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OF CARDIOVASCULAR ILLNESS AND DIVERSITY OF BIOLOGICAL RESPONSE

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Summary

Noise in gene expression (stochastic variation in the composition of the transcriptome in response to stimuli) may play an important role in maintaining robustness and flexibility, which ensure the stability of normal physiology and provide adaptability to environmental changes for the living system. Broad-based technologies have allowed us to study with unprecedented accuracy the molecular profiles of various states of health and cardiovascular disease. In doing so, we have observed a correlation between the degree of variation in gene expression and the state of health. Specifically, the stochastic variation in gene expression in response to environmental and physiological factors is found in healthy mice, and tends to disappear in mice with advanced disease states. Although further evidence is needed to draw a solid conclusion with respect to the significance of decreased transcriptional noise in the disease state as a whole, it is tantalizing to introduce the concept that stochasticity may be linked to the organism's adaptability to a changing environment, and the "quiet" states of gene expression may indicate the loss of diversity in the organism's response.

Noise in Gene Expression—A Requisite for Health?

Over the past couple of years, substantial progress has been made in the identification of genetic sources underlying expression differences between individual cells and organisms (Raser et al. 2005). The differences (noise) in gene expression refer to the stochastic variation in gene expression among cells and organisms within a homogeneous population in common environment. The potential sources for such a variation include: 1) inherent stochasticity of biochemical processes involving infrequent molecular events concerning small numbers of molecules; 2) differences in the internal states of a population of cells and organisms, such as chromatin modification (epigenetics); 3) subtle environment differences, such as morphogen gradients in multicellular development (such as regulatory small RNA molecules); 4) ongoing genetic mutation; and 5) topology of gene regulatory networks, such as presence and absence of functional feedback loops. Although fluctuations in gene expression as a result of intrinsic noise could be detrimental to cellular and organismal physiology, they have been found to be beneficial in many circumstances. Indeed, noise has been shown to provide a mechanism for single cell memory, facilitate differentiation and confer fitness advantage Acar et al. 2005a, Acar 2008b, Sigal et al. 2006, Kaufmann et al. 2007). For example, the extraordinary

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phenomenon that each murine olfactory neuron only expresses a single allele of one out of the ~1500 odorant receptor genes is thought to be due to a brief period of intrinsic noise that stochastically activates a single odorant promoter followed by inhibitory signaling to inactivate other receptor promoters within a cell. This allows for stochastic choice in stable monoallelic expression. The resulting heterogeneous population of olfactory neurons enables sensitive differentiation of odorant molecules; the stochastic nature of gene expression thus creates a functional sense of smell (Chess et al. 1994, Serizawa et al. 2004). Microorganisms are known to be exquisitely sensitive to environmental conditions, and depend on their ability to mutate or to undergo phenotypic switch for survival. For example, the phenotype of the soil bacterium *Bacillus subtilis* varies from dormant (vegetative) to competent states. Under certain conditions, only some bacteria exit a vegetative state and become competent (able to uptake DNA from the soil). The phenotypic switch is determined by the “off” or “on” expression state (bistable gene expression) of ComK, the master regulator of competence. ComK also spurs its own expression by acting as a transcription factor. This autoregulatory positive-feedback loop enables cells to stably reside at either a low or a high level of ComK expression, corresponding to vegetative and competent states respectively. Remarkably, the noise in ComK expression mainly comes from random production and/or degradation of comK mRNA, rather than from external factors (Maamar et al. 2007). That is, the naturally occurring noise in gene expression can increase population diversity and enhance survival in the face of environmental uncertainty. Recently, Acar et al. (Acar et al. 2008) have demonstrated that not only noise-induced stochastic transition among multiple phenotypes is crucial in determining the adaptability of cells to environmental fluctuations, but also the inter-phenotype switching rate will have to be tuned to the frequency of environmental changes to gain maximum fitness advantage. Hence, cell-to-cell variability in resonance with environmental changes plays an important role in the organism’s survival and fitness (Mettetal et al. 2007).

Gene Expression and the State of Cardiovascular Health

We have performed microarray analysis to study the molecular signature of several conditions (Karra et al. 2005, Nevins et al. 2003, Seo et al. 2004), including atherosclerosis (Karra et al. 2005, Seo et al. 2004). Mice on C57BL/6 background were used to reconstitute the human disease. Since atherosclerosis and its sequelae represent sensitive and vital responses to genetic and environmental risk factors and are often rate limiting for longevity in humans, this model is highly relevant to the study of human arterial degeneration (Benetos et al. 2002; Gardin et al. 2006). We specifically investigated the impact of four variables—age, gender, dietary fat intake and the apolipoprotein E (apoE) gene, a key genetic factor in atherogenesis—on arterial gene expression. ApoE is an important regulatory gene for fat metabolism and dietary molecular transport. The ApoE protein is a component of lipid/protein particles that participates in the transport of triglycerides and in cholesterol distribution among cells (Mahley 1988). ApoE deficiency is a major genetic risk factor for atherosclerosis, causing elevated serum cholesterol and triglyceride levels and leading to premature and advanced arterial disease (Plump et al. 1992). The apoE knockout (apoE^{-/-}) mouse model closely resembles human atherosclerosis, both in the spontaneous appearance and in the distribution of lesions in the arterial wall. Remarkably, wild-type (WT) mice of C57BL/6 and many other strain backgrounds possess intrinsic resistance to atherosclerosis.

Analysis of the large amount of data obtained from aortas of healthy and atherosclerotic mice required a “shrinkage approach”, which uses “point-mass mixture priors”, applying standard regression variable selection methodologies to problems of large-scale comparisons, with “sparsity modeling” of the expression data (Karra et al. 2005; Nevins et al. 2003; Seo et al. 2004). Such an analysis generated robust expression patterns, characterized by metagene (groupings of genes that share common changes in expression) signatures, for each specific stage of the atherosclerotic process (Karra et al. 2005). The derived metagenes provided not

only prognostic values for advancing disease state, but also mechanistic clues for the disease pathogenesis. Many genes encompassed in the metagenes have known functions in the development and progression of atherosclerosis, particularly as it relates to metabolic pathways, inflammatory responses and remodeling activities, characteristic of atherosclerosis (Karra et al. 2005; Nevins et al. 2003; Seo et al. 2004). Some genes, such as galectin 3—a member of the β -galactoside-binding gene family¹³, had not been directly implicated in atherosclerosis at the time of our report. Remarkably, a recent report confirmed the role of galectin 3 in atherosclerosis (Pricci et al. 2000). In arterial tissues, galectin 3 appears to play a protective role against the damage caused by advanced glycosylation end-products (AGE) (Iacobini et al. 2003). In galectin 3 knock-out animal models there is a substantial increase in damage from AGE deposition relative to control animals (Iacobini et al. 2003a; Iacobini et al. 2005b; Pricci et al. 2000). The activity of AGE-proteins is implicated in diabetes-associated tissue damage and also age-related vascular damage. This is just one example of a larger set of genes, discovered by our metagene approach, for which there are clear and defensible biological mechanisms implicating their involvement in atherogenesis. Yet, traditional studies were unable to unravel their link to atherosclerosis. This underscores the importance of gene expression profiling in gene discovery. It also supports the validity of our data.

Unpredictability of Gene Expression Pattern in Healthy Aortas

Instructively, we were unable to establish a discernable pattern in the gene expression profile of healthy mouse aortas, owing to intra-group variation in gene expression in aortas obtained from mice of the same gender, age, diet, and genetic background and housed in the same environment (Seo et al. 2004). Such inherent variation among individual animals within the same group was so substantial that it masked any organized gene expression changes in response to environmental factors and physiological determinants (inter-group changes). As a result, we were unable to identify gene expression patterns that differentiate the WT mice among different groups (different diet, age, and gender) (Seo et al. 2004). These data indicate that healthy aortas from isogenic mice exposed to homogeneous environment are associated with substantial variation (noise) in gene expression.

Aortas develop progressive atherosclerosis and age-related degeneration of the arterial tree in mice that lack the apoE gene. This process is further accelerated by high cholesterol diet and is associated with profound gene expression changes. Indeed, analysis of gene expression profiles allowed us to easily and with exquisite accuracy differentiate aortas between normal and diseased animals (Figure 1). Furthermore, these atherosclerotic aortas showed a surprising lack of variation in gene expression in individual mice within the same group. Even in the presence of modifiers (gender, diet, age), gene expression became markedly monotonous and lacked variability (Figure 2). Hence, stochastic differences (noise) in gene expression among different mice within the homogeneous population is lost regardless whether the environment is the same or not, once these mice develop a chronic illness such as atherosclerosis.

Loss of Variability as a Marker of Disease State

Recent studies have demonstrated phenotypic heterogeneity in genetically identical cells, human identical twins, and cloned cats (Raser et al. 2005). Interestingly, the stochastic variation or noise in gene expression underlies, at least in part, such phenotypic diversity, which affords cells/organisms within a population more choices and enhanced fitness to better survive environmental changes. This is consistent with the Chinese saying “do not cling to one tree branch for survival”. In the disease state, as we observed in atherosclerosis, the loss of noise in gene expression may decrease phenotypic diversity and render the organisms frailer, unable to respond to stress and environmental changes. Remarkably, there is a typical loss of degrees of freedom in the response of the tissue/organism to environmental changes in advanced disease

states. For example, the autonomic control of heart rate for a normal individual is exquisitely responsive to a variety of external stimuli (exercise, emotion, sleep). The variability, however, often disappears when the heart sustains substantial damage (La Rovere et al. 2008).

Indeed, the rate of contraction is rather constant and unaffected by environmental and other factors in advanced heart failure. Such a loss of variability in the heart rate may predispose the heart to rhythm complications leading to cardiac arrest (La Rovere et al. 2008). As another example, the range of water and electrolyte excretion is markedly narrowed in patients with advanced renal failure. Much work still remains to be done to understand why the gene expression profile of the atherosclerotic aortas in apoE^{-/-} mice becomes highly predictable and uninfluenced by factors that normally induce substantial gene expression variation in normal, healthy C57BL/6 mice with a competent apoE gene, and whether the loss of noise in gene expression is generalizable to other disease conditions. Indeed, Bahar and colleagues (Bahar et al. 2006) have shown increased cell-to-cell variation in gene expression in aging mouse heart. It is tempting to ponder that such a lack of variability in gene expression underlies the loss of degrees of freedom in phenotypic diversity typically seen in advanced chronic diseases. Characterization of the association will undoubtedly deepen our understanding of disease pathogenesis and provide clues for developing novel therapeutic strategies. Further work is also needed to understand why the variability in gene expression is increased in the heart with aging.

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Reference List

- Acar M, Becskei A, van Oudenaarden A. Enhancement of cellular memory by reducing stochastic transitions. *Nature* 2005;435:228–232. [PubMed: 15889097]
- Acar M, Mettetal JT, van Oudenaarden A. Stochastic switching as a survival strategy in fluctuating environments. *Nat. Genet* 2008;40:471–475. [PubMed: 18362885]
- Bahar R, Hartmann CH, Rodriguez KA, et al. Increased cell-to-cell variation in gene expression in ageing mouse heart. *Nature* 2006;441:1011–1014. [PubMed: 16791200]
- Benetos A, Waeber B, Izzo J, et al. Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: clinical applications. *Am. J. Hypertens* 2002;15:1101–1108. [PubMed: 12460708]
- Chess A, Simon I, Cedar H, Axel R. Allelic inactivation regulates olfactory receptor gene expression. *Cell* 1994;78:823–834. [PubMed: 8087849]
- Gardin JM, Arnold AM, Polak J, Jackson S, Smith V, Gottdiener J. Usefulness of aortic root dimension in persons > or = 65 years of age in predicting heart failure, stroke, cardiovascular mortality, all-cause mortality and acute myocardial infarction (from the Cardiovascular Health Study). *Am. J. Cardiol* 2006;97:270–275. [PubMed: 16442377]
- Iacobini C, Amadio L, Oddi G, et al. Role of galectin-3 in diabetic nephropathy. *J. Am. Soc. Nephrol* 2003;14:S264–S270. [PubMed: 12874444]
- Iacobini C, Oddi G, Menini S, et al. Development of age-dependent glomerular lesions in galectin-3/AGE-receptor-3 knockout mice. *Am. J. Physiol. Renal Physiol* 2005;289:F611–F621. [PubMed: 15870382]
- Karra R, Vemullapalli S, Dong C, et al. Molecular evidence for arterial repair in atherosclerosis. *Proc. Natl. Acad. Sci. U. S. A* 2005;102:16789–16794. [PubMed: 16275914]
- Kaufmann BB, Yang Q, Mettetal JT, van Oudenaarden A. Heritable stochastic switching revealed by single-cell genealogy. *PLoS Biol* 2007;5:e239. [PubMed: 17803359]
- La Rovere MT, Pinna GD, Raczak G. Baroreflex sensitivity: measurement and clinical implications. *Ann. Noninvasive Electrocardiol* 2008;13:191–207. [PubMed: 18426445]

- Maamar H, Raj A, Dubnau D. Noise in gene expression determines cell fate in *Bacillus subtilis*. *Science* 2007;317:526–529. [PubMed: 17569828]
- Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988;240:622–630. [PubMed: 3283935]
- Mettetal JT, van Oudenaarden A. Microbiology. Necessary noise. *Science* 2007;317:463–464. [PubMed: 17656711]
- Nevins JR, Huang ES, Dressman H, Pittman J, Huang AT, West M. Towards integrated clinico-genomic models for personalized medicine: combining gene expression signatures and clinical factors in breast cancer outcomes prediction. *Hum. Mol. Genet* 2003;12 Spec No 2:R153–R157. [PubMed: 12928487]
- Plump AS, Smith JD, Hayek T, et al. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell* 1992;71:343–353. [PubMed: 1423598]
- Pricci F, Leto G, Amadio L, et al. Role of galectin-3 as a receptor for advanced glycosylation end products. *Kidney Int. Suppl* 2000;77:S31–S39. [PubMed: 10997688]
- Raser JM, O'Shea EK. Noise in gene expression: origins, consequences, and control. *Science* 2005;309:2010–2013. [PubMed: 16179466]
- Seo D, Wang T, Dressman H, et al. Gene expression phenotypes of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol* 2004;24:1922–1927. [PubMed: 15297278]
- Serizawa S, Miyamichi K, Sakano H. One neuron-one receptor rule in the mouse olfactory system. *Trends Genet* 2004;20:648–653. [PubMed: 15522461]
- Sigal A, Milo R, Cohen A, et al. Variability and memory of protein levels in human cells. *Nature* 2006;444:643–646. [PubMed: 17122776]

Figure 1.

Gene expression predictors of aortas from apoE^{-/-} and WT mice of different ages. Rows depict standardized expression of genes across samples (columns). Blue represents relatively lower expression compared with red (higher expression).

Figure 2. Color map illustrating the composite genome wide gene expression variability of the aortas from wild type and apoE knockout mice. With increasing disease in the apoE mice, there is a decrease in gene expression variability.