Large-Scale Screening


Functional mapping of Toll/interleukin-1 signalling networks by expression cloning

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Abstract

Multiple cellular proteins have been identified as participating in Toll/interleukin-1 receptor-mediated inflammatory gene expression. The continuing isolation of novel components, based on sequence similarities, protein–protein interactions and protein purification, suggests that many elements of this signalling network remain to be identified. We report here the development of a high-throughput functional screening platform and its application for the identification of components of inflammatory signalling networks. Our results enable us to estimate that 100–150 gene products are involved in controlling the transcription of the human interleukin 8 gene. The approach, which is simple and robust, constitutes a general method for mapping signal transduction systems and for rapid isolation of a large number of signalling components based on the control of pathways leading to regulation of gene expression.

The emerging view of signal transduction networks is that of stable, self-organizing dissipative systems capable of robust self-regulation over a limited range of environmental conditions and subject to catastrophic cascading failure when pushed outside this range. A conceptual framework has been proposed for the analysis of such systems – HOT (highly optimized tolerance) [1]. These systems show many emergent properties, not directly inferable from ‘parts lists’ such as those being provided for an increasing number of species by genome sequencing. A characteristic property of HOT systems is that the number of elements with control functions far exceeds the number of elements involved in basic ‘engineering’ functions: in biological systems, the former is represented by protein phosphorylation and the latter by the tricarboxylic acid cycle, for instance. Thus genome sequencing has highlighted the need for novel, general and rapid methods for assigning a function to genes/gene products. In the absence of these, attempts to reverse engineer or produce realistic models of signal transduction networks will continue to be frustrated by missing key elements.

A main scientific interest at the cellular level relates to the rules of organization for intracellular signal sensing and signal-processing systems. The rapid progress in recent years has revealed close to the complete map of the euchromatic regions of the human genome with very significant advances being made in mapping the entire human transcriptome as well, a project that is being replicated in many other uni- and multicellular organisms. Since a near-comprehensive map of the human genome has become available, a number of genomics efforts have attempted to assign function to the large number of novel genes uncovered. Many of these annotations are based on identity, with all the potential pitfalls of this approach. To complement these efforts, we have recently developed a novel screening strategy to enable rapid functional assignment of proteins expressed from cDNA libraries [2–5]. The strategy exploits the observation that overexpression of most signalling intermediates mimics the effect of extracellular agonists [6–8]. Thus components of signalling networks can be identified by co-transfecting cells with cDNA expression libraries and an agonist-responsive reporter. The method has the capacity to screen entire cDNA expression libraries and has the potential of detecting all/most gene products that activate the reporter following overexpression. As a readout, a promoter construct of the pro-inflammatory cytokine-sensitive IL-8 (interleukin 8) chemokine gene was coupled with the reporters, destabilized

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Abbreviations used: HOT, highly optimized tolerance; IL-8, interleukin 8; MAPK, mitogen-activated protein kinase.

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EGFP (enhanced green fluorescent protein) or luciferase respectively. IL-8 is a major effector gene, whose expression is regulated upon activation of the Toll/IL-1 signalling pathways. It is therefore an ideal reporter to map pro-inflammatory signalling networks. By screening a cDNA library generated from peripheral blood mononuclear cells, we have isolated both repressors and activators of signalling. Statistical analysis of data from the screen shows a mean hit rate of approx. 1/7000 clones. Mathematical modelling of the hit-finding process suggests that screening 30% of the library (~1 million clones) will result in the isolation of approx. 90% of the detectable bioactive cDNA clones. After screening 11% of the library (~221,000 colonies), 133 positive cDNA pools have been isolated, the strongest 32 of which were broken down and the bioactive insert sequenced. The clones isolated to date corresponded to 28 genes, with some genes detected multiple times. We estimate, therefore, that screening the entire library (~3 million clones) will lead to the identification of at least 100–150 genes with IL-8 regulatory function. Our current data together with previous findings demonstrate that components with a wide variety of mechanisms of action can be isolated. Thus the hits include cytokines, intermediate regulators of redox, MAPK (mitogen-activated protein kinase) and NF-κB (nuclear factor κB) pathways and relevant transcription factors both with positive and negative effects. In addition, 10 out of the 28 identified genes encoded proteins with previously unknown function or with function not known to be related to inflammatory signalling, suggesting that many components of this signalling network remain to be identified. Initial characterization of one of the hits that encodes a novel protein, tribbles (which regulates MAPK signalling pathways), provides a proof of principle that entirely novel families of signalling regulators remain to be identified [5,9].

In summary, the screening approach we have recently developed and implemented to map inflammatory signalling networks provides an additional, fruitful experimental tool for functional genomics, systems biology and signalling research.

References