. By focusing on genomics and proteomics alone, there remains a correlation and predictive deficit in our disease models.

#### Mendelian diseases

Mendelian inherited and monogenic diseases have always been at the center of attention in the relation of genetic variation to diseases. Monogenic diseases served as a model to prove the use of genetic information to the development of a disease and the outcome of a disease process.

Phenotype–genotype relationships are complex even in the case of many monogenic diseases. Increasingly complex interactions have now been demonstrated in a number of monogenic Mendelian diseases (Nabholz CE, 2004). The (phenotypical and functional) expression and development of even a monogenic disease depends on its context, which comprises both other genes and environmental factors. These inter–gene and epigenetic interactions have a significant impact on the prediction of the age of occurrence, severity, and long–term prognosis of even 'genetic' diseases (Cajiao I, 2004; Hull J, 1998; Frank RE, 2004; Salvatore F, 2002; Sontag MK, 2004; Sangiuolo F, 2004).

The beta–thalassemias show a remarkable phenotypic diversity caused by the action of many secondary and tertiary modifiers, and a wide range of environmental factors (Weatherall DJ., 2001). Sickle cell anaemia and cystic fibrosis can serve as an example that genotype at a single locus rarely completely predicts phenotype (Summers KM., 1996). Although the gene defect in Huntington's disease is known for years, the contribution of the gene defect to the functional out come of the disease is not yet known (Georgiou–Karistianis N, 2003). Cell based research will help to elucidate the disease mechanism in Huntington's disease (Arrasate M, 2004).

In cystic fibrosis, the severity of the disease cannot be linked one–on–one to genetic variation in CFTR (Grody W, 2003). Cystic fibrosis is the most common autosomal recessive disorder in Caucasians, with a frequency of approximately 1 in 3000 live births, so finding a cure for this disease has a high im  
all cases of colorectal cancer (Kinzler, 1996). Genes which are involved in diabetes, such as GCK (glukokinase) , HNF1A and HNF4A (Hepatic Nuclear Factor) are linked to less than 5 percent of cases of diabetes (Edlund, 1998, Fajans, 2001).

On of the major emerging health problems of modern society is Alzheimer's disease (AD). This is not only because widely known people, such as the former president of the USA, Ronald Reagan, suffered from the disease in a long and unpleasant disease process. Today AD is still a chronic disease without a cure which causes patients to receive long–term care (Souder E, 2004).

Presently available drugs improve symptoms, but do not have a profound disease-modifying effect and fail to alter the course of AD, so it may be time to change the way we think about AD therapeutics (Crentsil V., 2004; Citron M., 2004; Kostrzewa RM, 2004)? Will we see a breakthrough in the understanding of the cellular and molecular alterations that are responsible for the degeneration of neurons in AD patients (Mattson MP., 2004)?

In Alzheimer's disease (AD), only a minority of cases can be linked to a single hereditary gene mutation, the complexity of the disease process extends beyond our present understanding and disease models (Selkoe DJ., 2001; Eikelenboom P, 2004). Neurodegeneration in AD may be caused by deposition of amyloid beta-peptide in plaques in brain tissue (Amyloid Hypothesis), but no causal treatment has come out of this in 10 years of hard work (Hardy J, 2002; Lee HG, 2004; Lee HG, 2004b). Little is understood about the dynamics of amyloid beta-peptide and its fundamental role in the disease process of AD (Regland B, 1992; Koo EH., 2002; LeVine H 3rd., 2004).

A complex disease requires studying and understanding a complex in-vivo pattern of a spatially and temporally changing metabolic process, which goes beyond studying gene expression profiles, either single or multiplexed. Studying the multi-scale spatial and temporal dynamics of a complex disease process in a long-term space-time continuum is a tremendous scientific challenge. Instead of focusing on individual (molecular) targets in drug research and therapy, complex diseases may require pathway-engineering or cell replacement to restore the appropriate dynamics of spatial and temporal patterns of intracellular molecular processes. Functional or structural protein (re-) modeling or restoration in-vivo may be a better approach for complex diseases than just docking a small molecule to an active binding site?

At this moment the cell is the target for many therapeutic efforts to come to a causal therapy of complex diseases, which we can now only treat with external substitution, such as diabetes. Many diseases are far more complex and multi-factorial than monogenic diseases and should be studied with more power at a higher biological level than the genome or proteome to capture the complexity of the disease process.

One of the most promising domains of research today is stem cell research (He Q, 2003; Doss MX, 2004). Since the isolation and growth in culture of proliferative cells derived from mouse embryos in 1981, stem cell research has come a long way (Evans MJ, 1981; Martin GR., 1981). Instead of treating complex disease processes with a multitude of drugs, each with its own spectrum of sometimes serious and cumulative side effects, failing components of the human cytome could be engineered or replaced by stem cells (adult or embryonic) differentiated into the appropriate cell type.

When the distortion of cellular metabolism goes beyond a mere dysfunction of a single protein, a complete replacement of the dysfunctional cells has a better chance to restore the complex and delicate balance and regulation of metabolic processing. The fine dynamics of spatial and temporal regulation

of cellula