

# A Systemic Network for *Chlamydia pneumoniae* Entry into Human Cells<sup>∇</sup>

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**Bacterial entry is a multistep process triggering a complex network, yet the molecular complexity of this network remains largely unsolved. By employing a systems biology approach, we reveal a systemic bacterial-entry network initiated by *Chlamydia pneumoniae*, a widespread opportunistic pathogen. The network consists of nine functional modules (i.e., groups of proteins) associated with various cellular functions, including receptor systems, cell adhesion, transcription, and endocytosis. The peak levels of gene expression for these modules change rapidly during *C. pneumoniae* entry, with cell adhesion occurring at 5 min postinfection, receptor and actin activity at 25 min, and endocytosis at 2 h. A total of six membrane proteins (chemokine C-X-C motif receptor 7 [CXCR7], integrin beta 2 [ITGB2], platelet-derived growth factor beta polypeptide [PDGFB], vascular endothelial growth factor [VEGF], vascular cell adhesion molecule 1 [VCAM1], and GTP binding protein overexpressed in skeletal muscle [GEM]) play a key role during *C. pneumoniae* entry, but none alone is essential to prevent entry. The combination knockdown of three genes (coding for CXCR7, ITGB2, and PDGFB) significantly inhibits *C. pneumoniae* entry, but the entire network is resistant to the six-gene depletion, indicating a resilient network. Our results reveal a complex network for *C. pneumoniae* entry involving at least six key proteins.**

*Chlamydia pneumoniae*, an obligate intracellular bacterium of the family *Chlamydiaceae*, is a highly prevalent pathogen implicated in severe human diseases such as respiratory tract infections, atherosclerosis, heart attacks (myocardial infarction), and asthma (10). These diseases, like other infectious diseases, are initiated by *C. pneumoniae* entry (10, 16, 17). Therefore, understanding the mechanism of *C. pneumoniae* entry is an important primary focus of *C. pneumoniae* studies.

Chlamydial entry is a multistep process, primarily including attachment, receptor binding, and endocytosis. The initial attachment of chlamydiae to cells is thought to occur through reversible electrostatic interactions with heparan sulfate-like glycosaminoglycans (10, 49). *C. pneumoniae* then binds to cellular receptors that trigger signal transduction, leading to endocytosis with cytoskeleton remodeling (10, 17). Several proteins have been proposed as cellular receptors critical for entry of the infectious elementary body (EB), such as epithelial membrane protein 2 (37), mannose 6-phosphate/insulin-like growth factor 2 receptor (33), platelet-derived growth factor receptor (19), and the Tom complex (18). However, antibody blocking of these receptors individually only partly inhibits chlamydial infection (19, 33), leading to a recent suggestion that multiple receptors and pathways are involved in EB entry (13, 17).

Two families of proteins are involved in cytoskeleton remodeling during endocytosis: ADP-19 ribosylation factor 6 and Rho GTPases (4, 17). Both of these mediate different steps of

actin reorganization during the internalization of chlamydiae. Downstream kinases following these two families of proteins include phosphatidylinositol 3-kinase, MEK-ERK kinases and ERK 1/2, and the adaptor protein Shc (14). Two other families of proteins are also activated during endocytosis of *C. pneumoniae* entry: focal adhesion kinase (FAK) and dynamin, which bind to several proteins interacting directly or indirectly with F-actin and connect the endocytic machinery to the actin cytoskeleton (10, 13, 33). The above proteins and pathways for attachment, receptor engagement, and endocytosis interact in a complex network that spatially and temporally mediates chlamydial entry, which remains largely elusive.

It is difficult for traditional genetics and biochemistry to characterize a system of this complexity, but a systems biology network approach (e.g., analysis of system-wide protein-protein interaction networks) can greatly facilitate this discovery. Protein interactions can be extracted from published databases and can be combined to form genome-wide comprehensive networks (8, 9, 12). Systemic analysis of these networks can simultaneously elucidate possible pathway components and the cross talk among these components in response to *C. pneumoniae* infection.

In this study, we used a systems biology approach to systematically elucidate a comprehensive systemic network response to EB attachment/entry. Key components in this entry network were also identified by *in silico* analysis and experimental validation. Our work provides a conceptual framework for further understanding of the fundamental molecular basis of EB entry.

## MATERIALS AND METHODS

***C. pneumoniae* propagation.** *C. pneumoniae* strain A03 (a gift from James Summersgill) was previously isolated from the coronary artery of a patient with coronary atherosclerosis (34). This clinical isolate was propagated in HEp-2 cells following standard laboratory protocols (30, 31, 34). Isolates were purified using 30% RenoCal centrifugation (27) to eliminate ~0.1% contamination from human cell material.

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**HCAEC culture and infection.** In order to compare human coronary artery endothelial cells (HCAEC) with other cell culture model systems for *C. pneumoniae*, we first performed an infectivity assay. The infectivity for HCAEC of *C. pneumoniae* was essentially equivalent to that for human peripheral blood monocyte leukemia (Thp1) cells, which are typically used to test protein-protein interactions (44). The infectivity was nearly half that for HEP-2 cells, which are routinely used to propagate the organism (27). Endothelial cell basal medium 2 with human epidermal growth factor (hEGF), hydrocortisone, GA-1000, fetal bovine serum (FBS), vascular endothelial growth factor (VEGF), human fibroblast growth factor—basic with heparin (hFGF-B), revitropin insulin-like growth factor (R3-IGF-1) and ascorbic acid [EGM-2-MV Bullet kit; Clonetics, East Rutherford, NJ] was used to grow HCAEC (Clonetics) according to the manufacturer's instructions. HCAEC cells were infected with *C. pneumoniae* at 0-min, 5-min, 25-min, and 2-h time points at a multiplicity of infection (MOI) of 0.5.

**RNA extraction, microarray hybridization, and array detection.** Cells were collected by centrifugation. RNA was purified using an RNeasy RNA purification kit (Qiagen Inc. Valencia, CA), including on-column treatment with DNase to eliminate all traces of DNA, according to the manufacturer's instructions. Affymetrix human genome U133 Plus 2.0 arrays (Affymetrix, Santa Clara, CA) were employed in this study. GeneChip one-cycle target labeling and control reagents (Affymetrix) were used for processing RNA and for hybridization to the microarrays following the manufacturer's protocols.

**RNAi knockdown.** To avoid cell line bias, RNA interference (RNAi) experiments were also performed in HEP-2 cells in addition to HCAEC. Cells were cultured to 60% confluence, and small interfering RNAs (siRNAs), including scrambled siRNA as a control, were transfected at a 75 nM final concentration, using 0.45% Oligofectamine (Invitrogen, Carlsbad, CA) in a 48-well format. After 36 h of siRNA-mediated gene knockdown, the medium was removed and the cells were infected with *C. pneumoniae* at an MOI of 0.5. After an additional 48 h of incubation, each experiment was analyzed for quantitative gene expression as described below. All experiments were performed in triplicate in each cell line, and the results are shown as averages and standard deviations.

**Quantitative RT-PCR.** Total RNA was extracted using an RNeasy RNA isolation kit (Qiagen, Valencia CA). DNA was digested with RQ DNase (Promega), and cDNA was generated using a reverse transcription kit (ABI). Quantitative reverse transcription-PCR (qRT-PCR) was carried out with a Power SYBR green PCR kit (ABI) following the manufacturer's instruction. The relative amount of target gene mRNA and 16S bacterial rRNA was normalized to host cell numbers using beta-actin mRNA.

**Network assembly.** We assembled a protein-protein interaction network by combining the existing network databases and using systems network approaches as previously adopted (8, 12). Briefly, our network database included proteins and interactions from BIND (<http://bond.unleashedinformatics.com/Action>), DIP (<http://dip.doe-mbi.ucla.edu/>), HPRD (<http://hprd.org/>), PreBIND (<http://www.blueprint.org/>), the curated inflammatory disease database, and the EMBL human database (9, 35, 36, 46).

**Network analysis.** The microarray data were analyzed using the Bioconductor in R project (22), the preliminary array quality assessment with the AFFYQCReport package, the background adjustment and normalization with the AFFY package, and the gene expression value estimation with the LIMMA package. Genes with *P* values of <0.05 and a fold change of >2 between infection and control were considered significantly altered by infection.

Genes with significant alterations in gene expression were used to overlap components in the protein interaction network as previously described (8, 12). These overlapped networks became the networks activated (up- and downregulated) during *C. pneumoniae* entry. The network was analyzed by using Network Analyzer (<http://med.bioinf.mpi-inf.mpg.de/netanalyzer/index.php>). The activated networks were decomposed into functional modules based on topological interconnection intensity and gene function (<http://www.geneontology.org/>) (3, 21, 28, 39, 40). Genes were classified according to the gene ontology database (<http://www.geneontology.org/>) (21).

## RESULTS

**A systemic network activated by *C. pneumoniae* entry.** To systematically decode the chlamydial entry network, we applied a protein-based network approach to analyze an expression profile altered by *C. pneumoniae* entry. Human coronary artery endothelial cells (HCAEC), a model cell line for studying atherosclerosis, were infected with *C. pneumoniae*, and a genome-wide gene expression profile of *C. pneumoniae* attach-

ment and entry was measured at 5 min (corresponding to *C. pneumoniae* attachment), 25 min (attachment and early entry), and 2 h (entry) (see Materials and Methods). We assembled a protein interaction network by integrating the known protein interaction databases as previously described (8, 9, 12) (see Materials and Methods).

To integrate the gene expression profiles with the combined network database, genes with significantly altered expression during *C. pneumoniae* entry were mapped to their corresponding proteins in the network, and the overlaid network became a network activated by *C. pneumoniae* entry (Fig. 1). This activated network was decomposed into functional modules using network topology and gene ontology databases (see Materials and Methods). Due to gene pleiotropy, only the primary functions were used to cluster these genes into modules, and genes were grouped primarily by their first biological functions/processes (Fig. 1). A total of 9 modules were activated during *C. pneumoniae* entry, including cell adhesion, receptor systems, actin-linked process, cell communication, cell-cell signaling, cell cycle, calcium transport, transcription, and endocytosis (Fig. 1B).

Consistent with previous reports about *C. pneumoniae* entry pathways (13, 16, 17), the network includes almost all known pathways and their components upregulated by *C. pneumoniae* entry. Such pathways included heparin binding and receptor activity (heparin-binding EGF-like growth factor [HBEGF]) in the receptor group, cell-cell adhesion (intercellular adhesion molecule 1 [ICAM1] [CD54]) in the cell adhesion group, RAS dexamethasone-induced 1 GTPase activity (RASD1) in the cell-cell communication group, Rho family GTPase 1 GTPase activity (RND1) in the actin-linked group, transcription factors located in the nucleus, cytokines located in the extracellular space, and components for calcium movement in the calcium transport group. These data reveal a complex cellular network involved in *C. pneumoniae* entry, which comprises several functional subnetworks that are functionally dominated by receptor activity, signal transduction and communication, cell adhesion, and transcription.

**Network modules dynamically activated for *C. pneumoniae* entry.** To examine how the network was dynamically activated to cope with *C. pneumoniae* entry, we calculated the sequential alteration of the mean of gene expression for each functional module against time ( $D_{\text{expression}}/D_t$ ) (Fig. 2). The modules of cell adhesion, calcium transport, and cell cycle were dramatically activated immediately after *C. pneumoniae* attached to cell surfaces (Fig. 2A). This was followed by receptor system, transcription, and actin-associated-process activation (Fig. 2B). Finally, endocytosis, cell communication, and cell signaling were activated (Fig. 2C). Throughout these processes, modules were distributed across the extracellular space to the nucleus (Fig. 1). This suggested that *C. pneumoniae* entry involves a delicate network in which functional modules ranging from cell adhesion to endocytosis coordinate precisely in time and space.

**Key proteins in the *C. pneumoniae* entry network.** Network hubs are likely to play essential roles in the biological networks (5, 26, 41, 48). Hubs are nodes with high connectivity to other nodes and can be identified via examining their contributions to network interconnectivity and diameter. This is determined

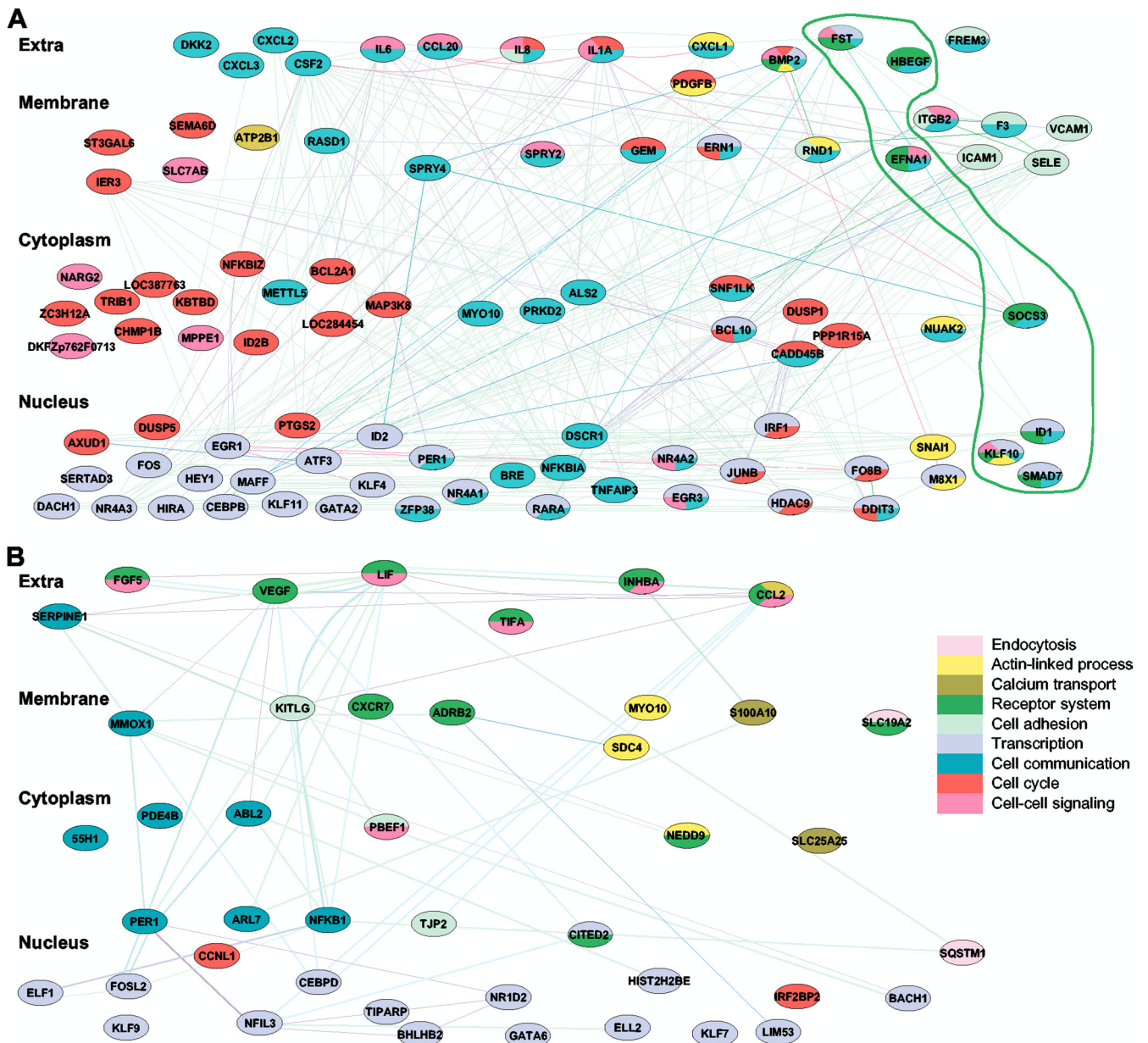


FIG. 1. Systemic network and modules involved in *C. pneumoniae* attachment/entry. Enhanced genes that were overlapped from *C. pneumoniae* attachment to entry (5 min, 25 min, and 2 h postinfection) were clustered into functional groups and combined into a network. This composition was treated as a *C. pneumoniae* entry network. Only the primary functions for each gene are indicated and colored in the index. Cellular components are shown on the left. (A) *C. pneumoniae* entry network, including the functional groups (highlighted). (B) Unique network activated at 2 h, in which genes are not overlapped with those upregulated at other time points.

by comparing knockouts and calculating changes in the average number of neighbors, which indicates interconnectivity and changes in the mean shortest path that measures the smallest number of links between selected nodes and essentially indicates network diameter. Node knockouts in a network decrease network interconnectivity, and knockouts of nodes with higher connectivity lead to greater decreases in network interconnectivity. The length of the network diameter is inversely related to its interconnectivity. Knocking out a hub would increase the network diameter because of the loss of short paths in a network.

To probe the key components in the *C. pneumoniae* receptor network, we first identified *in silico* the network hubs by measuring the contribution of individual network components to the network through knocking out *in silico* individual nodes. Special attention was paid to the network components located in the extracellular space and membrane (Fig. 1) because they play crucial roles in initial steps of *C. pneumoniae* entry. The component colony-stimulating factor 2 (CSF2) contributed most to network connectivity and diameter (Fig. 1 and 3A), indicating that it serves as a potential hub in the *C. pneumoniae* entry network. Similarly, chemokine C-X-C motif receptor 7

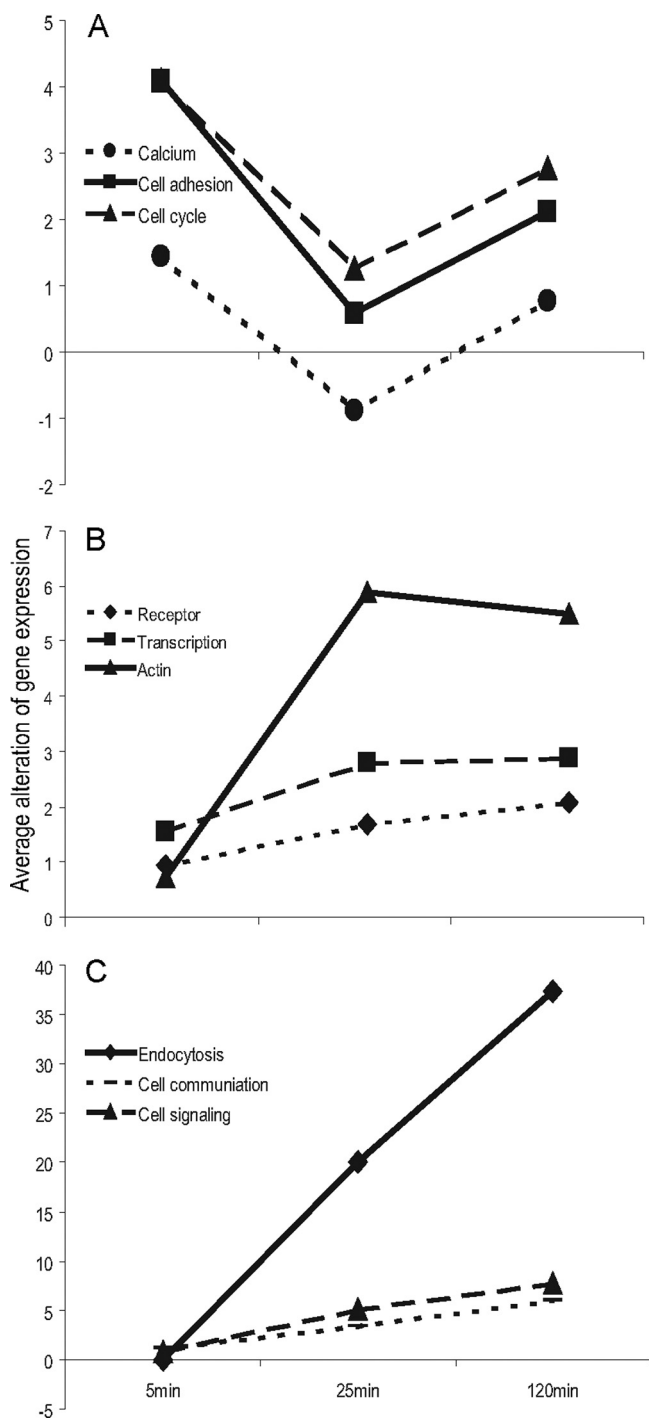


FIG. 2. Functional modules dynamically activated during *C. pneumoniae* entry. Average gene expression for each functional module altered by time ( $D_{expression}/D_t$ ) was plotted against time, and results were clustered into three groups (A to C) on the basis of their patterns. (A) Attachment; (B) activation; (C) endocytosis.

(CXCR7) and integrin beta 2 (ITGB2) are other potential network hubs (Fig. 1 and 3A).

To confirm our *in silico* prediction, we performed experiments to measure the effect of these hubs on *C. pneumoniae* infection by using RNAi knockdown hubs. We focused on hubs

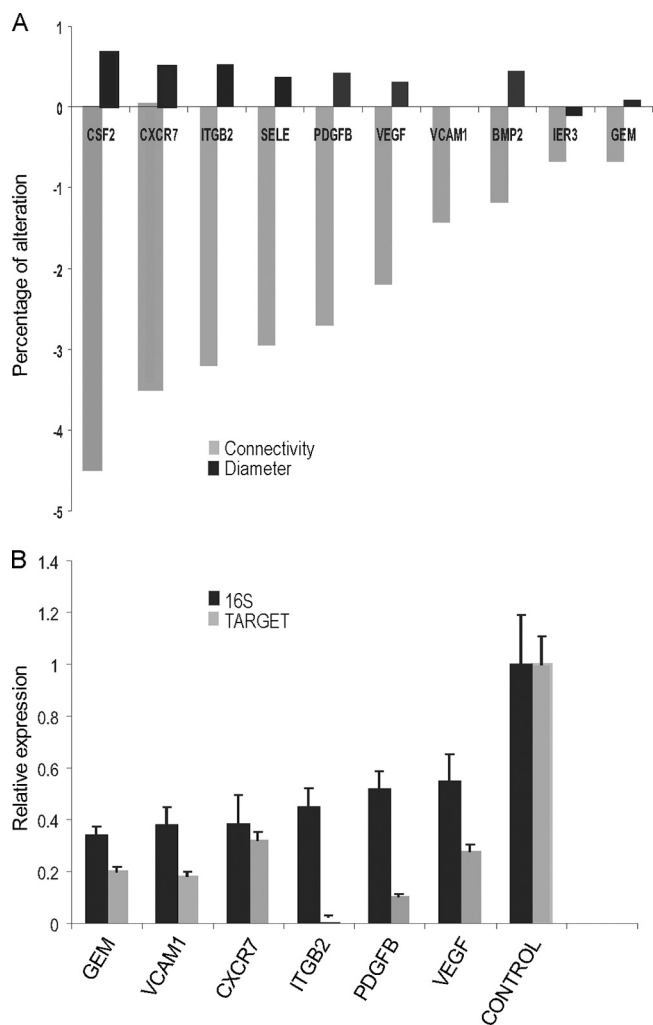


FIG. 3. Key proteins identified for *C. pneumoniae* entry. Key proteins in the network were predicted *in silico*, and then RNAi was used for knockdown of the predicted proteins. (A) Key proteins (network hubs) were predicted by using the percentage of alterations in the diameter, and the connectivity of the network individual hub was knocked out. (B) A total of six predicted hubs were successfully knocked down by RNAi, which significantly reduced *C. pneumoniae* infectivity. Data are averages of at least replicates of each cell line, as for Fig. 4.

functionally associated with cell adhesion, receptors, actin, and cell signaling because these categories of proteins are critical for bacterial entry and as potential *C. pneumoniae* receptors (13, 16, 17). These hubs included ITGB2, CXCR7, platelet-derived growth factor beta polypeptide (PDGFB), vascular cell adhesion molecule 1 (VCAM1), VEGF, and GTP binding protein overexpressed in skeletal muscle (GEM) (Fig. 1 and 3B). RNAi employed in this study successfully knocked down gene expressions of the corresponding targets (>75% [Fig. 3B]). After gene knockdown, alterations of *C. pneumoniae* infectivity affected by hub knockdown were quantitated by real-time RT-PCR using 16S rRNA primers specific for *C. pneumoniae* (see Materials and Methods) in two cell lines, HCAEC and HEP-2, to minimize the cell line bias. Knocking down these 6 individual hubs significantly, but not completely, inhibited *C. pneu-*

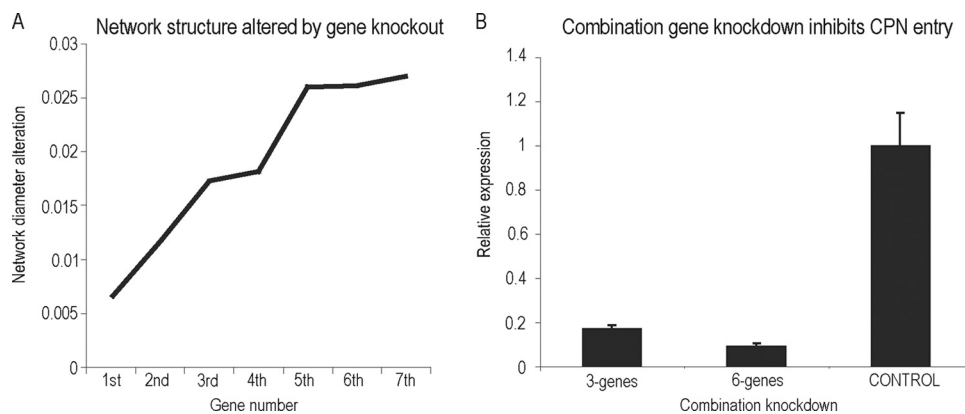


FIG. 4. Robustness of the *C. pneumoniae* entry network. (A) Gene combination knockout. The network diameter was dramatically changed after a 6-gene knockdown. (B) A combination of genes significantly reduced *C. pneumoniae* (CPN) infectivity. Knockdown of six genes almost entirely ablated *C. pneumoniae* infectivity, as measured by *C. pneumoniae* 16S rRNA expression using real-time qRT-PCR as described in Materials and Methods.

*moniae* infection (Fig. 3B). The inhibition ranged from 67% to 45% compared with controls (Fig. 3B). These results indicate that the potential hubs identified *in silico* are, in fact, important hubs in the *C. pneumoniae* entry network. The incomplete inhibition of *C. pneumoniae* entry by any single gene knockdown and the wide distribution of hubs throughout various functional modules, including cell adhesion (ITGB2 and VCAM1), receptor (VEGF and CXCR7), actin (PDGFB), and cell signaling (GEM), confirm that the *C. pneumoniae* entry network is very complex and includes multiple genes and modules.

**Robustness of network.** Knocking down a single gene only partially inhibited *C. pneumoniae* entry. We next investigated how many genes combined are sufficient to destroy the network and deplete *C. pneumoniae* infection. Thus, we examined the network robustness, or the resilience of the network. We first performed a sequential *in silico* combination gene knockout in the *C. pneumoniae* entry network and calculated the accumulating change in the network diameter after knocking out each node. A dramatic change of network diameter normally denotes a network disruption. Based on the contribution of each node to the network connectivity and diameter (Fig. 3A), we knocked out the selected genes *in silico* (Fig. 3B) in the following order: CXCR7, ITGB2, PDGFB, VEGF, VCAM1, and GEM. As expected, knocking out each node caused an increase in the accumulated network diameter, but the network diameter did not dramatically alter until 3-gene combination knockouts (CXCR7, ITGB2, and PDGFB) were performed. The entire network was set into a new state and was greatly disturbed (Fig. 4A). Furthermore, the entire network was almost destroyed by simultaneously knocking out all 6 genes (Fig. 4A). To validate this, we performed experiments using RNAi to knock down these genes simultaneously and measured *C. pneumoniae* growth by testing bacterial 16S rRNA levels. The combined knockdown of the first 3 genes (CXCR7, ITGB2, and PDGFB) significantly inhibits *C. pneumoniae* infectivity (>80%), and the combined knockdown of 6 genes (CXCR7, ITGB2, PDGFB, VEGF, VCAM1, and GEM) dramatically reduces *C. pneumoniae* infection (>90%) (Fig. 4B). This suggested that the entire *C. pneumoniae* receptor network exhibits strong resilience, and it would not be dramati-

cally destroyed until multiple genes (6 genes here) were simultaneously blocked.

## DISCUSSION

**Molecular complexity of *C. pneumoniae* entry.** The present study revealed the molecular complexity of *C. pneumoniae* entry using a systems biology network analysis of genome-wide gene expression profiles dynamically activated by *C. pneumoniae* entry. *C. pneumoniae* entry, which occurs within minutes of infection, alters the gene expressions of various pathway components, such as those involved in the immune response, calcium transport, and signal transduction (17, 33, 42, 45). These pathways, identified by traditional approaches, hypothetically interact to form a functional network in order to cope with *C. pneumoniae* stimulation. However, this network is too complex to be elucidated by traditional genetics, and it remains elusive. By taking advantage of the systems network approach, in which all known interactions and cross-talks among pathway components are included and interactions are treated as a complete network instead of linear circuits as in conventional approaches, we systematically elucidated a system network activated by *C. pneumoniae* entry (Fig. 1). The rich cross-talks between network components indicate that the network activated by *C. pneumoniae* entry is much more complex than previously thought.

Infectious agents can easily bind to cell surfaces via chemical interactions, but with low affinity. Microbe-specific receptors and coreceptors are required to strengthen these bindings, but they are not likely to be sufficient for a successful entry, which requires subtle contributions from other functional groups (modules). For instance, calcium transport and cytoskeletal movement are essential for surviving some receptor-ligand interactions and play crucial roles in strengthening microbe attachment to the cell surface (20). Similar roles exist for signal transduction, immune response, and chromatin remodeling (20). Therefore, a complex, coordinated network consisting of various network modules is required for microbe entry into cells (1, 23, 29, 32). Previous studies implied that *C. pneumoniae* entry requires an elaborate coordination of a series of

modules, including receptors, receptor tyrosine kinase signaling, and trafficking (11, 17, 18, 33, 42–43, 45). Here, we revealed that a successful *C. pneumoniae* entry requires nine modules, including receptor modules, endocytosis, actin-linked processes, calcium transport, cell adhesion, transcription, cell communication, cell cycle, and cell-cell signaling (Fig. 1). Since these modules contain all pathway components known to date to be related to *C. pneumoniae* entry, this network probably represents a typical network mediating *C. pneumoniae* entry.

Functional modules are regarded as the basic functional unit in biology and as more accurate than individual genes in describing functional patterns. Individual genes could be switched on or off in a short time, and they might not directly cause any change in biological functions, which might not lead to a change in phenotype. In contrast, functional modules normally perform specific functions and associate with a specific phenotype. Therefore, the dynamic pattern of functional modules instead of individual genes could be more representative of the complex process of *C. pneumoniae* entry. A total of nine network modules identified in this study dynamically switched their functions (Fig. 2) within a very short time (2 h) after *C. pneumoniae* infection. The major modules activated at 5 min serve in cell adhesion, calcium transport, and cell cycle, which facilitate bacterial attachment. At 25 min, modules function in receptor mediation, transcription, and actin remodeling, suggesting that these modules couple with *C. pneumoniae* binding and penetration into the membrane. At 2 h, modules serve in endocytosis, cell communication, and cell signaling, suggesting that *C. pneumoniae* passes through the membrane 2 h postinfection and translocates the membrane via endocytosis. These data indicated that bacterial entry requires a rapid cellular adjustment at the module level and that *C. pneumoniae* can complete an entry process in a very short time, from attachment via adhesion proteins to interaction with receptors and finally to entry via endocytosis.

Network module identification could be affected by network sources, and some identified modules might not be related to the stated biological functions (7). However, since the modules identified here were based on confident databases, network topology features, and gene functions from gene ontology databases, our set of modules in this study likely represents a featured class of protein complexes in a natural biological network stimulated by *C. pneumoniae* entry.

**Key cellular proteins for *C. pneumoniae* entry.** Many cellular proteins and their interaction partners are important for bacterial entry, but the central ones remain largely unknown. In *Chlamydia*, several individual proteins have been implicated in cell entry, such as mannose 6-phosphate/insulin-like growth factor 2 receptor for *C. pneumoniae* (33) and a platelet-derived growth factor receptor for *Chlamydia trachomatis* (19). However, inactivating each of them only partially reduces chlamydial entry (19, 33), suggesting that multiple proteins are responsible for *C. pneumoniae* entry, although they have not been systematically identified. Here, we systematically identified six proteins crucial for *C. pneumoniae* entry. Knocking them down individually using RNAi significantly inhibited *C. pneumoniae* entry (67% to 45%) (Fig. 3), but similar to previous observations, these depleting approaches did not completely block *C. pneumoniae* entry. This is consistent with a long-standing no-

tion in this field that there is no single receptor completely responsible for *C. pneumoniae* entry (13).

If no single protein is responsible for *C. pneumoniae* entry, multiple receptors should work together to account for *C. pneumoniae* entry, although there is a lack of studies to address this question. Here, we knocked out gene combinations *in silico*, leading to an alteration in the network structure (Fig. 4). Importantly, the network structure was strikingly destroyed when three or six genes were simultaneously knocked out. Experimental data also validated the *in silico* prediction that knocking down three or six key proteins dramatically inhibits *C. pneumoniae* entry (>80%) (Fig. 4), suggesting that *C. pneumoniae* entry requires multiple proteins and at least a triple combination of genes to dramatically block *C. pneumoniae* infection.

Among the genes identified above, most have been proven to mediate microbe entry into hosts. For example, CXCR7, ITGB2, and VCAM1 are important for virus entry; CXCR7 (RDC1) is a coreceptor for human immunodeficiency viruses (HIV) (38); VCAM-1 is a receptor for encephalomyocarditis virus (24); ITGB2 encodes the integrin beta chain beta 2; and integrin serves as receptor and coreceptor for virus entry (2, 47). The involvement of chemokine receptor (CXCR7), adhesion molecule (VCAM-1), and integrin (ITGB2) during *C. pneumoniae* entry indicated that the *C. pneumoniae* entry system involves multiple proteins. Additionally, this system is similar to that of viruses, suggesting that microbes can share some fundamental mechanisms during their entry into host cells.

In this study, we used gene expression data and overlapped a protein interaction database to enrich a *C. pneumoniae* entry network. Gene expression data may not be completely consistent with protein level data, but genomics data measured by the Affymetrix microarray employed here was generally consistent with proteomics data (15). Our result for the *C. pneumoniae* entry network should represent a natural protein interaction network triggered by *C. pneumoniae* entry, which generally parallels the recent observations showing that a complex network is involved in pathogen entry (6, 25). The multiple proteins for *C. pneumoniae* entry revealed here are also similar to the recent discoveries in viruses in which multiple receptors and coreceptors are required for virus entry (6). We actually expect more proteins crucial for *C. pneumoniae* entry to be uncovered in the near future with new concepts and technologies. For example, proteins downregulated during *C. pneumoniae* entry might also be important for *C. pneumoniae* entry because *C. pneumoniae* might inhibit them or hijack their functions in order to facilitate the entry process. Therefore, the results presented here represent only a basic *C. pneumoniae* entry network. Our results suggest that targeting one or two receptor proteins, as we and other have done (19, 33), may not efficiently block *C. pneumoniae* entry and prevent the spread of *C. pneumoniae* across cells. The rapid change in dynamic modules makes it challenging to develop an efficient strategy to block *C. pneumoniae* entry, but the key proteins and entry network identified here and the approach we have developed should lay a framework to further dissect the molecular complexity of *C. pneumoniae* entry and facilitate novel and efficient drug development.

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