Discovery and assessment of new target sites for anti-HIV therapies: an approach to utilize genome wide gene expression changes and computational models.

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Introduction.

Animal viruses as model systems to study activation of gene networks.

Viruses that infect mammalian cells have served as a good model system to study the regulation of gene expression. This is because the virus uses the host cell machinery to carry out its own processes therefore providing us with an opportunity to observe regulatory mechanisms operating in animal cells.

Human immunodeficiency virus type 1(HIV-1) is the etiological agent of AIDS. HIV-I is a member of the lentivirus subfamily of retrovirus. All retroviruses contain minimally three genes, gag, pol and env encoding the structural proteins and enzymes required for virus replication. Lentiviruses have a more complex genome than other retroviruses with HIV-1 containing six addition regulatory and accessory proteins (tat,rev, vif,vpu,vpr and nef) that are required for the integration of the virus into the human cell and its replication (Brass et al., 2008)

The remarkable feature of these types of viruses is that just 6 viral accessory proteins usurp hundreds of proteins in the host cell machinery for its own integration into the cell chromosome and replication of viral particles. A knock down screen using small RNA molecules have identified more than 250 human cell HIV-dependency factors are required for the virus life cycle (Brass et al., 2008). Previous to this study only about 40 host proteins were demonstrated to interact with the virus derived proteins to enable viral replication. The study by Brass et al (2008) significantly expands the potential number of target sites for therapeutic development.

In our studies (in collaboration with Dr. Uckun at the Parker Hughes Cancer Center), we interrogated more than 12,600 gene products using the Affymetrix gene-chip technology; these are microarrays that have small portions of genes bonded to an inert substrate to which a fluorescently labeled sample is added and visualized, to identify genes are switched on/off in the presence of the virus. Microarrays allow for the temporal sequence of gene induction and suppression to be followed and hence determining the key regulatory events leading to the cell response and then its destruction. We followed messenger RNA levels over three time periods (24 hrs, 48 hrs and 7 days). Out of the 284 genes identified by the Brass et al (2008) study 211 were represented on the Affymetrix chip and of these 100 showed significant changes across the three time points. In summary the Brass et al (2008) knocked down single mRNA transcripts while our study characterized the temporal sequence of gene up and down-regulation Identification of host cell proteins required for HIV replication presents us with a new strategy for treatment.

New strategies for anti-HIV therapy.

Current strategies for HIV therapy involves targeting of specific viral proteins and blocking their function. However, new drug resistance viral strains are constantly

emerging and the general unavailability of broad spectrum anti-virals render this approach very challenging. Newer approaches that utilize the genome-wide responses of host cell messenger RNAs are beginning to identify critical host genes for viral replication. In one approach, host cell genes can be altered or augmenting to aid anti-HIV therapy. For example, Interleukin-2 has been used to augment HAART in HIV infection, which accelerates normalization of CD4+ T-cell counts in infected individuals. In another approach, cellular processes like host-cell pathways and enzymes that are required for viral replication can be inhibited. Targets are discovered through basic knowledge of cellular enzymes and pathways that viral proteins interact with, or with genome-scale approaches like gene expression profiling to identify up-regulated parts of infected cell response. Functions of these upregulated genes include: 1) induced by virus to facilitate virus replication; 2) induced as part of cellular response to pathogen; 3) part of cellular response leading to pathogen clearance. Targeting genes in functions 1 and 2 would lead to reduced virus replication (for review see Kellam, 2006).

Both our studies and those performed by Brass et al (2008) suggested a role for the Nuclear factor kappa B (NF-KB) pathway for viral replication (IKKB, RelA). NF-KB is a ubiquitous rapid response transcription factor in cells involved in immune and inflammatory reactions, and exerts its effect by expressing cytokines, chemokines, cell adhesion moecules, growth factors, and immunoreceptors. NF-KB is also required for transcription of viral proteins once the viral DNA is inserted into the host chromosome. This pathway has been well characterized and modeled using ODEs.

Nuclear Factor Kappa B pathway as a model to assess target site for anti-HIV therapy.

Cytosolic calcium ([Ca2+]i) oscillations have been demonstrated to be necessary for signaling in all cells. Computational modeling and imaging studies showed that these oscillations contribute to the efficiency or specificity of cellular signaling (Dolmetsch et al., 1998) such that oscillations reduce the effective Ca2+ threshold for activating transcription factors, thereby increasing signal detection at low levels of stimulation. In addition, specificity is encoded by the oscillation frequency: rapid oscillations stimulate NF-AT, Oct/OAP transcription factors, whereas infrequent oscillations activate only NF-kappaB. This observation gave incentive to develop more complete models for NF-KB activation (Hoffmann et al, 2002; Nelson et al., 2004; Ihekwaba et al., 2004). Most detailed model contains 64 parameters and 26 variables, including steps in which the activation of the NF-kappaB transcription factor is intimately associated with the phosphorylation and ubiquitination of its inhibitor kappaB by a membrane-associated kinase, and its translocation from the cytoplasm to the nucleus (Ihekwaba et al., 2004).

Proposal Objectives:

1. Write and run the published models for NF-KB activation and compare with the range of dynamics already published for this pathway.
2. Gene expression changes suggest that HIV infection affects at least two components of this pathway (IKKB and RelA). Determine how the dynamics are affected by changing the levels of NF-KB and Rel A.
3. Screen for low-dimensional manifolds in this system of ODEs.

Objectives 1 and 2 will yield under what conditions changing the levels of RelA/IKKB will result in abolishing of NF-KB mediated transcription. Many other pathways are also affected by HIV infection so the initial study of the NF-KB pathway will serve as a model for investigations and screening other less well defined pathways. Also, establishing what parameters are required to perform these types of simulations will be an important outcome.

It will be interesting to determine if there are low-dimensional manifolds in the system of ODEs governing NF-KB activation. Existence of these manifolds will show which components of the pathway have a correlated response. This will be very useful in reducing the complexity of the dynamics and also show which components of the pathway are transmitting the signal to the gene promoter. Genome-wide gene expression changes are routinely analyzed for co-regulated genes. Genes that show a high degree of correlations for any given pathway could signature the presence of low-dimensional manifolds. The NF-KB pathway may serve as a model for identification of other pathways with low-dimensional manifolds that have not yet been characterized.

References and abstracts.

Brass AL, Dykxhoorn DM, Benita Y, Yan N, Engelman A, Xavier RJ, Lieberman J, Elledge SJ. (2008). Identification of Host Proteins Required for HIV Infection Through a Functional Genomic Screen. Science Jan 10 [Epub ahead of print].

Dolmetsch RE, Xu K, Lewis RS. Calcium oscillations increase the efficiency and specificity of gene expression. Nature. 1998 Apr 30;392(6679):933-6.

Cytosolic calcium ([Ca2+]i) oscillations are a nearly universal mode of signalling in excitable and non-excitable cells. Although Ca2+ is known to mediate a diverse array of cell functions, it is not known whether oscillations contribute to the efficiency or specificity of signalling or are merely an inevitable consequence of the feedback control of [Ca2+]i. We have developed a Ca2+ clamp technique to investigate the roles of oscillation amplitude and frequency in regulating gene expression driven by the proinflammatory transcription factors NF-AT, Oct/OAP and NF-kappaB. Here we report that oscillations reduce the effective Ca2+ threshold for activating transcription factors, thereby increasing signal detection at low levels of stimulation. In addition, specificity is encoded by the oscillation frequency: rapid oscillations stimulate all three transcription factors, whereas infrequent oscillations activate only NF-kappaB. The genes encoding the cytokines interleukin (IL)-2 and IL-8 are also frequency-sensitive in a way that reflects their degree of dependence on NF-AT versus NF-kappaB. Our results provide direct evidence that [Ca2+]i oscillations increase both the efficacy and the information content of Ca2+ signals that lead to gene expression and cell differentiation.

Hoffmann A, Levchenko A, Scott ML, Baltimore D. The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation. Science. 2002 Nov 8;298(5596):1241-5.

Nuclear localization of the transcriptional activator NF-kappaB (nuclear factor kappaB) is controlled in mammalian cells by three isoforms of NF-kappaB inhibitor protein: IkappaBalpha, -beta, and - epsilon. Based on simplifying reductions of the IkappaB-NF-kappaB signaling module in knockout cell lines, we present a computational model that describes the temporal control of NF-kappaB activation by the coordinated degradation and synthesis of IkappaB proteins. The model demonstrates that IkappaBalpha is responsible for strong negative feedback that allows for a fast turn-off of the NF-kappaB response, whereas IkappaBbeta and - epsilon function to reduce the system's oscillatory potential and stabilize NF-kappaB responses during longer stimulations. Bimodal signal-processing characteristics with respect to stimulus duration are revealed by the model and are shown to generate specificity in gene expression.

Ihekwaba AE, Broomhead DS, Grimley RL, Benson N, Kell DB. Sensitivity analysis of parameters controlling oscillatory signalling in the NF-kappaB pathway: the roles of IKK and IkappaBalpha. Syst Biol (Stevenage). 2004 Jun;1(1):93-103.Links

Analysis of cellular signalling interactions is expected to create an enormous informatics challenge, perhaps even greater than that of analysing the genome. A key step in the evolution towards a more quantitative understanding of signalling is to specify explicitly the kinetics of all chemical reaction steps in a pathway. We have reconstructed a model of the nuclear factor, kappaB (NF-kappaB) signalling pathway, containing 64 parameters and 26 variables, including steps in which the activation of the NF-kappaB transcription factor is intimately associated with the phosphorylation and ubiquitination of its inhibitor kappaB by a membrane-associated kinase, and its translocation from the cytoplasm to the nucleus. We apply sensitivity analysis to the model. This identifies those parameters in this (IkappaB)/NF-kappaB signalling system (containing only induced IkappaBalpha isoform) that most affect the oscillatory concentration of nuclear NF-kappaB (in terms of both period and amplitude). The intention is to provide guidance on which proteins are likely to be most significant as drug targets or should be exploited for further, more detailed experiments. The sensitivity coefficients were found to be strongly dependent upon the magnitude of the parameter change studied, indicating the highly non-linear nature of the system. Of the 64 parameters in the model, only eight to nine exerted a major control on nuclear NF-kappaB oscillations, and each of these involved as reaction participants either the IkappaB kinase (IKK) or IkappaBalpha, directly. This means that the dominant dynamics of the pathway can be reflected, in addition to that of nuclear NF-kappaB itself, by just two of the other pathway variables. This is conveniently observed in a phase-plane plot.

Kellam P.
Attacking pathogens through their hosts.
Genome Biol. 2006;7(1):201. Epub 2006 Jan 30. Review.

Nelson DE, Ihekwaba AE, Elliott M, Johnson JR, Gibney CA, Foreman BE, Nelson G, See V, Horton CA, Spiller DG, Edwards SW, McDowell HP, Unitt JF, Sullivan E, Grimley R, Benson N, Broomhead D, Kell DB, White MR. Oscillations in NF-kappaB signaling control the dynamics of gene expression. Science. 2004 Oct 22;306(5696):704-8.

Signaling by the transcription factor nuclear factor kappa B (NF-kappaB) involves its release from inhibitor kappa B (IkappaB) in the cytosol, followed by translocation into the nucleus. NF-kappaB regulation of IkappaBalpha transcription represents a delayed negative feedback loop that drives oscillations in NF-kappaB translocation. Single-cell time-lapse imaging and computational modeling of NF-kappaB (RelA) localization showed asynchronous oscillations following cell stimulation that decreased in frequency with increased IkappaBalpha transcription. Transcription of target genes depended on oscillation persistence, involving cycles of RelA phosphorylation and dephosphorylation. The functional consequences of NF-kappaB signaling may thus depend on number, period, and amplitude of oscillations.