peroxisome proliferation may be involved. Indeed, estragole increases liver weight at a dose lower than the carcinogenic dose.[19]

An important advantage in determining the actual average number of mRNA molecules per cell is that the responses obtained in different studies can be compared directly. As shown in Figures 11.6 and 11.7, the magnitude of the up-regulation of PPAR-alpha-inducible genes by estragole was comparable to that of clofibrate, since, at the same doses (*i.e.* 0, 10, 30, and 100 $mg \cdot kg^{-1}$) employed, estragole appears to be as potent as clofibrate in activating PPAR-alpha signaling.

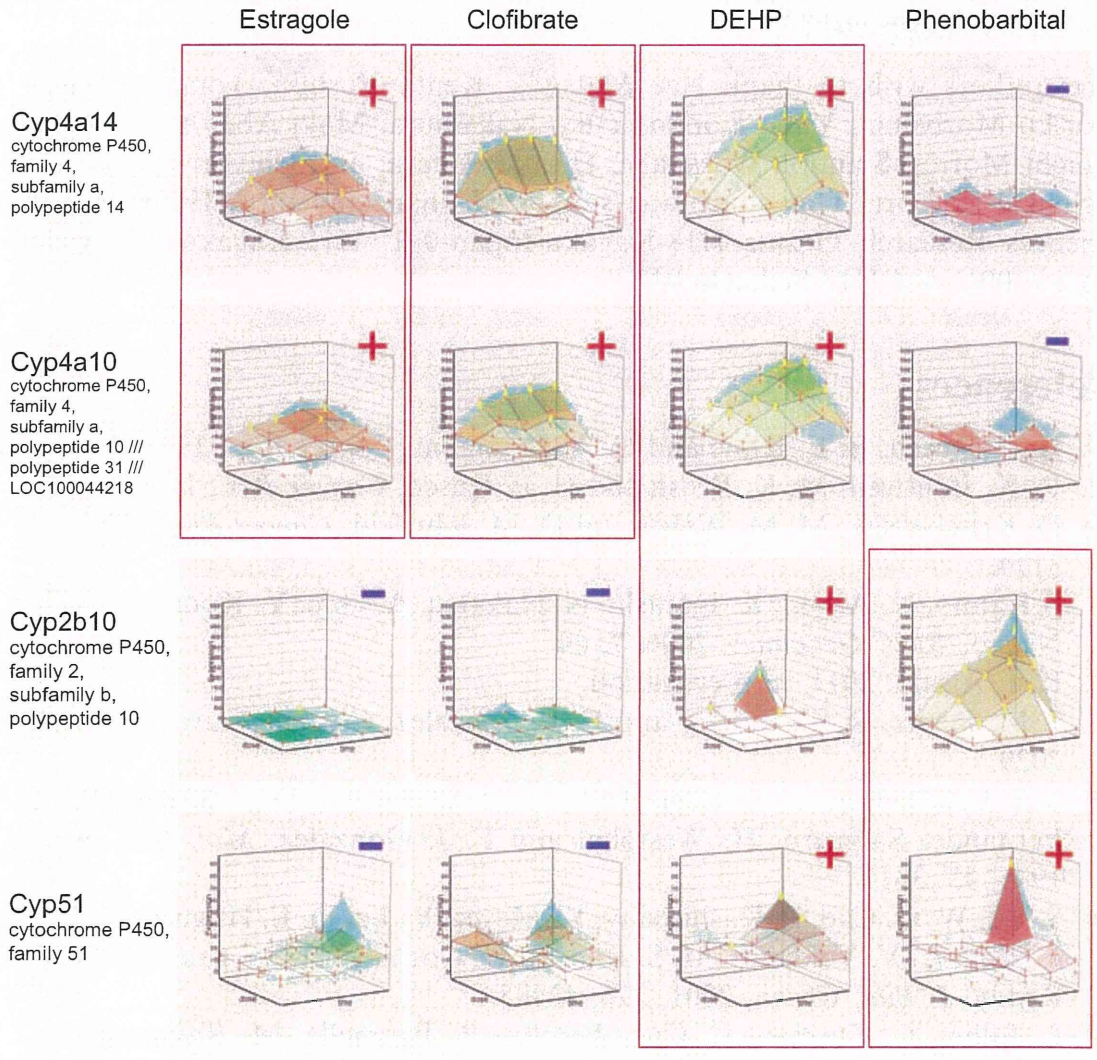

Figure 11.7 Percellome analysis of representative P450s induced by estragole, clofibrate, DEHP, and phenobarbital. DEHP appears to induce P450s *via* at least two different pathways, *i.e.* PPAR-alpha and CAR, whereas estragole, clofibrate, and phenobarbital induce only PPAR-alpha or CAR, respectively.

11.5 Conclusions

Our present observations that estragole appears to be as potent an agonist of PPAR-alpha as clofibrate (on a $mg \cdot kg^{-1}$ basis) should now be confirmed by actual binding and signaling studies. If confirmed, the hepatocarcinogenic potential of this compound should be reevaluated accordingly. Although recent reports on estragole carcinogenicity suggest involvement of its metabolites[20] or glucocorticoid pathways,[21] our Percellome data support neither the involvement of such pathways or pronounced genotoxicity (which can be monitored indirectly as an enhancement in DNA repair and responses to oxidative stress). Interestingly, DEHP and Wyeth 14,643, well-characterized non-genotoxic rodent hepatocarcinogens that evoke tumors through peroxisome proliferation, gave mutation in Lac Z transgenic mice.[22]

Acknowledgements

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References

1. H. P. Rusch, B. E. Kline and C. A. Baumann, *Cancer Res.*, 1945, **5**, 431.
2. R. K. Boutwell, M. K. Brush and H. P. Rusch, *Cancer Res.*, 1949, **9**, 741.
3. D. Kritchevsky, M. M. Weber and D. M. Klurfeld, *Cancer Res.*, 1984, **44**, 3174.
4. J. Kanno, K. Aisaki, K. Igarashi, N. Nakatsu, A. Ono, Y. Kodama and T. Nagao, *BMC Genomics*, 2006, **7**, 64.
5. K.-I. Aisaki, 2011, in preparation.
6. J. M. Peters, R. C. Cattley and F. J. Gonzalez, *Carcinogenesis*, 1997, **18**, 2029.
7. S. S. Lee, T. Pineau, J. Drago, E. J. Lee, J. W. Owens, D. L. Kroetz, P. M. Fernandez-Salguero, H. Westphal and F. J. Gonzalez, *Mol. Cell. Biol.*, 1995, **15**, 3012.
8. S. Yu, W. Q. Cao, P. Kashireddy, K. Meyer, Y. Jia, D. E. Hughes, Y. Tan, J. Feng, A. V. Yeldandi, M. S. Rao, R. H. Costa, F. J. Gonzalez and J. K. Reddy, *J. Biol. Chem.*, 2001, **276**, 42485.
9. L. Billiet, C. Furman, C. Cuaz-Perolin, R. Paumelle, M. Raymondjean, T. Simmet and M. Rouis, *J. Mol. Biol.*, 2008, **384**, 564.
10. M. Rakhshandehroo, G. Hooiveld, M. Muller and S. Kersten, *PLoS One*, 2009, **4**, e6796.
11. C. B. Brocard, K. K. Boucher, C. Jedeszko, P. K. Kim and P. A. Walton, *Tra c*, 2005, **6**, 386.

12. C. Kural, H. Kim, S. Syed, G. Goshima, V. I. Gelfand and P. R. Selvin, *Science*, 2005, **308**, 1469.
13. D. T. Odom, N. Zizlsperger, D. B. Gordon, G. W. Bell, N. J. Rinaldi, H. L. Murray, T. L. Volkert, J. Schreiber, P. A. Rolfe, D. K. Gifford, E. Fraenkel, G. I. Bell and R. A. Young, *Science*, 2004, **303**, 1378.
14. H. Ren, L. M. Aleksunes, C. Wood, B. Vallanat, M. H. George, C. D. Klaassen and J. C. Corton, *Toxicol. Sci.*, **113**, 45.
15. J. G. Dekeyser, E. M. Laurenzana, E. C. Peterson, T. Chen and C. J. Omiecinski, *Toxicol. Sci.*, **120**, 381.
16. C. Xu, C. Y. Li and A. N. Kong, *Arch. Pharm. Res.*, 2005, **28**, 249.
17. G. A. Francis, E. Fayard, F. Picard and J. Auwerx, *Annu. Rev. Physiol.*, 2003, **65**, 261.
18. M. Lemoine, J. Capeau and L. Serfaty, *PPAR Res.*, 2009, **2009**, 906167.
19. FAO/WHO, 69th joint meeting, *Safety Evaluation of Certain Food Additives*, WHO Food Additives Series, International Programme on Chemical Safety, World Health Organization, Geneva, 2009.
20. Y. Ishii, Y. Suzuki, D. Hibi, M. Jin, K. Fukuhara, T. Umemura and A. Nishikawa, *Chem. Res. Toxicol.*, **24**, 532.
21. V. I. Kaledin, M. Y. Pakharukova, E. N. Pivovarova, K. Y. Kropachev, N. V. Baginskaya, E. D. Vasilieva, S. I. Ilnitskaya, E. V. Nikitenko, V. F. Kobzev and T. I. Merkulova, *Biochemistry (Moscow)*, 2009, **74**, 377.
22. M. E. Boerrigter, *J. Carcinog.*, 2004, **3**, 7.

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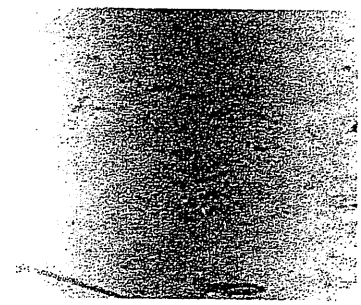
腎細胞癌の分子標的治療



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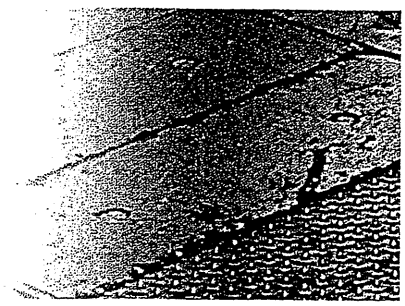

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AYUMI

腎細胞癌の分子標的治療

毒性学

Percellomeトキシコゲノミクスの進捗

Progress in percellome toxicogenomics

Percellomeトキシコゲノミクスプロジェクトとは

2006年に本誌の当欄にて，毒性学の高精度解析手法として開始した“Percellomeトキシコゲノミクスプロジェクト”を紹介させていただいた[1]．当毒性部の基本姿勢は変わらず，さまざまな物質が身体に取り込まれた際に生じる可能性のある毒性(有害性)を予測し，それらの使用に際しての被害を未然に防ぐのが毒性学の役割であるとの考えに立脚し，身のまわりにあり，体のなかに入ってくるすべての“もの”について，どのような場合に(胎児・新生児・小児など，吸い込む・飲み込むなど)，どのくらいの量で，どのような症状が現れるか(急性毒性，発癌を含む慢性毒性，遅発性毒性など)について研究を継続している．

具体的には実験動物の診断所見をヒトに外挿すべく実施しているが，従来法では種差や個体差は“安全係数”により量的な安全マージンをとることで勘案されてきた．しかし，サリドマイド奇形に代表されるように，これには科学的な限界があり，“毒性学の近代化”が必要である．医薬品の場合はヒトで治験を行える場合があるが，それも胎児や新生児には実施困難であり，一般的な物質の毒性を検討することを考えると現状では動物実験は不可避である．そこで，著者らはヒトの身代りとしての実験動物(遺伝子改変動物の活用を含む)を対象とした，Percellomeトキシコゲノミクス研究を開始した次第である．

これは生体というブラックボックスの中身を遺伝子発現ネットワークの面から解明することにより，生体反応メカニズムに基づいた分子毒性学を構築することを目的としている．その際，毒性を見落とさない“網羅性”を確保する必要性から，全遺伝子のトランスクリプトーム情報のなかから生物学的に有意と判断される反応ネットワークを網羅的に抽出するアプローチをとっている．複数の実験から得られる大量のデータを蓄積し横断的な解析を加えることが必須であることから，マイクロアレイデータの標準化と互換性確保のために“細胞1個当りのmRNAコピー数”を得るPercellome法[2]を開発し，プロジェクトを軌道に乗せたところまでを前回の記事でご紹介した．

最近の展開

その後の数年間に，100種類超(医薬品，一般化学物質，食品関連物質を含む)の化学物質によるマウス肝の初期応答データを含む，延べ3.5億遺伝子情報からなるPercellomeデータベースを得た．これは，基本的に投与後の時間，曝露用量，遺伝子発現量の3軸からなる三次元曲面データにより構成される(図1)．解析には，この三次元曲面の特徴抽出という独創的な方法をとり，解析ソフトウェア群(相崎健一ら)は独自開発である．また，動物実験レベルからのシステム管理により，高精細かつ高再現性を実現している．

得られたデータの例としては，アリル炭化水素受容体(AhR)に結合するダイオキシン(2, 3, 7, 8-TCDD)が比較的少数のAhR直下の遺伝子の発現を2時間目に誘導し，4, 8, 24と時間が経過する

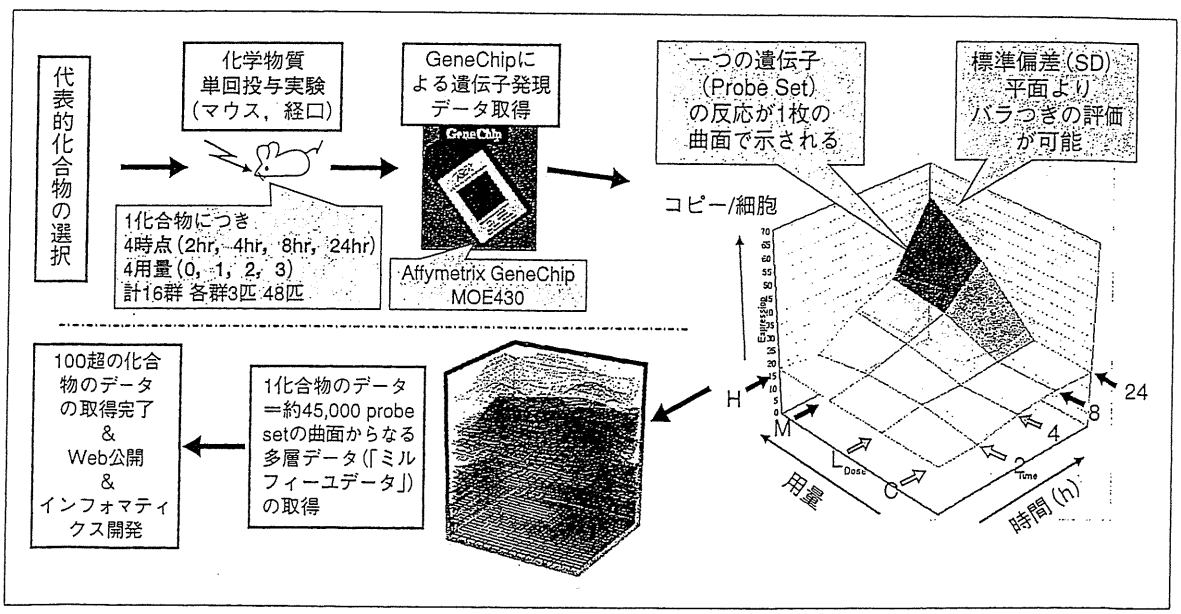

図1　Percellomeデータベースの概要

につれ数を増す状況が確認された．ダイオキシンの体内半減期が25時間であるにもかかわらず，2時間目のみの一過性発現のパターンをとるもの，持続的に発現が増加するものなどが観測されている．シックハウス症候群の指針値程度の，ごく低濃度域での吸入毒性トキシコゲノミクスも実施しており，ごく低濃度のホルマリン(0.1 ppm 付近)で肺の複数の遺伝子発現が明確に誘導されることをみている．サリドマイドは近年，癌治療薬として使用されていることから，複数の臓器における初期誘導を観測したところ，肺の2時間目に用量相関性をもって発現誘導のピークを示す遺伝子に，Cdkn1a (P21)が認められた．類似の発現パターンを示す初期応答遺伝子には，Fas，Foxo3a，Gata2など50あまりがあり，酸化的ストレスが誘発されることが推測された．実際，癌患者にサリドマイドが間質性肺炎を誘発する報告が増加しており，ヒトで確認された形となっている．

また，Percellome トキシコゲノミクスを発生毒性へも適用している．妊娠マウスにサリドマイドを投与し胎児で発現変動が認められた遺伝子のなかに，マウス胚の肢部形成に重要な分子が見出され(その遺伝子をノックアウトしたマウス胚にアザラシ肢症に類似の奇形が生じる)，サリドマイド奇形の標的分子検索の糸口が示唆された．さらに，胎生期～幼若期の発達中の脳に対する神経シグナル撹乱が脳構造や神経回路の形成に影響を及ぼし，成熟後に行動異常などの脳高次機能の障害として顕在化することを見出している．これについては，妊娠マウスへ神経伝達物質類似物質を投与し，生まれたマウスに誘発される遅発性中枢毒性と海馬の遺伝子発現異常の関連解析から標的ネットワークが示唆されつつある．

このほかにも投与した化学物質に関して，いままで報告のないあらたな遺伝子発現変動現象を多数見出し，そのいくつかには特定の毒性との連鎖を示唆する分子生物学的情報がみつかっていることから，それらを順次報告および一般公開する準備を進めている(http://www.nihs.go.jp/tox/TTG_Archive.htm；現在更新中．2010年度中再開予定)．

プロジェクトの今後

さらに，マイクロアレイのクロスハイブリダイゼーションを修正するアルゴリズムの開発を終え(特許出願準備中)，その実装準備中である(NTTデータおよび日本テラデータとの委託共同研究)．また，遺伝子ネットワークと毒性の動的な因果関係を導き出すインフォマティクスの構築研究やPercellomeデータの統合的提示方法の開発にも本格的に取り組んでおり(ソニーコンピュータサイエンス研究所との共同研究)，段階的に皆様にご披露できる予定である(厚生労働科学研究費補助金，環境研究総合推進費などによる)．

1) 菅野　純：毒性の高精細解析に向けてのトキシコゲノミクス．医学のあゆみ，218：1035-1036，2006．
2) Kanno, J. et al.：“Per cell” normalization method for mRNA measurement by quantitative PCR and microarrays. *BMC Genomics*, 7：64, 2006.

菅野　純／Jun KANNO
国立医薬品食品衛生研究所
安全性生物試験研究センター毒性部

循環器内科学

心筋トロポニンの高感度測定の有用性

Clinical utility of high-sensitivity cardiac troponin assay

従来の心筋トロポニン測定は検出感度が低いため，急性冠症候群の診療以外で用いられることはまれであった．最近，検出感度が5倍以上改善された高感度測定が臨床の場に登場した．この高感度測定は，従来測定では検出不可能であった小さな心筋障害を診断できる．そのため，超急性期の心筋梗塞診断の精度[1,2]や慢性心不全における予後予測の精度[3]を高めることが示されている．さらに，外来診療や検診・人間ドック分野へのあらたな展開も期待される．

急性冠症候群の診療

トロポニンが上昇している不安定狭心症は，突然死や急性心筋梗塞発症の危険度が高い．このトロポニンの上昇は，破砕したプラークや血栓が引き起した末梢の微小塞栓による微小心筋障害を反映している．そのため，2000年に公表されたヨーロッパ心臓病学会/アメリカ心臓病学会(ESC/ACC)の心筋梗塞の再定義[4]は，トロポニンが上昇している不安定狭心症を急性心筋梗塞に包括した．さらに，ヨーロッパ心臓病学会/アメリカ心臓病学会/アメリカ心臓協会/世界心臓協会(ESC/ACC/AHA/WHF)の共同タスクフォースは，2007年に急性心筋梗塞の診断基準の再改定[5]を公表した．新しい診断基準では，トロポニンの心筋梗塞診断における基準値を健常人の99thパーセンタイル値より大と定めた．一般に，測定値の相対的なばらつき(変動係数．coefficient of variation：CV)が小さいほど測定値の精度は高い．共同タスクフォースは試薬の精度にも言及しており，健常人の99thパーセンタイル値における変動係数が10%以下である試薬を用いることを推奨した．従来の試薬はこの条件を

SYSTEMS BIOLOGY AND SYNTHETIC BIOLOGY

Edited by

Pengcheng Fu

China University of Petroleum Beijing, China

Sven Panke

ETH Zurich, Institute of Process Engineering Zurich, Switzerland

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FOREWORD

The popular use of the term "systems biology" arose following the appearance of the first full genome sequences. These genome sequences suggested that we would have a full delineation of the molecular components of an organism. Expression profiling and proteomic data then could tell us when these components were actually used in a context-specific manner.

The need to track the interrelationship of all such components created the need to develop networks of the interactions of such components. Protein–protein interaction maps are one manifestation of this need, stoichiometric models are another; they are, however, amenable to rigorous mathematical analysis and prospective uses. Network reconstruction took center stage in systems biology, as networks describe the interactions between the gene products and the chemical compounds they make, provide context for high-throughput data mapping, and give the basis for mechanistic models that can compute phenotypic functions.

Having molecular manipulation tools and mathematical models in turn provides tools that allow the synthesis of biological components and biological functions. We thus witnessed the emergence of "synthetic biology." It is practiced on multiple scales, from component design, that is akin to classical molecular biology, to design of whole cell functions, such as metabolic engineering.

Thus, in retrospect we can state that genomics gave rise to systems, and systems biology in turn gave rise to synthetic biology. This of course is a simplified view, but provides a first-order approximation to the historical origin and appearance of these popularly used terms. This volume contains a series of chapters that highlight the development and status of the various aspects of systems and synthetic biology.

BERNHARD Ø. PALSSON

University of California, San Diego, CA

16

THE THEORY OF BIOLOGICAL ROBUSTNESS AND ITS IMPLICATION TO CANCER

Hiroaki Kitano

Sony Computer Science Laboratories, Inc., 3-14-13 Higashi-Gotanda, Shinagawa, Tokyo 141-0022, Japan

The Systems Biology Institute, 6-31-15 Jingumae, Shibuya, Tokyo 150-0001, Japan

Department of Cancer Systems Biology, Cancer Institute of Japanese Foundation for Cancer Research, Tokyo, Japan

16.1 INTRODUCTION

Systems biology aims at system-level understanding of biological systems [1,2]. Investigations of biological systems at system level are not a new concept and can be traced back to homeostasis by Walter Cannon, Cybernetics by Norbert Weiner [3], and the general systems theory by von Beltaranffy [4]. Numbers of approaches in physiology have also taken a systemic view of the biological subjects. Systems biology is gaining renewed interest today because of progress in genomics, molecular biology, nonlinear dynamics, computational science, and other related fields.

However, "system-level understanding" is a rather vague notion and is often hard to define. This is due to the fact that the system is not a tangible object. Genes and

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proteins are more tangible because they are identifiable matters. Although the system is composed of these matters and they are components of the system, the system itself cannot be made tangible. Often, diagrams of the gene regulatory networks and the protein interaction networks are shown as representations of systems. It is certainly true that such diagrams capture any one aspect of the structures of the system, but they are still only static slices of the system. The heart of the system lies within the dynamics it creates and the logic behind it. It is science on the dynamical state of affairs.

There are four distinct phases that lead us to system-level understanding at various levels. First, system structure identification enables us to understand the structure of the system. While this may be only a static view of the system, it is an essential first step. The structure shall then be identified, ultimately, in both physical and interaction structures. Interaction structures are represented as gene regulatory networks and biochemical networks that indicate how components interact within and in between cells. Physical details of specific regions of the cell, overall structure of cells, and organisms are also important because such physical structure imposes constraints on possible interactions and the outcome of interactions impacts the formation of physical structures. Nature of interaction could be different if proteins involved in interaction move by simple diffusion or under specific guidance from cytoskeleton.

Second, system dynamics needs to be understood. Understanding the dynamics of the system is an essential aspect of the study in systems biology. This requires integrative efforts of experiments, measurement technology development, computational model development, and theoretical analysis. Various methods, such as bifurcation analysis, have been used, but further investigations are necessary to handle the dynamics of systems with very high dimensional space.

Third, methods to control the system shall be investigated. One of the implications is to find a therapeutic approach based on system-level understanding. Many drugs have been developed through extensive effect-oriented screening. It is only recently that specific molecular targets have been identified and lead compounds are designed accordingly. Success in control methods of cellular dynamics may enable us to exploit intrinsic dynamics of the cell so that its effects can be precisely predicted and controlled.

Finally, designing the system that is to modify and construct biological system with designed features. Bacteria and yeast may be redesigned to yield desired properties for drug production and alcohol production. Artificially created gene regulatory logic could be introduced and linked to innate genetic circuits to attain desired functions [5].

Several different approaches can be taken within systems biology field. One may decide to carry out a large-scale, high-throughput experiment and try to find out the overall picture of the system at coarse-grain resolution [6–9]. Alternatively, working on precise details of specific signal transduction [10,11], cell cycle [12,13], and other biological issues to find out the logic behind them are a viable research approach. Both approaches are essentially complementary, and, together, can reshape our understanding of biological systems.

16.2 ROBUSTNESS IS THE FUNDAMENTAL ORGANIZATIONAL PRINCIPLE OF BIOLOGICAL SYSTEMS

Robustness is a property of the system that maintains a certain function despite external and internal perturbations that are ubiquitously observed in various aspects of biological systems [14]. It is distinctively a system-level property that cannot be observed by just looking at components. Specific aspects of the system, the functions to be maintained, and the types of perturbations that the system is robust against must be well defined to make solid arguments. For example, a modern airplane (system) has a function to maintain its flight path (function) against atmospheric turbulences (perturbations).

Bacteria chemotaxis is one of the most well-documented examples in which chemotaxis is a function maintained against the perturbations that are changes in ligand concentration and rate constants for the interactions involved [15–17]. The network for segmental polarity formation during *Drosophila* embryogenesis robustly produces repetitive stripes of differential gene expressions despite variations in initial concentration of substances involved, as well as kinetic parameters of interactions [18,19]. Various aspects of robustness of biological systems have been studied extensively, but more remains to be explored and formalized to create solid theoretical foundations.

Why is robustness so important? First of all, it is a feature that is observed to be so ubiquitous in biological systems; from such a fundamental process like phage fate decision switch [20] and bacteria chemotaxis [15–17] to developmental plasticity [18] and tumor resistance against therapies [21,22]. This implies that it may be a basis for principles that are universal in biological systems, as well as being opportunistic toward finding cures for cancer and other complicated diseases.

Second, robustness is a system-level property of the system in which interactions of components give rise to this feature. Robustness in this context refers to a feature of the system to maintain its function instead of structures or specific states. Structures or states can be dynamically changed if they lead to maintenance of the function of the system.

Third, robustness against environmental and genetic perturbation is essential for evolvability [23–25]. Evolvability requires generation of variety of nonlethal phenotype and genetic buffering [26,27]. Mechanisms that attain robustness against environmental perturbation may be used also for attaining robustness against mutations, developmental stability, and other features that facilitate evolvability [14,23–25].

Fourth, it is one of the features that distinguish biological systems and man-made engineering systems. Although some man-made systems, such as airplanes, are designed to be robust against the range of perturbations, most man-made systems are not as robust as biological systems. Some engineering systems that are designed to be highly robust entail mechanisms that are also present in life forms, which imply existence of the universal principle.

16.3 UNDERLYING MECHANISMS FOR ROBUSTNESS

16.3.1 System Control

First, extensive systems control is used, mostly saliently negative feedback loops but also feedforward and positive feedback controls, to make a system dynamically stable around the specific state of the system. An integral feedback is used in bacteria with chemotaxis as a typical example [15–17]. Due to integral feedback, bacteria can sense changes of chemoattractant and chemorepellant independent of absolute concentration so that proper chemotaxis behavior is maintained over a wide range of ligand concentration. In addition, the same mechanism makes it insensitive to changes in rate constants involved in the circuit. Positive feedbacks are often used to create bistability in signal transduction and cell cycle, so that the system is tolerant to minor perturbation in the stimuli [10,12,13].

16.3.2 Fault Tolerance (Redundancy and Diversity)

Second, fault tolerance mechanisms increase tolerance against components failure and environmental changes by providing alternative components or methods to ultimately maintain a function of the system. Sometimes there are multiple components that are similar to each other and are redundant. Other cases are different means that they are used to cope with perturbations that cannot be handled by the other means. This is often called phenotypic plasticity [28,29] or diversity. Redundancy and phenotypic plasticity are often considered as opposite things, but it is more consistent to view them as different ways to meet an alternative fail-safe mechanism.

16.3.3 Modularity

Third, modularity provides isolation of perturbation from the rest of the system. The cell is the most significant example. More subtle and less obvious examples are modules of biochemical and gene regulatory networks. Modules also play an important role during developmental processes that buffer perturbations so that proper pattern formation can be accomplished [18,30,31]. The definition of the module and the methods of how to detect such modules are still controversial, but the general consensus is that the module does exist and play an important role [32].

16.3.4 Decoupling (Buffering)

Fourth, decoupling isolates low-level noise and fluctuations from functional-level structures and dynamics. One example here is genetic buffering by Hsp90 in which misfolding of proteins due to environmental stresses is fixed, and thus effects of such perturbations are isolated from the functions of the circuits. This mechanism also applies to genetic variations where genetic changes in coding region that may affect protein structures are masked because protein folding is fixed by Hsp90, unless such masking is removed by extreme stress [24,33,34]. Emergent behaviors of complex

networks also exhibit such buffering properties [35]. These effects may constitute canalization proposed by Waddington [36]. A recent discovery by Uri Alon's group on oscillatory expression of p53 upon DNA damage may exemplify decoupling at signal-encoding level [37], because stimuli invoked pulses of p53 activation level, instead of gradual changes, effectively converting analogue into digital signal. Digital pulse encoding may indicate robust information transmission, although further investigations are clearly warranted to draw any conclusion at this moment.

An example of a sophisticated engineering system clearly illustrates how these mechanisms work as a whole system. An airplane is supposed to maintain a flight path following the command of the pilot against atmospheric perturbations and various internal perturbations, including changes in the center of gravity due to fuel consumption and movement of passengers, as well as mechanical inaccuracies. This function is carried out by controlling flight control surfaces (rudder, flaps, elevators, etc.) and a propulsion system (engines) by an automatic flight control system (AFCS). Extensive negative feedback control is used to correct deviations of flight path. The reliability of the AFCS is critically important for stable flight. To increase reliability, the AFCS is composed of three independently implemented modules (a triple redundancy system) all of which meet the same functional specification. Most parts of the AFCS are digitalized, so that low-level noise of voltage fluctuations is effectively decoupled from digital signals that define the function of the system. Due to these mechanisms, modern airplanes are highly robust against various perturbations.

16.4 INTRINSIC FEATURES OF ROBUST SYSTEMS: EVOLVABILITY AND TRADE-OFFS

For the system to be evolvable, it must be able to produce variety of nonlethal phenotypes [27]. At the same time, genetic variations need to be accumulated as a neutral network so that pools of genetic variants are exposed when the environment suddenly changes. Systems that are robust against environmental perturbations entail mechanisms such as system control, alternative, modularity, and decoupling that also support, by congruence, generation of nonlethal phenotype and genetic buffering. In addition, the capability to generate flexible phenotype and robustness requires the emergence of the bow tie structure as an architectural motif [38]. One of the reasons why robustness in biological systems is so ubiquitous is that it facilitates evolution, and evolution tends to select traits that are robust against environmental perturbations. This leads to successive addition of system controls.

Systems that acquire robustness against certain perturbations through design or evolution have intrinsic trade-offs between robustness, fragility, performance, and resource demands. Carlson and Doyle argued, using simple examples from physics and forest fire, that systems that are optimized for specific perturbations are extremely fragile against unexpected perturbations [39,40]. A system that has been designed, or evolved, optimally (either globally optimal or suboptimal) against certain perturbations is called a high optimized tolerance (HOT) system. Ceste and Doyle further

argued that robustness is a conserved quantity [41]. This means when robustness is enhanced against a range of perturbations, it must then be paid off by fragility elsewhere as well as compromised performance and increased resource demands.

Robust-yet-fragile trade-offs can be understood intuitively using the airplane example yet again. When comparing modern commercial airplanes with the Wright Flyer, modern commercial airplanes are, by a great magnitude, more robust against atmospheric perturbations than the Wright flyer, and are thus attributed to a sophisticated flight control system. However, such a flight control system fully relies on electricity. In a very unthinkable event of total power failure in which all electricity is lost in the airplane, the airplane cannot be controlled at all. Obviously, airplane manufacturers are well aware of this issue and take all possible counter measures to minimize such a risk. On the other hand, despite its vulnerability against atmospheric perturbations, the Wright flyer will never be affected by the power failure because there is no reliance on electricity. This extreme example illustrates that systems that are optimized for certain perturbations could be extremely fragile against unusual perturbations.

HOT model systems are successively optimized/designed (not necessarily globally optimized, though) against perturbations in contrast to self-organized criticality (SOC) [42] or scale-free networks [43] that are unconstrained stochastic additions of components without design or optimization involved. Such differences actually affect failure patterns of the system, and thus have direct implications on understanding the nature of disease and therapy design.

Unlike scale-free networks, HOT systems are robust against perturbations like removal of hubs as far as systems are optimized against such perturbations. However, systems are generally fragile against "Fail-on" type failure in which components failure results in continuous malfunction, instead of cease to function "Fail-off," so that incorrect signals are kept transmitted. This type of failure is known in the engineering field as the Byzantine Generals Problem [44], named after the problem in the Byzantine army composed of numbers of generals dispersed in the field, some of them traitors who sent incorrect messages to confuse the army.

Disease often reflects the systemic failure of the system triggered by the fragility of the system. Diabetes mellitus is an excellent example of how systems that are optimized for near-starving, intermittent food supply, high energy utilization lifestyle, and highly infectious conditions are fragile against unusual perturbations such as high energy containing foods, and a low energy utilization lifestyle [45]. Due to optimization toward a near-starving condition, the extensive control to maintain a minimum blood glucose level is acquired so that activities of central neural systems and innate immunity are maintained. However, no effective regulatory loop has been developed against excessive energy intake and feedback regulations work to reduce glucose uptake by adipocyte and skeletal muscle cells because it may reduce plasma glucose level below the acceptable level. These mechanisms lead to a state where blood glucose level is chronically maintained higher than the desired level, from the longer time scale that has not been optimized for, further leading to cardiovascular complications. Similar observations have been made for autoimmune disorders where the

evolution of robust immunity also entails proinflammatory and hyperactive immune system [46].

16.5 SELF-EXTENDING SYMBIOSIS

So far, robustness and its relationship with evolution have been argued within the framework of Mendel's genetics in a sense that mutation and crossover through mating has been considered as a mechanism for evolutionary innovations. Emergence of specific mechanisms for increasing robustness and enrichment of bow tie structure has been discussed within this paradigm. I have previously proposed that there may be other means of enhancing robustness through evolution, but by extending "self" with foreign biologic substances, a notation that I termed "self-extending symbiosis" [47]. Self-extending symbiosis is a phenomenon where evolvable robust systems continue to extend their system boundary by incorporating foreign biologic forms (genes, microorganisms, etc.) to enhance their adaptive capability against environmental perturbations, hence improving their survivability and reproduction potential. In other words, robust evolvable systems have consistently extended themselves by incorporating nonself into tightly coupled symbiotic states.

Looking at the history of evolutionary innovations, it has become clear that some of the major innovations are the result of acquisition of "nonself" into "self" at various levels. Horizontal gene transfer (HGT) facilitates evolution by exchanging genes of different species that have evolved for different optimization contexts, and was shown to be a frequently observed phenomenon in prokaryotes, archea, and unicellular eukaryotes [48,49]. Microorganisms acquire novel functions, mostly to enhance their robustness against environmental challenges, through horizontal exchange of genes. For example, it has been argued that global emergence of antibiotic- resistant bacteria may be caused by horizontal transfer of antibiotic genes [50–52]. In metazoan species, HGT has not been reported (at best, reported highly controversially) except in some rare instances on insect–bacteria symbiosis between the adzuki bean beetle *Callosobruchus chinensis* and *Wolachia* [53].

The serial endosymbiosis theory by Lynn Margulis [54,55] argues that eukaryotic cells have been created by acquiring bacteria as their organelles. This resulted in greater functionalities of eukaryotic cells, hence more robust against environmental challenges. Here, symbiosis resulted in incorporation of foreign biologic entity into cytoplasm as well as into its own genome.

While HGT and endosymbiosis resulted in incorporation of foreign biologic entity into genome and cellular structure, there are forms of symbiosis that do not directly alter genome but essential to the survival of the species. There are species that allow certain bacteria to be vertically inherited through the host's oocytes as observed in sponges, clams [56], and aphids [57]. Aphids, for example, are infected with the genus *Buchnera*, resulting in an endosymbiotic relationship and acquired dramatically improved energy utilization and terrain exploration capability. It was shown that aphids and *buchnera* undergo parallel evolution where the phylogeny trees of the host (aphids) and symbionts (genus *Buchnera*) are consistent [57]. A case

of parallel evolution has also been observed in endosymbiosis of *Psyllid* and *Candidatus* [58].

Apart from such tight coupling of host and symbiont, horizontal (environmental) acquisition of symbionts [59] is yet another approach in extending the self by incorporating a broader range of microbes, thereby allowing the host to be able to adapt to a broader range of environments and nutrients. Commensal bacterial flora are ubiquitously observed in various metazoan species, including termites [60], cockroaches [61], prawns [62], and mammalians, and have established inseparable relationships with the host organisms, and are even considered to have coevolved [63]. In human beings, the commensal bacterial flora in the gut consists of diverse microorganisms up to 500–1000 species, amounting to about 10^{14} bacteria weighing a total of 1.5 kg [64]. The human being as a symbiotic system consists of approximately 90 percent prokaryotes and 10 percent eukaryotes [65], and a random shotgun sequencing of the whole human symbiotic system would result in predominantly bacterial genome readouts of about 2 million genes with sporadic mammalian genes [66]. Such commensal intestinal bacteria play a critical role in various aspects of the host physiology. Mammalian bacterial flora has been considered to constitute an integral part of host protection by mutually beneficial symbiosis with the host immune system.

The line of observations point to the characteristic property of biological systems that the greater levels of robustness and functionalities is gained by incorporating foreign biologic entities into their own system in the form of different degree of symbiosis. HGT and endosymbiosis incorporate foreign entities into genome and cellular structures, where vertical inheritance based endosymbiosis do not directly alter the genome. Bacterial flora simply adds a layer of adaptive system that is symbiotically interacting with mucosal immune system of the host. A general tendency observed here is the continuous addition of external layers by symbiotic incorporation of foreign entities, and increased level of robustness against environmental perturbation is gained in this process.

16.6 CANCER AS A ROBUST SYSTEM

Cancer is a heterogeneous and highly robust disease that represents worse case scenario of system failure; a fail-on fault where malfunction components are protected by mechanisms that support robustness in normal physiology [21,22]. It is a robustness hijack. Survival and proliferation capability of tumor cells are robustly maintained against a range of therapies due to intratumoral genetic diversity, feedback loops for multidrug resistance, tumor–host interactions, and so on.

Intratumoral genetic heterogeneity is a major source of robustness in cancer cells. Chromosome instability facilitates generation of intratumoral genetic heterogeneity through gene amplification, chromosomal translocation, point mutations, aneuploidy, and so on [67–70]. Intratumoral genetic heterogeneity is one of the most important features of cancer that provides alternative, or fail-safe mechanisms for tumor to survive and grow again despite various therapies, because some tumor cells may have